

# EPPO Datasheet: *Xylella fastidiosa*

Last updated: 2020-05-12

## IDENTITY

**Preferred name:** *Xylella fastidiosa*

**Authority:** Wells, Raju, Hung, Weisburg, Parl & Beemer

**Taxonomic position:** Bacteria: Proteobacteria:

Gammaproteobacteria: Lysobacterales: Lysobacteraceae

**Other scientific names:** *Grapevine Pierce's disease agent*,  
*Xylella fastidiosa* subsp. *piercei* Schaad et al.

**Common names:** Anaheim disease, California vine disease, Pierce's disease of grapevine, citrus variegated chlorosis, dwarf disease of alfalfa (US), dwarf disease of lucerne (GB), leaf scorch of American sycamore, leaf scorch of almond, leaf scorch of coffee, leaf scorch of elm, leaf scorch of maple, leaf scorch of mulberry, leaf scorch of oleander, olive quick decline syndrome, peach phony disease, plum leaf scald

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**EPPO Categorization:** A2 list

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**EU Categorization:** Emergency measures, A2 Quarantine pest (Annex II B)

**EPPO Code:** XYLEFA



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## Notes on taxonomy and nomenclature

*X. fastidiosa* is a genetically diverse species that has been associated with a wide range of plant diseases (see common names above).

**Subspecies:** six different subspecies of *X. fastidiosa* have been proposed, namely:

- *X. fastidiosa* subsp. *fastidiosa*
- *X. fastidiosa* subsp. *multiplex*
- *X. fastidiosa* subsp. *pauca*
- *X. fastidiosa* subsp. *sandyi*
- *X. fastidiosa* subsp. *tashke*
- *X. fastidiosa* subsp. *morus*

However, of these subspecies only two, *fastidiosa* and *multiplex*, are currently officially considered as valid names by the International Society of Plant Pathology Committee on the Taxonomy of Plant Pathogenic Bacteria (ISPP-CTPPB) (Bull *et al.*, 2012). A pathovar subdivision within *X. fastidiosa* has also been proposed (Hendson *et al.*, 2001).

**Sequence types (STs):** Multilocus sequence typing (Maiden *et al.*, 1998) is a genetic typing methodology that is widely used to characterise *X. fastidiosa* as well as other bacteria (Scally *et al.*, 2005; Yuan *et al.*, 2010; Nunney *et al.*, 2014; Denancé *et al.*, 2017, 2019). Different STs have been identified in the different outbreaks in the EPPO region: ST53 (*X. fastidiosa* subsp. *pauca*) and ST87 (*X. fastidiosa* subsp. *multiplex*) in Italy; ST6 (*X. fastidiosa* subsp. *multiplex*), ST7 (*X. fastidiosa* subsp. *multiplex*) and ST53 in France; ST7 in Portugal; ST1 (*X. fastidiosa* subsp. *fastidiosa*), ST6, ST7, ST80 (*X. fastidiosa* subsp. *pauca*), and ST81 (*X. fastidiosa* subsp. *multiplex*) in Spain (Balearic Islands).

## HOSTS

*X. fastidiosa* is a polyphagous bacterium that can infect the xylem of a wide range of cultivated and wild host plants. A total of 595 plant species have been reported in the scientific literature (EFSA, 2020) as plant hosts of *X. fastidiosa*. From these, 343 were confirmed using at least two different molecular tests for the detection of the bacterium, and many of these plant species were found to have been infected in natural conditions.

*X. fastidiosa* is known to cause severe direct damage to several major crops including grapevine, almond, coffee, citrus, stone fruits, as well as forest, landscape and ornamental trees. The main and most common diseases caused by the bacterium are Pierce's disease (PD) of grapevine in North America, citrus variegated chlorosis (CVC) in South America, and, in recent years, olive quick decline syndrome (OQDS).

The OQDS is a devastating disease caused by *X. fastidiosa* that emerged in Southern Italy in 2013 (Saponari *et al.*, 2013) but that was first detected in the USA in the 2000s (Hernandez-Martinez, 2007; Krugner, 2011). The disease on olive (also called olive leaf scorch and quick decline) has also emerged in Argentina and Brazil (Haelterman *et al.*, 2015; Coletta-Filho *et al.*, 2016).

In the European Union territory, a list of host plants found to be susceptible to *Xylella fastidiosa* and its subspecies is maintained in a database (European Commission, 2020). It can be noted that no single strain of *X. fastidiosa* can infect all of the plant hosts and a large host range variation has been ascertained even within subspecies and among strains sharing the same ST (Nunney *et al.*, 2019).

