MECHANISMS OF PIERCE'S DISEASE TRANSMISSION IN GRAPEVINES: THE XYLEM PATHWAYS AND MOVEMENT OF *XYLELLA FASTIDIOSA*. COMPARISON OF THE XYLEM STRUCTURE OF SUSCEPTIBLE/TOLERANT GRAPEVINES AND ALTERNATE PLANT HOSTS

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ABSTRACT
In grape, there are very few xylem differences between susceptible and tolerant varieties, except for smaller vessel diameters and more parenchyma rays in the tolerant variety examined. These findings in the tolerant grape suggest a restriction to bacterial movement imposed at the level of the vessels. The systemic and non-systemic alternate hosts studied here showed relatively similar xylem characteristics with large variations. Overall, the comparison of the basic xylem characteristics (vessel length, diameter, stem-petiole-leaf connectivity) is not conclusive and more studies are needed. First, more species needs to be studied as *Xylella fastidiosa* (*Xf*) is found in numerous species. Second, *Xf* movement and patterns of colonization needs to be determined in more details (time, distance, location). Third, differences in the vessel network, vessel overlapping, spatial organization of the pit fields, structure (thickness, porosity) of the pit membrane, need to be considered to fully understand the role of the xylem network in bacterial movement.

INTRODUCTION
The capacity for *Xf* to move in the xylem differs greatly among species (Freitag, 1951), ranging from moving efficiently throughout the plant to instances where the bacterium moves only a few centimeter from the infection point (Hill and Purcell, 1995; Purcell and Saunders, 1999; Costa et al., 2004; Wistrom and Purcell, 2005; Baumgartner et al., 2005). Our lab has observed the presence of long xylem conduits from stem to leaves in chardonnay and cowart (Thorne et al., 2006; Chatelet et al, 2006) and we reported last year that these conduits seemed to be shorter in alternate hosts in which bacterial movement is limited. Other aspects of the xylem system, for example, a higher number of tracheids, shorter and narrower vessels, the spatial organization of the vessels, and the number and location of paratracheal parenchyma cells could be part of a passive strategy to limit bacterial movement. Another strategy in non-systemic species, where the bacteria do not move, could be to confine the bacteria to a limited area by a more timely production of tyloses or, in the case of asymptomatic species showing systemic bacterial movement, to limit the population size under a harmless threshold. The objective of this study was to study the comparative anatomy of different species of plants supporting a range of *Xf* population sizes and movement characteristics described by previous studies (Purcell and Saunders, 1999; Costa et al., 2004; Wistrom and Purcell, 2005; Baumgartner et al., 2005). Our hope is to understand how the xylem network might control bacterial movement in susceptible plants.

OBJECTIVES
1. Conduct an anatomical comparison of plant species that support high, medium and low population sizes of *Xf*.
2. Conduct an anatomical comparison of plant species that show systemic movement of *Xf* vs. those that do not.

A range of species chosen from previous studies (Purcell and Saunders, 1999; Costa et al., 2004; Wistrom and Purcell, 2005; Baumgartner et al., 2005) was examined:
1. grapevines with a low infection rate, medium bacterial population and showing very little to no bacterial movement (non-systemic): *Vitis vinifera* cv. Cabernet sauvignon and *Vitis vinifera* cv. Sylvaner.
2. grapevines with a high infection rate, high bacterial population and showing systemic movement: *Vitis vinifera* cv. Chardonnay and *Vitis vinifera* cv. Pinot noir,
3. other plant species with a high infection rate, medium bacterial population and showing systemic movement: *Ipomoea purpurea* (morning glory), *Vinca major* (periwinkle), *Citrus sinensis* (orange), *Prunus anygdalus* (almond),
4. plant species showing non-systemic movement: *Alnus rhombifolia* (white alder), *Umbellularia californica* (California laurel), *Artemisia douglasiana* (mugwort) and *Chenopodium quinoa* (quinoa), *Datura wrightii* (datura), *Eucalyptus globules* (eucalyptus).

