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EPPO STANDARD ON PHYTOSANITARY PROCEDURES

# PM 3/82 (3) 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 the 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. 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, which is considered to cause several diseases in a wide range of cultivated and wild host plants, especially in North, Central and South America (EFSA, 2015; Janse & Obradovic, 2010). 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 (Martelli et al., 2016; Saponari et al., 2013) 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*, namely subsp. *fastidiosa*, subsp. *pauca* and subsp. *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 *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 vector of Pierce's disease are also included in Annex IIA of the Commission Implementing Regulation (EU) 2019/2072 (EU, 2019) and in plant health provisions of other EPPO countries.

In the EPPO region, *Philaenus spumarius* has been confirmed as the main vector of *X. fastidiosa* to olive, and likely other host plants, in the Southern Italian outbreak of the bacterium (Cornara et al., 2017a, 2017b; Saponari et al., 2014). 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 potential vectors (EFSA, 2019a), although Cornara et al. (2020) found no evidence for a role of Cicadas in the epidemiology of *X. fastidiosa*. EFSA (2019a) lists potential European vectors drawn from the *Fauna Europaea* database (de Jong, 2013, see also Albre & Gibernau, 2019; Morente et al., 2018a, 2018b).

## 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 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 Mediterranean 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 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 infected plants. 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 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 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).

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

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

Further guidance is given in ISPM no. 23 *Guidance for inspection* (IPPC, 2005), ISPM 31 *Methodologies for sampling of consignments* (IPPC, 2009) and ISPM 36 *Integrated measures for plants for planting* (IPPC, 2016).

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 places 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, strains and the host plant species or cultivars. In general, sampling should preferably be performed during the period of active growth of the plant to maximize the likelihood of detection (Hopkins, 1981). For tropical plant species grown indoors, such as coffee plants, sampling may be performed all year round.

Experience gathered in Europe provides the following information on different host plants:

a. For *O. europaea* and *N. 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, for example soon after frost periods (abiotic stresses);

   Nevertheless, sampling can be performed all year around with no decrease in the diagnostic sensitivity during the winter and spring seasons (evidence collected in the framework of the EU funded project XF-ACTORS, 2020). These observations are considered valid for the areas with mild winters.
b. For *Polygala* spp., sampling can be performed from late spring to early autumn.
c. For deciduous plant species (e.g. *Prunus* spp.) in Italy (Apulia region) symptoms were consistently recorded, together with a detectable bacterium concentration, in leaves collected during summer. Asymptomatic leaves collected earlier in the vegetative period from the same trees tested negative whereas, as also shown in Spain (Alicante province) and more recently in Israel in the same period detection has been possible on 1-year twigs of almond trees as well as during dormancy (Roselló, pers. comm., 2019; Zecharia et al., 2021). These observations are considered valid for the areas with mild winter.
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 grapevine or deciduous trees, e.g. cherry and almond, that have been infected for some time, the bacterium is not detected into the new season's growth until the middle of summer, when symptoms may also become visible. For example, the most suitable time for searching 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 the place of production should be carried out. From the outcome of the inventory, the host plants which are most likely to show symptoms of the pest in the EPPO region should be selected and these plants should be included in the inspection of the place of production. Inspection of host plants in the immediate vicinity to the place of production, if present, may also be required. The web link to the European Commission database detailing host plants found to be susceptible to *X. fastidiosa* in the EU is referred to in Section 2.

For inspecting for symptoms and for testing for asymptomatic plants ISPM 31[1] gives guidance on the sample size.

### 3.1 | Selection of plants for inspection

An adequate proportion of plants should be subjected to a systematic examination to achieve the desired level of confidence of detecting the presence or signs of *X. fastidiosa* in the place of production.

For the purpose of inspection, a lot should be defined as a number of plants which are identifiable as being the

[1]ISPM 31 provides information on the number of units to be sampled, which is considered useful to determine sample sizes for both consignments and places of production.

same variety or clone, with propagating material from the same origin, 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* 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 example, 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 visible and are uniformly distributed and provided that the plants are selected at random or higher risk plants are targeted, e.g. those at the outer edge of the nursery. 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.

The EFSA survey card on *X. fastidiosa* (EFSA, 2019b) provides key elements to consider for a risk-based (e.g. place of production location, origin of plants, susceptibility of hosts, presence of vectors) and statistically sound survey design.

### 3.2 | Sampling of plant material for laboratory testing

Visual observations alone are not 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.

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.

## 4 | PLANT SAMPLE COLLECTION

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).

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*) individual leaves and petioles can be sampled.

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

The sample should consist of branches/cuttings representative of the symptoms seen on the plant(s) and containing at least 10 to 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.

Symptomatic plants should always be sampled as part of the inspection.

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

In the case of no symptomatic finding, sampling of asymptomatic plants should be considered based on the risk. Testing of asymptomatic plants is recommended for host plants near to a plant showing symptoms.

