

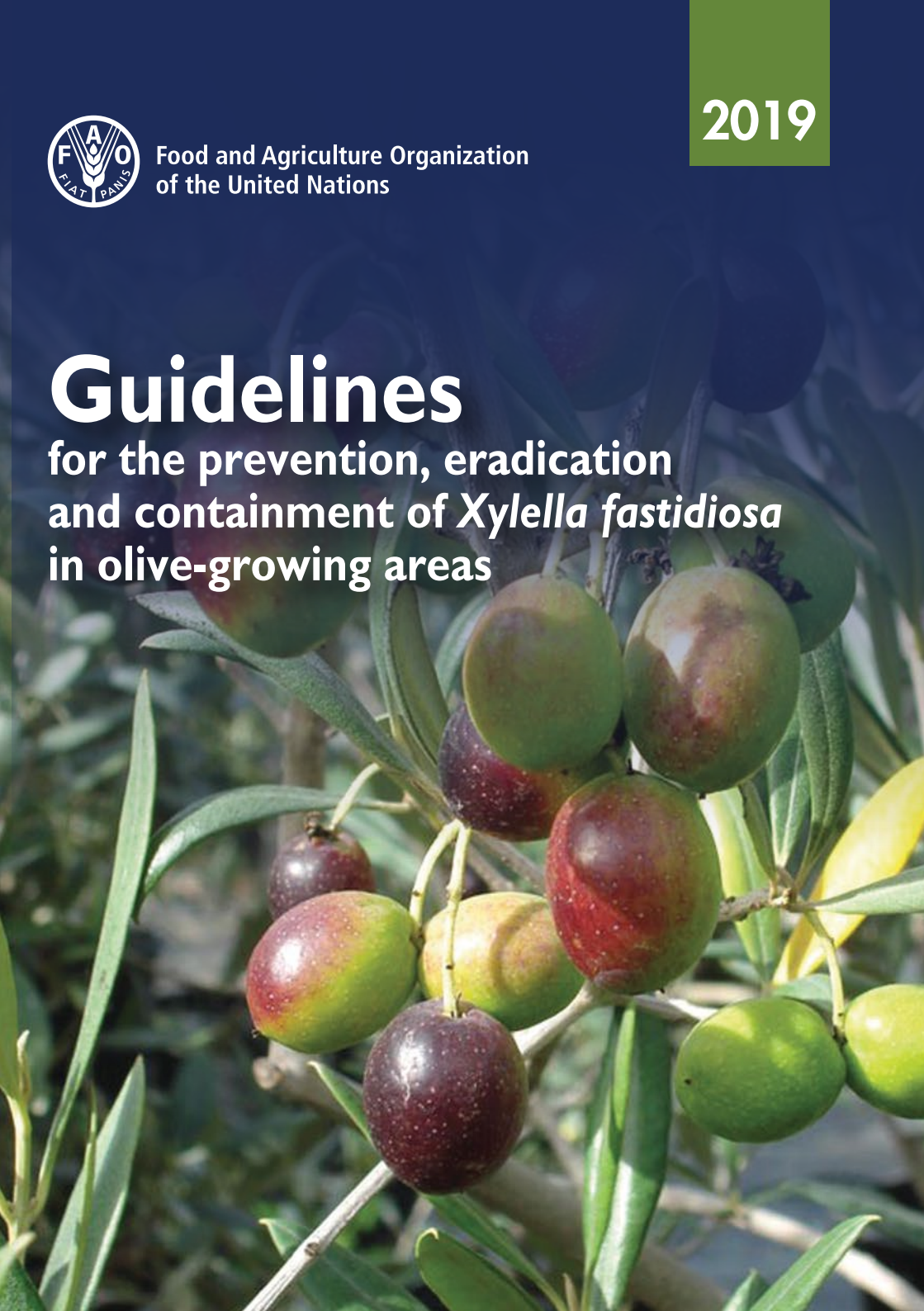


Food and Agriculture Organization  
of the United Nations

2019

# Guidelines

for the prevention, eradication  
and containment of *Xylella fastidiosa*  
in olive-growing areas



# **Guidelines for the prevention, eradication and containment of *Xylella fastidiosa* in olive-growing areas**

**Luigi Catalano**

Centro Interprofessionale per le Attività Vivaistiche (CIVI-ITALIA), Rome

**Shoki Al-Dobai**

Crop Protection Officer  
FAO Regional Office for the Near East and North Africa

**Donato Boscia**

Istituto di Protezione Sostenibile delle Piante, Consiglio Nazionale  
delle Ricerche (IPSP-CNR), UOS Bari, Italy

**Giovanni P. Martelli**

Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti  
(DiSSPA), Università Aldo Moro, Bari, Italy

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# Foreword

These guidelines are published by the Food and Agriculture Organization of the United Nations (FAO) in response to the new and emerging threat of the olive quick decline syndrome (OQDS) caused by the bacterial pathogen *Xylella fastidiosa*. The disease poses an imminent threat to the entire Mediterranean basin, where 95 percent of the world's olive trees are grown.

The guidelines: (i) provide detailed information on the disease, its symptoms and vectors, outlining specific strategies for the implementation of a contingency programme, including biological and chemical control, nutrition management and best cultural practice; (ii) target the national plant protection organizations (NPPOs), technical officers, olive-tree growers and other crucial stakeholders, who will play an integral role in the implementation of the national and regional contingency programmes; (iii) represent a useful simplified tool that provides growers and field advisors with the technical information needed to assist them in defining specific measures for preventing the introduction and spread of the disease in their growing areas.

The guidelines are based on: (i) the *Xylella fastidiosa* Pest Risk Assessment conducted by the European Food Safety Authority (EFSA, 2015); (ii) the Commission Implementing Decision (EU) 2015/789; (iii) articles published by Italian scientists; (iv) the national action plan elaborated by the Italian Ministry of Agricultural, Food and Forestry Policies (MIPAAF) comprising the technical guidelines for the containment of the spread of *X. fastidiosa* spp. pauca strain CoDiRO (complesso del disseccamento rapido dell'olivo, the former name of OQDS); (v) the containment of OQDS by the phytosanitary service of the Apulian region of south-east Italy. All these documents concern preventive measures on the introduction, spread and control of *X. fastidiosa*.

# Acronyms

ALS	almond leaf scorch
CLS	coffee leaf scorch
CoDiRO	complesso del disseccamento rapido dell'olivo
CVC	citrus variegated chlorosis
DiSSPA	Department of Soil, Plant and Food Sciences, University of Bari
DSF	diffusible signalling factor
DTBIA	direct tissue blot immunoassay
EFSA	European Food Safety Authority
ELISA	enzyme-linked immunosorbent assay
EPPO	European and Mediterranean Plant Protection Organization
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
IAMB	Mediterranean Agronomic Institute of Bari
IF	immunofluorescence
IPSP-CNR	Institute of Sustainable Plant Protection of the National Research Council of Italy
MEIF	membrane entrapment immunofluorescence
MIPAAF	Ministero delle Politiche Agricole Alimentari e Forestali
NAC	N-acetylcysteine
NGS	next generation sequencing
NPPO	national plant protection organization
OCPC	Ordinance Civil Protection Department Commission
OLS	oleander leaf scorch
OQDS	olive quick decline syndrome

PACA	Provence, Alps, Cote d'Azur
PAFF	EU Standing Committee on Plants, Animals, Food and Feed
PCR	polymerase chain reaction
PD	Pierce's disease
PLS	plum leaf scald
POnTE	Pest Organisms Threatening Europe
PPD	phony peach disease
RAPD	random-amplified polymorphic DNA
RFLP	restriction fragment length polymorphism
RLK	receptor-like kinases
RLP	receptor-like proteins
UNIBA	Università degli Studi di Bari Aldo Moro
XF	<i>Xylella fastidiosa</i>
XF-ACTORS	<i>Xylella fastidiosa</i> Active Containment through a Multidisciplinary-oriented Research Strategy





## Olive growing in the Mediterranean basin

Over 750 million olive trees are cultivated worldwide, 95 percent of which are concentrated in the Mediterranean region. Most of the global production of olives comes from Southern Europe, North Africa and the Near East. Spain, Italy and Greece, in particular, contribute 93 percent of the European production. On the other hand, Spain, Greece, Italy and Tunisia account for 65 percent of the olive-growing areas, 76 percent of the olive trees in production, and 74 percent of the total olive yield (Table 1). As for olive oil production, Spain, Italy and Greece contribute 36, 24 and 17 percent, respectively, of global production. Other important producing countries are Turkey, the Syrian Arab Republic and Morocco.

**Table 1. Surface given over to olives in Mediterranean basin countries and yield relative to 2013 (from <http://faostat3.fao.org>)**

Country	Surface (ha)	Yield (t/ha)
Spain	2 507 000	3.7
Tunisia	1 822 820	0.6
Italy	1 146 863	2.6
Greece	918 100	2.1
Morocco	922 235	1.3
Turkey	861 070	1.9
Syrian Arab Republic	697 442	1.2
Algeria	348 196	1.7
Portugal	351 771	1.9
Libya	210 000	0.7
Jordan	62 390	2.1
Lebanon	53 600	1.8
Egypt	61 711	8.8
Palestine	51 000	1.5
Albania	37 941	2.4
Israel	33 700	2.3
Croatia	18 590	1.8
France	17 174	1.6
Cyprus	10 653	1.2
Bosnia and Herzegovina	110	1.4
Malta	6	0.8

Olive cultivation has a social connotation, as it employs abundant labour and involves many small producers, and in many areas has additional value as it contributes to the land management of extensive territories that would otherwise be abandoned. Production, however, is seasonal and this has repercussions on job conditions and availability of by-products. Like all woody crops, olive trees are exposed to the attacks of a long list of pathogens and pests to which, in recent times, a new entry, *Xylella fastidiosa*, has been added.

### ***Xylella fastidiosa*: morphological traits and taxonomic position**

*Xylella fastidiosa* is a Gram-negative gamma-proteobacterium with rod-shaped cells 1.0–4.0 x 0.25–0.50 mm in size, deprived of flagella, and showing a characteristically rippled cell wall (Wells *et al.*, 1987) (Figure 1). It has the following taxonomy:

Kingdom:	Bacteria
Phylum:	Proteobacteria
Class:	Gammaproteobacteria
Order:	Xanthomonadales
Family:	Xanthomonadaceae
Genus:	<i>Xylella</i>
Species:	<i>Xylella fastidiosa</i>

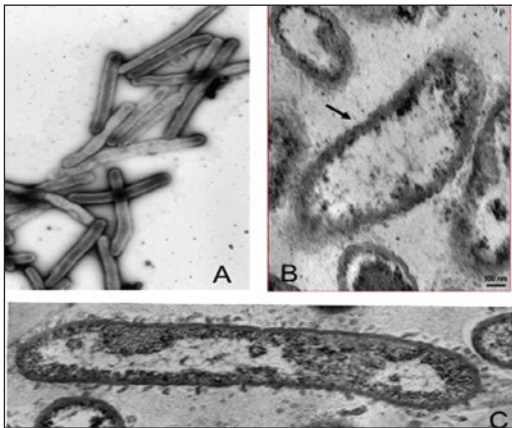


Figure 1. A: *X. fastidiosa* cells from a pure culture of the Salentinian strain (CoDiRO) of the bacterium; B and C: electron microscope views of *X. fastidiosa* cells (arrow in B points to the rippled cell wall).

