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Xylem structure of four grape varieties and 12 alternative hosts to the xylem-limited bacterium *Xylella fastidious*

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• *Background and Aims* The bacterium *Xylella fastidiosa* (*Xf*), responsible for Pierce's disease (PD) of grapevine, colonizes the xylem conduits of vines, ultimately killing the plant. However, *Vitis vinifera* grapevine varieties differ in their susceptibility to *Xf* and numerous other plant species tolerate *Xf* populations without showing symptoms. The aim of this study was to examine the xylem structure of grapevines with different susceptibilities to *Xf* infection, as well as the xylem structure of non-grape plant species that support or limit movement of *Xf* to determine if anatomical differences might explain some of the differences in susceptibility to *Xf*.
• *Methods* Air and paint were introduced into leaves and stems to examine the connectivity between stem and leaves and the length distribution of their vessels. Leaf petiole and stem anatomies were studied to determine the basis for the free or restricted movement of *Xf* into the plant.
• *Key Results* There were no obvious differences in stem or petiole vascular anatomy among the grape varieties examined, nor among the other plant species that would explain differences in resistance to *Xf*. Among grape varieties, the more tolerant 'Sylvaner' had smaller stem vessel diameters and 20 % more parenchyma rays than the other three varieties. Alternative hosts supporting *Xf* movement had slightly longer open xylem conduits within leaves, and more connection between stem and leaves, when compared with alternative hosts that limit *Xf* movement.
• *Conclusions* Stem–leaf connectivity via open xylem conduits and vessel length is not responsible for differences in PD tolerance among grape varieties, or for limiting bacterial movement in the tolerant plant species. However, it was found that tolerant host plants had narrower vessels and more parenchyma rays, possibly restricting bacterial movement at the level of the vessels. The implications of xylem structure and connectivity for the means and regulation of bacterial movement are discussed.

Key words: Grape, grapevine, *Vitis vinifera*, host, leaf, stem, xylem, Pierce's disease, *Xylella fastidiosa*.

INTRODUCTION

Xylella fastidiosa (*Xf*) is a xylem-limited bacterium that lives as a harmless endophyte in most plants species, but various subspecies and strains of *Xf* are differentially pathogenic in several agriculturally important crops such as coffee, citrus and grapevine (Hopkins and Purcell, 2002). The bacterium is transmitted by xylem sap-feeding sharpshooter leafhoppers (Redak *et al.*, 2004), which acquire *Xf* while feeding on the xylem of infected plants (Houston *et al.*, 1947). In susceptible cultivars of grapevine, *Xylella fastidiosa* subsp. *Piercei* infection results in leaf scorch, premature leaf senescence, petiole 'matchsticks', incomplete periderm development and eventually death (Stevenson *et al.*, 2005); a suite of symptoms collectively referred to as Pierce's disease (PD).

PD symptoms were traditionally thought to result from the accumulation of bacteria and its associated gum within the xylem vessels, causing vascular occlusions and water deficit (Hopkins, 1989; Purcell and Hopkins, 1996). This vascular occlusion hypothesis implies a positive correlation between symptom severity and pathogen concentration. Indeed, some studies showed correlations between high *Xf* populations and the apparent susceptibilities of grapevine genotypes (Raju and Goheen, 1981; Hopkins and Thompson, 1984; Fry and Milholland, 1990; Krivanek and Walker, 2005). However, the relationship between PD symptoms and bacterial populations is more complex. First, Thorne *et al.* (2006*a*) showed that visual symptoms of PD were qualitatively and quantitatively different from those of various water deficits, although water deficits exacerbate the development of PD symptoms and, at times, localized water deficits are possible (Gambetta *et al.*, 2007; Choat *et al.*, 2009). Secondly, multiple studies showed that the overall proportion of vessels occluded by *Xf* and associated gums was very low (Hopkins, 1989; Newman *et al.*, 2003; Alves *et al.*, 2004; Krell *et al.*, 2006), and was unlikely to induce water deficit. Finally, more recently, Gambetta *et al.* (2007) demonstrated, via a novel, robust quantitative PCR (qPCR) assay to quantify *Xf in planta*, that there was very little correlation between *Xf* concentrations in leaves and symptom severity. That study showed that the *Xf* populations were patchily distributed across whole leaves, and that leaves could exhibit severe leaf scorch symptoms with

low bacterial concentrations. An alternative to the occlusion hypothesis is that disease symptoms result from a systemic plant response to the presence of the gums and tyloses (Stevenson *et al.*, 2004), via a higher rate of ethylene production in the infected leaves (Sun *et al.*, 2006, 2007; Perez-Donoso *et al.*, 2007). Phytotoxins and programmed cell death are also considered in the induction of PD symptoms (Gilchrist and Lincoln, 2006; Reddy *et al.*, 2007).

Pierce's disease is presently controlled in California by reducing vector populations through habitat management (Purcell *et al.*, 1999) and insecticide applications, although development of *Vitis vinifera* cultivars resistant or tolerant to *Xf* remains an active area of research. *Vitis vinifera* cultivars vary in their susceptibilities to PD, while other *Vitis* species are tolerant of *Xf* colonization (Purcell, 1981; Raju and Goheen, 1981; Hopkins and Thompson, 1984; Fry and Milholland, 1990; Krivanek and Walker, 2005). In addition to grapevine, *Xf* multiplies harmlessly within numerous plant species (Freitag, 1951). Most of those alternative hosts of *Xf* do not show PD symptoms, despite allowing bacterial proliferation and movements beyond the inoculation point, although at much lower levels than in grapevine (Hill and Purcell, 1995; Purcell and Saunders, 1999; Costa *et al.*, 2004; Baumgartner *et al.*, 2005; Wistrom and Purcell, 2005). However, most of these studies recorded plant longevity, the appearance and severity of the symptoms, and the *Xf* populations in the plant. More detailed investigations of the nature of the plant–pathogen interaction are necessary to identify possible reasons for differences in plant susceptibility to *Xf* colonization. Since *Xf* is a xylem-limited bacterium, further investigation of the xylem structure in resistant and susceptible plants is needed. For example, since *Xf* must digest the pit membrane that separates vessels (Newman *et al.*, 2003; Scarpari *et al.*, 2003), shorter and narrower vessels, and internal separation of xylem tissue by rays of non-conducting tissue, could limit *Xf* movement. Conversely, the presence of long open conduits would allow fairly quick movement of *Xf* over long distances (Chatelet *et al.*, 2006; Thorne *et al.*, 2006*b*).

There are three known xylem-limited bacterial species: *Xylella fastidiosa*, *Clavibacter xyli* and *Pseudomonas syzygii*. *Xylella fastidiosa* is the most economically important and the most studied; *C. xyli* subsp. *xyli* is the agent of ratoon stunting disease of sugar cane (Davis *et al.*, 1980); *C. xyli* subsp. *cynodontis* causes stunting disease of Bermuda grass (Davis and Augustin, 1984); and *P. syzygii* causes Sumatra disease of cloves (Bennett *et al.*, 1985; Roberts *et al.*, 1990). Similar to *Xf*, the other two xylem-limited bacteria are found in a multitude of plant hosts (Kamiunten and Wakimoto, 1976; Davis *et al.*, 1983; Roberts *et al.*, 1990). However, it is not known how these bacteria spread within the xylem system, and how the plants respond to this invasion. The same applies for exogenous bacteria that can also invade xylem tissues, such as *Erwinia stewartii*, the agent of corn wilt (Pepper, 1967), *Xylophilus ampelinus* (ex *Xanthomonas ampelina*), the agent of grapevine bacterial necrosis (Panagopoulos, 1969; Grall and Manceau, 2003), or *Ralstonia solanacearum*, responsible for bacterial wilt of solanaceous crop plants (Hayward, 1991). A better understanding of the propagation of these bacteria within the xylem may reveal multiple methods of bacterial movement and of plant defences.

General grapevine anatomy and its vascular tissue have been investigated by various researchers (Pratt, 1974; Mullins *et al.*, 1992; Gerrath *et al.*, 2001). Several anatomical aspects related to PD development have also been documented (Esau, 1948; Hopkins, 1976; Mollenhauer and Hopkins, 1976; Milholland *et al.*, 1981; Stevenson *et al.*, 2004, 2005; Chatelet *et al.* 2006; Thorne *et al.*, 2006*b*). However, generally these studies were limited to a few plants and/or a few xylemic characters. The objective of this study was to expand the scope of these studies by examining the xylem structure of grapevines with different susceptibilities to *Xf* infection, as well as the xylem structure of non-grape plant species that do and do not support the movement of *Xf* to determine if anatomical differences might explain some of the differences in susceptibility to *Xf*.

MATERIALS AND METHODS

Plant materials

Grapevines (*Vitis vinifera*, varieties 'Sylvaner', 'Cabernet Sauvignon', 'Pinot Noir' and 'Chardonnay') were propagated from seed or cuttings. These four cultivars are tolerant ('Sylvaner'), susceptible ('Cabernet Sauvignon') and highly susceptible ('Pinot Noir' and 'Chardonnay') to PD (Purcell, 1977, 1980; Raju and Goheen, 1981; Hopkins and Purcell, 2002; Krivanek *et al.*, 2005). Twelve plant species that varied in their ability to support *Xf* were chosen for anatomical characterization (Costa *et al.*, 2004; Baumgartner *et al.*, 2005; Wistrom and Purcell, 2005). Species supporting *Xf* movement included *Ipomoea purpurea* (Convolvulaceae), *Vinca major* (Apocynaceae), *Citrus sinensis* (Rutaceae), *Prunus amygdalus* (Rosaceae), *Helianthus annuus* (Asteraceae) and *Nicotiana sanderae* (Solanaceae). Species that supported limited *Xf* movement included *Umbellularia californica* (Lauraceae), *Alnus rhombifolia* (Betulaceae), *Datura meteloides* (Solanaceae), *Eucalyptus globulus* (Myrtaceae), *Artemisia douglasiana* (Asteraceae) and *Chenopodium quinoa* (Amaranthaceae). *Ipomoea purpurea*, *H. annuus*, *D. meteloides*, *C. quinoa* and *N. sanderae* were grown from seeds (Lake Valley Seed, Boulder, CO, and Botanical Interests, Inc., Broomfield, CO, USA), while cuttings of other plants were collected on the UC Davis campus. All greenhouse-grown plants were grown in 3·78 L pots containing U.C. Davis Mix (equal parts peat moss, coarse sand and nitrolysed redwood sawdust) in the greenhouse (30/20 $\pm$ 3 °C; 40/70 $\pm$ 10 % relative humidity; natural light), and watered daily with modified Hoagland's nutrient solution (Wada *et al.*, 2008). Grapevines, *A. douglasiana*, *I. purpurea*, *H. annuus*, *D. meteloides*, *C. quinoa* and *N. sanderae* were trained vertically as a single cane or stem, with lateral branches removed. Grape canes and other plants were approx. 2–4 months old when sampled, and had intact terminal tips.

