Department of Primary Industries
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NSW GOVERNMENT

Xylella fastidiosa: on-farm preparedness guide for vineyards

NSW PRIMARY INDUSTRIES PLANT BIOSECURITY AND PRODUCT INTEGRITY

Leonie Martin

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Xylella fastidiosa: on-farm guide for vineyards

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A healthy vineyard. Photo: Andrew McMillan, PIXNIO.

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Xylella fastidiosa: on-farm preparedness guide for vineyards

Lead author: Leonie Martin
Plant Biosecurity Officer
NSW Department of Primary Industries
and Regional Development
Plant Biosecurity and Product Integrity
105 Prince Street, Orange, NSW 2800
M: 0428 569 822
E: leonie.martin@dpi.nsw.gov.au

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Background

Xylella fastidiosa is one of the most significant emerging plant disease threats worldwide. So far, we are fortunate that it is not currently in Australia. The overall aim is to keep Australia free of bacterial pathogens of the *Xylella* genus, as it is a devastating disease in many plant species, and there is no cure once a plant is infected.

Xylella fastidiosa has been categorised as Australia's number one National Priority Plant Pest (NPPP) due to its recognised potential to severely affect Australia's plant industries and the environment. It is known to infect approximately 712 plant species and could affect more than 20 plant industries if it were to become established in Australia. At-risk crops include olives, almonds, viticulture (dried, table and wine grapes), citrus, berries, cherries, coffee, macadamias, nursery and garden, pecans, and summer fruit (includes canned fruit).

Xylella fastidiosa infection goes by several names depending on the affected plant. It is often referred to as bacterial leaf scorch, but in grapes, it is known as Pierce's disease, California vine disease or measles, and Anaheim disease. In olives, it is known as olive quick decline syndrome; in peaches, it is called phoney disease; and in citrus, it is known as citrus variegated chlorosis.

Xylella fastidiosa is a xylem-limited bacterial infection transmitted to plants by sap-feeding insects such as spittlebugs and sharpshooters. The bacteria does not affect human health but it can kill plants by blocking their xylem, preventing the water transport system, and causing water stress, which presents as leaf-scorching symptoms. Water stress then leads to yellowing leaves, premature loss of leaves and dieback, reduced fruit size and quality and fruit failing to ripen. Progressive weakening of the plant over time causes vine death.

Xylella fastidiosa was recorded in California in the late 1800s when it devastated southern California vineyards. Further outbreaks in California in the 1930s and early 1940s revealed that sharpshooter leafhoppers and spittlebugs were vectors (Houston et al. 1947; Severin 1949, 1950).

It was finally confirmed as a bacterial infection in the late 1970s and has since spread around the world with the movement of asymptomatic plants. Infection has now been confirmed in the Americas, Middle East, Asia, and Europe. It has been causing issues for agriculture in Italy, France, Germany, Switzerland, and Spain over the last 10 years.

Introduction

This guide provides practical advice to assist growers with preventing and preparing for an *X. fastidiosa* outbreak in Australia. It includes information on:

- how to best prevent and manage the risks associated with *X. fastidiosa*
- what it is
- how it is likely to arrive in Australia
- what symptoms to look for
- how to conduct ongoing surveillance
- how to collect and send samples for testing.

IF YOU SEE ANYTHING UNUSUAL, CALL THE EXOTIC PLANT PEST HOTLINE

1800 084 881

The best chance of managing an *X. fastidiosa* outbreak in Australia is through early detection and reporting anything unusual to the **Exotic Plant Pest Hotline on 1800 084 881**. It is important to know what insects in Australia are known or have the potential to vector *X. fastidiosa*. Regular ongoing surveillance and sweep netting for insect vectors is a vital early warning tool to reduce the timeframe between infection and detection.

The California epidemic of *X. fastidiosa* in grapevines highlighted several key points that complicate managing the spread of the disease:

1. From an economic perspective, there is a point where significant levels of infection make maintaining the vineyard unprofitable. Determining the economic threshold has its challenges, as grape variety, yield, region, year, and relative contribution of primary and secondary spread for the vineyard must all be considered and for wine grapes, whether a vineyard is owned or operated by a winery.

2. *Xylella fastidiosa* is a serious pathogen of some species but can coexist with many other organisms without causing them harm. Hence, many plants, including natives, ornamentals, and crops, might be long-term point sources from which the pathogen is acquired by vectors and continues to spread.
3. Insect vectors have a wide host range and can disperse long distances in short hopping flights. They can be very persistent in various climates and are often able to overwinter as adults and feed on dormant grapevines. Once a vector acquires *X. fastidiosa* as an adult, it can inoculate plants through the remainder of its life. Some common host plant species are lavender, *Polygala myrtifolia*, oleander, magnolia, *Acacia cultriformis* and Marguerite daisy. Refer to '*Xylella fastidiosa* in other hosts' on page 13 for details on other hosts.

Entry and transmission pathways

Entry into Australia

The most likely ways that *X. fastidiosa* could arrive in Australia are by:

- The importation of infected plants or planting material such as budwood, cuttings or rootstock; these plants are unlikely to be displaying symptoms at the time of import.
- Insects infected with the disease. Two exotic plant-feeding insects of concern are the meadow spittlebug (*Philaenus spumarius*) and the glassy-winged sharpshooter (*Homalodisca vitripennis*). These are also subject to biosecurity measures to keep them out of Australia.

More efficient means of transport mean that the frequency of movement and likely establishment of pathogens in Australia are increasing. Australia has biosecurity officers, detector dogs and X-ray machines at the borders to ensure that host plants and insects that carry *X. fastidiosa* do not arrive with cargo, mail or passengers.

