

Pathogen profile

Xylella fastidiosa: an examination of a re-emerging plant pathogen[‡]

[‡]In memory of Professor Bruce Kirkpatrick, respected scholar, mentor and friend. You will always be missed.

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SUMMARY: *Xylella fastidiosa* is a Gram-negative bacterial plant pathogen with an extremely wide host range. This species has recently been resolved into subspecies that correlate with host specificity. This review focuses on the status of *X. fastidiosa* pathogenic associations in plant hosts in which the bacterium is either endemic or has been recently introduced. Plant diseases associated with *X. fastidiosa* have been documented for over a century, and much about what is known in the context of host–pathogen interactions is based on these hosts, such as grape and citrus, in which this pathogen has been well described. Recent attention has focused on newly emerging *X. fastidiosa* diseases, such as in olives.

TAXONOMY: Bacteria; Gammaproteobacteria; family *Xanthomonadaceae*; genus *Xylella*; species *fastidiosa*.

MICROBIOLOGICAL PROPERTIES: Gram-negative rod (0.25–0.35 × 0.9–3.5 μm), non-flagellate, motile via Type IV pili-mediated twitching, fastidious.

HOST RANGE: *Xylella fastidiosa* has a broad host range that includes ornamental, ecological and agricultural plants belonging to over 300 different species in 63 different families. To date, *X. fastidiosa* has been found to be pathogenic in over 100 plant species. In addition, it can establish non-symptomatic associations with many plants as a commensal endophyte. Here, we list the four distinct subspecies of *X. fastidiosa* and some of the agriculturally relevant diseases caused by them: *X. fastidiosa* ssp. *fastidiosa* causes Pierce's disease (PD) of grapevine (*Vitis vinifera*); *X. fastidiosa* ssp. *multiplex* causes almond leaf scorch (ALS) and diseases on other nut and shade tree crops; *X. fastidiosa* ssp. *pauca* causes citrus variegated chlorosis (CVC) (*Citrus* spp.), coffee leaf scorch and olive quick decline syndrome (OQDS) (*Olea europaea*); *X. fastidiosa* ssp. *sandyi* causes oleander leaf scorch (OLS) (*Nerium oleander*). Significant host specificity seemingly exists for some of the subspecies, although this could be a result of technical biases based on the limited number of plants tested,

whereas some subspecies are not as stringent in their host range and can infect several plant hosts.

DISEASE SYMPTOMS: Most *X. fastidiosa*-related diseases appear as marginal leaf necrosis and scorching of the leaves. In the case of PD, *X. fastidiosa* can also cause desiccation of berries (termed 'raisining'), irregular periderm development and abnormal abscission of petioles. In olive trees affected with OQDS, leaves exhibit marginal necrosis and defoliation, and overall tree decline occurs. Plants with ALS and OLS also exhibit the characteristic leaf scorch symptoms. Not all *X. fastidiosa*-related diseases exhibit the typical leaf scorch symptoms. These include CVC and Phony Peach disease, amongst others. In the case of CVC, symptoms include foliar wilt and interveinal chlorosis on the upper surfaces of the leaves (similar to zinc deficiency), which correspond to necrotic, gum-like regions on the undersides of the leaves. Additional symptoms of CVC include defoliation, dieback and hardening of fruits. Plants infected with Phony Peach disease exhibit a denser, more compact canopy (as a result of shortened internodes, darker green leaves and delayed leaf senescence), premature bloom and reduced fruit size. Some occlusions occur in the xylem vessels, but there are no foliar wilting, chlorosis or necrosis symptoms.

USEFUL WEBSITES: <http://www.piercesdisease.org/>; <https://pubmlst.org/xfastidiosa/>; <http://www.xylella.incc.br/>; <https://nature.berkeley.edu/xylella/>; https://ec.europa.eu/food/plant/plant_health_biosecurity/legislation/emergency_measures/xylella-fastidiosa_en

Keywords: citrus, grapevine, olive, Pierce's disease, tyloses, xylem.

INTRODUCTION

In the late 1800s, Newton B. Pierce described a severe grapevine disease occurring in Anaheim, California. This disease was initially called Anaheim disease (Pierce, 1892) and was first attributed to a viral infection because: (i) no causal microorganism could be

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cultured from the infected vines; and (ii) the disease was graft transmissible. However, electron micrographs indicated bacterial-like bodies in the xylem of infected plants (Hopkins and Mollenhauer, 1973), and a bacterium was subsequently cultured from infected plants (Davis *et al.*, 1978). Koch's postulates were then completed, ultimately confirming *Xylella fastidiosa* as the causal agent of what was eponymously named Pierce's disease (PD). In addition to PD, this bacterium has been implicated as the causal agent of many other significant plant diseases (Fig. 1). The severity of the citrus variegated chlorosis (CVC) epidemic in Brazil (Bove and Ayres, 2007) and PD epidemic in southern California (Siebert, 2001) prompted sequencing of the 9a5c CVC strain genome (Simpson *et al.*, 2000), followed by the Temecula 1 PD strain of *X. fastidiosa* (Van Sluys *et al.*, 2003). Notably, *X. fastidiosa* was the first plant-pathogenic bacterium to have its genome sequenced. Subsequently, genomes of other *X. fastidiosa* isolates have been sequenced (Chen *et al.*, 2010, 2016; Giampezzuzzi *et al.*, 2015; Guan *et al.*, 2014), providing a robust database for genome mining and comparative genomics.

Xylella fastidiosa colonizes two environments: the plant host xylem and the mouthparts of its insect vector. Much of what is known about *X. fastidiosa* biology has been performed with California isolates of the subspecies *fastidiosa*, the grapevine host and two prominent insect vectors in California: *Homalodisca vitripennis*, the glassy-winged sharpshooter (GWSS), and/or *Graphocephala atropunctata*, the blue-green sharpshooter (BGSS). Thus, the bulk of the literature base on *X. fastidiosa* is in the context of the PD pathosystem in California. What has been learned in the grape-GWSS/BGSS pathosystem may be applicable to other plant systems. However, we must be mindful that there may be important differences in the way in which *X. fastidiosa* interacts with plant hosts and insect vectors found in different environments. Thus, it may be difficult or impossible to extrapolate findings from one pathosystem to another, or one epidemic to another, without taking into consideration the ecological context of each scenario (Almeida and Nunney, 2015). This article focuses primarily on the PD pathosystem in California (Fig. 2).

Historically, *X. fastidiosa* was limited to the Americas; however, recently, it has been identified in Europe, most notably in olives (*Olea europaea*) in the Apulia region of southern Italy. The spread of *X. fastidiosa* and its associated diseases is primarily attributed to human activity that introduces infected plant material, or effective insect vectors, to a new region (Almeida and Nunney, 2015). There is also evidence which suggests that *X. fastidiosa* has a marked capacity to engage in interstrain recombination that can effectively result in new strains with different host ranges from the original parent strains (Nunney *et al.*, 2014).