Air movement in petioles and stems

One petiole and attached leaf each from nodes 3, 7, 12, 16 and 20 were carefully removed from stems at the base of the petioles under water. Petioles were attached to a rubber tube under water, and air was infused into the cut ends at a pressure of 35 kPa controlled by a pressure regulator. This pressure is 7

% that of the lowest air-seeding threshold reported for grapevine (Sperry *et al.*, 1987) and about 20 % that of the lowest value reported for trees (Choat *et al.*, 2003; Hacke *et al.*, 2004; Sperry and Hacke, 2004). After a few seconds under pressure, the major veins and their secondary veins were cut with a razor blade every few millimetres starting from the margin, and moving toward the base of the leaf. The incisions were made until a stream of air bubbles appeared at the cut. The distance from where the bubbles first appeared to the air loading point and the distance from the leaf margin to the loading point were measured. Leaf lengths were variable between species, ranging from 20 to 360 mm. In order to compare plant species, the farthest position of bubble appearance was reported as a percentage of the total length of their leaves. Air injection in leaves was replicated five times for each selected node for both grapes and alternative hosts. Data are expressed as calculated means and their standard error ($n = 5$). Statistical analysis was performed using analyis of variance (ANOVA). $P < 0{\cdot}05$ was considered statistically significant.

For stems, plants were brought to the lab and the stems were cut under water, connected to a plastic tube and pressurized air was pushed into the stem base at 35 kPa. Starting from the stem apex, the primary veins of each leaf were cut as previously described until a stream of air bubbles appeared. If air did not appear in the vein, the leaf lamina was separated from the petiole below the lamina/petiole junction. Air exiting at the apical end of the petiole signified that the lamina/petiole junction was blocking the air. If no air exited the apical end of the petiole, it was cut at its base to verify whether air was able to travel within the petiole. The distance from the loading point in the stem to the node where air appeared in leaves was measured, and was calculated as a percentage of the total length of the stem. Finally the stem was cut every few millimetres starting from the apex toward the base until a stream of air bubble appeared at the cut, and the distance from the loading point to the appearance of the bubble was measured. The stem lengths were very variable, ranging from 6 to 240 cm. Therefore, for comparison, the farthest position attained by the air in the stem of every species was calculated as a percentage of the total length of their stem. Air was injected in five different stems for both grapes and alternative hosts. Data are expressed as calculated means and their standard error ($n = 5$) and the statistical analysis was performed using ANOVA.

Vessel length distribution in stems and petioles

Grape canes and other plants were approx. 2 months old when sampled, and had intact terminal tips. Plants were kept in a cool, dark location overnight prior to measurement, and thoroughly watered to decrease transpiration and xylem tension. The length, diameter and number of nodes were measured for each stem or cane. Vessel length was measured according to the technique of Ewers and Fisher (1989), modified to infuse paint under pressure. Stems were cut underwater, and submerged until attached to the paint infusion apparatus. Latex paint (ACE Royal High Gloss Clean Red Enamel; ACE Hardware, Oak Brook, IL, USA) was diluted 1:300 in deionized water, filtered through Whatman #1 filter paper, and degassed prior to use. A modified stainless steel sprayer (B and G Equipment Co., Jackson, GA, USA) was filled with 3·5 L of diluted paint and pressurized with compressed air to 100 kPa, so the paint solution flowed out of the sprayer and into clear plastic tubing. Air was removed from the system prior to attachment of the stem to the tube. Stems were forced into tubing underwater and secured with wire. All leaves were removed and the stem was placed inside a plastic bag. Paint was infused into the stem for approx. 96 h, until liquid ceased emerging from the distal end. Paint infusion in leaves and stems was replicated five times for both grapes and alternative hosts. Vessel length distribution was calculated using Excel (Microsoft, Redmond, WA, USA), and statistical analysis was performed using ANOVA.

Paint particles filled vessels, but were stopped by pit membranes, indicating one continuous xylem vessel element. Particles in the latex paint solution were larger than 0·22 μm in diameter, since they did not pass through a sterilizing filter (Millipore Corporation, Billerica, MA, USA). Pigment and additive particles in latex paint measured between 0·3 and 7·5 μm in diameter (Croll, 2002), but pores in vessel pit membranes measured between 0·005 and 0·17 μm, depending on the plant species (Siau, 1984).

In a first experiment, vessel lengths were calculated for stems of each plant species or grapevine cultivar. The paint solution was loaded into stems as described above. Thin (~1 mm) cross-sections were cut by hand with a Platinum Injector razor blade (Longs Corporation, Walnut Creek, CA, USA) every 1 cm and placed on a glass slide in 50 % glycerol. Sections were photographed with an Olympus Vanox-AHBT (Olympus America, Melville, NY, USA) compound light microscope linked to a Pixera 600ES digital camera. Vessels with paint were counted from the digital image of each section.

In a second experiment, vessel length distribution was calculated for leaves from node 3, 7, 12, 16 and 20 from the stem apex with the same technique. The leaves were excised under water and their petiole was connected to silicone tubing linked to the reservoir filled with the paint solution. The leaves were kept under water and were infused with the paint suspension at a pressure of 35 kPa until the paint solution stopped moving. At the end of the infusion time, thin freehand cross-sections were cut every 5 mm with a razor blade. The petiole and major veins were sectioned every 5 mm starting from the paint infusion point, and progressing toward the margin of the leaf. The vessels with paint were counted in each section and vessel length distribution was calculated as described for stems.

Tylose formation

Dental paste and a 0·40 mm hypodermic needle were used to grossly imitate the wounds left by a feeding sharpshooter (Leopold *et al.*, 2003). Five stems of similar age from each species mentioned above were selected to evaluate the tylose formation after wounding. A drop of dental paste (CutterSil Mucosa, Heraeus Kulzer, Inc., Armonk, NY, USA) was placed above the first mature leaf proximal to the shoot apex. The hypodermic needle filled with dental paste was driven four times through the dental paste drop, a few millimetres into the stem xylem. The dental paste sealed the

wound upon withdrawing the needle. Stem segments were collected at 0, 1, 3 and 6 d after wounding, and the presence of tyloses in the vessels was observed in cross-sections made within the wounded area, 5, 10 and 100 mm above the wound. For each distance, the proportion of vessels with tyloses was calculated.

Vessel diameter distribution at the base of the stem and petiole

Five stems from each species were cross-sectioned at the base. For each stem, five zones of the xylem were randomly selected and the number and diameter of the vessels within each zone were counted and measured. From the same plants, five mature leaves were collected and cross-sectioned at the base of the petiole. For each section, all the vessels were counted and their diameter was measured. The diameter distributions of the vessels, at the base of the stem, and in the petiole of a mature leaf, were calculated for each species.

Anatomical comparisons among grape cultivars and other plant species

Segments of 1 cm from the base of the stem and petiole were sectioned with a sliding microtome (AO-860, American Optical, Buffalo, NY, USA) in transverse, tangential and radial planes with a section thickness of 25 μm. The sections were dehydrated through an ethanol series (Ruzin, 1999). Each step lasted 1 h, except for the 4 h step in 50 % ethanol with 1 % safranin O, and the 1 min 95 % ethanol step with 0·5 % fast green FCF. Sections were further dehydrated and cleared in an ethanol–xylene series (2:1, 1:1, 1:2) followed by two xylene rinses of 10 min each. Sections were mounted with coverslips in Permount (Fisher Scientific, Fair Lawn, NJ, USA), and photographed with a Olympus Vanox-AHBT (Olympus America, Melville, NY, USA) compound light microscope linked to a Pixera 600ES digital camera. The total numbers of vessels, bundles, rays and paratracheal parenchyma cells were counted. Data are expressed as calculated means and their standard error ($n = 5$). Statistical analysis was performed using ANOVA ($P < 0{\cdot}05$ was considered statistically significant).

RESULTS

Air movement in the leaves

The farthest distance travelled by air in the plant species tested ranged from 20 to 86 % of the total length of the vascular path from the petiole base to individual leaf vein endings (Table 1). This range indicated that the lengths of open, continuous xylem vessels (conduits) are highly variable, and that it is possible for bacteria to move passively from the base of the petiole toward the tip of the leaves.

In grapevine, air travelled up to 70 % of the leaf length in tolerant 'Sylvaner', moderately susceptible 'Cabernet Sauvignon' and highly susceptible 'Pinot Noir'. In highly susceptible 'Chardonnay', air only travelled 47–60 % of the leaf length. Overall, air travelled >50 % of the total leaf length in species allowing *Xf* movement, compared with 30–40 % of the leaf length in plants limiting *Xf* movement. Although *V. major* is able to support extensive *Xf* movement, air only moved into the first third of its leaves. Conversely, in the *Xf* movement-limiting hosts *D. meteloides* and *C. quinoa*, air moved as far as in leaves of non-limiting species. In sum, the lengths of the open xylem conduits did not correspond with the tolerant/susceptible category in grapevine and the observed ability of alternative hosts to limit *Xf* movement.

