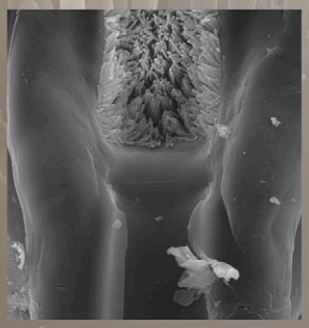
How Do Plant Diseases Caused by *Xylella fastidiosa* Emerge?



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In 2002, Don Hopkins and Sandy Purcell wrote a Feature article for Plant Disease on diseases caused by the bacterium *Xylella fastidiosa* (Hopkins and Purcell 2002). They began by proposing that this plant pathogen posed "some of the most significant new disease threats in the Americas." Hopkins and Purcell also discussed how "a relatively obscure plant pathogen" became, 2 years earlier, the first bacterial plant pathogen to have its genome sequenced. Their review came on the heels of two emerging epidemics, that of citrus variegated chlorosis (CVC) in Brazil and Pierce's disease of grapevines in southern California. One decade later, the bacterium *X. fastidiosa* has risen from obscurity, new diseases have emerged, and the diseases it causes have become worldwide threats. In addition, there were only a handful of peer-reviewed manuscripts yearly published on *X. fastidiosa* until the early 2000s, and that has changed significantly during the last decade. As a consequence, our understanding of *X. fastidiosa* and the diseases it causes has improved substantially.

Our goal in this Feature article is to provide a critical overview of the main processes that have led to the emergence of *X. fastidiosa* diseases. We steered away from inferences on the expansion or contraction of regions subject to specific diseases, or any approaches to forecast disease emergence. We believe, with few exceptions, that there are not sufficient biological and ecological data available for meaningful modeling of these processes, as has been evidenced by recent *X. fastidiosa* epidemics. Broadly speaking, the emergence of *X. fastidiosa* diseases so far appears to follow a limited set of pathways, upon which we will focus from ecological and evolutionary perspectives. Molecular mechanisms determining host plant specificity have not been elucidated and the topic is not addressed here. We also take advantage of this opportunity to raise various questions we believe should be addressed by the wider scientific community. We invite readers interested in other *X. fastidiosa*-related topics to explore recent reviews (Chatterjee et al. 2008; Purcell 2013; Retchless et al. 2014).

Biology of a Plant and Insect Colonizer

X. fastidiosa is a bacterium that colonizes two distinct habitats, the xylem network of host plants and the foregut of xylem-sap feeding insects (Chatterjee et al. 2008) (Fig. 1). Processes leading to plant colonization by the bacterium are yet to be fully understood. Movement of *X. fastidiosa* between proximal vessels occurs through intact and/or damaged pit membranes, which is a necessary process for successful intraplant movement of *X. fastidiosa* within plants (Baccari and Lindow 2011; Chatelet et al. 2006; Newman et al. 2003). The specific mechanisms leading to disease remain poorly understood, but recent studies addressing this question from a host plant perspective suggest that symptom development is, initially, a consequence of physiological responses initiated by water deficit responses (Choi et al. 2013; Daugherty et al. 2010b; Sun et al. 2013).

Research on *X. fastidiosa* focuses on its role as a plant pathogen; however, to understand its ecology and evolution we propose that a broader view is necessary, recognizing that disease is the outcome of interactions between specific pathogen genotypes and host species (Casadevall and Pirofski 2014). Infection dynamics of *X. fastidiosa* are influenced by the extensive list of host plant species that can be infected (at least temporarily), the plant host specificity of different pathogen genotypes, and the wide range of potential insect vectors. *X. fastidiosa* is capable of persisting at the inoculation site in many plant species under greenhouse and field conditions after either insect or mechanical inoculation (Purcell and Saunders 1999); it can also be recovered from a wide range of weeds in infected agricultural crop (e.g., Lopes et al. 2003). Two decades ago, Hill and Purcell (1995) compiled published data on *X. fastidiosa* host range and concluded that plants in 29 families were hosts of this bacterium. A more recent report listed 309 plant species in 63 families as hosts of *X. fastidiosa* (EFSA Panel on Plant Health 2015). *X. fastidiosa* does not appear to cause disease in most of these plant species; however, the available data suggest that these asymptomatic infections typically declined over time (Purcell and Saunders 1999). Thus, colonization of plants by *X. fastidiosa* does not necessarily sum to disease development.

Even though *X. fastidiosa* is a plant pathogen of considerable economic importance, mechanisms of host plant-pathogen specificity remain unknown. The limited genomic structural variability within *X. fastidiosa* suggests that phylogenetic groups colonizing different host plants have similar pathogenicity mechanisms (Van Sluys et al. 2003). The only study to address the mechanisms of host-plant specificity experimentally showed that an isolate of *X. fastidiosa* could expand its host range if a cell-to-cell signaling-based gene regulation system was disrupted, suggesting that alleles or gene regulation were associated with specificity (Killiny and Almeida 2011). But the approach used did not allow for the identification of candidate loci for future testing, and therefore the question of pathogen specificity remains largely unanswered.

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Disease is the outcome of complex *X. fastidiosa*-plant-environment interactions. But it is not known what proportion of contacts between *X. fastidiosa* and host plants in natural ecosystems result in disease. This leaves open the possibility that disease may represent a relatively small proportion of these interactions, leading to the suggestion that *X. fastidiosa* may be considered primarily an endophyte rather than a pathogen (Chatterjee et al. 2008). It may be notable that for *X. fastidiosa*, cell-cell signaling regulates limited virulence to plants while promoting vector plant-to-plant transmission (Newman et al. 2004). Transmission rates can also be modulated by the vector's response to disease symptoms. In two *X. fastidiosa*-induced disease systems studied, sharpshooter leafhoppers did not avoid infected yet asymptomatic plants or healthy plants painted to mimic disease symptoms, but discriminated against infected and symptomatic plants (Daugherty et al. 2011; Marucci et al. 2005). This behavior may be advantageous for these insects: water stressed and *X. fastidiosa*-infected plants have some shared physiological characteristics, of which xylem sap under high tension is of paramount relevance. Increased tension in a water column leads to a food source that is energetically expensive for insects, resulting in the ingestion of less xylem sap (Andersen et al. 1992; Miranda et al. 2013) and promoting the movement of vectors to another host (Krugner et al. 2012). Since symptomatic plants are heavily colonized by *X. fastidiosa* (Newman et al. 2003), avoidance of infected plants by vectors may act to reduce transmission rates when disease incidence is low (Sisterson 2008; Zeilinger and Daugherty 2014) and select for decreased bacterial virulence. This effect could be important when transmission rates are low; however, if vectors are common and transmission rates high, rapid bacterial growth leading to increased virulence may be favored, a pattern often observed in diseases that are vector-transmitted between hosts (e.g., malaria; de Roode et al. 2005).

