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European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes PM 3/82 (1)

Phytosanitary procedures
Procédures phytosanitaire

PM 3/82 (1) Inspection of places of production for *Xylella fastidiosa*

Specific scope

This Standard describes the procedures for inspection of places of production of plants for planting which are susceptible to *Xylella fastidiosa*. All potential host plants have been considered as well as insects which are vectors of the pest. The scope of a place of production inspection may be for export or for internal country movements of materials or as an element of a national survey. Further inspections would be needed to determine freedom of a country or area from the pest concerned. The Standard does not cover eradication or containment measures in infected areas, or measures needed to establish and maintain pest-free places of production within areas where the pest is known to occur.

Specific approval and amendment

First approved in 2016-09.

1. Introduction

Xylella fastidiosa (EPPO code: XYLEFA) (Wells *et al.*, 1987) is listed as an EPPO A1 pest, and is a regulated pest in the European Union (EU, 2000), and in several EPPO countries (EPPO, 2015). *Xylella fastidiosa* is a xylem-limited plant pathogen, which is considered to cause several diseases in a wide range of cultivated and wild host plants, especially in North, Central and South America (Janse & Obradovic, 2010; EFSA, 2015). Outside the Americas, diseases associated with *X. fastidiosa* have been reported in Taiwan, causing pear leaf scorch, and symptoms of Pierce's disease in commercial vineyards (*Vitis vinifera*) (EFSA, 2016). Symptoms similar to Pierce's disease were reported from vineyards and almond orchards in several provinces of Iran in 2014 (Amanifar *et al.*, 2014). Since 2013, the bacterium has been found in aged olive trees (*Olea europaea*) affected by extensive leaf scorch and dieback and in a range of other hosts in the Salento Peninsula (Puglia region, Southern Italy) (Nigro *et al.*, 2013; Saponari *et al.*, 2013). In 2015, *X. fastidiosa* was recorded in France, on the island of Corsica, first on *Polygala myrtifolia* (EPPO, 2015) and subsequently on a number of other plant species (see the section 'Host plants concerned') (NPPO FR – Corsica, 2015). The organism has since also been found in a limited number of locations in continental Southern France in the region of Provence-Alpes Côte d'Azur (NPPO FR – PACA, 2015).

There are three accepted subspecies of *X. fastidiosa*, namely *fastidiosa*, *pauca* and *multiplex* (Schaad *et al.*, 2004), based on DNA–DNA hybridization data, although only two, subspecies *fastidiosa* and *multiplex*, are so far considered valid names by the International Society of Plant Pathology Committee on the Taxonomy of Plant Pathogenic Bacteria (ISPP-CTPPB) (Bull *et al.*, 2012). The subspecies cause different diseases on different plants and have different geographical distribution (EFSA, 2015). The bacterium is the causal agent for Pierce's disease of grapevine, almond leaf scorch, alfalfa dwarf, oak leaf scorch, maple leaf scald, sycamore leaf scorch, mulberry leaf scorch, periwinkle wilt, pecan leaf scorch, elm leaf scorch, oleander leaf scorch, phony peach, plum leaf scald, citrus variegated chlorosis and coffee leaf scorch (Hopkins & Purcell, 2002). Various subspecies of the bacterium have been genetically identified and sequenced, and some strains including the CoDiRO strain of *X. fastidiosa* subsp. *pauca* found on *Olea europaea* and other species in Puglia (IT) have been completely sequenced (Giampetruzzi *et al.*, 2015). To date the findings (except interceptions on consignments of *Coffea* plants) in France have been the subspecies *multiplex* mainly on *Polygala myrtifolia*.

1.1 Vectors of *X. fastidiosa*

Xylella fastidiosa is vectored by insects that feed on xylem fluid (EFSA, 2015). In the Americas, numerous species of xylem sap-sucking Hemiptera from the families Cicadellidae,

Aphrophoridae and Cercopidae (Auchenorrhyncha) are known to be vectors of *X. fastidiosa* (Redak *et al.*, 2004). The non-European species *Carneocephala fulgida*, *Draeculacephala minerva*, *Graphocephala atropunctata* and *Homalodisca vitripennis* are known to be vectors of *X. fastidiosa* and the latter is listed as an EPPO A1 pest. Non-European Cicadellidae known to be vector of Pierce's disease are also included in Annex I of Council Directive 2000/29/EC (EU, 2000) and in plant health provisions of other EPPO countries.

In Southern Italy, the highly polyphagous and widely spread *Philaenus spumarius* (Aphrophoridae) is the only species currently identified in Europe as a vector of *X. fastidiosa* (Saponari *et al.*, 2014). Although native to Europe, *Philaenus spumarius* has been introduced into North America and has been identified as a vector in California (Purcell, 1980). Cicadidae and Tibicinidae species in the EPPO region should also be considered potential vectors (EFSA, 2015). EFSA (2015) lists potential European vectors drawn from the Fauna Europaea database (de Jong, 2013).