HOST RANGE

Xylella fastidiosa's broad host range includes both monocots and dicots [European Food Safety Authority (EFSA), 2016]. Although

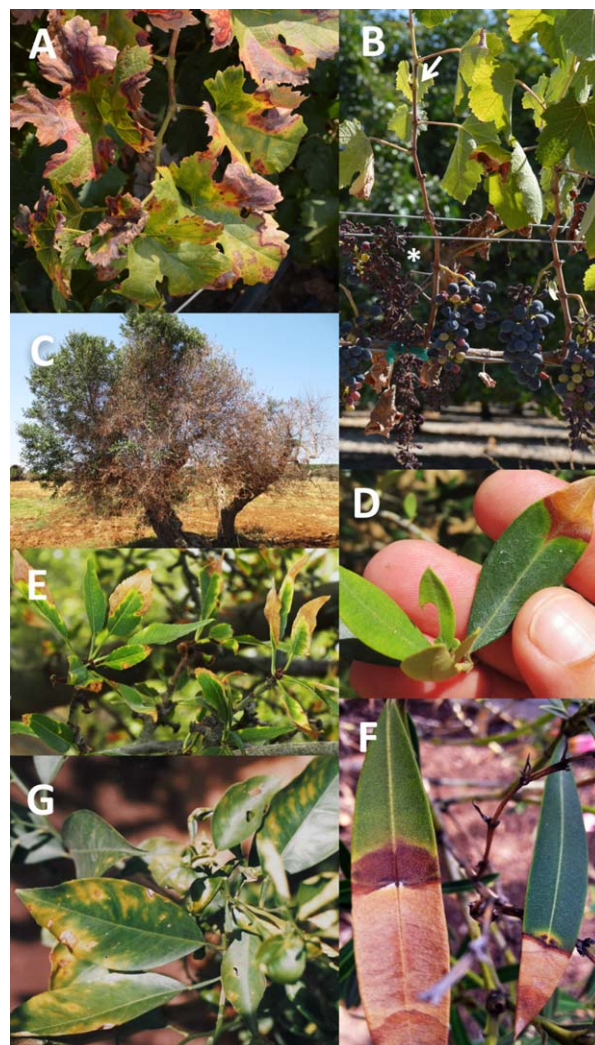


Fig. 1 Host symptoms associated with *Xylella fastidiosa* infection. (A) Leaf scorching. (B) Berry desiccation or 'raising' (asterisk) and green islands (white arrow) associated with Pierce's disease (PD). (C) Limb dieback and (D) foliar symptoms in olive quick decline syndrome (OQDS). (E) Foliar symptoms of almond leaf scorch. (F) Foliar symptoms of oleander leaf scorch (OLS). (G) Foliar symptoms of citrus variegated chlorosis (CVC). Photo credits: (A, B) Philippe Rolshausen, University of California, Riverside, CA, USA; (C, D, G) Rodrigo Kruegner, US Department of Agriculture Agricultural Research Service; (E) David Doll, University of California Cooperative Extension, Merced, CA, USA.

all *X. fastidiosa* isolates were initially assigned to the same species, they have been further classified to the subspecies level (Sally *et al.*, 2005; Schuenzel *et al.*, 2005). Three clades of *X. fastidiosa* have been identified in North America, corresponding to the different subspecies: *X. fastidiosa* ssp. *fastidiosa*, which is found in grapevines, almond and alfalfa; *X. fastidiosa* ssp. *multiplex*, which can be found in almond, peach, plum and oak; and *X. fastidiosa* ssp. *sandyi*, which, thus far, has only been found in

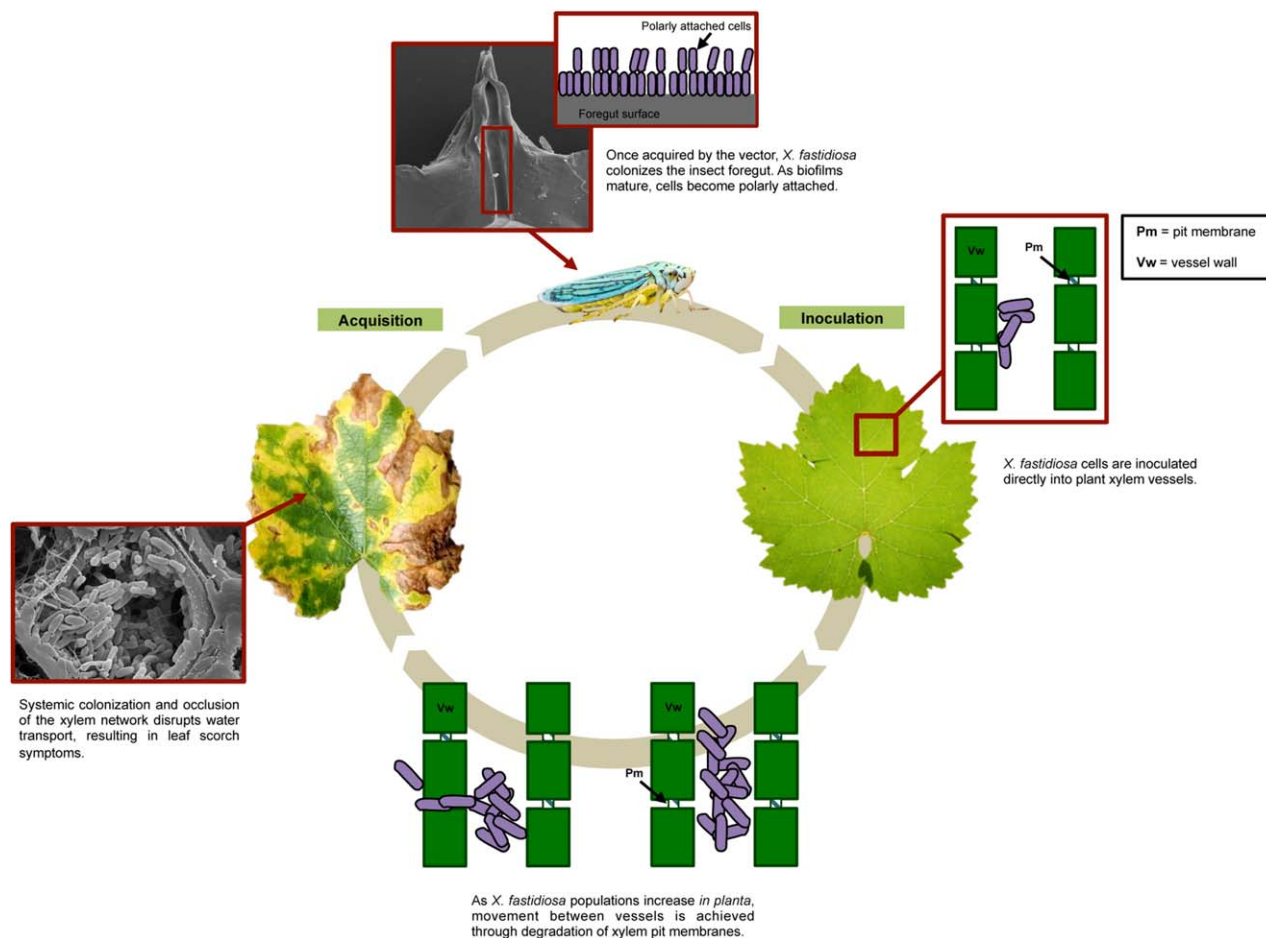


Fig. 2 Pierce's disease cycle.

oleander. *Xylella fastidiosa* ssp. *pauca* is another subspecies which is primarily found in citrus and coffee in South America (Sally *et al.*, 2005; Schuenzel *et al.*, 2005). The strain associated with olive quick decline syndrome (OQDS) in Italy, aptly referred to as the CoDiRo strain (an abbreviation of the Italian name for OQDS), is genetically related to the *pauca* subspecies (Giampetruzzi *et al.*, 2015; Marcelletti and Scortichini, 2016), and is the current subject of intense research focus on *X. fastidiosa* in Europe. Furthermore, the bacterium can reside in a wide range of plants as a harmless endophyte and has been detected in hundreds of asymptomatic plant species (Chatterjee *et al.*, 2008a; Hopkins and Purcell, 2002; Raju *et al.*, 1980). Most *X. fastidiosa* strains do not move systemically in symptomless hosts (Hill and Purcell, 1995a; Purcell and Saunders, 1999), but these plants could still serve as sources of inoculum (Hill and Purcell, 1997). Because of the large number of hosts, the list of plant species described provides prominent examples of plant hosts in which *X. fastidiosa* has a pathogenic interaction and is not meant to be a comprehensive list.

