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European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes PM 3/81 (1)

Phytosanitary procedures
Procédures phytosanitaire

PM 3/81 (1) Inspection of consignments for *Xylella fastidiosa*

Specific scope

This Standard describes the procedures for inspection of consignments for detection of *Xylella fastidiosa* on host plants. All potential host plants have been considered as well as insects which are vectors of the pest. The Standard can be applied to phytosanitary import inspection, including sampling and identification of symptoms. The Standard does not state what phytosanitary action should be taken in response to finding the pest, but may indicate where, for example, a consignment should be held pending a test result (EPPO, 2009).

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 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, as well as 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*: *fastidiosa*, *pauca* and *multiplex* (Schaad *et al.*, 2004) on the basis of 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 distributions (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 being listed as an EPPO A1 pest. Non-European Cicadellidae known to be vectors of Pierce's disease are also included in Annex I of Council Directive 2000/29/EC (EU, 2000) and in the 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 as 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), the CoDiRO strain of *X. fastidiosa*, 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 native Mediterranean and European plant species.

General trees, shrubs or perennial host plants 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, 2015) (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 particular 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, yellow spots on leaves, chlorotic foliage (often together with pronounced yellow discoloration 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, sun burn, 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 in 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 inspections of consignments

Useful information referring to phytosanitary inspections to be carried out for imported consignments is given in the 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 database, 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 most relevant pathway for introduction of *X. fastidiosa* is the importation of plants for planting and infectious insects (vectors) originating from areas where the pest is present. Plants for planting are generally considered to present a high risk of pest introduction especially as:

- the pest can survive, and multiply, on living hosts
- once at their destination plants will remain planted or be replanted. The pest may survive on the plant it was introduced with and might then transfer to a suitable host if the conditions are suitable, especially if the plants are grown outdoors.

Xylella fastidiosa has recently been detected on plants for planting imported into or moved between EU countries, particularly on coffee (*Coffea* spp.). A list of imported host plants is available from the European Food Safety Authority (EFSA, 2015).

Many plants are susceptible to *X. fastidiosa*, and many EPPO countries require that import consignments of plants or parts of plants are free from the pest. Inspections on import consignments are focused on verifying the compliance of the export certificate and the compliance of the exported material (e.g. country of origin, that plants are dormant and that appropriate treatments and production conditions have been applied to prevent the introduction of *X. fastidiosa* and its vectors).

Adequate facilities will be needed at the point of entry or approved place of inspection to set out plants so that a sufficient number can be inspected.

Plants in a dormant state which are imported from countries where *X. fastidiosa* is known to occur may need to be sampled and tested for latent infection, or to be held in contained conditions for inspection during the following growing season.

Dormant plants with bare stems will show no symptoms and therefore visual inspection of these stocks is not worthwhile until they are in the vegetative period of growth.

3. Inspection of plants

3.1 Selection of plants for visual inspection

When plants are imported or moved in active growth, with leaves, an adequate proportion of plants of a consignment should be subjected to a systematic examination in order to detect the presence or signs of pests in a lot.

Consignments may consist of one or more commodities or lots. Where a consignment comprises more than one commodity or lots, the inspection should consist of several separate visual examinations (IPPC, 2005). A lot should be considered as a number of units of a single commodity, identifiable by its homogeneity of composition, origin, etc., forming part of a consignment (IPPC, 2015).

The size of the unit of inspection, or sample (as 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 the lowest possible level of infestation. All lots which include symptomatic plants should be sampled for testing, with the sample including a representative range of symptoms.

For consignments originating from countries where *X. fastidiosa* is not known to occur, 448 plants should be inspected from a lot of 10 000 which will provide 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.

Where the consignment originates from countries where *X. fastidiosa* is known to occur, the objective should be to detect by visual inspection an infection level of 0.1% or more with a confidence level of at least 99%. For a consignment of 10 000 plants this would require inspection of 3689 plants, provided the infection is uniformly distributed and the plants are selected at random. For small lots the numbers required will mean that all plants should be inspected. In case of symptoms, symptomatic plants are preferentially selected.

4. Sample collection

Samples for laboratory testing should preferably be composed of branches or cuttings with attached leaves. The sample should include mature leaves and 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 sources for diagnosis as they contain higher numbers 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 penduncles (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

Visual observations are not always sufficient for the detection of *X. fastidiosa* due to the fact that latent infection could be present and secondary infection caused by other organisms may hide the symptoms of the pest. As *X. fastidiosa* can be present without symptoms, random sampling for detection of latent infection should be included in the inspection procedure, at least for consignments originating in areas/countries where *X. fastidiosa* is known to occur (see EFSA, 2015).

Sampling of symptomatic and/or non-symptomatic branchlets, shoots and/or leaves (petioles included), according to the most reliable and feasible level of confidence, is appropriate for testing for the presence of *X. fastidiosa*. The infection may be present at very low densities or only locally within a plant and not present in all parts.

The sample should be representative of the entire aerial part of the plant.

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

There is limited experience of testing samples comprising 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]

Dormant material should be sampled according to the criteria provided for asymptomatic plants.

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

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

5. How to preserve and transport samples

The preservation and transport of samples should be conducted according to the following procedure:

- place samples in a 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 during a day off, and to inform the laboratory in advance that the sample(s) will be sent.