Taxonomically, *X. fastidiosa* is a single species. However, it comprises strains that differ genetically, biologically (host range) and in geographical distribution, which are retained as subspecies: *X. fastidiosa* spp. *fastidiosa*, *X. fastidiosa* spp. *multiplex*, *X. fastidiosa* spp. *sandyi*, *X. fastidiosa* spp. *pauca* (Table 2). A fifth recently proposed putative subspecies (*X. fastidiosa* spp. *morus*) is under scrutiny, the same as a newly described *Xylella* species denoted *Xylella taiwanensis* (Su *et al.*, 2016).

**Table 2. Currently known *X. fastidiosa* spp., their putative origin and main hosts**

Subspecies	Putative geographical origin	Main hosts
<i>X. fastidiosa fastidiosa</i>	Central America	grapevine, almond
<i>X. fastidiosa multiplex</i>	Southern United States of America	stone fruits, shade trees, olive (United States of America)
<i>X. fastidiosa sandyi</i>	Undetermined	oleander, magnolia
<i>X. fastidiosa pauca</i>	South America	citrus, coffee, olive (Italy, Argentina, Brazil, insular Spain)

## ***Xylella fastidiosa* as a plant pathogen**

*X. fastidiosa* is the elicitor of devastating diseases of agricultural crops in a number of countries. In fact, members of the *X. fastidiosa* subspecies and their strains are pathogens to more than 300 host plant species (EFSA, 2013; 2016), in which they cause diseases of economic importance, e.g. grapevine Pierce’s disease (PD); citrus variegated chlorosis (CVC); phony peach disease (PPD); plum leaf scald (PLS); leaf scorch of oleander (OLS), almond (ALS), coffee (CLS), and a number of forest and shade trees. *X. fastidiosa* also infects cultivated crops (e.g. *Medicago sativa*/alfalfa dwarf) and wild weeds, plus a number of shrubs. Many wild plants, i.e. grasses, sedges and trees, may carry the pathogen without showing symptoms.

In infected hosts *X. fastidiosa* occurs as: (i) motile forms, i.e. bacterial cells that move from vessel to vessel in upward and downward directions and multiply; (ii) sticky cells that produce a sort of mucilage in which they are immersed, the biofilm, which is responsible for clogging the vessels and impairing the upward flow of crude sap. The virulence of the pathogen largely

depends on the fine balance between these two bacterial forms that is regulated by a diffusible signalling factor (DSF, i.e. 2-Z-tetradecenoic acid, and cis-11-methyl-2-dodecenoic acid) which, by increasing the adhesiveness of *X. fastidiosa* cells, decreases its ability to move within the host. Virulence factors required for pathogenesis in grapevines have recently been discovered, consisting in the production of: (i) the enzyme complex LesA (type II secreted lipase/esterase) which is responsible for the initial scorching of vine leaves (Nascimento *et al.*, 2016); (ii) a homologue of a temperature-independent cold-shock protein (csp1) that may have an important function during host colonization and in response to cellular stress (Burbank and Stenger, 2016); (iii) an antivirulence secreted protease (PrfA) that controls bacterial cell growth, biofilm formation and pathogenicity (Gouran *et al.*, 2016). However, whether this applies to all bacterial subspecies and hosts other than grapevines has yet to be established.

## Pierce's disease

*X. fastidiosa* was identified in the United States of America in 1987 as the cause of Pierce's disease (PD) of grapevines (Wells *et al.*, 1987), a disorder known in California since 1884 (Pierce, 1892), an upsurge of which is now threatening the Californian grape industry because of the introduction of a very efficient vector, the glassy-winged sharpshooter (*Homalodisca vitripennis*) (Purcell and Feil, 2001). This vector is a serious new menace to California's vineyards, as it moves faster and farther than native sharpshooters. It inhabits citrus and some woody ornamentals in unusually high numbers, making vineyards more vulnerable to PD and increasing the risk of the introduction of the CVC strain of *X. fastidiosa* into the United States of America. The most characteristic symptom of primary infection of *Xf* spp. *fastidiosa* in grapevines is leaf scorch. This disorder, denoted Pierce's disease, shows as an early sign a sudden drying of the leaf margin which turns brown and desiccates, while the adjacent tissues turn yellow or red (Figure 2). The desiccation spreads over the rest of the leaf blade, so that the whole leaf may shrivel and drop, leaving only the petiole attached to the cane. Diseased canes mature irregularly, showing patches of brown and green tissue. In later years, infected vines push late and produce stunted chlorotic shoots. Survival after infection depends on the vine species and the cultivar. European grapes (*Vitis vinifera*) are far more susceptible than the American *Vitis* and *Muscadinia* species, most of their cultivars dying within two to five years.



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Figure 2. Pierce's disease of the grapevine induced by *X. fastidiosa* spp. *fastidiosa*.

### Citrus variegated chlorosis

In Central and South America *X. fastidiosa* has become very noxious due to the rapid expansion of citrus variegated chlorosis (CVC) in citrus (Brazil), and coffee leaf scorch (CLS) in *Coffea arabica* in Central America, both caused by *Xf pauca*. Citrus trees can show CVC symptoms from nursery size. Younger trees become systemically affected by the bacterium, whereas trees older than 15 years usually may not be totally affected, but have one or two scaffold branches showing symptoms. Affected trees show foliar chlorosis resembling zinc deficiency with interveinal chlorosis (Figure 3).



© courtesy of H. Della Coletta Filho

Figure 3. Citrus variegated chlorosis induced by *X. fastidiosa* spp. *pauca*.

The chlorosis appears on young leaves as they mature and persists on older leaves. Newly affected trees show a sectorial distribution of the symptoms, whereas trees affected for a longer period show 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 of the blades, corresponding to yellow chlorotic areas on the upper side. Fruit size is greatly reduced and the rind turns so hard that it can damage juicing machines. Sugar content of affected fruit is higher than that of non-affected fruit. Blossom and fruit set occur at the same time on healthy and infected trees, but normal fruit thinning does not occur on affected trees, the fruits remain small but ripen earlier. Infected trees show stunting and slow growth rate; twigs and branches die back and the canopy thins, but they do not die.

### ***Xylella fastidiosa* in Europe**

In Europe *X. fastidiosa* has been included in the European and Mediterranean Plant Protection Organization (EPPO) A1 list of quarantine pathogens since 1981 and is regulated as such in the EU under Council Directive 2000/29/EC, i.e. a plant health directive that sets out Member States' legal obligations, once the organism has been detected in their territory and irrespective of the symptoms to take all necessary measures to eradicate it or, if that is impossible to restrain its further spread. In the last few years the presence of *X. fastidiosa* has repeatedly been recorded in Europe.

As a record of *X. fastidiosa* affecting a grapevine stand in Kosovo (Berisha *et al.*, 1998) has never been confirmed, Italy remains the first European site of an epidemic outbreak of this quarantine bacterium. In fact, in mid-October 2013, a strain of *X. fastidiosa* pauca was observed in the west coast of the Salento peninsula of Apulia (south-east Italy) and identified as the main agent of a decline condition of olive trees (*Olea europaea*), a detailed description of which is given below. This disease, which at the time of first detection was confined to a small area of Lecce province (Figure 4B), by 2016 had expanded throughout the province, penetrating the neighbouring provinces of Brindisi and Taranto (Figure 5).



Figure 4. A: Salento peninsula (encircled); B: site of the first *X. fastidiosa* outbreak in the Salento peninsula.

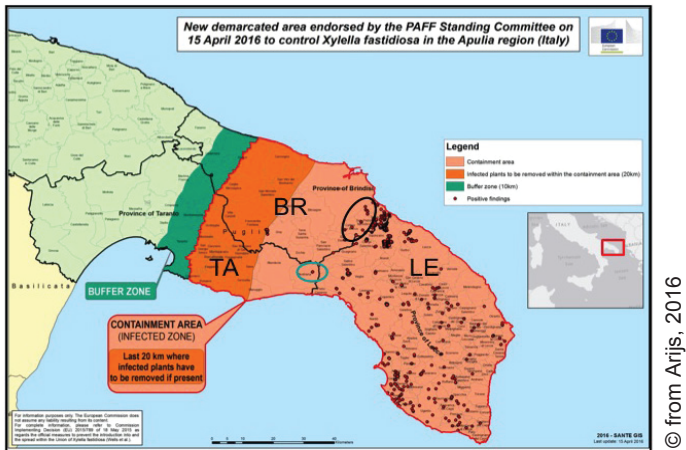
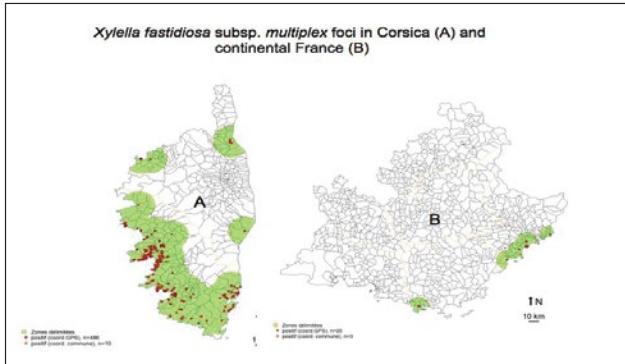


Figure 5. Distribution (summer 2016) of olive quick decline syndrome in the Salento peninsula. The infected area comprises the provinces of Lecce (LE) and the southern borders of Brindisi (BR) and Taranto (TA) (infection foci in Brindisi and Taranto encircled).



Since 2012, multiple interceptions of *X. fastidiosa pauca* and *X. fastidiosa sandyi* took place in imported plants in France when, in October 2015, a strain of *X. fastidiosa multiplex* was discovered in the field, first in the island of Corsica (282 foci, spring 2016) then in the mainland (PACA = Provence, Alps, Cote d’Azur, 14 foci) (Figure 6) (Anonymous, 2016a).



