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"Xylella fastidiosa (Wells et al., 1987), the dangerous pathogen in Europe"

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Abstract

This bibliographical work tries to study the biology, importance and effects of the pathogen *Xylella fastidiosa* (*Wells et al., 1987*). This pathogen is common on the American continent, although the recent discovery of an outbreak in the South of Italy has raised the alert in Europe. This work aims to understand both its geographical distribution, as their vectors, host plants and the methods of control on the crops.

Keywords: Xylella fastidiosa, olive, Cicadellidae, xylem, grapevine

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1. Introduction

Xylella fastidiosa (Wells et al., 1987) is a vector-transmitted bacterial plant pathogen associated with important diseases in a wide range of plants. It causes Pierce's disease in grapevine (Vitis vinifera), which is described as a major constrain for commercial grapevine production in parts of the USA and tropical America. Numerous species of xylem sap-sucking insects (leafhoppers/Cicadellidae) are known to be vectors of this bacterium.

Xylella fastidiosa is a regulated harmful organism in the European Union, listed in Annex I, Part A, Section I to Council Directive 2000/29/EC as a harmful organism not known to occur in any part of the Union, whose introduction into, and spread within, all Member States is banned. Non-European Cicadellidae known to be vectors of Pierce's disease, caused by *Xylella fastidiosa*, are also listed in Annex I, Part A, Section I to Council Directive 2000/29/EC.

2. Identity of the pathogene

Xylella fastidiosa is the causal agent of Pierce's disease of grapevine, phony peach disease, plum leaf scald, almond, elm, oak, American sycamore, mulberry and maple leaf scorch, and citrus variegated chlorosis disease, among other diseases. The causal agents of those diseases were previously considered to be different pathogens, but *Xylella fastidiosa* is now considered to be the unique causal agent.

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Gamma Proteobacteria
Order: Xanthomonadales
Family: Xanthomonadaceae
Genus: Xylella

Species: X. fastidiosa

Therefore, the valid scientific name is *Xylella fastidiosa* (Wells et al., 1987).

2.1. Taxonomy

Xylella fastidiosa is a gammaproteobacterium in the family Xanthomonadaceae. It was initially thought to be a virus, but in the 1970s it was shown to be a bacterium (Purcell, 2013). It was first described and named in 1987 (Wells et al., 1987). To date, the genus *Xylella* consists of only one species, *X. fastidiosa*. Nevertheless, *X. fastidiosa* has substantial genotypic and phenotypic diversity, and a wide host range (Schuenzel et al., 2005; Nunney et al., 2013).

There are four accepted subspecies of *X. fastidiosa* — *fastidiosa*, *pauca*, *multiplex* and *sandyi* (Schaad et al., 2004; Schuenzel et al., 2005)—although only two, subspecies *fastidiosa* and subspecies *multiplex*, are so far considered valid names by the International Society of Plant Pathology Committee on the Taxonomy of Plant Pathogenic Bacteria (ISPP-CTPPB) (Bull et al., 2012).

Subspecies *fastidiosa* is the best-characterised group, and the only genetic group causing disease in grapevines in the USA (Pierce's disease) (Nunney et al., 2010). The subspecies *fastidiosa* is more diverse in Central America; thus, it has been suggested that its presence in the USA is the consequence of an introduction (Nunney et al., 2010). The introduction of ssp. *fastidiosa* in Taiwan has led to an epidemic in grapevine (Su et al., 2013).

Isolates within ssp. *pauca* causing citrus variegated chlorosis in Brazil are reasonably well characterised (Nunney et al., 2012a). The genotype present in Italy is a recombinant of alleles within subspecies *pauca* (Maria Saponari and Donato Boscia, National Research Council, Institute for Sustainable Plant Protection, Bari, Italy, personal communication, 2014; Cariddi et al., 2014).

The subspecies *multiplex* appears, so far, to have the widest host range in terms of plant species expressing disease symptoms (Nunney et al., 2013). It is subdivided into various subgroups, which are mostly associated with specific host plants (Nunney et al., 2013). The presence of subspecies *multiplex* in Brazil is considered to be the result of an introduction from the USA associated with plums (Nunes et al., 2003; Almeida et al., 2008; Nunney et al., 2012b). Interestingly, Nunney et al. (2012b) raised the hypothesis of a recent inter-subspecies recombination between the sympatric *X. fastidiosa* subsp. *pauca* and subsp. *multiplex* in South America to explain why host plants such as citrus or coffee, which have been cultivated there for about 250 years, have been affected for only the last 25 years.

Isolates from the subspecies *sandyi* are poorly characterised and their biology is not well understood (Yuan et al., 2010).

3. Symptoms, detection and identification

The symptoms associated with the presence of *Xylella fastidiosa* in plants vary from asymptomatic associations to plant death, due to the large number of different host affected by the bacteria, pathogen diversity, and partly because of the wide range of climatic conditions in areas where the pathogen is found.

Most host plants infected with *X. fastidiosa* do not express any symptom. Symptoms often consist of a rapid drying of leaf margins, with scorched leaves. The different names given to the disease illustrate this heterogeneity of symptoms: "Pierce's disease" on grapevine, "alfalfa dwarf', "almond leaf scorch", "phony peach disease", "plum leaf scald", "citrus variegated chlorosis" or "leaf scorch" of elm, coffee, oak, sycamore and oleander (Figure 1). In Taiwan, pear leaf scorch was also reported on *Pyrus pyrifolia* (Japanese pear) and *P. serotina* (Asian pear) (Chen et al., 2006).

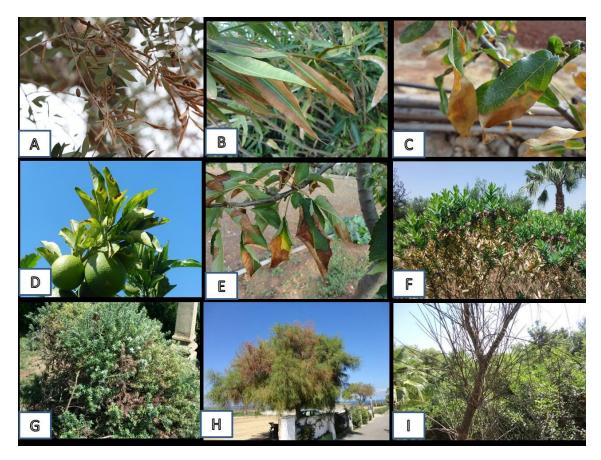


Figure 1. Xylella fastidiosa symptoms on various host plant species. (A) Olive trees (B) Oleander (C) Almond leaf scorch disease (D) Citrus variegated chlorosis symptoms on leaf (never found infected in Apulia) (E) Cherry (F) Polygala myrtifolia (G) Westringia fructicosa (H) Acacia saligna I: Spartium junceum. Photographs courtesy of Donato Boscia, CNR—Institute for Sustainable Plant Protection (A, B, C, E, F, G, H and I) and Helvecio Della Coletta Filho, Centro de Citricultura Sylvio Moreia – IAC Cordeiropolis, SP, Brazil (D).

