Is *Xylella fastidiosa* a serious threat to European forests?

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The recent emergence of Olive Quick Decline Syndrome in Italy, caused by *Xylella fastidiosa*, has drawn attention to the risks posed by this vector-borne bacterium to important crops in Europe (especially fruit trees and grapevine). Comparatively very little is known on actual and potential impacts of this pathogen in forests, in the native (North American) and introduced (European) regions, respectively. The present review aims to address important questions related to the threat posed by *X. fastidiosa* to European forests, such as the following: What are the symptoms, hosts and impact of bacterial leaf scorch caused by *X. fastidiosa* on trees in North America? Which forest tree species have been found infected in the introduction area in Europe? How does *X. fastidiosa* cause disease in susceptible hosts? Are there any *X. fastidiosa* genotypes (subspecies and sequence types) specifically associated with forest trees? How is *X. fastidiosa* transmitted? What are the known and potential vectors for forest trees? How does vector ecology affect disease? Is the distribution of *X. fastidiosa*, especially the strains associated with trees, restricted by climatic factors? Is disease risk for trees different in forest ecosystems as compared with urban settings? We conclude by pointing to important knowledge gaps related to all these questions and strongly advocate for more research about the *Xylella*-forest pathosystems, in both North America and Europe.

Introduction

The recent epidemics of Olive Quick Decline Syndrome (OQDS or CoDiRO) in Apulia, southern Italy, has attracted much attention to *Xylella fastidiosa* (Wells *et al.,* 1987) in Europe (Saponari *et al.,* 2013; Almeida, 2016). This bacterium, previously only known from the Americas, its putative area of origin, has a very wide host range, reaching 650 plant species in 82 different plant families (EFSA, 2018a, b). In addition to olive trees, it is considered a major threat to fruit crops such as grapevine, citrus, coffee, peach or almond (Rapicavoli *et al.,* 2018; Sicard *et al.,* 2018; EFSA, 2018b, 2019). Comparatively very little is known about *X. fastidiosa* on forest trees, although the seminal work leading to the first description of the bacterium included strains isolated from American elm (*Ulmus americana* L.), American sycamore (*Platanus occidentalis* L.) and northern red oak (*Quercus rubra* L.). These trees showed symptoms of what is now known as bacterial leaf scorch (BLS) of shade and landscape trees (Wells *et al.,* 1987; Lashomb *et al.,* 2002; Sinclair and Lyon, 2005; Sherald, 2007; Harris and Balci, 2015).

Historically, *X. fastidiosa* was first associated with Pierce's disease (PD) of grapevine, a decline disease that has caused considerable damage in American vineyards since the late 19th century. The etiology of the disease remained unexplained for almost one century until the causal bacterium could eventually be cultured (Davis *et al.,*1978). It was later shown that strains causing PD formed a homogeneous group with other insect-vectored xylem-limited bacteria that had similar morphology and nutritional requirements (e.g. difficult to grow on synthetic media, hence 'fastidiosa') and were associated with a range of diseases on various hosts, such as phony disease of peach or leaf scorch of almond. Based on DNA hybridization studies and serological and biochemical analyses, these bacteria were described as a new species in a new genus (Wells *et al.,* 1987). *Xylella fastidiosa* was the first non-viral plant pathogen to have its complete genome sequenced (Simpson *et al.,* 2000). Extensive samplings and phylogenetic analyses in the past decades have shown that *X. fastidiosa* is a genetically diverse species, now split into several subspecies (Denancé *et al.,* 2019; see https://www.bacterio.net/ for updated taxonomy). Most *X. fastidiosa* diseases

have been reported from regions with Mediterranean, tropical and sub-tropical climates (EFSA, 2018a, b, 2019; EPPO, 2019). However, the BLS of trees has been primarily reported through out the Eastern half of the US, including Northeastern areas with cold winters (Lashomb *et al.,* 2002).

The catastrophic OQDS epidemics in Apulia prompted intensive surveys in Italy and other European olive-producing countries. These surveys revealed the presence of *X. fastidiosa* in mainland Spain and the Balearic Islands, another region of Italy (Tuscany), Portugal (as transient), Germany (outbreak considered as eradicated) and France, on various plant species (EPPO, 2019; EFSA, 2019). For example, a large diversity of plants, including some trees (*Acer pseudoplatanus* L., *Quercus ilex* L. and *Q. suber* L.) was found infected in natural settings in the island of Corsica (Denancé *et al.,* 2017). While only one strain was associated with OQDS and found in other host plants in Apulia (Loconsole *et al.,* 2016), different strains belonging to different sub-species were later found in Tuscany (Saponari *et al.,* 2019), France and Spain, suggesting multiple introduction events (Denancé *et al.,* 2017; Landa *et al.,* 2020). The date of introduction of *X. fastidiosa* into Corsica was estimated to be either 1985 or 2001, according to different epidemiological models (Soubeyrand *et al.,* 2018). The pathogen is therefore well established in Europe, at least in Corsica, Southern Italy (Strona *et al.,* 2017), and the Balearic Islands (Moralejo *et al.,* 2019).

So far, *X. fastidiosa* has been mainly seen as a threat to agriculture and horticulture due to the severe diseases it causes on high-value fruit crops (EFSA, 2015, 2019) and, to a lesser extent, to ornamental plants and amenity trees (Lashomb *et al.,* 2002; Baldi and Porta, 2017). No serious impact of *X. fastidiosa* has been reported in forests in its native range. However, the impact of introduced strains of *X. fastidiosa* in new areas could not have been predicted from what is known in other areas, as illustrated by the emergence of OQDS in Italy while little damage had been reported on olive trees in California (Krugner *et al.,* 2014). The introduction and establishment of *X. fastidiosa* in some areas in Europe therefore raises the question of its potential future impact on European forests. In complement to previous general risk assessments (EFSA, 2015, 2018b, 2019), the present review aims to provide a thorough assessment and discussion of the specific risk posed by *X. fastidiosa* in forest settings and includes (1) an up-to-date, comprehensive summary of what is known about *X. fastidiosa* on forest trees, both in its native area (BLS disease in North America) and in Europe; (2) a synthesis of the current understanding of the ecology, biology and genetics of *X. fastidiosa* and the epidemiology of diseases it causes, acquired from other pathosystems and most relevant to assess the threat to European forests and (3) the identification of knowledge gaps regarding the threat posed by *X. fastidiosa* to European forests, some practical recommendations and suggestions for future research.

Vulnerability of forest tree species

What are the symptoms of BLS of forest trees in North America?

In North America, *X. fastidiosa* has been associated with the disease referred as BLS of shade and landscape trees (Sinclair and Lyon, 2005). The attribution of the symptoms to a bacterium similar to that causing grapevine PD was proposed for the first time in 1980, based on microscopic and immunological observations of symptomatic oak, elm and sycamore leaves (Hearon *et al.,* 1980; Sherald, 2007). Koch's postulates were later fulfilled on red oaks (Chang and Walker, 1988). The symptoms of BLS typically occur as foliar burns or scorches, affecting the margin and interveinal areas of the leaf blade. Quite often, the necrotic area is separated from the green part by a yellow- or red- coloured halo (Hammerschlag *et al.,* 1986; Gould and Lashomb, 2007) (Figure 1). The symptoms may be confused with drought effects or early senescence, which may explain why these symptoms were previously attributed to abiotic or viral causes (Hammerschlag *et al.,* 1986; Sherald, 2007; Purcell, 2013).

Leaf symptoms in susceptible species usually appear in late summer and increase during the fall. In pin oak (*Quercus palustris* Muenchh.), symptoms appear earlier than in other oak species and early defoliation of infected trees is observed (Harris *et al.,* 2014). BLS symptoms first appear in a single branch or part of the crown and then spread to the entire crown in several years (Hammerschlag *et al.,* 1986; Gould and Lashomb, 2007; Sherald, 2007) (Figure 1). Once infected the parts of the trees do not recover (Hammerschlag *et al.,* 1986). Positive detection of the bacterium in a tree is frequently associated with positive detection the following year (Barnard *et al.,* 1998; Harris *et al.,* 2014). Branches infected with the bacterium generally have a later budburst, with smaller leaves than in healthy trees (Zhang *et al.,* 2011). The list of symptoms may also include decrease in fruit production, premature leaf abscission, stunting or reduced growth, decline in vigour, dieback and eventual death (Hartmann *et al.,* 1995; Sinclair and Lyon, 2005; Barnard, 2007). Most reports of crown dieback associated with *X. fastidiosa* relate to pin oak and northern red oak, *Q. rubra* (Harris *et al.,* 2014). The spatial distribution of the disease in an area seems random, with some apparently healthy trees co-existing with trees showing severe symptoms (Gould and Lashomb, 2007).