1.2 Host plants concerned

Xylella fastidiosa has an extensive natural host range, which includes many herbaceous and woody plants, cultivated crops and weeds. The range includes the following woody plants: species of *Citrus*, *Juglans*, *Magnolia*, *Olea*, *Prunus* and *Vitis*. The host range covers 75 families, 204 genera and 359 species (EFSA, 2016), but the presence of *X. fastidiosa* does not always cause visible symptoms in many of them. In Salento (Southern Puglia region, Southern Italy), *X. fastidiosa* CoDiRO strain, has been detected on olive trees and other hosts, such as oleander (*Nerium oleander*), almond (*Prunus dulcis*) and cherry (*Prunus avium*), including both ornamental and wild plants. In France, *X. fastidiosa* subsp. *multiplex* has been detected on *Polygala myrtifolia* and many other Mediterranean and European native plant species.

General trees, shrubs or perennial host plant species have a high risk for introduction and spread of the disease. A detailed list of plants known to be susceptible to the European and non-European isolates of *X. fastidiosa* is reported in Annex I of Commission Implementing Decision (EU) 2015/789 (EU, 2015a) (see http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32015D0789&from=EN) as updated and amended and in a database from EFSA (2016). A European Commission database detailing host plants found to be susceptible to *X. fastidiosa* in the European Union is available (see http://ec.europa.eu/food/plant/plant_health_biosecurity/legislation/emergency_measures/xylella-fastidiosa/susceptible_en.htm).

1.3 Symptom description

Symptoms depend on the combination of host and *X. fastidiosa* strain. As the bacterium invades xylem vessels it blocks the transport of mineral nutrients and water. Generally, symptoms include leaf scorching, wilting of the foliage, defoliation, chlorosis or bronzing along the leaf margin and dwarfing. Bacterial infections can be so severe as to lead to the death of infected plants. The bronzing may intensify before browning and drying (Janse & Obradovic, 2010). Symptoms usually appear on just a few branches but later spread to cover the entire plant. Depending on the plant species, the presence of yellow spots on leaves, chlorotic foliage often together with pronounced yellow discolouration between healthy and necrotic tissues, irregular lignification of bark, stunting, premature leaf drop, reduction of production and dimension of fruits, fruit distortion, crown dieback or a combination of symptoms may occur. Symptoms can be confused with those caused by other biotic or abiotic factors (other pathogens, environmental stresses, water deficiency, salt, air pollutants, nutritional problems, sunburn, etc.); illustrations of possible confusions can be seen at http://agriculture.gouv.fr/sites/minagri/files/xylella_fastidiosa_symptomes_et_risques_de_confusions_biotiques_et_abiotiques_dgal-1.pdf

Symptoms on various hosts can be seen at https://gd.eppo.int/taxon/XYLEFA/photos. Symptoms of diseases associated with *X. fastidiosa* in Europe and in the Americas are presented in Appendix 1 (in alphabetical order of disease name).

2. General elements for phytosanitary inspections

Useful information referring to phytosanitary inspections to be carried out for imported consignments are given in EPPO Standard PM 3/72 (2) *Elements common to inspection of places of production, area-wide surveillance, inspection of consignments and lot identification* (EPPO, 2009). Additional information can be found via the EU emergency control measures by species: http://ec.europa.eu/food/plant/plant_health_biosecurity/legislation/emergency_measures/xylella-fastidiosa/susceptible_en.htm. A further referral guideline is ISPM no. 23 *Guidelines for Inspection* (IPPC, 2005).

The requirement for the production and movement of plants for planting from a place of production free from *X. fastidiosa* is one of the most effective measures to prevent the spread of the pest with trade. The procedures described in this Standard are mainly specific to inspection of place of production, but may also be applicable for export inspection when the requirements of the importing country are similar, or for internal movement of plants for planting or surveys.

2.1 Inspection and sampling period

The concentration of the bacterium in a plant depends upon environmental factors, strain and host plant species. To maximize the likelihood of detection, inspections and sampling should be performed during the period of active

growth and after warm periods. For tropical plant species grown indoors, such as coffee plants, sampling may be performed all year round.

For outdoor plants in Europe this period is usually between late spring and autumn. Sampling after warm periods (e.g. late summer–early autumn) increases the probability of accurate bacterial detection (EU, 2015b):

(a) for *Polygala* spp., sampling can be performed from late spring to early autumn;
(b) for *Olea europaea* and *Nerium oleander*, observations conducted in Italy (Apulia region) indicated that:
    - withering, desiccation and leaf scorching symptoms associated with *X. fastidiosa* infections are more strongly expressed in summer, although persistent during the entire year
    - in some cases, symptoms were, also observed during winter at the start of the new vegetative growth;
(c) for deciduous plant species (e.g. *Prunus* spp.) in Italy (Apulia region) symptoms were consistently recorded, together with a detectable bacterial concentration, in leaves collected during summer. Asymptomatic leaves collected earlier in the vegetation period from the same trees tested negative;
(d) if necessary, dormant plants can be sampled by taking mature branches (e.g. woody cuttings), from which the xylem tissue is recovered and processed for detection of *X. fastidiosa*.