PIERCE'S DISEASE IN CALIFORNIA: AN EPIDEMIOLOGICAL CASE STUDY

Xylella fastidiosa is spread by xylem-feeding insect vectors belonging to the sharpshooter subfamily of leafhopper (Cicadellidae) and spittlebug (Cercopidae) families (Hewitt *et al.*, 1946). These vectors can feed on both woody and herbaceous hosts. There is no evidence of vector specificity; presumably, an implicated vector in any region is capable of transmitting any strain of *X. fastidiosa*, whether it is endemic or invasive (Almeida and Nunney, 2015). Because some sharpshooters fly long distances and feed on many different hosts, they can introduce *X. fastidiosa* into an array of host plants. *Xylella fastidiosa* has a persistent and propagative (but non-circulative) relationship in adult sharpshooter insects, which is the only described vector–microbe relationship of this sort. An adult insect remains infective throughout its life, whereas infective nymphs lose the ability to transmit when they moult and shed the cuticular lining of the foregut (Purcell and Finlay, 1979). These sharpshooters acquire *X. fastidiosa* by feeding on infected

hosts and can immediately transmit *X. fastidiosa* to a new plant host with no latent period (Fig. 2). A possible explanation for the absence of a latent period is the potential ability of sharpshooters to transiently hold a column of fluid containing *X. fastidiosa* cells in their stylets without swallowing it, allowing the fluid to be immediately egested during feeding (Backus *et al.*, 2015). Over time, the bacteria colonize the insect foregut (namely the cibarium and precibarium regions) by attaching to the cuticular lining and forming biofilms (Bransky *et al.*, 1983). Inoculation occurs through a combined egestion/salivation mechanism that dislodges the bacteria from the cuticular lining of the foregut.

Because the bulk of the epidemiological data has been collected in the context of PD in California, this section focuses primarily on the dynamics of PD epidemiology in California as a case study, with the caveat that the epidemiology of *X. fastidiosa* diseases in other areas may not mirror that of PD in California. Vector and strain behaviour and differences in climate and plant species must be taken into account in other ecological contexts. PD has been in California for over a century, but it spread slowly and did not cause significant damage in most viticultural areas of the state. However, in the late 1990s, PD became an acute concern in California. To understand why the disease incidence increased to epidemic levels, one must consider the behaviour of the insect vectors. Historically, BGSS was the most important vector in California coastal vineyards, whereas the red-headed (*Carneocephala fulgida*) and green (*Draeculacephala minerva*) sharpshooters were most significant in the San Joaquin Valley (Hewitt *et al.*, 1949). These native vectors overwinter in riparian habitats (e.g. stream banks) and, in the spring and summer, move into vineyards where they feed on grapevines. Native sharpshooters exclusively feed on succulent new growth (Purcell, 1975). Once in the plant, the bacteria can move acropetally from the point of inoculation into new growth, as well as basipetally down to the vine cordon and trunk, where they overwinter. Infections that occur in spring or early summer become chronic because the bacteria have adequate time to migrate to these permanent parts of the vine (i.e. the cordon and trunk are not pruned annually). Infections occurring late in the season do not have a chance to become chronic because severe winter pruning removes infected parts of the vine before *X. fastidiosa* can migrate into the cordon to overwinter.

Because they fly from their preferred habitats only into the edge of vineyards, native sharpshooters probably do not spread PD from one vine to another (Purcell, 1974, 1975). In the northern coastal regions of California, PD persists mainly in 'PD hot spots' along parts of the vineyard adjacent to riparian areas. The dominant vector in this region is the native BGSS, which enters vineyards from these riparian areas in the spring and summer, following overwintering, to feed and reproduce. PD incidence is highest in these hot spots, creating what is known as the PD

'edge effect' (Hopkins and Purcell, 2002), meaning that infection remains primarily along the edges of the vineyard. The lack of spread within vineyards and limited persistence in vines from year to year were essential features of PD epidemiology, because this limited occurrence and distribution of the disease (Hopkins and Purcell, 2002). Under these conditions, PD is considered to be a monocyclic disease.

The epidemiology of PD changed drastically in the southern regions of California as a result of the introduction of the polyphagous GWSS. It is hypothesized that GWSS was introduced into California from Texas on nursery stock. GWSS and *X. fastidiosa* are endemic to Texas and the southeastern USA, and are the primary reasons why these regions lack a major *Vitis vinifera* grape industry. GWSS was first spotted in 1990 in southern California. Soon after, PD reached epidemic proportions, causing millions of dollars of damage to Temecula Valley vineyards (Blua *et al.*, 1999). Furthermore, the presence of GWSS brought about a new *X. fastidiosa*-related disease in southern California called oleander leaf scorch (OLS). The disease was first noticed in the Palm Springs–Indio area of Riverside County in 1994 and spread to adjacent areas in the following years (Blua *et al.*, 1999).

This change in PD epidemiology is a result of differences in the feeding, flying and breeding behaviour of GWSS. Unlike native sharpshooters, GWSS can feed on both succulent new growth and woody stems. Woody stems are closer to the cordons and main trunk, and bacteria introduced here require less time to reach the cordon and trunk to overwinter. GWSS can also feed on dormant vines, which can introduce bacteria directly into the permanent part of the vine. Therefore, the feeding behaviour of GWSS increases the incidence of chronic infections. In addition, GWSS is a much stronger flier than the native sharpshooters and can fly faster and further into the vineyard. The final property of GWSS that contributes to the increase in PD is its breeding behaviour. In contrast with native sharpshooters, which preferentially feed and reproduce on riparian plants, GWSS can reproduce on numerous plant species, with special preference for citrus. Citrus groves support enormous populations of these insects and, in southern California, many citrus groves are planted adjacent to vineyards (Perring *et al.*, 2001). This unfortunate situation allows GWSS easy access to vineyards and increases the spread and incidence of PD. With the establishment of GWSS, PD became so severe in southern California that it threatened to eliminate viticulture in some areas. Its potential impact in central and northern grape-growing regions of California remains unknown. Most of the economically important diseases caused by *X. fastidiosa* occur mainly in parts of North America that do not experience cold winters (Hopkins and Purcell, 2002). Therefore, if GWSS becomes established in northern California, the incidence and distribution of PD could reach the same levels as experienced in the southern part of the state.

EPIDEMIOLOGY OF *X. FASTIDIOSA* IN EUROPE

Xylella fastidiosa was recently identified in olive trees affected with OQDS in Salento (Lecce district) in the Apulia region in southern Italy. The disease has rapidly spread throughout the region. This represents the first confirmed detection of *X. fastidiosa* in the European Union (EU) (Loconsole *et al.*, 2014). An understanding of OQDS epidemiology is just emerging, but, thus far, the meadow spittlebug, *Philaenus spumarius*, has been identified as the primary insect vector (Saponari *et al.*, 2014). Based on multi-locus sequence tagging analyses, the strain found in Italy is most closely related to subspecies *pauca* and was probably introduced in contaminated plant material originating from Costa Rica (EFSA Panel on Plant Health, 2015; EFSA, 2016). *Xylella fastidiosa* is a regulated quarantine pathogen in the EU and, since 2015, coffee plant imports from Costa Rica and Honduras have been forbidden (Almeida, 2016). Following the initiation of a monitoring programme, *X. fastidiosa* has also been identified in Corsica, Mallorca and the south of France, although there has not been a widespread epidemic in these areas (Almeida, 2016). Three isolates were obtained in France from coffee plants propagated in glasshouses. One of the isolates was characterized as being closely related to both the *sandyi* and *fastidiosa* subspecies. The other two isolates were most closely related to subspecies *pauca* implicated in OQDS in Apulia, but with some divergence (Jacques *et al.*, 2015). In Corsica and the south of France, isolates were found to be different from subspecies *pauca* (Almeida, 2016). Following regulations set forth by the EU, countries infested with *X. fastidiosa* have been directed to eradicate infected plant material and suppress insect populations. However, because of the widespread epidemic in Apulia and the geographical distribution of olive plantings, complete eradication is probably not possible in this area (Strona *et al.*, 2017). Moreover, the implementation of these regulations in Italy has proven to be difficult because of a cultural and scientific gridlock that has occurred, caused by the cultural significance of olive trees in Italy (Abbott, 2015; Almeida, 2016; Stokstad, 2015). OQDS threatens to not only cause enormous economic damage to the olive industry, but to also reshape the cultural landscape in Italy's olive-growing regions. The list of additional vectors and plant hosts in southern Italy is unknown, and epidemiological modelling indicates that southern Italy is becoming a reservoir for *X. fastidiosa* (Strona *et al.*, 2017). All known xylem-feeding insects in Europe are considered as potential vectors of the bacterium (EFSA Panel on Plant Health, 2015), further reiterating the risk of *X. fastidiosa* spread into other countries and plant hosts in Europe.