There are also many research projects to support early detection mechanisms for *X. fastidiosa* if it were to arrive in Australia. It is only through industry, government and the community working together that we have the best chance of detecting *X. fastidiosa* early.

Natural habitats are being turned into agroecosystems and monocultures by humans, and farming practices that place susceptible host populations close to each other further increase the effects of pathogens once introduced.

Sentinel hosts such as lavender and polygala could be used as an early warning system. They are highly susceptible and die quickly, which would assist with earlier detection.

Good general biosecurity practices

Do not buy plants or seeds online. It is important to know where your plants and seeds have come from and what they are intended for. Always buy seed and plant material from reputable Australian suppliers. If you are buying plants or seeds from overseas suppliers, then you must comply with the Biosecurity Import Conditions System (BICON) and get an import permit if required.

'Plate it, do not plant it' is an initiative from the Australian Department of Agriculture, Fisheries and Forestry encouraging people not to plant food products that are intended for eating (Table 1). When you purchase fresh fruit and vegetables meant to be eaten, make sure you eat them and do not plant them in the garden.

Fresh fruit and vegetables from the supermarket are intended for eating. By planting them, you could unknowingly introduce exotic pests and diseases into the Australian environment. These pests could damage your home garden and then spread to native vegetation and rural areas, threatening our environment and agricultural industries.

Plant-based goods (fruit, herbs, vegetables, spices, seeds) are imported into Australia for a variety of purposes and end uses and the import conditions will reflect this. Goods for growing, such as seeds, are different from foods and have a higher risk of carrying pests and diseases, so they have much tighter import conditions.

Table 1. 'Plate it, do not plant it' is an initiative from the Australian Department of Agriculture, Fisheries and Forestry encouraging people not to plant food products that are intended for eating.

Seeds from fresh fruit or vegetables	Seeds from dried fruit or vegetables	Seeds of spices and herbs
		
Fresh herbs and sprouts	**Fresh fruit or vegetables**	**Food scraps leftover from fresh fruit or vegetables**
		

Source: 'Plate it. Don't plant it', Department of Agriculture, Fisheries and Forestry (DAFF), https://www.agriculture.gov.au/biosecurity-trade/pests-diseases-weeds/protect-animal-plant/dont-plant-it.

Transmission pathways

Xylella fastidiosa can be transmitted and spread via grafting (Figure 1). This pathway can lead to rapid and widespread transmission (Smith et al. 1997) as the bacterium is directly transferred between infected and clean plant material.

Xylella fastidiosa is not transmitted by contaminated pruning shears. It is considered quite a benign pathogen as it does not survive for long outside of a live plant host. Likewise, it does not survive in cut plant material such as pruned branches. Seed transmission is also being investigated but is yet to be confirmed.

Various factors influence the level of bacterial transmission of *X. fastidiosa*, such as the density of bacterial populations in host plants, insect vector species, insect vector host range, plant preference of insect vectors, seasonal conditions, individual plant susceptibility and climate. The spread of *X. fastidiosa* depends on the population density of suitable xylem feeding vectors.

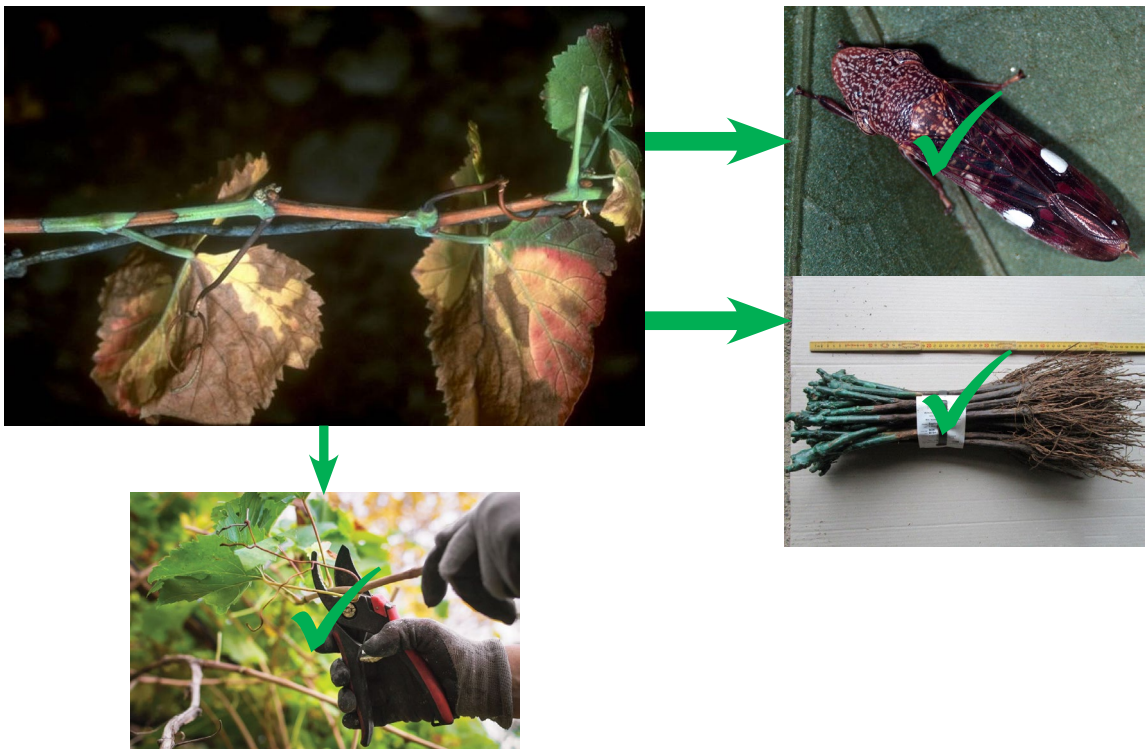

Figure 1. *Xylella fastidiosa* transmission pathways. Photos: top left, *Xylella fastidiosa* in grapevine (J Clark, University of California, Berkeley, US). Top right: glassy-winged sharpshooter (Reyes Garcia III, USDA Agricultural Research Service, Bugwood.org). Bottom right: grafted vines (Derek Pritchard, Dunkery Vineyard). Bottom left: pruning shears (The Tree Center).

