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EPPO STANDARD ON PHYTOSANITARY PROCEDURES

# PM 3/81 (3) 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. Revision approved in 2020–09 and 2022–09. The revisions of this protocol have been prepared based on the outcome of different EU funded projects (XF-ACTORS, 2020, PONTE) as well as Euphresco projects.

Authors and contributors are given in the Acknowledgements section.

## 1 | INTRODUCTION

*Xylella fastidiosa* (EPPO Code XYLEFA) (Wells et al., 1987) is listed as an EPPO A2 pest and is a regulated pest in the European Union (EU, 2019/2072), and in several EPPO countries (EPPO, 2022). *X. 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 symptoms of Pierce's disease in commercial vineyards (*Vitis vinifera*) (Su et al., 2014). 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). The outbreak of *X. fastidiosa* in olive trees in Southern Italy (Saponari et al., 2013; Martelli et al., 2016) and the presence of the bacterium in plant species in several Mediterranean countries constituted an important expansion of its geographical distribution and also added new host plants.

There are three accepted subspecies of *X. fastidiosa*, subsp. *fastidiosa*, subsp. *pauca* and subsp. *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 *O. europaea* and other species in Puglia (IT) as well as *X. fastidiosa* subsp. *pauca* strains found in intercepted consignments of *Coffea* plants in the Netherlands, have been completely sequenced (Giampetruzzi et al., 2015; Potnis et al., 2019). For the occurrence of the different subspecies in all affected countries in the EU refer to EU (2020) (https://ec.europa.eu/food/plant/plant_health_biosecurity/legislation/emergency_measures/xylella-fastidiosa/latest-developments_en).

### 1.1 | Vectors of *X. fastidiosa*

Insects belonging to the order Hemiptera, suborder Auchenorrhyncha (Redak et al., 2004), that feed on xylem sap (Chatterjee et al., 2008) are considered as potential vectors of *X. fastidiosa*. Vectors that acquire *X. fastidiosa* as adults remain infective for life (Purcell et al., 2014).

In the Americas, numerous species of xylem sap-sucking Hemiptera from the families Cicadellidae, Aphrophoridae and Cercopidae 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 vectors of Pierce's disease are also included in Annex IIA of the Commission Implementing Regulation (EU) 2019/2072 (EU, 2019) and in the plant health provisions of other EPPO countries.

In the EPPO region, *Philaenus spumarius* has been confirmed as a vector of *X. fastidiosa* to olive, and likely other host plants, in the Southern Italian outbreak of the bacterium (Saponari et al., 2014; Cornara et al., 2017a, 2017b). More recently, *Philaenus italosignus* and *Neophilaenus campestris* have also been confirmed to be vectors of *X. fastidiosa* subsp. *pauca* ST53 to olive plants under experimental conditions (Cavalieri et al., 2019). Cicadidae and Tibicinidae species in the EPPO region should also be considered as potential vectors (EFSA, 2019), although Cornara et al. (2020) found no evidence for a role of Cicadas in the epidemiology of *X. fastidiosa*. EFSA (2019) lists potential European vectors drawn from the Fauna Europaea database (de Jong, 2013, see also Albre & Gibernau, 2019; Morente et al., 2018).

### 1.2 | Host plants concerned

*X. 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 EFSA database (EFSA, 2021) includes 638 plant species reported to be infected by *X. fastidiosa*, of which for 312 plant species the infection has been determined with at least two different detection tests. These species cover hundreds of host plant genera in 82 botanical families (61 botanical families when considering only records with at least two different detection methods). The list of hosts in Europe is regularly updated with the results of official surveys (EU, 2019). The presence of *X. fastidiosa* does not always cause visible symptoms in host plants. 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 (*P. dulcis*) and cherry (*P. avium*), including both ornamental and wild plants. In France, *X. fastidiosa* subsp. *multiplex* has been detected on *Polygala myrtifolia* and many other ornamentals and European plant species. In Portugal (Porto), *X. fastidiosa* subsp. *multiplex* has been detected on olive and plant species typical for the Mediterranean area. In Spain, in the Balearic Islands, all three subspecies have been detected and host plants include wild and cultivated olive trees, vines and almond. In mainland Spain (Alicante), almond and host plants typical to the Mediterranean area have been reported as hosts. In Israel, *X. fastidiosa* subsp. *fastidiosa* has been reported on almond.