ECONOMIC IMPACT

Because *X. fastidiosa* causes diseases in economically valuable crops, the occurrence of these epidemics is typically accompanied by substantial economic consequences. The California table, raisin and wine grape industries were valued at \$4.95 billion in 2015

[California Department of Food and Agriculture (CDFA), 2015]. Following the introduction of GWSS into southern California, losses were estimated to be \$37.9 million annually (Siebert, 2001). Despite the presence of the PD/GWSS Control Program, established in 2001, PD costs producers over \$100 million per year in crop loss and replanting expenses. This amount excludes an additional \$50 million per year spent on preventative measures, including vector control (Alston *et al.*, 2015). Alston *et al.* (2013) evaluated the costs and benefits of PD research in the wine grape industry, and estimated that PD would cost an additional \$189 million per year if the PD Control Program (PDCP) ended. Therefore, the benefits of PD research clearly outweigh the costs in terms of preventing another major outbreak.

Similar to the effect of PD on commercial grape production, CVC affects all major commercial sweet orange cultivars (Goncalves *et al.*, 2012). It is considered to be one of the most important diseases affecting the Brazilian citrus industry, which accounts for 30% of sweet orange production and 85% of exports of frozen orange juice concentrate worldwide (Goncalves *et al.*, 2014; Rodrigues *et al.*, 2013). Currently, 40% of citrus plants in Brazil are affected by CVC, and economic losses caused by the disease can reach \$120 million annually (Goncalves *et al.*, 2012, 2014).

The recent outbreak of OQDS in Italy presents a formidable threat to European agriculture, with olive farming in southern Italy's Apulia region accounting for nearly 40% of Italy's olive oil production (Strona *et al.*, 2017). EFSA recently stated that the dissemination of *X. fastidiosa* to other European countries is very likely, as many other olive-growing regions (e.g. Spain, Portugal, Greece) match the climatic conditions of Apulia (Luvisi *et al.*, 2017). Currently, nearly 10 000 ha of olive trees have been destroyed, which amounts to millions of dollars in damage (Martelli *et al.*, 2016). Thus, the identification and evaluation of risk reduction options are crucial (Pereira, 2015).

HOST COLONIZATION

Generally speaking, the ability to form a robust surface-attached community (biofilm) directly correlates with virulence for many bacterial pathogens (Danhorn and Fuqua, 2007). The formation of a biofilm plays an important role in the colonization of the insect and plant environments for *X. fastidiosa*. The surface in this case is either the wall of the xylem (Fig. 3A) or the cuticular lining of the insect foregut. *Xylella fastidiosa* has never been found outside of the plant host or its insect vector. In the insect environment, it is clear that the attachment and formation of biofilm-like structures within the foregut are tightly associated with the acquisition, retention and, ultimately, transmission of *X. fastidiosa* by sharpshooters (Killiny and Almeida, 2014). However, the role of biofilm formation in the plant environment is much more nuanced. In heavily infected plants, *X. fastidiosa* biofilms are abundant (Fig. 3A) (Newman *et al.*, 2003; Roper *et al.*, 2007a); thus, attachment to

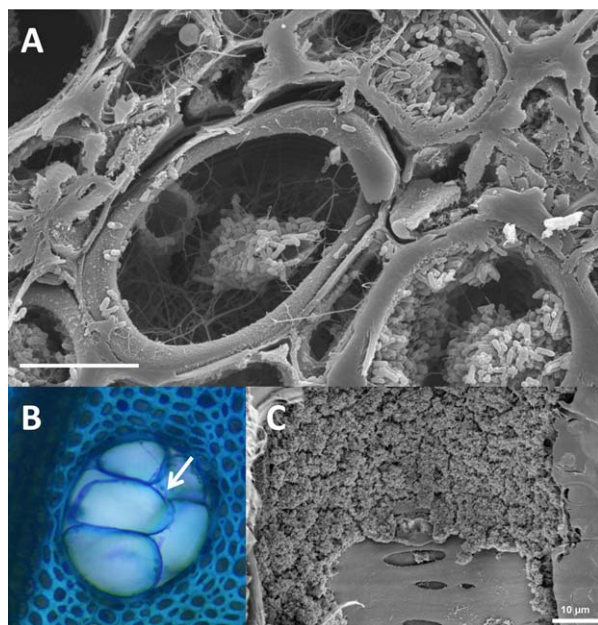


Fig. 3 Vascular occlusions and depositions associated with Pierce's disease (PD) in grapevine. (A) Bacterial aggregates in xylem of petioles. (B) Tyloses fully occluding grapevine xylem (white arrow). (C) Amorphous gels coating xylem vessel walls in PD-infected grapevines. Photo credit: (C) Qiang Sun, University of Wisconsin, Madison, WI, USA.

the xylem wall is a key aspect of establishment *in planta*. However, to move systemically in the plant, the bacteria must enter an exploratory, motile state. There are several examples of mutants that are defective in surface/cell–cell attachment, but display hypervirulent phenotypes (Guilhabert and Kirkpatrick, 2005). It has been proposed that *X. fastidiosa* forms biofilms partially to attenuate its own virulence by controlling rampant systemic colonization. Logically, it would benefit the bacterium not to kill its host immediately, and this model also explains how *X. fastidiosa* can exist as a commensal endophyte in many plant species (Chatterjee *et al.*, 2008a; Guilhabert and Kirkpatrick, 2005; Roper and Lindow, 2014). To be effectively acquired from the plant by the insect, the bacterium must be in the adhesive state. Taken together, this requires that *X. fastidiosa* fluctuate between an exploratory (non-adhesive) state and an insect-acquirable (adhesive) state. In this article, we focus on the latest information regarding the factors governing plant and insect colonization. [For previous reviews on the topic of *X. fastidiosa* plant and insect colonization, particularly with regard to adhesion, see Chatterjee *et al.* (2008a) and Roper and Lindow (2014).]

Plant colonization

Xylella fastidiosa produces prolific amounts of outer membrane vesicles (OMVs) relative to other bacterial species. For *X. fastidiosa*, OMVs hinder attachment to glass and xylem

surfaces, suggesting that they serve to prevent attachment during the exploratory phase of xylem colonization. Blocking attachment is a novel function described for OMVs in bacteria (Ionescu *et al.*, 2014). Afimbrial haemagglutinin adhesins, HxfA and HxfB, facilitate cell–cell attachment (Guilhabert and Kirkpatrick, 2005) and are found in both the outer membrane of *X. fastidiosa* cells and as part of OMVs that are shed from the cells (Voegel *et al.*, 2010). Loss-of-function mutations in both the *hxfA* and *hxfB* genes result in hypervirulent phenotypes after inoculation into grapevines, supporting the hypothesis that *X. fastidiosa* uses adhesion to control the dynamics of systemic colonization to attenuate its own virulence. XatA, an autotransported protein related to surface adhesion and cell–cell aggregation, is also found in OMVs (Matsumoto *et al.*, 2012), but a knockout mutation in *xatA* resulted in a significant decrease in virulence, rather than the hypervirulent phenotype that resulted from mutations in the haemagglutinin adhesins. It is tempting to speculate that the alteration of OMV composition and OMV production is a mechanism used by *X. fastidiosa* to adjust its adhesiveness during plant colonization to either facilitate systemic plant movement or enable insect acquisition.