6. Sampling of vectors

Insects can be analysed to detect *X. fastidiosa*. Xylem-sucking hemipterans are efficient carriers of *X. fastidiosa*, and there is a risk of introducing infected specimens with plants or fruits. Foliage, branchlets, leaves and all accessible container surfaces, including floor or walls, should be examined to look for live vectors. Adults can be collected using aspirators and if they cannot be processed immediately, they should be stored under 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). The Panel on Phytosanitary Inspections and the Panel on Diagnostics in Bacteriology reviewed this protocol.

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Appendix 1 – Specific procedures – detection

As stated in the section 'Host plants concerned', over 300 plant species are host to *X. 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, yellow spots on leaves, chlorotic foliage often together with pronounced yellow discoloration 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 these 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, sun burn, 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) than 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 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 tree decline. Usually, a narrow band of yellow (chlorotic) tissue 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 in most varieties nut production is severely reduced within a few years.

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).

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).

1.3 Bacterial leaf scorch of blueberry

The first symptom of bacterial leaf scorch of blueberry is a 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 uniformly distributed throughout the foliage.

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

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 independently 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 grape clusters. The growth rate of affected trees is greatly reduced and twigs and branches may wilt. Trees in nurseries can show symptoms of variegated chlorosis, as do trees aged over 10 years. Young trees (1–3 years) become systemically colonized by *X. fastidiosa* faster than older trees. Trees more than 8–10 years old are usually not totally affected, but rather have symptoms on the extremities of branches.

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 side) than fruits from healthy trees (right side). Small raised lesions appear on the underside of leaves. Courtesy M.M. Lopez Instituto Valenciano de Investigaciones Agrarias, Valencia (ES).

1.6 Coffee leaf scorch

Symptoms of coffee leaf scorch appear on new growth of field plants as large marginal and apical scorched

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).

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 has been 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).

1.7 Olive leaf scorching and quick decline

Infections of *X. fastidiosa* in olive 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 called '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. The 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, 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 discoloration 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

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

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

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 the 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 general loss of plant vigour followed by death of part of or the entire 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.

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).

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 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 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 trees never display the typical of leaf scorching seen on infected plum trees. Leaves affected by plum leaf scald have 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).

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).

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 symptoms of leaf scorch. 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 typically develops on the leaf margins (see Fig. 17). As in olive, infections may lead to the death of infected plants.

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

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

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).

Appendix 2 – Short procedure for inspectors

Inspectors should be well equipped and trained to recognize symptoms of *X. fastidiosa* and similar diseases, and should have access to all necessary sets of information to aid identification and determine susceptible host plants. Lot identification and selection of material for inspection has to be performed according to the characteristics of the host plants and the associated risk. Controls should not exclusively consist of visual inspection, as latent infection is possible.

Xylella fastidiosa host plants are detailed in Annex I of the Commission Implementing Decision (EU) 2015/789 (EU, 2015), as updated and amended.

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

Plants showing visual symptoms should be sampled for laboratory testing. Collecting some samples of asymptomatic host plants for laboratory testing is recommended if no symptoms are seen. The petiole and midrib recovered from leaf samples are the best sources for sampling as they contain more xylem vessels.

When plants are moved in active growth, with leaves, an adequate proportion of plants of a consignment should be subjected to systematic examination in order to detect the presence or signs of *X. fastidiosa* in a lot.

Consignments may consist of one or more commodities or lots. Where a consignment comprises more than one commodity or lot the inspection should consist of several separate visual examinations (IPPC, 2005).

A lot should be considered as a number of units of a single commodity, identifiable by its homogeneity of composition, origin, etc., forming part of a consignment (IPPC, 2015).

The size of the unit of inspection, or sample (as 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.

For consignments originating from countries where *X. fastidiosa* is not known to occur, 448 plants should be inspected from a lot of 10 000 which will provide a 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.

For consignments originating from countries where *X. fastidiosa* is known to occur, the objective should be to detect by visual inspection an infection level of 0.1% or more with a confidence level of at least 99%. For a consignment of 10 000 plants this would require inspection of 3689 plants, provided the infection is uniformly distributed and the plants are selected at random.

1. Symptomatic plants

The sample should consist of branches/cuttings representative of the symptoms seen on the plant and containing at least 10–25 leaves depending on the leaf size.

2. Asymptomatic plants

As *X. fastidiosa* can be present without symptoms, random sampling for detection of latent infections should be considered in the inspection procedure, particularly for consignments originating in countries where *X. fastidiosa* is known to occur (see EFSA, 2015).

Sampling of symptomatic and/or non-symptomatic branchlets, shoots and/or leaves (petioles included), according to the most reliable and feasible level of confidence, is appropriate for testing the presence of *X. fastidiosa*. The infection may be present at very low densities or only locally within a plant and not present in all parts.

Dormant material should be sampled according to the criteria provided for asymptomatic plants.

The sample should be representative of the entire aerial part of the plant (canopy, etc.).

For testing individual asymptomatic plants, at least 4–10 branches should be collected, depending on the host and the size of the plant.

All samples for laboratory testing should be clearly labelled for traceability of information, with plant species, identification of lot, sampling date, parts or part of plants sampled and symptoms (possibly with images). Samples should be sent to the laboratory as soon as possible after collection.