Experience in temperate areas in other parts of the world shows that in vines or deciduous trees, e.g. cherry and almond, that have been infected for some time, the bacteria do not move into the new season's growth until the middle of summer, when symptoms may also become visible. For example, the most suitable time to search for symptoms in grapevine is late summer to early autumn when weather conditions are predominantly hot and dry or when grape plants are exposed to drought stress (Galvez *et al.*, 2010).

3. Inspection of plants

An initial inventory of the plants growing in and near the place of production should be carried out and compared with those host plants which are most likely to show symptoms of the pest in the EPPO region. Those plants should then be included in the visual inspection of the place of production. A European Commission database detailing host plants found to be susceptible to *X. fastidiosa* in the EU is available.

3.1 Selection of plants for visual inspection

An adequate proportion of plants should be subjected to a systematic examination in order to detect the presence or signs of pests in the place of production.

For the purpose of visual inspection a lot should be defined as a number of plants which are identifiable as being the same variety or clone, with propagating material from the same origin of, cultivated in the same field and treated in the same way and at the same time.

The size of the unit of inspection (the minimum number of individuals to be examined) to be selected for inspection at a specified level of infection in a specified lot size is indicated in Tables 1, 3 and 4 of ISPM no. 31 *Methodologies for sampling of consignments* (IPPC, 2009). For *X. fastidiosa* the level of confidence should allow reliable detection of a level of infestation which is as low as possible. All lots which include symptomatic plants should be sampled for testing, with the sample including a representative range of symptoms.

If 448 plants are inspected from a lot of 10 000 this provides 99% confidence of detecting evident symptoms present in 1% of the plants, provided that symptoms are seen and are uniformly distributed and the plants are selected at random or higher-risk plants are targeted, e.g. those at the outer edge of the nursery. This may be sufficient as part of a national survey. If 3689 plants are inspected from a lot of 10 000 this provides 99% confidence of detecting evident symptoms present in 0.1% of the plants, provided the symptoms are seen and are uniformly distributed and the plants are selected at random. This level of inspection may be more appropriate, for example, in supporting the issue of a phytosanitary certificate.

For small lots (fewer than 1000 plants), all plants should be inspected.

Inspection of whole rows randomly or chosen evenly across the field is usually carried out.

Where possible, inspections should be undertaken during overcast days as symptoms may be obscured by bright sunlight.

3.2 Sampling of plant material for laboratory testing

Visual observations alone are not always sufficient for the detection of *X. fastidiosa*, due to the fact that latent infections could be present and secondary infections caused by other organisms may hide the symptoms of the pest.

4. Sample collection

Samples for laboratory testing should preferably be composed of branches or cuttings with attached leaves. The sample should include mature leaves; young growing shoots should be avoided. For small plants the entire plant can be sent to the laboratory. For sclerotic leaves (e.g. *Coffea*) individual leaves and petioles can be sampled.

As *X. fastidiosa* is confined to the xylem tissue of its hosts, the petiole and midrib recovered from leaf samples are the best source for diagnosis as they contain larger amounts of xylem vessels (Hopkins, 1981). However, other sources of tissue include small twigs and roots of peach (Aldrich *et al.*, 1992), blueberry stem and roots (Holland *et al.*, 2014) and *Citrus* fruit peduncles (Rossetti *et al.*, 1990).

4.1 Symptomatic plants

The sample should consist of branches/cuttings representative of the symptoms seen on the plant(s) and containing at least 10–25 leaves (depending on leaf size). Symptomatic plant material should preferably be collected from a single plant; however, a pooled sample may also be collected from several plants showing similar symptoms.

4.2 Asymptomatic plants

Testing of asymptomatic plants is recommended for host plants near to an outbreak area or in the framework of trace back and forward activities.

For asymptomatic plants, the sample should be representative of the entire aerial part of the plant. Recent experimental data on detection of *X. fastidiosa* in monumental and ancient olive trees showed that detection was more reliable when sampling the medium to upper part of the canopy.

For testing individual asymptomatic plants, at least 4–10 branches must be collected, depending on plant size.

There is limited experience of testing samples composed of leaves (including their petioles) collected from several asymptomatic plants. However, *X. fastidiosa* has been detected from samples of 100–200 leaves (including their petioles) collected from consignments of asymptomatic coffee plants (NRC, NL unpublished data).[1]

ISPM 31 (IPPC, 2009) provides useful information on the number of plants to be sampled.

Collection of shoot portions in active growth should be avoided as concentrations of the bacterium are lower near the growing points.