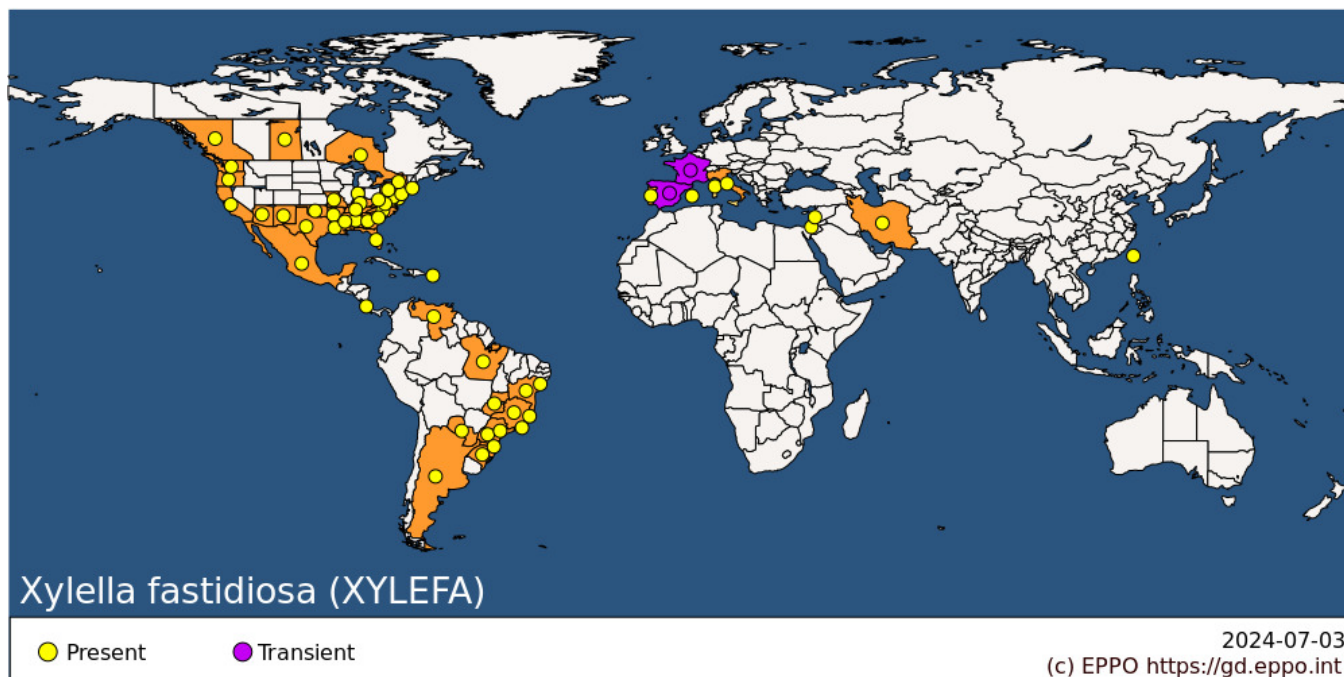
**Host list:** *Acacia cultriformis*, *Acacia dealbata*, *Acacia saligna*, *Acer pseudoplatanus*, *Acer rubrum*, *Amaranthus retroflexus*, *Anthyllis hermanniae*, *Arbutus unedo*, *Argyranthemum frutescens*, *Artemisia arborescens*, *Artemisia sp.*, *Asparagus acutifolius*, *Bidens pilosa*, *Calicotome sp.*, *Calicotome spinosa*, *Calicotome villosa*, *Campsis radicans*, *Carya illinoensis*, *Catharanthus*, *Cercis siliquastrum*, *Chenopodium album*, *Cistus albidus*, *Cistus creticus*, *Cistus monspeliensis*, *Cistus salviifolius*, *Cistus x incanus*, *Citroncirus*, *Citrus trifoliata*, *Citrus x aurantium var. sinensis*, *Citrus*, *Coffea*, *Convolvulus cneorum*, *Coprosma repens*, *Coronilla valentina subsp. glauca*, *Coronilla valentina*, *Cyperaceae*, *Cytisus scoparius*, *Cytisus villosus*, *Dimorphotheca ecklonis*, *Dimorphotheca fruticosa*, *Diospyros kaki*, *Dodonaea viscosa*, *Elaeagnus angustifolia*, *Elaeagnus x submacrophylla*, *Eremophila maculata*, *Erica cinerea*, *Erigeron bonariensis*, *Erigeron karvinskianus*, *Erigeron sp.*, *Erigeron sumatrensis*, *Eriocephalus africanus*, *Erysimum*, *Euphorbia chamaesyce*, *Euphorbia terracina*, *Euryops chrysanthemoides*, *Euryops pectinatus*, *Ficus carica*, *Fortunella*, *Fraxinus angustifolia*, *Genista balearica*, *Genista corsica*, *Genista ephedroides*, *Genista hirsuta*, *Genista scorpius*, *Genista tricuspidata*, *Genista x spachiana*, *Gleditsia triacanthos*, *Grevillea juniperina*, *Hebe elliptica*, *Hebe*, *Helichrysum italicum*, *Helichrysum sp.*, *Helichrysum stoechas*, *Heliotropium europaeum*, *Hibiscus sp.*, *Hypericum perforatum*, *Ilex aquifolium*, *Juglans regia*, *Laurus nobilis*, *Laurus sp.*, *Lavandula angustifolia*, *Lavandula dentata*, *Lavandula latifolia*, *Lavandula stoechas*, *Lavandula x chaytorae*, *Lavandula x heterophylla*, *Lavandula x intermedia*, *Lepidium ruderales*, *Ligustrum sinense*, *Liquidambar styraciflua*, *Lolium multiflorum*, *Lonicera japonica*, *Magnolia x soulangeana*, *Medicago arborea*, *Medicago sativa*, *Metrosideros excelsa*, *Morus alba*, *Morus rubra*, *Myoporum insulare*, *Myrtus communis*, *Nerium oleander*, *Olea europaea*, *Parthenium hysterophorus*, *Pelargonium graveolens*, *Pelargonium x fragrans*, *Persea americana*, *Phagnalon saxatile*, *Phagnalon sp.*, *Phillyrea latifolia*, *Phlomis fruticosa*, *Phlomis italica*, *Pistacia vera*, *Plantago major*, *Platanus occidentalis*, *Poaceae*, *Polygala myrtifolia*, *Prunus angustifolia*, *Prunus armeniaca*, *Prunus avium*, *Prunus cerasifera*, *Prunus cerasus*, *Prunus domestica*, *Prunus dulcis*, *Prunus persica*, *Prunus salicina*, *Prunus serotina*, *Quercus palustris*, *Quercus pubescens*, *Quercus rubra*, *Quercus suber*, *Raphanus sativus*, *Retama monosperma*, *Rhamnus alaternus*, *Robinia pseudoacacia*, *Rosa Cluster-flowered bush hybrids*, *Rosa canina*, *Rosa multiflora*, *Rubus ulmifolius*, *Rumex sp.*, *Salvia rosmarinus*, *Santolina chamaecyparissus*, *Solanum americanum*, *Sorghum halepense*, *Spartium junceum*, *Strelitzia reginae*, *Streptocarpus*, *Teucrium capitatum*, *Ulex europaeus*, *Ulex minor*, *Ulmus americana*, *Vaccinium corymbosum*, *Vaccinium darrowii*, *Vaccinium virgatum*, *Vernonia sp.*, *Viburnum tinus*, *Vinca minor*, *Vinca*, *Vitex agnus-castus*, *Vitis labrusca*, *Vitis riparia*, *Vitis vinifera*, *Vitis*, *Westringia fruticosa*, *Westringia glabra*, *x Citrofortunella microcarpa*