Experimentally identified insect vectors of *X. fastidiosa* belong to two insect groups, the sharpshooter leafhoppers (Cicadellidae, Cicadellinae) and spittlebugs (superfamily Cercopoidea, with five species of Aphrophoridae and two species of Clastopteridae identified) (Almeida et al. 2005a; EFSA Panel on Plant Health 2015). In addition, there are two reports of cicadas (Cicadidae) transmitting *X. fastidiosa* (Krell et al. 2007; Paião et al. 1996), which need to be confirmed through additional experiments. Colonization of these insects by *X. fastidiosa* occurs in a noncirculative, persistent manner (Purcell and Finlay 1979), with the bacterium colonizing the foregut of insect vectors (Purcell et al. 1979). Consequently, there is no transovarial or transtadial transmission (Almeida and Purcell 2003; Freitag 1951; Purcell and Finlay 1979). Colonization of regions in the foregut called cibarium and precibarium were first shown microscopically (Brlansky et al. 1983; Purcell et al. 1979), and later correlated with insect inoculation of plant hosts during feeding (Almeida and Purcell 2006). So far, no other plant pathogen is known to be transmitted in a similar manner, with the possible exception of *Ralstonia syzigii*, which is transmitted by spittlebugs in the Machaerotidae (Eden-Green et al. 1992).

Transmission efficiency of *X. fastidiosa* by vectors increases with both the time an insect feeds on an infected host plant (acquisition) and the subsequent time it feeds on an uninfected host (inoculation), up to 48 to 96 h (Almeida and Purcell 2003; Purcell and Finlay 1979). Presumably, a longer feeding time increases the likelihood of insect vectors reaching colonized xylem vessels in the case of acquisition, and performing specific probing behaviors in the case of inoculation. The colonization of a vector by the bacteria is a critical part of acquisition and is a complex process, similar to biofilm formation on surfaces, which has been explored in some detail (e.g., Killiny and Almeida 2009a, b, 2014). Specific probing behaviors involved in inoculation are yet to be determined; however, the inoculation of *X. fastidiosa* into dormant grapevines with positive xylem sap pressure (positive root pressure) indicates that vector behaviors are required for the inoculation of bacterial cells into plants (Almeida et al. 2005b).

One important aspect of *X. fastidiosa* transmission relevant to the emergence of new diseases is the lack of vector specificity (Almeida et al. 2005a). The insect groups that transmit *X. fastidiosa* are distributed worldwide in tropical and temperate climates, and all insect species belonging to the above-mentioned groups should be considered as potential vectors until proven otherwise. For example, one vector species has been shown to transmit *X. fastidiosa* isolates belonging to four different *X. fastidiosa* subspecies (Almeida and Purcell 2003; Purcell et al. 1999; Sanderlin and Melanson 2010; Saponari et al. 2014). And an *X. fastidiosa* subspecies originally from South America has been transmitted by various vectors in South America, one in North America, and another in Europe (Brlansky et al. 2002; Damsteegt et al. 2006; Marucci et al. 2008; Saponari et al. 2014). This lack of specificity increases the likelihood that newly introduced *X. fastidiosa* isolates, when reaching a novel environment, will be transmitted by an endemic vector species. However, while the ability to transmit *X. fastidiosa* is not limited to a few insect species, transmission efficiency is highly variable and dependent on a range of vector-plant-pathogen interactions (Lopes et al. 2009). Transmission efficiency may vary for different vector species on the same host plant species (Daugherty and Almeida 2009; Lopes et al. 2009), or the same vector species feeding on different tissues of the same plant (Daugherty et al. 2010a); however, observations suggest that the general mechanisms of transmission are conserved among vectors. The one caveat is that most of the research on *X. fastidiosa* transmission has been conducted with two vector species (*Graphocephala atropunctata* and *Homalodisca vitripennis*) and one *X. fastidiosa* subspecies (subsp. *fastidiosa*), so it is important that a broader range of taxa be studied to confirm these results. Until that is done, the effectiveness of individual sharpshooter leafhopper species in transmitting *X. fastidiosa* should not be extrapolated from epidemic to epidemic without considering the novel ecological context.

A Plant Generalist or Not: Revisiting *Xylella fastidiosa* Systematics

X. fastidiosa currently is the sole species in the genus *Xylella*; *Xanthomonas* spp. are sister taxa to *X. fastidiosa* (Retchless et al. 2014). As noted earlier, *X. fastidiosa* has traditionally been referred to as having a "wide host range" or as a "generalist." This is accurate in the sense that a very large number of plant species have been demonstrated to sustain *X. fastidiosa* infections; however, there is mounting evidence suggesting that while this description is accurate, it is misleading in terms of disease and epidemic development. Specifically, very few of these plants sustain long-term infections and become symptomatic. Furthermore, it is now clear that specific symptomatic hosts are only susceptible to isolates in one or a limited number of *X. fastidiosa* phylogenetic clades, with the result that specific clades of *X. fastidiosa* have a small number of symptomatic host plant species (Nunney et al. 2013). Such insights are of great relevance in understanding disease outbreaks.

We revisit *X. fastidiosa* taxonomy in the context of recently available genetic diversity data, with two important caveats. First, it is fully expected that new clades of *X. fastidiosa* will be reported in the future (e.g., Nunney et al. 2014b). Second, many of the plant species listed as hosts of *X. fastidiosa* should in fact be considered putative hosts, since most associations studied so far derive from symptomatic plant tissue, but without experimental work to confirm the pathogenicity of isolates. Although associations are relevant, the fulfillment of Koch's postulates is a requirement to demonstrate that individual genotypes are pathogenic to specific host plant species. The importance of experimental work to determine the host range of pathogens remains paramount. We emphasize the importance of experimentally determining plant species susceptibility to *X. fastidiosa*, as there are plant species-pathogen genotype associations that do not lead to disease. Moreover, even when symptoms eventually develop, a delay of several months following infection is not uncommon. These issues are especially relevant given the economic and quarantine importance of this bacterial species. In this context, it important to note that to date, no native plant hosts of *X. fastidiosa* in South and Central America have been identified. In contrast, a number of native hosts (primarily trees) of the North American subsp. *multiplex* have been identified (Fig. 2), including several oak species (*Quercus* spp.), American elm (*Ulmus americana*), American sycamore (*Platanus occidentalis*), sweetgum (*Liquidambar styraciflua*),

and pecan (*Carya illinoinensis*) (for a more complete list see Table 2 in Nunney et al. 2013).