Non-proteinaceous, cell surface, carbohydrate-based molecules are also important for the association of *X. fastidiosa* with plants, specifically lipopolysaccharide (LPS) and exopolysaccharide (EPS). The LPS molecule comprises a conserved lipid A-core oligosaccharide component and a variable O-antigen. Truncation of the long-chain, two-linked, rhamnose-rich O-antigen affects the net surface charge (measured as the zeta potential) of the *X. fastidiosa* cell and has a significant impact on surface attachment and cell–cell aggregation in both the plant and insect environments (Clifford *et al.*, 2013; Rapicavoli *et al.*, 2015). In plants, it was deduced that truncation of the O-antigen caused a hyperattaching phenotype, but a defect in cell–cell attachment. The consequences were cells that could attach to a proxy plant surface (glass), but were unable to build upon themselves to form a robust biofilm. This resulted in a thinner and topographically rougher biofilm than the wild-type *X. fastidiosa* biofilm. Furthermore, this truncation severely impaired virulence and colonization in grapevine (Clifford *et al.*, 2013).

Xylella fastidiosa produces an EPS, termed fastidian gum. Fastidian gum is predicted to be similar in structure to xanthan gum (da Silva *et al.*, 2001), but the polymer has yet to be fully characterized. Spatiotemporal localization of EPS during the different stages of biofilm formation indicated that there is very little EPS associated with the initial biofilm stages (microcolony formation), but EPS can be seen intercalating the mature biofilm structure. Thus, EPS provides a greater contribution to the maintenance of three-dimensional biofilm architecture, and stability of the mature biofilm, as opposed to initial surface attachment (Roper *et al.*, 2007a). Findings by Souza *et al.* (2006) corroborate this, as EPS mutants in the CVC strain of *X. fastidiosa* were not affected in

surface attachment, but showed a reduced capacity to form biofilms. Immunolocalization in PD-infected grapevines showed that an EPS matrix was associated with *X. fastidiosa* cell aggregates in the plant xylem vessels, where it also contributed to xylem vessel occlusion (Roper *et al.*, 2007a). *Xylella fastidiosa* mutants compromised in EPS production showed reduced virulence in grapes (Killiny *et al.*, 2013).

Systemic movement *in planta*

The xylem in grapevines comprises many interconnected vessels that are finite, but variable in length, and any one vessel rarely spans the entire length of the stem (Thorne *et al.*, 2006b; Tyree and Zimmermann, 2002). These xylem vessels are connected to each other by scalariform bordered pits that separate vessel elements from one another via pit membranes, which are composed of primary plant cell wall material (cellulose microfibrils embedded in a meshwork of hemicellulose and pectin) (Buchanan *et al.*, 2000; Choat *et al.*, 2004). The pores within the pit membrane range from 5 to 20 nm in diameter and allow for the flow of water and small solutes from one vessel to another, but block air embolisms and pathogen movement (Stevenson *et al.*, 2004; Tyree and Zimmermann, 2002). *Xylella fastidiosa* is too large to passively move through the pores of the pit membranes (Mollenhauer and Hopkins, 1974), and uses enzymatic degradation of the pit membranes as its primary means of achieving systemic colonization. *Xylella fastidiosa* utilizes an endo-polygalacturonase (endo-PG) to digest polymers with a pectin backbone and requires this endo-PG to systemically colonize grapevines. A deletion mutation in the single copy gene encoding endo-PG resulted in a complete loss of pathogenicity (Roper *et al.*, 2007b). Interestingly, effective degradation of the pit membranes requires a concerted effort with an endo-PG and at least one endoglucanase (EGase), EngXCA2, indicating that effective degradation of the plant cell wall meshwork is a cooperative effort between at least two classes of cell wall-degrading enzyme (CWDE) (Perez-Donoso *et al.*, 2010). EngXCA2 is a prototypical EGase that degrades both carboxymethylcellulose and xyloglucan substrates (Perez-Donoso *et al.*, 2010). *engXCA1* (PD1856) and *egl* (PD2061) putatively encode two additional EGases, but these have not been fully characterized. *Xylella fastidiosa* also possesses three genes that encode S8 subtilisin-like serine protease autotransporters (PD0218, PD0313 and PD0950) (Tripathi and Sowdhamini, 2008), and their role in systemic movement *in planta* is still being investigated.

Xylella fastidiosa lacks flagella, but uses Type IV pili as its only currently known means of motility in the xylem. This surface-based motility allows the bacterium to migrate basipetally against the flow of xylem sap, which is quite remarkable given the high shear rate of fluid in the xylem that can occur during plant transpiration (De La Fuente *et al.*, 2007; Meng *et al.*, 2005). Calcium enhances the twitching motility via PilY1, a Type IV pilus structural protein. It

has been proposed that calcium concentrations help to regulate the twitching motility in the xylem as ionic concentrations of calcium fluctuate in the xylem sap (Cruz *et al.*, 2012, 2014). Movement in the acropetal position could be entirely passive if the bacterium uses the transpiration stream to move towards the tip of the plant.

Regulation of host colonization

It is estimated that only 10%–15% of the vessels are heavily colonized by *X. fastidiosa* (Newman *et al.*, 2003) and that the majority of colonized vessels contain only a few cells. Too many cells in one vessel would block or limit the flow of xylem sap in that particular vessel and, presumably, the nutrients necessary for bacterial survival (Newman *et al.*, 2003). The current model of cell density-regulated host colonization (Chatterjee *et al.*, 2008a) indicates that, when vessels become heavily occluded, the Rpf high cell density system down-regulates genes conducive to systemic spread (i.e. motility and CWDEs) via the accumulation of a diffusible signalling factor (DSF) (Newman *et al.*, 2004). This accumulation of DSF activates the RPF signalling system and enhances the production of various adhesins that increase cell 'stickiness' (Chatterjee *et al.*, 2008a). This would allow the cells in heavily occluded vessels to enter the insect-acquirable state to increase the chances of acquisition and, subsequently, transmission to a new plant by an insect vector. OMV production, which is also linked to adhesiveness, is regulated by cyclic di-guanosine monophosphate (di-GMP) signalling via the Rpf DSF (Ionescu *et al.*, 2014). Disruption of the Rpf system results in hyper-vesiculation (Ionescu *et al.*, 2014), making *X. fastidiosa* less adhesive and, subsequently, hypervirulent *in planta* (Newman *et al.*, 2004). [Please see Chatterjee *et al.* (2008b) and Roper and Lindow (2014) for reviews on the gene regulation hierarchy in *X. fastidiosa*.]

Insect colonization (acquisition and transmission)

Xylella fastidiosa is the only known insect-transmitted plant pathogen that is persistent within the sharpshooter vector, but non-circulative (Backus and Morgan, 2011). It is semi-persistent in nymphs, i.e. the bacterium is lost after each vector moult, but persistent in non-moulting adult vectors (Backus and Morgan, 2011; Chatterjee *et al.*, 2008a). *Xylella fastidiosa* is also propagative within vectors, allowing the insects to transmit the pathogen for months after acquisition from an infected plant (Hill and Purcell, 1995b). There is no evidence of trans-stadial or trans-ovarial transmission, meaning that the pathogen does not transfer through insect life stages or to offspring, respectively (Janse and Obradovic, 2010). In contrast with *X. fastidiosa* strain specificity in plant hosts, there is a lack of vector–*X. fastidiosa* strain specificity (Damsteegt *et al.*, 2006).

The transmission of *X. fastidiosa* consists of three steps: (i) pathogen acquisition from an infected host plant; (ii) retention of the pathogen within the vector; and (iii) transmission of the

pathogen to a susceptible plant host (Killiny and Almeida, 2014). Vectors can acquire *X. fastidiosa* and immediately transmit it to a new host, indicating that there is no latent period required for transmission (Purcell and Finlay, 1979). On acquisition, *X. fastidiosa* attaches to and forms biofilms within the foregut of the insect (Killiny and Almeida, 2009, 2014). Cells initially attach laterally, which increases the cell surface area in contact with the insect cuticle. In the later stages of biofilm formation, the cells become polarly attached to the foregut cuticle, presumably to allow for maximal cell surface exposure to the nutrient-dilute xylem sap (Almeida and Purcell, 2006; Chatterjee *et al.*, 2008a; Killiny and Almeida, 2009). *Xylella fastidiosa* colonizes two major regions of the foregut: the precibarium and the cibarium. Although the cibarium is probably the reservoir for bacterial inoculum within the foregut, transient colonization of the precibarium is associated with the transmission of *X. fastidiosa* to plants (Almeida and Purcell, 2006; Backus and Morgan, 2011; Rapicavoli *et al.*, 2015).