RESULTS
Stem-petiole-leaf lamina connectivity - Grape shoots have open xylem conduits that allow the passive movement of GFP-*Xf* from the stem to 50-60% of the leaf length through the primary xylem (Rost et al., PD symposium report 2005; Chatelet et al., 2006). However, there was no difference between the resistant and susceptible grapevine varieties (Table 1). Also, despite large variations, the alternate species categorized as supporting the systemic spread of *Xf* seemed to have longer open conduits compared to the non-systemic plants (Table 1). Another important difference between systemic and non-systemic

species resides in the continuity of these conduits between the stem and the leaves. In most of the non-systemic hosts, air or paint moved only into the first leaf above the stem loading point as opposed to several leaves in systemic species.

Table 1: Xylem vessel lengths measured as the total distance (mm) from the base of the petiole to the leaf margin divided by the distance moved by low pressured air and paint and expressed as a percentage for primary laminar veins, in grapevine varieties and in plants allowing or not the movement of *Xf*.

	Node 3	Node 7	Node 12	Node 16	Node20
Grapevine					
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]
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]
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]
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]
Systemic					
Morning glory		67.1 (2.0)[a,b]	73.1 (2.1)[a,b]	77.7 (1.6)[b]	86.6 (1.6)[a]
Periwinkle	30.6 (1.6)[c]	34.9 (2.2)[d,e]			
Orange	69.5 (1.6)[a]	68.9 (2.4)[a,b]			
Almond	52.7 (2.6)[b]	53.4 (3.5)[c]	53.9 (1.8)[c]	54.5 (2.9)[c,d]	54.8 (2.7)[b]
Sunflower		59.0 (3.4)[b,c]	55.9 (3.6)[c]	58.8 (5.9)[c]	58.9 (2.9)[b]
Tobacco			42.3 (4.2)[d]	50.0 (4.5)[d]	56.1 (2.7)[b]
Non-systemic					
Laurel	45.4 (2.9)[b]	39.6 (3.2)[d]			
White alder	30.2 (2.4)[c]	27.9 (2.1)[e]	29.7 (2.7)[e]		
Datura	70.9 (2.7)[a]	76.6 (2.6)[a]	70.3 (1.9)[b]	84.1 (2.5)[f]	
Eucalyptus	34.1 (4.2)c	32.2 (8.6)[d,e]	35.3 (2.3)[d,e]	34.8 (1.9)[a,b]	
Mugwort		19.9 (1.1)[f]	20.0 (2.2)[f]	20.8 (1.1)[e]	21.1 (1.4)[c]
Quinoa	66.1 (4.3)[a]	72.6 (4.5)[a]	79.9 (1.8)[a]	86.8 (1.4)[a]	80.8 (2.4)[a]

Leaf vessel length distribution (Figure 1, left) - The species with limited *Xf* movement have slightly shorter vessels. With the exception of morning glory (systemic) and quinoa (non-systemic), at least 40% of the vessels are less than 3cm. The longest vessels [24-27cm] were found in systemic plants: morning glory, sunflower and tobacco. The vessel lengths in grapevine leaves are within the range of those from alternate hosts.

Stem vessel length distribution (Figure 1, right) - The vessel length distributions in stems of systemic or non-systemic hosts are similar. In all alternate hosts except orange, 30 to 80% of the vessels were less than 3cm. In contrast in grapevine, the majority of the vessels (55%) were less than 6cm. Also, the longest vessel measured in alternate hosts was about 30cm (eucalyptus), while in grapevines it was about 100cm.

Vessel diameter distribution at the base of the stem (Figure 2, left)- The diameter distributions of the vessels at the base of the stem of the alternate hosts are similar, ranging mostly from 10 to 35µm, except for the vessels from sunflower (systemic) and datura (non-systemic) whose diameter ranged from 40 to 65µm. In contrast, the grapevines have most of their vessels ranging from 150 to 400µm at the exception of the resistant Sylvaner whose vessels are slightly smaller and mostly ranged from 80 to 250µm.