The reliable detection and identification of *X. fastidiosa* is very important not only because of its quarantine status, but also because the different subspecies are markedly different in host range and, therefore, in terms of plant disease significance. Another reason is the fact that *X. fastidiosa* infects a wide range of host plant species asymptomatically. Symptom development depends on host plant species—*X. fastidiosa* genotype (Almeida and Purcell, 2003) and is usually correlated with high bacterial populations within plants (Hill and Purcell, 1995; Newman et al., 2003). Because bacterial populations within plants are correlated with pathogen acquisition efficiency by vectors (Hill and Purcell, 1997), plant species infected with low populations of *X. fastidiosa* may serve as an inefficient reservoir for vectors to acquire the bacterium (Almeida et al., 2005).

Many analyses are culture dependent and rely on isolation using non-selective media (Raju et al., 1982; Davis et al., 1983; Wells et al., 1983; Chang and Walker, 1988; Hill and Purcell, 1995; Almeida et al., 2004, Lopes and Torres, 2006). Detection must be performed under good laboratory conditions as isolates may take one to four weeks to develop colonies on solid media owing to their slow growth. Potential difficulties during *in vitro* cultivation include low bacterial densities in plant tissue, heterogenity of bacterial distribution within the plant and potential growth inhibitors extracted during tissue grinding for culturing. Moreover, other pathogenic agents may be present at the same time in samples and may hinder the detection of *X. fastidiosa*.

Several methods have been used to identify *X. fastidiosa* directly in petiole or stem cross-sections, including electron microscopy (French et al., 1977) and immunofluorescence (Carbajal et al., 2004; Buzkan et al., 2005). Serologically based methods such as enzyme-linked immunoassay (ELISA) or immunofluorescence have been used extensively, but are sometimes considered less sensitive than the isolation approach (French et al., 1978; Sherald and Lei, 1991). Those methods could also lead to false-negative or -positive detections. The EPPO protocol (EPPO, 2004) states that, for official purposes, a strain should be isolated and pathogenicity tests should give positive responses.

Numerous polymerase chain reaction (PCR)-based methods have been proposed for *X. fastidiosa* detection, with different objectives, including general detection, quarantine purposes (Chen et al., 2000; Minsavage et al., 1994; Harper et al., 2010), subspecific detection targeting an *X. fastidiosa* subspecies or a given plant species for high-throughput methods (Pooler and Hartung, 1995; Oliveira et al., 2002; Huang, 2009; Guan et al., 2013; Li et al., 2013; Ouyang et al., 2013), *in situ* detection methods (Ouyang et al., 2013; Schaad et al., 2002) or loop-mediated isothermal amplification (LAMP) and Ex Razor procedures (Harper et al., 2010; Ouyang et al., 2013).

Identification of putative *X. fastidiosa* colonies is best achieved by molecular methods. These include sequence-based analyses targeting housekeeping genes. Such analyses target either single gene portions or, better, multiple genes by a method known as MLST or multilocus sequence analysis (MLSA) (Almeida et al., 2014; Nunney et al. 2010; Parker et al., 2012), which better addresses identification at the subspecies level due to the presence of homologous recombination among genotypes. Other techniques, such as quantitative real time PCR (Bextine and Child, 2007, Brady et al., 2012) and variable tandem repeat analysis (Coletta-Filho et al., 2001), have also been used for typing purposes, although they provide varying levels of genetic resolution.

4. Biology of the pathogen

4.1. Host plant colonisation

X. fastidiosa colonises the xylem network of plants, where it can move up- and downstream (Almeida et al., 2001; Meng et al., 2005). Populations of X. fastidiosa restrict water movement in the xylem, and high frequencies of blocked vessels are associated with disease symptom development (Newman et al., 2003). X. fastidiosa colonises many host plants that remain symptomless, and serve as a source of inoculum for vectors (Hopkins and Purcell, 2002). The colonisation of different host species (by different X. fastidiosa genotypes) ranges from successful infections resulting in plant death within months to persistent yet non-symptomatic infection (Purcell and Saunders, 1999). Therefore, colonisation patterns are complex and depend upon host plant species and genotype of the pathogen.

Despite the large variability of symptoms, there is a consistent association of symptoms with plant physiological responses to water stress. An important aspect of plant susceptibility is the ability of *X. fastidiosa* to move within the xylem network and reach high bacterial populations. Movement and the size of bacterial populations are

correlated with the severity of disease symptoms. Importantly, they are also correlated with the efficiency with which *X. fastidiosa* is acquired by insect vectors. In other words, hosts that harbour larger bacterial populations distributed throughout the plant are more likely to result in infection of insects than hosts with low bacterial populations, which usually do not become systemic. Therefore, the importance of alternative hosts (i.e. not focal crop; plants such as weeds) in disease epidemiology is highly variable and dependent on their capacity to harbour large populations of the pathogen, in addition to being feeding hosts of the vector.

4.2. Vector transmission

Xylella fastidiosa is a xylem-limited bacterium that is exclusively transmitted by xylem sap-feeding insects belonging to the order Hemiptera, sub-order Auchenorrhyncha (Redak et al., 2004).

The transmission of X. fastidiosa by insects is peculiar in that it does not require a latent period, yet the bacteria are persistently transmitted (Almeida et al., 2005). Vectors (both nymphs and adults) acquire the bacteria by feeding in the xylem of an infected plant and can inoculate the pathogen to healthy plants immediately after acquisition. Bacteria are restricted to the alimentary canal and do not systemically infect the insect body. They adhere to and multiply in the pre-cibarium and cibarium (parts of the foregut). This implies that vectors lose infectivity with moulting, as the foregut is of ectodermal origin and is renewed with moulting. Therefore, newly emerged adults must feed on an infected plant to become infectious and spread X. fastidiosa. Once infected, adult vectors can transmit during their whole lifetime, as the bacterium multiplies and persists in the vector foregut (Almeida et al., 2005). The bacterium is not transovarially transmitted to the progeny of the vector (Freitag, 1951). Winged adults, because of their high mobility, are mostly responsible for X. fastidiosa spread. It is important to remember that, since the bacterium is restricted to the foregut (Purcell and Finlay, 1979), the number of bacterial cells per insect is low (very few live bacterial cells in the vector's foregut are required for transmission: Hill and Purcell, 1995) and therefore a sensitive diagnostic tool, such as PCR, is needed to detect the presence of X. fastidiosa in the vector insects. ELISA is not sensitive enough for detection of X. fastidiosa in the vector insects. Importantly, even PCR (or qPCR and other related methods) have so far not been shown to provide robust results in insects.