Studies of genetic and phenotypic diversity for *X. fastidiosa* have historically been confusing and inconclusive, despite the efforts of a small and dedicated group of scientists. We argue that the main determinants for conflicting results can be summarized under four headings. First, isolates from a small range of host plants and geographic distribution have been used for studies, over-representing a limited and narrow sampling of genetic diversity. Second, procedures for typing have relied on within-study comparisons of the previously mentioned small number of available isolates with methods that provided inadequate phylogenetic resolution. Third, methodological differences in the typing of isolates limited comparisons among studies, a problem that is disappearing now that sequencing technology is easily available worldwide. Lastly, *X. fastidiosa* is naturally competent (Kung and Almeida 2011); gene flow deeply impacts the systematics and evolution of bacteria (Polz et al. 2013).

The current view of *X. fastidiosa* genetic diversity has overcome most of these limitations, largely through the use of a portable, multilocus sequence typing (MSLT) approach (Maiden et al. 1998). MLST for *X. fastidiosa* was first introduced by Scally et al. in 2005 (Scally et al. 2005) and refined by Yuan et al. five years later into the form currently employed (Yuan et al. 2010). MLST has been successfully used to study *X. fastidiosa* diversity at the species/subspecies level, and to infer the phylogenetic placement of newly identified isolates. Together with Richard Stouthamer, one of us (LN) established a public MLST database at www.pubmlst.org. These data have resulted in a robust taxonomy for the species (Fig. 3). Furthermore, the MLST classification of isolates into sequence types (STs) (unique genotypes based on the seven loci used in MLST) has provided insights about *X. fastidiosa* evolution and host specificity. For example, comparing subsp. *pauca* STs found on coffee and citrus, it has been shown that in general they are reciprocally host specific (Almeida et al. 2008; Nunney et al. 2012). Similar patterns have been seen in subsp. *multiplex*. For example, comparing native oaks and sycamore, data from Nunney et al. (2013) and Harris and Balci (2015) show that ST9 is almost entirely restricted to oaks (84/85 isolates mostly from DC (63/64), but also KY = 14, FL = 4, GA = 2, TN = 1) with just one isolation from sycamore, while ST8 reversed this pattern with 17/21 isolates from sycamore (DC = 14, TX = 2, KY = 1) and just 1 from oak (in KY).

Based on current knowledge, *X. fastidiosa* is primarily a species of the Americas (Fig. 4). A distant relative is found in Taiwan (Su et al. 2014), but should probably be classified as a separate species (see Fig. 3). Two other exceptions that must yet be confirmed and for which no genetic information is available, are reports from Iran (Amanifar et al. 2014) and Turkey (Guldur et al. 2005). Lastly, the recent introduction of *X. fastidiosa* into Italy is an important change to its geographical distribution (Saponari et al. 2013). The American representatives were initially divided into three subspecies, subsp. *fastidiosa*, *multiplex*, and *pauca*, based on DNA-DNA hybridization data (Schaad et al. 2004). MLST sequence data confirmed the status of these subspecies, and suggested a fourth, subsp. *sandyi*, which was not present among the earlier strains that were tested (Scally et al. 2005). Subsequent sampling and analysis based on MLST has indicated that these subspecies evolved in geographical isolation with subsp. *pauca* native to South America (Nunney et al. 2012), subsp. *multiplex* native to temperate and subtropical North America (Nunney et al. 2012, 2014a), subsp. *fastidiosa* is found in Costa Rica and is presumed to be native to southern Central America (Nunney et al. 2010), and subsp. *sandyi* has only been detected in southern regions of the United States (Yuan et al. 2010). Subspecies *morus* represents a new proposal and is discussed below. Historical geographic isolation of the original four subspecies is consistent with the known biology of *X. fastidiosa*: this bacterium can only invade a new region by long-distance dispersal of infected insects or infected plants. In the absence of human intervention, the

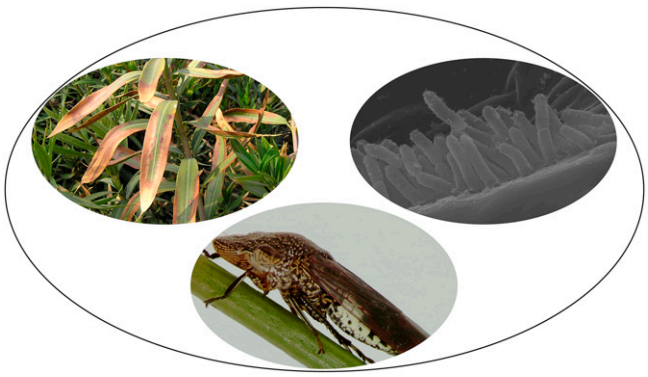

Fig. 1. Diseases caused by the bacterium *Xylella fastidiosa* are the consequence of complex biological and ecological interactions over short and long time periods. Leafhopper vectors such as the invasive *Homalodisca vitripennis* are generally polyphagous and associated with many hosts that may be susceptible to *X. fastidiosa* infections, such as oleander (*Nerium oleander*). The bacterium multiplies in both insect and plant hosts (cells colonizing an insect are shown here), and although there is host plant-bacterium genotype specificity, that does not appear to be the case for insect vector species-bacterium genotype. Photograph of *H. vitripennis* kindly provide by Rodrigo Krugner, with permission.

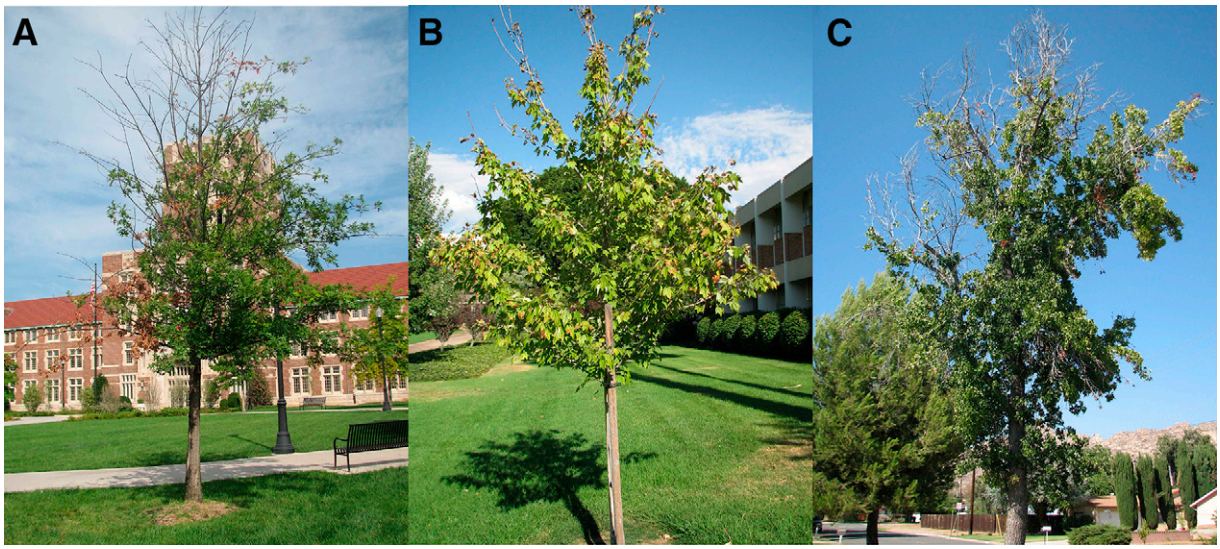

Fig. 2. Symptomatic native hosts of *Xylella fastidiosa* subsp. *multiplex* in the United States. **A,** Oak (*Quercus* sp.) tree with large amount of defoliation; **B** and **C** show symptomatic young and established sweetgum (*Liquidambar styraciflua*) trees.

former is very unlikely and the latter is close to impossible. However, it has become apparent that in the recent past, human-mediated invasion is the primary driver of economically costly *X. fastidiosa* introductions. We discuss three main pathways leading to the emergence of *X. fastidiosa* diseases, following examples available in the literature.