## GEOGRAPHICAL DISTRIBUTION

*X. fastidiosa* is known to occur over a wide range of climatic zones in tropical and subtropical areas, as well as in more temperate or even continental climate regions. Until the 2010s, *X. fastidiosa* was known to occur in the Americas only. In Asia, a bacterium causing pear leaf scorch disease in Taiwan and first identified as *X. fastidiosa*

(Su *et al.*, 2013) has later been reported as being a new species *X. taiwanensis* (Su *et al.*, 2016). In the EPPO region, the outbreak of *X. fastidiosa* in olive trees in Apulia, Southern Italy (Saponari *et al.*, 2013) and the presence of the bacterium in Mediterranean plant species in the natural and urban landscapes of Italy (Apulia, Toscana), France (Corsica and Provence-Alpes-Côte d'Azur), Spain (Balearic Islands, Comunidad Valenciana and Madrid) and Portugal (Porto), constituted a major change to its geographical distribution. Records from Turkey (Güldür *et al.*, 2005), Lebanon (Temsah *et al.*, 2015; Habib *et al.*, 2016) and Kosovo (Berisha *et al.*, 1998; EPPO, 1998) are considered invalid.

The distribution below is given for all *X. fastidiosa* subspecies.



**EPPO Region:** France (mainland, Corse), Israel, Italy (mainland), Portugal (mainland), Spain (mainland, Islas Baleares)

**Asia:** Iran, Israel, Lebanon, Taiwan

**North America:** Canada (British Columbia, Ontario, Saskatchewan), Mexico, United States of America (Alabama, Arizona, Arkansas, California, Delaware, District of Columbia, Florida, Georgia, Indiana, Kentucky, Louisiana, Maryland, Mississippi, Missouri, New Jersey, New Mexico, New York, North Carolina, Oklahoma, Oregon, Pennsylvania, Rhode Island, South Carolina, Tennessee, Texas, Virginia, Washington, West Virginia)

**Central America and Caribbean:** Costa Rica, Puerto Rico

**South America:** Argentina, Brazil (Bahia, Espirito Santo, Goias, Minas Gerais, Para, Parana, Rio de Janeiro, Rio Grande do Sul, Santa Catarina, Sao Paulo, Sergipe), Paraguay, Venezuela

## BIOLOGY

### Interaction between *X. fastidiosa* and its host plants

*Xylella fastidiosa* is a xylem-limited fastidious bacterium that in nature is exclusively transmitted by xylem-fluid feeding insects. The bacterium therefore proliferates only in xylem vessels, in roots, stems and leaves. Symptom expression is usually linked to the occlusion of these vessels by bacterial aggregates, as well as by tyloses and gums formed by the plant. Therefore, symptoms of *X. fastidiosa* often resemble those caused by water stress. In some cases, the infection results in rapid death of the host plant. However, many plant species if not the majority may not even express any symptoms (EFSA, 2015a, 2018, 2019b). The symptomless plants often serve as a source of inoculum for vectors (Hopkins and Purcell, 2002). In some host plants, bacterial cells may remain restricted to parts of the plant or even at the point of entrance. In susceptible plants, they move systemically through the xylem vessels, mainly upstream but also downstream, and may invade all plant organs. The time period between infection and the appearance of symptoms (incubation period) is highly variable and ranges from a few months to even more than one

year, depending on the *X. fastidiosa* genotype, the host species, the age of the plant and growing conditions (EFSA, 2019b, 2020).

Winter climate is usually a key factor in delimiting the areas where *X. fastidiosa* can persist from one season to the next. Pierce's disease and phony disease only occur in areas with a mild winter, presumably in relation to survival of the bacterium in dormant plants (Purcell, 1989). Experimental cold therapy of diseased grapevines suggests that freezing temperatures can eliminate the bacterium directly in the plants (Purcell, 1980). Although the bacterium preferably overwinters in infected plants, it may also survive in its insect vectors. *X. fastidiosa* is efficiently acquired by vector insects, with no latent period, and it multiplies and persists in infective adults indefinitely (Severin, 1950). Therefore, if these infected adults overwinter, they can maintain *X. fastidiosa* during the adverse season. This is the case of sharpshooters (Cicadellidae, Cicadellinae) in the American continent. However, the European sharpshooters and most of the European spittlebugs (Aphrophoridae and a few Cercopidae) overwinter as eggs and therefore, if infected, these vectors cannot sustain overwintering of *X. fastidiosa*, since transovarial transmission of *X. fastidiosa* does not occur (Freitag, 1951).