Vessel diameter distribution at the base of the petiole (Figure 2, right) - Petioles of the four grapevine varieties showed similar vessel diameter distributions with about 70% of the vessels ranging from 10 to 45µm. Alternate hosts (systemic and non-systemic) showed a lower vessel diameter distribution, in the range 0-25µm, with the exception of sunflower (systemic) and datura (non-systemic) whose diameter ranged from 30 to 55µm (Sunflower) and 15 to 45µm (datura).

Anatomical differences from stem cross-sections - Among grapevines, the only difference resides in the number of rays. The resistant grapevine (Sylvaner) has significantly more rays separating the vessels (Table 2) than the other grapevines in which bacterial movement is more efficient. Regarding the other plant species hosting *Xf*, no discernable differences between systemic and non-systemic were observed.

Table 2: Anatomical comparisons of stems of similar age

	Nb vessel at cane / stem base (SE)	Vessel density (SE)	% vessel ≤ 3cm (SE)	Longest vessel (SE)	Nb of rays / stem base (SE)
Grapevine					
Sylvaner	513 (38)[a]	12 (2)[a]	17 (5)[a]	69 (9)[a]	40 (2)[a]
Cabernet Sauvignon	487 (27)[a]	14 (1)[a]	24 (2)[a]	60 (3)[b]	34 (1)[b]
Pinot Noir	474 (27)[a]	13 (2)[a]	20 (3)[a]	64 (9)[a]	34 (2)[b]
Chardonnay	433 (19)[a]	10 (1)[a]	26 (2)[a]	72 (9)[a]	35 (1)[b]
Systemic					
Morning glory	298 (26)[g]	15 (2)[f,g]	66 (5)[b,c]	13 (2)[e,f]	84 (3)[f]
Periwinkle	584 (5)[b,c]	58 (5)[c]	84 (2)[a]	17 (1)[c,d,e]	82 (2)[f]
Orange	446 (4)[d,e]	75 (5)[b]	21 (1)[g]	12 (1)[f]	137 (1)[d]
Almond	731 (11)[a]	28 (2)[e]	70 (2)[a,b]	18 (1)[b,c,d]	146 (2)[c]
Sunflower	314 (22)[f,g]	8 (2)[g]	42 (7)[e,f]	21 (2)[b]	19 (1)[i]
Tobacco	474 (23)[d,e]	6 (1)[g]	64 (7)[b,c]	15 (2)[d,e,f]	116 (1)[e]
Non-systemic					
Laurel	434 (19)[d,e]	14 (1)[f,g]	37 (4)[f]	20 (1)[b,c]	56 (2)[g]
White alder	657 (18)[a,b]	87 (7)[a]	71 (3)[a,b]	6 (1)[g]	170 (3)[b]
Datura	485 (14)[d,e]	25 (1)[e,f]	45 (2)[d,e,f]	27 (1)[a]	32 (2)[h]
Eucalyptus	507 (5)[c,d]	65 (3)[b,c]	53 (2)[c,d,e]	28 (1)[a]	198 (3)[a]
Mugwort	489 (52)[d,e]	40 (4)[d]	58 (5)[b,c,d]	12 (1)[f]	18 (1)[i]
Quinoa	391 (20)[e,f]	20 (3)[e,f,g]	35 (3)[f]	18 (1)[b,c,d]	30 (2)[h]

CONCLUSIONS

From this study, it appears that the comparison of the basic xylem characteristics (vessel length, diameter, stem-petiole-leaf connectivity) in relation to the limitation of bacterial movement is not conclusive. In grape, the only differences consisted of smaller vessel diameters and higher number of rays in the tolerant grapevine variety, suggesting a restriction of the bacterial movement imposed at the level of the vessel elements. T he alternate hosts showed similar xylem features with a lot of variability. Overall, the comparison of the basic xylem characteristics (vessel length, diameter, stem-petiole-leaf connectivity) is not conclusive and needs to be extended. First, more species needs to be studied as *Xf* is found in numerous species. Second, *Xf* movement and patterns of colonization needs to be determined in more details (time, distance, location). Third, differences in the vessel network, vessel overlapping, spatial organization of the pit fields, structure (thickness, porosity) of the pit membrane, need to be considered to fully understand the role of the xylem network in bacterial movement.