The vector foregut is an extremely turbulent environment, as xylem sap flow rates have been estimated to reach nearly 8 cm/s (Retchless *et al.*, 2014). Therefore, attachment to the foregut is not a trivial process, and relies on numerous factors at the vector–pathogen interface. Several surface-associated bacterial components, such as Type I pili, haemagglutinin adhesins and fimbriae, contribute to the adhesive properties of *X. fastidiosa* that are necessary for colonization in insect vectors (Chatterjee *et al.*, 2008b; Feil *et al.*, 2007; Killiny and Almeida, 2009; Newman *et al.*, 2004). In addition, cell surface polysaccharides, namely LPS and EPS, also play a role in the acquisition process. The major surface-associated polysaccharide, LPS, contributes to the early stages of acquisition of *X. fastidiosa* by BGSS. A mutant strain with a truncated O-antigen was impaired in attachment to the cuticular lining of the insect foregut, and thus acquisition of the pathogen was disrupted (Rapicavoli *et al.*, 2015). EPS has also been implicated in the insect transmission of *X. fastidiosa* by BGSS, as mutants impaired in EPS production were poorly transmitted (Killiny *et al.*, 2013). It is not clear whether this inefficiency is a result of defects in the initial acquisition of *X. fastidiosa* or the retention of cells over time.

SECRETION SYSTEMS

Unlike many other phytopathogenic bacteria, *X. fastidiosa* does not possess a Type III secretion system, a system commonly used to secrete protein effectors that enhance virulence and/or suppress plant defences (Dow and Daniels, 2000). However, *X. fastidiosa* does possess operons containing the genes necessary to produce components of the Type I, II, IV and V secretion systems (Simpson *et al.*, 2000; Van Sluys *et al.*, 2003). The Type I secretion system is a general secretory pathway that has many functions, including multidrug resistance efflux and the secretion of various proteases,

hydrolases and toxins (Delepelaire, 2004). The Type I translocator channel is ATP dependent and comprises two inner membrane proteins that attach to TolC, a periplasm/outer membrane protein which is highly conserved amongst many phytopathogenic bacteria (Holland *et al.*, 2005). A mutation in *tolC* caused a loss of pathogenicity (Reddy *et al.*, 2007). However, bacteria could not be isolated from grapevines inoculated with the mutant strain, suggesting that the *tolC* mutation is lethal *in planta*. The mutant strain also displayed a high level of sensitivity to several phytochemicals, detergents and crude plant extracts, highlighting the necessity of TolC for multidrug efflux and the viability of *X. fastidiosa* in plants.

Xylella fastidiosa possesses a 12-protein Type II secretion system (T2SS) that is closely related to those found in *Xanthomonas campestris* pv. *campestris* and *Xanthomonas oryzae* pv. *oryzae* (Jha *et al.*, 2005). The T2SS is made up of three parts: the outer membrane pore, the inner membrane complex and the pseudopilin complex (Sandkvist, 2001). Pseudopilin formation is driven by XpsE, an ATPase that facilitates the stacking of pseudopilin subunits that push the loaded protein out into the extracellular environment (Jha *et al.*, 2005). Proteins secreted by T2SS are often proteases and plant CWDEs that utilize a secretion signal to direct their transport (Sandkvist, 2001). The majority of the proteases and CWDEs in *X. fastidiosa* are predicted to be secreted via the T2SS. Mutations in *xpsE* in *X. fastidiosa* cause a non-pathogenic phenotype similar to the *pglA* mutant (M. C. Roper, unpublished data), indicating a strong connection between T2SS and the secretion of critical CWDEs in *X. fastidiosa*.

The Type V secretion system comprises a family of autotransporter proteins that transport themselves out of the cell without requiring any energy input (van Ulsen *et al.*, 2014). *Xylella fastidiosa* has six genes predicted to encode members of the AT-1 autotransporter family, one of which is XatA (Matsumoto *et al.*, 2012). Its C-terminal helper domain interacts with the bacterial outer membrane and with OMVs, and its passenger domain is released into the extracellular environment. A *xatA* mutant formed smaller bacterial aggregates and produced less biofilm relative to the wild-type parent strain, indicating an important role for XatA in *X. fastidiosa* adhesion. When inoculated into grapevines, the mutant strain did not induce symptoms of PD, or systemically colonize plants, and was compromised in colonization at the point of inoculation (Matsumoto *et al.*, 2012). The Type IV secretion system in *X. fastidiosa* has not been characterized and currently nothing is known regarding its function.

A list of known *X. fastidiosa* virulence/pathogenicity factors can be found in Table 1.

PLANT HOST RESPONSES TO *X. FASTIDIOSA*

The obstruction of xylem vessels by gums and tyloses is a common plant response designed to restrict the movement and

Table 1 List of virulence/pathogenicity factors for *Xylella fastidiosa*.

PD number	Gene	Function	Mutant phenotype in virulence/pathogenicity	Reference(s)
Adhesins				
PD0058	<i>fimF</i>	Fimbrial adhesin: cell–cell aggregation, biofilm formation	Reduced	Feil <i>et al.</i> (2007)
PD0062	<i>fimA</i>	Fimbrial adhesin: cell–cell aggregation, biofilm formation	Reduced	Feil <i>et al.</i> (2007)
PD0731	<i>xadA</i>	Afimbrial adhesin: cell surface attachment, biofilm formation	Reduced	Feil <i>et al.</i> (2007)
PD0986		Putative haemagglutinin-like protein	Reduced	Zhang <i>et al.</i> (2015)
PD1792	<i>hxfB</i>	Haemagglutinin: cell–cell aggregation	Reduced Enhanced	Feil <i>et al.</i> (2007) Guilhbert and Kirkpatrick (2005)
PD2118	<i>hxA</i>	Haemagglutinin: cell–cell aggregation	Enhanced	Guilhbert and Kirkpatrick (2005)
Quorum sensing				
PD0233	<i>rpfB</i>	DSF synthesis, quorum sensing	Reduced	Almeida <i>et al.</i> (2012)
PD0279	<i>cgsA</i>	Cyclic di-GMP synthase A, quorum sensing	Reduced	Chatterjee <i>et al.</i> (2010)
PD0406	<i>rpfC</i>	Quorum sensing	Reduced	Chatterjee <i>et al.</i> (2008b)
PD0407	<i>rpfF</i>	Quorum sensing	Enhanced	Chatterjee <i>et al.</i> (2008b)
Secretion systems				
PD0732	<i>xpsE</i>	Type II secretion system: ATPase	Loss	M. C. Roper (unpublished data)
PD1964	<i>tolC</i>	Type I secretion system	Loss	Reddy <i>et al.</i> (2007)
Polysaccharide synthesis				
PD0814	<i>wzy</i>	Lipopolysaccharide synthesis	Reduced	Clifford <i>et al.</i> (2013)
PD1391	<i>gumH</i>	Exopolysaccharide synthesis	Loss	Killiny <i>et al.</i> (2013)
PD1394	<i>gumD</i>	Exopolysaccharide synthesis	Loss	Killiny <i>et al.</i> (2013)
Hydrolytic enzymes				
PD0956	<i>prtA</i>	Serine protease	Enhanced	Gouran <i>et al.</i> (2016)
PD1485	<i>pglA</i>	Polygalacturonase: pit membrane degradation	Loss	Roper <i>et al.</i> (2007b)
PD1703	<i>lesA/lipA</i>	Lipase/esterase	Reduced	Zhang <i>et al.</i> (2015) and Nascimento <i>et al.</i> (2016)
PD1826	<i>chiA</i>	Chitinase	Loss	Labroussaa <i>et al.</i> (2017)
PD1851	<i>engXCA2</i>	Endoglucanase: pit membrane degradation	Reduced	M. C. Roper (unpublished data)
PD1856	<i>engXCA1</i>	Endoglucanase: pit membrane degradation	Reduced	M. C. Roper (unpublished data)
Membrane proteins				
PD0528	<i>xatA</i>	Cell–cell aggregation, biofilm formation	Reduced	Matsumoto <i>et al.</i> (2012)
PD0843	<i>tonB1</i>	Iron and vitamin B12 transport	Reduced	Cursino <i>et al.</i> (2009)
Stress response				
PD1380	<i>csp1</i>	Cold shock protein	Reduced	Burbank and Stenger (2016)
Metabolism				
PD1311		Acyl-coenzyme A (acyl-CoA) synthetase	Loss	Hao <i>et al.</i> (2017)
Toxins				
PD0928	<i>zot</i>	Putative Zot-like toxin	Reduced	Zhang <i>et al.</i> (2015)
Regulatory systems				
PD0848	<i>pill</i>	Type IV pilus: regulates twitching	Reduced	Cursino <i>et al.</i> (2011)
PD1099	<i>dinJ/relE</i>	Toxin–anti-toxin system, regulatory system	Enhanced	Burbank and Stenger (2017)
PD1100				
PD1284	<i>algU</i>	Alternate sigma factor: pathogenicity-related genes, stress response genes	Reduced	Shi <i>et al.</i> (2007)
PD1386	<i>xhpT</i>	Two-component regulatory system: pathogenicity-related genes, EPS synthesis	Reduced	Voegel <i>et al.</i> (2013)
PD1671		Two-component regulatory system: pathogenicity-related genes	Enhanced	Cursino <i>et al.</i> (2015)
PD1678	<i>phoQ</i>	Two-component regulatory system: pathogenicity-related genes, survival genes	Loss	Pierce and Kirkpatrick (2015)
PD1679	<i>phoP</i>	Two-component regulatory system: pathogenicity-related genes, survival genes	Loss	Pierce and Kirkpatrick (2015)
PD1984	<i>gacA</i>	Two-component regulatory system: pathogenicity-related genes	Reduced	Shi <i>et al.</i> (2009)