In oaks, symptoms of BLS are close to those of wilting caused by *Bretziella* (*Ceratocystis*) *fagacearum* (Bretz) Z.W. de Beer, Marinc., T.A. Duong and M.J. Wingf in North America ("oak wilt"). However, in the latter case, they appear earlier in the season and tree wilt occurs in a few months (Gould and Lashomb, 2007), while decline of BLS-infected trees occurs over several years, often lasting 5 to more than 10 years (Hartman *et al.,* 1995). In addition, unlike with oak wilt, trees with BLS do not exhibit brown or black stripes in the functional xylem (Sherald, 2007). Leaves with browning (BLS) often remain on the tree (Sherald and Kotska, 1992; Sinclair and Lyon, 2005).

Which forest tree species are affected in the native area (America)?

BLS disease of trees was reported mostly in association with native American species of oak, elm and sycamore (Lashomb *et al.,* 2002). Oaks appear as an especially susceptible group, with at least 18 oak species reported as natural hosts in the *Lepidobalanus* (white oaks) and *Lobatae* (red oaks) sections of the *Quercus* genus (Gould and Lashomb, 2007). The latter are the most frequently reported as affected by the disease (Barnard, 2007), in particular pin oak and northern red oak (Sherald, 2007;

Figure 1 Symptoms of BLS on trees: close-up of leaf symptoms with the typical BLS discoloration on northern red oak (top left), elm (middle left), *Ginkgo biloba* (top right); general view of infected trees (pin oaks) showing early leaf senescence and drop (bottom). This figure appears in colour in the online version of *Forestry*.

Mc Elrone *et al.,* 2008; Harris and Balci, 2015). Disease reports on oaks come from many states in the eastern half of the US: Florida, South and North Carolina, Tennessee, Oklahoma, Kentucky, Missouri, Indiana, Pennsylvania, New Jersey, New York and Washington DC (Sherald and Kotska, 1992; Hartman *et al.,* 1995; Sinclair and Lyon, 2005; Sherald, 2007; Nunney *et al.,* 2013; Harris *et al.,* 2014; Overall and Rebek, 2015). There is no mention of the disease on oaks in California, although "oak-type isolates"

(according to the terminology of Nunney *et al.,* 2013; see below) are detected in other trees. Although most of the reports of *X. fastidiosa* in the US referred to native tree species, Nunney *et al.* (2013) isolated a strain of *X. fastidiosa* from the European English oak (*Q. robur* L.) in Kentucky. The disease was described in other broadleaf trees, such as mulberry (*Morus* spp.), red maple (*Acer rubrum* L.) or California buckeye (*Aesculus californica* (Spach) Nutt.) (Sinclair and Lyon, 2005). A case of very high incidence of BLS was reported on London plane trees (*Platanus x acerifolia* (Aiton) Willd.), hybrids between the American sycamore *P. occidentalis* and the Eurasian oriental plane *P. orientalis* L., in North Carolina (Lashomb *et al.,* 2002). Other broadleaved trees showing BLS symptoms and found infected by *X. fastidiosa* include ash (*Fraxinus* spp.) (McGaha *et al.,* 2007; Nunney *et al.,* 2013), *Alnus rhombifolia* Nutt., *Carya* spp., *Cercis* spp., *Juglans* sp. (Wong *et al.,* 2004; Hernandez-Martinez *et al.,* 2007; Yuan *et al.,* 2010; Melanson *et al.,* 2012; Nunney *et al.,* 2013) as well as *Fagus crenata* Blume, as bonsai (Huang *et al.,* 2003). *Salix* sp. and *Eucalyptus* sp. were reported as positive for *X. fastidiosa* but description of symptoms is missing (EFSA, 2018a). The pathogen was reported as infecting a few gymnosperm species (Wong *et al.,* 2004; McGaha *et al.,* 2007), but these reports were not confirmed. In general, conifers are considered as non-hosts (EFSA, 2019) and might be resistant to *X. fastidiosa* because they lack the xylem vessels that the pathogen typically colonizes (McGaha *et al.,* 2007).

The complete list of plant species naturally infected by *X. fastidiosa,* for which both symptoms and positive detection of the bacterium were recorded, can be found in the EFSA database (EFSA, 2018; Delbianco *et al.,* 2019). This database categorizes *Xylella* host plant species based on the number and type of detection methods applied for each finding.

What is the impact of Xylella fastidiosa in forests in the native area?

Regardless of the tree species involved, infected trees do not die immediately but tree life is shortened and the aesthetic quality may be significantly reduced (Sherald and Kostka, 1992; Hartman *et al.,* 1995; Gould and Lashomb, 2007). This aesthetic impact may provide a clue to interpret the reason why BLS on trees is mainly mentioned in urban areas, on ornamental trees (Sherald, 2007; Zhang *et al.,* 2011; Harris *et al.,* 2014). In general, affected trees may decline to the point where they must be removed (Hearon *et al.,* 1980; Hartman *et al.,* 1995). Damage to ornamental trees can be significant (Harris and Balci, 2015). In some cities in New Jersey, US, 30–35 per cent of red oaks showed symptoms (Gould and Lashomb, 2007). The loss of value and the amount of replacement costs for older trees affected by this disease were estimated at $8,000 per tree (Gould and Lashomb, 2007). In addition, *X. fastidiosa* has been reported to cause typical symptoms of leaf scorch on red oak and box-elder (*Acer negundo*) in a commercial nursery in Maryland (Huang, 2007), indicating that the disease may occur in nurseries and may affect the production and trade of plants intended for planting.

While most of the disease reports refer to urban areas and amenity trees, symptoms of BLS were also detected in severely declining oak trees (*Quercus spp.*) in several state forests in Delaware and New Jersey since the early 2000s (Griffiths, 2013). Hopkins and Purcell (2002) deemed that BLS could

have a significant environmental and economic impact in the southeastern US. However, only one relatively old study in Florida suggested the presence of *X. fastidiosa* in both natural and urban environments on eight oak species, particularly on turkey oak (*Q. laevis)*, the most frequent species in the area (Barnard *et al.,* 1998). Nunney *et al.* (2013) reported that liquidambar is severely infected in urban areas of California, while there is no mention of symptoms in its natural range in the southeastern US. Whether trees growing in urban areas may be more susceptible to the disease than trees in the forests remains an open question (see some hypotheses in a following section). One hypothesis could be an under-estimation of the impact of *X. fastidiosa* in forests. Despite a few characteristics, the symptoms can be easily confused with other foliar browning agents or factors and a reliable diagnosis can only be established on the basis of the detection of the bacterium. Molecular methods currently provide highly specific and sensitive ways to test a sample for *X. fastidiosa,* and have been continuously improved (Baldi and Porta, 2017; Supplementary data 1) but detection is complicated by the irregular distribution of the bacteria in the plant. The difficulties of diagnosis can be illustrated by results of a survey in the central US, where a very low detection rate of 5 per cent was obtained from trees apparently suffering from BLS (Adams *et al.,* 2013). Possible causes are both misdiagnosis (incorrect assignment of symptoms to *X. fastidiosa*) or false negatives (true infection but not detected, due to insufficient sensitivity of the method or to inadequate sampling).

Which forest tree species have been found infected in Europe?

In Apulia and Tuscany, small trees or shrubs typical of the Mediterranean maquis, such as Rhamnus or Cistus, were reported among hosts of *X. fastidiosa* (EFSA, 2018b; Saponari *et al.,* 2019). In an intensive survey of forestry plants growing in nurseries, public and private gardens potentially exposed to high inoculum pressure in the Salento peninsula (Apulia), none of the tested species were found to be infected by *X. fastidiosa* (Potere *et al.,* 2015).

In France, intensive samplings following the OQDS emergence in Italy led to the first report of *X. fastidiosa* in 2015 from Corsica on *Polygala myrtifolia* L. and some other plant species (Denancé *et al.,* 2017). The bacterium was later found in mainland France, on the French Riviera in a few urban areas. Since then, extensive surveys were conducted in these areas and more than 45 000 thousand samples of many different plant species were analyzed. The bacterium was detected in ~3 per cent of these samples, from 49 different plant species, including mainly shrubs but also a few forest trees, *Acer pseudoplatanus*, *Prunus avium* L., *Quercus suber* and *Q. ilex* (http://draaf.paca.agriculture.gouv.fr, Denancé *et al.,* 2017, Jacques unpublished results).