On one hand, *X. fastidiosa* transmission is restricted to xylem sap-feeding insects; on the other hand, insect transmission of *X. fastidiosa* is known to be poorly specific and therefore all xylem sap-feeding insects are considered vectors, which has not been disproven so far (Frazier, 1944; Purcell, 1989; Almeida et al., 2005). However, transmission efficiency varies substantially depending on insect species, host plant and *X. fastidiosa* genotype (Redak et al., 2004; Lopes et al., 2010).

4.3. Ecology

The ecology of *X. fastidiosa* diseases is the outcome of complex biotic and abiotic interactions. Although general insights from one disease system are useful for another, ecological parameters are not necessarily transferable.

Despite the fact that *X. fastidiosa* has a notoriously large alternative host plant range, the epidemiological importance of such hosts varies. The spring spread of *X. fastidiosa*

from host plants in riparian habitats (i.e. along creeks/rivers) into vineyards in coastal areas of northern California is well established (Purcell, 1974). Although there is vector spread of *X. fastidiosa* from grapevine to grapevine in late summer and autumn, only the spring spread from alternative hosts to grapevine is of epidemiological importance (reviewed in Hopkins and Purcell, 2002). A similar scenario occurs in the Central Valley of California, where insect vectors move to vineyards for brief flights from alfalfa fields, but there is no spread from grapevine to grapevine (Purcell and Frazier, 1985). The opposite scenario occurs with citrus variegated chlorosis in Brazil. In that case, *X. fastidiosa* is also known to colonise a wide range of weeds associated with citrus orchards (Lopes et al., 2005), but pathogene spread occurs primarily from citrus to citrus tree (Laranjeira et al., 1998). Alternative hosts, in this case, may be important for maintenance of the pathogen in the environment, and provide a habitat for insect vectors, but their epidemiological impact is deemed to be low.

Similarly, epidemics of Pierce's disease of grapevines in California, USA, may also have distinct characteristics if vector species are different. In coastal northern California, spread is driven by adult *Graphocephala atropunctata* leafhoppers that overwinter in riparian areas adjacent to vineyards. In spring they migrate to vineyards and infect vines, leading to a disease distribution limited to the overwintering habitat of vectors. After the introduction of the invasive species *Homalodisca vitripennis* to southern California, Pierce's disease epidemics had devastating consequences for vineyards in Temecula Valley, where entire vineyards were found to be symptomatic (i.e. no edge effect). In this case, insect vectors overwintered on adjacent citrus plants, reaching extremely large populations (one to two million per hectare) (Coviella et al., 2006). Vectors were found distributed throughout vineyards in very large numbers (Perring et al., 2001), leading to higher rates of disease spread.

In the Americas, in most diseases caused by *X. fastidiosa* that have been studied, the vectors are leafhoppers. In Europe, spittlebugs are much more abundant and diverse than sharpshooter leafhoppers, and not as much is known about their biology, ecology and role as vectors. In addition, agricultural practices and environmental conditions, including the landscape and climate, are extremely variable in the EU. Research will certainly be necessary to establish the basics of *X. fastidiosa* ecology in the EU.

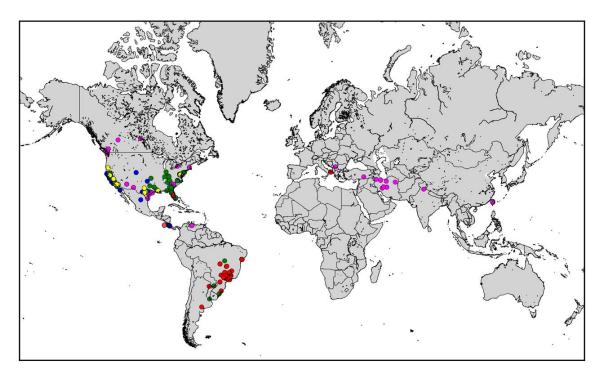
5. Current distribution

5.1. Global distribution

Diseases caused by *X. fastidiosa* occur in tropical, subtropical and temperate areas, mainly in the Americas. The geographical distribution based on the coordinates of the the host plants is as follows (Figure 2):

• North America: *X. fastidiosa* has been reported in Canada (on elm in southern Ontario (Goodwin and Zhang, 1997), British Columbia (FIDS, 1992) and Saskatchewan (Northover and Dokken-Bouchard, 2012); on maple in Alberta (Holley, 1993)). Mexico and the USA (Alabama, Arizona, Arkansas, California, Delaware, District of Columbia, Florida, Georgia, Indiana, Kentucky, Louisiana, Maryland, Mississippi, Missouri, Montana, Nebraska, New Jersey, New Mexico, New York, North Carolina, Oklahoma, Oregon, Pennsylvania, South Carolina, Tennessee, Texas, Virginia, Washington, West Virginia: EPPO PQR, 2014).

- Central America and Caribbean: *X. fastidiosa* has been reported in Costa Rica (Nunney et al., 2014) and Mexico (Legendre et al., 2014). In addition it has been intercepted in consignments imported into Europe from Honduras (EUROPHYT, online).
- **South America**: *X. fastidiosa* has been reported in Argentina (Leite et al., 1997; de Coll et al., 2000), Brazil (Bahia, Espirito Santo, Goias, Minas Gerais, Parana, Rio Grande do Sul, Rio de Janeiro, Santa Catarina, São Paulo, Sergipe), Ecuador (Legendre et al., 2014), Paraguay and Venezuela.
- Asia: *X. fastidiosa* has been reported in Iran (Amanifar et al., 2014), India (Jindal and Sharma, 1987: this report remains uncertain, detection based mostly on symptom observation and coloration of xylem), Lebanon (Temsah et al., 2015: this report remains uncertain, further analysis is needed to confirm the report based only on ELISA detection and scanning electron microscopy observations), Taiwan (Leu and Su, 1993), and Turkey (Güldür et al., 2005: this report remains uncertain, detection based on ELISA and electron microscopy observations; no further reports or studies published).
- **Africa:** *X. fastidiosa* has not been reported.
- **Europe:** An outbreak of *X. fastidiosa* in Kosovo was reported by Berisha et al. (1998), but this report was not confirmed by further studies. France reported the eradication of a confirmed case on coffee plantlets kept in contained glasshouse facilities. These coffee plants were received from Ecuador (*Coffea arabica*) and Mexico (*Coffea canephora*) (Legendre et al., 2014). Also they have reported an outbreak of *X. fastidiosa* subsp. *multiplex* in plants of *Polygala myrtifolia* (EPPO, 2015). Recently, a field outbreak of *X. fastidiosa* has been recorded in the Apulia region of Italy (EPPO, 2013).