TABLE 1. *Farthest position reached by air in leaf primary veins expressed as a percentage of the total distance from the beginning of the petiole to the margin of the leaf*

	Node 3	Node 7	Node 12	Node 16	Node 20
Grapevine					
V. vinifera 'Sylvaner'	71·9 (2·9)^{a}	68·6 (2·1)^{a}	71·1 (2·4)^{a}	71·5 (2·6)^{a}	69·5 (1·9)^{a}
V. vinifera 'Cabernet Sauvignon'	71·7 (2·8)^{a}	69·9 (2·3)^{a}	73·6 (3·1)^{a}	70·6 (2·6)^{a}	69·4 (2·9)^{a}
V. vinifera 'Pinot Noir'	71·8 (2·9)^{a}	64·3 (3·8)^{a}	68·7 (4·0)^{a}	71·2 (2·9)^{a}	69·9 (2·9)^{a}
V. vinifera 'Chardonnay'	52·9 (4·2)^{b}	47·0 (2·9)^{b}	54·9 (4·3)^{b}	61·2 (2·0)^{b}	62·5 (2·4)^{b}
Movement					
I. purpurea		67·1 (2·0)^{ab}	73·1 (2·1)^{ab}	77·7 (1·6)^{b}	86·6 (1·6)^{a}
V. major	30·6 (1·6)^{c}	34·9 (2·2)^{de}			
C. sinensis	69·5 (1·6)^{a}	68·9 (2·4)^{ab}			
P. amygdalus	52·7 (2·6)^{b}	53·4 (3·5)^{c}	53·9 (1·8)^{c}	54·5 (2·9)^{cd}	54·8 (2·7)^{b}
H. annuus		59·0 (3·4)^{bc}	55·9 (3·6)^{c}	58·8 (5·9)^{c}	58·9 (2·9)^{b}
N. sanderae			42·3 (4·2)^{d}	50·0 (4·5)^{d}	56·1 (2·7)^{b}
Limited movement					
U. californica	45·4 (2·9)^{b}	39·6 (3·2)^{d}	29·7 (2·7)^{e}		
A. rhombifolia	30·2 (2·4)^{c}	27·9 (2·1)^{e}	70·3 (1·9)^{b}	84·1 (2·5)^{f}	
D. meteloides	70·9 (2·7)^{a}	76·6 (2·6)^{a}	35·3 (2·3)^{de}	34·8 (1·9)^{ab}	
E. globulus	34·1 (4·2)^{c}	32·2 (8·6)^{de}	20·0 (2·2)^{f}	20·8 (1·1)^{e}	21·1 (1·4)^{c}
A. douglasina		19·9 (1·1)^{f}	79·9 (1·8)^{a}	86·8 (1·4)^{a}	80·8 (2·4)^{a}
C. quinoa	66·1 (4·3)^{a}	72·6 (4·5)^{a}			

Measurements were made in leaves of four grapevine varieties as well as in plant species that do and do not support the movement of *Xylella fastidiosa* beyond the inoculation site.

Data are average means (s.e.) of five leaves for each node. Values in columns with different letters are significantly different at the 95 % confidence level according to ANOVA (Turkey–Kramer test).

Air movement within stems and from stem to leaves

The farthest position travelled by air within the stem of the different species was also highly variable, ranging from 30 % to almost 100 % of the stem length (Fig. 1). The farthest position travelled by air in the stem before it branched off into a leaf was also very variable, ranging from 10 % to almost 100 % of the stem length. In all species, air travelled up the stem beyond the last detection point in the leaves. In the four grapevine varieties tested, air travelled 25–30 % of the length of the stem before going into a leaf, but was found up to about 60 % of the total length of the stems. In alternative hosts, the farthest position that air travelled in the stem before entering a leaf depended upon the plant species tested, ranging from 10–15 % in *I. purpurea* and *C. quinoa* to almost 100 % of the total length of the stem in *C. sinensis*, *P. amygdalus* and *C. quinoa*. Likewise, the farthest position attained by air in the stem alone was very variable, ranging from 30 % in *I. purpurea* and *A. douglasiana* to almost 100 % of the total length of the stem in *V. major*, *C. sinensis*, *P. amygdalus*, *U. californica* and *C. quinoa*. Again, the length of the open xylem conduits and the connections between stem and leaves did not correspond to any significant difference between tolerant/susceptible grapevine and between alternative host groups.

Leaf and stem vessel length distribution

Vessel length distributions were similar in leaves of the four grapevine varieties tested. Most of the vessels were <18 cm and roughly 50 % of them were shorter than 3 cm (Fig. 2A). These are similar to vessel lengths in alternative hosts (Fig. 2B, C), where at least 40 % of the vessels in alternative *Xf* hosts were <3 cm, except for *I. purpurea* and *C. quinoa*. The longest vessels, 24–27 cm, were found in species supporting *Xf* movement: *I. purpurea*, *H. annuus* and *N. sanderae*.

In grapevine stems, most of the vessels were <50 cm, with 55 % of them being <6 cm long and the longest measuring about 1 m (Fig. 2D). In contrast, in all alternative hosts, except *C. sinensis*, most of the vessels of the stems were shorter than 27 cm, with 30–80 % of them being <3 cm long (Fig. 2E, F). The vessel length distributions in stems of alternative hosts were similar. The longest vessel measured in alternative hosts was about 30 cm (*E. globulus*).

Petiole and stem vessel diameter distribution

Vessel diameters were similar in petioles of the four grapevine varieties tested, with about 70 % of the vessels ranging from 10 to 45 μm (Fig. 3A). Alternative hosts (Fig. 3B, C) had smaller vessel diameters, mostly <25 μm, with the exception of *H. annuus*, whose vessels were between 30 and 55 μm, and *D. meteloides*, whose vessels were between 15 and 45 μm in diameter.

Grapevine stem vessel diameters ranged from 150 to 400 μm, except for 'Sylvaner', which had slightly smaller vessels, between 80 and 250 μm (Fig. 3D). In contrast, the vessel diameters at the base of the stem of the alternative hosts were similar to vessel diameters in petioles (Fig. 3E, F), ranging mostly from 10 to 35 μm, except for the vessels from *H. annuus* and *D. meteloides* whose diameter was between 40 and 65 μm.

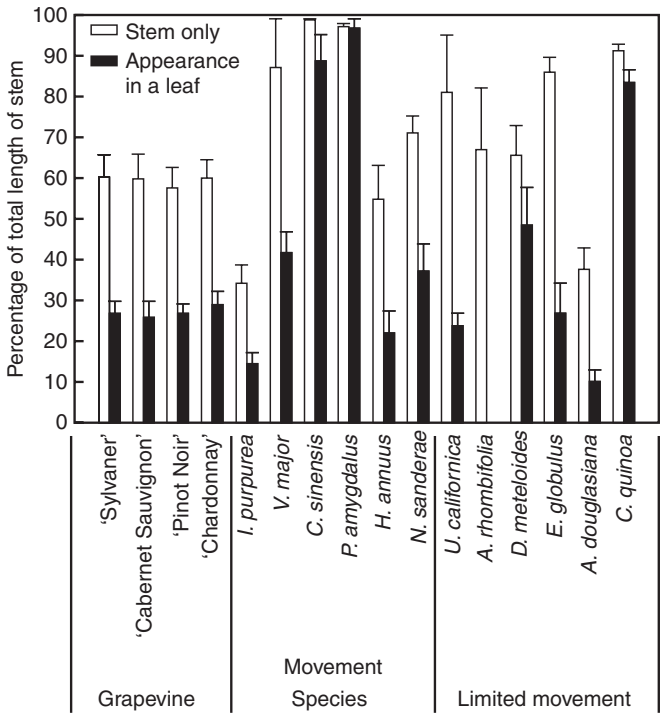

FIG. 1. Farthest position attained by air infused into stems (white) and into stems with adjacent leaves (black), expressed as a percentage of the total length of the stem. Data are the mean + s.e., $n = 5$ stems.

Tylose development

There were no major differences in tylose production between grapevine varieties and between alternative hosts (Fig. 4A–C) except for *P. amygdalus*, *V. major* and *A. rhombifolia* that did not produce any tyloses in response to wounding. In all species, the production of tyloses was greatest near the wound. Six days after wounding, between 5 and 15 % of the vessels had tyloses at the wounding site and the amount of tyloses decreased as the distance from the wound increased, until the vessels became eventually free of them. For example, no tyloses were observed in *U. californica* past 10 mm from the wound and, in all grapevine varieties and *A. douglasiana*, the vessels became free of tylose at 100 mm. In all remaining species, some tyloses were still present at 100 mm.

Anatomical comparison of stem and leaf cross-sections

There were no significant differences in the total vessel number, the proportion of short vessels or the longest vessels between resistant and susceptible grape varieties for greenhouse-grown canes of similar length, age and diameter (Table 2). The average grape cane measured 240 cm, with 24–36 nodes. The only significant xylem anatomy difference noted among grape cultivars was the number of rays in 'Sylvaner', with about 20 % more rays compared with the other grapevine varieties (Table 2). The average longest