DSF, diffusible signalling factor; EPS, exopolysaccharide; GMP, guanosine monophosphate.

systemic infection of vascular pathogens (Clerivet *et al.*, 2000; Sun *et al.*, 2008; Tyree and Zimmermann, 2002; Yadeta and Thomma, 2013). Tyloses are outgrowths of xylem-associated

parenchyma cell walls into the neighbouring vessel and are found in abundance in the late stages of *X. fastidiosa* infection (when disease symptoms are evident) (Fig. 3B). Tyloses result from the

weakening of pit membranes by the activity of CWDEs in the vessel lumen and/or the realignment of new cellulose microfibrils that are being added to the plant cell wall as it expands in the presence of ethylene (Esau, 1977). High ethylene levels have been reported in grape tissues infected with *X. fastidiosa* (Perez-Donoso *et al.*, 2007; Sun *et al.*, 2007), which can partially explain the extensive induction of tylose formation during PD. Amorphous gels also occur in the xylem of PD-infected grapevine (Fig. 3C) and it is presumed that these are of plant origin.

Tyloses can efficiently block *X. fastidiosa* movement in the stem vasculature and prevent systemic infection if the disassembly of pit membranes in the vessel–xylem parenchyma interface occurs more rapidly than the breakdown of the intervessel pit membrane system (Sun *et al.*, 2013). Tyloses form more rapidly in PD-resistant species of grapevine, such as species of native muscadine and wild grapevines (i.e. *V. rotundifolia* and *V. arizonica*, respectively), than in susceptible species (*V. vinifera*). The formation of these occlusions restricts the movement of *X. fastidiosa* into vessel segments beyond the inoculation point (Fry and Milholland, 1990a,b; Mollenhauer and Hopkins, 1976; Sun *et al.*, 2013). Conversely, it appears that the enzymatic breakdown of pit membranes facilitated by *X. fastidiosa* takes place more quickly in the PD-susceptible genotype, which allows the pathogen to move systemically before tyloses seal the vessels. Although tylose formation could be a successful mechanism for the restriction of bacterial spread, it has the negative side effect of blocking water flux within the affected xylem vessel (Stevenson *et al.*, 2004). Indeed, whether tyloses in *X. fastidiosa*-infected grapevines contribute to disease resistance (i.e. prevention of pathogen movement) or susceptibility (i.e. by reduction of water movement) continues to be a matter of debate (Cantu *et al.*, 2016). Numerous observations have confirmed that extensive tylose formation in compatible *X. fastidiosa*–host interactions has an impact on plant water transport, which, in turn, worsens the symptoms of the disease (Choi *et al.*, 2013; Machado and Tyree, 1994; Perez-Donoso *et al.*, 2007; Sun *et al.*, 2013). In addition, *X. fastidiosa* cells and/or their secretions may also contribute to the blockage of vascular tissues (Fig. 3A). It is well established that plants affected by *X. fastidiosa* infections are more vulnerable to water stress, possibly as a result of the decrease in xylem hydraulic conductivity, a consequence of stomatal closure and blockage of the vessels (de Souza *et al.*, 2009).

It has been proposed that PD symptoms result from the water deficit caused by xylem occlusions, instead of the bacterial infection itself. However, this hypothesis has been challenged in several studies. For example, Thorne *et al.* (2006a) exposed 'Chardonnay' grapevines to severe water stress, inoculation with *X. fastidiosa* and a combination of both treatments to determine whether symptoms of PD mimicked those of water deficit. The results showed that visual symptoms of PD were qualitatively and quantitatively different from those caused by water stress. *Xylella*

fastidiosa-inoculated vines showed several PD-specific symptoms, such as green islands, matchstick petioles and patchy leaf necrosis, whereas vines exposed to water deficit did not develop any of these hallmark symptoms. It is worth noting that vines used in this comparative study were subjected to moderate and severe water stress conditions for a relatively short period of time (91 days); some of the similarities between PD and drought symptoms could be more evident when studying vines under chronic low-level water deficit. In another study, Choi *et al.* (2013) evaluated the transcriptional response of 'Cabernet Sauvignon' grapevines to *X. fastidiosa* inoculation, water deficit and a combination of the two stresses. This report indicated that gene expression changes associated with bacterial inoculation were clearly distinct from those related to water stress. Notably, vines experiencing both pathogen infection and water deficit presented more pronounced expression changes of genes involved in *X. fastidiosa*-unique responses, which supports the idea of a direct interaction between PD and grapevine water stress.

Plants can respond to pathogen attack by the induction of defence responses, including phytoalexins, pathogenesis-related (PR) proteins, oxidative reactions and structural reinforcements (i.e. callose and suberin deposition). Phytoalexins are generally low-molecular-weight secondary metabolites with antimicrobial properties that accumulate on pathogen perception. Phenolics, a class of secondary metabolites that are derived from the shikimic acid and phenylpropanoid pathways, are considered to be important phytoalexins in many plant species (Nicholson and Hammerschmidt, 1992). Maddox *et al.* (2010) examined the induction of phenolic compounds in 'Thompson Seedless' grapevines inoculated with *X. fastidiosa* during a period of 6 months. At 2 months, they observed that *X. fastidiosa*-inoculated vines showed increased levels of phenolics (e.g. catechins, procyanidins and stilbenoids) in the xylem sap and neighbouring tissues compared with uninoculated controls. In addition, higher levels of lignin and condensed tannin precursors were found in the xylem cell walls of the inoculated vines. Amongst the detected phenolic compounds, catechol, caffeic acid and resveratrol showed stronger anti-*X. fastidiosa* activities when evaluated using *in vitro* agar dilution assays. However, phenolic accumulation in the grapevines was transient because, at 4 months, the levels of these compounds were equal between *X. fastidiosa*-inoculated and control vines, and, at 6 months, when the inoculated vines showed severe PD symptoms, their phenolic levels had dropped significantly. The study concluded that phenolic production in grapevine corresponds to an early response to *X. fastidiosa* and that it may delay or limit systemic infections, but is not sufficient to successfully control the pathogen. These observations may partially explain why particular grapevine cultivars that fail to induce phenolics to high concentrations, such as 'Chardonnay' or 'Crimson Seedless', display PD symptoms much sooner than other cultivars (Wallis

and Chen, 2012). The accumulation of phenolic compounds has also been reported as a response to *X. fastidiosa* in CVC-resistant citrus (de Souza *et al.*, 2007a,b). The induction of genes involved in phenylpropanoid and flavonoid biosynthesis occurs as quickly as 1 day after inoculation in 'Ponkan' mandarin (*Citrus reticulata* Blanco), a CVC-resistant citrus variety (Rodrigues *et al.*, 2013).