Following good hygiene procedures is important when collecting samples for the laboratory; in particular tools should be disinfected between sample collections.

Samples should be sent to the laboratory as soon as possible after collection.

5. How to preserve and transport plant samples

Preservation and transportation of samples should be carried out according to the following procedures:

- Shake samples to ensure that no vectors are moved with the plant material (e.g. adult vectors will fly away when leaves or twigs are shaken). It is important to check that the sample does not contain any adult or juvenile vector species, in order to prevent their escape outside the collecting site.
- Place samples in closed container (e.g. plastic sealable bags, etc.).
- Keep at cool temperatures to avoid exposing samples to stress conditions.
- Transport samples to the diagnostic laboratory as soon as possible, before the plant tissues deteriorate. It is important to make sure that the samples will not be received by the laboratory on a non-working day, and to inform the laboratory of when they are likely to arrive.
- Samples should be sent to the laboratory as soon as possible after collecting.

6. Sampling of vectors

Insects can be analysed to detect *X. fastidiosa*. Monitoring of Hemiptera which are vectors of *X. fastidiosa* may be a complementary activity to visual inspection and host plant testing at a place of production. Vectors that acquire *X. fastidiosa* as adults remain infective for life (Purcell *et al.*, 2014).

Adult vectors should preferably be collected with sweeping nets (adults) or aspirators. Sticky traps are not usually effective for xylem feeders (Purcell *et al.*, 2014), but insects may be trapped accidentally and specimens collected from sticky traps can be used for testing. A combination of methods will increase the number and diversity (of species) captured. The presence of hemipteran larvae may be an indication that the plant is infected.

Vectors can be removed from the traps using small forceps/pincers and a suitable solvent. After removal from the traps, insects should be rinsed in ethanol/acetone. Traps should be serviced on a weekly basis.

Sampling for insects should preferably be done from late spring until early autumn to maximize the likelihood of detection of the bacterium.

If insects cannot be processed immediately, they should be stored in 95–99% ethanol or at −20°C. Sticky traps can also be stored at −20°C.

Appendix 2 provides a short procedure for inspectors.

7. Acknowledgements

This Standard was first drafted by Mr Governatori (IT). It was reviewed by the Panel on Phytosanitary Inspections and the Panel on Diagnostics in Bacteriology.

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[1]The Panel on Diagnostics in Bacteriology is aware that this sampling recommendation is under revision.

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Appendix 1 – Specific procedures – detection

As stated in the section 'Host plants concerned', over 300 plant species are hosts of *Xylella fastidiosa*. However, the bacterium does not appear to cause disease in many of these plant species. Colonization is frequently asymptomatic in many hosts for a long time after inoculation and does not necessarily result in the development of disease. There are also significant differences in susceptibility between hosts.

1. Disease symptoms

Symptoms depend on the combination of host and *X. fastidiosa* strain. As the bacterium invades xylem vessels it blocks the transport of mineral nutrients and water. Generally, symptoms include leaf scorching, wilting of the foliage, defoliation, chlorosis or bronzing along the leaf margin and dwarfing. Bacterial infections can be so severe as to lead to the death of the infected plant. The bronzing may intensify before browning and drying (Janse & Obradovic, 2010). Symptoms usually appear on just a few branches but later spread to cover the entire plant. Depending on the plant species, the presence of yellow spots on leaves, chlorotic foliage, often together with pronounced yellow discolouration between healthy and necrotic tissues, irregular lignification of bark, stunting, premature leaf drop, reduction of production and dimension of fruits, fruit distortion, crown dieback or a combination of symptoms may occur. Symptoms can be confused with those caused by other biotic or abiotic factors (other pathogens, environmental stresses, water deficiency, salt, air pollutants, nutritional problems, sunburn, etc.); illustrations of possible confusions can be seen at http://agriculture.gouv.fr/sites/minagri/files/xylella_fastidiosa_symptomes_et_risques_de_confusions_biotiques_et_abiotiques_dgal-1.pdf

Symptoms on various hosts can be seen at https://gd.eppo.int/taxon/XYLEFA/photos. Symptoms of diseases associated with *X. fastidiosa* in Europe and in the Americas are presented below (in alphabetical order of disease name).

1.1 Alfalfa dwarf

The main symptom is stunted regrowth after cutting. This stunting may not be apparent for many months after initial infection. Leaflets on affected plants are smaller and often slightly darker (often with a bluish colour) compared to uninfected plants, but are not distorted, cupped, mottled or yellow. The taproot is of normal size, but the wood has an abnormally yellowish colour, with fine dark streaks of dead tissue scattered throughout. In recently infected plants the yellowing is mostly in a ring beginning under the bark, with a normal white-coloured cylinder of tissue inside the yellowed outer layer of wood. Unlike in bacterial wilt, *Clavibacter michiganensis* subsp. *insidiosus*, the inner bark is not discoloured, nor do large brown or yellow patches appear. Dwarf disease progressively worsens over 1–2 years after the first symptoms and eventually kills infected plants. Noticeable dwarfing requires 6–9 months after inoculation in the greenhouse and probably longer in the field (http://alfalfa.ucdavis.edu).