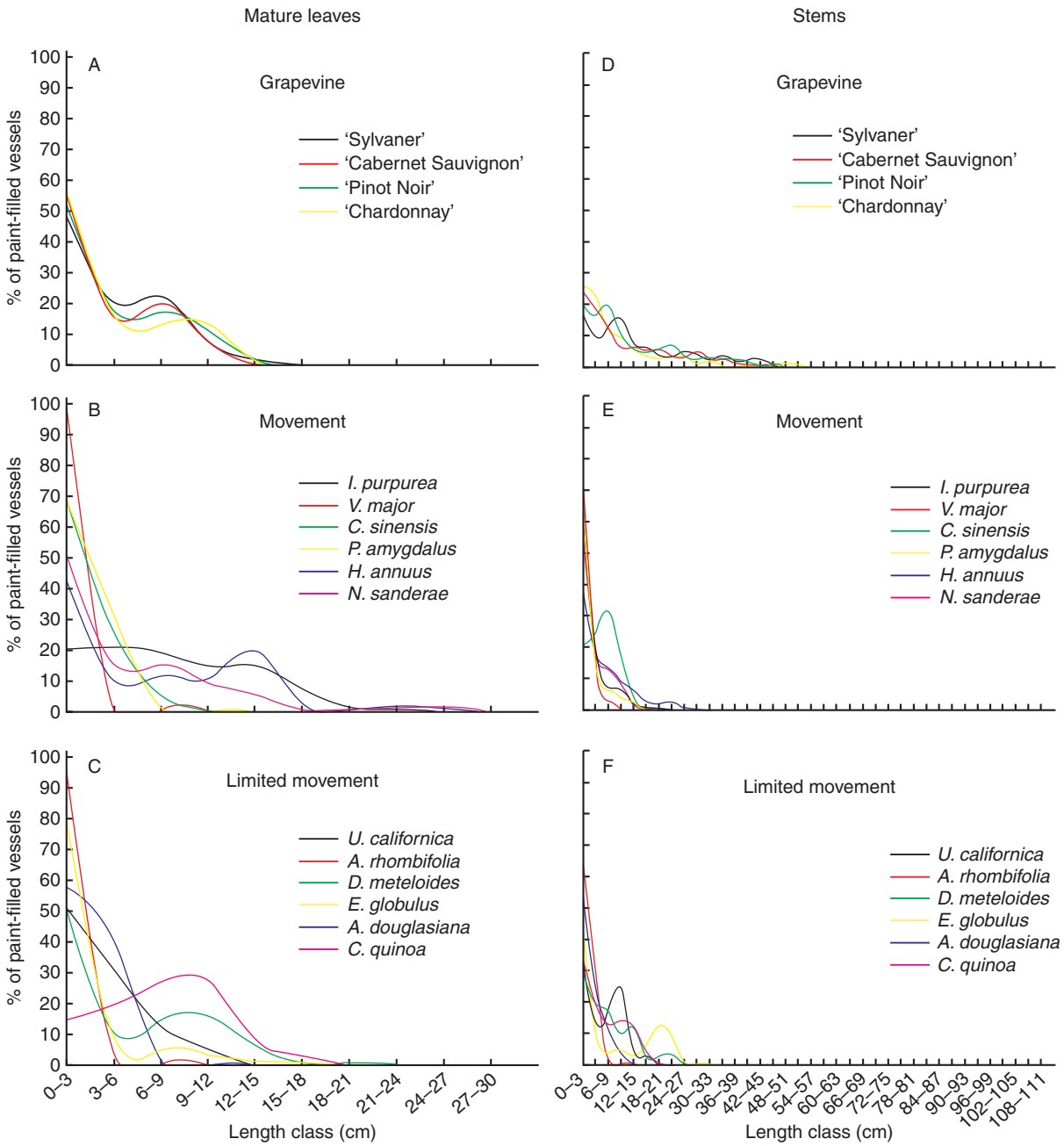

FIG. 2. Vessel length distribution in mature leaves (A–C) and stems (D–F) of grapevines and alternative hosts of *Xylella fastidiosa*. For each length class, the number of paint-infused vessels was calculated as a percentage of the total number of painted vessels at the base of the petiole or stem. $n = 5$ leaves, 5 stems.

vessel measured by paint and air infusion was 72 cm, but most of the vessels were <15 cm long in all cultivars. Stems from the alternative host plants were between 6 and 150 cm long, depending on the species. The longest vessel measured in any alternative host was 28 cm long, in *E. globulus*, and the percentage of vessels <3 cm long ranged from 21 to 84 %. We observed no discernible differences in vessel density, vessel length or number of rays between alternative hosts limiting and allowing *Xf* movement. Likewise, when the xylem structure of the petiole was compared, there were no significant differences among the four grapevine varieties or between the alternative hosts (Table 3).

This was also true for the paratracheal parenchyma cells (Table 4). With the exception of *V. major*, *P. amygdalus* and *A. rhombifolia*, where they were absent, paratracheal parenchyma cells were scanty to vasicentric. In the four grapevine cultivars, vessels had 6–7 paratracheal parenchyma cells, whereas 2–3 cells were present in most of the alternative hosts. *Helianthus annuus* and *E. globulus* were the exceptions, with 13·5 and ten cells, respectively. In longitudinal section,

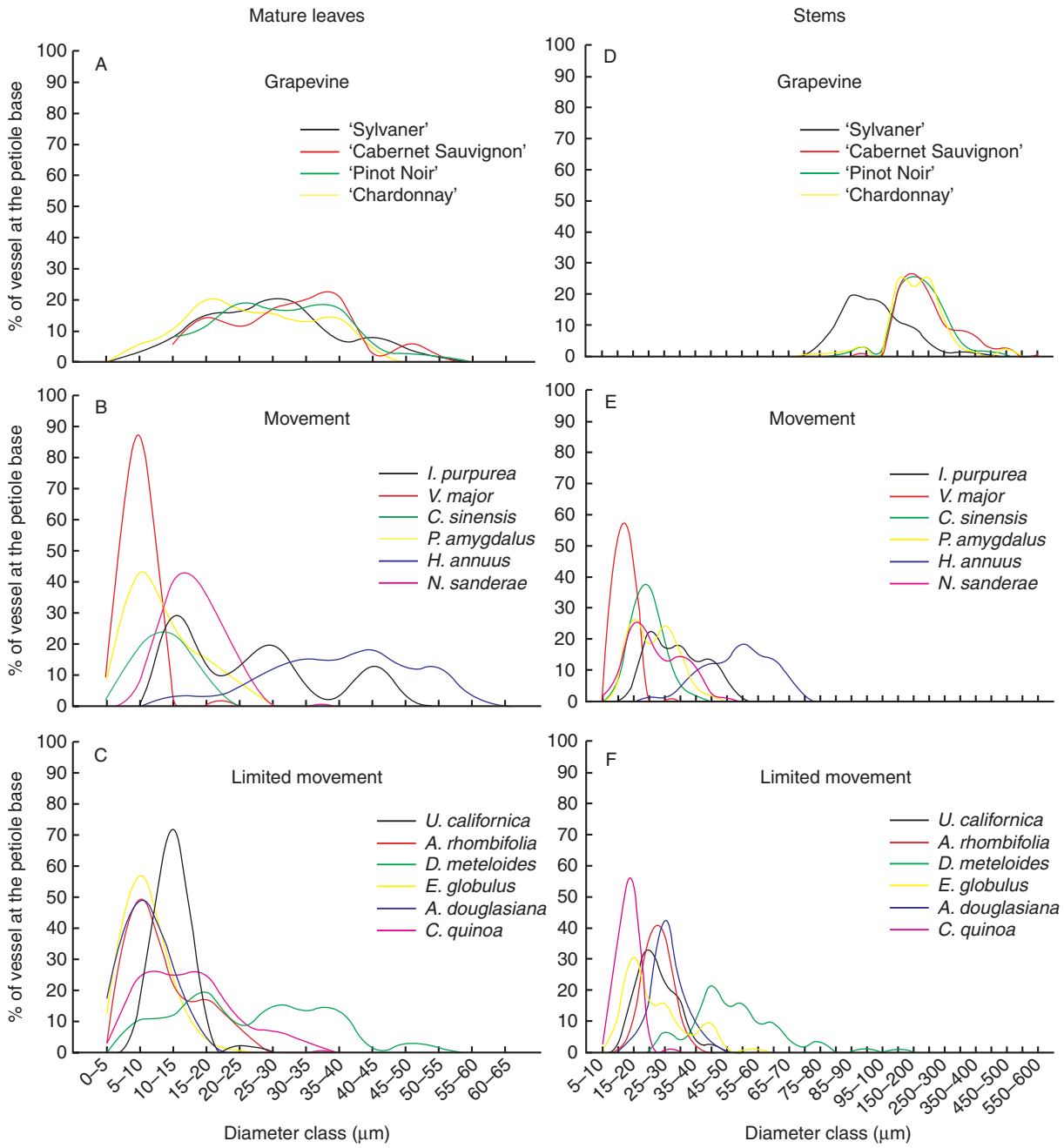

FIG. 3. Vessel diameter distribution at the base of the petiole of mature leaves (A–C) and stems (A–F) from grapevines and alternative host species of *Xylella fastidiosa*. $n = 5$ leaves, 5 stems.

strands of paratracheal parenchyma cells had up to ten cells for grapevines, while it was slightly less for the alternative species, mostly 1–4 cells.

DISCUSSION

This study examined four varieties of grapes with different susceptibilities to *Xf* infection, and 12 alternative host plant species categorized into two groups: those that allow *Xf* movement, and those that limit *Xf* movement, to determine whether gross xylem physical characteristics had a role in *Xf* colonization. The results showed that there were few or minor differences among the grapevine varieties or between the alternative hosts. There was only one grape varietal difference: the stem of the tolerant variety 'Sylvaner' had smaller vessel diameters and 20 % more parenchyma rays than the other three varieties. Among the alternative hosts, the xylem in leaves, and the xylem connecting the stem to the leaves was slightly more open in the hosts, allowing more bacterial movement as compared with hosts in which bacterial movement was

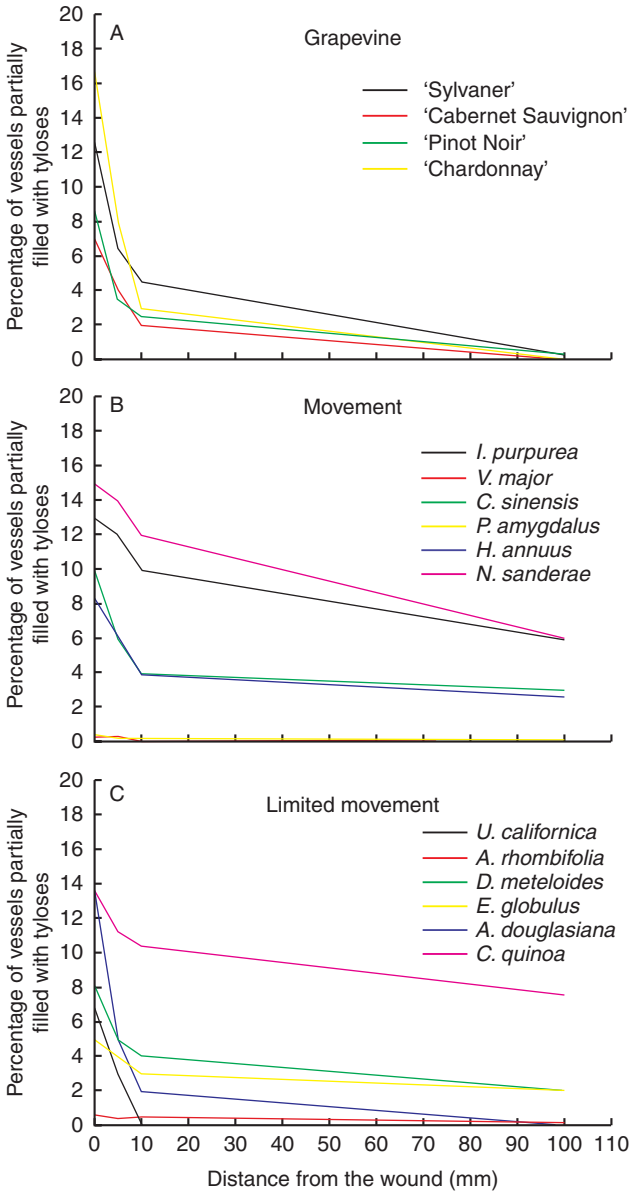

FIG. 4. Percentage of stem vessel with tyloses distal from a simulated sharpshooter feeding site, 6 d after wounding with a needle. $n = 5$ stems.