Introduction of Exotic Genotypes

The most common pathway leading to *X. fastidiosa* epidemics is the introduction of exotic genotypes into environments that are ecologically prone to the maintenance of the bacterium in the plant community, primarily due to the availability of endemic insect vectors and host plant species susceptible to persistent infections. Although the introduction of insect vectors carrying *X. fastidiosa* represents a potential pathway, only one vector species is considered invasive (*Homalodisca vitripennis*, Cicadellidae, a sharpshooter leafhopper), and another is distributed beyond its region of origin (*Philaneus spumarius*, Aphrophoridae, a spittlebug). The expansion in the geographic range of these species has not been associated with the spread of *X. fastidiosa*; therefore, we consider this an unlikely route. The main dispersal pathway would then be the movement of infected, and potentially asymptomatic, plant material from areas where the pathogen occurs. A recent report by the European Food Safety Authority evaluating the risk of *X. fastidiosa* introductions into the European Union reached similar conclusions (EFSA Panel on Plant

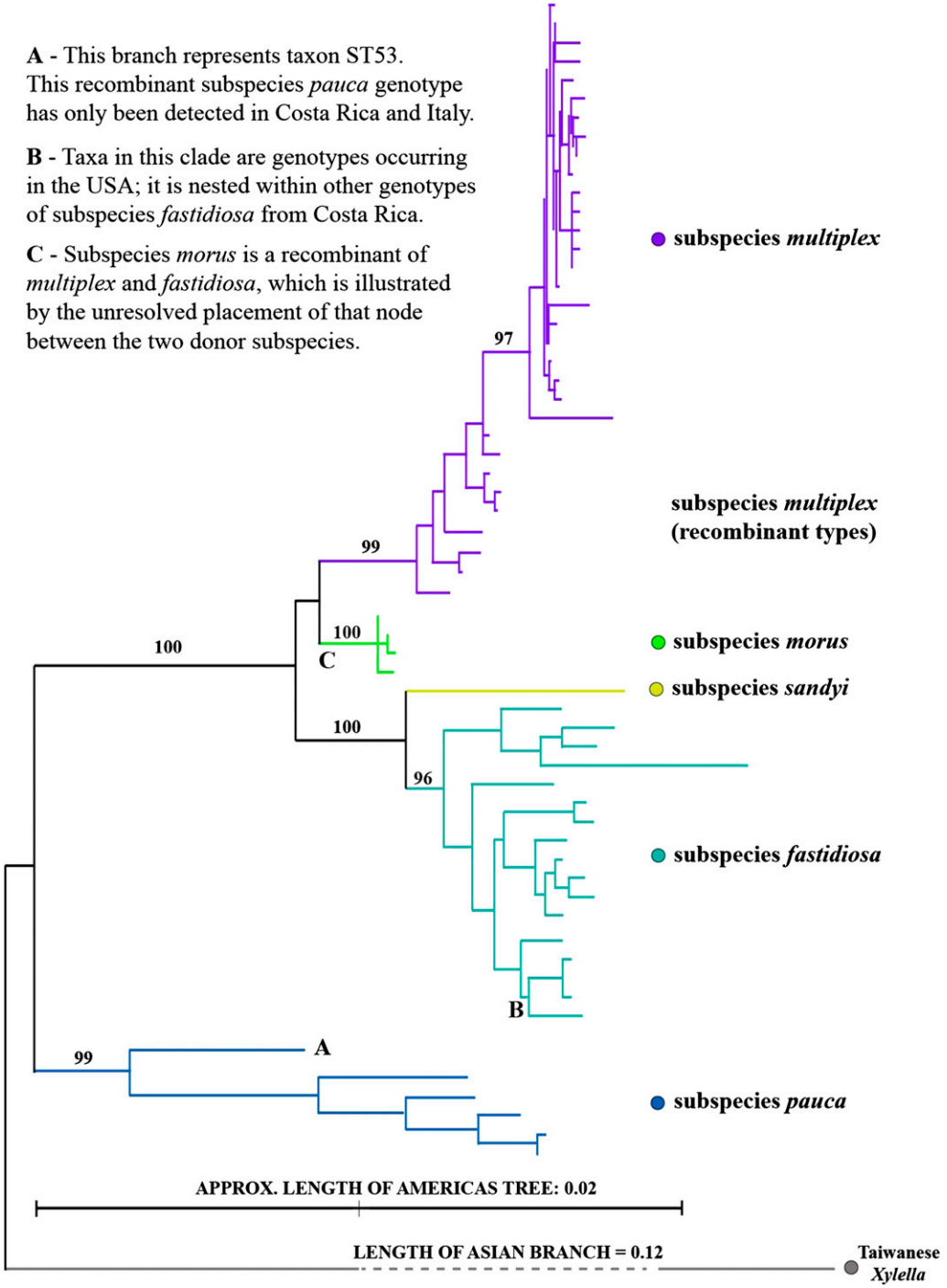

Fig. 3. A phylogenetic tree based on genetic distances of all available *Xylella fastidiosa* sequence types identified using multilocus sequence typing. The five currently and generally accepted subspecies are labeled with different colors, while the Taiwanese genotype causing pear leaf scorch is shown in gray at the bottom of the tree, with a not-to-scale branch due to its dissimilarity to the other taxa. We highlight three specific branches of interest with letters **A, B,** and **C,** with associated descriptions on the figure itself. Despite the fact that homologous recombination affects tree topology, including branch length, all subspecies are statistically well supported; both topology and branch support for the subspecies are also equivalent to this figure when other approaches are used (redraw from Fig. 1 in Nunney et al. 2014b).

Health 2015) with a much more detailed and systematic analysis of potential pathways. Here we discuss examples with conclusive evidence from the available literature.