Vector dispersal capacity is also an important factor in the spread of disease and is a determinant of vector population density and pathogen distribution among host plants and vectors (Purcell 1985). There is very little information available on dispersal abilities for many sharpshooter vectors. Glassy-winged sharpshooters (GWSS) actively move among several habitats and host plants, which allows for the rapid spread of *X. fastidiosa* over a wide area. Blue-green sharpshooters (BGSS) are more limited to riparian vegetation and neighbouring vineyards (Blua et al. 2001; Redak et al. 2004).

Vector species are not always considered important in crops but might still play a role in maintaining the infection cycle outside the affected crops (Purcell 1982). Outside sources of inoculum are important when epidemics are generated mainly by primary spread, as was observed in northern coastal areas and in the Central Valley of California, where adjacent riparian woods, lucerne fields and pastures served as a major reservoir of *X. fastidiosa* for Pierce's disease spread into vineyards (Hewitt et al. 1942; Purcell 1975).

There are risks associated with native vectors as well. While they might not be considered as pests, they could be important vectors. The number of potential species of native vectors is yet to be confirmed, and much is still to be learned about their biology, life cycles (Figure 2), feeding habits and distribution within New South Wales/Australia. Further research is required in this area.

Xylella fastidiosa insect vectors

All sucking insects that feed on xylem sap are potential vectors for *X. fastidiosa* (Table 2). Sharpshooters and leafhoppers (Hemiptera: Cicadellidae: Cicadellinae) were the first known vectors. However, the vector species list is increasing with the global spread of *X. fastidiosa*, with Hemiptera *Auchenorryncha* species belonging to the superfamily Cercopoidea (froghoppers and spittlebugs) now recognised as a major vector group of concern (Cornara et al. 2016).

An insect vector of major concern is the glassy-winged sharpshooter (*Homalodisca vitripennis*). The glassy-winged sharpshooter is an exotic pest, i.e. it is not present in Australia. A recent study has indicated there are Australian native species of froghoppers of concern, such as *Bathyllus albicinctus*. These insects are highly polyphagous, meaning they feed on many different plant species, which in the case of *X. fastidiosa*, increases the risk of disease transmission. Work continues to identify other possible Australian native vectors.

Table 2. Exotic and native vectors of *Xylella fastidiosa*.

Scientific name	Common name	Image	Photo source
Not currently in Australia			
Homalodisca vitripennis, formerly known as *H. coagulate*	Glassy-winged sharpshooter (GWSS)		Reyes Garcia III, USDA Agricultural Research Service, Bugwood.org
Oncometopia nigricans	Black-winged sharpshooter (BWSS)		Susan Ellis, Bugwood.org
Graphocephala atropunctata	Blue-green sharpshooter (BGSS)		Alex H Purcell, University of California, Berkeley, Bugwod.org
Clastoptera spp.	Spittlebugs		A cluster of immature spittlebugs on Gambel oak leaf. Photo: Melissa Schreiner, Colorado State University, Bugwood.org
Philaenus spumarius	Meadow froghopper/ meadow spittlebug		Anevrisme, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=2542344
Native to Australia			
Bathyllus albicinctus	Froghopper		TimL, NatureMapr, https://canberra.naturemapr.org/sightings/4258237