TABLE 2. *Anatomical comparisons of stems of similar age from four grape cultivars and the alternative host species of* Xylella fastidiosa

	No. of vessels at cane/stem base	Vessel density	% vessel ≤3 cm	Longest vessel	No. of rays/ stem base
Grapevine					
V. vinifera	513 (38)^{a}	12 (2)^{a}	17 (5)^{a}	69 (9)^{a}	40 (2)^{a}
'Sylvaner'					
V. vinifera	487 (27)^{a}	14 (1)^{a}	24 (2)^{a}	60 (3)^{b}	34 (1)^{b}
'Cabernet Sauvignon'					
V. vinifera	474 (27)^{a}	13 (2)^{a}	20 (3)^{a}	64 (9)^{a}	34 (2)^{b}
'Pinot Noir'					
V. vinifera	433 (19)^{a}	10 (1)^{a}	26 (2)^{a}	72 (9)^{a}	35 (1)^{b}
'Chardonnay'					
Movement					
I. purpurea	298 (26)^{g}	15 (2)^{fg}	66 (5)^{bc}	13 (2)^{ef}	84 (3)^{f}
V. major	584 (5)^{bc}	58 (5)^{c}	84 (2)^{a}	17 (1)^{cde}	82 (2)^{f}
C. sinensis	446 (4)^{de}	75 (5)^{b}	21 (1)^{g}	12 (1)^{f}	137 (1)^{d}
P. amygdalus	731 (11)^{a}	28 (2)^{e}	70 (2)^{ab}	18 (1)^{bcd}	146 (2)^{c}
H. annuus	314 (22)^{fg}	8 (2)^{g}	42 (7)^{ef}	21 (2)^{b}	19 (1)^{i}
N. sanderae	474 (23)^{de}	6 (1)^{g}	64 (7)^{bc}	15 (2)^{def}	116 (1)^{e}
Limited movement					
U. californica	434 (19)^{de}	14 (1)^{fg}	37 (4)^{f}	20 (1)^{bc}	56 (2)^{g}
A. rhombifolia	657 (18)^{ab}	87 (7)^{a}	71 (3)^{ab}	6 (1)^{g}	170 (3)^{b}
D. meteloides	485 (14)^{de}	25 (1)^{ef}	45 (2)^{def}	27 (1)^{a}	32 (2)^{h}
E. globulus	507 (5) ^{cd}	65 (3)^{bc}	53 (2)^{cde}	28 (1)^{a}	198 (3)^{a}
A. douglasina	489 (52)^{de}	40 (4)^{d}	58 (5)^{bcd}	12 (1)^{f}	18 (1)^{i}
C. quinoa	391 (20)^{ef}	20 (3)^{efg}	35 (3)^{f}	18 (1)^{bcd}	30 (2)^{h}

Data are average means (s.e.) of five stems. Values in columns with different letters are significantly different at the 95 % confidence level according to ANOVA (Turkey–Kramer test).

limited. Therefore, the results show that gross xylem structure and organization are not responsible for genotypic differences in susceptibility to *Xf* infection and PD.

Earlier studies on different plant species showed that particle movement was limited by the frequency of vessel endings, especially at the stem–leaf junction, where most vessels were thought to end, except for the few vessels crossing the junction (Larson and Isebrands, 1978; Wiebe *et al.*, 1984; André, 1999, 2002; Martre *et al.*, 2000; Tyree and Zimmermann, 2002). Such vascular arrangement was thought to act as a safety mechanism against embolism (Zimmermann, 1983; Aloni and Griffith, 1991; Tyree and Ewers, 1991; Choat *et al.*, 2005) and bacterial movement (Zimmermann, 1983; Tarbah and Goodman, 1987; Bové and Garnier, 2002). Indeed the tracheids and short vessels in these junctions would stop the propagation of air from the leaf to the stem, or vice versa, and are thought to facilitate the shedding of embolized leaves (Tyree *et al.*, 1993; Rood *et al.*, 2000). Although the long open conduits described here would allow free movement of air into the leaf blade if they became embolized, this can be expected to have a limited impact on overall leaf hydraulic conductance because they are so few in number (1–2 % of all vessels) and because water can bypass the obstruction easily through the finely reticulate vein network of a leaf (Wylie, 1938; Roth-Nebelsick *et al.*, 2001; Salleo *et al.*, 2001; Cochard *et al.*, 2004). However, allowing a few bacterial cells to pass unimpeded from stem to leaves or leaves to stems via these open conduits could have a considerably greater impact on pathogenesis by allowing the bacteria to move rapidly throughout the infected plant, particularly because *Xf* can degrade and traverse pit membranes (Roper *et al.*, 2007; Pérez-Donoso *et al.*, 2010) unlike inert gas embolisms.

The presence of long xylem vessel conduits connecting leaves to the stem several internodes below the leaf in all the species we examined (Chatelet *et al.* 2006; Thorne *et al.* 2006*b*; this study) suggests that most plant species with vessels could have such characteristics. Since bacterial infection affects many different species, an interesting question arises as to how such paths might be exploited by invading bacteria. Thorne *et al.* (2006*b*) and Chatelet *et al.* (2006) speculated on the importance of these conduits for the

TABLE 3. *Anatomical comparisons of petioles of mature leaves from four grape cultivars and 12 alternative host plant species of* Xylella fastidiosa

	No. of vessels at cane/petiole base	Vessel density	% vessel ≤1 cm	Longest vessel	No. of rays/petiole base
Grapevine					
V. vinifera 'Sylvaner'	130 (5)^{a}	22 (3)^{a}	40 (3)^{a}	8 (1)^{a}	17 (1)^{b}
V. vinifera 'Cabernet Sauvignon'	133 (7)^{a}	22 (2)^{a}	45 (3)^{a}	9 (1)^{a}	18 (1)^{ab}
V. vinifera 'Pinot Noir'	129 (3)^{a}	22 (4)^{a}	47 (4)^{a}	8 (1)^{a}	19 (1)^{a}
V. vinifera 'Chardonnay'	103 (9)^{b}	33 (8)^{a}	42 (6)^{a}	9 (1)^{a}	18 (1)^{ab}
Movement					
I. purpurea	158 (21)^{ef}	14 (3)^{gh}	6 (1)^{h}	16 (2)^{a}	10 (1)^{h}
V. major	47 (2)^{h}	45 (2)^{e}	68 (3)^{b}	2 (1)^{c}	14 (1)^{g}
C. sinensis	134 (6)^{fg}	50 (1)^{de}	17 (2)^{g}	6 (1)^{b}	44 (1)^{c}
P. amygdalus	151 (8)^{efg}	85 (4)^{c}	42 (2)^{d}	6 (1)^{b}	34 (1)^{d}
H. annuus	174 (8)^{de}	2 (1)^{h}	36 (3)^{def}	14 (1)^{a}	11 (1)^{h}
N. sanderae	204 (19)^{cd}	72 (7)^{cd}	41 (2)^{de}	14 (2)^{a}	23 (1)^{e}
Limited movement					
U. californica	181 (16)^{cde}	32 (3)^{efg}	34 (5)^{ef}	8 (1)^{b}	54 (1)^{b}
A. rhombifolia	595 (10)^{a}	375 (21)^{a}	49 (2)^{c}	4 (1)^{c}	66 (1)^{a}
D. meteloides	217 (9)^{c}	44 (2)^{ef}	39 (2)^{de}	15 (1)^{a}	21 (1)^{e}
E. globulus	404 (25)^{b}	259 (21)^{b}	75 (2)^{a}	8 (1)^{b}	53 (1)^{b}
A. douglasina	24 (1)^{h}	19 (1)^{fgh}	32 (1)^{f}	2 (1)^{c}	17 (1)^{f}
C. quinoa	117 (6)^{g}	57 (3)^{de}	7 (1)^{h}	15 (1)^{a}	10 (1)^{h}

Data are average means (s.e.) of five stems. Values in columns with different letters are significantly different at the 9 5 % confidence level according to ANOVA (Turkey–Kramer test).

TABLE 4. *Paratracheal parenchyma cells in the stem of the grape cultivars and the alternative host species of* Xylella fastidiosa

	Paratracheal parenchyma cell arrangement	Cell number/ vessel element CS	Average cell number/ vessel element	Cell number/ strand
Grapevine				
V. vinifera 'Sylvaner'	Scanty to vasicentric	Up to 14	6·5 (0·5)	Up to 10
V. vinifera 'Cabernet Sauvignon'	Scanty to vasicentric	Up to 18	6·7 (0·6)	Up to 8
V. vinifera 'Pinot Noir'	Scanty to vasicentric	Up to 15	6·8 (0·4)	Up to 9
V. vinifera 'Chardonnay'	Scanty to vasicentric	Up to 13	6·4 (0·5)	Up to 8
Movement				
I. purpurea	Scanty	Up to 5	2·9 (0·5)	2–3
V. major	Absent			
C. sinensis	Scanty	Up to 5	3·0 (0·2)	1–2
P. amygdalus	Absent			
H. annuus	Vasicentric	Up to18	13·5 (0·4)	1–2
N. sanderae	Scanty	Up to 3	2·1 (0·1)	2–4
Limited movement				
U. californica	Scanty	Up to 5	2·9 (0·2)	2–4
A. rhombifolia	Absent			
D. meteloides	Scanty to vasicentric	Up to 5	2·7 (0·2)	2–4
E. globulus	Scanty to vasicentric	Up to 15	10·0 (0·4)	Up to 8
A. douglasina	Scanty to vasicentric	Up to 4	2·6 (0·1)	1–2
C. quinoa	Scanty	Up to 4	2·6 (0·2)	1–2

Data are mean (s.e.), $n = 5$ stems.
CS, cross-section.

passive systemic spread of pathogens via the xylem and their role in the development of diseases such as PD in grapevine. Similarly, it was suggested that the inability to restrict bacterial movement by the xylem was a key determinant of the appearance of symptoms (Fry and Milholland, 1990; Krivanek and Walker, 2005). However, in this study, the interconnectedness of the organs and the vessel length distribution profiles were similar between plant varieties and species that have been reported to differ in susceptibility to PD, and to limit and not limit *Xf* movement. These observations indicate that susceptibility to PD is not controlled by physical limitations in xylem organization to bacterial movement and imply that factors other than maximum open conduit length and vessel length distribution determine the extent of bacterial movement.