The most recent case of an introduction is the outbreak of rapid olive decline in the Apuglia region in southern Italy, first reported in October 2013 (Saponari et al. 2013); see Box 1 and Figure 5. While the distribution and consequences of this introduction are yet to be determined, it is known that this outbreak is associated with a strain of *X. fastidiosa* subsp. *pauca* classified as ST53 (Elbeaino et al. 2014). Subspecies *pauca* is of South American origin but this ST so far has not been found in South America; however, it has been detected in Costa Rica infecting primarily oleander (Nunney et al. 2014b). Thus, this particular ST of subsp. *pauca* has been introduced into two regions, where it infects novel hosts. While olive is currently considered the primary host in the Italian outbreak, infection of oleander has also been observed, illustrating a common feature of *X. fastidiosa*: oleander and olive are hosts of the same strain, and yet they are in different Orders (Gentianales versus Lamiales). As a result, given our current knowledge, it is not possible to predict potential hosts following an invasion.

Yet another introduction involved the best studied *X. fastidiosa* disease, Pierce's disease of grapevines. It was first proposed that the Gulf Coast Plain area of the United States was the center of origin of the etiological agent of the disease based on the fact that species of grapevines (*Vitis* spp.) native to the United States were tolerant to infection, while the exotic European grapevine (*Vitis vinifera*) was susceptible (Hewitt 1958). With the recent availability of larger datasets on the genetic diversity of *X. fastidiosa*, we now know that the genotype causing disease in grapevines in the United States originated from Central America (Nunney et al. 2010). The lack of genetic diversity among isolates belonging to this clade in the United States is evidence of a relatively recent introduction (Yuan et al. 2010), and it has been proposed that the introduction into the United States of a single genotype was via an infected coffee plant, a known host of *X. fastidiosa* in Central America (Nunney et al. 2010). Isolates derived from this single genotype are now widely distributed through grape-growing regions of the United States, from Florida to California. Interestingly, an isolate from this same almost monomorphic clade found in the United States has now been reported causing Pierce's disease of grapevines in Taiwan (Su et al. 2013), suggesting that *X. fastidiosa*-infected plant material originating from the United States was inadvertently introduced into the country, eventually leading to an epidemic.

A similar scenario appears to have occurred with the emergence of plum leaf scald in Argentina, Paraguay, and Brazil (French and Kitajima 1978; Kitajima et al. 1975). The disease in plum and other *Prunus* species were known in the southeast United States, but the origin of the *X. fastidiosa* genotype(s) causing plum leaf scald in South America remained unidentified until Nunes et al. (2003) studied the gene content of several isolates. They determined that the tested plum isolate from Brazil grouped with North instead of South American isolates (i.e., belonging to subsp. *multiplex*), demonstrating yet another introduction, this time from the United States to South America. These examples illustrate the challenges of limiting the inadvertent transportation of *X. fastidiosa*-infected plant material from one country, or continent, to another.

Introduction of an Invasive Vector

To our knowledge, there is only one example of an *X. fastidiosa* vector being considered invasive, spreading over vast geographical distances and reaching large populations at various environmental conditions (Grandgirard et al. 2006; Petit et al. 2008). *Homalodisca vitripennis* (Cicadellidae, Cicadellinae) is native to the southeastern United States (Turner and Pollard 1959; Young 1958) and invaded California sometime in the late 1980s (Stenger et al. 2010) but was not detected until 1990 (Sorensen and Gill 1996). Subsequent growth

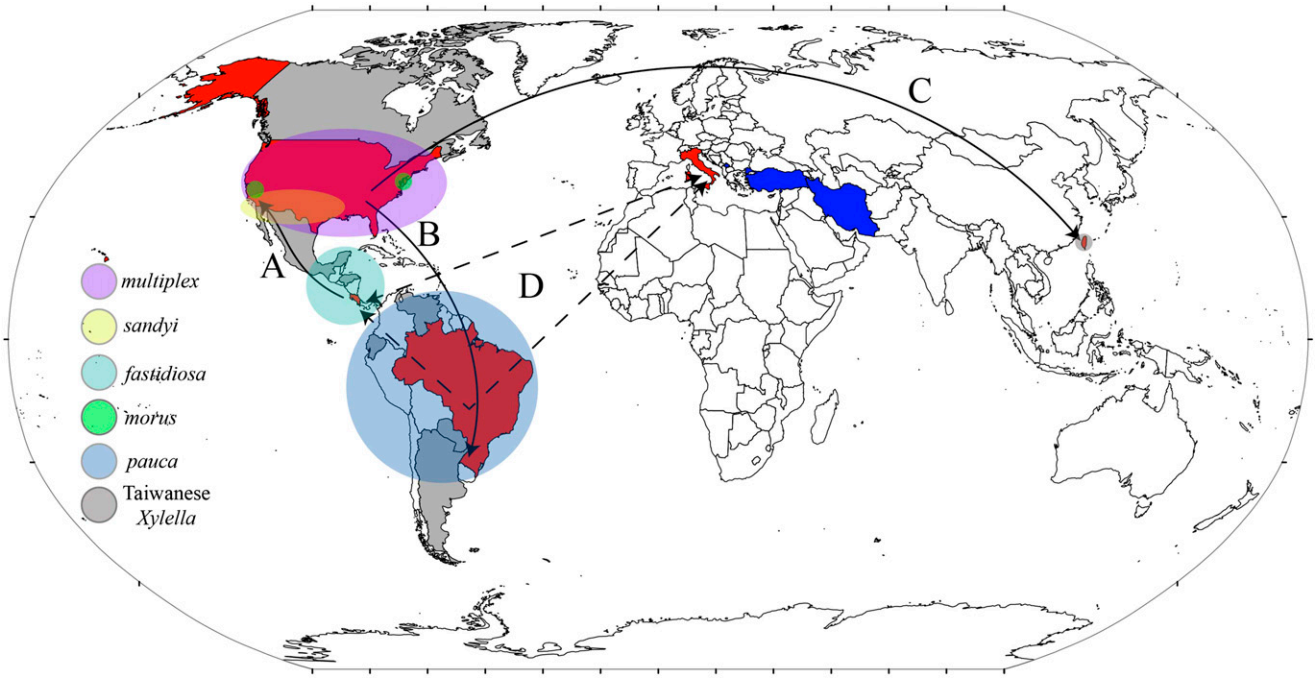

Fig. 4. World map illustrating the proposed endemic distribution of *Xylella fastidiosa* subspecies, and introduction events that have expanded the geographic range of some subspecies. *X. fastidiosa* is generally thought of as a pathogen of the Americas; the only other strongly supported example of an endemic genotype comes from Taiwan. The bacterium has also been reported from Kosovo, Turkey, and Iran; however, these reports do not include sufficient data to determine if these were introductions or endemic genotypes associated with plant diseases (countries labeled in blue). Gray countries represent those where *X. fastidiosa* is known to occur, but for which there are no genetic data. Red countries, with the exception of Italy, are countries with endemic genotypes of *X. fastidiosa*, but have also been subject to introductions. **A,** Genotypes causing Pierce's disease of grapevine in the United States are nested within a clade of Costa Rican genotypes. **B,** Plum leaf scald in Brazil is caused by subsp. *multiplex*, which is endemic in North America and also associated with plums in that region. **C,** Pierce's disease emerged in Taiwan in the early 2000s, the genotype matches those causing the same disease in the United States. **D,** Subsp. *pauca* was reported for the first time outside of South America in 2014, with exactly the same genotype associated with diseases in Costa Rica and Italy. We interpret the later case as the genotype originating from South America, but current data do not allow one to determine if invasions to both countries originated from South America, or if the genotype went first to Italy and then Costa Rica, or Costa Rica and then Italy. The endemic range of subsp. *sandyi* remains uncertain since the genetic data are consistent with a recent introduction into the United States.