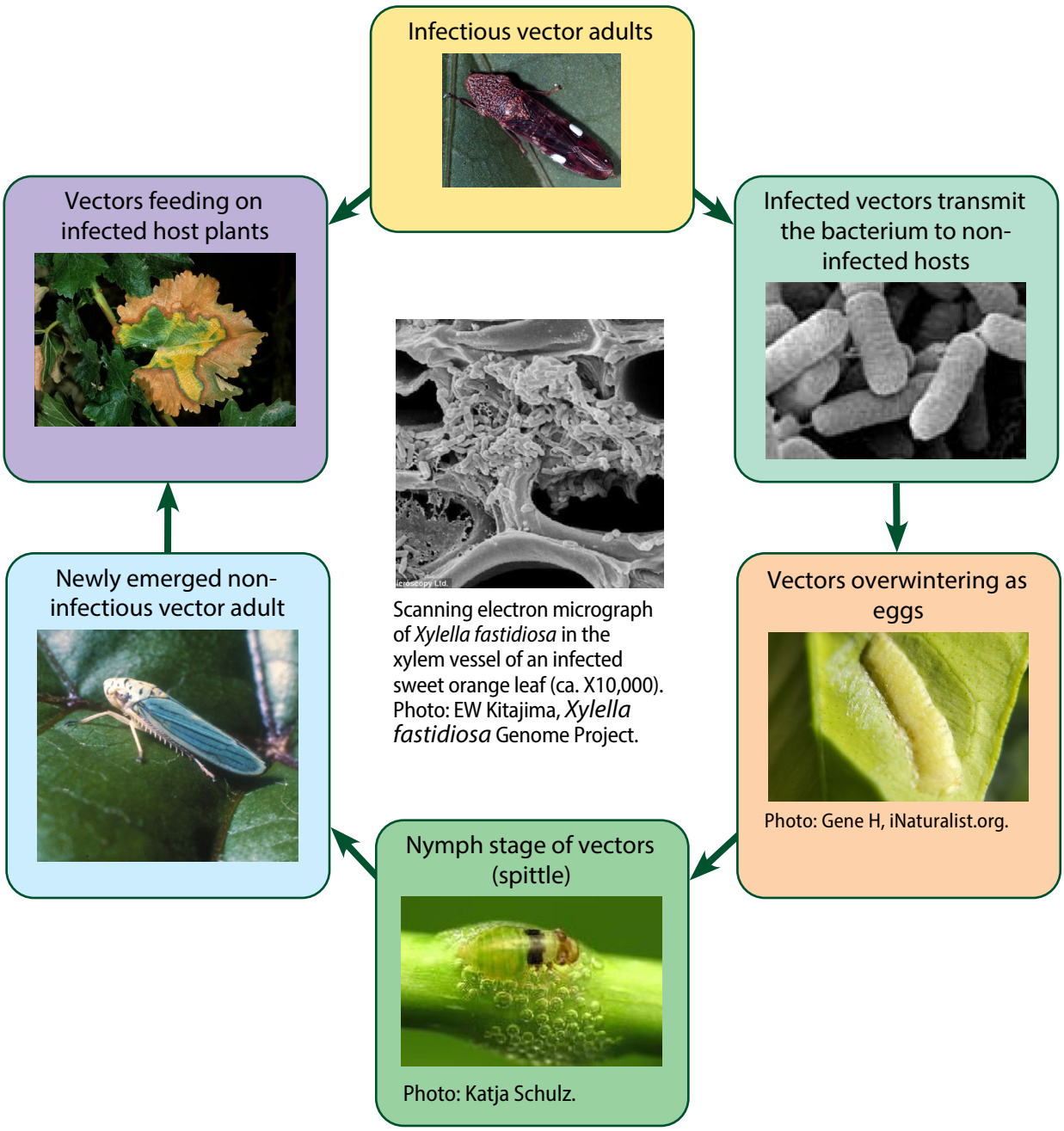

Figure 2. Overview of the *Xylella fastidiosa* life cycle. Source: adapted from Frem et al. (2021).

Detection

Detection of *X. fastidiosa* is difficult to predict and manage. Most often, when symptoms are seen in host plants, it is too late to control as the disease might have been present for some time. Plants can be asymptomatic for several months up to 2–3 years after contracting the infection (Almeida and Purcell 2005; FAO 2018). Early symptoms might resemble water stress or physiological disorders, delaying confirmation that *X. fastidiosa* is responsible.

Regular surveillance for symptoms and checking for *X. fastidiosa* vectors, such as the glassy-winged sharpshooter and native froghoppers, are essential to minimise the time between detection and implementing control activities. Timing is essential for managing this disease and its vectors if eradication or containment is to be successful.

The bacterium flourishes in the xylem of infected hosts and spreads to the shoots and root system systemically. It might relocate into the root system during colder weather and re-emerge in fresh growth once the plant actively begins to grow in the spring. In-field detection can be complicated

as some hosts do not express symptoms (i.e. they are asymptomatic), and some symptoms can be confused with biotic and abiotic factors such as water stress, nutrient deficiency, and failure to thrive.

The other thing to consider is the latency period, i.e. the time between contracting the infection and displaying symptoms. This can vary between host species and even within species depending on their level of *X. fastidiosa* susceptibility (Table 3). *Xylella fastidiosa* symptoms can vary between host species and the subspecies of *X. fastidiosa* infecting them.

Table 3. The latency period of *Xylella fastidiosa* in infected hosts. Source: Luck et al. (2010).

Pathogen species	Plant host	Latency period
Xylella fastidiosa subsp. *fastidiosa*	*Vitis vinifera*	2–3 months
Xylella fastidiosa subsp. *multiplex*	*Polygala myrtifolia*	3–4 months
Xylella fastidiosa subsp. *pauca*	*Polygala myrtifolia*	6–9 months
Xylella fastidiosa subsp. *pauca*	*Citrus* spp.	8–12 months
Xylella fastidiosa subsp. *pauca*	*Olea europaea*	9–14 months

Asymptomatic pathogen reservoirs

Xylella fastidiosa can be asymptomatic in a large number of host species. Some hosts might never develop symptoms, which makes detection difficult. This can facilitate the establishment and spread in a new environment for a long time before we know it is there. Despite not displaying symptoms, asymptomatic hosts can act as reservoirs for *X. fastidiosa*, where vectors can still acquire infection and spread the disease to new plants.

Climatic factors

Climate is an important factor in *X. fastidiosa* disease epidemiology. It can influence the distribution and size of vector populations, where and how host plants are grown, pathogen transmission and plant-pathogen infection dynamics.

Studies have shown that higher temperatures result in:

- greater feeding rates and survival of sharpshooter vectors
- higher transmission efficiency
- higher *X. fastidiosa* multiplication rate within the plant
- shorter incubation period and latency in plants
- greater persistence of infections in plants
- extreme weather conditions such as drought can exacerbate or bring on symptoms in infected plants and make symptoms more severe.

These can all translate to changes in the field for plant infection. For example, lower winter temperatures are associated with reduced *X. fastidiosa* infection levels and recovery of some infected plants. Alternatively, a combination of higher temperatures and rainfall is associated with higher vector population densities in the field, which poses a greater risk for *X. fastidiosa* establishment or spread (Sicard et al. 2018).