The lack of differences in vessel length distributions or open paths among the alternative hosts characterized in the literature as 'systemic' and 'non-systemic' is interesting. There are at least two possible implications. First, the characteristics measured here are not the pertinent ones to ascertain the potential for *Xf* movement. However, in our previous studies, experiments tested for open pathways in several ways including measurements of the movement of light-emitting bacteria, green fluorescent protein (GFP)–*Xf*, air and fluorescent beads, and all produced similar results quantifying long, open pathways in shoots, petioles and leaf lamina (Chatelet *et al.*, 2006; Thorne *et al.*, 2006*b*). Thus, the potential for passive movement of *Xf* is probably reflected in the measurements reported here. However, it is clear that the bacterium is motile (Meng *et al.*, 2005; De La Fuente *et al.*, 2007), and it may well be that the environment within the xylem of different genotypes is important to that motility. Secondly, this classification did not account for the difference in *Xf* strain specificity with the plant host. Strains of *Xf* differ greatly in their abilities to move and colonize various host plant

species systemically. For example, oleander leaf scorch strains of *Xf* colonized and caused disease in oleander (*Neerium oleander*) but not in grape, whereas the reverse was true for grape strains (Purcell *et al.*, 1999). Almond strains of *Xf* were weakly systemic and non-pathogenic in grape, but grape strains were systemic and pathogenic to grape and almond (Almeida *et al.*, 2003). However, all the alternative hosts in this study were chosen from previous work that had used the *Xf* strain specific to PD, thereby eliminating genetic differences in virulence and intra-plant movements. Thirdly, there may have been false negatives in earlier work using the less sensitive enzyme-linked immunosorbent assay (ELISA) compared with a more sensitive qPCR assay, leading to 'non-systemic' interpretation for some species (Gambetta *et al.*, 2007). This possibility raises the further question of the role of bacterial distribution and population in the development of PD.

Although high bacterial populations were previously thought necessary for symptom development (Hopkins, 1985; Fry and Milholland, 1990; Hill and Purcell, 1995; Krivanek and Walker, 2005), that is evidently not the case for *Xf* in leaves (Alves *et al.*, 2004; Krell *et al.*, 2006; Gambetta *et al.*, 2007). Thus, the movement of small amounts of bacteria through a limited number of open conduits may be important in disease development. The presence of *Xf* is patchy in infected susceptible plants (Newman *et al.*, 2003; Krell *et al.*, 2006; Gambetta *et al.*, 2007), and not correlated with symptoms (Krell *et al.*, 2006; Gambetta *et al.*, 2007). In the present study, there were no significant differences in open pathways between susceptible and tolerant species. These results point toward a systemic response of susceptible grapevines to the presence of *Xf* in its xylem sap, possibly involving ethylene (Hopkins 1985; Thorne *et al.*, 2006*a*; Pérez-Donoso *et al.*, 2007) and programmed cell death (Gilchrist and Lincoln, 2006).

Sun *et al.* (2007) demonstrated that ethylene is necessary for tylose development in wounded grapevine stems. Tyloses are produced by paratracheal parenchyma cells, outgrowing into the vessel lumen via vessel–parenchyma pits and eventually occluding the vessel (Esau, 1977). They occur naturally in a wide range of species and can be induced by wounding and pathogen infection (Wallis and Truter, 1978; Beckman and Talboys, 1981; Biggs, 1987; Bonsen and Kučera, 1990; Cochard and Tyree, 1990; Pearce, 1991; Saitoh *et al.*, 1993; Schmitt and Liese, 1993; Clerivet *et al.*, 2000; Salleo *et al.*, 2002; Sun *et al.*, 2006). These vascular occlusions that develop in response to infection are often thought to be involved in the isolation of pathogens for disease defence. In grapevines infected with *Xf*, tyloses are observed in primary and secondary xylem (Esau, 1948; Hopkins and Mollenhauer, 1975; Stevenson *et al.*, 2004). However, their role in PD of grapevine is not clear as some studies reported their frequency in PD-infected grapevines to be greater in resistant genotypes (Mollenhauer and Hopkins, 1976), greater in susceptible genotypes (Krivanek *et al.*, 2005) and unrelated to the susceptibility of the grape genotype (Fry and Milholland, 1990). Our results showed that the amount of tylose produced in response to needle wounding as well as the type and number of paratracheal parenchyma cells was similar among the grapevine varieties and among the alternative hosts, regardless of their ability to limit *Xf* movement. This is in accordance with the low fraction of vessels being occluded observed in PD studies (Hopkins, 1989; Newman *et al.*, 2003; Alves *et al.*, 2004; Krell *et al.*, 2006) and the wounding study by Sun *et al.* (2006). These results provide further evidence that tyloses may be unable to limit *Xf* movement and spread in tolerant plants. The mechanisms for *Xf* tolerance observed in many species are still not resolved.

The only significant differences between the tolerant and susceptible grapevines in this study were the smaller vessel diameters and the higher number of rays in the stem of the tolerant 'Sylvaner' cultivar compared with the more susceptible grapevine varieties. A smaller vessel diameter suggests that *Xf* would self-aggregate more easily, possibly leading to a more rapid maturation of biofilms. However, the role of biofilm formation in disease development is unclear (Hopkins, 1989; De Souza *et al.*, 2003, 2004; Koide *et al.*, 2004; Newman *et al.*, 2004; Guilhabert and Kirkpatrick, 2005; Feil *et al.*, 2007; Chatterjee *et al.*, 2008*a*, *b*). In addition to narrower vessels, the higher number of rays in the tolerant cultivar could also slow down the bacterial infection through active secretion of defence chemical compounds. Several studies suggested that the xylem sap composition influences the expression of genes involved in growth, aggregation, attachment and virulence of *Xf* (Bi *et al.*, 2007; Reddy *et al.*, 2007; Zaini *et al.*, 2009; Shi *et al.*, 2010). Recently, Basha *et al.* (2010) showed differences in the xylem sap composition (amino acids, sugars and proteins) of susceptible and tolerant *Vitis* species. Although more work is needed to clarify those differences, a higher number of parenchyma cells around the xylem could certainly facilitate the secretion of antimicrobial compounds in the stem xylem of the tolerant *Vitis* cultivar as a response to infection by *Xf*. In addition, the higher number of rays in the tolerant cultivar suggests that rays could also play a role in limiting the lateral spread of the bacteria. Rays are composed of one to several layers of dense living ray parenchyma cells, without tracheids or vessel elements (Esau, 1977), and they separate the water-conducting xylem into longitudinal zones. These rays would present a barrier to bacteria moving exclusively in the xylem. The lateral spread of bacteria from one zone to another can only occur when vessels bridge the two zones, either by crossing the ray or where a ray ends and two contiguous zones merge. In any case, lateral movement would be limited by the degree of connectivity of the vessels within a zone as bacteria move from vessel to vessel by digesting the pit membrane.

The work by Zimmermann and Brown (1971) and Newbanks *et al.* (1983) demonstrated that there are many lateral pit field connections between vessels along their length. The connectivity of vessels is presumed to be a key determinant in the vulnerability of plants to the spread of embolisms between vessels (Wheeler *et al.*, 2005). Indeed the more surface area of pit membrane existing between vessels, the greater the chance of having a large pore that will allow the movement of gas to the next vessel. A similar logic could be applied to the movement of *Xf* in the xylem of grapevine genotypes. The xylem of susceptible grapevines could have greater intervessel pitting, thus allowing *Xf* access to adjacent vessels via pit membranes, while the

xylem of tolerant grapevine would have more isolated vessels, preventing bacterial movement to other vessels. Differences in pit structure could also produce a difference between tolerance and susceptibility by presenting pit membranes that are susceptible to different degrees to digestion and breaching.

Conclusions

Our investigation of the xylem structure of PD-susceptible and tolerant grapevines, and of alternative host species that allow or restrict *Xf* movement produced no evidence that the gross xylem anatomy (vessel length or number, or organ connectivity) was responsible for tolerance or for limiting bacterial movement. However, the narrower vessels and higher numbers of parenchyma rays found in tolerant compared with susceptible plants imply that bacterial movement might be restricted by more subtle differences in vessel morphology. More subtle differences in the vessel network, such as the degree of intervessel pitting or the thickness of the pit membrane, coupled with the secretion of defensive chemical compounds may play a role in limiting *Xf* movement and disease development. Another important result of this study is the observation of long, open conduits in all examined species. The role of such conduits in bacterial colonization of plants is largely unstudied. Future analyses of the role of the xylem in bacterial movement should examine vessel overlapping, spatial organization of the pit fields, and pit membrane thickness and porosity.

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LITERATURE CITED

Almeida RPP, Purcell AH. 2003. Biological traits of *Xylella fastidiosa* strains from grapes and almonds. *Applied and Environmental Microbiology* **69**: 7447–7452.

Aloni R, Griffith M. 1991. Functional xylem anatomy in root–shoot junctions of six cereal species. *Planta* **184**: 123–129.

Alves E, Marucci CR, Lopes JRS, Leite B. 2004. Leaf symptoms on plum, coffee and citrus and the relationship with the extent of xylem vessels colonized by *Xylella fastidiosa*. *Journal of Phytopathology* **152**: 291–297.

André JP. 2002. *Organisation vasculaire des angiospermes: une vision nouvelle*. Paris: Institut Nationale de la Recherche Agronomique (INRA).

André JP, Catesson AM, Liberman M. 1999. Characters and origin of vessels with heterogenous structure in leaf and flower abscission zones. *Canadian Journal of Botany* **77**: 253–261.

Basha SM, Mazhar H, Vasanthaiah HKN. 2010. Proteomic approach to identify unique xylem sap proteins in Pierce's Disease-tolerant *Vitis* species. *Applied Biochemistry and Biotechnology* **160**: 932–944.

Baumgartner K, Warren JG. 2005. Persistence of *Xylella fastidiosa* in riparian hosts near northern California vineyards. *Plant Disease* **89**: 1097–1102.

Beckman CH, Talboys PW. 1981. Anatomy of resistance. In: Mace ME, Bell AA, Beckman CH. eds. *Fungal wilt diseases of plants*. New York: Academic Press, 487–521.

Bennett CPA, Hunt P, Asman A. 1985. Association of a xylem-limited bacterium with Sumatra disease of cloves in Indonesia. *Plant Pathology* **34**: 487–494.