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**Figure 6. *X. fastidiosa* spp. multiplex outbreaks on the island of Corsica and continental France (spring 2016).**

**Table 3. Hosts of *X. fastidiosa* spp. multiplex found in France**

Latin name	Common name
<i>Acer pseudoplatanus</i> L.	sycamore maple
<i>Artemisia arborescens</i> L.	tree wormwood
<i>Asparagus acutifolius</i> L.	wild asparagus
<i>Calicotome villosa</i> (Poir.) Link.	thorny broom
<i>Cistus monspeliensis</i> L.	Montpellier cistus or narrow-leaved cistus
<i>Cistus salvifolius</i> L.	sage-leaved rock rose or salvia cistus
<i>Coronilla valentina</i> L.	scorpion vetch
<i>Cytisus racemosus</i>	sweet broom
<i>Cytisus scoparius</i> (L.) Link.	Scotch broom
<i>Genista corsica</i> (Loisel.) DC.	broom
<i>Genista ephredoides</i> DC.	broom
<i>Hebe</i>	shrubby veronica

Latin name	Common name
<i>Helichrysum italicum</i> (Roth) G.Don	curry plant
<i>Lavandula angustifolia</i> Mill.	common lavender
<i>Lavandula dentata</i> L.	toothed lavender
<i>Lavandula stoechas</i> L.	French lavender
<i>Lavandula x allardii</i>	lavender allardii
<i>Metrosideros excelsa</i> Sol. ex Gaertn.	New Zealand Christmas tree
<i>Myrtus communis</i> L.	common myrtle
<i>Pelargonium graveolens</i> L'Hér.	rose geranium or sweet-scented geranium
<i>Phagnalon saxatile</i> (L.) Cass.	Mediterranean phagnalon
<i>Polygala myrtifolia</i> L.	myrtle-leaf milkwort
<i>Prunus cerasifera</i> Ehrh.	cherry plum, myrobalan
<i>Quercus suber</i> L.	cork oak
<i>Rosa x floribunda</i>	rose
<i>Rosmarinus officinalis</i>	rosemary
<i>Spartium junceum</i>	Spanish broom

Until July 2016, over 20 alternative hosts (Table 3) had been discovered in French territories. Although this strongly indicates that the bacterium is spreading, no vector has apparently been identified so far.

In autumn 2014, an unidentified subspecies of *X. fastidiosa* was intercepted in the Netherlands in coffee plant consignments from Costa Rica and Honduras (Bergsma-Vlami *et al.*, 2015).

In September 2015, *X. fastidiosa* was detected in four symptomless coffee plants in Switzerland. One of them, in a tropical plant centre in Wolhusen (canton of Lucerne) was infected by *X. fastidiosa sandyi*, while in a garden centre at Dürnten (canton of Zürich) one plant was infected by the same subspecies (*Xf sandyi*) and two plants hosted *Xf pauca* (EPPO Reporting Service No. 10 – 2015 Num. article: 2015/181).

In June 2016, another European record came from Germany, where infection by a strain of *X. fastidiosa* was found in a potted oleander plant in a greenhouse of a small nursery in Saxony. Reportedly, “one potted plant of *Olea europaea* from the same greenhouse also showed symptoms but was tested negative” (Anonymous, 2016b).

In October 2016/February 2017, infections by *Xf fastidiosa*, *Xf pauca* and *Xf multiplex* were detected in insular Spain (Balearic Islands) in the following hosts: *Acacia saligna*, *Lavandula dentata*, *Nerium oleander*, *Olea europaea*, *Olea europaea sylvestris*, *Polygala myrtifolia*, *Prunus domestica* and *Prunus dulcis* (Anonymous, 2017).

This latter record establishes that, of the four different *X. fastidiosa* subspecies introduced in the last six to eight years in Europe, three (*Xf pauca*, *Xf multiplex* and *Xf fastidiosa*) have given rise to field outbreaks in Italy, France and Spain. This, together with the repeated interceptions of *Xylella*-infected coffee plants imported from Central America (see also Loconsole *et al.*, 2016a) provides ample evidence of the peril to which Old World crops have been exposed due to the unchecked introduction of plant materials from risky areas. Now that the Mediterranean region is facing the threat of a veritable *Xylella*-induced pandemic, supported by the favourable climatic conditions for the spread of this pathogen (Purcell, 1997; Bosso *et al.*, 2016a; 2016b), coordinated efforts need to be made at the regional, national and international levels to enforce control measures and regulations for the management and containment of disease spread.

## *Xylella fastidiosa* outbreak in Apulian olive orchards

In 2013, Saponari *et al.* (2013) reported the presence of *X. fastidiosa* in Apulian olive trees affected by a disease characterized by leaf scorching, scattered desiccation of twigs and branches that starts at the top of the canopy then extends to the rest of the crown, conferring a burned aspect on the affected plants (Figure 7).



**Figure 7. Burned aspect of a *Xylella*-infected young olive tree due to extensive desiccation of the canopy.**

Tissue desiccation starts at the tip of the leaves and progresses towards the petiole, spreading to the whole blade (Figure 8).



**Figure 8. Initial leaf yellowing that precedes apical scorching, which is followed by desiccation of twigs and branches.**

Dead leaves remain attached to the twigs and are shed following winter rains. These symptoms recall those of severe attacks of “brusca” (olive scorch), a recurrent long-known physiological disorder (Sanzani *et al.*, 2012) reported since the end of the eighteenth century in the same areas where the new disease is expanding (reviewed by Frisullo *et al.*, 2015). Thus “brusca” was included among the causes that were tentatively identified by local growers and farm advisors as possible elicitors of the decline: phytotoxicity due to groundwater pollution, severe attacks of *Colletotrichum* spp. (the agent of olive anthracnose), poor management of the groves, or heavy infestations of the lepidopteron *Zeuzera pyrina* (leopard moth). None of these, however, kills the plants, contrary to the agent of this novel disorder, whose attacks culminate with the death of the trees within a few years from the onset of symptoms.

The most severely and impressively affected olives are the centuries-old trees of the local highly susceptible cultivars *Cellina di Nardò* and *Ogliarola salentina* (Figure 9), which the growers unsuccessfully tried to save through drastic rejuvenation pruning to stimulate new growth. In fact, the new vegetation pushed out by these skeletal-looking trees would soon wither and desiccate (Figure 10, Figure 11).



**Figure 9. Progressive stages of the olive quick decline syndrome from incipient signs of infection to plant death.**



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**Figure 10.** Skeletal-looking centuries-old trees, severely pruned to favour a new flush of vegetation, where the few shoots produced are already dead.



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**Figure 11.** The fate of the “Gigante di Alliste”, a monumental tree in the Italian province of Lecce with an estimated age of 1 500 years. The first signs of infection appeared in March 2015, the tree was heavily pruned in winter and by June 2016 the new vegetation was desiccated and the decline dramatic. This tree was exposed to treatment with various products (resistance inducers, biostimulants, copper compounds) to no avail.

Many of these trees also show a variously extended browning of the sapwood of twigs, branches and trunks associated with the presence of fungal species of the genera *Phaeoacremonium*, *Phaemoniella*, *Pleumostomophora* and *Neofusicoccum* (Nigro *et al.*, 2013; 2014), whose penetration is favoured by leopard moth galleries (Figure 12).



© DiSSPA

**Figure 12.** Cross-sectioned olive branches showing necrotic wood colonized by different fungal species. Necroses start from galleries drilled by the larvae of the leopard moth, *Zeuzera pyrina*.

This disease, which was originally known as complesso del disseccamento rapido dell'olivo (CoDiRO) because of the concomitant presence of the above-listed putative agents of damage (moths, mycetes and *X. fastidiosa*). In declining ancient trees, was later renamed olive quick decline syndrome (OQDS) when extensive field observations disclosed that the presence of the moth was incidental and the fungi were largely absent in *X. fastidiosa*-infected and symptomatic young trees. Thus, the fungi are now retained as disease aggravators when present together with *Xylella*.

In line with the behaviour of all subspecies and strains of *X. fastidiosa*, strain CoDiRO colonizes the xylem vessels of the host plants, where it moves up and downstream and multiplies. Bacterial colonies and the associated biofilm clog the vessels, which results in a restriction of the upward movement of xylem fluid, hence in a physiological response of the plant akin to water stress. Leaf scorch and the ensuing desiccation of twigs and branches are primarily due to blocking of the vessels. However, as reported above, it has recently been discovered that in the case of Pierce's disease of the grapevine several virulence factors play a role in pathogenicity (Nascimento *et al.*, 2016; Burbank and Stenger, 2016; Gouran *et al.*, 2016).



## Current knowledge of the *xylella fastidiosa* strain CoDiRO

As soon as experimental evidence was obtained that the declining olive trees of the Salento Peninsula hosted a strain of *X. fastidiosa* (Saponari *et al.*, 2013), intensive investigations were initiated by a group of plant pathologists of the: (i) Department of Soil, Plant and Food Sciences, University of Bari (DiSSPA); (ii) Bari outfit of the Institute of Sustainable Plant Protection of the National Research Council of Italy (IPSP-CNR) (iii) Mediterranean Agronomic Institute of Bari (IAMB). The following are the current major research achievements:

- (i) Finalization of serological (ELISA, DTBIA, immunofluorescence) and molecular (PCR, real time PCR, PCR-LAMP) procedures for the reliable identification of *Xf* in host plants and vector (Loconsole *et al.*, 2014a; Yaseen *et al.*, 2015; Cariddi *et al.*, 2014).
- (ii) Isolation in axenic culture of strain CoDiRO from olives on other naturally infected plant species (Cariddi *et al.*, 2014; Elbeaino *et al.*, 2014).
- (iii) Identification of CoDiRO, as a strain of *Xf pauca*. Molecular evidence of its identity with a bacterial isolate (ST53) of the same subspecies present in Costa Rica, a country from which it may have landed in Salento with an unidentified ornamental plant (Loconsole *et al.*, 2014c; Giampetruzzi *et al.*, 2015a).
- (iv) Complete sequence of the genome of strain CoDiRO, a DNA molecule of 2.46 MB (Giampetruzzi *et al.*, 2015a).
- (v) Identification of the spittlebug *Philaelenus spumarius* (family Aphrophoridae) as a vector of strain CoDiRO, and determination of its biological cycle (Saponari *et al.*, 2014; Cornara *et al.*, 2016).
- (vi) Electron microscopic detection and identification by gold immunolabelling of the bacterium in xylem vessels of infected plants and in the foregut of the spittlebug vector (Cariddi *et al.*, 2014; Cornara *et al.*, 2016).
- (vii) Identification of 24 alternative hosts of strain CoDiRO (Table 4) in Lecce province out of more than 600 trees and shrubs and over 200 weed species of 50 botanical families analysed, including grapevines and citrus (Potere *et al.*, 2015; 2016).