1.2 Almond leaf scorch

The most characteristic symptoms of almond leaf scorch are leaf scorching followed by decreased productivity and general decline of the tree. A narrow band of yellow (chlorotic) tissue usually develops between the brown necrotic tissue and the green tissues of the leaves; however, when the sudden appearance of leaf scorch symptoms is prompted by hot weather the narrow chlorotic band may not develop. As the disease progresses, affected twigs on branches die back from the tip (Mircetich *et al.*, 1976). Even highly susceptible varieties take many years to die, but nut production is severely reduced within a few years in most varieties.

Leaf scorching symptoms have been also reported on almond in late summer/autumn in Italy (Fig 1).

Fig. 1 Leaf scorch symptoms on almond. Courtesy D. Boscia, CNR-Institute for Sustainable Plant Protection (IT).

1.3 Bacterial leaf scorch of blueberry

The first symptom of bacterial leaf scorch of blueberry is marginal leaf scorching (Fig. 2). The scorched leaf area may be bordered by a darker band (Brannen *et al.*, 2016). In the early stages of disease progression, symptoms may be localized, but over time symptoms can become

Fig. 2 Scorch symptoms with distinct leaf burn surrounded by a dark line of demarcation between green and dead tissue. Courtesy P. M. Brennan University of Georgia (US).

Fig. 3 Infected plants with yellow stems and a 'skeletal' appearance. Courtesy P. M. Brennan University of Georgia (US).

uniformly distributed throughout the foliage. Newly developed shoots can be abnormally thin with a reduced number of flower buds. Leaf drop occurs and twigs and stems have a distinct 'skeletal' yellow appearance (Fig. 3). Following leaf drop the plant typically dies during the second year after symptoms are observed (Chang *et al.*, 2009).

1.4 Bacterial leaf scorch of shade trees

Symptoms of bacterial leaf scorch are similar on different tree hosts such as *Acer* spp., *Cornus florida*, *Celtis occidentalis*, *Liquidambar stryraciflua*, *Morus alba*, *Platanus* spp., *Quercus* spp. and *Ulmus americana* (Gould & Lashomb, 2007). In most cases the disease is identified by a characteristic marginal leaf scorch where affected leaves have marginal necrosis and may be surrounded by a chlorotic (yellow) or red halo. Generally, symptoms progress from older to younger leaves, and as the disease progresses branches die and the tree declines. Symptoms first appear in late summer to early autumn. Some plant species may be killed by the disease. More information and pictures of symptoms are available in Gould & Lashomb (2007; available online).

1.5 Citrus variegated chlorosis

The first symptoms of citrus variegated chlorosis to appear on leaves are small chlorotic spots on the upper surface that correspond to small gummy brown spots on the underside of the leaf. Symptoms are most obvious on developed leaves independent of plant age and mainly on sweet orange cultivars (Figs 4 and 5).

Affected trees show foliar interveinal chlorosis on the upper surface, resembling zinc deficiency. Sectoring of symptoms in the canopy occurs on newly infected trees. However, citrus variegated chlorosis generally develops throughout the entire canopy on old infected trees. Affected trees are stunted and the canopy has a thin appearance because of defoliation and dieback of twigs and branches. Blossom and fruit set occur at the same time on healthy and affected trees, but normal fruit thinning does not occur on affected trees and the fruits remain small (Fig. 6), have a hard ring and ripen earlier. The plants do not usually die, but the yield and quality of the fruit are severely reduced (Donadio & Moreira, 1998). On affected trees of cv. Pera and other orange cultivars, fruits often occur in clusters of 4–10, resembling clusters of grapes. The growth rate of affected trees is greatly reduced and twigs and branches may wilt. Trees in nurseries can show symptoms of

Fig. 4 Citrus variegated chlorosis (CVC): typical spots caused on sweet orange leaves. Courtesy M. Scortichini, Istituto Sperimentale per la Frutticoltura, Rome (IT).

Fig. 5 Small raised lesions appear on the underside of leaves.

Fig. 6 Citrus variegated chlorosis (CVC): fruits are smaller and mature earlier (left) than fruits from healthy trees (right). Small raised lesions appear on the underside of leaves. Courtesy M. M. Lopez Instituto Valenciano de Investigaciones Agrarias, Valencia (ES).

variegated chlorosis, as do trees older than 10 years. Young trees (1–3 years) become systemically colonized by *X. fastidiosa* faster than older trees. Trees older than 8–10 years are usually not totally affected, but rather have symptoms on the extremities of branches.