For early detection, it is best to target both plant sampling and vector collection during the warmer months as this will be the best time to detect *X. fastidiosa* in the field. The bacterium can retreat into plant roots during cold winter months, so it could go undetected in leaves and stems. The bacterium will then return to the growing parts of the plant, making it easier to sample. Insect populations tend to decrease in cold weather but peak in warmer months, so sampling from spring to autumn is best for field detection.

What to look for – symptoms

Xylella fastidiosa in grapevines

Muscadinia and native American cultivars display milder symptoms than those of *Vitis vinifera*. On *V. vinifera*, the initial symptoms are chlorotic spots (yellow spots) on areas of the leaf lamina (leaf blade), in particular along the margins, with a sudden drying of leaf edges often surrounded by a yellowish or a reddish halo (Hopkins and Purcell 2002).

Symptoms of *X. fastidiosa* vary depending on the *Vitis* species, cultivar, and local climatic conditions. *X. fastidiosa* subsp. *fastidiosa* has been the only subspecies reported to cause disease in grapevines (Nunney et al. 2010).

The characteristic symptom of *X. fastidiosa* in grapevines is leaf scorch (Figure 3). Leaves become yellow around the leaf margins or between the veins. The outer leaf area might dry suddenly while the rest of the leaf remains green. Affected leaves are less vigorous and smaller than healthy leaves. Concentric zones of discoloured and dead tissue (necrosis) are seen. The whole leaf might shrivel and drop, leaving only the leaf stalk attached.

Diseased stems often mature irregularly, with patches of brown and green tissue. These are known as 'green islands' (Figure 4). Flower clusters on infected vines might set berries but these usually dry up before reaching maturity.

It is just as important to look for symptoms in other plant species nearby such as olive, citrus, and almond trees, and ornamentals as early detection and reporting are essential.

Figure 3. Symptoms of *Xylella fastidiosa* on grapevine, left Chardonnay, right Merlot. Photos: Agriculture Service, Government of the Balearic Islands.

Figure 4. Symptoms of *Xylella fastidiosa* on grapevines. Left, match sticks; middle, affected grapes; right, green islands. Photos: Agriculture Service, Government of the Balearic Islands.

If you suspect you may have *Xylella fastidiosa*, then please report it to the **Exotic Plant Pest Hotline on 1800 084 881** or submit the online form. Ensure you provide good quality images of the plant symptoms and or the insect vectors and provide accurate contact details. Make sure you note the accurate location.

Infection severity

Xylella fastidiosa can kill grapevines by blocking the plant's water-conducting system. Susceptible grapevine cultivars can die within 1–2 years of the initial infection, although in several species and cultivars, they can live considerably longer. Symptoms are rarely seen in one-year-old plants.

Leaf symptoms vary with the grapevine species and cultivar. Grape varieties such as Pinot Noir and Cabernet Sauvignon show regular zones of progressive leaf margin discolouration and drying. Discolouration and scorching in the Thompson Seedless variety occurs in sectors of the leaf rather than as rings around the margins. Symptoms are usually more obvious in grapevines that are stressed by high temperatures or drought conditions. Climatic differences between regions can affect the timing and severity of the symptoms but not the type of symptoms.

In late summer and autumn, the necrotic leaf edges coalesce to form concentric rings that extend from the outer edge towards the centre. Subsequently, the leaf turns dry on the edges but remains turgid. The lamina might shrivel and drop while the petiole remains attached to the branch (the so-called 'match stick' effect; Figure 5). The latter is a characteristic symptom of Pierce's disease late in the season. Fruit clusters shrivel or turn into raisins, branches and twigs usually start wilting from the tip, and infected stems mature irregularly, showing patches of green tissue called 'green islands'. Buds on infected plants sprout later than those on healthy plants, and they are stunted, growing slowly, which can also present as uneven budburst (EPPO 2018).

Figure 5. Typical symptoms of *Xylella fastidiosa* in grapes. Left, leaf scorch and right, match sticks. Photos: Jack Kelly Clark, University of California, and Dr Lisa Overall, Rogers State University, respectively.

In the following year some canes might fail to bud out. New leaves are yellow and older leaves appear scorched. Infected vines might grow at a normal rate, but the quantity of new growth is less than that of healthy vines. In late summer, leaf scorching symptoms reappear. In later years, infected grapevines develop late and produce stunted yellow shoots.

Xylella fastidiosa in other hosts

Xylella fastidiosa has been detected on several hosts in the recent European outbreaks. Most symptomatic plants display typical leaf scorching symptoms. On *Nerium oleander*, necrosis develops on leaf margins and infection might lead to the death of entire plants (EPPO 2018). *Polygala myrtifolia* (myrtle-leaf milkwort) was one of the most susceptible hosts in the recent European

outbreaks. Infected plants show scorched leaves, with desiccation starting from the tip and progressing to the entire blade (EPPO, 2018; Figure 6).

Xylella fastidiosa also infects other hosts such as olives (Figure 7) and almonds (Figure 8), while *Xylella taiwanensis* infects pears (Figure 9). As these species are often in home gardens and landscaping around production areas, it is important to watch for symptoms on them.

Figure 6. Symptoms of *Xylella fastidiosa* on myrtle-leaf milkwort (*Polygala myrtifolia*).

Xylella fastidiosa in olives

Olive quick decline syndrome (OQDS) is caused by a distinct strain of *X. fastidiosa*. The bacterium lives and multiplies in the sap of olive trees, blocking water and nutrient uptake.

Olive quick decline is characterised by leaf scorching and twig death throughout the upper part of the canopy (Figure 7). Leaf scorching begins at the tip of the leaf, spreading towards the base until the whole leaf is covered. Dead leaves remain attached to the branches until dislodged by rain.