### **Interaction between *X. fastidiosa* and its vectors**

In theory all xylem-fluid feeding insects are considered vectors, until proven otherwise (Frazier, 1944; Purcell, 1989; Almeida *et al.*, 2005), but transmission efficiency varies substantially depending on insect species, host plant, and *X. fastidiosa* genotype (Redak *et al.* 2004; Lopes *et al.*, 2010). All *X. fastidiosa* insect vectors belong to Auchenorrhyncha (Hemiptera) and are distributed within the superfamilies Cercopoidea, Cicadoidea and Membracoidea. Each of the three superfamilies include xylem-fluid feeding groups, but, while all Cercopoidea (known as spittlebugs or froghoppers) and Cicadoidea (cicadas) are regarded as xylem-fluid feeders, in Membracoidea only the subfamily *Cicadellinae* (known as sharpshooters) within the family *Cicadellidae* are actually xylem-fluid feeders.

Acquired bacteria adhere to the precibarium and the cibarium of the foregut and do not systemically colonize the insect body (Purcell and Finlay, 1979). This implies that nymphs lose infectivity when moulting, as the foregut is of ectodermal origin and is renewed, and that newly emerged adults must again acquire *X. fastidiosa* to become infectious. Normally very few live bacterial cells are sufficient for successful transmission (Hill and Purcell, 1995, 1997) which is neither transovarial nor transstadial. Temperature has a key role in the interaction between the bacterium and its vectors: it influences the pathogen multiplication in source plants (Feil and Purcell, 2001); the pathogen multiplication in vectors (Dohm *et al.*, 2002); the successful establishment of the pathogen in the new host (Chu and Volety, 1997), and the vector behaviour (Su and Mulla, 2001).

In Californian vineyards, *Homalodisca vitripennis* (= *H. coagulata*), *Xyphon fulgidum* (= *Carneiocephala fulgida*), *Draeculacephala minerva*, and *Graphocephala atropunctata* are considered to be the most important vectors of Pierce's disease. In Brazilian citrus orchards, *Acrogonia terminalis*, *Dilobopterus costalimai*, *Oncometopia facialis* are considered to be the most important vectors of citrus variegated chlorosis.

In the EPPO region, the only confirmed vectors of *X. fastidiosa* are *Philaenus spumarius* (Cercopoidea: Aphrophoridae), and in experimental conditions, *Neophilaenus campestris* and *Philaenus italosignus* (Aphrophoridae) (Cavaliere *et al.*, 2018; Saponari *et al.*, 2014, Cornara *et al.*, 2017b). *P. spumarius* (meadow spittlebug), the only effective vector of *X. fastidiosa* under natural conditions, is a highly polyphagous and univoltine species. Nymphs and adults have a different host range. The nymphs prefer tender plant parts and herbaceous plants, in particular plants of the Asteraceae, Fabaceae, and Apiaceae families (Di Serio *et al.*, 2019; Dongiovanni *et al.*, 2018b) which often grow as cover crops or invasive plants, in the olive groves, on field hedges, and in natural and semi-natural areas. The adults, by contrast, tend to feed on woody plant species (EFSA, 2019c).

In Southern Italy (Apulia) adults of *P. spumarius* emerge between the end of April and the beginning of May and move from the herbaceous vegetation of olive groves to the canopy of olive trees and other evergreen or deciduous trees and shrubs in late spring to early summer (Di Serio *et al.*, 2019; Cornara *et al.*, 2017a; Bodino *et al.*, 2017). This movement can also be observed if the grass cover persists over the summer. At the end of summer and early autumn, adults return to herbaceous plants for egg laying and overwintering (Weaver and King, 1954; Cornara *et al.*, 2018). The host range of *X. fastidiosa* subsp. *pauca* strain ST53, which causes the OQDS in Italy, overlaps with that of *P. spumarius* (Weaver and King, 1954; Cornara *et al.*, 2017b), suggesting that some of these host species may serve as natural reservoirs and possibly as inoculum sources for primary spread of the pathogen to the affected crops.

Climatic conditions in different parts of the EPPO region can affect the life cycle of *P. spumarius*. For example, in the Mediterranean climate, adults are abundant soon after emergence and then later after aestivation in October. In the Netherlands, by contrast, *P. spumarius* can be detected from June onwards to October (Noordijk *et al.*, 2019), with a peak in the adult population in June and July.

In general, in the context of surveillance and considering the above, it is preferable to collect insect samples late in the season, as insects collected during this period might have fed on multiple host plants and the chances to be infected are therefore higher.

## DETECTION AND IDENTIFICATION

The detection and identification of *Xylella fastidiosa* relies on the combination of visual examination, sampling and testing of insect vectors and plant material.

### Symptoms

Symptoms of *Xylella*-diseases are highly variable and their expression depends on the combination of host plant and bacterial strain, as well as on environmental conditions, including the specific growing conditions of the individual plant and its phenological stage. These symptoms include leaf scorching, wilting of the foliage, (premature) defoliation, chlorosis or bronzing along the leaf margin, stunting and dieback of shoots and twigs, formation of new malformed (asymmetric) leaves and dwarfing (EPPO, 2023). Considering the large host range of *X. fastidiosa*, descriptions of symptoms are provided below only for its main hosts.

#### *Grapevine*

The most characteristic symptom of primary infection is leaf scorch. An early sign is the sudden drying of part of a green leaf, which then turns brown while adjacent tissues turn yellow or red. The desiccation spreads and the whole leaf may shrivel and drop, leaving only the petiole attached. Diseased stems often mature irregularly, with patches of brown and green tissue. In later years, infected plants develop late and produce stunted chlorotic shoots. They rarely survive more than a year or two, even if there are signs of recovery.