- (viii) Experimental evidence that upon mechanical inoculation with bacterial cultures, strain CoDiRO does not infect grapevines (cv. Cabernet Sauvignon) and citrus (orange Madame Vinous, mandarin, grapefruit Duncan, Naveline, citranges Carrizo, Troyer and C35), whereas it multiplies readily in oleander, olive seedlings and rooted cuttings of cv. *Cellina di Nardò* and to a much lesser extent in other olive cultivars (Coratina, Frantoio, Leccino), (Saponari *et al.*, 2014; 2016; EFSA, 2015).
- (ix) Complete sequence of the genome of CO33, a coffee-infecting isolate of *X. fastidiosa* intercepted in northern Italy, a DNA molecule of 2.68 MB (Giampetruzzi *et al.*, 2015b).
- (x) In olive plants exposed to infective *Philaenus spumarius*, *X. fastidiosa* was detected by laboratory assays in still symptomless plants as soon as six months after caging with the vector (Saponari *et al.*, 2016).
- (xi) Among bait plants of the young trees of olive, oleander, citrus, grapevine and almond planted in diseased olive orchards for exposure to infective vectors, only olives and oleanders became infected within 12 months and started to show symptoms 16–18 months after planting (Saponari *et al.*, 2016).
- (xii) Realization that under natural disease conditions some olive cultivars (e.g. Leccino and Frantoio) appear to be less affected than the severely diseased cvs *Ogliarola salentina* and *Cellina di Nardò*, and comparative analysis of the transcriptome of cvs Leccino and *Ogliarola salentina* was carried out to investigate the reasons for this differential behaviour (Giampetruzzi *et al.*, 2016).

## International Research

Although the above-listed studies have provided a wealth of information on the extant situation of the Apulian outbreak, fear that the contagion could spread to other regions of Italy and, from there, to other Mediterranean countries. This has prompted the European Union to launch international research programmes as part of Horizon 2020, in the framework of which two projects denoted POnTE (Pest Organisms Threatening Europe) and *Xf*-ACTORS (*Xylella fastidiosa* Active Containment Through a Multidisciplinary -Oriented Research Strategy) have been envisaged with the coordination of D. Boscia and M. Saponari, respectively, both researchers at IPSP-CNR Bari.

Participants in POnTE are 15 research units from Italy (two), France (two), United Kingdom (two), Spain (two), and one each from Austria, Finland, Norway, the Netherlands, Serbia, Israel and Costa Rica. The Mediterranean units and Costa Rica will deal with *X. fastidiosa*.

Research units participating in *Xf-ACTORS*, which is entirely devoted to *X. fastidiosa*, are 21 from Italy (four), Spain (three), Belgium (three), France (two), United Kingdom (two), Portugal (two), one each from Greece, Germany, the Netherlands, Costa Rica, Brazil, United States of America, Taiwan, plus other partners to a total of 29.

## Epidemiology

As mentioned, *X. fastidiosa* is exclusively transmitted by xylem sap-feeding insects belonging to the order Hemiptera, suborder Auchenorrhyncha. Vectors acquire the bacterium by feeding on the xylem of an infected plant and can inoculate it to healthy plants immediately after acquisition. The bacteria localize in the alimentary canal, where they adhere to and multiply in parts of the foregut (precibarium and cibarium). This implies, that the nymphal stages of the vector lose infectivity after moult, as the foregut is of ectodermal origin and is renewed with moulting. Thus, newly emerged adults must feed on an infected plant to acquire the bacterium and spread it. The bacterium is not trans-ovarially transmitted to the progeny of the vector (Freitag, 1951). Winged adults, because of their mobility, are mostly responsible for *X. fastidiosa* spread.

All xylem fluid-feeding insects in Europe and the Mediterranean region should be regarded as potential vectors, but some species are more likely candidate vectors than others, owing to their wide geographical distribution, abundance and range of hosts on which they feed. As members of the families Cicadellidae (leafhoppers), Aphrophoridae (spittlebugs) and Cercopidae (froghoppers) are vectors in the Americas, all members of these three families should be considered as potential vectors in the Mediterranean area also, where congenial climatic conditions exist.

Saponari *et al.* (2014) and Cornara *et al.* (2016), have experimentally determined that *Philaenus spumarius*, the meadow spittlebug (Figure 13) is the only vector of OQDS identified so far. Transmission with spittlebug adults testing positive to *X. fastidiosa* from 25 to 71 percent of the population, carried out on young plants of olive, oleander, citrus, grapevine, GF677 (*Prunus persica* x *Prunus amygdalus*) and periwinkle showed that *P. spumarius* transmitted the bacterium to all plant species except grapevines. Stone fruits and citrus were infected locally, with no systemic invasion (Cavaliere *et al.*, 2016).

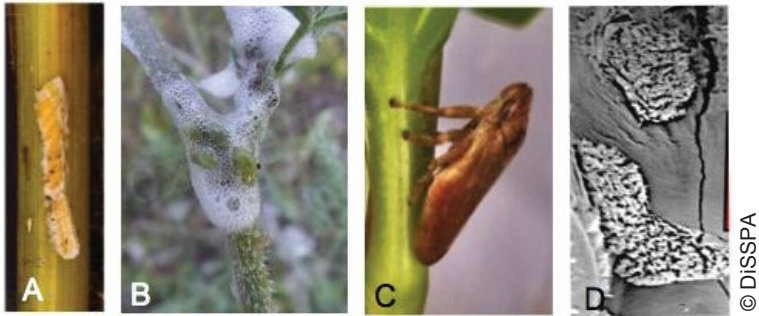


Figure 13. A: *Philaenus spumarius* eggs on a weed stem; B: *P. spumarius* juveniles in a foam nest; C: adult *P. spumarius*; D: *X. fastidiosa* strain CoDiRO colonies in the foregut of *P. spumarius* adult.

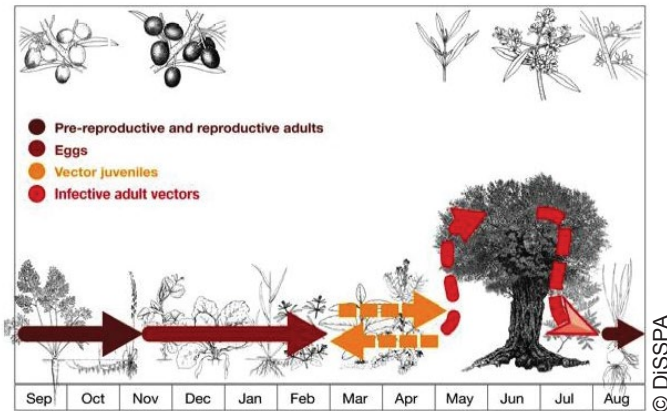


Figure 14. Biological cycle of *Philaenus spumarius* in the Salento peninsula.

The biological cycle of *P. spumarius* in the Salento area has also been elucidated (Cornara and Porcelli, 2014), as shown diagrammatically in Figure 14. Spittlebug adults thrive on olive trees from late spring until mid-summer, acquire the bacterium while feeding and may move it to neighbouring trees. In mid-summer, when the olives stop vegetating, the adults move to the ground on hosts (shrubs rather than weeds, as these have

dried out by that time) that are still green. Mating takes place in autumn, eggs are laid and the juveniles that emerge in late winter/early spring feed on weeds protected by the foam nests. Moulting takes place in late spring and the emerging adults move to the olive canopy where, if the tree is infected, they acquire the bacterium and retain it for the rest of their life.

As mentioned, the natural spread of *X. fastidiosa* is mediated by insects (leafhoppers) in whose foregut the pathogen can persist and multiply throughout their lifespan. Vectors can fly relatively short distances (up to 100 m – EFSA, 2013), but they can be transported by wind over much longer distances. This behaviour, in addition to the density and composition of host plants in the landscape, plays a significant role in vector dispersal and disease spread from plant to plant. However, other methods of spreading exist that are human-mediated and of paramount importance for the establishment of novel infection foci in places out of vector reach. Among these is vegetative propagation through grafting, which is widely used for a great many plant species, including several cultivated *X. fastidiosa* hosts. This is an insidious way for disease dissemination over medium and long distances through the transport and trading of infected plant material, nursery productions in particular.

Transport of *Xylella*-contaminated vectors by vehicles (cars, trucks, buses, agricultural implements) and humans (Figure 15) is an additional method of disease spreading. In fact, the sudden appearance in 2015 of an OQDS focus in the countryside of Oria, a village in the province of Brindisi at a flight distance of around 50 km from the infection front in Lecce province, was attributed to passive transport of infective vectors. To avoid this kind of unwelcome event, a few simple rules must be observed after visiting an infected spot, e.g. brush hair and clothes to which the insects adhere before boarding vehicles; keep windows closed during parking in, or driving through, infected areas; refrain from collecting weed and wild plants from infected and surrounding areas.



**Figure 15.** *Philaenus spumarius* adult on a woman's hair (A); on the body of a car (B) parked near an infected olive orchard.

The two main pathways of pathogen entry into non-infected areas have been addressed by EFSA (2015).

**Plants for planting.** The risk posed by these plants is high because of the ability of *X. fastidiosa* to survive within the hosts during transport and the fact that infected plants can be symptomless, thus they remain undetected. Furthermore, the very large number of hosts, i.e. 359 plant species, including hybrids, from 204 genera and 75 different botanical families (EFSA, 2016a), facilitates the spread and establishment of infection in vector-infested areas.

**Infective insect vectors.** The vectors can move through two main routes: association with plants or plant parts or travelling on their own as stowaways. However, for both options, the probability of entry and becoming established is moderate, because the ability of infective vectors to survive transport or storage is low, depending on the transport conditions and the pest management procedures applied at the origin of the planting material.

## Detection

Serological and molecular tests are suitable for screening large numbers of samples. The listed techniques are detailed in the European and Mediterranean Plant Protection Organization reports (EPPO, 2010; 2014).

**Serology.** Serological tests that were developed over the years include enzyme-linked immunosorbent assay (ELISA) (Sherald and Lei, 1991); membrane entrapment immunofluorescence (MEIF) (Hartung *et al.*, 1994); direct tissue blot immunoassay (DTBIA); Western blotting (Lee *et al.*, 1992; Chang *et al.*, 1993) and immunofluorescence (Carbajal *et al.*, 2004). Immunofluorescence (IF) can be used as a screening test for the diagnosis and detection of *X. fastidiosa* in plant material and identification of pure cultures. Instructions to perform ELISA and IF tests are provided in EPPO Standards PM 7/101.