In Spain, especially in the Balearic Islands, 18 species have been recognized as hosts, including the forest tree species *Fraxinus angustifolia* Vahl., *Juglans regia* L., *Prunus avium* (EFSA, 2018b).

Finally, among the 96 forest tree species (either European native or not) with significant abundance in European forests according to pooled National Forest Inventories (Mauri *et al.,* 2017), 15 are listed as natural hosts of *X. fastidiosa* according

to the EFSA database, among which nine have been recorded as infected in Europe (Table 1). Some species affected by BLS in America and readily planted throughout Europe, such as *A. platanoides* L. or *Q. rubra*, were not found infected. However, *X. fastidiosa* is currently restricted to Mediterranean Europe where these species are not common.

Xylella fastidiosa pathogenicity

How does Xylella fastidiosa cause disease in susceptible hosts? A bacterium with various lifestyles

Historically, *X. fastidiosa* was primarily associated with devastating diseases on various important crops such as grapevine and citrus. However, studies on the epidemiology of these diseases have shown that *X. fastidiosa* can live as a harmless endophyte in many plant species (Gambetta *et al.*, 2007; Chatterjee *et al.*, 2008; Purcell, 2013; Roper *et al.*, 2019). This is especially the case in the putative native area of *X. fastidiosa* in America, where co-evolutionary processes with native plant species may be involved. For example, *Vitis rotundifolia* Michx (Muscadine grape), which can be infected by *X. fastidiosa* with only mild symptoms, is native to the southeastern region of North America and may have evolved resistance, contrary to *Vitis vinifera* L. (Ruel and Walker, 2006). Several authors suggested that the diseases caused by *X. fastidiosa* may be the exception rather than the rule, corresponding to encounters of particular bacterial genotypes with some host plants (Sicard *et al.*, 2018; Roper *et al.*, 2019). In many (most?) cases, *X. fastidiosa* appears as a plant commensalist.

In susceptible hosts, the bacterium exhibits different phenotypes during the course of infection. In the first phase upon inoculation of bacteria into the xylem vessels by vectors, bacterial cells are planktonic and move acropetally with the sap flow or make use of Type IV pili to circulate against the sap flow. During this systemic plant colonization phase or exploratory lifestyle, bacteria multiply and secrete the diffusible signaling factor (DSF) (Newman *et al.*, 2004). Accumulation of DSF up-regulates the secretion of adhesins enhancing cell stickiness and exopolysaccharide production, while genes involved in type IV pilus and other functions are down-regulated. Consequently, in a second phase, large bacterial biofilms are formed, contributing to vessel occlusion. The flow of xylem sap is blocked, symptoms may appear (after a latent period of several weeks to months), and *X. fastidiosa* cells can be efficiently acquired and retained by insect vectors. The balance between the two bacterial phenotypes (adhesive vs. non-adhesive) could be critical to explain commensalism or parasitism of *X. fastidiosa* in different host plants (Roper *et al.*, 2019). Late recognition of the pathogen by the plant immune system, when the bacterium has already colonized a large part of the xylem system, could explain why downstream plant reactions, especially formation of tyloses, are ineffective to block the pathogen and eventually lead to extensive vascular blockage and possibly death in susceptible plants (Roper *et al.*, 2019). Pathogenesis and virulence factors have been especially well studied in the case of PD of grapevine and were reviewed in Chatterjee *et al.* (2008), Rapicavoli *et al.* (2018) and Roper *et al.* (2019). Symptoms are generally thought to result mainly from water transport disruption after vessel occlusion by bacterial biofilms but also tyloses and gums produced by plants

in reaction to the infection. The effects of virulence factors are also likely involved (Ionescu *et al.*, 2014; Nacimento *et al.*, 2016; Gambetta *et al.*, 2018), as it was shown that grapevines infected with *X. fastidiosa* exhibit unique symptoms, not produced in water-deficit treatments (Thorne *et al.*, 2006).

Vascular colonization in forest trees

In several species of oak, American sycamore and red maple, microscopic studies showed an accumulation of bacteria in the vessels of petioles and leaf veins (Hearon *et al.*, 1980; Hartman *et al.*, 1995). In a study on red and pin oak, the intensity of leaf symptoms appeared to be positively related to ELISA values in tested foliage, thus presumably indicative of larger *X. fastidiosa* populations (Harris *et al.*, 2014). In red oak, *X. fastidiosa* was isolated from the sap of symptomatic tree branches, but only from August to January (Chang and Walker, 1988). The bacterium was also detected in some cases from asymptomatic parts of infected trees or from completely asymptomatic trees (Harris *et al.*, 2014). In sycamore, the bacterium was detected and isolated from sap collected from roots and stems almost all year round, with a generally higher frequency in stems (Henneberger *et al.*, 2004). The bacteria present in the conductive tissues of the branches were believed to colonize the petioles and veins of the leaves at the time of bud break (Chang and Walker, 1988; Henneberger *et al.*, 2004; Zhang *et al.*, 2011).

Mc Elrone *et al.* (2008) studied the functional and structural characteristics of xylem in different organs of both healthy and *X. fastidiosa*-infected trees of several American oak species. A high rate of embolism was observed in *X. fastidiosa* infected petioles, preceding a sharp decrease of hydraulic conductivity and occurrence of leaf symptoms in infected trees as season progressed. The occurrence of large pit membrane pores (greater than the diameter of individual *X. fastidiosa* cells) was demonstrated in several *Quercus* species and was suggested to contribute to the higher susceptibility to BLS of these species by facilitating systemic colonization of the secondary xylem.

Interaction of Xylella fastidiosa and other stressors in trees

Since *X. fastidiosa* infection in susceptible plants results in some dysfunction of the vascular system, potentially affecting tree's ability to respond to water demand, it is not surprising that Xylella infection may be a contributing factor of decline in addition to other stress factors, especially drought (McElrone *et al.*, 2003). Hammerschlag *et al.* (1986) even suggested that the presence of BLS was an indicator of stress and Barnard (2007) questioned the primary pathogenicity of the bacterium for oaks. Xylella infections may act as a predisposing or aggravating factor associated with other decline factors (Sherald and Kotska, 1992, Hartman *et al.*, 1995, Hanneberger *et al.*, 2004). Symptoms are generally expressed at the end of summer when water demand is the highest in plants (Chatterjee *et al.*, 2008). A significant association was observed between Xylella infection and decline in *Q. laevis*, where shoots on Xylella-positive trees were ~38 per cent shorter than those on paired Xylella-negative trees. However, the role of *X. fastidiosa* in oak decline was not firmly established and

Table 1 European forest tree species reported as natural hosts of *X. fastidiosa* according to the EFSA database (EFSA, 2018a; Delbianco *et al.,* 2019).

Genus	Species	Common name	Geographical origin	Natural infection	Xf subspecies	Comment
Acer	*A. platanoides* L.	Norway maple	EUR	USA, CAN	Multiplex	Reports on 7 other *Acer* species in USA (multiplex + one fastidiosa)
	A. pseudoplatanus L.	Sycamore maple	EUR	FR	Multiplex	
Aesculus	*A. hippocastanum* L.	European horse chestnut	EUR	CAN	Unknown	BLS report – not retained in EFSA database
Celtis	*C. orientalis* L.		EUR-As			Infections on *C. occidentalis* in the USA (multiplex)
Ficus	*F. carica*	Common fig	As	USA, FR, SP	Multiplex	Intercepted in Italy
Fraxinus	*F. angustifolia* L.	Narrow-leaved ash	EUR	SP	Multiplex	Reports on 3 other *Fraxinus* species in Canada and USA (multiplex)
Juglans	*J. regia* L.	Common walnut	EUR-As	SP	Fastidiosa	Reports on 2 other *Juglans* species in the USA (subsp unknown)
Liquidambar	*L. styraciflua* L.	American sweetgum	Am	USA	Multiplex	
Olea	*O. europaea* L.	Olive	EUR	USA, ARG, BRA, IT, SP FR	Multiplex, pauca	Mainly pauca in Europe, only multiplex in USA
Platanus	*P. orientalis* L.	Oriental plane	EUR			Numerous reports in USA on *P. occidentalis* (multiplex and 1 fastidiosa) BLS report on *P. x acerifolia* (London plane) not retained in EFSA database
Prunus	*P. avium* L.	Wild cherry	EUR-As	USA, IT, SP, FR	Fastidiosa, multiplex, pauca	Also numerous reports on *P. dulcis* in America, and Europe
	P. cerasifera Ehrh.	Cherry plum	EUR-As	USA, FR	Multiplex	
Quercus	*Q. ilex* L.	Holm oak	EUR	FR	Pauca	Very few positive samples
	Q. robur L.	Pedunculate oak	EUR	USA	Multiplex	Single report
	Q. rubra L.	Red oak	Am	USA	Multiplex	Numerous reports; also on other oak species
	Q. suber L.	Cork oak	EUR	FR	Multiplex	Single report
Ulmus	*U. glabra* Huds.	Scots elm	EUR	USA	Unknown	Numerous reports on other *Ulmus* species in USA