#### *Peach*

Young shoots are stunted and bear greener, denser foliage than healthy trees. Lateral branches grow horizontally or droop, so that the tree seems uniform, compact and rounded. Leaves and flowers appear early, and leaves remain on the tree longer than on healthy trees. Affected trees yield increasingly fewer and smaller fruits until, after 3-5 years, they become economically worthless.

#### *Citrus*

Trees can start showing the symptoms of variegated chlorosis from nursery size up to 7-10 years of age. These younger trees become systemically affected by *X. fastidiosa*. Trees more than 15 years old are not usually totally affected, but rather have one or two major scaffold branches showing symptoms. Affected trees show foliar chlorosis resembling zinc deficiency with interveinal chlorosis. The chlorosis appears on young leaves as they mature and may also occur on older leaves. Newly affected trees show sectoring of symptoms, whereas trees which have been affected for a period of time show the variegated chlorosis throughout the canopy. As the leaves mature, small, light-brown, slightly raised gummy lesions (becoming dark-brown or even necrotic) appear on the underside, corresponding to the yellow chlorotic areas on the upper side.

Fruit size is greatly reduced; its sugar content is higher than in non-affected fruit, and the rind may become so hard that can cause damage to juicing machines. Blossom and fruit set occur at the same time on healthy and affected trees, but physiological fruit thinning does not occur on affected trees and the fruits remain small. Because of this, total production is not greatly reduced. On affected trees of cv. Pera and other orange cultivars, fruits often occur in clusters of 4-10, resembling grape clusters. Affected trees show stunting and slow growth rate; twigs and branches die back, although usually the plant does not die, and the canopy thins.

## *Olive*

The disease on olive plants is characterized by the appearance of scattered desiccation of twigs and small branches. Leaves are the first to be affected. Scorching starts at their tip and progresses towards the petiole, extending to the whole blade. Dead leaves remain attached throughout summer to the twigs, which are also desiccating, and begin to drop with the first rains in autumn. Symptoms are first localized in the upper part of the crown, then they extend to the rest of the canopy. Trees of susceptible cultivars, decline and die within a few years from the appearance of symptoms. These trees, especially the centuries-old ones, are often pruned heavily, forcing them to push new growth which, eventually, will wither and desiccate (Martelli, 2016).

## *Polygala myrtifolia*

This is one of the major susceptible hosts in the current European outbreak. Infected plants show scorched leaves, with desiccation starting from the tip and progressing to the entire blade.

## Morphology

### *Xylella fastidiosa*

*X. fastidiosa* is a Gram-negative bacterium, rod-shaped with distinctive rippled cell walls. It is non-flagellate, does not form spores and measures 0.1-0.5 x 1-5  $\mu\text{m}$ .

### Vectors

Adult sharpshooters are distinguished from other leafhopper subfamilies by the possession of highly 'inflated' or 'swollen' faces caused by the massive musculature required by the cibarial (pharyngeal) pump to suck in large quantities of xylem (Young, 1968). Sharpshooters are typically among the largest of leafhoppers. The smaller species (e.g. *Xyphon fulgidum*) may be about 4 mm in length, while larger species such as *Homalodisca vitripennis* may exceed 15 mm. Nymphs generally resemble adults in form but usually differ markedly in colouration and markings. The subfamily is quite diverse, especially in the American tropics, with over 210 genera (Young, 1977). Colouration varies from uniformly green (e.g. *Draeculacephala* spp.) or other cryptic colouration to combinations of bright-red, orange, yellow and blue tropical species. Sharpshooters typically have broad plant host ranges. Even those species that are found on a single or few host species are capable of prolonged survival in captivity on unusual hosts. The rhododendron sharpshooter, *Graphocephala fennahi*, is a North American species now established in Europe, where it occurs not only on *Rhododendron* but on many other woody plants. Female sharpshooters and spittlebugs insert their eggs into plant tissues. The eggs of some sharpshooter species remain dormant over winter.

Cercopidae may be distinguished from leafhoppers, which possess one or more rows of spines along the hind tibia, by the smooth hind tibia with a few or a circlet of stout spines at the apex of the tibia. The body shape of froghoppers is generally stouter than that of leafhoppers.

The meadow spittlebug *Philaenus spumarius* belongs to the order Hemiptera, superfamily Cercopoidea, family Aphrophoridae. The name spittlebug came from the shell built up by the nymphs mixing fluid voided from the anus and a secretion produced by glands located between the 7th and the 8th abdominal sternites. Air bubbles are introduced within the spittle by mean of caudal appendages and a ventral tube formed by abdominal tergites bent downward (Cornara *et al.*, 2018).

*P. spumarius*, that has never been considered an agricultural pest in Europe before the introduction of *X. fastidiosa*, is extremely variable in colour, ranging from light grey to blackish although the most typical form is yellow-green with indistinct dark lines.

*P. spumarius* is widely distributed (temperate regions of Europe, Asia, North America and also North Africa) and occurs in most terrestrial habitats (meadows, abandoned fields, waste ground, roadsides, banks of streams, hayfields, marshlands, parks, gardens and cultivated fields; Yurtsever, 2000) on hundreds of host plants ranging from grasses to trees, including meadow crops, herbs, thistles, garden plants, shrubs, and conifers. However, nitrogen fixing legumes and other plants with high amino acids concentration in the xylem sap (*Medicago sativa*, *Trifolium* sp., *Vicia* spp.,

and *Xanthium strumarium*) are the preferred hosts.