In general, trees, shrubs or perennial host plant species are 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 the consolidated version of Commission Implementing Regulation (EU) 2020/ 1201, which can be accessed following this link https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32020R1201 and in a database from EFSA (2021).

### 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 water and mineral nutrients. Symptoms observed 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. Symptoms usually appear on just a few branches but later spread to cover the entire plant. Symptoms can be confused with those caused by other biotic or abiotic factors (other pathogens, environmental such as, water deficiency, salt, air pollutants, nutritional problems, sunburn, etc.); illustrations of possible confusions can be downloaded following this link (https://agriculture.gouv.fr/telecharger/85855?token=9f22e2e6c496c32d8195cb9e164470bde14d654153cdc47f57cf04094ff14b4f).

Symptoms most commonly seen in the EPPO region include bronzing, which may intensify before browning and drying (Janse & Obradovic, 2010). 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 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).

Additionally, illustrations of possible symptoms can be seen at: https://www.ponteproject.eu/category/symptom-xylella/.

## 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 (https://ec.europa.eu/food/plants/plant-health-and-biosecurity/legislation/control-measures/xylella-fastidiosa_en).

Further guidance is given in guidelines ISPM no. 23 *Guidelines for Inspection* (IPPC, 2005) and ISPM no. 31 *Methodologies for sampling of consignments* (IPPC, 2009).

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, in living hosts
- once at their destination plants will remain planted or be replanted. The pest may survive in 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.

*X. fastidiosa* has been detected on plants for planting imported into or moved between EU countries, particularly on coffee (*Coffea* spp.).

Many EPPO countries require that import consignments of plants or parts of plants are free from *X. fastidiosa*. 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 inspection of these stocks is not worthwhile until they are in the vegetative period of growth.

## 3 | INSPECTION OF HOST PLANTS

### 3.1 | Selection of plants for inspection

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

When host plants are imported or moved in active growth, with leaves, plants of a consignment should be subjected to a systematic examination to achieve the desired level of confidence of detecting the presence or signs of *X. fastidiosa* in a lot.

A consignment may consist of one or more lots. Where a consignment comprises more than one lot, the inspection to determine compliance may have to consist of several separate visual examinations, and therefore the lots will have to be sampled separately (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*, a high confidence and the capacity to detect a low level of infection are required. The level of confidence and the detection level are parameters which are normally defined by the NPPO. 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 a sample of 448 plants from a lot of 10 000 plants 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.

When consignments originate from countries where *X. fastidiosa* is known to occur, the objective could be to detect by 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

As *X. fastidiosa* is confined to the xylem tissue of its hosts, the petiole and/or midrib recovered from leaf samples are the best material 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).

Samples for the laboratory should be composed of branches/cuttings with attached leaves. The sample should include mature leaves. Young growing shoots should be avoided. Studies conducted in the EU funded project XF-ACTORS (2020) showed that in infected olive trees, the bacterium was more consistently detected in twigs than in leaves, especially when samples

are collected from resistant olive cultivars (i.e. with low bacterial population).

For small plants the entire plant can be sent to the laboratory.

For sclerotic leaves (e.g. *Coffea* spp.) individual leaves and petioles can be sampled.

Sampling of symptomatic and/or asymptomatic branchlets, shoots and/or leaves (petioles included) 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.

After taking samples, they should be sent to the laboratory as soon as possible.

### 4.1 | Symptomatic plants (if present)

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.