PR proteins are rapidly induced in response to biotic stress and have a variety of antimicrobial properties (van Loon *et al.*, 2006), e.g. contact toxicity, defence signalling and the inhibition of pathogen enzymatic activity. Yang *et al.* (2011) analysed differential protein accumulation after *X. fastidiosa* inoculation in two siblings [9621–67 (highly PD resistant) and 9621–94 (highly PD susceptible)] from a cross of *V. rupestris* × *V. arizonica*. This study detected a strong induction of a thaumatin-like protein (TLP, PR protein class 5) at 12 weeks post-inoculation only in the PD-resistant genotype, suggesting a role of this protein in limiting *X. fastidiosa* infection. A separate study also identified the presence of TLPs in the xylem sap of a PD-tolerant *Vitis* species in response to *X. fastidiosa* inoculation (Basha *et al.*, 2010). Strong up-regulation of several PR proteins (e.g. PR protein class 1) was observed at 30 days post-inoculation with *X. fastidiosa* in 'Ponkan' mandarin when compared against a CVC-susceptible genotype, indicating that these proteins may be necessary for disease resistance in citrus (Campos *et al.*, 2007).

CONTROL OF PD

The most widely used methods of control for PD are: (i) severe pruning and roguing of infected vines; and (ii) control of the insect vector via insecticide applications. These insecticides are critical for the control of PD in California and are based primarily on systemic conventional insecticides (i.e. imidacloprid). Coordinated area-wide insecticide sprays for *H. vitripennis* were able to restore grape production to economically viable levels in the Temecula Valley. Growers often apply within-vineyard applications of the insecticide imidacloprid to complement the area-wide control applications to control *H. vitripennis*. It is not clear whether

within-vineyard applications are necessary for the control of PD or if a grower can rely solely on area-wide management for sufficient PD control. One study indicated that disease incidence was less in vineyards in which within-vineyard applications were applied, but these benefits may take several growing seasons to become apparent (Daugherty *et al.* 2015). In addition, modelling of vector behaviour in the context of climate change suggests that warmer temperatures would favour an increase in PD incidence (Daugherty *et al.*, 2017).

A robust grapevine breeding programme has also been established to evaluate germplasm for PD resistance. Although no *V. vinifera* genotypes resistant to PD have been identified, genetic sources of disease resistance are present in the *Vitis* germplasm. Wild *Vitis* populations with varying degrees of PD resistance or tolerance have been identified in the southeastern USA and northern Mexico (Krivanek *et al.*, 2005). Marker-assisted selection has been successfully used to introduce PD defence genes from wild *Vitis* genotypes into economically important *V. vinifera* varieties. One of the genes responsible for the non-*vinifera* resistance was mapped on chromosome 14 and named 'Pierce's disease resistance 1' (*PdR1*) (Krivanek *et al.*, 2006). Marker-assisted selection has been used to introgress this resistance trait into *V. vinifera* genotypes. The idea is that these PD-resistant vines can be planted in PD hotspots in northern California.

The availability of genetic transformation approaches for *V. vinifera* has opened up the possibilities of using biotechnology to improve PD resistance in susceptible genotypes (Aguero *et al.*, 2005; Dandekar *et al.*, 2012; Lindow *et al.*, 2014). Because of the negative perception of genetically modified organisms, various strategies have been suggested to use biotechnology for traditional breeding and viticulture practices. One example is transgrafting, which refers to the grafting of non-transgenic scions to transgenic rootstocks (Song *et al.*, 2015). This approach is based on the hypothesis that rootstocks expressing different PD resistance-associated transgenes could potentially confer resistance to the non-transgenic scion. A transgenic grapevine rootstock variety engineered to express *X. fastidiosa* DSF synthase

Box 1. Questions to be answered for *X. fastidiosa* and its hosts

From the plant perspective

- How does the plant immune system interface with *X. fastidiosa* in the xylem?
- What are the specific and/or shared mechanisms underlying the susceptibility of different hosts? How can we translate the knowledge we've gained in grapevines to other *X. fastidiosa* pathosystems?
- Many *X. fastidiosa* plant hosts are non-symptomatic. Can we block/remove these susceptibility factors in susceptible hosts? Can we learn from non-symptomatic interactions to make grape and other susceptible hosts tolerant?

From the pathogen perspective

- *X. fastidiosa* lacks a Type III secretion system and its effectors. Do the Type I, II, IV, and V systems secrete effectors that play role(s) in pathogenicity?
- How does *X. fastidiosa* interface with other microbes in the host plant's phytobiome?

developed fewer PD symptoms and decreased the systemic movement of the bacterium within the plant in glasshouse trials (Lindow *et al.*, 2014). As discussed above, the Rpf DSF cell–cell signalling system regulates adhesiveness (amongst other *X. fastidiosa* phenotypes), and it is hypothesized that overexpression of the signalling factor that influences adhesion locks the bacteria in the adhesive state and decreases systemic movement of the pathogen. In addition, a transgenic rootstock variety expressing a pear polygalacturonase-inhibiting protein (PGIP) (which presumably inhibits the PG used by *X. fastidiosa* to move systemically in the vine) showed tolerance to PD, but not complete resistance, in glasshouse trials. Transgenic rootstock vines expressing a protein chimera consisting of the lytic antimicrobial peptide, cercoprin B, linked to human neutrophil elastase, which binds to the *X. fastidiosa* outer membrane protein (MopB), exhibited fewer PD symptoms, but were not completely resistant to PD (Dandekar *et al.*, 2012). Moreover, stacking the resistance genes (or transgenes) mentioned above could also be accomplished by crossing plants of the same rootstock variety. Therefore, if transgenic rootstock strategies for PD defence prove to be effective, it is likely that only a few particular rootstock genotypes would be needed for deployment with numerous *V. vinifera* grape scion varieties (Cantu *et al.*, 2016).

FUTURE PERSPECTIVES

As a result of its capacity to undergo interstrain recombination and its incredibly wide host range, *X. fastidiosa* remains a formidable threat to many susceptible agricultural crops and, perhaps, in plant species in which a pathogenic relationship has yet to be described. Because there are no effective control measures that target the bacterium itself, this is an open area of research. Current efforts are turning towards the natural chemistries produced by grapevine-associated microbes as potential control measures for PD-infected vines (Aldrich *et al.*, 2015). This research is centred on understanding the grapevine's dependence on its natural microbiota and how that affects pathogen invasion and disease outcomes. This requires a forward-thinking, holistic vision of sustainable crop productivity that can only be arrived at through a systems-level understanding of the diverse interacting components in any given pathosystem.

In addition, there is very little known about how *X. fastidiosa* interfaces with the plant host immune system. Current work is focused on LPS as a pathogen-associated molecular pattern (PAMP) and how grapevines differentially perceive full-length and truncated versions of the O-antigen molecule with respect to PAMP-triggered immunity (J. Rapicavoli *et al.*, unpublished data). There are still many unanswered questions regarding *X. fastidiosa* biology and its interactions with its plant hosts, and several pertinent ones are summarized in Box 1.

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