

**MECHANISMS OF PIERCE'S DISEASE TRANSMISSION IN GRAPEVINES: THE XYLEM PATHWAYS AND  
MOVEMENT OF XYLELLA FASTIDIOSA. XYLEM STRUCTURE AND CONNECTIVITY IN GRAPEVINE  
SHOOTS PROVIDES A PASSIVE MECHANISM FOR THE SPREAD OF BACTERIA IN GRAPE PLANTS  
INFECTED WITH PIERCE'S DISEASE**

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**ABSTRACT**

This progress report shows that there is a difference in the structure of xylem pathways available for *Xylella fastidiosa* (Xf) movement in host plants known to support systemic bacterial movement and those that don't. In addition to a reduced inter-organ connectivity, non-systemic species also show a shorter path available for bacterial movement in the leaves. However, systemic and non-systemic species show similar rates of tylose formation, signifying that tyloses don't seem to be responsible for the lack of Xf movement in the non-systemic plants. To be more conclusive, more xylem characteristics from the different hosts are being examined

**INTRODUCTION**

*Xylella fastidiosa* (Xf) capacity to move in plants differs greatly among species (Purcell, 2004), ranging from moving everywhere in the stem and leaves to only a few centimeters from the infection point. Our lab showed the presence of long xylem conduits from stem to leaves in grape cultivars chardonnay and cowart (Thorne et al., 2006; Chatelet et al, in press) and we recently reported that these conduits seemed to be shorter in alternate hosts in which bacterial movement is limited. A higher number of tracheids, shorter and narrower vessels, spatial organization of the vessel and of the paratracheal parenchyma cells could be a passive strategy to limit bacterial movement. Another strategy of the non-systemic species 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 objectives of this study are to carefully study the comparative anatomy of different species of plants which support a range of Xf population sizes and movement characteristics. Our hope is to understand how the xylem network might control bacterial movement in susceptible plants.

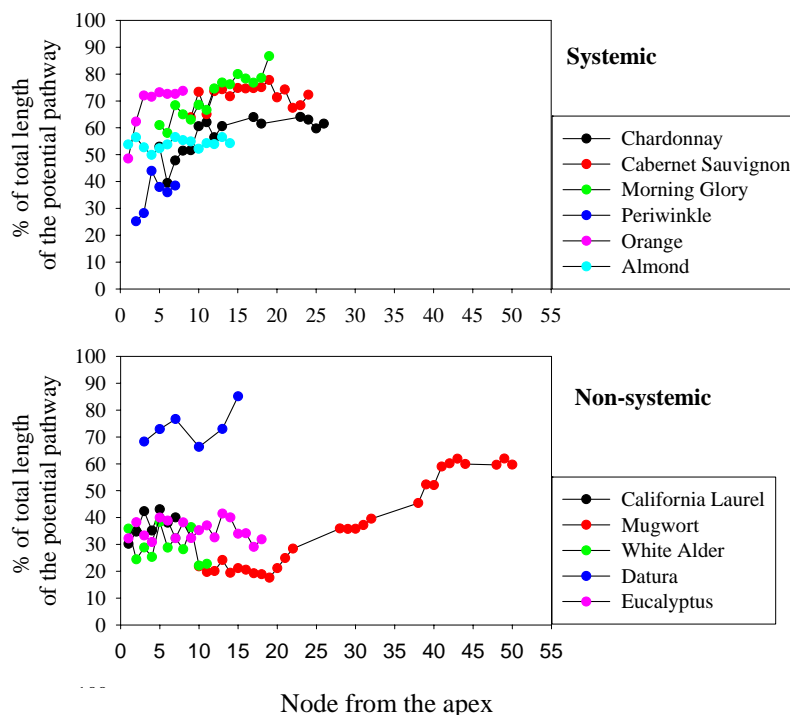
**OBJECTIVES**

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.

**RESULTS**

A range of species was examined: with a high infection rate, high bacterial population and showing systemic movement: *Vitis vinifera* cv. Chardonnay and *Vitis vinifera* cv. Cabernet sauvignon; one species with a high infection rate, medium bacterial population and showing systemic movement: *Ipomoea purpurea* (morning glory), *Vinca major* (periwinkle), *Citrus sinensis* (Orange), *Prunus amygdalus* (Almond), and 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).

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., in press). The xylem of several different plant species harboring Xf was examined using air and paint injection to determine if similar xylem conduits exist. When loaded at the base of the petiole, air and paint traveled to various extents into the leaf blade of all examined species (Figure 1).