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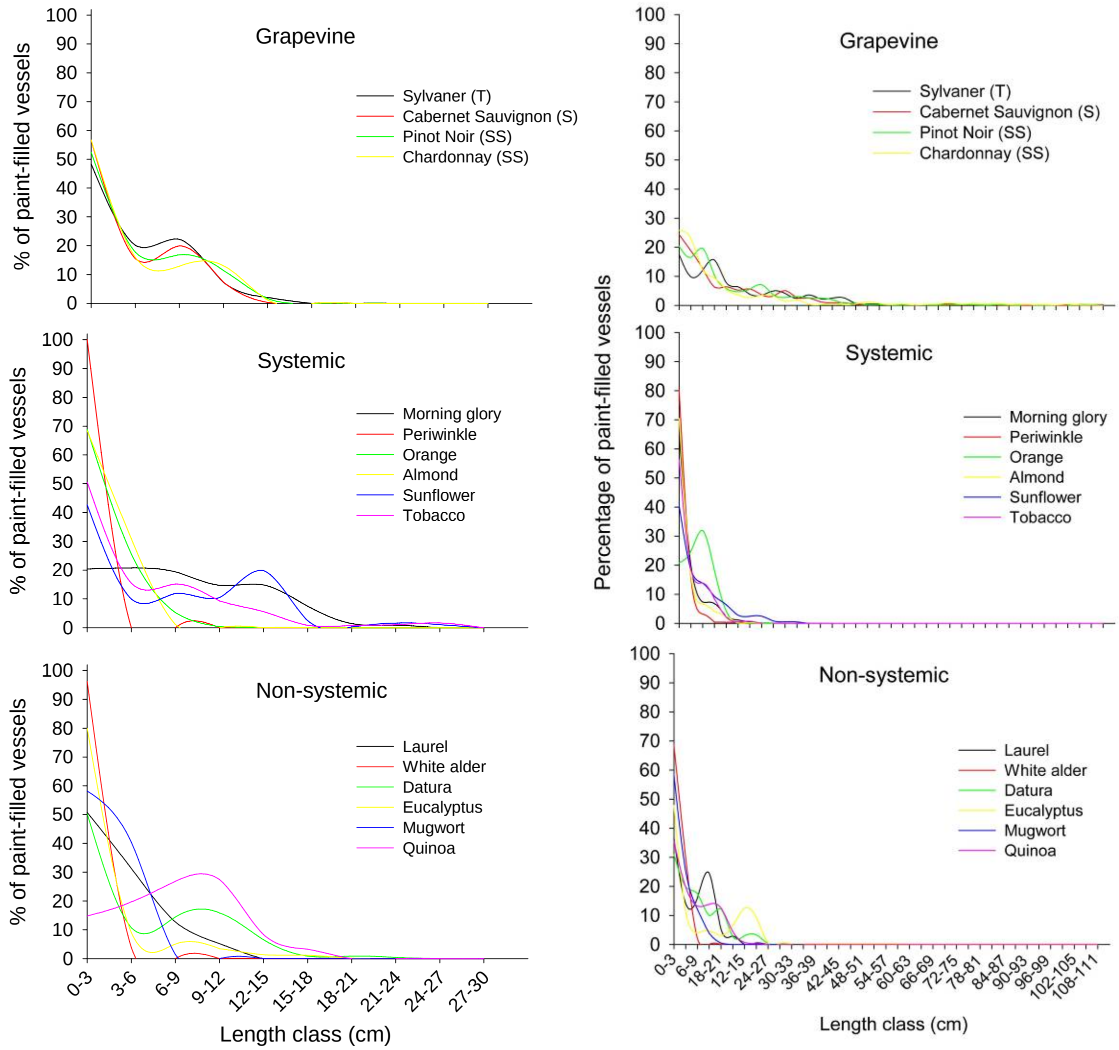

Figure 1: Vessel length distribution in the mature leave (left) and in the stem (right) of grapevines and alternate hosts to *Xf*. For each length class, the number of painted-vessels was calculated as a percentage of the total number of painted vessels at the petiole or stem base.