1.6 Coffee leaf scorch

Symptoms of coffee leaf scorch appear on new growth of field plants as large marginal and apical scorched areas on recently developed leaves (Fig. 7). Affected leaves drop prematurely, shoot growth is stunted and apical leaves are small and chlorotic. Symptoms may progress to shoot dieback. Infection of coffee plants by *X. fastidiosa* can also lead to the 'crespera' disease which was reported from Costa Rica (Fig. 8). Symptoms range from mild to severe curling of leaf margins, chlorosis and deformation of leaves, asymmetry (see Fig. 8), stunting of plants and shortening of internodes (Montero-Astúa *et al.*, 2008).

Fig. 7 Leaf scorch symptoms on *Coffea* sp. Courtesy M. Bergsma-Vlami, NPPO (NL).

Fig. 8 'Crespera' symptoms on *Coffea* sp., including curling of leaf margins, chlorosis and deformation (asymmetry). Courtesy M. Bergsma-Vlami, NPPO (NL).

1.7 Olive leaf scorching and quick decline

Infections of olive by *X. fastidiosa* were first reported by Krugner *et al.* (2014) in trees exhibiting leaf scorch or branch dieback symptoms in California (US), where infections were found to be associated with *X. fastidiosa* subsp. *multiplex*. However, a poor correlation was found between the symptoms and the presence of *X. fastidiosa*.

More recently a new olive disorder, consisting of olive plants showing leaf scorching and desiccated branches (including partial defoliation and shoot death) and associated with the presence of *X. fastidiosa*, has been reported in Southern Italy (Saponari *et al.*, 2013; Giampetruzzi *et al.*, 2015), Argentina (Haelterman *et al.*, 2015) and Brazil (Coletta-Filho *et al.*, 2016). The *X. fastidiosa* strains in all these cases were closely related genetically to the subspecies *pauca*.

In Southern Italy, this new olive disorder has been termed 'olive quick decline syndrome'. *Xylella fastidiosa* (CoDiRO strain), *Phaeoacremonium* spp., *Phaeomoniella* spp. and *Zeuzera pyrina* have been found in association with this syndrome in ancient olive trees. Olive quick decline syndrome is characterized by leaf scorching and scattered desiccation of twigs and small branches which, in the early stages of the infection, are mainly observed on the upper part of the canopy. Leaf tips and margins turn dark yellow to brown,

Fig. 9 Symptoms of quick olive decline syndrome. Courtesy D. Boscia, CNR-Institute for Sustainable Plant Protection (IT).

Fig. 10 Symptoms of quick olive decline syndrome. Courtesy D. Boscia, CNR-Institute for Sustainable Plant Protection (IT).

eventually leading to desiccation (Fig. 9). Over time, symptoms become increasingly severe and extend to the rest of the crown, which acquires a blighted appearance (Fig. 10). Desiccated leaves and mummified drupes remain attached to the shoots. Trunks, branches and twigs viewed in cross-section show irregular discolouration of the vascular elements, sapwood and vascular cambium (Nigro *et al.*, 2013). Rapid dieback of shoots, twigs and branches may be followed by death of the entire tree. *Xylella fastidiosa* has also been detected in young olive trees with leaf scorching and quick decline.

There are limited data on *X. fastidiosa* infecting olives, but evidence indicates that pathogen genotype defines pathogenicity. While *X. fastidiosa* is associated with but does not cause disease in olives in the USA (Krugner *et al*., 2014), Koch's postulates have been fulfilled in Italy (Saponari *et al*., 2016); pathogenicity data are not available from Brazil or Argentina. Nonetheless, a strong correlation between leaf scorching symptoms and presence of *X. fastidiosa* has been observed in three distant regions around the world (Southern Italy, Argentina and Brazil) (Coletta-Filho *et al*., 2016).

1.8 Pierce's disease of grapes

On grapevine, the most characteristic symptom of primary infection is leaf scorch. An early sign of infection is a sudden drying of part of a green leaf, which then turns brown while adjacent tissues turn yellow or red (see Fig. 11). The leaf symptoms can be confused with fungal diseases, in particular with the Rotbrenner, a fungal disease of grapevine caused by *Pseudopezicula tracheiphila* (Müll.-Thurg.) Korf & W.Y. Zhuang (1986) (Fig. 12). The desiccation spreads over the whole leaf causing it to shrivel and drop, leaving only the petiole attached (Fig. 13).

Diseased stems often mature irregularly, with patches of brown and green tissue. Chronically infected plants may have small, distorted leaves with interveinal chlorosis (Fig. 14) and shoots with shortened internodes. Fruit clusters shrivel. In later years, infected plants develop late and produce stunted chlorotic shoots. Symptoms involve a

Fig. 11 Yellowing and desiccation of grapevine leaves and wilting of bunches in the Napa Valley, California (US). Courtesy ENSA-Montpellier (FR).

Fig. 12 Symptoms caused by *Pseudopezicula tracheiphila*. Courtesy H. Reisenzein, AGES (AT).