CAN = Canada, FR = France, SP = Spain, IT = Italy, ARG = Argentina and BRA = Brazil.

the authors also suggested a possible role of root rot caused by *Armillaria tabescens* Scop. (Barnard *et al.*, 1998).

Similarly, infection by *X. fastidiosa* on elms was suggested to predispose trees to attacks by elm bark beetles vectoring *Ophiostoma ulmi* (Buisman) Nannf. and *Ophiostoma novo-ulmi* Brasier, thereby favouring the establishment and spread of Dutch elm disease (Stipes and Campana, 1981). In a survey carried out in the District of Columbia, US, the disease was ~12 times more common in scorch-affected elms than in other elms. Furthermore, over 40 per cent of all cases of Dutch elm disease occurred in trees already affected by BLS (Sinclair and Lyon, 2005).

On American sycamore, leaf scorch is a chronic disease and it may take years before the occurrence of tree death (Gould and Lashomb, 2007). However, *X. fastidiosa*-infected sycamore trees may become attacked by secondary pathogens (e.g. Botryosphaeria spp.) causing cankers and xylem discoloration, eventually leading to death, similar to those caused by the vascular pathogen *Ceratocystis platani* (J.M. Walter) Engelbr. & T.C. Harr. (Britton *et al.*, 1998; Harrington, 2013). It should be noted that a number of Botryosphaeriaceae species have been recently identified in Europe in association with declining and dying London plane trees (Pelleteret *et al.*, 2017), suggesting that an interaction between *X. fastidiosa* and those fungal pathogens may be likely should *X. fastidiosa* spread to London plane trees in Europe.

In general terms, tree decline leading to death may occur as a result of the interaction between *X. fastidiosa* infection and other secondary pathogens and pests whose establishment or

activation is favoured by the weakening of the tree by *X. fastidiosa*, including root rot pathogens, endophytic pathogenic fungi and bark beetles.

Are there any Xylella fastidiosa genotypes (sub-species and sequence types) specifically associated with forest trees?

Since the description of *X. fastidiosa* by Wells and colleagues in 1987, only one other species, including strains causing leaf scorch of nashi pear tree in Taiwan, has been described in the genus (Su *et al.*, 2016). All other strains responsible for diseases on hundreds of different plant species share at least 95 per cent of DNA identity, satisfying the criteria for being placed in a unique species, *X. fastidiosa* (Denancé *et al.*, 2019).

The species has been split into several subspecies, based on both host-range and genetic/genomic data. Three subspecies are widely accepted: subsp. fastidiosa, subsp. multiplex, both taxonomically valid, and subsp. pauca (not yet formally valid in the absence of a type strain deposited in two international collections). Other subspecies have been proposed and this classification is not yet fixed (Almeida and Nunney, 2015; Denancé *et al.*, 2019). The subspecies fastidiosa includes the agent of PD on grapevine but also strains causing almond, alfalfa and maple leaf scorches. The subspecies pauca groups strains responsible for the olive decline in Italy and also strains associated with Citrus variegated chlorosis (CVC) and coffee leaf scorch (Almeida *et al.*, 2008). The subspecies multiplex has the widest host range (Nunney *et al.*, 2013). It has been associated with almond leaf scorches and most of the documented reports on forest trees (cf Table 1; Schuenzel *et al.*, 2005; Barnard, 2007; Nunney *et al.*, 2010). Within each sub-species, sequence types (ST) were further identified based on the polymorphism of seven housekeeping genes (Yuan *et al.*, 2010). A total of 87 STs have been described so far that include 39 STs within the multiplex subspecies (http://pubmlst.org/xfastidiosa/ last updated: 3 June 2019).

Only subspecies multiplex has been found in native American trees (Nunney *et al.*, 2013), with the exception of one report on maple (Acer sp.) associated with subsp. fastidiosa (Yuan *et al.*, 2010). Within subspecies, the typing of isolates from various hosts and locations in the US provides only limited evidence of strong host specificity. In a study with 143 isolates collected from various plants in California, Texas, Tennessee, Kentucky, Florida and Georgia, Nunney *et al.* (2013) showed a fairly high diversity with 32 STs identified within the subspecies multiplex. Most STs were isolated from several host species belonging to different botanical families. A cluster of strains (ST 08, 09, 24, 24, 37, 38, 39, 44, 45, 49, 51), described as the "oak type", were mainly associated with oaks and some other indigenous tree species (liquidambar, plane and elm), by contrast with two other clusters ("peach type" and "almond type") associated with fruit trees (almond, olive, peach, plum, apricot). Strains associated with oaks belonged to four STs (ST08, ST09, ST23 and ST44), but only two of these STs (ST09 and ST44) were strictly associated with oaks. Harris and Balci (2015) also found ST09 associated with oaks (*Q. palustris*, *Q. rubra*, *Quercus coccinea*, *Quercus phellos*), both in symptomatic and non-symptomatic tissues, in Washington DC. A few studies with reciprocal inoculations suggested that strains isolated from a given host are more aggressive on

plants on the same species than on other species. This was shown for *X. fastidiosa* subsp. multiplex strains isolated from either pecan or sycamore that failed to induce strong symptoms on the other host, although the strains were phylogenetically close (Sanderlin, 2017). Recently in an extensive study, Nunney *et al.* (2019) showed that *X. fastidiosa* subsp. multiplex strains are generally specialized on their host of isolation while some hosts (plum and almond) can be infected by a large range of strains of this subspecies. Interestingly, two strains presenting the same ST displayed different host ranges, possibly explained by the occurrence of specialized genotypes within the same ST. Recently, it has been suggested that STs, at least ST6, may not be monophyletic (Landa *et al.*, 2020).

None of the "oak type" strains (sensu Nunney *et al.*, 2013) of subspecies multiplex associated with native American forest trees have been reported in Europe so far, despite extensive sampling campaigns organized in the *X. fastidiosa*-infected countries, mostly on symptomatic plants (EFSA, 2018a, b, 2019). However, it should be noted that the ST7-Griffin-1 strain isolated from a red oak in Georgia, US (Chen *et al.*, 2013) was also isolated in Spain and in France. In Italy, the causal agent associated with OQDS in Apulia is *X. fastidiosa* subsp. pauca ST53 strain. The strain detected in Tuscany belongs to multiplex ST87 (Saponari *et al.*, 2019). In Spain, the three main subspecies, i.e. fastidiosa, pauca and multiplex, have been detected, with one (ST1), one (ST80) and three STs (ST6, ST7 and ST81), respectively (EFSA, 2018a, b ; Landa *et al.*, 2020). ST6, ST7 and ST79 of subsp. multiplex, ST53 of subsp. pauca and ST76 of subsp. fastidiosa were also found in France (Denancé *et al.*, 2017).

It is remarkable to note that strains identified in Europe show a wider host range than usually reported in the native area for a single ST (Nunney *et al.*, 2013). For example, ST53 has been detected on 34 plant species in Italy (Saponari *et al.*, 2019), not including *Q. ilex* which was found infected by this strain in France (Denancé *et al.*, 2017). The ST6 and ST7 strains have been reported on 54 plant species, including some forest tree species (*Acer pseudoplatanus*, *Prunus avium* and *Q. suber*) in France (Denancé *et al.*, 2017). Several tree species were found infected by *X. fastidiosa* subsp. multiplex ST81 in Spain, including almond, olive and wild olive, plum and fig tree (*Ficus carica* L.), narrow-leafed ash (*Fraxinus angustifolia*), and acacia (Acacia sp.) (EFSA, 2018b).