and expansion of *H. vitripennis* during the 1990s eventually led to epidemics of oleander leaf scorch (Purcell et al. 1999) and Pierce's disease in southern California (Hopkins and Purcell 2002). Estimates suggested 1 to 2 million insects per hectare in the region (Coviella et al. 2006), populations which are thought to have allowed for its transportation to French Polynesia (Grandgirard et al. 2006), where biological control successfully controlled very large populations that developed in those tropical regions (Grandgirard et al. 2008). It is

Box 1. The European outbreak of *Xylella fastidiosa* associated with a severe disease of olive

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In 2013, an outbreak of *Xylella fastidiosa* was first identified in Apulia (southeastern Italy) in olive trees severely affected by a novel disease denoted Olive Quick Decline Syndrome (*Complesso del Disseccamento Rapido dell'Olivo*, CoDiRO in Italian). CoDiRO was first widely observed in 2010 and is characterized by extensive scorching and desiccation of leaves and twigs. At a preliminary examination, many of such trees appeared to be impacted by the presence of three different agents: (i) the leopard moth (*Zeuzera pyrina*), which drills galleries in the branches and trunks of olives; (ii) a set of xylem-inhabiting fungi of different genera (*Phaeoacremonium* and *Phemoniella*, in particular) (Nigro et al. 2014), which invade the sapwood taking also advantage of the moth galleries; and (iii) the bacterium *X. fastidiosa.* As time passed and a better insight into the disease was gained with field and laboratory observations, it became evident that the role of the leopard moth is minor, whereas the fungi could play the role of aggravators. Although the etiological role of *X. fastidiosa* is yet to be determined, the finding of this quarantine bacterium in the European Union prompted urgent investigations to address the many open questions and to enforce actions for its containment to avoid further spread in the neighboring areas and in other countries.

The disease.
CoDiRO is a destructive disorder. Infected trees initially display canopy desiccation followed by tree collapse. Centenarian olive trees seem to be the most seriously affected. These large trees are heavily pruned by the growers to promote sprouting of uninfected vegetation. Removal of infected branches appears to be unsuccessful as new shoots are infected and soon decline.

Detection and isolation of *X. fastidiosa*.
The initial identification of the bacterium in the CoDiRO-affected olives was achieved through specific PCR assays, followed by sequence analysis (Saponari et al. 2013). Axenic cultures of *X. fastidiosa* were also obtained, initially from oleander and periwinkle, then from olive and other alternative hosts found in the infected area (Saponari et al. 2014b). Electron microscope observations of thin-sectioned olive leaf petioles disclosed in the treachery elements accumulations of bacterial cells with the thick and rippled cell wall that characterizes *X. fastidiosa.* Validation of serological (ELISA) and molecular (PCR) assays allowed the finalization of diagnostic procedures for a sensitive and reliable detection of the bacterium in olive tissues, which were adopted for large-scale surveys in the Apulian region (Loconsole et al. 2014).

Spread.
Surveys confirmed that *X. fastidiosa* is restricted to Lecce Province, where a major outbreak that expanded from the first focus, initially estimated at 8,000 ha, extended up to 23,000 ha after just a few months. The situation is currently worse (as of March 2015), with the emergence of a myriad of small outbreaks, mainly in the central and southern parts of Lecce Province, in addition to a small outbreak 30 km away in the neighboring province of Brindisi (north of Lecce).

Genetics and biology of the bacterial strain.
Molecular data have provided strong evidence that the olive isolate of *X. fastidiosa* is related to the subsp. *pauca*, but is clearly distinct from the *pauca* strains so far characterized, so that a new sequence type (ST) profile was assigned to it based on multilocus sequence typing analysis. The distinctiveness of the olive isolate from the known *pauca* strains strongly supports the notion that it represents a novel entity, for which the name *X. fastidiosa* strain CoDiRO has been proposed. The surveys carried out in the contaminated Apulian area have shown that olive, oleander, almond, cherry, *Polygala myrtifolia*, *Westringia fruticosa*, *Acacia saligna*, *Spartium junceum, Myrtus communis, Rosmarinus officinalis, Rhamnus alaternus,* and *Vinca minor* are susceptible hosts under natural field infections (Saponari et al. 2014a). An extensive survey to assess the presence of *X. fastidiosa* infections in grapevines and citrus trees growing in the heavily contaminated area has excluded the presence of infections in both hosts.

Identification of the vector(s).
A survey of hemipterans thriving in affected olive groves initiated in November 2013 is still underway. Among the few potential vector species identified the most common was *Philaenus spumarius* L., the meadow spittlebug. Transmission tests with field-collected insects showed that *P. spumarius* transmits *X. fastidiosa* strain CoDiRO not only to periwinkle plants, as initially ascertained, but also to olive and other host plants.

Legislative provisions.
Actions aimed at the containment of the pathogen have been enforced. Specifically: (i) surveys including visual inspections, sampling, and analysis of garden and nursery productions of the province of Lecce destined for marketing, (ii) prohibition of the movement of plant species that are alleged hosts of *X. fastidiosa* from Lecce Province, (iii) guidelines for the safe production and marketing of grapevines and their propagative materials, and (iv) adoption of the EU "*Commission Implementing Decision of 23 July 2014 as regards measures to prevent the introduction into and the spread within the Union of Xylella fastidiosa.*" An area of 2,310 km^2, where approximately nine million olive trees are grown, has been demarcated as "infected zone." This represents the entire province of Lecce, from the Adriatic to the Ionian coast, where infection foci are randomly dispersed but the presence of *X. fastidiosa* has been ascertained only in a minor part of the territory. However, due to the recent progression of the disease, demarcation of the infected area is being updated. Meanwhile, an area-wide vector control program was initiated, as well as monitoring and eradication of infected hosts, in an attempt to reduce disease spread within the boundaries of the currently infected zone.