Colour code: blue = X. fastidiosa subsp. fastidiosa; green = X. fastidiosa subsp. multiplex; red = X. fastidiosa subsp. pauca; yellow = X. fastidiosa subsp. sandyi; fuchsia = X. fastidiosa subsp. unidentified)

Figure 2. World distribution of Xylella fastidiosa subspecies.

There are uncertainties associated with reports that incompletely describe the detection methods that were used. The tedious isolation process of *X. fastdidosa*, the difficulty in fulfilling Koch's postulates and the need also to understand the vector's role are certainly part of the explanation why the identification process has sometimes been stopped or performed inadequately. Furthermore, it should be stressed that, since infected plants might be missed because they are asymptomatic or show symptoms that could be due to drought, the known distribution can be linked only to areas where the disease has provoked clearly visible symptoms, and usually epidemics.

5.2. Situation in Italy

In mid-October 2013, the NPPO of Italy informed the EPPO Secretariat of the first detection of *Xylella fastidiosa* (bacterium included on the EPPO A1 List since 1981) on its territory. In Southern Italy (near Lecce, Salento peninsula, Puglia region), quick decline symptoms were observed on olive trees (*Olea europea*). Investigations showed that symptomatic olive trees were generally affected by a complex of pathogens: *X. fastidiosa*, several fungal species belonging to the

genus *Phaeoacremonium* and *Phaemoniella*, and *Zeuzera pyrina* (leopard moth).

In Italy the disease has been called 'complesso del disseccamento rapido dell'olivo'. Although an unconfirmed record of *X. fastidiosa* in Kosovo was published in 1996, the presence of this bacterium had never previously been confirmed in Europe. Following the reporting of an extensive leaf scorch and dieback of olive trees, spreading rapidly in the area of Salento (Puglia region), the Regional Plant Protection Service promptly initiated investigations to identify the possible causal agent. These surveys were carried

out in collaboration with experts from the University of Bari and the Consiglio Nazionale delle Ricerche (CNR). The systematic screening of samples taken from symptomatic olive trees (many of them were a century-old), revealed the presence of extensive brown discoloration of the vascular system. Portions of xylem tissue taken from symptomatic olive trees were subjected to mycological analysis by isolation on different growing media. Fungal colonies were obtained and identified by morphological and molecular tests. The results showed the constant presence of fungal species belonging to the genus *Phaeoacremonium*, the most frequently found species was P. parasiticum followed by P. rubrigenum, P. aleophilum and P. alvesii. Species of the genus *Phaeomoniella* were also isolated. According to the NPPO, this is the first time that P. parasiticum and P. alvesii have been detected on O. europaea in Italy. In addition, these samples from olive trees were subjected to molecular analysis using specific primers for X. fastidiosawhich gave positive results. The analysis was extended to almond (*Prunus dulcis*) and oleander (*Nerium oleander*) plants which were growing in the vicinity of affected olive trees and showing symptoms of leaf scorch. The results were also positive. Further serological tests (DAS-ELISA with 2 commercial kits) confirmed the presence of *X. fastidiosa*. The NPPO stressed that the definitive identification of the bacterium still awaits its isolation in pure culture in order to perform pathogenicity tests. In addition, further investigations are on-going to identify the bacterial strain, to evaluate its pathogenicity and identify the putative local insect vector(s). It is recalled that *X. fastidiosa* has an extensive natural host range (more than 200 species), including olive from which the bacterial genotype A (pathogenic to oleander and almond but not to grapevine) has been isolated in California (US). Surveys are being carried out in Puglia to delimit the extent of the infected area. It is prohibited to move propagation material of any susceptible host species from the infected area. For the control of the disease, which does not seem to be exclusively due to X. fastidiosa, the adoption of further phytosanitary measures is currently being evaluated. In Italy, it has been shown that the subspecies that is occurring on olive trees is X. fastidiosa subspecies pauca. (EPPO, 2013)

5.3. Situation in France

In July 2015, the presence of the bacterium was reported for the first time by France. *X. fastidiosa* was detected on a few ornamental plants (*Polygala myrtifolia*) planted in a commercial area in Propriano (Corse). Other foci were then detected on the island (both in Corse-du-Sud and Haute-Corse departments), mainly on *P. myrtifolia* (but otherplants were also found to be infected). On the 13th of October 2015, the bacterium was discovered for the first time on the mainland. It was detected in the municipality of Nice (Alpes-Maritimes department - Provence-Alpes-Côtes-d'Azur region) in one plant of *P. myrtifolia*. In France, the subspecies which is occurring on *P. myrtifolia* plants is *X. fastidiosa* subsp. *multiplex* (thus differing from Italy, where it is *X. fastidiosa* subsp. *pauca* that is occurring on olive trees). At the end of October 2015, more infected *P. myrtifolia* plants were found in Nice, and another focus was detected in the municipality of Mandelieu la Napoule (1 infected *P. myrtifolia* plant). At the end of 2015, several foci were found in Alpes-Maritimes and Var departments. In all cases, eradication measures have immediately been implemented in accordance with a contingency plan.

5.4. Situation in Spain

Although *X. fastidiosa* still has not been detected throughout the Spanish territory, different associations of farmers are demanding the Government to take preventive measures to avoid the appearance of new foci in Europe. (Antonio Trapero, 2016).