*P. spumarius* overwinter as eggs. Eggs are oviposited in stubble, herbs, dead parts of plants, plant residues, cracks and tree trunk barks, or in the leaf litter (but the majority of eggs are laid close to the ground between two apposed surfaces) at the end of summer (in Apulia, oviposition was achieved in semi-artificial conditions in October). Hatching occurs in the following spring. The larvae, not very mobile, feed on the sap present in the xylem by sticking their stylets into the plant. Larval development has five instars. Adults appear in April and live until autumn (but they may survive until the successive spring in case of mild winters: Saponari *et al.* 2014). They are not very active and exhibit a jumping behaviour when they are disturbed.

Although the nymphs live inside the spittle, they can actively crawl over short distances, thus moving from one herbaceous plant to another (Bodino *et al.*, 2017). Adults are much more mobile, both actively and passively. Passive dispersal over great distances is mediated by wind and human activities (transportation by cars can also occur).

## Detection and inspection methods

### Sampling

Samples for the laboratory should be collected during summertime and be composed of branches or cuttings with (mature) leaves attached. Sampling of young growing shoots briefly after emergence should be avoided because the bacteria may be difficult to detect in the new season's flush. For small-sized plants, the entire plant can be sent to the laboratory. In the case of detection surveys, it is usually recommended to sample multiple plants and test a pooled sample from the survey site. For example, to test for the presence of *X. fastidiosa* in olive trees, a laboratory sample may consist of up to 20 g of leaf petioles, corresponding to about 800-900 leaf petioles, while 4 leaf petioles are taken per plant. This would result that up to 200-225 plants are then tested in a single pooled sample.

Vector sampling is considered to be an efficient practice to detect the bacterium in a given area. Currently, sweep nets are commonly used to collect adult insects of *P. spumarius* (Cruaud *et al.*, 2018; Cornara *et al.*, 2018). Other trapping methods, such as minicage (biocenometers), pitfall traps, sticky traps, aerial suction traps, beat trays and tanglefoot bands have been proven to be less effective than sweep nets. To maximise the likelihood of detection, insect samples should be collected when the adults are abundant in the field and after they have fed on multiple hosts at the end of the summer or after aestivation.

More details about sampling can be found in the EPPO Standard PM 7/24 (EPPO, 2023) and the EFSA Pest survey card (EFSA, 2019a).

### Laboratory testing

Given the difficulty of isolating *X. fastidiosa*, various serological and molecular diagnostic protocols have been validated to be applied directly on plant tissue or insect vectors extracts. Given their lower sensitivity, the use of serological tests is advised only for screening large numbers of symptomatic plants as well as asymptomatic material from an outbreak area or a buffer zone around an outbreak, as the concentration of the bacterium in the infected tissues is likely to be higher. Molecular methods based on the use of PCR, are generally retained to be more sensitive and specific, although the presence of inhibitors may be a problem with some matrices. Their use is recommended in particular for detecting *X. fastidiosa* in asymptomatic plants, insect vectors and for subspecies assignment directly or by sanger sequencing the amplicons of at least two and up to seven, housekeeping gene fragments (EPPO, 2023). For areas where the pathogen is known to be present, the result of a single test is considered sufficient (EPPO, 2023) but in areas considered to be pest free, for a positive detection to be considered valid, a minimum of two positive screening tests should be undertaken for plant samples. These tests should either differ in the underlying biological principles or in the genomic sequence they target. Remote sensing techniques and hyperspectral imaging methods are becoming more reliable for the early detection of infected trees, particularly for the detection of the pre-visual symptoms (Kumar *et al.*, 2012; Li *et al.*, 2014). In Zarco-Tejada *et al.* (2018), airborne imaging spectroscopy and thermography revealed the presence of *X. fastidiosa* in olive trees prior to symptom expression.

Real-time PCR (Harper *et al.*, 2010, erratum 2013) is recommended for detection of *X. fastidiosa* in vector samples. In order to reduce the number of samples to be tested in the laboratory, it is possible to pool the samples of the same species. For small vectors (e.g. *Philaenus*), 1–5 heads can be pooled, while for large vectors (e.g. *Cicada orni* or *Aphrophora*

spp.) a single insect's head can be used (EPPO, 2023).

More details about laboratory testing can be found in the EPPO Standard PM 7/24 and ISPM 27/DP 25 (EPPO, 2019a; IPPC, 2018).

## PATHWAYS FOR MOVEMENT

*X. fastidiosa* is unable to spread in the environment autonomously either by contact or by air diffusion. It also appears that *X. fastidiosa* is not seed-transmitted. Natural spread of *X. fastidiosa* is mainly ensured by its insect vectors that generally fly short distances (on average up to 100-150 metres) but can be transported by wind over longer distances. The main pathway for entry and spread of *X. fastidiosa* is the trade, or movement, of plants for planting. Infectious vectors can also be carried over short and long distances on plants, plant parts, or as hitchhikers (i.e. through indirect means, such as clothing or body parts of people, vehicles, onto which they can be passively transported). Fruit, cut flowers, ornamental foliage and wood (not for plant propagation purposes) are considered as unlikely pathways for *X. fastidiosa*.

## PEST SIGNIFICANCE

### Economic impact

In the USA, within the main areas where *X. fastidiosa* occurs naturally (coastal plains of the Gulf of Mexico), *Vitis vinifera* and *V. labrusca* cannot be cultivated because they are rapidly infected due to high rates of natural spread. As a consequence, only selections of *V. rotundifolia* (muscadine) and specially bred resistant hybrids can be cultivated. The same situation exists throughout tropical America. Pierce's disease is thus a major constraint on grapevine production in the USA and tropical America. In California alone, the value of grapevine losses to Pierce's disease, including the costs of roguing and replanting diseased vines, is on average of 56.1 million USD/year (wine, table and raisin grapes). Moreover, since another 48.3 million USD are spent by nurseries, citrus growers, and government agencies to limit the populations of *H. vitripennis*, the estimated cost of Pierce's disease is approximately of 104.4 million USD per year (Tumber *et al.*, 2014).