**Nucleic acid-based tests.** Polymerase chain reaction (PCR)-based tests (Minsavage *et al.*, 1994; Rodrigues *et al.*, 2003; Huang *et al.*, 2006; Huang, 2009), PCR RFLP (restriction fragment length polymorphism) and RAPD (random-amplified polymorphic DNA) analysis (Mehta *et al.*, 2001), as well real-time PCR and loop-mediated isothermal amplification (LAMP) (Oliveira *et al.*, 2002; Guan *et al.*, 2013), have been used to detect the bacterium in grapevine, citrus, almond, olive and other hosts. Immunocapture-PCR (IC-PCR) and immuno-PCR (I-PCR) tests are quick and very sensitive methods for screening *X. fastidiosa*, with the advantage of not requiring any concentration or DNA purification steps (Peroni *et al.*, 2008). Nested PCR has also been used to detect the bacterium in insect vectors (Bextine *et al.*, 2004). A multiplexed lateral flow microarray assay has also been developed for the detection of *X. fastidiosa* in citrus, which seems to provide sensitive and specific detection (Cary and Stubben, 2011). PCR-based techniques (conventional PCR, real time quantitative PCR, LAMP PCR) are generally more sensitive than serological methods and have high specificity and powerful discriminatory capabilities to detect small numbers of bacteria in plants and insect vectors. Although, several PCR tests effectively detect *X. fastidiosa* DNA template once purified, it should be kept in mind that a recurrent problem in PCR tests of environmentally-collected tissue samples is the contamination of the DNA template with PCR inhibitors (Bextine *et al.*, 2004; Chen *et al.*, 2008; Fatmi *et al.*, 2005).

Procedures for detecting the CoDiRO strain of *X. fastidiosa* in olives, other host plants and vectors are both serological (ELISA, DTBIA) and molecular (various PCR protocols, including LAMP) (Loconsole *et al.*, 2014b; Djelouah *et al.*, 2014) and continue to be satisfactory. For example, bacterial detection threshold in olive tissue extracts by ELISA and conventional PCR was up to a dilution of  $10^5$ , whereas quantitative real-time PCR was 100-fold more sensitive than either method (Loconsole *et al.*, 2014a; 2014b). A comparative evaluation of the performance of different routine testing methods disclosed that: (i) all the diagnostic procedures tested were able to detect the pathogen in symptomless plants; (ii) LAMP using crude sap appears to be a rapid and reliable screening test; (iii) real-time quantitative PCR has the highest diagnostic and analytical sensitivity; (iv) although DTBIA has a lower sensitivity than other procedures, it becomes useful when applied to materials to be moved under a phytosanitary regulation regime, e.g. inspections of plant consignments for export/import, controls in nurseries (Loconsole *et al.*, 2016).

Updated protocols for laboratory identification of *X. fastidiosa* have been published in a recent issue of the EPPO Bulletin (Anonymous, 2016c).

The advent of innovative techniques such as next generation sequencing (NGS) has allowed the in-depth study of genomic data of both pathogen (different *X. fastidiosa* strains) and its major hosts. In fact, the draft genome sequences of the CoDiRO strain isolated from olive plants in southern Italy and of an intercepted coffee-infecting bacterial isolate have been determined by Giampetrucci *et al.* (2015a; 2015b). Furthermore, the genetic bases of the disease resistance shown in the field by plants of cv. Leccino (Figure 16) have been investigated through the comparative transcriptome analysis of this cultivar and that of the highly susceptible cv. *Ogliarola salentina*. It was ascertained that in cv. Leccino there is an up-regulation of genes coding for receptor-like kinases (RLK) and receptor-like proteins (RLP) involving signal transduction, hence plant defence responses (Giampetrucci *et al.*, 2016), and much less *Xylella* (133 267 bacterial cells/ml of tissue extract) than in cv. *Ogliarola salentina* (2 094 000 bacterial cells/ml of tissue extract) (Martelli, 2016).





Figure 16. Adjacent rows of olive trees of cvs Leccino and Ogliarola salentina showing the remarkable differential response to natural infection by the CoDiRO strain of *X. fastidiosa*. Both rows are infected but the Leccino trees show very mild symptoms, are productive and do not decline, so this cultivar is genetically equipped to resist *Xylella* infection to some extent.

## EU legislative provisions

The deliberation of Council Directive 2002/89/EC of 28 November 2002 amended Directive 2000/29/EC, updating the protective measures against introduction into the European Community (EU) of organisms harmful to plants or plant products, and against their spread within the Community. Specifically, the confirmed presence at the end of 2013 of *X. fastidiosa* (a quarantine pathogen) in Lecce province and its spread in olive trees and several other plants (Table 4) required new rules and measures.

Table 4. Hosts of *X. fastidiosa* spp. *pauca* strain CoDiRO found in Italy (Apulia)

Latin name	Common name
<i>Acacia saligna</i> (Labill.) Wendl.	coojong or golden wreath wattle, orange wattle, blue-leafed wattle, Western Australian golden wattle, Port Jackson willow (Africa)
<i>Asparagus acutifolius</i> L.	wild asparagus
<i>Catharanthus</i> sp.	rosy periwinkle or Madagascar periwinkle
<i>Cistus creticus</i> L.	rock rose or Cretan rockrose
<i>Dodonaea viscosa</i> Jacq.	hopbush
<i>Eremophila maculata</i> F.Muell.	spotted emu bush or spotted fuchsia bush
<i>Euphorbia terracina</i> L.	false caper



Latin name	Common name
<i>Grevillea juniperina</i> L.	juniper or juniper-leaf grevillea or prickly spider-flower
<i>Laurus nobilis</i> L.	bay tree
<i>Lavandula angustifolia</i> Mill.	common lavender
<i>Myoporum insulare</i> R.Br.	boobiialla
<i>Myrtus communis</i> L.	common myrtle
<i>Nerium oleander</i> L.	oleander
<i>Olea europaea</i> L.	olive
<i>Phillyrea latifolia</i> L.	green olive tree or mock privet
<i>Polygala myrtifolia</i> L.	myrtle-leaf milkwort
<i>Prunus avium</i> L.	sweet cherry
<i>Prunus dulcis</i> (Mill.) D.A.Webb	almond
<i>Rhamnus alaternus</i> L.	Italian buckthorn
<i>Rosmarinus officinalis</i> L.	rosemary
<i>Spartium junceum</i> L.	Spanish broom
<i>Vinca</i> sp.	periwinkle
<i>Westringia fruticosa</i> (Willd.) Druce	coastal or native rosemary
<i>Westringia glabra</i> L.	violet Westringia

The first European document to specifically regulate *X. fastidiosa* was the “Commission Implementing Decision 2014/497/EU as regards measures to prevent the introduction into and the spread within the Union of *Xylella fastidiosa* (Wells and Raju)”, of 23 July 2014. Since then, various emergency measures have been implemented regarding territorial management in response to numerous requests from the European Commission.

On 10 February 2015, the Italian Council of Ministers declared, for the first time in the country, a state of emergency due to a phytosanitary problem posed by the *X. fastidiosa* outbreak. This was followed immediately by an ordinance from the head of the Italian Ordinance Civil Protection Department Commission, appointing a Commissioner to deal with the emergency (OCPDC 225 of 11 February 2015).

Later that year, the European Commission deliberated a “Commission Implementing Decision 2015/789/EU of 18 May 2015 as regards measures to prevent the introduction into and the spread within the Union of *Xylella fastidiosa* (Wells *et al.*)”, which defines the measures to be implemented by Member States, i.e. “*Any person who suspects or becomes aware of the presence of the specified organism shall immediately inform the responsible official body and provide it with all relevant information concerning the presence, or suspected presence, of the specified organism. The responsible official body shall immediately record such information. Member States shall ensure that any person having under its control plants which may be infected with the specified organism is immediately informed of the presence or the suspected presence of the specified organism, of the possible consequences and risks and of the measures to be taken*”.

This decision was implemented by the Italian Ministry of Agricultural, Food and Forestry Policies (MIPAAF) under Decreto Ministeriale 19 Giugno 2015 that established the phytosanitary measures for the prevention and control of *X. fastidiosa* subject to specific mandatory rules within Italy.

## Management of the olive quick decline syndrome (OQDS)

Although various curing strategies against *X. fastidiosa* are being experimented, primarily in the United States of America with special reference to Pierce’s disease (Hopkins, 2014), as yet no treatment is available for curing *Xyella*-infected plants.

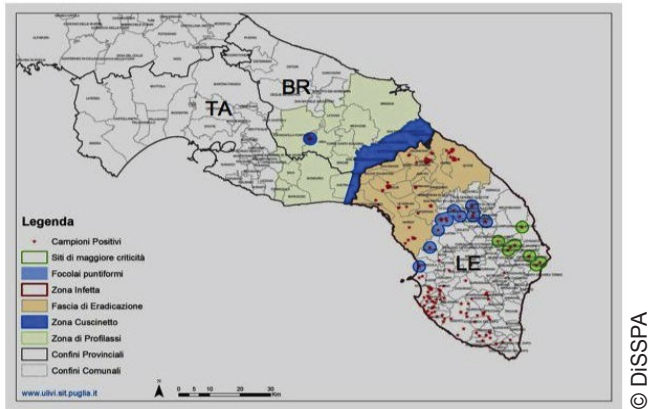
However, control strategies are available encompassing: (i) pathogen and vector exclusion (quarantine) which, as previously specified is difficult to implement because of the globalization of plant trading; (ii) use of systemic insecticides against vectors to control secondary spreading within a crop. Results are temporary unless treatments are repeated over time; (iii) cropping in areas that are climatically unfavourable to the pathogen (geographic control); (iv) elimination of infected primary and alternative hosts of the pathogen and its vector(s); (v) minimizing stresses due to drought, weeds, overproduction, other diseases, primarily those affecting the wood; (vi) use of resistant or tolerant plants.

Whereas some of these are being implemented against OQDS, as specified below, the time does not seem to be ripe for seeking any of the future control strategies: (i) genetic improvement through cysgenesis or genome editing via the CRISPR-Cas system for the addition of desirable (e.g. resistance) genes to susceptible hosts; (ii) biocontrol, i.e. a sort of cross-protection mediated by the use of a non-pathogenic bacterial strain (EB92-1) that, when inoculated into a host, inhibits the growth of pathogenic strains (Hopkins, 2014); (iii) plant transformation with the *rpfF* gene from *X. fastidiosa*, encoding the diffusible signal factor that regulates the growth of bacterial colonies (Lindow, 2014).

Prevention and control are therefore the appropriate measures that have to be relied upon for minimizing the impact of *X. fastidiosa* outbreaks and restraining disease spread. Basic information to pursue these goals in southern Italy is provided by the above-listed results of the work carried out at Bari by researchers from UNIBA, CNR and IAMB. These studies have laid the groundwork for the implementation of a strategy for disease containment, based primarily on the accurate surveillance of the territory north of the infected area, elimination of inoculum sources and control of vectors. To this end, the Italian experience gained from *Xylella*-induced OQDS is to be considered relevant for the entire Mediterranean area, whose climatic conditions are comparable with those of southern Italy.