The basis for host specificity of *X. fastidiosa* strains, if any, is still mostly undetermined, whether it is an interaction with the host plant or vector genotype (Harris and Balci, 2015). Recombination, within and among subspecies, is recognized to be the major driver of diversity in *X. fastidiosa*, potentially facilitating shifts to novel host plants (Nunney *et al.*, 2013, 2014; Gambetta *et al.*, 2018; Denancé *et al.*, 2019; Vanhove *et al.*, 2019; Landa *et al.*, 2020). The subspecies multiplex appears as the lineage that is most prone to recombination (Vanhove *et al.*, 2019). For example, the emergence of coffee and citrus diseases in South America has been suggested to be associated with new pathogenicity acquired by the endemic subspecies pauca via inter-subspecific homologous recombination (IHR) with *X. fastidiosa* subsp. multiplex after the introduction of the latter in the region (Nunney *et al.*, 2012). The ST53 lineage of subspecies pauca that is responsible for the outbreak of OQDS in southern Italy also shows evidence of IHR, with subspecies fastidiosa (Vanhove *et al.*,

2019). The evolutionary history of *X. fastidiosa* is also driven by the acquisition of genetic material from heterologous origin, such as plasmids from other bacterial species or genomic regions of bacteriophage origin (Denancé *et al.*, 2019). These horizontal transfers may also contribute to adaptation, including to new hosts.

Transmission of *Xylella fastidiosa* by insect vectors

How is X. fastidiosa transmitted?

Any insect feeding predominantly from xylem is considered a potential vector of *X. fastidiosa* (Almeida, 2016). Upon acquisition from an infected xylem vessel, bacterial cells are retained into the vector foregut. No latent period is required (or less than 1 h) between acquisition and transmission to another plant (Purcell and Finlay, 1979; see also Severin, 1950; Purcell *et al.*, 1979; Hill and Purcell, 1995; Esteves *et al.*, 2019 for more details on transmission). Low bacterial populations inside the vector foregut, even below detection thresholds (100 CFU/insect head), are enough for efficient inoculation to occur (Hill and Purcell, 1995; Cornara *et al.*, 2016). However, acquisition efficiency by vectors is likely favoured by high bacterial load in the source plant (Almeida *et al.,* 2005). High numbers of infective individuals feeding on a plant increase the transmission probability and reduce the incubation time for symptoms appearance (Daugherty and Almeida, 2009; Cornara *et al*., 2018, 2019). Transmission is neither transstadial, i.e. the pathogen is lost at each molt (Purcell and Finlay, 1979), nor transovarial, i.e. from parent to offspring (Freitag, 1951).

What are the known and potential vectors of Xylella fastidiosa on trees?

Insects that feed predominantly from xylem vessels belong to two superfamilies, i.e. Cercopoidea (froghoppers and spittlebugs) and Cicadoidea (cicadas), and one subfamily, i.e. Cicadellidae Cicadellinae (sharpshooters) of the order Hemiptera (Novotny and Wilson, 1997).

Most of the knowledge on *X. fastidiosa* epidemiology and vector–bacterium relationship comes from studies carried out on PD epidemics in California, and CVC in Brazil; in both cases, sharpshooters play the key role in *X. fastidiosa* natural spread (Rapicavoli *et al.*, 2018). A few studies have considered potential vectors of *X. fastidiosa* in trees in North America. The bacterium was detected in seven leafhopper species captured on elm and oak, some of which were known as vectors of *X. fastidiosa* in other hosts, and three species of treehopper, not known to be competent vectors (Sherald, 2007). Zhang *et al.* (2011), considering both insect infection and abundance, suggested that the sharpshooter *Graphocephala versuta* Say. could be the main species involved in BLS spread in oaks in central New Jersey. This species has also been putatively associated with the spread of *X. fastidiosa* in Oklahoma forest nurseries (Overall and Rebek, 2015). Unfortunately, no transmission tests to forest trees to determine vectors' competence and efficiency have been performed, and most of the assumptions on BLS natural spread are based on identification of candidate vectors, estimates of their

Figure 2 Meadow spittlebug, *Philaenus spumarius*: on the top, typical nymph spittle; on the bottom, adult insect. This figure appears in colour in the online version of *Forestry*.

relative abundance, and molecular detection of *X. fastidiosa* in the insects. However, detection of *X. fastidiosa* in an insect does not necessarily determine that the insect will be a vector. For example, some phloem feeders may occasionally ingest the bacterium but they are unable to transmit it (Purcell, 1980; Cavalieri *et al.*, 2019).

While at least in North America some preliminary indications about possible vectors of *X. fastidiosa* to forest trees are available, very little is known regarding possible vectors in Europe. Few sharpshooter species are present in Europe and spittlebugs are deemed to play the key role in *X. fastidiosa* natural spread in European ecosystems (EFSA, 2015; Almeida, 2016; Cornara *et al.*, 2019). The meadow spittlebug *Philaenus spumarius* L. (Figure 2) is currently considered the only significant vector of *X. fastidiosa* across all the European outbreaks detected so far (Cornara *et al*., 2017a, b; Cruaud *et al.*, 2018; Moralejo *et al.*, 2019). Two other species, *Neophilaenus campestris* Fallén and *P. italosignus* Drosopoulos & Remane, were found to be competent vectors of the bacterium on cherry, olive, and myrtle-leaf milkwort but only under experimental conditions (Cavalieri *et al.*, 2019). In contrast with previous studies in the US and Brazil (Paião *et al.*, 2002; Krell *et al.*, 2007), Cornara *et al.* (2020) suggested cicadas, at least the widespread Mediterranean species *Cicada orni* L., have no or very negligible role in *X. fastidiosa* transmission to olive.

The meadow spittlebug *P. spumarius* has a very wide distribution in Europe, ranging from the Mediterranean to Lapland and it is a highly polyphagous species. It has the potential to live under different environmental conditions, from moist to relatively dry,

as long as the host plant is actively growing and not subjected to severe water stress (Cornara *et al.*, 2018). Oaks, such as *Q. ilex* and *Quercus crenata*, seem to be among preferred hosts of adults *P. spumarius* among woody plants (Di Serio *et al.*, 2019; Bodino *et al.*, 2020). Beside oaks, other forest trees have been reported to be hosts for *P. spumarius*, as for example *Acer campestre* L. (Mazzoni, 2005). Other xylem-feeders reported on forest trees in Europe, and therefore candidate vectors of BLS, include: *P. tarifa* Remane & Drosopoulos, *Aphrophora alni* Fallén and *Aphrophora corticina* Germar, *Cercopis arcuata* Fieber and *Haematoloma dorsata* Ahrens, whose adults can be found on oak plants; *Cicadella viridis* L., whose adult females may lay their overwintering eggs on ash; *A. alni* and *N. campestris* that can be collected on elm; (Cornara *et al.*, 2019; Bodino *et al.*, 2020). Maple is reported as host of the sharpshooter *Graphocephala fennahi* Young (Sergel, 1987). This latter species, native to Nearctic region and originally associated only with Rhododendron spp., extended its geographic range and host plant spectrum in Europe, and has been reported on several tree and shrub species (Arzone *et al.*, 1986; Sergel, 1987).

How disease is affected by vector ecology

Xylella transmission and plant infection strongly depend on each vector's biology, ecology, host-range and behaviour (Redak *et al.*, 2004; Sicard *et al.*, 2018; Markheiser *et al.*, 2020). For example, the timing of inoculation in the growing season, depending on vector's phenology, ecology and life cycle, was shown to be critical for the establishment of host–plant chronic infections in PD. A dramatic increase in PD severity occurred in California in the 1990s following the introduction of a new vector (*Homalodisca vitripennis* Germar) from the Southeastern States (Blua *et al.*, 1999).