Browning and branch death spreads throughout the canopy giving the tree a burned appearance. These leaf scorch and die back symptoms can be easily confused with several other olive tree diseases and environmental disorders. Laboratory tests are necessary to confirm the actual cause of symptoms.

Figure 7. Symptoms of *Xylella fastidiosa* on olives. Photos: European and Mediterranean Plant Protection Organisation (EPPO).

Xylella fastidiosa in almonds

The most common symptoms of almond leaf scorching disease are leaf scorching followed by decreased productivity and general decline. In early summer, leaves appear with marginal leaf scorch (brown, necrotic (dead) leaf tissue). Usually, a narrow band of yellow (chlorotic) tissue occurs between the dead tissue and the part of the leaf that is still green. When the sudden appearance of leaf scorch symptoms is prompted by hot weather, the narrow chlorotic band might not develop. As the disease progresses, affected twigs on limbs die back from the tip (Mircetich et al. 1976). Even highly susceptible varieties take many years to die completely, but nut production is severely reduced within a few years in most varieties. This disease is commonly refered to as 'golden death', which describes the golden yellow of the canopy of a severely infected tree.

Figure 8. Symptoms of *Xylella fastidiosa* on almonds.

Xylella taiwanensis in pears

Xylella taiwanensis (Xt), a sister pathogen to *X. fastidiosa*, causes pear leaf scorch disease in pear trees. The disease causes water stress, early defoliation and dieback, loss of tree vigour and reduced fruit yield and quality.

Figure 9. Pear leaf scorch caused by *Xylella taiwanensis*. Photos: Chih-Horng Kuo (https://twitter.com/chihhorngkuo/status/1157214575512547328).

Sampling procedures

The distribution of *X. fastidiosa* in a plant can vary, therefore, it is important to collect samples at the right time of the year. Spring and autumn are the best times to sample for *X. fastidiosa* when the plant is actively growing and the temperature is not too hot or cold. *Xylella fastidiosa* cannot survive extreme low or high temperatures well in the sensitive growing tips of the plant so the bacteria retreat to the roots and lower extremities of the plant, making collection of infected material much harder during intense cold snaps and heatwaves (Luck et al. 2010).

If you suspect you may have *Xylella fastidiosa*, then please contact the **Exotic Plant Pest Hotline on 1800 084 881** before collecting samples to discuss the situation with NSW DPIRD staff. They will provide you with advice about sample collection and submission. **Do not** collect and send samples in without first talking to NSW DPIRD staff.

Xylella fastidiosa pathogen

Visual surveillance, complemented by sampling where required, is used for *X. fastidiosa* surveillance. Target known symptomatic hosts displaying symptoms for the highest chance of interception. Stems with leaves attached are the preferred plant samples for *X. fastidiosa* diagnostics.

IF YOU SEE ANYTHING UNUSUAL, CALL THE EXOTIC PLANT PEST HOTLINE

1800 084 881

Late summer to autumn is the best time to sample for *X. fastidiosa* in grapevines. In chronically infected vines, bacteria do not move into the new season's growth until mid-summer. Leaves attached to the cane generally give the most reliable result. Certain hosts have recommended times of year for sampling (refer to Table 4).

Assess the entire vine for symptoms. The disease usually starts from the top of the canopy. For large vines, only take lower limb samples if the upper canopy is showing significant disease symptoms.

Table 4. Host sampling recommendations.

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Crop cycle	Fruiting – veraison – harvest				Leaf drop – pruning				Budburst – flowering – fruit set			
Vector activity												
Pathogen activity												
Grapevines	Sample								Sample			
Polygala, citrus	Sample								Sample			
Prunus, oleander	Sample											
Olives	Sample								Sample			
Blueberry		Sample										

Before sampling:

1. photograph the entire plant you are sampling
2. take clear, focused, close-up photos of the symptomatic regions
3. record the GPS coordinates of the photos/samples taken
4. tag the row and the suspect vine for future reference in case you need to return to that location.