6. Host plants

Regarding to the hosts, the list of known host of X. fastidiosa is summarised in Table 1

Table 1. The list of host plants genera known from literature to be hosts of Xylella fastidiosa ssp. fastidiosa, multiplex, pauca, sandyi and unattributed subspecies

Subspecies	Plant family	Plant genus
fastidiosa	Adoxaceae	Sambucus
•	Amaranthaceae	Alternanthera,
		Chenopodium
	Anacardiaceae	Rhus, Toxicodendron
	Apiaceae	Conium, Datura, Daucus,
	_	Oenanthe
	Apocynaceae	Nerium, Vinca
	Araliaceae	Hedera
	Asteraceae	Ambrosia, Artemisia,
		Baccharis, Callistephus,
		Conyza, Franseria,
		Helianthus, Lactuca,
		Solidago, Sonchus,
		Xanthium
	Betulaceae	Alnus
	Boraginaceae	Amsinckia
	Brassicaceae	Brassica
	Cannaceae	Canna
	Caprifoliaceae	Lonicera
		Symphoricarpos
	Convolvulaceae	Convolvulus, Ipomoea
	Cyperaceae	Cyperus
	Fabaceae	Acacia, Chamaecrista,
		Cytisus, Genista, Lathyrus,
		Lupinus, Medicago,
		Melilotus, Spartium,
		Trifolium, Vicia
	Fagaceae	Quercus
	Juglandaceae	Juglans
	Lamiaceae	Callicarpa, Majorana,
		Melissa, Mentha,
		Rosmarinus, Salvia,
	Lauraceae	Persea, Umbellularia
	Magnoliaceae	Magnolia

Subspecies	Plant family	Plant genus
	Malvaceae	Malva
	Myrtaceae	Eucalyptus, Eugenia, Metrosideros
	Oleaceae	Fraxinus, Syringa
	Onagraceae	Epilobium, Fuchsia, Godetia, Oenothera
	Pittosporuceae	Pittosporum
	Platanaceae	Platanus
	Poaceae	Avena, Bromus, Cynodon, Digitaria, Echinochloa, Eragrostis, Eriochola, Festuca, Holous, Hordeum, Lolium, Paspalum, Pennisetum, Phalaris, Phleum, Poa, Setaria, Sorghum, Erodium, Pelargonium
	Polygonaceae	Persicaria, Polygonum, Rheum, Rumex
	Portulaceae	Montia, Portulaca
	Resedaceae	Reseda
	Rhamnaceae	Rhamnus
	Rosaceae	Cotoneaster, Fragaria, Photinia, Prunus, Rosa, Rubus
	Rubiaceae	Coffea, Coprosma
	Rutaceae	Citrus
	Salicaceae	Populus, Salix
	Sapindaceae	Acer, Aesculus
	Scrophulariaceae	Veronica
	Simmondsiadaceae	Simmondsia
	Solanaceae	Datura, Lycopersicon, Nicotiana, Solanum
	Urticaceae	Urtica
	Verbenaceae	Duranta
	Vitaceae	Ampelopsis, Parthenocissus, Vitis
multiplex	Altingiaceae	Liquidambar
1	Apocynaceae	Catharanthus, Vinca
	Araliaceae	Hedera
	Asteraceae	Ambrosia, Encelia, Helianthus, Iva, Pluchea, Ratibida, Senecio, Solidago, Sonchus, Xanthium
	Betulaceae	Alnus
	Brassicaceae	Capsella, Sisymbrium
	Caryophyllaceae	Stellaria

Subspecies	Plant family	Plant genus
_	Celastraceae	Celastrus
	Cornaceae	Cornus
	Ericaceae	Vaccinium
	Fabaceae	Cassia, Cercis, Gleditsia,
		Lupinus, Medicago
	Fagaceae	Fagus, Quercus
	Ginkgoaceae	Ginkgo
	Juglandaceae	Carya
	Lamiaceae	Salvia
	Lythraceae	Lagerstroemia
	Magnoliaceae	Liriodendron
	Malvaceae	Malva
	Moraceae	Morus
	Oleaceae	Chionanthus, Fraxinus,
		Ligustrum, Olea
	Plantaginaceae	Veronica
	Platanaceae	Platanus
	Poaceae	Poa, Erodium, Sorghum
	Rosaceae	Prunus, Rubus
	Rutaceae	Citrus
	Sapindaceae	Acer, Aesculus,
	ang manasan	Koelreuteria, Sapindus
	Ulmaceae	Celtis, Ulmus
	Urticaceae	Urtica
	Vitaceae	Ampelopsis, Vitis
раиса	Amaranthaceae	Alternanthera
	Apocynaceae	Catharanthus, Nerium
	Asteraceae	Acanthospermum, Bidens
	Commelinaceae	Commelina
	Convolvulaceae	Іротоеа
	Euphorbiaceae	Euphorbia, Phyllanthus
	Fabaceae	Acacia, Medicago, Senna
	Lamiaceae	Westringia
	Malvaceae	Hibiscus, Sida
	Oleaceae	Olea
	Poaceae	Brachiaria, Cenchrus,
		Cynodon, Digitaria,
		Echinochloa, Panicum
	Polygalaceae	Polygala
	Portulaceae	Portulaca
	Rosaceae	Prunus
	Rubiaceae	Coffea, Richardia,
		Spermacoce
	Rutaceae	Citrus
	Solanaceae	Nicotiana, Solanum
	Verbenaceae	Lantana
	Vitaceae	Vitis
sandyi	Apocynaceae	Catharanthus, Nerium

Subspecies	Plant family	Plant genus
_	Bignoniaceae	Jacaranda
	Magnoliaceae	Magnolia
	Moraceae	Morus
	Xanthorrhoeaceae	Hemerocallis
NA	Adoxaceae	Sambucus
	Altingiaceae	Liquidambar
	Amaranthaceae	Salsola
	Anacardiaceae	Pistachia, Schinus
	Apocynaceae	Catharanthus, Nerium
	Aquifoliaceae	Ilex
	Araliaceae	Hedera
	Arecaceae	Phoenix
	Asteraceae	Ambrosia, Baccharis,
		Conyza, Lactuca,
		Ratibida, Senecio,
		Silybum, Sonchus,
		Xanthium
	Bignoniaceae	Chitalpa
	Brassicaceae	Brassica, Capsella,
		Coronopus
	Caprifoliaceae	Lonicera
	Caryophyllaceae	Stellaria
	Convolvulaceae	Convolvulus
	Cyperaceae	Carex, Cyperus
	Cypressaceae	Juniperus
	Fabaceae	Albizia, Chamaecrista,
		Medicago, Spartium
	Fagaceae	Quercus
	Geraniaceae	Erodium, Geranium
	Ginkgoaceae	Ginkgo
	Juglandaceae	Carya, Juglans
	Lamiaceae	Lavandula, Marrubium,
	Lamitaceae	Rosmarinus
	Magnoliaceae	Magnolia
	Malvaceae	Hibiscus, Malva
	Moraceae	Ficus, Morus
	Oleaceae	Chionanthus, Fraxinus,
	Oreaceae	Olea
	Onagraceae	Ludwigia
	Pinaceae	Pinus
	Plantaginaceae	Plantago
	Platanaceae	Platanus
	Poaceae	Agrostis, Avena, Bromus,
	1 ouceue	Cynodon, Echinochloa,
		Eriochloa, Hordeum,
	Polygongogg	Lolium, Poa, Setaria
	Polygonaceae Portulaceae	Polygonum, Rumex Portulaca

Subspecies	Plant family	Plant genus
_	Ranunculaceae	Ranunculus
	Rosaceae	Heteromeles, Prunus,
		Pyrus, Rubus
	Rubiaceae	Coffea
	Rutaceae	Citrus
	Salicaceae	Salix
	Sapindaceae	Acer
	Solanaceae	Datura, Solanum
	Ulmaceae	Ulmus
	Verbenaceae	Callicarpa, Lippia,
		Verbena
	Vitaceae	Ampelopsis, Vitis
NA: Data not availa	ble regarding subspecies.	