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notable that these invasions were not associated with the introduction of *X. fastidiosa*, supporting our view that the primary mechanism of *X. fastidiosa* invasions is the movement of infected live plants. In the case of California, the introduction of *H. vitripennis* had several important consequences; we focus here on the emergence of *X. fastidiosa* diseases alone. Newton Pierce, after whom Pierce's disease was later named, studied the first known outbreak of the disease that was in southern California (Pierce 1892). Since that time, *X. fastidiosa* has been regularly reported in grapevines, almonds, and alfalfa, indicating it has been continuously present. However, disease outbreaks were primarily limited to small areas, apparently due to habitat specific of endemic vectors and the broader ecological context.

There were two main consequences associated with the extremely large populations of *H. vitripennis* in southern California in the two decades subsequent to its introduction (Almeida 2008; Sisterson et al. 2008). The first was the development of a Pierce's disease epidemic, where very large populations of a relatively inefficient vector (*H. vitripennis* is not an efficient vector on grapevines when compared with other species [Daugherty and Almeida 2009]) led to the effective spread of the pathogen to a focal crop under new ecological conditions, decimating the vineyards of the Temecula region (Hopkins and Purcell 2002). Chemical control of *H. vitripennis* populations in the region has led to the restoration of the local wine industry to economically profitable levels (M. Daugherty, *personal communication*). The second consequence is based on associations rather than conclusive epidemiological data, yet the contention is well supported by field observations. We contend that the introduction of the highly polyphagous *H. vitripennis* led to the establishment of various *X. fastidiosa* diseases in southern California, notably oleander leaf scorch (Purcell et al. 1999) and scorch diseases of a range of trees (Hernandez-Martinez et al. 2007, 2009). A large list of diseases associate with *X. fastidiosa* has been generated, albeit Koch's postulates have only been fulfilled for a few of them (e.g., Hernandez-Martinez et al. 2009; Purcell et al. 1999). We suggest that *X. fastidiosa* genotypes had been widely established in southern California ahead of the *H. vitripennis* invasion, albeit restricted to disease cycles with endemic vectors and asymptomatic hosts or associated with species where it caused disease rarely enough to be overlooked. The presence of *H. vitripennis* resulted in increments of such rare events due to its large populations, or in the displacement of genotypes from endemic cycles to disease cycles that incorporated hosts of this invasive vector. The lack of vector-pathogen specificity is the trait most responsible for this outcome. In fact, *H. vitripennis* is the only vector species shown to transmit *X. fastidiosa* belonging to all currently accepted subspecies (*fastidiosa*, *multiplex*, *sandyi*, and *pauca*), although this should be expected from all known and potential *X. fastidiosa* vector species.

Recombination and Adaptation to New Plant Hosts

The anthropogenic introduction of *X. fastidiosa* subspecies into new regions can have two effects: the emergence of a known disease in a new area, and/or the emergence of a new disease involving a new plant host. In this section we focus on the second of these possibilities. One example of *X. fastidiosa* invading a new host is the case of mulberry leaf scorch. It was first noted in the early 1980s in Washington DC, and subsequent sampling revealed infected trees (the native *Morus rubra*) along the eastern seaboard as far north as southern New York (Kostka et al. 1986). Within a few years the disease was found on the west coast with infected trees (the introduced *Morus alba*) observed in California (Hernandez-Martinez et al. 2007). Initial genetic typing showed that the mulberry isolates always grouped together, but their relationship to the other subspecies was marker dependent. The reason for this ambiguity was revealed using MLST: the genome is a roughly equal mix of genetic material from subsp. *fastidiosa* and *multiplex*, such that an examination of the seven MLST loci revealed three alleles from subsp. *fastidiosa*, three from subsp. *multiplex*, and one chimeric allele containing sequence from both subspecies and consequently a recombination breakpoint (Nunney et al.

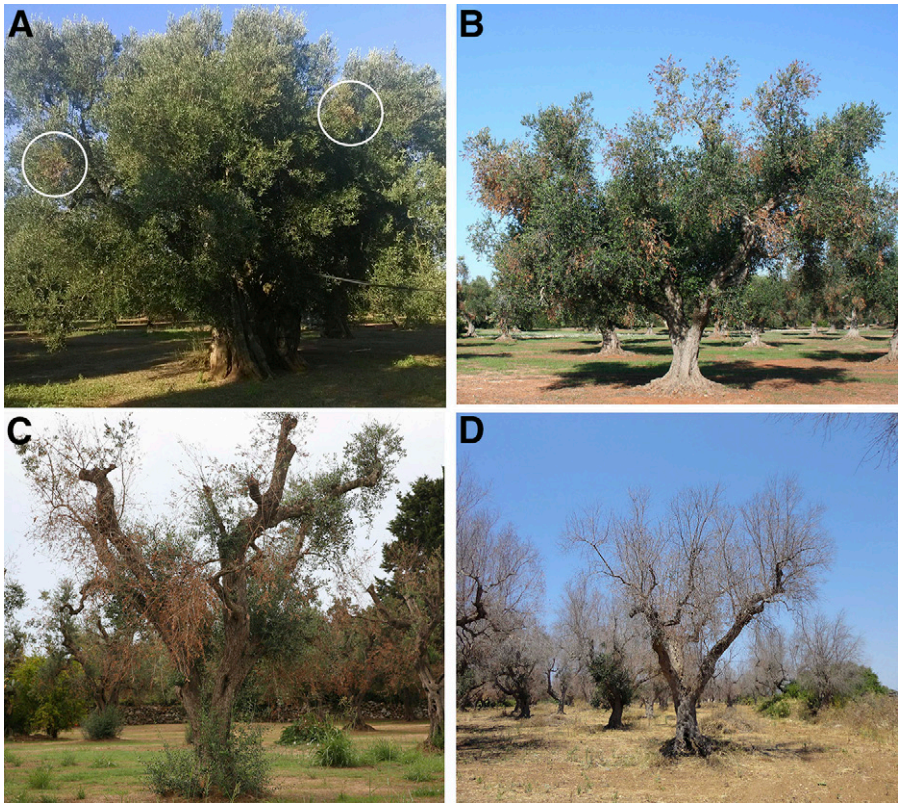

Fig. 5. Scale of symptoms in olive trees in southern Italy associated with *Xylella fastidiosa*. **A,** Early symptoms limited to isolated branches, likely due to independent infection events. **B,** Larger number of symptomatic branches, leading to **C,** heavily symptomatic plants with severe pruning used as an unsuccessful cultural practice to eliminate infections. **D,** Dead trees, which are often cut to stumps and/or uprooted. We note that, as of late 2014, Koch's postulates were not yet fulfilled for the *X. fastidiosa*-olive association in Italy; therefore, the etiological agent(s) of this olive disease remained to be conclusively identified.

2014c). All other forms of *X. fastidiosa* are genetically very distinct from the mulberry type, which themselves show almost no genetic variability. Since they do not group with any pre-existing subspecies, and since they appear to be unique in naturally infecting mulberry, it's been proposed that they define a new subspecies (subsp. *morus*), which was created by one or more massive genetic exchanges between subsp. *fastidiosa* and *multiplex* that created a chimeric genome via intersubspecific homologous recombination (IHR) (Nunney, Yuan, Russell, and Stouthamer, *in prep*).