For asymptomatic plants, the sample should be representative of the entire aerial part of the plant. Studies conducted in the EU funded project XF-ACTORS (2020) showed that in olive orchards sampling in the upper part of the olive canopy is more reliable. It was also shown in this project that sampling plants along the first two boundary rows of a field (PS) is an effective method for detecting

**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.

*X. fastidiosa* even in conditions of low prevalence of infection. As mentioned in section 4, the bacterium was more consistently detected in twigs than in leaves.

For testing individual asymptomatic plants, the number of branches to be collected is at least 4 to 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.

## 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 to prevent their escape outside the collecting site.
- Place samples in 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 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*. Monitoring of Hemiptera which are vectors of *X. fastidiosa* may be a complementary activity to inspection and host plant testing at a place of production. Adult vectors should preferably be collected with sweeping nets or aspirators.

Effective methods for quantitative *P. spumarius* sampling have been developed by Morente et al. (2018a, 2018b). Adult *P. spumarius* should preferably be collected with sweep nets or aspirators. A video on insect collection has been published by EFSA and is available at https://www.youtube.com/watch?v=Rjh7FFQCtg8.

Sticky traps are usually not as effective as active sampling for xylem feeders, but remain valuable (Cornara et al., 2018) and insects may be trapped accidentally. Specimens collected from sticky traps can be used for testing.

Sticky traps can be sent to the laboratory for further processing or vectors can be removed from the traps using small forceps/pincers and a suitable solvent such as vegetal xylene, Bio-Clear, kerosene, regular fuel (Purcell et al., 2014) or rapeseed oil. It should be noted that some solvents may be dangerous to human health. A third option is to use scissors to cut around the vector on the sticky trap and place the sticky trap with the vector in a tube. After removal from the traps, insects should be sent in a tube with ethanol. Traps should be serviced checked on a weekly basis.

Sampling for insects should preferably be done from late spring until the end of autumn to maximize the likelihood of detection of the bacterium. However, in Corsica and PACA, detection of *X. fastidiosa* has been obtained in insects collected in winter (December and January) (Cunty et al., 2020).

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.

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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 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 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, 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 such as, water deficiency, salt, air pollutants, nutritional problems, sunburn, etc.); illustrations of such symptoms can be seen via this https://agriculture.gouv.fr/telecharger/85855?token=9f22e2e6c496c32d8195cb9e164470bde14d654153cdc47f57cf04094ff14b4f

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 of 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 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 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 symptom associated with *X. fastidiosa* on almond is 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, 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 A1).


**FIGURE A1** 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 A2). 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 A3). Following leaf drop, the plant typically dies during the second year after symptoms are observed (Chang et al., 2009).


**FIGURE A2** 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 A3** Infected blueberry plant with yellow stems and a '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 independent of plant age and mainly on sweet orange cultivars (Figures A4 and A5).

Affected trees show foliar interveinal chlorosis on the upper surface, resembling zinc deficiency. Sectoring of 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


**FIGURE A4** 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 A5** Small raised lesions on the underside of a *citrus* sp. leaf caused by *X. fastidiosa* infection.

**FIGURE A6** Citrus variegated chlorosis: *Citrus* fruit from infected trees are smaller and mature earlier (left) than fruits from healthy trees (right). Small raised lesions appear on the underside of leaves. EPPO global database: Courtesy of M.M. Lopez Instituto Valenciano de Investigaciones Agrarias, Valencia (ES).


**FIGURE A7** Leaf scorch symptoms on *Coffea* sp. EPPO global database: Courtesy of M. Bergsma-Vlami, NPPO (NL).


**FIGURE A8** 'Crespera' symptoms on *Coffea* sp., including curling of leaf margins, chlorosis and deformation (asymmetry). EPPO global database: Courtesy of M. Bergsma-Vlami, NPPO (NL).

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 A6), 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 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 (Figure A7). 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 (Figure A8). Symptoms range from mild to severe curling of leaf margins, chlorosis and deformation of leaves, asymmetry (see Figure A8), stunting of plants and shortening of internodes (Montero-Astúa et al., 2008).

### 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 (Giampetruzzi et al., 2015; Saponari et al., 2013), 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', *X. fastidiosa* (CoDiRO strain). 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 A9). Over time, symptoms become increasingly severe and extend to the rest of the crown, which acquires a blighted appearance (Figure A10). 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. *X. fastidiosa* has also been detected in young olive trees with leaf scorching and quick decline.


**FIGURE A9** Symptoms of quick olive decline syndrome (on leaves of an *Olea europaea*). EPPO global database: Courtesy of D. Boscia, CNRInstitute for sustainable plant protection (IT).


**FIGURE A10** Symptoms of quick olive decline syndrome (whole plant of *Olea europaea*). EPPO global database: Courtesy of D. Boscia, CNRInstitute for sustainable plant protection (IT).

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 (Southern 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 a sudden drying of a part of a green leaf, which then turns brown while adjacent tissues turn yellow or red (see Figure A11). The leaf symptoms can be confused with fungal diseases, in particular with the '*Rotbrenner*',


**FIGURE A11** Yellowing and desiccation of leaves and wilting of bunches in a *Vitis* sp. plant in the Napa Valley, California (US). EPPO global database: Courtesy of M. Scortichini, Istituto Sperimentale per la Frutticoltura, Rome (IT).