7. Vectors

X. fastidiosa is exclusively transmitted by xylem sap-feeding insects (order Hemiptera, sub-order Auchenorrhyncha: Redak et al., 2004). They have sucking mouthparts (mandibular and maxillary stylets) that allow them to reach the xylem of their host plants, from which they ingest sap. Owing to the very poor nutritional value of xylem fluid, xylem fluid feeders ingest large amounts of sap and produce large amounts of honeydew. They are generally not direct pests unless present at very high population levels. Within the Cicadomorpha, the three superfamilies, Cercopoidea, Cicadoidea and Membracoidea, include xylem fluid-feeding groups but, whereas all Cercopoidea (known as spittlebugs or froghoppers) and Cicadoidea (cicadas) are regarded as xylem fluid feeders, the superfamily Membracoidea includes a single xylem fluid-feeding subfamily, the Cicadellinae (known as sharpshooters). Only these three groups of 'specialists' in xylem fluid feeding have been shown to be vectors of X. fastidiosa. Some phloem sap feeders also feed marginally to the xylem, however tests for X. fastidiosa transmission capacity on one of these species were negative (Purcell, 1980). Spittlebugs, cicadas and sharpshooters are heterometabolous insects that develop through egg, five nymphal stages and adult (winged) stage. Nymphs of cicadas and of spittlebugs of the family Cercopidae are subterranean root feeders, whereas nymphs of spittlebugs of the family Aphrophoridae and of sharpshooters develop on the parts of host plants above the ground. All adults feed and live on the aerial parts of host plants (Ossiannilsson, 1981; Tremblay, 1995; Redak et al., 2004).

7.1. Non European vectors of X. fastidiosa

Because *X. fastidiosa* has been found and studied primarily in the Americas, and causes disease in different crops in the Nearctic and Neotropic regions, its vectors have been identified and studied in these biogeographical areas only. Almost all known vectors of *X. fastidiosa*, all of them sharpshooters (Cicadellinae) or spittlebugs (Cercopoidea), are listed by Redak et al. (2004).

Besides the above-mentioned insects, cicadas are also xylem fluid feeders, but their role in transmitting *X. fastidiosa* is still largely hypothetical. There are only two reports of

the possible role of cicadas (e.g. *Diceroprocta apache* Davis) in *X. fastidiosa* transmission (Paiaõ et al., 2002; Krell et al., 2007), providing very limited data, which makes the uncertainty very high.

Table 2 lists the known vectors in the Americas. The geographical distribution, host plants and feeding preference of the American vector species, and their relative role in *X. fastidiosa* transmission, are well documented (Redak et al., 2004). Most of the vector species spread in subtropical and tropical ecosystems and therefore develop and breed throughout the year. However, some North American sharpshooter species, e.g. *Draeculacephala minerva*, *Graphocephala atropunctata*, *Xyphon fulgida* and *Homalodisca vitripennis*, are known to overwinter as adult (http://www.cnr.berkeley.edu/xylella/insectVector/insectVector.html) and therefore *X. fastidiosa* can survive the winter in the vector, as well as in the infected plants.

Table 2. Vectors of X. fastidiosa in the Americas: main insect groups and most important vector species

Insect group	Most important species	Distribution	Role as vector	Role as vector: criteria
Sharpshooters (Cicadellidae, Cicadellinae): 38 spp.	Bucephalogonia xanthophis (Berg)	Neotropical: Argentina, Bolivia, Brazil, Paraguay	High in citrus	Common, abundant on ornamental plants, citrus and nursery stocks
	Dilobopterus costalimai Young	Neotropical: Brazil	High in citrus	Common, abundant on ornamental plants and citrus
	Graphocephala atropunctata (Signoret)	USA and Central America	High in grapevine	Common in diverse ecosystems, on grapevine and ornamental plants
	Homalodisca vitripennis (Germar)	USA (southern states), Mexico (northern part), French Polynesia, Easter Island	High in grapevine	Common and abundant in diverse ecosystems, on grape, ornamentals, citrus and nursery stock

Insect group	Most important species	Distribution	Role as vector	Role as vector:
	species		Vector	criteria
Spittlebugs	Philaenus	USA	Low	Not
(Cercopoidea):	spumarius L.	Including		associated
six species		Hawaii,		with disease
		Mexico,		epidemics
		Tahiti		
Cicadas	Diceroprocta	Mexico,	Doubtful	Missing
(Cicadoidea):	<i>apache</i> Davis	Arizona,		information
two species	Dorisiana viridis	Utah,		on
	(Olivier)	Nevada,		transmission
		California		capacity

7.2. Potential European vectors of *X. fastidiosa*

Following Frazier (1944) and Purcell (1989), all the xylem fluid feeders should be considered to be potential vectors. With the exception of *Philaenus spumarius* (Aphrophoridae), an Old World species introduced in North America and identified as a vector of *X. fastidiosa* in California (Purcell, 1980), all the American vector species are absent from Europe according to the Fauna Europaea database (de Jong, 2013). *X. fastidiosa* has never previously established in Europe and, in the case of the current Apulian outbreak of *X. fastidiosa*, only one species, *P. spumarius*, has so far been proved to be able to transmit the strain of *X. fastidiosa* involved (Saponari et al., 2014). This species is the only vector identified so far in Europe.

Sharpshooters (Cicadellidae, subfamily Cicadellinae) are by far the most important vectors of *X. fastidiosa* in the Americas, but only a few species are present in Europe (Wilson et al., 2009). One species, *Cicadella viridis*, is widespread in Europe, but is common only in humid areas.