If wilting plants are observed, without a clear cause, it is recommended to test plants to confirm the presence or absence of *X. fastidiosa*.

### 4.2 | Asymptomatic plants

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 of host plants originating in areas/countries where *X. fastidiosa* is known to occur (see EFSA, 2015).

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

Evaluations performed in the framework of XF-ACTORS (2020), aiming at verifying the minimum amount of tissues to be collected from a plant to get consistent and reliable detection, have provided detailed information regarding sampling procedures for many plants (Loconsole et al., 2021).

Details on sampling for testing samples composed of large amount of tissue is presented in Table 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.

**TABLE 1** Guidance on sampling for lots of plants for selected species and tissue to be recovered when testing samples composed of large amounts of tissue[a] (e.g. composite samples from consignment/places of production of plants for planting)

| Host | Minimum number of leaves/twigs/stems to be collected per plant | Number of plants that can be pooled |
|---|---|---|
| *Olea europaea*[b] | 4 (leaves) | Up to 225 |
| *Nerium oleander* | 2 (leaves) | Up to 100 |
| Herbaceous plantlets | 1 (plantlet) | Up to 200 |
| *Polygala myrtifolia*[c] | 2 (twigs) | Up to 125 |
| *Lavandula* spp.[d] | 2 (stems) | Up to 100 |
| *Prunus dulcis*/*P. avium* | 2 (twigs) | Up to 100 |
| *Coffea* spp. | 2 (leaves) | Up to 50 |
| *Helichrysum italicum* | 2 (stems) | Up to 50 |

[a]The indications contained in this table are based on the data published by Bergsma-Vlami et al. (2017) for coffee; National Institute of Biology, SI; Loconsole et al. (2018) for other plants. Validation data available in the EPPO Diagnostic Database.

[b]When sampling plants from a lot, at least four leaves/plant should be collected.

[c]Tests performed on leaves repeatedly failed to detect the bacterium.

[d]Leaves should be removed either by detaching them from the stem or by cutting out the leaf blade.

## 5 | HOW TO PRESERVE AND TRANSPORT SAMPLES

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

- Place the samples in a closed container along with an absorbent component (e.g. plastic sealable bags, etc.).
- Keep at cool temperatures to avoid exposing samples to stress conditions.
- Transport the samples to the diagnostic laboratory as soon as possible, before the plant tissues deteriorate.
- It is important to make sure that the laboratory starts appropriate procedures immediately upon reception, therefore the laboratory should be informed about the foreseen date of arrival and number of samples.

## 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. Adults can be collected using aspirators or sticky traps, 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.

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## APPENDIX 1 - SPECIFIC PROCEDURES: SYMPTOMS OF *X. FASTIDIOSA* INFECTION ON THE MAIN HOSTS

As stated in the section 'Host plants concerned', over 600 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 and between varieties and types of the same host.

### 1. Disease symptoms

Symptoms depend on the combination of host and *X. fastidiosa* strain and the susceptibility of the host variety. As the bacterium invades xylem vessels it blocks the transport of water and mineral nutrients. Symptoms usually appear on just a few branches but later spread to cover the entire plant. 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). 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 such as, water deficiency, salt, air pollutants, nutritional problems, sunburn, etc.); illustrations of such symptoms can be seen via this link.

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).

Additionally, illustrations of possible symptoms can be found at: https://www.ponteproject.eu/category/symptom-xylella/.

#### 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 tis sue 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 the case of bacterial wilt, caused by *Clavibacter 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 symptom associated with *X. fastidiosa* on almond is 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, instead a brown wavy line can be observed in the middle of the desiccated tissue area. 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 southern Europe (Figure 1).


**FIGURE 1** Leaf scorch symptoms on *Prunus dulcis* (almond). EPPO global database: Courtesy of D. Boscia, CNR Institute for sustainable plant protection (IT).