By contrast, in peaches, phony disease does not kill trees or cause dieback, but it does significantly reduce the size and number of fruits. An analysis of biophysical effects on peach trees has been made by Anderson & French (1987). The disease was extremely important in the south-eastern USA in the 1940s, when 5-year-old orchards were often found to be 50% affected and older orchards entirely so. However, the efficient control methods now available (insecticides, destruction of infected trees, elimination of wild host plants around orchards) allow a high degree of control, except in areas where incidence is very high.

In citrus, variegated chlorosis has been described by Roistacher as 'the world's most destructive disease of sweet orange'. It has spread rapidly through large areas of southern Brazil and appears that it cannot be controlled. In a survey made in São Paulo State in June 1990, it was reported that 13 sites out of 920 had infected trees. In a follow-up survey in August 1991, 72 out of the 920 sites were infected (a 5-fold increase in a period of just 14 months). By 2000, it was estimated that the disease affected 34% of the 200 million sweet orange trees (Bové and Ayres, 2008), and some growers in São Paulo state are now planting mangoes instead of citrus.

In olive, the most serious epidemic has developed in Apulia (Southern Italy). The bacterium was diagnosed for the first time in 2013, in the municipal area of Gallipoli (Province of Lecce), and the 'focus' of infection was already about 8 000 hectares. Since then the disease has advanced by an average of 2 km a month, and now it has reached the municipal area of Monopoli (Province of Bari), 140 kilometres from Gallipoli. As of 2019, it has been estimated that in the Provinces of Lecce, Brindisi and Taranto approximately 11 million plants were infected, over an area of about 50 000 ha.

Damage concerned olive trees in production as well as nurseries of olive plants and have been estimated at a total of 1.2 billion EUR. An indirect impact concerned the export from Italy of any species of plants susceptible to *X. fastidiosa*. Finally, the damage caused by *X. fastidiosa* to the landscape of the 'Salento' Peninsula in Apulia and to the Mediterranean cultural heritage associated to olive trees is unquantifiable.



## Control

The control of *X. fastidiosa* relies on different methods and can have different targets: the vector, the bacterium, both the vector and the bacterium, and host plants that may function as reservoirs of *X. fastidiosa*. Control strategies against insect vectors (mostly *P. spumarius* in the case of OQDS) should take into account the interactions of the vectors with the pathogen (transmission characteristics), the host plants (of the pathogen and vector) and the environment, as well as the inoculum sources and types of spread (primary and secondary) (Almeida *et al.*, 2005; Lopes *et al.*, 2016).

The management strategies that are detailed below mainly apply to OQDS and are based on currently available information; however, they are likely to evolve as new knowledge becomes available. The objective is to reduce population density of *P. spumarius* and to avoid its further spread. Primary (from weeds to olives) and secondary spread (among olive trees within and between orchards) is ensured by *P. spumarius*, and most likely by adult *P. spumarius*.

The most effective active substances to control adults of *P. spumarius* on olive trees are neonicotinoids (i.e. acetamiprid) and pyrethroids (deltamethrin) (Dongiovanni *et al.*, 2018a). Application of insect growth regulators (buprofenzin and spirotetramat) against spittlebug nymphs showed very low efficacy. Preliminary data suggest that citrus oil is more effective than pyrethrins, however both products are not persistent. For the control of the juveniles, the neonicotinoids and pyrethroid products cause significant reduction in nymphs on the sprayed vegetation.

In the EPPO region, no antibacterial compounds are routinely applied to perennial crops, except copper, which is unable to cure plants of *X. fastidiosa* or even to prevent transmission by insects. However, a compound containing zinc, copper and citric acid has been shown to reduce the multiplication of *X. fastidiosa* in olive tissues (Scortichini *et al.*, 2018).

Another selective and potential control method is the application of particle film technology (e.g. kaolin) to interfere with the vector-host plant selection. When applied on grapes, kaolin particles change the colour of the tree canopy, thus inhibiting landing by sharpshooter vectors and reducing pathogen transmission (Puterka *et al.*, 2003; Tubajika *et al.*, 2007). However, it seems that this method does not work in the case of OQDS.

With regard to biological control, very little is currently known on the use of natural enemies (parasitoids of eggs, nymphs, adults; or entomopathogenic micro-organisms) against *X. fastidiosa* vectors.

Elimination of weeds within and around olive groves may help in reducing the vector populations. For example, in Apulia tillage performed in winter and spring significantly reduced (almost to zero) the abundance of both *P. spumarius* and *N. campestris* on olive trees and ground vegetation.

Sowing of *Lolium* spp. and *Hordeum vulgare* as a cover crop in winter decreased *P. spumarius* juvenile populations.

The most promising control measures against diseases caused by *X. fastidiosa* rely on the use of resistant germplasm. Various degrees of resistance, tolerance and susceptibility have been observed in economically relevant crops such as grape, citrus, almond, and more recently, olive. In this last case extended surveys and experimental infectivity studies identified cv. Leccino as being tolerant to *X. fastidiosa* subsp. *pauca* ST53 infections based on lower incidence, lower bacterial levels, and symptom severity when compared to cv. Ogliarola salentina (Boscia *et al.*, 2017). It is also hoped that tolerance or resistance traits can also be found in other olive varieties: one of these is the cultivar FS-17 also known as 'Favolosa'. In California, grape selections carrying a genetic trait that confers resistance/tolerance to Pierce's disease of *Vitis* spp. have been released to grapevine nurseries for propagation and resistant varieties should become soon available for commercial use (Walker *et al.*, 2017). Moreover, transgenic grapevine rootstocks, expressing a pear polygalacturonase inhibitory protein (PGIP) or a chimeric antimicrobial protein (CAP), have showed resistance to *X. fastidiosa*. The search for resistant germplasm also continues in the case of *Prunus* and *Citrus* spp.