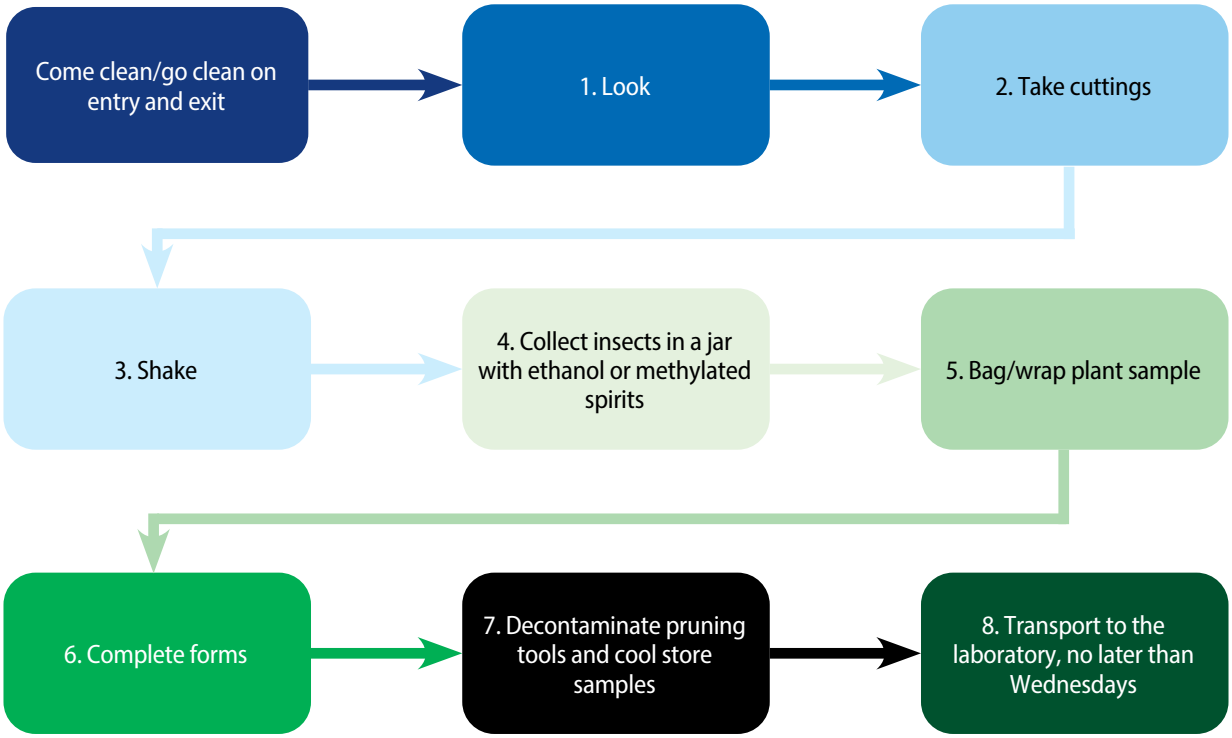

Figure 10. *Xylella fastidiosa* plant sample collection process.

Collect samples as follows:

1. Look for stems with symptomatic leaves. The laboratory will need xylem tissue for diagnosis, so the stem is the best tissue to sample. A stem size of approximately 10 mm in diameter is preferred. Collect samples close to symptoms, preferably stems with mature symptomatic leaves with petiole (Figure 11) and woody twigs attached. Avoid collecting dead (necrotic) tissue (advanced stages of infection). The stem material needs to be green (refer to Figure 12).

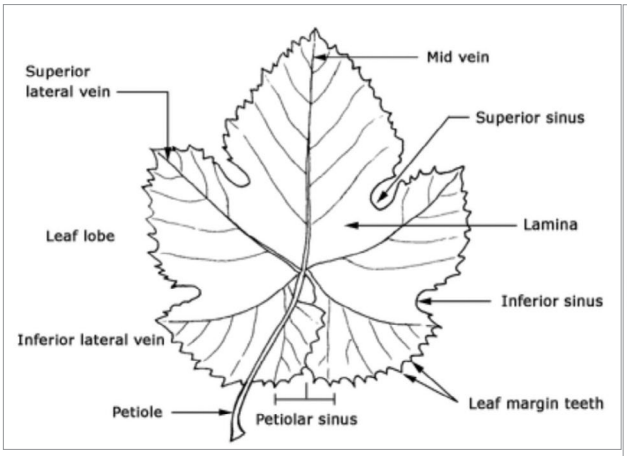

Figure 11. Grapevine leaf biology. Source: Irrpublic.clli.net.nsw.edu.au.

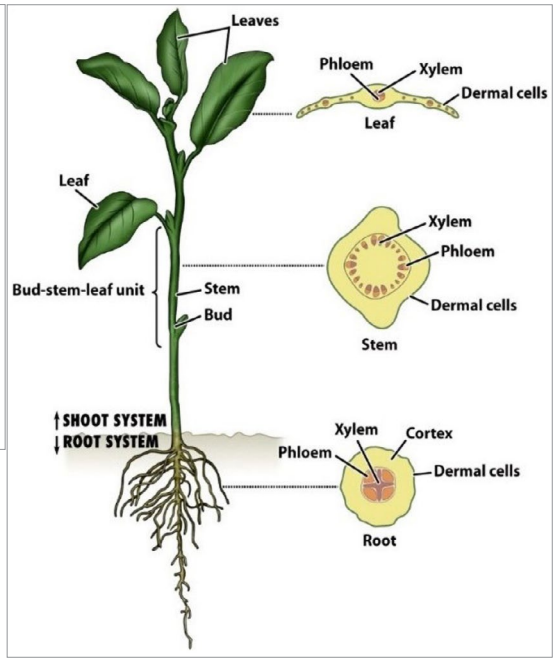

Figure 12. Xylem location within a plant. Cain et al. (2006).

2. Take a stem cutting approximately 150–200 mm long, comprising the symptomatic and healthy leaves (Figure 13). The stem cutting must contain a green stem, not a dead stem. If the symptomatic leaves are on new growth, include some older stems as the bacteria might not be detectable in the new growth (e.g. on a grapevine, include the older cane the symptomatic stem is growing from). Do not remove leaves.

Figure 13. *Xylella fastidiosa* sample type and quantity. The left indicates a section for sampling from a grapevine. The right picture is from an olive tree, showing the quantity and type of material that should be collected.

3. Repeat step 2 to collect at least 8 stem cuttings from each vine.
4. Shake samples to release any insects into a plastic bag or directly into a jar of ethanol.
5. Wrap cutting samples in some paper towel, seal them into a ziplock bag, and then seal them inside a second ziplock bag for full containment. On the inner bag, write the collection location and date with a permanent marker.