The life-cycle and ecology of *P. spumarius* has been particularly studied in relation to OQDS in Italy (for a detailed description, see Cornara *et al.*, 2018 and Bodino *et al.*, 2019, 2020). Briefly, eggs (the overwintering form) are laid close to the ground, usually on plant debris or directly on the basal portion of herbaceous plants. Upon hatching in early spring, the nymphs crawl to reach a succulent herbaceous host, where they form the characteristic spittle (Figure 2), with a preference for plants belonging to Asteraceae and Fabaceae (Bodino *et al.*, 2020). After emergence, adults tend to disperse toward woody hosts (olive trees and wild trees such as oaks, lentisk, myrtle, etc.) in late spring. Once on infected olive trees, spittlebugs acquire *X. fastidiosa* and spread it from tree-to-tree within the olive orchard, and possibly to other trees and shrubs (Cornara *et al.*, 2017b). In the end of summer, spittlebugs go back to herbaceous hosts for oviposition (Cornara *et al.*, 2017b, 2018). This dispersal trend from herbaceous plants to woody hosts and back to ground cover vegetation was found to be less pronounced in North-Western Italian olive orchards than in Apulia (Bodino *et al.*, 2020). In Corsica, *Cistus monspeliensis* L. was reported as the main host plant for both nymphs and adults (Cruaud *et al.*, 2018). Spittlebugs likely do not disperse over long distances during their lifetime; nymphs tend to remain close to the place where they hatched (Morente *et al.*, 2018), and adults might move just a few hundred metres during the search for oversummering hosts and oviposition places (Weaver and King, 1954; Cornara *et al.*, 2018, 2019). Hence, olive groves appear

to be very suitable habitats, probably sustaining *P. spumarius* all along its life cycle with ground cover vegetation providing oviposition sites in fall and hosts for nymph development in spring while olive trees provide food, shelter and mating sites during summer (Santoiemma *et al.*, 2019). Favourable habitats can potentially support high population levels of vectors, and ultimately, if *X. fastidiosa* is present, be associated to high probability of disease transmission. The landscape composition of the close surrounding of the olive groves (125–250 m) was suggested to play a significant role in *X. fastidiosa* epidemics by affecting vector populations. In agreement with *P. spumarius* preference for oaks, a species distribution model (SDM) of *X. fastidiosa* based on occurrence data in Apulia identified evergreen and deciduous oak woodlands as the most favourable habitats among forested areas (Bosso *et al.*, 2016b).

Is the distribution of Xylella fastidiosa, especially subsp. multiplex, restricted by climatic factors?

Most diseases caused by *X. fastidiosa* were reported from regions with tropical, sub-tropical or Mediterranean climates. However, there are also reports from regions with temperate and even continental climates (EFSA, 2019). This wide distribution might be explained by the evolutionary history of the different subspecies in the Americas. Northernmost records of *X. fastidiosa* are always associated with subspecies multiplex (EFSA, 2015). Phylogenetic and genomic studies suggest that *X. fastidiosa* subspecies may have evolved in different environments. In particular, the evolution of *X. fastidiosa* subsp multiplex in a temperate environment in North America is supported by its rate of substitutions and observed nucleotide polymorphisms (Nunney *et al.*, 2010). It probably diverged from more tropical forms related to *X. fastidiosa* subsp. fastidiosa, with a probable origin in Central America, and *X. fastidiosa* subsp. pauca, with a probable origin in South America (Nunney *et al.*, 2010, 2014). Observation and experimental data have provided evidence of the detrimental effect of low temperatures on survival of *X. fastidiosa*, especially for PD, with "winter (cold) curing" limiting the establishment of the disease in cold areas and of chronic infections in California (Lieth *et al.*, 2011; Purcell, 2013). However, experimental data are lacking to support subspecies multiplex or specific strains recovered from northern areas as more cold tolerant.

BLS in forest tree species is mostly associated with *X. fastidiosa* subsp multiplex and has been reported in several regions with relatively cold winters, such as British Columbia, southern Ontario and Saskatchewan in Canada, north-eastern states of the US, and in Argentina (Sherald, 2007; Gould and Lashomb, 2007; Harris *et al.*, 2014). Adams *et al.* (2013) found that the northern limit of BLS in trees corresponded to the area where the average annual minimum temperatures over 10–24 years was between −18 and −23°C. The survival of the bacterium in these cold conditions might be explained by the fact that the bacterium could overwinter in protected parts of the tree such as the trunk or roots (Chang and Walker, 1988; Henneberger *et al.*, 2004; Gould and Lashomb, 2007; Adams *et al.*, 2013). In plane trees, Henneberger *et al.* (2004) showed a very strong negative correlation between the accumulation of temperatures below −5°C and bacterial populations detected or isolated in the sap

of the stems, while these temperatures were not being reached in the roots.

The EFSA Panel on Plant Health recently produced maps that represent climatic suitability for establishment of *X. fastidiosa* in Europe, taking subspecies into consideration (EFSA, 2019). Ensemble predictions were based on various SDM techniques, using available records in US and Europe. Subspecies multiplex had the widest climatic envelope compared with that predicted for subsp. fastidiosa or pauca, with suitable climatic conditions over a large part of the EU territory with the exception of central and northern Europe. The highest suitability for each of the three subspecies was found in Mediterranean and south Atlantic areas, characterized by warm temperatures during the summer-dry period, mild winter temperatures and well-defined rainy season. In an independent study, Godefroid *et al.* (2019) also predicted a large extent of climatically suitable areas in Europe, especially for subspecies multiplex and again mostly in circum-Mediterranean and Atlantic areas. Irrespective of climate change, the introduced strains have thus high potential for expansion. Additionally, new areas might be at risk under the ongoing climate change characterized by increasing minimum winter temperatures, since the three subspecies appear to be limited by cold (with subsp. pauca the most sensitive and subsp. multiplex the least). However, hotter summer temperatures and changing precipitation regimes might make other regions less favourable to *X. fastidiosa* establishment. Bosso *et al.* (2016a) suggested that climate change, under several greenhouse gas emission scenarios, would not affect the future potential distribution of *X. fastidiosa* in the Mediterranean Basin.

Is disease risk for trees different in forest ecosystems as compared with urban settings?

The fact that the BLS of trees in North America is mainly observed in urban areas while most affected tree species are native and also present in forests is not explicitly discussed in the literature. Nunney *et al.* (2013) and Harris *et al.* (2014) point to the lack of information on the presence of *X. fastidiosa* outside urban areas. The nature of the symptoms (leaf browning at the end of the season, early defoliation) could explain why they are more easily noticed for ornamental trees and overlooked in the forest environment. Landscape and amenity trees are observed daily by many people, thus BLS could be easily and more often noticed and reported in urban settings, while surveillance of forest trees is more sporadic. Some environmental factors could also contribute to the greater severity of the disease in urban environments, such as the existence of more frequent and intense plant stressors, which could exacerbate the expression of symptoms (Barnard, 2007; Nunney *et al.,* 2013; Harris *et al.,* 2014). The disease is often mentioned on trees that are already well developed or even old (McElrone *et al.,* 2008) but can also affect trees shortly after planting (Sherald and Kotska, 1992).

On the other hand, Sherald (2007) proposed that the highest prevalence of the disease in parks and gardens could be explained by ecological factors. For example, the presence of reservoir host species, i.e. host plants of *X. fastidiosa* acting as a source of inoculum for trees, could be more commonly found in open areas than in forests. Similarly, some authors suggested

that vectors of the disease could be particularly abundant in urban areas (Zhang *et al.,* 2011; Harris *et al.,* 2014). The abundance of vectors in residential landscaping might be favoured by irrigation practices (Almeida *et al.,* 2005) since sap-feeders are usually attracted by plants in a good hydric status (Yurtsever, 2000). As indicated earlier, spittlebugs generally share their life-cycle between herbaceous plants and woody hosts (Figure 3). Parks with trees surrounded by well-watered garden plants and shrubs might constitute an especially favourable habitat for vectors, by sustaining their development during their life cycle. By contrast, closed-canopy forests with a poor understory may be less favourable for the nymphal stages, and would not allow the built-up of dense populations of *X. fastidiosa* vectors. The crucial role of understory vegetation has been demonstrated for the Saratoga spittlebug, *Aphrophora saratogensis* Fitch., which can be an important pest in young red pine plantations. Herbicidal treatments targeting overstory vegetation in pine plantations were shown to provide effective protection of susceptible pines from serious damage, by decreasing spittlebug populations (Linnane and Osgood, 1976). Weed management was also suggested as a control measure reducing resident vector populations in infected olive and almond orchards in Italy and Spain (Dongiovanni *et al.,* 2018; Bodino *et al.,* 2019; Moralejo *et al.,* 2019).