**FIGURE A12** Symptoms caused by *Pseudopezicula tracheiphila* on *Vitis* sp. leaves. Courtesy of H. Reisenzein, AGES (AT).

a fungal disease of grapevine caused by *Pseudopezicula tracheiphila* (Müller-Thurg.) Korf & W.Y. Zhuang (1986) (Figure A12). The desiccation spreads over the whole leaf, causing it to shrivel and drop, leaving only the petiole attached (Figure A13).

Diseased stems often mature irregularly, with patches of brown and green tissue. Chronically infected plants may have small, distorted leaves with interveinal chlorosis (Figure A14) 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 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 the chronic infection for more than 5 years.


**FIGURE A13** Pierce's disease of grapevine: Persistent petioles. EPPO global database: Courtesy of J. Clark & a.H. Purcell, University of California, Berkeley (US).

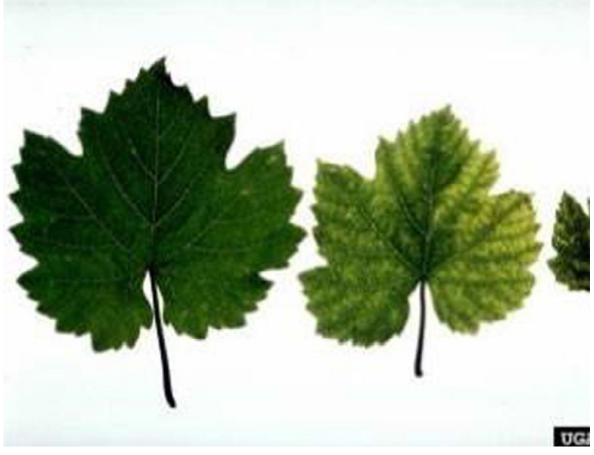

**FIGURE A14** Pierce's disease of grapevine: Spring symptoms in cultivar chardonnay (healthy leaf on the left). Courtesy of a.H. Purcell, University of California, Berkeley (US).

### 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 A15). Lateral branches grow horizontally or droop. 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, 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


**FIGURE A15** 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 A16** Plum leaf scald: Typical scorched symptom on plum leaf caused by *X. fastidiosa*. Reproduced from Mizell et al. (2015).

earlier than normal. The leaves of infected peach never display the typical leaf scorching seen on infected plum trees. Symptoms of plum leaf scald on leaves are a typical scorched and scalded appearance (Figure A16). Plum leaf scald also increases the susceptibility of the tree to other problems.

### 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.

**FIGURE A17** Marginal leaf scorch symptoms caused by *X. fastidiosa* subsp. *pauca* on oleander. Courtesy of D. Boscia, CNR-Institute for sustainable plant protection (IT).


**FIGURE A18** Symptoms on *Polygala myrtifolia*. Courtesy of B. Legendre, Anses, plant health laboratory (FR).

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 developing on the leaf margins is typical (see Figure A17). As in olive, infections may lead to death of the infected plants.

*Polygala myrtifolia* is one of the major susceptible hosts in the outbreaks in the Mediterranean area. Infected plants show scorched leaves, with desiccation starting from the tip and progressing to the entire blade (see leaf tip desiccation in Figure A18). An infected plant is shown in Figure A19. Leaf scorching symptoms have been also reported on cherry (Figure A20) in late summer/autumn in Italy.


**FIGURE A19** Infected *Polygala myrtifolia*. EPPO global database: Courtesy of B. Legendre, Anses, plant health laboratory (FR).


**FIGURE A20** Leaf scorch symptoms caused by *X. 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 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 examination, as latent infection is possible.

The inspections should take place during the period of active growth of the plants. Where possible, inspections should be undertaken during overcast days as symptoms may be obscured by bright sunlight.

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.

The inspection 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 online.[2]

Plants showing 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.

The level of confidence and the detection level are parameters which as 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.

If 448 plants are inspected from a lot of 10 000 this can provide a 99% confidence of detecting evident symptoms present in 1% of the plants, provided that symptoms are visible and are uniformly distributed and provided that the plants are selected at random or higher-risk plants are targeted, e.g. those at the outer edge of the nursery. 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.

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 tree or herbaceous plant and to the conditions to ensure that the selection of plants for 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 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.

The sample should consist of branches/cuttings representative of the symptoms seen on the plant(s) and containing at least 10 to 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.

In the case of no symptomatic finding, sampling of asymptomatic plants should be considered based on the risk. Testing of asymptomatic plants is recommended for host plants near to a plant showing symptoms.

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 culturally important and ancient olive trees showed that detection was more reliable when sampling the upper part of the canopy.

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

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.

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

Monitoring of Hemiptera which are vectors of *X. fastidiosa* may be a complementary activity to the inspection and host plant testing at a place of production. Vectors should preferably be collected with sweep nets or aspirators. Live insects for analysis can be killed and stored in 95–99% ethanol or at −20°C.

Yellow sticky traps can be used (but is not a recommended method because the removal of specimens is often problematic), 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). Sticky traps can be stored at −20°C.

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