6. Complete a sample submission form (Plant Health Diagnostic Service Specimen Advice Form) and slip it into the outer ziplock bag. The outer ziplock bag will now contain the form (clean and dry) and the inner ziplock bag with the plant sample secured inside. The sample should be contained, labelled, and ready to be dispatched to the laboratory for testing. Samples should be treated so they arrive at the laboratory in a fresh, well-preserved state.
   - The sample submission form includes contact details of both the sending and receiving officers, important sample information relating to plant species/parts affected, the location of the affected plant, symptoms, and photos.
   - **Samples should be collected and sent early in the week, preferably no later than Wednesday morning, to ensure they arrive at the laboratory during weekday business hours.**
   - Samples should be securely sealed to prevent loss, contamination, or tampering and be sent to Elizabeth Macarthur Agricultural Institute (EMAI) for diagnostics. Place the packaged samples in a cool box or esky for transport. Deliver the samples immediately or not later than one day after the sample is collected. If dispatch is delayed, pack the sample with an ice pack if temperatures exceed 25 °C, otherwise they can be sent without an ice pack. Do not store samples below 4 °C as lower temperatures can induce sample deterioration.
7. Pruning tools and equipment must be decontaminated (e.g. bleach – sodium hypochlorite) with 70% alcohol before taking a sample from a new plant.
   - There is no specific disinfectant required for *X. fastidiosa* – bleach, ethanol, and Virkon are all suitable options to decontaminate tools and equipment. Even though *X. fastidiosa* cannot be easily transmitted via contaminated tools, other diseases can be, and precautionary measures should be taken to minimise the spread of any disease.
8. Samples must be transported to the laboratory within 3 days of collection. Samples should ideally be stored between 4–15 °C; however, they can survive temperatures up to 35 °C. They should not be frozen. *Xylella fastidiosa* samples should be stored in a cool, dry box or esky, ensuring they stay within the required temperature parameters until they can be refrigerated or shipped to the laboratory.
   - Where possible, a courier should be used to transport the samples as this helps get samples to the laboratory quickly. If the sample has to be posted, use express post and avoid posting on a Thursday or Friday. You do not want plant samples sitting at a postal distribution centre over the weekend, lengthening the time needed for the samples to reach the laboratory. Dispatch early in the week is best.
9. The laboratory will then assess the samples and perform the necessary diagnostic steps. This might take several steps to complete. The laboratory will then contact you with the results.

Note: negative test results do not necessarily mean that *Xylella fastidiosa* is absent, more that it has not been detected in that sample, as the bacteria might be unevenly distributed through the vine and unevenly distributed in the plant over the course of the year. It is important to sample symptomatic material at the right time of year.

It is also helpful to provide digital photos where possible as part of the sample submission so the diagnosticians can clearly see the symptoms you are observing in the field. Symptoms on the plant samples may be less clear post collection especially if dispatch to the laboratory takes a couple of days.

Xylella fastidiosa vectors

Visual surveillance using sweep netting, shaking of plants and possibly light trapping could be used for *X. fastidiosa* vectors. Spittlebugs, froghoppers and glassy-winged sharpshooters are 3 key insect vectors, and hand collection methods for these have been provided below.

Visual surveillance (refer to Table 2 for images of the insect vectors).

Glassy-winged sharpshooter (*Homalodisca vitripennis*) adults are large (12 mm long) and prolific flyers.

Meadow spittlebugs (*Philaenus spumarius*) spend most of their life cycle in the herbaceous layer (such as clover and medic). Adult meadow spittlebugs are very small (5–7 mm long).

Froghopper (*Bathyllus albicinctus*), also referred to as spittlebugs, are native to Australia and are highly polyphagous as they consume a wide variety of plants and should be included in any surveillance program.

To conduct visual inspection:

1. Visually inspect host trees for adult vectors from late spring to early summer.
2. Target ground cover (clovers and medics) by sweep netting for adults from late summer to early autumn. Use an insect aspirator/pooter to collect small insects from sweep netting.
3. Observe ground cover and tree branches for signs of spittlebug froth. If froth is observed, inspect for nymphs.
4. Tip insects out of nets or aspirators and store insect samples in sealed plastic containers with 95–99% ethanol or at –20 °C without ethanol. The insects must be dead before being sent to the laboratory for diagnosis.
5. A completed sample submission form (Plant Health Diagnostic Service Specimen Advice Form) must accompany the sample (https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0020/710912/PHDS-Specimen-advice-form-October-2024-update-OUT24-16532.pdf).
6. Samples should be securely sealed to prevent loss, contamination or tampering. All samples should be treated in a manner that allows them to arrive at the laboratory in a fresh, well-preserved state. Samples will be sent to Elizabeth Macarthur Agricultural Institute (EMAI) for diagnostics.

Sample submission

If you suspect you may have *Xylella fastidiosa*, then please contact the **Exotic Plant Pest Hotline on 1800 084 881** before collecting samples to discuss the situation with NSW DPIRD staff. They will provide you with advice about sample collection and submission. **Do not** collect and send samples in without first talking to NSW DPIRD staff.

Via courier:
Plant Health Diagnostic Service
Elizabeth Macarthur Agricultural Institute
Woodbridge Road
MENANGLE NSW 2568

Via Australia Post:
Plant Health Diagnostic Service
Elizabeth Macarthur Agricultural Institute
Private Bag 4008
NARELLAN NSW 2567

All samples must be accompanied by a completed Plant Health Diagnostic Service Specimen Advice Form (https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0020/710912/PHDS-Specimen-advice-form-October-2024-update-OUT24-16532.pdf).

Slip the completed sample submission form into the outer ziplock bag. Your outer ziplock bag will now contain the form (clean and dry) and the inner ziplock bag with the plant sample secured inside. Your sample is now contained, labelled and ready for dispatch to the laboratory for testing.

For more information on submitting samples, see the NSW DPIRD Plant Health Diagnostics Sending Samples Information page (https://www.dpi.nsw.gov.au/about-us/services/laboratory-services/sample-submission/submitting-samples-to-nsw-animal-and-plant-health-laboratories?SQ_VARIATION_1414050=0).

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Notes

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Xylella fastidiosa: on-farm preparedness guide for vineyards