Another possible factor explaining differences of disease risk between forest and urban trees (or fruit trees) could be linked to the genetic diversity of plant populations. Urban trees, and even more fruit trees and grapevine, largely belong to few vegetatively propagated cultivars/varieties. If a *X. fastidiosa* strain is introduced in a region where susceptible cultivars are widely planted, the transmission of this strain will be highly facilitated. This is probably one explaining factor of the epidemics of OQDS in the Apulia region, where 85 per cent of olive trees belong to two traditional cultivars ("Cellina di Nardo" and "Ogliarola salentina"), which turned out to be highly susceptible to the ST53 strain of *X. fastidiosa* subsp. *pauca*, both in terms of bacterial population levels and expression of symptoms (D'Attoma *et al.,* 2019). More heterogeneous populations or landscapes, as generally occurs in European forests, might be comparatively less favourable for epidemic spread and evolution towards high virulence levels (Stukenbrock and Mc Donald, 2008; Sicard *et al.,* 2018). Moreover, homogeneous plantings, as may occur in tree alignments along streets and landscape settings, could also favour pathogen spread through root grafts, although there is no current evidence of such tree-to-tree transmission in tree species other than *Citrus* spp. and of any epidemiological significance of such mechanism (He *et al.,* 2000).

Conclusions

Risk of Xylella fastidiosa disease emergence in forests

The recent "Update of the scientific opinion on the risks to plant health posed by Xylella fastidiosa in the EU territory" (EFSA, 2019) concluded that "the spread of *X. fastidiosa* to forest areas could lead to impact on oaks, elms, maples and other tree species known to be affected in North America, but with high uncertainty to the level of this impact, especially for native European species absent in North America". No new significant finding since then can notably change this statement.
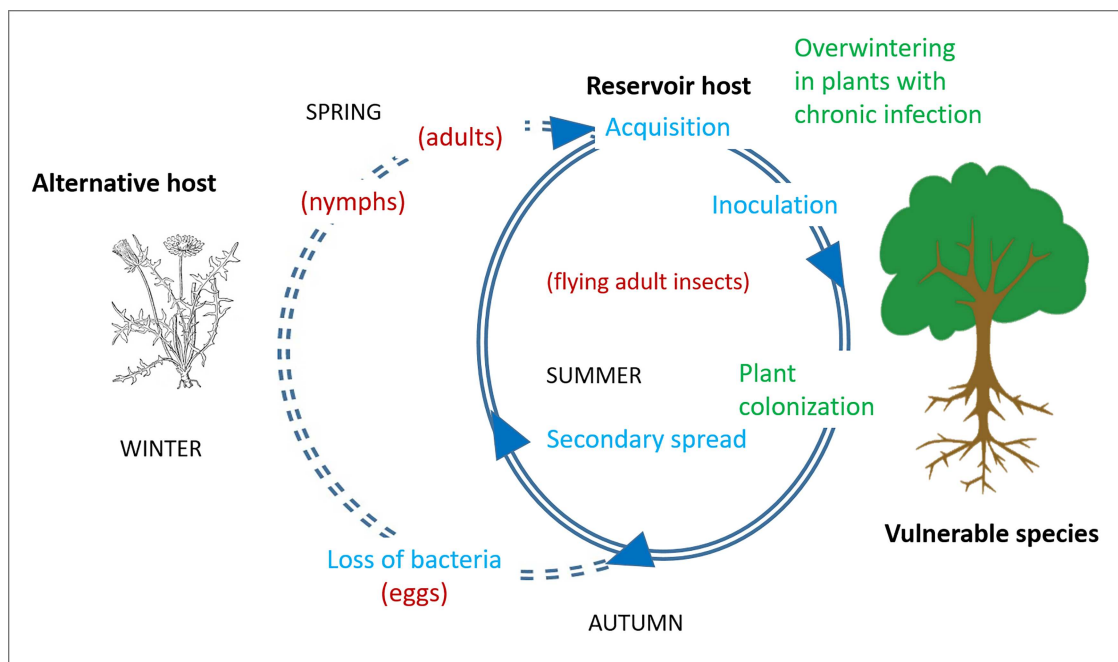
Figure 3 Hypothetical *Xylella fastidiosa* disease cycle in forest trees: processes indicated in blue are linked to insect (e.g. spittlebug) vectoring of the bacteria; processes in green are within plant; insect stages are indicated in brown. Here, the vulnerable species is a tree, both susceptible and economically or environmentally important. The alternative host is used by vectors for oviposition and nymph development. Acquisition of bacteria by vectors (emerging healthy adult insects) in spring is from chronically infected host plants (reservoir hosts), either the vulnerable species itself, other woody species or possibly the alternative host. Some vectors (in some species, or for a given species, according to climatic conditions) may overwinter as infected adults or make several generations per year (see main text for further explanations). This figure appears in colour in the online version of *Forestry*.

The current situation of *X. fastidiosa* in North America may suggest a relatively low threat to forests since little damage has been reported in these ecosystems. Although American native forest tree species have been found to be susceptible to *X. fastidiosa*, especially the subspecies multiplex, disease reports and damage have been mostly restricted to urban or peri-urban environments, in parks and gardens. This moderate impact may reflect a long ecological and evolutionary history of *X. fastidiosa* with temperate forest ecosystems in North America (Almeida and Nunney, 2015), a situation for which large-scale epidemics with severe impacts are not expected to occur (Jousimo *et al.*, 2014). In particular, some level of resistance or tolerance to the bacterium may have evolved in native North American tree populations (Purcell, 2013), resulting in a low frequency of highly susceptible genotypes and the necessity of stressful conditions for the expression of severe symptoms. The same author suggested that natural regulation of the bacterial populations by microbial antagonisms (resistance induced by low virulent strains or phages) could also be involved.

Extrapolating a low risk for European forests would be too hasty, however. The history of emerging diseases associated with *X. fastidiosa* in different parts of the world, on different plants, and with different subspecies consistently demonstrates that unpredictable host-*X. fastidiosa* interactions with high impact can occur as soon as a non-native component is included in the host–pathogen–vector system. The most common pathway leading to emergence of *X. fastidiosa* diseases has been the introduction of strains of the bacterium into new areas, through the movement of infected, potentially asymptomatic, plant material (Almeida and Nunney, 2015; EFSA, 2019). Examples include the introduction of *X. fastidiosa* subsp. fastidiosa in California in the late 19th century, possibly through the importation of coffee plants from Central America (Nunney *et al.*, 2010). The Central American origin of the ST53 strain of *X. fastidiosa* subsp. pauca causing OQDS in Italy is strongly supported by pangenomic studies (Giampetruzzi *et al.*, 2017), although ST53 may not be native to Central America but originating from a single pauca genotype introduced from South America (Nunney *et al.*, 2014). The scenario suggested for the emergence of CVC in Brazil involved a change in the virulence of the local native *X. fastidiosa* subsp. pauca strains so that they were able to infect Citrus after inter-subspecific recombination with introduced strains of *X. fastidiosa* subsp. multiplex, possibly from plum plants (Nunes *et al.*, 2003; Nunney *et al.*, 2012). Even in an area where the bacterium is already present, subsequent introductions therefore represent a significant risk with the possibility of intra- or inter-subspecies recombination events changing the host range of the pathogen, as was emphasized for other plant pathogens (Brasier, 2000). Indeed, a characteristic of the bacterium, which complicates risk prediction is its very wide actual and potential host range, albeit with no apparent phylogenetic signal. Contrary to the general rule observed in plant–pathogen interactions (Gilbert and Webb, 2007) the known host range of *X. fastidiosa* cannot be used to make predictions of host jump to another

plant species based on the phylogenetic relatedness with plants known as hosts (Nunney *et al.*, 2019). In the same line, Sicard *et al.* (2018) showed the absence of congruence between phylogenetic trees of the host plants and of the STs of the pathogen.