### 1.3 Bacterial leaf scorch of blueberry

The first symptoms caused by the bacteria in blueberry result in marginal leaf scorching (Figure 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. 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 (Figure 3). Following leaf drop the plant typically dies during the second year after symptoms are observed (Chang et al., 2009).


**FIGURE 2** Scorch symptoms on blueberry plant with distinct leaf burn surrounded by a dark line of demarcation between green and dead tissue. EPPO global database: Courtesy of P.M. Brennan University of Georgia (US).


**FIGURE 3** Infected blueberry plant with yellow stems and 'skeletal' appearance. EPPO global database: Courtesy of P.M. Brennan University of Georgia (US).

### 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 styraciflua*, *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 (Figures 4 and 5).

Affected trees show foliar interveinal chlorosis on the upper surface, resembling zinc deficiency. Symptoms can occur in some parts of the canopy 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 (Figure 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


**FIGURE 4** Citrus variegated chlorosis: Typical spots caused on sweet orange (*citrus* sp.) leaves. EPPO global database: Courtesy of M. Scortichini, Istituto Sperimentale per la Frutticoltura, Rome (IT).


**FIGURE 5** Small raised lesions on the underside of a *citrus* sp. leaf caused by *X. fastidiosa* infection.

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

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.

### 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 (Figure 7). Affected leaves drop prematurely, shoot growth is stunted and apical leaves are small and chlorotic. Symptoms may progress to shoot die back. Infection of coffee plants by *X. fastidiosa* can also lead to the '*crespera*' disease which has been reported from Costa Rica (Figure 8). Symptoms range from mild to severe curling of leaf margins, chlorosis and deformation of leaves, asymmetry


**FIGURE 7** Leaf scorch symptoms on *Coffea* sp. EPPO global database: Courtesy of M. Bergsma-Vlami, NPPO (NL).


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

(see Figure 8), stunting of plants and shortening of internodes (Montero-Ast'ua 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'. *X. fastidiosa* (CoDiRO strain). 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 (Figure 9). Over


**FIGURE 9** Symptoms of quick olive decline syndrome (on leaves of an *Olea europaea*). EPPO global database: Courtesy of D. Boscia, CNR Institute for sustainable plant protection (IT).


**FIGURE 10** Symptoms of quick olive decline syndrome (whole plant of *Olea europaea*). EPPO global database: Courtesy of D. Boscia, CNR Institute for sustainable plant protection (IT).

time, symptoms become increasingly severe and extend to the rest of the crown, which acquires a blighted appearance (Figure 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. *X. 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 different subspecies can infect olive (subsp. *pauca* and subsp. *multiplex*). While *X. fastidiosa* is associated with but does not cause disease in olives in the United States (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 (South ern Italy, Argentina and Brazil) (Coletta-Filho et al., 2016).

### 1.8 Pierce's disease of grapes

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

Diseased stems often mature irregularly, with patches of brown and green tissues. Chronically infected plants may have small, distorted leaves with interveinal chlorosis (Figure 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


**FIGURE 11** Yellowing and desiccation of leaves and wilting of bunches in a *Vitis* sp. plant). EPPO global database: Courtesy of M. Scortichini, Istituto Sperimentale per la Frutticoltura, Rome (IT).

**FIGURE 12** Symptoms caused by *Pseudopezicula tracheiphila* on *Vitis* sp. leaves. Courtesy of H. Reisenzein, AGES (AT).


**FIGURE 13** Pierce's disease of grapevine: Persistent petioles. EPPO global database: Courtesy of J. Clark & a.H. Purcell, University of California, Berkeley (US).


**FIGURE 14** Pierce's disease of grapevine. Spring symptoms in cultivar chardonnay (healthy leaf on the left). Courtesy of a.H. Purcell, University of California, Berkeley (US).

mature vines. More tolerant cultivars may survive the 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 (Figure 15). Lateral branches grow horizontally or droop. 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, rounded, 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. The leaves of infected peach trees never display the typical leaf scorching seen on infected plum trees. Leaves affected by plum leaf scald have a typical scorched and scalded appearance (Figure 16). Plum leaf scald also increases the susceptibility of the tree to other problems.