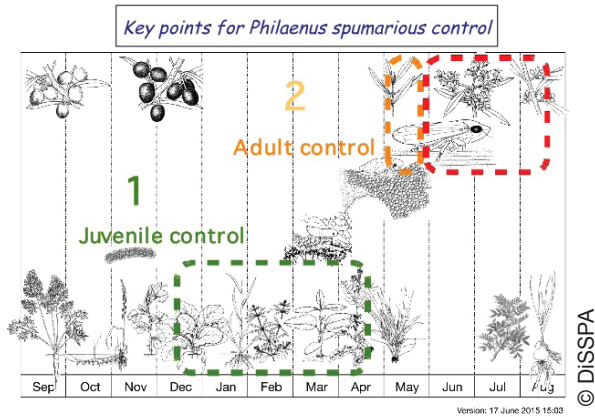
## Italian OQDS containment plan

The OQDS containment plan was conceived in 2015, and its execution was entrusted to an Extraordinary Commissioner. As shown in Figure 17, three demarcated areas – Zona Infetta (infected area), Zona Cuscinetto (buffer zone) and Zona di Profilassi (containment area) – were identified in the Salento peninsula, comprising the provinces of Lecce, Brindisi and Taranto.



**Figure 17. The Italian disease containment plan envisaged in 2015 for immediate application.**

The following actions were to be implemented in the containment area and the buffer zone: (i) verification of no infection through the extensive and continuous monitoring of vectors, olives and alternative hosts for the presence of *X. fastidiosa*, immediate removal of infected plants should the disease enter the area; (ii) preservation of the health status of the olives and other susceptible hosts through chemical treatment against vectors (adults) and mechanical weeding against vector juveniles (nymphal stages) at specific times, as diagrammatically illustrated in Figure 18.



**Figure 18. Diagrammatic representation of strategy for controlling *Philaenus spumarius*: weeding against the nymphal stages of the vector, and insecticides against the adults.**

Although newly emerged adults and their larval stages are not infective (M. Saponari and D. Cornara, personal communication, see also Ben Moussa *et al.*, 2016), chemical applications and weed control will reduce the inoculum potential with the aim of curtailing the vector population, i.e. the primary cause of disease spreading, as will elimination of alternative hosts from highways, canals, green areas, etc.

The steps defining vector control are detailed below, as derived from the recent scientific acquisitions on the life cycle of *P. spumarius* (Figure 14), i.e. an insect with a single generation a year and a high reproductive capacity in favourable environments like those largely characterizing the Mediterranean climate. Thus, in the climatic conditions of southern Italy, where the winter temperatures are generally above freezing, the *P. spumarius* life cycle can be summarized as follows:

**January–April.** Juvenile forms, hatched from overwintering eggs, station on herbaceous plants and shrubs under the protection of foam nests. In this period, the following mechanical or chemical measures are required: (i) tilling of the soil in olive orchards; (ii) destruction of the herbaceous hosts on which the vector nymphs thrive; (iii) if weeds hosting vector nymphs are not easily accessible, herbicides or spot application of insecticides may be applied, following specific operator safety measures.

**May–August.** During moult, spittlebug nymphal stages lose the infectivity that can be acquired by feeding on infected plants. In any case, infection risk is very low or zero, for it was ascertained that no *X. fastidiosa* is detectable at ground level (weeds) in the surveyed areas of Salento (Potere *et al.*, 2016). Therefore, the non-infective adults move from herbaceous plants to other hosts, attracted by the tender spring vegetation. This migration is usually massive. If the tree on which adult spittlebugs land is infected, the insects acquire the bacterium, thus becoming a source of inoculum for neighbouring trees. In the period described, vector adults must be targeted with insecticides using the appropriate products (Table 7) both in olive groves and non-cultivated surfaces, i.e. spotted treatments in greenish areas with weeds that can potentially be *X. fastidiosa* hosts. This “two-front war”, against juveniles and adults, is expected to progressively reduce the vector population, hence the inoculum potential and the progression of the contagion into neighbouring groves.

Actions envisaged by the 2015 plan in the infected areas were directed at impairing disease spread to the still untouched groves. Surveys were therefore to be carried out for the identification of the number and size of infection foci, accompanied by both the elimination of alternative hosts and infected olives (whenever feasible), and vector control (chemical treatments and weeding).

In the newly discovered OQDS foci the EU Commission required the felling of infected olives and the surrounding trees, including the healthy ones, in a radius of 100 m. This measure was strongly opposed by the owners of the groves, whose complaints produced judicial interventions that stopped the execution of the plan. This was a great pity, because it was estimated that where mechanical weeding (the only measure that could be implemented in 2016) was done, the *P. spumarius* juvenile population was reduced by around 70 percent (F. Porcelli, personal communication).

Subsequent to the judicial impasse, the EU Standing Committee on Plants, Animals, Food and Feed (PAFF) identified new more extended demarcated areas (see Figure 5) in which to enforce a strategy in line with the Italian containment plan. This ensures that the plan has to be resumed as soon as a new edition is promulgated by the Apulian regional authorities.

The infected area is the most problematic, as OQDS is spreading rapidly there and the above-listed interventions can, at most, slow down the disease progression. The repeatedly mentioned unavailability of cures cancels current hopes of saving infected plants, a great number of which are centuries-old monumental trees that characterize the landscape and are greatly valued by local residents. One way of addressing the problem is to promote strategies that would allow the coexistence of olives with the pathogen by prolonging the life of declining trees, while waiting for therapeutic remedies.

In the last couple of years, field experiments have been carried out in Lecce province in which a number of products, ranging from plant protectors to growth promoters and a range of other compounds (foliar fertilizers, biological antagonists, resistance inducers, fungicides, humic acids), have been administered to infected trees either as foliar sprays or soil drenches. Of interest are the experiments carried out by Carlucci *et al.* (2016) and Scortichini (2016), who reported that although none of the experimental products had a bactericidal effect, thus *X. fastidiosa* persisted in the treated plants, nevertheless these plants responded to most of the chemicals used by pushing new green growth.

These experiments were reviewed by the Plant Health Panel of EFSA (2016b), which came to the following conclusion:

*“This opinion addresses the question of the efficacy of current treatment solutions to cure Xylella fastidiosa-diseased plants, and discusses the experimental treatments under evaluation by two research groups in Apulian olive orchards infected by the CoDiRO strain. The increasing problems from newly emerging vascular bacterial diseases and the limited success to cure plants from such infections have stimulated numerous studies on treatments with chemical and biological compounds. Under field conditions, various formulations of copper and zinc as spray or root drench are currently used while further options, for example the application of bioactive substances, are at an experimental stage. In Apulia, preliminary results from intensive treatments with such formulations, in combination with the use of good crop management practices, reported more vigorous new growth of diseased trees. However, results provided so far confirmed the continued presence of Xylella fastidiosa after the treatments under evaluation. This is in agreement with current knowledge that there are no means to cure plants from this*

*bacterial disease, in the sense of eliminating the pathogen from plant tissues. The reported positive response of the treated olive trees is most probably due to the effect of micronutrients and other bioactive compounds that, together with soil cultivation and agronomical practices, improve the vigour of the plants and their resilience to stress caused by bacterial infections. Notwithstanding the preliminary status of these findings, the Panel acknowledged the potentially positive effects of such treatments in prolonging the productive phase of olive trees and their putative relevance for the management of olive orchards, particularly in the containment area where eradication of the pathogen is considered no longer possible. The Panel also concluded that long-term studies are needed to confirm that the reported positive effects on crop performance can be sustained over many years.”*

The result is that the above-mentioned field treatments only allow a precarious host-pathogen coexistence that needs to be nursed with repeated applications.

Another way of relieving the consequences of *X. fastidiosa* infection is being experimented in Brazil against CVC. Infected citrus plants exposed to N-acetylcysteine (NAC) absorbed from a slow-release fertilizer react with significant symptom remission accompanied by reduced bacterial replication rate (Muranaka *et al.*, 2013). NAC has a mucolytic activity as it breaks the disulfide bridges of sulfur-containing amino acids of the bacterial biofilm, which turns fluid, facilitating the upward flowing of crude sap. NAC has recently been administered to olives affected by OQDS in the field (C. Dongiovanni, personal communication), but time is needed to evaluate the results.

As mentioned, looking for resistant cultivars is a most desirable approach in view of a possible reconstitution of the Salentinian olive industry, based on a set of cultivars that can substitute for the largely predominant and highly susceptible *Ogliarola salentina* and *Cellina di Nardò*. Two of these, cvs Leccino (see Figure 16) and FS-17, have already been found, while others are being sought by field scouting and exposure of a wide number of olive accessions to natural infection in experimental plots. To this end, a germplasm plot has been established in a heavily infected area of Lecce province (Figure 19) where 30 different cultivars have already been transplanted and 50 more, of Italian and foreign origin, are scheduled for addition.



A serious source of distress for the people of Salento is the unacceptable loss of their very ancient and majestic trees (see Figure 11), devastating what used to be an admirable landscape. To address this problem an ingenious procedure, i.e. patch-grafting of tissues from the resistant cv. Leccino on the trunk of badly compromised but still living trees, has been devised by some local growers (Figure 20).



Figure 19. Looking for sources of resistance. A newly established olive germplasm orchard in which cultivars of different types and origin are exposed to natural infection.

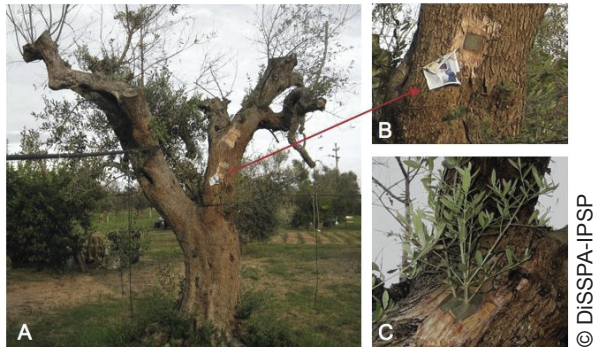


Figure 20. Attempts to reconstruct the canopy of a declining tree. A successful patch-graft of an ancient infected olive tree (A) made with tissues from the resistant cv. Leccino (B) is pushing out new shoots (C).

Successful grafts develop into shoots that grow vigorously, as it can be witnessed in the field, and are still green several months post-grafting. This ensures that if multiple grafts are made on the branches of heavily pruned large trees, the reconstruction of the canopy, hence the survival of the tree, can be expected. To verify this, trees of three large and heavily infected olive groves, some 10 ha in size altogether, are being patch-grafted with material from some 240 different cultivars (P. La Notte, personal communication). Time will tell whether and to what extent this attempt will succeed.

## Good crop management practices

Whatever the strategy deployed to keep *X. fastidiosa* infections under control, the enforcement of “good crop management practices” should not be overlooked. In fact, relinquishing such practices is thought to have facilitated the establishment and rapid spread of *X. fastidiosa* in Salento. Although this is not enough to preserve plants from the infection, proper management of the crop (e.g. soil tillage, protection measures against insect vectors, pruning, fertilization and irrigation) may have an impact on OQDS, if aimed at reducing the habitats of vector populations and thus their transmission capability.