While introductions of pathogen strains and vectors (as shown for PD) clearly represent the main driver of *X. fastidiosa* disease emergence, the risk of establishment of the disease is strongly affected by environmental factors, especially climate and landscape features which drive pathogen survival, disease transmission and vector population levels (Sicard *et al.*, 2018; EFSA, 2019). Xylella pathosystems are typical examples illustrating the "epidemiological triangle" (here with additional layers of complexity leading to more than three components), i.e. the fact that the outcome in terms of disease and impact is the result of very specific interactions between host–pathogen–vector-populations and the environment, both biotic and abiotic (Almeida *et al.*, 2005). Compared with other situations where severe Xylella outbreaks occurred, European forests present some features potentially limiting risk, such as the occurrence of genetic and species diversity. However, diversity has not proven to be sufficient to limit the impacts of other introduced pathogens, especially with a large host-range, as exemplified by sudden oak death caused by *Phytophthora ramorum* Werres, de Cock, and Man in't Veld in California, in very diverse woodlands (Rizzo *et al.*, 2005). Moreover, as a vascular pathogen, the impact of Xylella may be strongly increased in trees already weakened by drought stress and other stressors, including pathogens and insect pests. Even if native European forest trees are not very susceptible to the strains that have been already introduced, *X. fastidiosa* may become an additional factor contributing to tree declines. Mediterranean ecosystems, with high climatic suitability for establishment of the bacterium (EFSA, 2019; Godefroid *et al.*, 2019) and potentially exposed to frequent drought stress can therefore be considered as higher risk areas. The fact that a low prevalence and no important impact has been observed on tree species, especially oaks (mainly represented by holm oak) in Corsica until now despite long presumed presence of *X. fastidiosa* may be a matter of hope. However, biological invasions are generally characterized by a lag phase corresponding to the build-up of populations until they reach levels associated with potential high impact (Aikio *et al.*, 2010). Bacterial populations may not have reached sufficient levels in introduced areas in Europe to reveal the full range of susceptible species. This phenomenon is well exemplified by the history of *Phytophthora ramorum* in the UK, which was first only observed on ornamentals in nurseries, but after several years caused devastating epidemics in larch plantations, and has more recently emerged on chestnut (Brasier and Webber, 2010; Webber, 2017).

Knowledge gaps and recommendations

Given the great uncertainty on potential impacts of *X. fastidiosa* on European forests, the first recommendation is to strictly apply quarantine, eradication and containment measures to avoid any new introduction or further spread of the pathogen (and potential alien vectors). Any introduced strain, including ST09 particularly associated with oaks in the US, could represent a risk,

in view of the unpredictable outcome of interactions with non-coevolved hosts and the potential of recombination with already present strains. Surveillance is also crucial and should be developed for forest and urban trees, especially oaks, and seedlings in nurseries. Since the *multiplex* subspecies, preferentially affecting forest tree species in America, shows lower sensitivity to cold than other subspecies, surveillance may be considered widely and should not only focus to the Mediterranean region. Improvement of detection methods, especially for trees, is still an important issue given the high concentration of tannins and difficulties linked to heterogeneous distribution of the bacterium in trees (Supplementary data 1). The design of surveillance schemes could benefit from recent modelling approaches, as developed by White *et al.*, (2017) and Martinetti and Soubeyrand (2019), while acquired surveillance data may help refining the models for more accurate predictions at a larger scale.

Many unknowns persist about the biology and epidemiology of the disease, especially as far as forest trees, especially European species, are considered. A deep understanding of the ecology of the disease, in a perspective of prevention, is essential when addressing risks to forests since control measures, both for the vector and the hosts, are much more limited or difficult to implement than in an agricultural context (Desprez-Loustau *et al.*, 2016). Extrapolating knowledge from other pathosystems in the native area or other pathosystems in Europe (e.g. OQDS in Italy) may have strong limitations since *X. fastidiosa* diseases have appeared as the outcome of complex interactions between the bacterial strains, the vectors, the host plants and the environmental conditions (Almeida and Nunney, 2015). Understanding the complexity of the *Xylella*-forest pathosystems will require an integrated, multidisciplinary approach, with strong collaborations between forest pathologists, ecophysiologists and entomologists (Jactel et al., 2020).

Processes of bacterial colonization within trees are still poorly studied. Understanding the factors conditioning the distribution of the bacterium in different parts of the tree is particularly important to assess the vulnerability of tree species and tree genotypes within species. The mortality of trees is likely related to the ability of *X. fastidiosa* to establish chronic infections, and thus successfully overwinter. Seasonal dynamics of bacterial colonization within trees, with potential survival in trunks and roots during winter, is a crucial process to be investigated, especially in colds areas. Specific response of *X. fastidiosa* strains to temperature, especially of *multiplex* subspecies from different geographical origins, should also be studied. The phenological status of the trees at the time of vectors flight could be a factor affecting inoculation success (Ghelardini and Santini, 2009). The existence of anatomical features of xylem vessels affecting bacterial colonization and disease expression is another interesting field to be investigated. It is generally admitted that insect vectoring constitutes the only natural means of *X. fastidiosa* spread. However, few studies, mostly on Citrus, have been conducted about possible horizontal (rootgrafts) or vertical (seed-to-seedling) transmission from tree-to-tree. The subspecies *pauca* has been detected in different parts of the seed from CVC-affected sweet orange but no transmission of the bacterium to seedlings seems to occur (EFSA, 2015). It would be of utmost importance to exclude (or confirm) possible transmission from root-graft or seeds for forest trees, in an epidemiological but also regulatory perspective.

Generally speaking, a better knowledge of the distribution of the bacteria within trees could also improve sampling procedures for detection.

One of the most relevant and pending question relates to the susceptibility of European forest tree species to the different *X. fastidiosa* subspecies and sequence types, which clearly needs further investigation. This could start by screening a range of bacterial strains on a range of tree genotypes of different species, primarily focusing on oaks which appear to be especially susceptible in North America. This is, however, not an easy question since high virulence towards a particular host may be associated with a single strain as shown for OQDS, the emergence of which in Italy was unpredictable based on the few reports of *X. fastidiosa* in California (where only subsp *multiplex* was detected, with low impact). More generally, the determinants of host specificity and pathogenicity in *X. fastidiosa*, i.e. why a specific bacterial strain will cause severe disease on a particular host while being a harmless endophyte in another is not yet understood. We advocate that oaks be considered as model plants, in addition to grapevine, to investigate pathogenesis of *X. fastidiosa*. Such studies may take advantage of the development of a miniaturized system to investigate systemic transcriptomic changes in oak during biotic interactions (Maboreke *et al.,* 2016) and of the availability of a well-annotated genome sequence (Plomion *et al.,* 2018).

Another important field of investigation should focus on the vectors, since they are one of the major drivers of establishment and spread of *X. fastidiosa*. While since 2014 a large (but still insufficient) amount of data has been produced on the ecology of spittlebugs in European agroecosystems, almost nothing is known with regard to European forests. This scientific gap rather awaits a quick response. Surveys in forest habitats, starting by European regions affected by disease outbreaks, are required to gather data on vectors' presence, seasonal abundance and host preference, and vectors' infection by *X. fastidiosa*. Molecular methods allowing the detection of *X. fastidiosa* in insects have already provided interesting results but still require improvements (Cruaud *et al.,* 2018). Such preliminary step should be followed by detailed studies on vectors' population dynamics, spatial distribution, dispersal, feeding habits and behaviour on forest trees. Comparison of data gathered in different climatic conditions would permit to deepen our knowledge and refine models for risk assessment of BLS spread in European forests, eventually aimed at improving surveillance. These studies should proceed in parallel with transmission tests to forest trees found to be infected with *X. fastidiosa* (under controlled conditions for quarantine reasons). Knowledge on Apulian OQDS outbreak should not be generalized and applied to other pathosystems, since *Xylella*-pathosystems should be treated on a case-by-case basis. Therefore, besides *P. spumarius*, all the xylem-feeders merit investigation regarding their potential role in BLS epidemiology in European forests, including species that proved to be non-relevant in OQDS spread, such as *Neophilaenus campestris* or *Cicada orni*. Of particular relevance, considering its distribution and host range, would be *Graphocephala fennahi*, a sharpshooter of American origin which has extended its host and geographical range in Europe during the last decades.

Current and future studies conducted in Europe may help understand aspects of BLS that have not been clarified in North America. However, some questions are currently difficult to address in a European context and experiments can only be carried out in quarantine facilities. Further investigations about BLS in North American forests, starting by the study of prevalence and severity of symptoms, are highly needed. Testing different hypotheses could explain why a lower impact has been reported so far in forests as compared with landscape trees. This could shed light on the underlying mechanisms, especially related to the ecology of vectors, the genetic variation in susceptibility of tree species and other natural disease regulation processes, such as microbial antagonisms, that could be of great interest to maintain or increase resilience of worldwide forests to *X. fastidiosa*.

Supplementary data

Supplementary data are available at *Forestry* online.

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Conflict of interest statement

None declared.

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