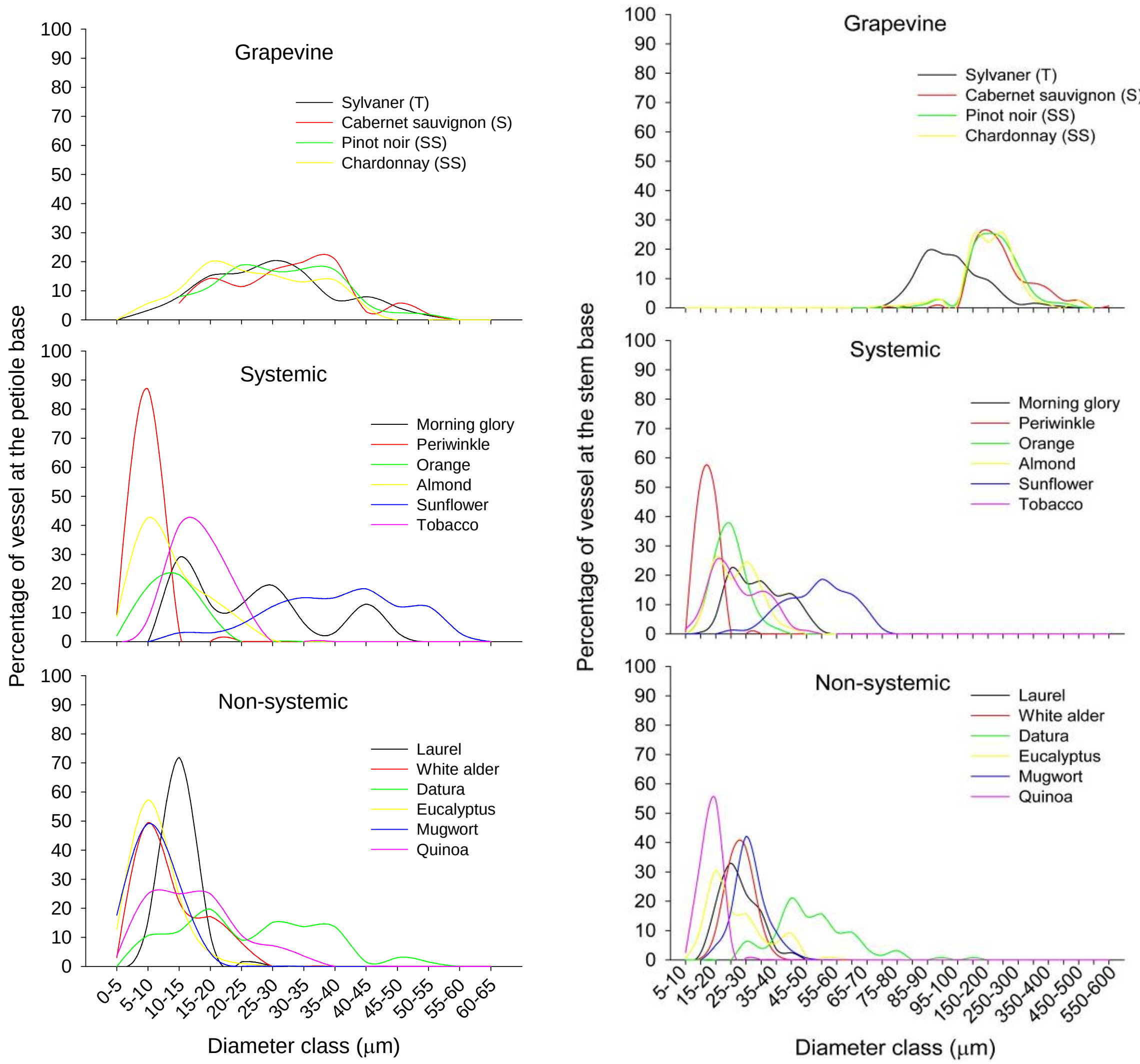

Figure 2: Vessel diameter distribution at the base of the petiole of mature leaves (left) and at the base of stems (right) from grapevines and alternate hosts to *Xf*.