**Figure 1.** Air and paint position in the leaf veins calculated as percentage of the total length of the vascular pathway from the base of the petiole to the tip of the leaf. Air and paint were loaded at the base of the petiole of leaves from different nodes.

Although variations from the mean were large, species categorized as supporting the systemic spread of *Xf* seemed to have longer open conduits compared to the non-systemic plants. 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. A more detailed investigation of the anatomy of the stem-leaf connection is currently undertaken.

### Leaf shape and vascular network

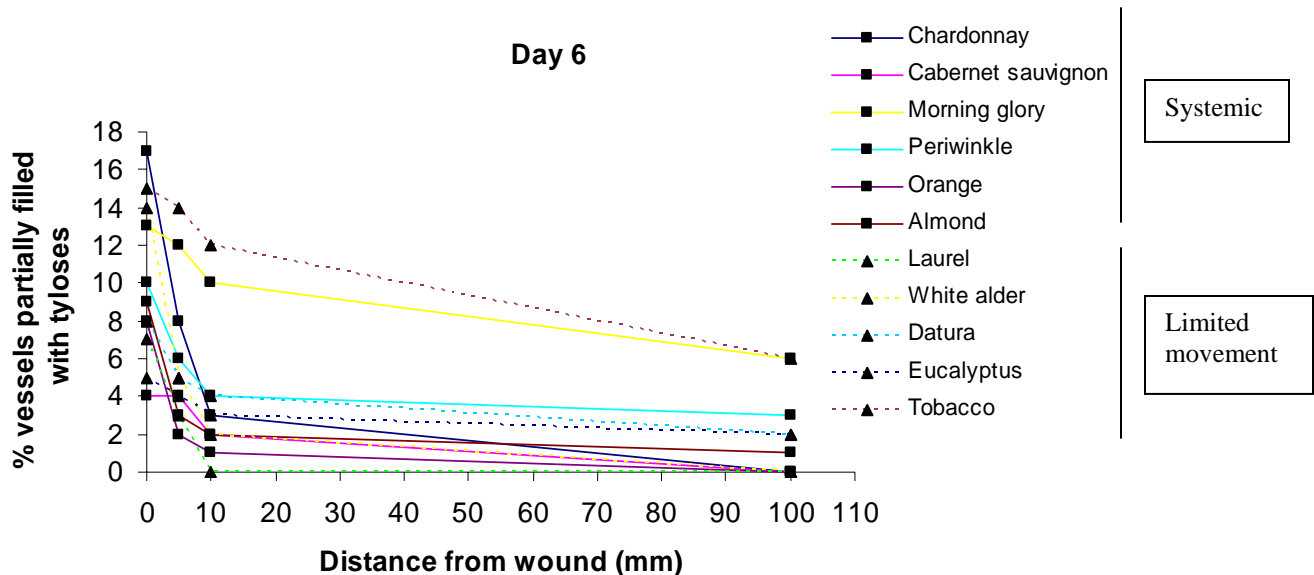
In addition to the importance of the stem-leaf connection, the shape and venation pattern of the leaves might also be important criteria to the movement of *Xf* into the leaves. The comparison of leaves of the different hosts showed a variety of shape and venation pattern and didn't reveal any obvious relation between bacterial movement and leaf morphology.

### Xylem structure of the stem and the petiole

Similarly, xylem structure was variable among species. There was no evident relation between the stem and petiole xylem structure and bacterial movement. The only difference could be in the size and number of vessels. This is currently being investigated.

### Tyloses formation

In addition to the xylem connectivity (see previous reports), comparison of tylose formation and development between grapevine and alternate hosts was studied. Bacterial movement in the alternate hosts could be impeded by rapid tylose formation as opposed to grapevine where tylose development occurs after colonization by the bacteria. Stems of similar age from the species mentioned above were wounded with a needle to imitate the insect. Stem segments were collected at 0, 1, 3 and 6 days after wounding and the presence of tylose in the vessels was observed in cross-sections made within the wounded area, 5 mm above it, 10 mm above and 100 mm above the wound. For each distance, the proportion of vessels partially filled or completely occluded by tyloses was calculated. The results showed that there are no differences between systemic and non-systemic plants.



**Figure 2.** Percentage of vessels partially filled with tyloses (A, C, E) six days after the wounding.

## CONCLUSION

Our results show that there are differences in xylem structure between systemic and non-systemic species. Indeed it seems that non-systemic species have shorter and narrower vessels in the stem, a lower connectivity between stem and leaves and a shorter path within the leaves. In addition, tylose formation didn't seem to be the cause for the limited *Xf* movement in the non-systemic plants. The species examined didn't produce more tyloses or faster.