Finally, control of *X. fastidiosa* also consists in minimizing other sources of stress to the host plant (e.g. drought, overproduction, other diseases). The effect of some agricultural practices on *Xylella* infections, such as pruning, irrigation and fertilisation, have also been investigated with encouraging results.

## Phytosanitary risk

From 1981 to 2017, *X. fastidiosa* was included in the EPPO A1 List of pests recommended for regulation as quarantine pests, but following its introduction in the EPPO region, it was transferred to the EPPO A2 List in 2017. In 2019, *X. fastidiosa* was included in the list of priority pests of the European Union, in the framework of its new plant health legislation (EU, 2019). The introduction of *X. fastidiosa* subsp. *pauca* strain ST53 and different ST of *X. fastidiosa* subsp. *multplex* in the EPPO region represents a high risk to economically important crops and to the environment. In particular, the grapevine strain of *X. fastidiosa* had and still has the potential to kill large numbers of grapevines and to make areas unfit for growing *V. vinifera*. Its North American vectors do not occur in the EPPO region, but vector capacity is so non-specific that one could certainly expect European Cicadellinae (e.g. *Cicadella viridis*) or Cercopidae to transmit the bacterium if introduced. The main threat in the long term is that *X. fastidiosa* could become established in natural vegetation which would then act as a reservoir for infection of vineyards.

Models have been used to estimate the potential distribution of *X. fastidiosa*, and have revealed that the following areas in Southern Europe and the Mediterranean area were climatically suitable for the establishment of *X. fastidiosa* and were more particularly at risk: Atlantic regions of Southern Portugal and Southwestern Spain, coastal areas in Southern France, Italy and Croatia as well as all their islands and archipelagos. Moreover subsp. *multplex* could possibly establish further north in the EU compared to other subspecies (EFSA, 2019b). However, as the potential disease distribution may depend on the biology of its potential vectors, it is accordingly rather difficult to assess.

The presence in the EPPO region of different populations and subspecies of the bacterium may allow the appearance of recombining strains, resulting from crosses between the different subspecies. These events may increase the risk of appearance of new pathogenic strains that are more virulent or pathogenic to new hosts (Vanhove *et al.*, 2019). The likelihood of such crosses between different strains could be favoured by the existence of an overlapping host range. For example, it has been determined that in the European Union territory, out of the 43 plant species reported as host of subspecies *pauca*, 23% were also shown to be host of another subspecies, most frequently subsp. *multplex*, and that 35% may be host for all the three subspecies (European Commission, 2020).

## PHYTOSANITARY MEASURES

The most effective method for preventing the introduction and spread of *X. fastidiosa* into uninfected areas is to impose strict phytosanitary measures and perform inspections (EPPO, 2016b) on imported host plant material, as well as to survey the health status of potential host plants and vectors, in particular in orchards and nurseries (EPPO, 2016c). In this respect, correct and timely disease identification is crucial for the appropriate application of any phytosanitary measure. Rapid detection (and destruction) of new foci of infection are also of the greatest importance.

Within the EPPO region, phytosanitary regulations are in place to prevent the entry and spread of *X. fastidiosa*. For the EU Member States, phytosanitary measures are detailed in the Decision (EU) 2015/789 (EU, 2015).

Considering the serious threat that *X. fastidiosa* represents for grapevine and citrus cultivation, prohibitions to import plants for planting of citrus, grapevine and coffee plants from third countries where *X. fastidiosa* occurs have been imposed by many EPPO member countries. In addition, several countries require that host plants for planting from infected third countries are either grown in a pest free area, or under protected conditions with inspection, sampling and testing for the absence of the bacterium prior to their export. For certain host plants (e.g. *Vitis* plants for planting), hot water treatment (50°C for 45 min) can be considered effective to sanitize plants for planting coming from an infected area (EFSA, 2015b). Plants for planting can also be grown under complete physical isolation (EPPO, 2016d) to exclude insect vectors.

Countries are also recommended to carry out regular surveys to verify the absence or presence of *X. fastidiosa* on their territory. Within the European Union territory, specific annual surveys should be carried out by all Member States and lists of specified host plants have been defined to that effect (European Commission, 2015, 2020; EFSA, 2019a). Detailed information regarding the disease spread capacity is of course fundamental when determining the extent of the area to survey. In this respect, the use of spread models could facilitate the decision-making process. For example, a model has been used to determine the disease spread within an olive orchard. It was estimated that the median short-distance spread was of approximately 150 m per year and that the median long-distance spread was

of approximately 10 km per year (EFSA, 2019b). However, the above values may change according to several parameters, such as the density, spatial distribution and number of different of host plants species, abundance, mobility and biological characteristics of insect vectors.

In the event of a new outbreak of *X. fastidiosa*, eradication or containment measures should be applied. These include the delimitation of infected and buffer zones where appropriate official measures are applied with varying intensity according to the zone concerned. These measures include the destruction of infected plants and potential host plants located in their vicinity, specific surveys (including intensive sampling and testing), restrictions on the movement of host plants, as well as appropriate control measures against the vectors and plants that may host those vectors. So far, it has to be recognized that no eradication attempts have been successful in outdoor conditions, and in areas where the disease has established.

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