**Soil management.** Soil management practices for warm-dry climate areas characterized by low organic matter content have the following purposes: (i) improving water conservation by reducing soil evapotranspiration; (ii) increasing soil macro-porosity to improve water infiltration and storage; (iii) enhancing soil aeration; (iv) maintaining the soil free of weeds, thus reducing water/nutritional competition; (v) incrementing P, K and organic matter fertilizers. Soil tillage must avoid root injuries that could constitute predisposing factors to infection by pathogens living in the soil. The soil must be tilled with sufficient moisture in order to avoid the formation of a hard compact layer, large lumps, or excessive pulverization and loss of soil structure. When properly conducted, all this contributes positively to containing soil erosion.

**Pruning.** Olive orchards must be pruned at least every two years in order to insure the best growing conditions by favouring canopy aeration and lighting. The following precautions should, however, be observed: (i) spray cut surfaces with copper formulates shortly after pruning, while protective mastic must be applied on pruned branches. These practices reduce insect and pathogen

penetration which could debilitate the trees; (ii) cut and burn the smallest canopy pruning residues, ensuring avoidance of any contact with vectors. Chopped branches and trunks can be moved (Figure 21) as vectors cannot acquire the bacteria from corky tissues; (iii) although no ultimate evidence exists that *X. fastidiosa* can be transmitted by surface-contaminated pruning tools, it may be precautionary to disinfect them with sodium hypochlorite or quaternary ammonium salts before moving to another tree; (iv) if early symptoms of infection are detected, prompt and targeted pruning of the infected shoots/branches may slow down the infection progression. Timely and targeted pruning of citrus has been reported from Brazil as a procedure capable of saving citrus plants from systemic infection by *X. fastidiosa pauca*, the CVC agent (De Souza *et al.*, 2014). However, whether the same procedure is applicable to olives remains to be established.



**Figure 21.** A load of chopped olive trunks and branches for disposal.

**Irrigation.** Stress may be a determining factor in increasing plant susceptibility to infectious diseases. Where olive trees are managed in dry conditions, the application of all the precautions and techniques of dry farming are required. Nevertheless, irrigation is recommended whenever available to avoid drought stress. For this reason, growers may apply micro-irrigation systems as drip or sub-irrigation. Irrigation may avoid some of the undesirable effects described, considering for each phenological phase the consequences of water stress (Table 5).

**Table 5. Description of the effects of water stress on olive-tree growth**

Time of year	Phenological phases	Effects of water stress
February–April	differentiation and development of flower buds beginning of shoot growth	lower inflorescence increase of abnormal flowers reduced shoot growth
May–June	flowering fruit setting fruit growth (cell division)	incomplete flowers reduced fruit size due to lower cell number reduced shoot growth
July–August	stone hardening beginning of fruit growth by cell enlargement	slight reduction of fruit size and vegetation
September–October	fruit growth (cell enlargement)	reduction of size, pulp/stone ratio and storage substances in the plant organs poor quality of inflorescence and vegetation the following year

**Fertilizers.** Ensuring the correct growth of olive trees throughout the season requires the annual input of organic and inorganic fertilizers suitably rationalized during the year, as suggested in Table 6.

The use of organic-based fertilizers improves soil structure and is more advisable than the use of chemicals.

**Table 6. Annual amount of fertilizers required for olive-tree growth. Data reported represent the optimal distribution of nutrient during the olive-growing season (percentage unit of fertilizer: Disciplinare Produzione Integrata Regione Puglia 2015)**

Element	Pre-blossom	After fruit setting	Fruit growing	Post harvest
nitrogen	40	30	30	20
phosphorus	20	–	–	50
potassium	35	–	–	45
micro-elements	15	10	–	–

## Surveillance

Surveillance is a process whereby information on a particular pest for a specific area is collected from several sources, in order to permit their use by the national plant protection organization. Detailed guidelines are described in ISPM 6 (Anonymous, 2006).

Surveillance programmes for *X. fastidiosa* should adhere to the specifications issued by the NPPO responsible for the area. In particular, surveillance should address the monitoring of risk aspects to the environment over the entire production and trade chains, considering (i) genetic resources (mother plants, varietal collections); (ii) nursery material ready to be distributed for planting; (iii) monitoring of the phytosanitary status of the environment (crops, unmanaged fields, natural environments, gardens and parks).

### Specifically:

- Monitoring and assessment of the sanitary status of mother plants are important as they could be used as sources of plant propagating material.
- Control for the absence of *X. fastidiosa* from plant propagating materials, which is multiplied in the nursery for further distribution in the agricultural and natural environments, is a crucial measure. This could be done by applying good agricultural practices such as the use of healthy material for propagation, the control of insect vectors, and of weeds, shrubs and other susceptible plants that could host the pathogen as well as its vector.
- Assessment of the healthy status of the environment, for which it is essential to know whether: (i) the pathogen has been introduced; (ii) it is spreading; (iii) putative insect vectors are present.

Annual surveys are advisable within each country of the Mediterranean basin site of olive cropping. A survey initially consists of visual examination for the presence of *X. fastidiosa* symptoms on assessed and non-host plants. The detection of suspected *X. fastidiosa*-infected plants should require immediate sampling and analysis. Should the presence of *X. fastidiosa* be confirmed, the establishment of “demarcated areas” is required. The size of these areas will vary according to the situation. In Italy, for example, the demarcated areas comprise the entire Salento peninsula (Figure 5) over an extent of some 130 km, whereas these areas are much smaller and scattered in Corsica and continental France (Figure 6).

Considering that field outbreaks of *X. fastidiosa* occur only in Italy (*Xf pauca* CoDiRO strain), France (*Xf multiplex*), and insular Spain (*Xf fastidiosa* and *multiplex* in Mallorca; *Xf pauca* in Ibiza) the following procedures and guidelines refer to these specific experiences.

When a demarcated area has been established, it is advisable to perform surveys within a radius of 200 m around infected plants (primary host), to detect other plants of the same species, plants of the same genus as the primary host, and all other plants showing symptoms of infection using a sampling scheme to confirm with 99 percent reliability that the level of presence of the specified organism in these areas around infected plants is below 0.1 percent.

As the host range of *X. fastidiosa* is very wide, and potential vectors are in principle numerous (e.g. all xylem sap-feeding insects) and widely represented in the Mediterranean basin, disease eradication requires drastic measures to be applied as soon as possible to the infected crop, to wild, unmanaged and ornamental plants that may host the bacterium, and to vectors in the infected plots and their vicinity. The history of *Xylella*-induced diseases in new areas shows that once the bacterium is firmly established in the environment it cannot be eradicated (Purcell, 2013).

The observations made in infected Apulian olive groves in the course of the outbreak on olive trees and other plants, notified by the Italian authorities at the end of 2013, show the difficulty of early detection of *X. fastidiosa* in areas and hosts with no previous record of infection by this pathogen. No wonder, then, that the disease of olive trees was initially linked with other possible causal agents (Martelli *et al.*, 2016). It was only following laboratory assays (ELISA, confirmed by PCR) that the presumptive causal agent was identified (Saponari *et al.*, 2013). Moreover, it is not possible to rely only on visual observations for the unequivocal identification of symptoms caused by *X. fastidiosa*. There is a period during which infected plants may be a source of inoculum for secondary infections although they do not display symptoms.

In a situation where no outbreak has been known to occur, surveillance should be risk-based, focusing on the maintenance of the phytosanitary status of the genetic resources and on the most risky import pathways, especially targeting import lines from countries where the pathogen is known to occur. As symptoms are not always easy to recognize or discriminate from

those of other diseases or disorders, and as symptomless infections are possible, visual inspections must be carried out by highly trained inspectors and laboratory testing by trained specialists. Owing to the significant role of asymptomatic infection, symptomless plants should also be selected and subjected to diagnostic testing for early detection (rather than using diagnostic tests only to confirm visual symptoms). Laboratories are obliged to notify immediately any identification of the quarantine pathogen to the competent authority and should preferably have to prove that they have the capacity to identify *X. fastidiosa* according to the highest standards (accreditation according to norm ISO17025, participation to proficiency testing, etc.).

An adequate number of samples of each host plant must be taken (see Annex I), and the number of host plants sampled at each location should be such as to allow a sufficiently high probability of detection and should be guided by statistical sampling methods (Madden and Hughes, 1999).

**Table 7. Active ingredients of the common insecticides, authorized or not, under EU rules for olive protection, which may be effective against *X. fastidiosa* adult vectors**

Active ingredient	Mode of action and optimal period of application	Efficacy on spittlebugs (or froghoppers)	Reordered and admitted use in EU rules on olive groves
imidacloprid	systemic; avoid any application during blooming	++++	yes
etofenprox	contact and ingestion on adults	+++	no
buprofezin	growth inhibitor during spring on young stages	+++	yes
dimethoate	contact and ingestion, cytotoxic, good persistence	++	yes
deltamethrin	contact and ingestion on adults	++	yes
lambda-cyhalothrin	contact and ingestion on adults	++	yes
chlorpyrifos-methyl	contact and ingestion, low cytotoxic effect	++	no

## Targeted risk-based selection of sites

Distance to known outbreak sites clearly contributes to the risk at a particular location. As mentioned, bacterial dissemination is primarily performed by leafhoppers, which fly short distances but can be dispersed over much longer distances by passive transport (e.g. wind, vehicles). Consequently, suitable locations several kilometres from known outbreak sites may also be considered at high risk. This is particularly so where there is relatively unbroken host availability between a specific location and a known outbreak site. In this case, as in the Salento peninsula, host plantings in between act as “stepping stones”, connecting host locations in terms of disease spread.

Aerial photographs and crop maps offer an additional tool for surveying large areas and for the early identification of potential outbreaks, providing that field observations and sampling are carried out in zones suspected to be infected, i.e. high-risk areas (D’Onghia *et al.*, 2014; Santoro *et al.*, 2014). For example, Gualano *et al.* (2014) showed how high resolution aerial images processed by visible and near-infrared data could be used to identify trees showing damage by *X. fastidiosa* infection.

As risk-based selection of survey locations is subject to error, a certain proportion of targeted survey effort should also be allocated to random searches (Anonymous, 2006). The spread of infectious vectors and planting material by humans over long distances also requires surveillance in areas that are far from known outbreak sites but where the host, vector and climatic conditions are suitable for their establishment. One way of addressing these issues is to prioritize a survey based on risk but also to allow for a sampling coverage in some lower-risk areas by stratified sampling. A region is split into regular strata and each stratum is given a risk value. The number of sites surveyed in each stratum is then weighted by the relative risk value of the stratum. Clearly, sites where no host or vector is present and where climatic conditions are unsuitable carry a risk value of zero and are not surveyed.

Non-targeted random surveys are also required to establish unbiased estimates of disease incidence and distribution to inform pest risk assessment and provide epidemiological information (Anonymous, 2006).

In areas where an outbreak has occurred, intensive detection surveys should be performed to identify all infested sites. In such cases, it is particularly important to target surveillance efforts based on maps of disease risk. Investigations should be organized to trace back the outbreaks from audit lines and distribution records, to draw dissemination lines and to identify plots at risk.