Bi JL, Dumenyo CK, Hernandez-Martinez R, Cooksey DA, Toscano NC. 2007. Effect of host plant xylem fluid on growth, aggregation, and attachment of *Xylella fastidiosa*. *Journal of Chemical Ecology* **33**: 493–500.

Biggs AR. 1987. Occurrence and location of suberin in wound reaction zones in xylem of 17 tree species. *Phytopathology* **77**: 718–725.

Bonsen KJM, Kucera LJ. 1990. Vessel occlusions in plants: morphological, functional and evolutionary aspects. *International Association of Wood Anatomists Bulletin* **11**: 393–399.

Bové JM, Garnier M. 2002. Phloem- and xylem-restricted plant pathogenic bacteria. *Plant Science* **163**: 1083–1098.

Chatelet D, Matthews MA, Rost TL. 2006. Xylem structure and connectivity in grapevine (*Vitis vinifera* L.) shoots provides a passive mechanism for the spread of bacteria in grape plants. *Annals of Botany* **98**: 483–494.

Chatterjee S, Almeida RPP, Lindow SE. 2008*a*. Living in two worlds: the plant and insect lifestyles of *Xylella fastidiosa*. *Annual Review of Phytopathology* **46**: 243–271.

Chatterjee S, Newman KL, Lindow SE. 2008*b*. Cell-to-cell signaling in *Xylella fastidiosa* suppresses movement and xylem vessel colonization in grape. *Molecular Plant-Microbe Interactions* **21**: 1309–1315.

Choat B, Ball M, Luly J, Holtum J. 2003. Pit membrane porosity and water stress-induced cavitation in four co-existing dry rainforest tree species. *Plant Physiology* **31**: 41–48.

Choat B, Lahr E, Melcher PJ, Zwieniecki MA, Holbrook NM. 2005. The spatial pattern of air seeding thresholds in mature sugar maple trees. *Plant, Cell and Environment* **28**: 1082–1089.

Choat B, Gambetta GA, Wada H, Shackel KA, Matthews MA. 2009. The effects of Pierce's disease on leaf, petiole hydraulic conductance in *Vitis vinifera* cv. 'Chardonnay'. *Physiologia Plantarum* **136**: 384–394.

Clérivet A, Déon V, Alami I, Lopez F, Geiger J-P, Nicole M. 2000. Tyloses and gels associated with cellulose accumulation in vessels are responses of plane tree seedlings (*Platanus* × *acerifolia*) to the vascular fungus *Ceratocystis fimbriata* f.sp *platani*. *Trees* **15**: 25–31.

Cochard H, Tyree MT. 1990. Xylem dysfunction in *Quercus*: vessel sizes, tyloses, cavitation, and seasonal changes in embolism. *Tree Physiology* **6**: 393–408.

Cochard H, Nardini A, Coll L. 2004. Hydraulic architecture of leaf blades: where is the main resistance? *Plant, Cell and Environment* **27**: 1257–1267.

Costa HS, Raetz E, Pinckard TR, *et al*. 2004. Plant hosts of *Xylella fastidiosa* in and near southern California vineyards. *Plant Disease* **88**: 1255–1261.

Croll SG. 2002. DLVO theory applied to TiO_2 pigments and other materials in latex paints. *Progress in Organic Coatings* **44**: 131–146.

Davis MJ, Augustin BJ. 1984. Occurrence in Florida of the bacterium that causes bermudagrass stunting disease. *Plant Disease* **68**: 1095–1097.

Davis MJ, Gillaspie AG, Harris RW, Lawson RH. 1980. Ratoon stunting disease of sugarcane – isolation of the causal bacterium. *Science* **210**: 1365–1367.

Davis MJ, Raju BC, Brlansky RH, *et al*. 1983. Periwinkle wilt bacterium: axenic culture, pathogenicity, and relationships to other gram-negative, xylem-inhabiting bacteria. *Phytopathology* **73**: 1510–1515.

De La Fuente L, Burr TJ, Hoch HC. 2007. Mutations in type I and type IV pilus biosynthetic genes affect twitching motility rates in *Xylella fastidiosa*. *Journal of Bacteriology* **189**: 7507–7510.

De Souza AA, Takita MA, Coletta-Filho HD, *et al*. 2003. Analysis of gene expression in two growth states of *Xylella fastidiosa* and its relationship with pathogenicity. *Molecular Plant-Microbe Interactions* **16**: 867–875.

De Souza AA, Takita MA, Coletta-Filho HD, *et al*. 2004. Gene expression profile of the plant pathogen *Xylella fastidiosa* during biofilm formation *in vitro*. *FEMS Microbiology Letters* **237**: 341–353.

Esau K. 1948. Anatomic effects of the viruses of Pierce's disease and phony peach. *Hilgardia* **18**: 423–482.

Esau K. 1977. *Anatomy of seed plants*. New York, NY: John Wiley and Sons.

Ewers FW, Fisher JB. 1989. Techniques for measuring vessel lengths and diameters in stems of woody plants. *American Journal of Botany* **76**: 645–656.

Feil H, Feil WS, Lindow SE. 2007. Contribution of fimbrial and afimbrial adhesins of *Xylella fastidiosa* to attachment to surfaces and virulence to grape. *Phytopathology* **97**: 318–324.

Freitag JH. 1951. Host range of Pierce's disease virus of grapes as determined by insect transmission. *Phytopathology* **41**: 920–934.

Fry SM, Milholland RD. 1990. Multiplication and translocation of *Xylella fastidiosa* in petioles and stems of grapevine resistant, tolerant and susceptible to Pierce's disease. *Phytopathology* **80**: 61–65.

Gambetta GA, Fei J, Rost TL, Matthews MA. 2007. Leaf scorch symptoms are not correlated with bacterial populations during Pierce's disease. *Journal of Experimental Botany* **58**: 4037–4046.

Gerrath JM, Posluszny U, Dengler NG. 2001. Primary vascular patterns in the *Vitaceae*. *International Journal of Plant Sciences* **162**: 729–745.

Gilchrist D, Lincoln J. 2006. Resistance to Pierce's by transgenic expression of plant-derived anti-apoptotic genes. In: *Proceedings of the 2006 Pierce's disease research symposium*. San Diego, CA: California Department of Food and Agriculture.

Grall S, Manceau C. 2003. Colonization of *Vitis vinifera* by a green fluorescence protein-labeled, gfp-marked strain of *Xylophilus ampelinus*, the causal agent of bacterial necrosis of grapevine. *Applied and Environmental Microbiology* **69**: 1904–1912.

Guilhabert MR, Kirkpatrick BC. 2005. Identification of *Xylella fastidiosa* antivirulence genes: hemagglutinin adhesins contribute to *X. fastidiosa* biofilm maturation and colonization and attenuate virulence. *Molecular Plant-Microbe Interactions* **18**: 856–868.

Hacke UG, Sperry JS, Pittermann J. 2004. Analysis of circular bordered pit function. II. Gymnosperm tracheids with torus-margo pit membranes. *American Journal of Botany* **91**: 386–400.

Hayward AC. 1991. Biology and epidemiology of bacterial wilt caused by *Pseudomonas-solanacearum*. *Annual Review of Phytopathology* **29**: 65–87.

Hill BL, Purcell AH. 1995. Multiplication and movement of *Xylella fastidiosa* within grape and four other plants. *Phytopathology* **85**: 1368–1372.

Hopkins DL. 1976. Pierce's disease of grapevines. *American Wine Society Journal* **8**: 26–27.

Hopkins DL. 1985. Physiological and pathological characteristics of virulent and avirulent strains of the bacterium that causes Pierces disease of grapevine. *Phytopathology* **75**: 713–717.

Hopkins DL. 1989. *Xylella-fastidiosa* – xylem-limited bacterial pathogen of plants. *Annual Review of Phytopathology* **27**: 271–290.

Hopkins DL, Mollenhauer HH. 1975. Tylose and gum formation in the xylem of Pierce's disease infected grapevines. *Proceedings of the American Phytopathological Society* **2**: 65 (abstract).

Hopkins DL, Purcell AH. 2002. *Xylella fastidiosa*: cause of Pierce's disease of grapevine and other emergent diseases. *Plant Disease* **86**: 1056–1066.

Hopkins DL, Thompson CM. 1984. Seasonal concentration of the Pierce's disease bacterium in 'Carlos' and 'Welder' muscadine grapes as compared with 'Schuyler' bunch grape. *Horticultural Sciences* **19**: 419–420.

Houston BR, Hewitt WE, Esau K. 1947. The mode of vector feeding and the tissues involved in the transmission of Pierce's disease virus in grape and alfalfa. *Phytopathology* **37**: 247–253.

Kamiunten H, Wakimoto S. 1976. Coryneform bacteria found in xylem of the ratoon stunting diseased sugarcane. *Annals of the Phytopathological Society of Japan* **42**: 500–503.

Koide T, Zaini PA, Moreira LM, *et al*. 2004. DNA microarray-based genome comparison of a pathogenic and a nonpathogenic strain *Xylella fastidiosa* delineates genes important for bacterial virulence. *Journal of Bacteriology* **186**: 5442–5449.

Krell RK, Perring TM, Farrar CA, Park YL, Gispert C. 2006. Intraplant sampling of grapevines for Pierce's disease diagnosis. *Plant Disease* **90**: 351–357.

Krivanek AF, Walker MA. 2005. *Vitis* resistance to Pierce's disease is characterized by differential *Xylella fastidiosa* populations in stems and leaves. *Phytopathology* **95**: 44–52.

Larson PR, Isebrands JG. 1978. Functional significance of nodal constricted zone in *Populus-deltoides*. *Canadian Journal of Botany* **56**: 801–804.

Leopold RA, Freeman TP, Buckner JS, Nelson DR. 2003. Mouthpart morphology and stylet penetration of host plants by the glassy-winged sharpshooter, *Homalodisca coagulata*, (Homoptera: Cicadellidae). *Arthropod Structure and Development* **32**: 189–199.

Martre P, Durand JL, Cochard H. 2000. Changes in axial hydraulic conductivity along elongating leaf blades in relation to xylem maturation in tall fescue. *New Phytologist* **146**: 235–247.

Meng Y, Li Y, Galvani CD, *et al*. 2005. Upstream migration of *Xylella fastidiosa* via pilus-driven twitching motility. *Journal of Bacteriology* **187**: 5560–5567.