In contrast, a relatively high number of spittlebug species (Cercopoidea: Aphrophoridae and Cercopidae), which are less important vectors in America, occur in Europe and some, such as *Philaenus spumarius*, are very common, but are generally associated with herbaceous plants. Since, apart from *P. spumarius*, potential European native vectors have been very poorly studied so far (Lopes et al., 2014), their role in spreading *X. fastidiosa* is difficult to assess.

Figure 3 shows the species with the highest potential for X. fastidiosa spread, based on three criteria: polyphagy, abundance and frequency in different environments.

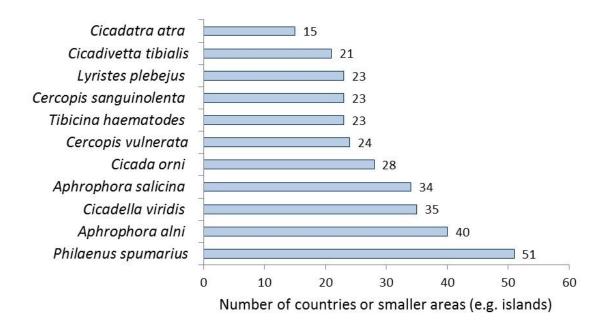


Figure 3. Reported presence of the most widespread species of xylem fluid feeders in Europe (from Fauna Europaea; de Jong, 2013)

As stated earlier, cicadas are xylem-fluid feeders and are also expected to be potential vectors, although their role in *X. fastidiosa* transmission is still unclear. In Italy, 18 species of cicadas are known, in the families Cicadidae and Tibicinidae, while 53 species are reported in Europe, most having a very restricted area of distribution (de Jong, 2013). Based on the two reports of cicadas as vectors of *X. fastidiosa* (Paiaõ et al., 2002; Krell et al., 2007), it is considered that the potential role of cicadas as vectors of *X. fastidiosa* in Europe to be of high relevance (although the uncertainty is high), owing to the large populations of cicadas, particularly in southern EU regions, in addition to the wide host range of plant species utilised by these insects.

Table 3 show the most important potential insect vector species in the EU and their distribution. It should be noted that, whereas the sharpshooters in America overwinter as adult and when infected can maintain *X. fastidiosa* during winter, the European sharpshooters (Cicadellidae, Cicadellinae) and most of the European spittlebugs (Aphrophoridae, with the exception of a few Cercopidae) overwinter as egg (Nickel and Remane, 2002) and, therefore, if infected, cannot sustain overwintering of *X. fastidiosa*, since transovarial transmission of *X. fastidiosa* does not occur (Freitag, 1951).

 $Table\ 3.\ Current\ and\ potential\ vector\ species\ of\ X.\ fastidiosa\ in\ Europe:\ main\ insect\ groups\ and\ most\ important\ potential\ vector\ species$

Insect group	Most common species	Distribu tion	Potential role as vector	Potential role as vector: criteria
Sharpshooters	Cicadella viridis	All	Moderate to	Very common, wide
(Cicadellidae,	(Linnaeus 1758)	Europe	high	host range but
Cicadellinae): seven		1		hygrophilous
species				
Spittlebugs	Aphrophora alni	All	Moderate to	Common, wide host
(Cercopoidea): 34	(Fallen 1805)	Europe	high	range
species	Aphrophora	All	Moderate	Common, oligophagous
	salicina (Goeze 1778)	Europe		
	Philaenus	All	High	Very common and
	spumarius (L.)	Europe		abundant in diverse
				ecosystems
				Identified as a vector in
				Apulia (Saponari et al., 2014)
	Cercopis	Not	Moderate	Many host plants but
	vulnerata Rossi	present		mainly associated with
	1807	in		herbaceous plants
		northern		
		Europe		
Cicadas	Cicada orni	Not	Doubtful	Missing information on
(Cicadoidea): 54	Linnaeus	present		transmission capacity
species		in		
		northern		
		Europe		
	Cicadatra atra	Balkans,	Doubtful	Missing information on
	(Olivier)	Italy and		transmission capacity
		France		
	Lyristes plebejus	Not	Doubtful	Missing information on
	(Scopoli)	present		transmission capacity
		in		
		northern		
		Europe	5 1.01	3.5
	Cicadivetta	Not	Doubtful	Missing information on
	tibialis (Panzer)	present		transmission capacity
		in		
		northern		
	Tibicina	Europe	Doubtful	Missing information and
		Not	Doubliui	Missing information on
	haematodes	present in		transmission capacity
	(Scopoli)	northern		
		Europe		
		Europe		

7.3. Conclusion about vectors

All xylem fluid-feeding insects in Europe should be regarded as potential vectors, but some species are more likely candidate vectors, owing to their wide geographical distribution, abundance and host plant range. Members of the families Cicadellidae, Aphrophoridae and Cercopidaeare are vectors in the Americas and, hence, all members of these three families should be considered as potential vectors in Europe. With regards to the reports previously mentioned (Paiaõ et al., 2002; Krell et al., 2007), the Cicadidae and Tibicinidae should also be considered potential vectors. *P. spumarius* has been shown to transmit the local strain of *X. fastidiosa* to an indicator plant, *Catharanthus roseus* (Saponari et al., 2014). A preliminary report indicates that *P. spumarius* also transmits the local strain of *X. fastidiosa* to olive (Cornara and Porcelli, 2014; Martelli, 2014). Figure 4 shows the spatial distribution of this most important potential vector species of *X. fastidiosa* throughout Europe.

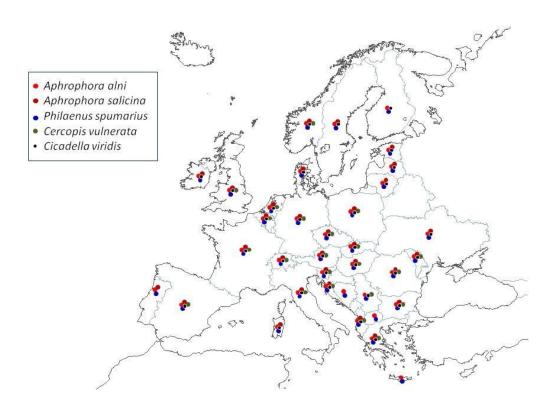


Figure 4. Reported presence in Europe of the most important potential vector species of X. fastidiosa (data from http://www.faunaeur.org; de Jong, 2013)

8. Pathogen effects

8.1. Negative effects on crop yield and/or quality to cultivated plants

The impact of *X. fastidiosa* on crops in the Americas is variable, depending on host plant, geographical region, epidemiological constraints and management options. The yield of most infected symptomatic plant species is negligible or not commercially acceptable; plants often die within years of infection

Grapevine production in the south-eastern USA (e.g. Florida, Georgia) is considered to be economically unfeasible because *X. fastidiosa* is endemic and experimental vineyards are destroyed within years of planting (Anas et al., 2008). In California, on the other hand, grapevine production is differentially affected in different regions, depending on vector ecology. In central California (e.g. Napa and Sonoma valleys), where an endemic vector occurs at low densities, losses are low but regular, while in southern California, a decade ago, prior to the widespread use of pesticides to control the invasive vector *H. vitripennis*, *X. fastidiosa* caused the collapse of the local wine industry.