## Monitoring of demarcated areas

The demarcated areas should be inspected twice in the course of the year, at the most appropriate periods, through visual inspections and the collection of plant and insect samples (see Annex I and Annex II).

Surveys are to be carried out in the infected area and in the buffer zone based on the following criteria:

**Infected area.** Monitoring is performed through the visual inspection of all tree host plants. Significant sampling is carried out of tree and herbaceous host plants, on both symptomatic and symptomless plant material, and on vectors.

**Buffer zone.** Monitoring is performed through visual inspection of all the host plants (trees and shrubs), accompanied by sampling of symptomatic and symptomless host plant material, and vectors.

## Surveillance of nursery activities

A specific surveillance programme to look for the harmful organism must be carried out in any nurseries in the demarcated areas.

Surveys should be conducted at least twice a year in each nursery, including inspections of lots of host plants as well as monitoring of host plants in the vicinity of the nurseries. Moreover, phytosanitary analyses must be performed by accredited laboratories on one percent of the plants present in each well-identified lot for no more than 100 plants per lot.

In compliance with the EFSA Plant Health Panel provisions, samples should also be taken from species that are not considered to be host to the harmful organism, in order to acquire additional confirmatory data.

Monitoring of the host plants is performed through the following procedure:

- visual inspection of all host plants;
- collection of samples from symptomatic and symptomless plants;
- collection of samples of vectors by sweep (entomological) net and/or yellow sticky traps.

For each holding, a survey fact sheet reporting all the results of the inspections and the corresponding analyses must be prepared.

## Requirements for movement of plant material from demarcated areas

It is forbidden to move plants for planting out of the delimited areas if they have been determined as hosts of the pathogen, unless they are certified nursery productions. It is also forbidden to collect propagating material from plants inside the demarcated area.

The host plants intended for planting can only be transported outside the delimited area provided the following conditions are met:

- They have been grown throughout their cropping cycle in a production site free from the harmful organism, in compliance with ISPM 10, under a structure providing isolation and external protection to exclude the entry of insect vectors.
- In this site plants have officially been inspected twice in the most appropriate periods and no symptoms of the harmful organism have been observed.
- Just before marketing the plants must be submitted to accurate inspection and sampled for laboratory tests.

In case of pathogen detection, the marketing of host plants must be immediately suspended and the whole lot destroyed. Moreover, all existing lots of host plants in nurseries must be sampled in order to ensure the absence of the pathogen. Only after these checks can the nursery again be authorized.

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# ANNEX I

## Methods of sampling plant material

**Sampling of plant material.** *X. fastidiosa* is confined to the xylem tissue of its hosts, thus the petiole and midvein recovered from leaf samples with symptoms are the best source for diagnosis, as they contain a higher amount of xylem vessels. However, other sources of infected tissue include small twigs and roots and fruit petioles.

**Sampling period.** Late summer to autumn is in general the best period to sample for *X. fastidiosa* diagnosis. For example, in chronically infected vines or deciduous trees (e.g. cherry and almond) the bacteria do not move into the new season's growth until the middle of summer, when symptoms become evident. Leaves attached to the canes or branches should preferably be sampled. In evergreen species, the sampling is possible throughout the year but it is based on the specific climatic conditions of the area and fluctuation of the bacteria in a given host plant. In cases where sampling is carried out in summer, no collection should be made during the hottest hours of the day.

## Sample collection

**Perennial evergreen hosts.** In these host species, with suspicious or ascertained symptoms, the samples (leaves or cuttings with attached leaves) should be collected from symptomatic branches/twigs. For both symptomatic and symptomless hosts, the sample should be representative of the entire aerial part of the plant (canopy), collecting an appropriate number of leaves or cuttings with attached leaves. At least 10–12 mature leaves should be picked or, alternatively, eight twigs 15–20 cm in size around the canopy, avoiding the vegetating apices. Experimental data pertaining to *X. fastidiosa* detection in monumental ancient olive trees in the Apulian outbreak (Southern Italy), showed that more reliable detection can be achieved by sampling the middle to the upper part of the canopy.

**Perennial deciduous hosts.** Samples are of the same type and size as specified above, i.e. at least eight leafy twigs 15–20 cm in size. Alternatively, from September onwards, 10–12 mature leaves from woody twigs.

**Annual hosts (weeds).** Samples should include mature portions of the plants (stems, leaves, roots, etc.), or the entire plant whenever feasible.

**Shrubs.** Leafy twigs 15–20 cm in size or, alternatively, 15–20 mature leaves from woody twigs.

Samples can be collected by hand whenever feasible (e.g. individual leaves, small accessible twigs) or with appropriate tools (ordinary pruning shears, telescopic pruning shears). It is advisable to disinfect all tools with sodium hypochlorite or quaternary ammonium salts before moving to another tree to be sampled.

It is of paramount importance to make sure that there are no potential or alleged insect vectors on the collected plant material. Thus a vigorous shaking of the samples is advised prior to placing them in a bag, which will then be sealed in a second bag. Bags are to be labelled with data for sample traceability, e.g. (i) collection data; (ii) name of collector; (iii) site of collection; (iv) latitude and longitude; (v) species sampled; (vi) presence/absence of symptoms, and transported as soon as possible to the laboratory for analysis.

### Sample preparation for analysis

For each sample, at least 0.5–0.8 g of tissue (petioles and midribs or basal leaf portions) should be recovered from 5–10 leaves (depending on the leaf size and consistency) and used for the preparation of the plant extract regardless of the methods of detection. Samples should be inspected for symptoms and, if present, symptomatic leaves (showing leaf scorching and necrosis) should be selected and processed (removing the necrotic and dead tissue). If no symptoms are visible, leaves to be processed should be as representative of the entire sample as possible.

# ANNEX II

## Equipment, supplies and insect storage

The equipment necessary to conduct surveys and monitoring of Auchenorrhyncha is relatively simple. Note that juveniles or delicate non-sclerotized collections must be stored in 75–80 percent alcohol (EtOH), as must all insects smaller than 0.5 cm. EtOH-preserved specimens may be easier to maintain and store, resulting in better-preserved collections, even if they are reasonably larger than 0.5 cm. It is advisable to place a pencil-written label inside the vial.

**Forceps.** Fine, lightweight forceps are recommended. If sharp-pointed forceps are used, care must be taken not to puncture specimens. If possible, grasp specimens with the part of the forceps slightly behind the points. A brush may serve as an affordable, delicate alternative to forceps.

**Vials** containing abundant (liquid volume  $30 >$  insect volume) 75–80 percent EtOH.

**Small containers** for storing specimens after their removal from killing bottles. These may be made of cardboard, plastic, or metal but should not be airtight to avoid mould, and need to be partly filled with soft tissue or cloth to keep specimens from rolling about. Do not use cotton because specimens become entangled in the fibres and may be difficult or impossible to extricate without damage. Each insect should be “papered” by purpose-made envelopes (see *The Complete Field Guide to Butterflies of Australia*, or similar references), or the operator can use small letter envelopes with pencil-written data.

## Plastic bags for storing material and hand lens

**Sweeping net.** This tool is similar to a butterfly net but has a strong handle and a durable bag to withstand being dragged through dense vegetation. Sweeping over prickly Mediterranean vegetation, such as brambles, could easily damage the tissue. Efficient use of a net is gained only with experience. Collection from weeds has to be performed with a pendulum-like movement, with the rim of the net as close as possible to the soil, to prevent the specimen from escaping. The same movement can be applied to the canopy of the

plant to be sampled. Swing the net rapidly to capture the specimen then follow through to force the insect into the very bottom of the bag. The contents of the net can be emptied into a plastic bag, remembering to write on each bag the date, locality and sweep site (for example, olive canopy, weeds, etc.).

**Aspirator.** This is an effective device for collecting small and highly mobile insects able to crawl and fly. There are two types of aspirator that can be used for the present purposes, mouth aspirator or motorized suction device.

**Sticky traps.** This type of trap, a board, piece of tape, panel of glass, piece of wire net, cylinder or other object, often yellow, is coated with a sticky substance and suspended from a tree branch or other convenient support. Insects landing on the sticky surface are glued on and unable to escape. The sticky material is later dissolved with a suitable solvent but, for molecular analysis, it is advisable to gently detach the insects with forceps, avoiding the use of chemicals.

**Frappage.** Insects can be collected from the canopy by shaking branches over a white sheet stretched below the sampling site. Then they can be picked up with the aspirator.

**Storage.** Insects can be killed and preserved in EtOH 75–80 percent. Samples should be covered with an EtOH volume 30 times the volume of the insect. Another possibility is to use a smaller amount of EtOH with meniscus just above the sample, changing it at least three times over the following two days. Another way is to kill insects in a jar with ether or ethyl acetate, or carbon dioxide. Collected samples for molecular analysis must be preserved in a coolbox until they reach the laboratory.

Field-collected insects can be analysed for the presence of *X. fastidiosa* by PCR and real-time PCR. If the insects cannot be processed immediately after capture, they should be stored at  $-20^{\circ}\text{C}$  for short periods or preserved in 95–99 percent EtOH or acetone for longer periods. Living insects for analysis can be killed by freezing or by exposure to carbon dioxide or ethyl acetate. Insects from sticky traps can be removed from the traps using small forceps/pincers with the help of a proper solvent. After removal from the traps, insects should be rinsed in ethanol/acetone and stored under ethanol/acetone.

As *X. fastidiosa* only colonizes the foregut and does not systemically spread into the body, only the head of the insect should be used for DNA extraction, thus avoiding the extraction of several contaminants that may inhibit the enzymatic reactions. Total DNA can be extracted from single insect heads, or using a number of DNA extraction kits.

## Precautions to prevent spread of insect vector

All operators and technicians have to pay attention to cleaning their own clothes, vehicles, tools and instruments used for sampling. In each field sector selected for the sampling procedure, cleaning points are required (quaternary ammonium salts or similar products are used for the disinfection of scissors, saws and other instruments used for cutting infected plant material).

In order to avoid the migration or accidental transport of potentially infected insect vectors, vehicle windows must be closed during the entire monitoring procedure.

Operators and technicians in charge shall follow specific training courses organized by the officers of national plant protection organizations concerning: (i) the quarantine measures laid down by laws or decrees aimed at avoiding the spread of quarantine pests from infected to healthy areas; (ii) the phytosanitary risk that any given Mediterranean country runs upon introduction of a quarantine pest in a bordering country; (iii) the knowledge of symptoms, transmission pattern and spreading of *X. fastidiosa* in olive trees and other plant hosts; (iv) the knowledge of sampling methods for plant and vectors, storage and transport of samples to the laboratories for analysis; (v) the updating of sampling procedure, i.e. methods based on georeferencing techniques to guarantee the traceability of sampling, storage and transport of collected material.





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