Milholland RD, Huang PY, Clayton CN, Jones RK. 1981. Pierce's disease on muscadine grapes in North Carolina USA. *Plant Disease* **65**: 73–74.

Mollenhauer HA, Hopkins DL. 1976. Xylem morphology of Pierce's disease-infected grapevines with different levels of tolerance. *Physiological Plant Pathology* **9**: 95–100.

Mullins MG, Bouquet A, Williams LE. 1992. *Biology of the grapevine*. Cambridge: Cambridge University Press.

Newbanks D, Bosch A, Zimmermann MH. 1983. Evidence for xylem dysfunction by embolization in Dutch elm disease. *Phytopathology* **73**: 1060–1063.

Newman KL, Almeida RPP, Purcell AH, Lindow SE. 2003. Use of a green fluorescent strain for analysis of *Xylella fastidiosa* colonization of *Vitis vinifera*. *Applied and Environmental Microbiology* **69**: 7319–7327.

Newman KL, Almeida RPP, Purcell AH, Lindow SE. 2004. Cell–cell signaling controls *Xylella fastidiosa* interactions with both insects and plants. *Proceedings of the National Academy of Sciences, USA* **101**: 1737–1742.

Panagopoulos CG. 1969. The disease of Tsilik Marasi of grapevine – its description and identification of the causal agent *Xanthomonas-ampelina* new species. *Annals of the Institute of Phytopathology Benaki* **9**: 59–81.

Pearce RB. 1991. Reaction zone relics and the dynamics of fungal spread in the xylem of woody angiosperms. *Physiology and Molecular Plant Pathology* **39**: 41–56.

Pepper EH. 1967. *Stewart's bacterial wilt of corn. Monographs of the American Phytopathological Society No. 4*. St. Paul, MN: American Phytopathological Society.

Pérez-Donoso AG, Greve LC, Walton JH, Shackel KA, Labavitch JM. 2007. *Xylella fastidiosa* infection and ethylene exposure result in xylem and water movement disruption in grapevine shoots. *Plant Physiology* **143**: 1024–1036.

Pérez-Donoso AG, Sun Q, Roper CM, Greve CL, Kirkpatrick B, Labavitch JM. 2010. Cell wall-degrading enzymes enlarge the pore size of intervessel pit membranes in healthy and *Xylella fastidiosa*-infected grapevines. *Plant Physiology* **152**: 1748–1759.

Pratt C. 1974. Vegetative anatomy of cultivated grapes – a review. *American Journal of Enology and Viticulture* **25**: 131–150.

Purcell AH. 1977. Cold therapy of Pierce's disease of grapevines. *Plant Disease Reporter* **61**: 514–518.

Purcell AH. 1981. Vector preference and inoculation efficiency as components of resistance to Pierce's disease in European grape cultivars. *Phytopathology* **71**: 429–435.

Purcell AH, Hopkins DL. 1996. Fastidious xylem-limited bacterial plant pathogens. *Annual Review of Phytopathology* **34**: 131–151.

Purcell AH, Saunders SR. 1999. Glassy-winged sharpshooters expected to increase plant disease. *California Agriculture* **53**: 26–27.

Purcell AH, Saunders SR, Norberg E, McBryde JR. 1999. Reductions of Pierce's disease vector activity by management of riparian woodlands. *Phytopathology* **89**: S62.

Raju BC, Goheen AC. 1981. Relative sensitivity of selected grapevine cultivars to Pierce's disease bacterial infections. *American Journal of Enology and Viticulture* **32**: 155–158.

Redak RA, Purcell AH, Lopes JRS, Blua MJ, Mizell RFIII, Andersen PC. 2004. The biology of xylem fluid-feeding insect vectors of *Xylella fastidiosa* and their relation to disease epidemiology. *Annual Review of Entomology* **49**: 243–270.

Reddy JD, Reddy SL, Hopkins DL, Gabriel DW. 2007. ToIC is required for pathogenicity of *Xylella fastidiosa* in *Vitis vinifera* grapevines. *Molecular Plant-Microbe Interactions* **20**: 403–410.

Roberts SJ, Edengreen SJ, Jones P, Ambler DJ. 1990. *Pseudomonas-syzygii*, *sp-nov*, the cause of Sumatra disease of cloves. *Systematic and Applied Microbiology* **13**: 34–43.

Rood SB, Patino S, Coombs K, Tyree MT. 2000. Branch sacrifice: cavitation-associated drought adaptation of riparian cottonwoods. *Trees* **14**: 248–257.

Roper MC, Greve LC, Warren JG, Labavitch JM, Kirkpatrick BC. 2007. *Xylella fastidiosa* requires polygalacturonase for colonization and pathogenicity in *Vitis vinifera* grapevines. *Molecular Plant-Microbe Interactions* **20**: 411–419.

Roth-Nebelsick A, Uhl D, Mosbrugger V, Kerp H. 2001. Evolution and function of leaf venation architecture: a review. *Annals of Botany* **87**: 553–566.

Ruzin SE. 1999. *Plant microtechnique and microscopy*. New York: Oxford University Press.

Saitoh T, Ohtani J, Fukazawa K. 1993. The occurrence and morphology of tyloses and gums in the vessels of Japanese hardwoods. *International Association of Wood Anatomists Journal* **14**: 359–371.

Salleo S, Lo Gullo MA, Raimondo F, Nardini A. 2001. Vulnerability to cavitation of leaf minor veins: any impact on leaf gas exchange? *Plant, Cell and Environment* **24**: 851–859.

Salleo S, Nardini A, Lo Gullo MA, Ghirarrdelli LA. 2002. Changes in stem and leaf hydraulics preceding leaf shedding in *Castanea sativa* L. *Biologia Plantarum* **45**: 227–234.

Scarpari LM, Lambais MR, Silva DS, Carraro DM, Carrer H. 2003. Expression of putative pathogenicity-related genes on *Xylella fastidiosa* grown at low and high cell density conditions *in vitro*. *FEMS Microbiology Letters* **222**: 83–92.

Schmitt U, Liese W. 1993. Response of xylem parenchyma by suberization in some hardwoods after mechanical injury. *Trees* **8**: 23–30.

Shi X, Bi J, Morse JG, Toscano NC, Cooksey DA. 2010. Differential expression of genes of *Xylella fastidiosa* in xylem fluid of citrus and grapevine. *FEMS Microbiology Letters* **304**: 82–88.

Siau JF. 1984. *Transport processes in wood.* Berlin: Springer-Verlag.

Sperry JS, Hacke UG. 2004. Analysis of circular bordered pit function. I. Angiosperm vessels with homogenous pit membranes. *American Journal of Botany* **91**: 369–385.

Sperry JS, Holbrook NM, Zimmermann MH, Tyree MT. 1987. Spring filling of xylem vessels in wild grapevine. *Plant Physiology* **83**: 414–417.

Stevenson JF, Matthews MA, Greve LC, Labavitch JM, Rost TL. 2004. Grapevine susceptibility to Pierce's disease II: progression of anatomical symptoms. *American Journal of Enology and Viticulture* **55**: 238–245.

Stevenson JF, Matthews MA, Rost TL. 2005. The developmental anatomy of Pierce's disease symptoms in grapevines: green islands and matchsticks. *Plant Disease* **89**: 543–548.

Sun Q, Rost TL, Matthews MA. 2006. Pruning-induced tylose development in stems of current-year shoots of *Vitis vinifera* (Vitaceae). *American Journal of Botany* **93**: 1567–1576.

Sun Q, Rost TL, Reid MS, Matthews MA. 2007. Ethylene and not embolism is required for wound-induced tylose development in stems of grapevines. *Plant Physiology* **145**: 1629–1636.

Tarbah F, Goodman RN. 1987. Systemic spread of *Agrobacterium tumefaciens* biovar 3 in the vascular system of grapes. *Phytopathology* **77**: 915–920.

Thorne ET, Stevenson JF, Rost TL, Labavitch JM, Matthews MA. 2006*a*. Pierce's disease symptoms: comparison with symptoms of water deficit and the impact of water deficits. *American Journal of Enology and Viticulture* **57**: 1–11.

Thorne ET, Young BM, Young GM, *et al*. 2006*b*. The structure of xylem vessels in grapevine and a possible passive mechanism for the systemic spread of bacterial disease. *American Journal of Botany* **93**: 497–504.

Tyree MT, Ewers FW. 1991. The hydraulic architecture of trees and other woody plants. *New Phytologist* **119**: 345–360.

Tyree MT, Zimmermann MH. 2002. *Xylem structure and the ascent of sap*, 2nd edn. New York: Springer.

Tyree MT, Cochard H, Cruiziat P, Sinclair B, Ameglio T. 1993. Drought-induced leaf shedding in walnut: evidence for vulnerability segmentation. *Plant, Cell and Environment* **16**: 879–882.

Wada H, Shackel K, Matthews M. 2008. Fruit ripening in *Vitis vinifera*: apoplastic solute accumulation accounts for pre-veraison turgor loss in berries. *Planta* **227**: 1351–1361.

Wallis FM, Truter SJ. 1978. Histopathology of tomato plants infected with Pseudomonas solanacearum with emphasis on ultrastructure. *Physiological Plant Pathology* **13**: 307–318.

Wheeler JK, Sperry JS, Hacke UG, Hoang N. 2005. Inter-vessel pitting and cavitation in woody Rosaceae and other vesselled plants: a basis for a safety versus efficiency trade-off in xylem transport. *Plant, Cell and Environment* **28**: 800–812.

Wiebe HH, Greer RL, Vanalfen NK. 1984. Frequency and grouping of vessel endings in alfalfa (*Medicago sativa*) shoots. *New Phytologist* **97**: 583–590.

Wistrom C, Purcell AH. 2005. The fate of *Xylella fastidiosa* in vineyard weeds and other alternate hosts in California. *Plant Disease* **89**: 994–999.

Wylie RB. 1938. Concerning the conductive capacity of the minor veins of foliage leaves. *American Journal of Botany* **25**: 567–572.

Zaini PA, De La Fuente L, Hoch HC, Burr TJ. 2009. Grapevine xylem sap enhances biofilm development by *Xylella fastidiosa*. *FEMS Microbiology Letters* **295**: 129–134.

Zimmermann MH, Brown CL. 1971. *Trees: structure and function*. New York: Springer-Verlag.

Zimmermann MH. 1983. *Xylem structure and the ascent of sap*. Berlin: Springer-Verlag.