The genetic exchange that created subsp. *morus* has also resulted in a group of genotypes ("recombinant multiplex") that cluster with subsp. *multiplex*, presumably due to repeated "backcross" exchanges with the native subspecies (*multiplex*) (Nunney et al. 2014a). Of interest is that the isolates from diseased blueberry plants (from Georgia and Florida) were all of only two sequence types, both of which were recombinant subsp. *multiplex*. No non-recombinant subsp. *multiplex* have yet been isolated from blueberry, strongly suggesting that we have a second example of genetic mixing between an introduced and native subspecies resulting in the infection of a new host. The involvement of IHR in the genesis of subsp. *morus*, and the subsequent formation of the group of recombinant subsp. *multiplex*, might seem like a special event unlikely to be repeated; however, we now have evidence that a similar genetic exchange occurred in South America. Studies of citrus and coffee *X. fastidiosa* isolates from Brazil have provided evidence of IHR from subsp. *multiplex* to subsp. *pauca* (Almeida et al. 2008; Nunney et al. 2012). Based on MLST data, Nunney et al. (2012) estimated that about half of the genome was polymorphic for subsp. *multiplex* sequence, suggesting that, as in the case of subsp. *morus*, one or more major genetic exchanges had occurred. However, non-recombinant subsp. *pauca* has not been found, although it seems probable that more thorough sampling away from agricultural areas will eventually isolate it. These examples highlight the important question of the consequences of gene flow on the emergence of *X. fastidiosa* diseases. We propose that the introduction of novel allelic diversity into countries/regions where *X. fastidiosa* is already present poses a significant risk and should be a major concern to regulatory bodies around the world.

In addition to host species switches induced by IHR, genetic exchange within subspecies occurs (Almeida et al. 2008; Nunney et al. 2013). This, together with IHR, may be highly relevant in determining the ability of *X. fastidiosa* to adapt to resistant plant genotypes. Specifically, the breeding programs that are developing resistant plant material for various *X. fastidiosa* hosts (notably wine grapes) should take into account the potential for *X. fastidiosa* to adapt. The groups of bacterial genes that are frequently exchanged and maintained in a population and those that are quickly purged have not been identified. Similarly, general patterns of short- and long-term genome evolution have so far not been analyzed. These are essential components for the robust deployment of resistant plant material, transgenic or not, as the strong selective pressure on *X. fastidiosa* populations due to the use of new technologies will eventually lead to the selection of novel pathogen variants that are capable of breaking down resistance. This process is equivalent to antibiotic resistance strains of human pathogens, such as tuberculosis, or loss of *Bacillus thuringiensis*-derived plant resistance to pests. Our argument is not that new technologies will not be successful; our argument is that the evolution of *X. fastidiosa* needs to be considered and incorporated into management practices aimed at prolonging the use of such plant lines. That, however, cannot be accomplished given the very superficial and limited knowledge currently available.

Last Thoughts

X. fastidiosa is no longer a plant pathogen limited to a few countries in the Western Hemisphere, where its geographical distribution ranges from Canada to Argentina. The long-term presence of *X. fastidiosa* in Taiwan raises questions about its potential distribution in Asia. Its introduction into Europe and a recent report from Iran will dramatically broaden its geographic range. Is this bacterium present elsewhere? And, as shown recently in Central America (Nunney et al. 2014b), how much of the genetic diversity of *X. fastidiosa* populations remains to be described? Old and unaddressed questions are now more relevant than ever, especially for Europe and the Mediterranean basin, where the host plant communities have, as far as we know, not been exposed to *X. fastidiosa*. One of the most relevant pending questions is what drives host specificity in this pathogen; in other words, why do pathogen genotypes cause disease in one plant species and not another, while still being able to colonize various plant species with different degrees of success but without inducing symptom expression? Finally, we still know very little about *X. fastidiosa* outside of its crop hosts. We are strong believers that much would be gained from studies of *X. fastidiosa* in natural environments, not only in regards to its biology, ecology, and evolution, but also on how to better manage diseases it causes in crops of economic importance.

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Rodrigo P. P. Almeida

Dr. Almeida first started working with *X. fastidiosa* in 1995 as an undergraduate student in the laboratory of Dr. João Lopes in Brazil, under whose supervision he obtained an M.Sc. degree in Entomology. In 2002, he was awarded a Ph.D. for his work studying *X. fastidiosa* vector transmission with Dr. Alexander Purcell. After three years as an assistant professor at the University of Hawaii at Manoa, Dr. Almeida was recruited by the University of California, Berkeley, where he is now an Associate Professor in the Department of Environmental Science, Policy and Management. Research interests in his group center on the biology, ecology, and evolution of insect-transmitted plant pathogens; although he has worked with various pathogens, his group today focuses on *X. fastidiosa* and grapevine ampeloviruses.

Leonard Nunney

Dr. Nunney is an evolutionary geneticist. He obtained his Ph.D. in genetics in 1977 from the University of Nottingham and held postdoctoral positions at the University of Edinburgh and Princeton University before taking a permanent position in the Biology Department at the University of California Riverside. He first started working on *X. fastidiosa* in 2002, attracted by the possibility of using genomic resources to understand its phylogeography and host specificity. The focus of his research group is the application of evolutionary theory to applied problems, which, in addition to work on *X. fastidiosa*, includes a study of the evolution of cancer suppression and research into the conservation biology problem of how to maximize the ability of small populations to adapt to a changing environment.

Maria Saponari

Dr. Saponari is a plant pathologist working on invasive plant pathogens of woody crops (olive and citrus). She obtained her Ph.D. in crop protection in 2001 from the University of Bari (Italy), and soon after she was recruited as researcher by the National Research Council (CNR). Her main interests are to develop innovative tools for the identification, detection, and quantification of plant pathogens, and to develop genomic information that can help to elucidate host-pathogen interactions. She has contributed to define the genetic relatedness of the CoDiRO strain to the *X. fastidiosa* subsp. *pauca* and to identify the first ascertained vector of *X. fastidiosa* in Italy (*Philaenus spumarius*).

Donato Boscia

Dr. Boscia is a plant virologist with nearly 30 years of experience in the study of some of the major virus and virus-like diseases of Mediterranean woody crops (mainly grapevine and stone fruits) and in the development of antisera and monoclonal antibodies for diagnosis and characterization of economically important plant viruses. Senior Scientist at the National Research Council of Italy, he is currently the head of the Unit of Bari of the Institute for Sustainable Plant Protection. In the last few years (since 2013) he moved his interest to investigations on the hotspot of *Xylella fastidiosa* associated with the quick decline of olive in Italy, for which he has contributed to the first identification of the bacterium and to the development of the main research program now ongoing in Apulia on *X. fastidiosa*.

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