Fig 13 Pierce's disease of grapevine: persistent petioles. Courtesy J. Clark & A. H. Purcell, University of California, Berkeley (US).

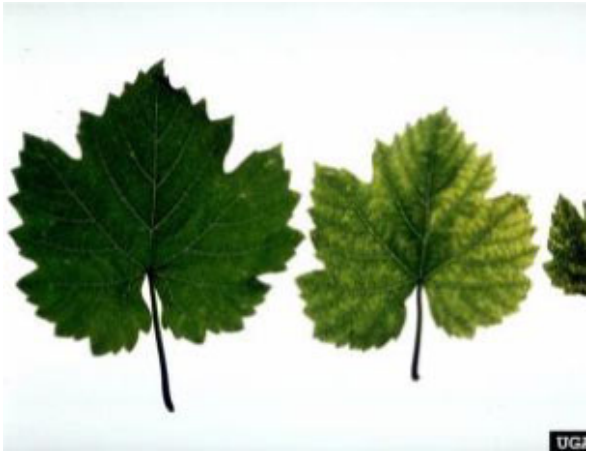

Fig. 14 Pierce's disease of grapevine: spring symptoms in cultivar Chardonnay (healthy leaf on the left). Courtesy A. H. Purcell, University of California, Berkeley (US).

general loss of plant vigour followed by death of part of or the whole vine. Highly susceptible cultivars rarely survive more than 2–3 years, although signs of recovery may be seen early in the second growing season. Young vines succumb more quickly than mature vines. More tolerant cultivars may survive chronic infection for more than 5 years.

1.9 Phony peach disease and plum leaf scald

On infected peach trees, young shoots are stunted and bear greener, denser foliage than healthy trees (Fig. 15). Lateral branches grow horizontally or droop, so that the tree seems uniform, compact and rounded. Leaves and flowers appear early, and remain on the tree for longer than on healthy trees. Early in summer, because of shortened internodes, infected peach trees appear more compact, leafier and darker green than normal trees. Affected trees yield increasingly fewer and smaller fruits until, after 3–5 years, they become economically worthless. Fruits may also be more strongly coloured and will often ripen a few days earlier

Fig. 15 Phony peach: typical 'phony peach' symptom on peach leaves caused by *Xylella fastidiosa.* Courtesy M. Scortichini, Instituto Sperimentale per la Frutticoltura, Rome (IT).

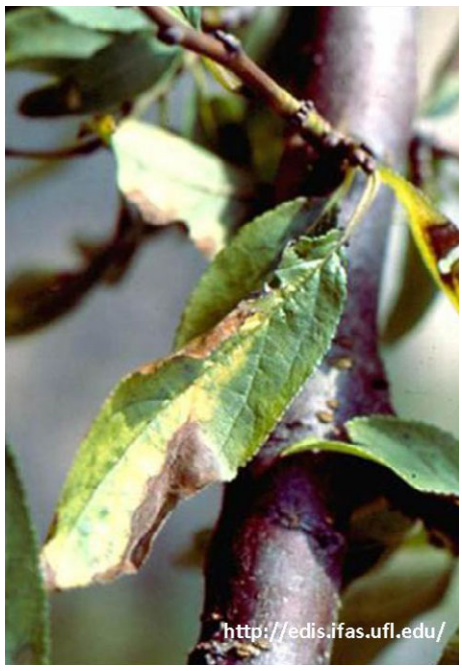

Fig. 16 Plum leaf scald: typical scorched symptom on plum leaf caused by *Xylella fastidiosa.* Reproduced from Mizell *et al*. (2015).

than normal. Infected peach and plum trees bloom several days earlier than healthy trees and tend to hold their leaves later into the autumn. The leaves of infected peach never display the typical of leaf scorching seen on infected plum trees. Symptoms of plum leaf scald on leaves are a typical scorched and scalded appearance (Fig. 16). Plum leaf scald also increases the susceptibility of the tree to other problems. Phony peach disease and plum leaf scald can limit the life of peach and plum orchards (Mizell *et al.*, 2015).

1.10 Other hosts: leaf scorching symptoms seen in other hosts in Europe

For a general description of symptoms see above. Besides olive, *X. fastidiosa* has been detected in different hosts

under natural conditions in the current European outbreak areas. Most of these findings refer to symptomatic plants, which display typical leaf scorching symptoms. A list of hosts in which *X. fastidiosa* has been detected in Europe is available and regularly updated at http://ec.europa.eu/food/plant/plant_health_biosecurity/legislation/emergency_measures/xylella-fastidiosa/susceptible_en.htm.

On oleander, necrosis developing on the leaf margins is typical (see Fig. 17). As in olive, infections may lead to death of the infected plants.

Polygala myrtifolia is one of the major susceptible hosts in the current European outbreaks. Infected plants show scorched leaves, with desiccation starting from the tip and progressing to the entire blade (see leaf tip desiccation in Fig. 18). An infected plant is shown in Fig. 19.