**FIGURE 15** Phony peach: Typical 'phony peach' symptom on peach leaves caused by *X. fastidiosa*. EPPO global database: Courtesy of M. Scortichini, Instituto Sperimentale per la Frutticoltura, Rome (IT).


**FIGURE 16** Plum leaf scald: Typical scorched symptom on plum leaf caused by *X. 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 https://ec.europa.eu/food/plants/plant-health-and-biosecurity/legislation/control-measures/xylella-fastidiosa/database-susceptible-host-plants_en.

On oleander, necrosis typically develops on the leaf margins (see Figure 17). As in olive, infections may lead to the death of infected plants.

*Polygala myrtifolia* is a major susceptible host in out breaks in the Mediterranean area. Infected plants show scorched leaves, with desiccation starting from the tip and progressing to the entire blade (see Figure 18). An illustration of a whole infected plant is given in Figure 19. Leaf scorching symptoms have been also reported on cherry (Figure 20) in late summer/autumn in Italy.

**FIGURE 17** Marginal leaf scorch symptoms caused by *X. fastidiosa* subsp. *pauca* on oleander. Courtesy of D. Boscia, CNR-Institute for sustainable plant protection (IT).


**FIGURE 18** Symptoms on *Polygala myrtifolia*. EPPO global database: Courtesy of B. Legendre, Anses plant health laboratory (FR).


**FIGURE 19** Infected *Polygala myrtifolia*. EPPO Global Database: Courtesy of B. Legendre, Anses Plant Health Laboratory (FR).


**FIGURE 20** Leaf scorch symptoms caused by *Xylella fastidiosa* on cherry. EPPO global database: Courtesy of 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 examination, as latent infection is possible.

A European Commission database detailing host plants found to be susceptible to *X. fastidiosa* in the European Union is available online.[1]

Following good hygiene procedures is important when collecting samples for the laboratory. Inspectors should take appropriate precautions during inspection and sampling, such as wearing protective clothes (coat, overshoes, gloves, etc.). Good hygiene procedures when collecting samples for the laboratory should be followed by decontaminating tools and hands.

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.

A consignment may consist of one or more lots. Where a consignment comprises more than one lot, the inspection to determine compliance may have to consist of several separate visual examinations, and therefore the lots will have to be sampled separately (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).

For *X. fastidiosa*, a high confidence and the capacity to detect a low level of infection are required. The level of confidence and the detection level are parameters which are normally defined by the NPPO. 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 a sample of 448 plants from a lot of 10 000 plants 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.

[1] https://ec.europa.eu/food/plants/plant-health-and-biosecurity/legislation/control-measures/xylella-fastidiosa/database-susceptible-host-plants_en

When consignments originate from countries where *X. fastidiosa* is known to occur, the objective could be to detect by 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.

Sampling of symptomatic and/or asymptomatic 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.

### Symptomatic plants

The sample should consist of branches/cuttings with observed symptoms and should contain 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.

If wilting plants are observed, without a clear cause, plants is recommended to be tested to confirm the presence or absence of *X. fastidiosa*.

### Asymptomatic plants

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 of host plants originating in areas/countries where *X. fastidiosa* is known to occur (see EFSA, 2015).

For testing individual asymptomatic plants, the number of branches to be collected is at least 4–10 depending on the host and plant size. Samples for the laboratory should be composed of branches/cuttings with attached leaves.

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

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.

### 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. Adults can be collected using aspirators or sticky traps, and if they cannot be processed immediately, they should be stored under 95%–99% ethanol or at −20°C. Sticky traps are usually not as effective as active sampling for xylem feeders but remain valuable. Sticky traps can also be stored at −20°C.