## REFERENCES

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- Thorne ET, Young BM, Young GM, Stevenson JF, Labavitch JM, Matthews MA, Rost TL. 2006b. The structure of xylem vessels in grapevine and a possible passive mechanism for the systemic spread of bacterial disease. *American Journal of Botany* 93(4): 497-504.

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# MAGNETIC RESONANCE IMAGING: A NON-DESTRUCTIVE APPROACH FOR DETECTION OF XYLEM BLOCKAGES IN *XYLELLA.FASTIDIOSA*-INFECTED GRAPEVINES

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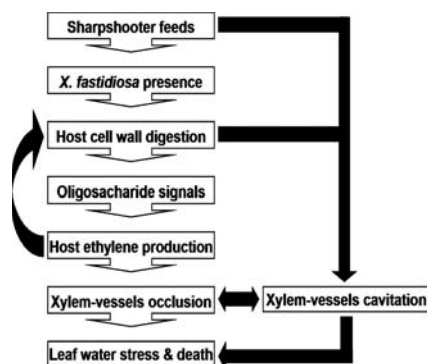
**Reporting Period:** The results reported here are from work conducted October 1, 2005 to September 30, 2006.

## ABSTRACT

Our report in the 2005 Pierce's Disease Research Symposium (Shackel and Labavitch, 2005) demonstrated the value of using MRI to follow the development of cavitations in grapevine xylem following introduction of *Xylella fastidiosa* (*Xf*) via needle inoculation. Strong correlations between increasing proportions of stem xylem conduits that were cavitated, visualized using MRI, and decreased water conductance. Similar observations were made on stems after treatment of vines with ethylene, supporting, but not proving, the suggestion of our Pierce's disease (PD) development model (Labavitch et al., 2005) that vine ethylene production was an important factor in the development of *Xf* infection. This year's effort has been devoted primarily to the replication of the observations of the first two years of this project. In addition, we have continued with the development of conventional, destructive anatomical approaches that we will use to define the nature of the more permanent xylem obstructions, gels and tyloses, whose presence in the water conduits of infected vines may be associated with the occurrence of cavitations. Finally, after considerable delay, we can report that our collaboration with colleagues in the University of California, Davis (UCD) NMR Facility to develop an NMR probe designed for ease of use in grapevine imaging and greater resolution appears to be on course.

## INTRODUCTION

Results from several Pierce's Disease (PD) research programs reported in the 2001 to 2005 PD research symposia in San Diego have supported the idea that obstructions in the grapevine's water-transporting xylem tissue develop rapidly post-inoculation, before an appreciable bacterial population has been established. Thus, careful analysis of the timing of changes in xylem element anatomy and function relative to *Xylella fastidiosa* (*Xf*) introduction and the appearance of the external symptoms of disease development, is important for establishing reliable indicators of the "stage" of PD development. Because the more conventional destructive analyses of xylem function made it impossible to fully understand the progression of internal symptoms and loss of grapevine water-conducting capacity with symptom appearance, we began testing the possibility of using MRI to follow xylem function in individual vines over time. In the course of this study we have developed imaging techniques for obtaining quantitative information about xylem function in individual vine internodes over time, defined the limitations of these techniques, and demonstrated that both PD infection and ethylene treatment trigger decreases in vine water-moving capacity (Shackel et al., 2005; Pérez-Donoso, 2006; Pérez-Donoso et al., 2006). The techniques we have developed also have been used in our tests of a model for PD development (Figure 1; Labavitch et al., 2005). We are currently attempting to define the relationship of the tracheid and vessel cavitations revealed by MRI and other, less transient occlusions that develop in the xylem of *Xf*-infected grapevines.



**Figure 1.** Hypothetical model for PD development. PD starts with infection caused by the glassy-winged sharpshooter's introduction of *Xf* locally (i.e., into one or a few vessels). Once *Xf* is in the xylem the bacteria become systemic, which implies that *Xf* must be able to cross (digest away?) the cell wall in the pit membranes that separate two neighboring vessels. The digestion of the cell wall by bacterial enzymes would generate transient oligosaccharides with biological activity. The presence of these oligosaccharides is detected by the plant triggering a series of defensive responses, including an increase in ethylene production. Ethylene has been shown to induce tylose formation. Cavitation of vessels may be also important for the disruption of water transport in the plant. Cavitations may happen during insect feeding or during PD progression. The "bottom line" of our thinking is that PD is primarily caused by the grapevine's responses (local and systemic) to *Xf* presence.