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.

In Brazil, approximately 40 % of 200 million citrus plants in Sao Paulo State show disease symptoms due to infection with *X. fastidiosa* (Almeida et al., 2014). There, small growers have been eliminated from the industry, orchards are replanted more frequently because of *X. fastidiosa* infections and the increased costs of controlling vector populations and surveying for vectors and symptomatic plants have substantially changed the Brazilian citrus industry. Economic losses due to tree removal alone are estimated to be very severe (Bove and Ayres, 2007). However, in the case of the citrus industry in Brazil, it is difficult to discern the economic impact of citrus variegated chlorosis, caused by *X. fastidiosa*, from that of citrus greening, caused by *Liberibacter* spp.

In Argentina, the disease killed 500 000 plum trees between 1935 and 1940 and was therefore considered to be a plague of national importance (http://www.agromeat.com/156985/inta-y-senasa-detectaron-la-bacteria-xylella-fastidiosa-en-olivos).

8.1.1. Magnitude of the negative effects on crop yield and/or quality of cultivated plants in the infected area of Salento (Lecce province) in the absence of control measures.

Preliminary studies conducted in the infected area of Salento showed that the local strain of *X. fastidiosa* (CoDiRO strain, subspecies *pauca*) can infect, besides olive, stone fruits like almond and cherry, oleander and some other ornamentals (Saponari et al., 2013, 2014b). In contrast, *X. fastidiosa* has not been detected from citrus and grapevine, and until now preliminary transmission experiments have consistently failed

to infect citrus and grapevine (Maria Saponari, CNR, Bari, Italy, and Donato Boscia, CNR—Institute for Sustainable Plant Protection, personal communication 2014). In the absence of control measures in the infected area of Salento, the negative effects on crop yield of olive are dramatic, as documented by the extended area with olive dieback. Although almond and cherry orchards are of of less importance than olive in Salento, these crops are more economically important in other areas. Other known hosts of the local strain of *X. fastidiosa* are of landscape value, and therefore *X. fastidiosa* is also an important threat to these ornamentals. The populations of the known vector, *P. spumarius*, are locally very high, and therefore there is a much higher risk of continuous epidemic spread of the disease to the susceptible host plants with dramatic damages to olive orchards and to landscape ornamental species. Olive is a very important landscape tree in the area, in addition to being an economically important crop, and therefore a massive negative impact on the Salento landscape is expected.

8.2. Environmental effects

There has been identified two different categories of environmental consequences: the direct and indirect impact on the host plants themselves, and the indirect impact caused by the control methods implemented against the disease, in particular insecticide treatments.

Most of the *X. fastidiosa* diseases studied affect agricultural crops, but some forest trees are also affected (Sinclair and Lyon, 2005). In some areas, it is no longer possible to grow some host plants, e.g. grapevine in southern Florida, because of the intensity of the disease. The floristic composition of some cultivated, semi-natural or natural landscapes is thus likely to change, as well as the associated faunistic composition, leading to wide ecosystemic, agricultural and socio-economic consequences. A change of crop is likely to modify the historical and cultural image of the land, as well as the local economic activity in a very broad sense (agriculture, agro-industry, trade, tourism).

The intensive use of insecticide treatment to limit the disease transmission and control the insect vector may have direct and indirect consequences for the environment by modifying whole food webs with cascading consequences, and hence affecting various trophic levels. For example, the indirect impact of pesticides on pollination is currently a matter of serious concern (EFSA, 2013b). In addition, large-scale insecticide treatments also represent risks for human and animal health.

9. Control methods

With regard to control measures, although there are some ongoing research lines, there is not yet an effective control method of the pathogen applicable in the field. Control of *X. fastidiosa* is therefore currently achieved by removing sources of inoculum, using healthy plant propagation material and controlling the vector(s).

Use of resistant or tolerant varieties to *X. fastidiosa* can also play an important role in the disease management. Each genotype of *X. fastidiosa* is different in terms of host range (whereas the general biology of the bacterium will remain the same), therefore host range of a new genotype cannot be derived from literature. However, due to vector preference for host plants, there will be a difference between the artificial host range inferred from laboratory studies and the actual host range determined by vectors for a given strain and region.

10.Conclusion

X. fastidiosa presents a risk to the EU territory because it has the potential to cause diseases in the risk assessment area once it establishes, as hosts are present and the environmental conditions are favourable. X. fastidiosa may affect several crops in Europe, such as citrus, grapevine and stone fruits (almond, peach, plum), but also several tree species and ornamental plants, such as oak, sycamore and oleander. X. fastidiosa has a very broad host range, including many cultivated and spontaneous plants common in Europe.

There is some host differentiation among the generally accepted four subspecies of *X*. *fastidiosa* with regard to symptomatic hosts, but many plants could be infected and remain asymptomatic. There is, however, high uncertainty with regard to the potential host range of *X*. *fastidiosa* in the European flora as a range of European wild plant species have never met the bacterium and it is not known if they would be hosts, and symptomatic or asymptomatic. In addition, there is limited published information on the biology of *X*. *fastidiosa* subspecies that have been recently described. The biology of these subspecies is not yet fully understood. The impact of *X*. *fastidiosa* in forest is more difficult to assess owing to a general lack of data.

All xylem fluid-feeding insects in Europe are considered to be potential vectors. Members of the families Cicadellidae, Aphrophoridae and Cercopidae are vectors in the Americas and, hence, should also be considered as potential vectors in Europe. The Cicadidae and Tibicinidae should also be considered to be potential vectors. However, there are uncertainties with regards to their potential contribution to an epidemic in Europe.

The environmental conditions required for establishment are met in many places, as demonstrated by the detection of *X. fastidiosa* in Apulia, Italy. There is a potential for consequences in the EU territory, as shown by the impact on olive in Apulia and as illustrated by the impact of Pierce's disease in California and citrus variegated chlorosis in Brazil.

X. fastidiosa is present in Europe with a distribution restricted to part of the Lecce province in the Italian region of Apulia and is under official control.

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