Fig. 17 Marginal leaf scorch symptoms caused by *Xylella fastidiosa* subsp *pauca* on oleander. Courtesy D. Boscia, CNR-Institute for Sustainable Plant Protection (IT).

Fig. 18 Symptoms on *Polygala myrtifolia*. Courtesy B. Legendre, Anses, Plant Health Laboratory (FR).

Fig. 19 Infected *Polygala myrtifolia*. Courtesy B. Legendre, Anses, Plant Health Laboratory (FR).

Fig. 20 Leaf scorch symptoms caused by *Xylella fastidiosa* on cherry. Courtesy D. Boscia, CNR-Institute for Sustainable Plant Protection (IT).

Leaf scorching symptoms have been also reported on cherry (Fig. 20) in late summer/autumn in Italy.

Appendix 2 – Short procedure for inspectors

Inspectors should be well equipped and trained to recognize the symptoms of *Xylella fastidiosa* and similar diseases, and should have access to all the necessary sets of information to aid identification and determine susceptible host plants. Lot identification and selection of material for inspection have to be performed according to the characteristics of the cropping area and the associated risk. Controls should not exclusively consist of visual inspections, as latent infection is possible. Distance to known outbreak sites and the origin of planting material clearly contribute to the risk at a particular location.

The inspections should take place during times of active growth, between late spring and autumn and after periods of warm temperatures. Where possible, inspections should

be undertaken during overcast days as symptoms may be obscured by bright sunlight.

It is important to follow good hygiene procedures when collecting samples for the laboratory, in particular disinfecting tools between sample collections.

Visual inspections should be concentrated on host species which have shown symptoms in the EPPO region. A European Commission database detailing host plants found to be susceptible to *X. fastidiosa* in the European Union is available.

Host plants of *X. fastidiosa* are detailed in the annexes of Commission Implementing Decision (EU) 2015/789 (EU, 2015a), as updated and amended.

Plants showing visual symptoms should be sampled for laboratory testing. If no symptoms are seen, it is recommended that some samples of asymptomatic host plants are collected for laboratory testing.

A map of the area should include species and cultivar names, locations and the estimated total number of plants. Host plants at the place of production which are likely to show symptoms should be included in the survey.

If 448 plants are inspected from a lot of 10 000 this provides 99% confidence of detecting evident symptoms present in 1% of the plants, provided the symptoms are uniformly distributed and the plants are selected at random. This may be sufficient as part of a national survey. If 3689 plants are inspected from a lot of 10 000 this provides 99% confidence of detecting evident symptoms present in 0.1% of the plants, provided symptoms are seen and are uniformly distributed and the plants are selected at random. This level of inspection may be more appropriate for supporting the issue of a phytosanitary certificate, for example.

For large lots (>10 000 plants) as part of a national survey it is normally sufficient to inspect a sample of 500 plants. Greater numbers (up to 4000 plants) might need to be inspected to support the issue of a phytosanitary certificate, for example. In either case it is recommended to target plants growing as close as possible to sources of infection, for example near uncultivated ground, hedgerows, gardens or sites where plants are traded.

In general, every row of plants should be walked but this can be varied according to the conditions to ensure that the selection of plants for visual inspection is representative. Inspection of rootstock beds and hedges is achieved by walking between two rows and inspecting either side to ensure that all the stock may be inspected. Plants in two or three rows close together may be inspected together. If it is necessary, the inspector may move across rows to check plants in a neighbouring row. A marker of some sort should be left to ensure return to the correct location for continuation. Large mother trees should be inspected individually all around the tree and also inside where the foliage may be denser.

Test results are highly dependent on the quality of the sample which arrives at the laboratory.

All samples for laboratory testing should be clearly labelled for traceability of information, with identification by location (possibly with GPS coordinates), plant species, sampling date, parts or part of plants sampled, symptoms (possibly with images), the owner's details and the name of the sampler. Plants from which samples have been taken should be marked, to enable follow-up in the case of positive test results.

Sampling and testing of the most abundant weed species which are susceptible to *X. fastidiosa* may be carried out. Any samples should be collected separately, especially in the case of weeds showing symptoms.

All sampled material should be stored in a cooler, in a manner that allows it to arrive at the laboratory in a fresh condition, without overheating or desiccation.

Monitoring of Hemiptera which are vectors of *X. fastidiosa* may be a complementary activity to visual inspection and host plant testing at a place of production. Vectors should preferably be collected with sweep nets, beating trays or aspirators. Live insects for analysis can be killed by freezing or by exposure to carbon dioxide or ethyl acetate.

Yellow sticky traps can be used, even if some Hemiptera are not attracted to yellow. The quality of the dead insects from sticky traps mainly depends on the period of time for which the traps have been hanging in the field (the shorter the period, the better the sample).

Samples should be sent to the laboratory as soon as possible after collection.