

**MECHANISMS OF PIERCE'S DISEASE TRANSMISSION IN GRAPEVINES:
THE XYLEM PATHWAYS AND MOVEMENT OF *XYLELLA FASTIDIOSA*.
PROGRESS REPORT NUMBER TWO: GREEN ISLANDS AND MATCHSTICKS**

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ABSTRACT

During this period our focus was the comparative xylem anatomy of a resistant species, *Muscadinia rotundifolia* cv Cowart and a susceptible species, *Vitis vinifera* cv Chardonnay. When infected by *Xylella fastidiosa* both species produced tyloses (parenchyma ingrowths into tracheary elements) and gums; *M. rotundifolia* tended to have fewer tyloses. The resistant species also had narrower vessels, but otherwise xylem anatomy was similar to *V. vinifera*. Fluorescently tagged beads were loaded into both species. Beads traveled through the stem xylem in both, but did not move into petioles in these experiments. Tyloses were first apparent 24 hours after pruning in both species and most vessels were blocked in both after eight days of pruning. This suggests that the mechanism to form tyloses in both species is similar, although the resistant species tended to show fewer tyloses in response to *Xf*. Two symptoms, green islands and matchsticks are reported in this study. Green islands formed as a result of incomplete initiation of the phellogen. In regions of the stem where a phellogen and subsequent periderm arose, immediately exterior tissue was cut off, causing it to brown. In regions of the stem where no periderm is formed, the exterior tissues remained green. Consequently, the stem is mottled with both green living epidermis and brown dying epidermis as determined by the presence or absence of an underlying periderm. Matchsticks formed when the leaf lamina separated from the petiole, and the petiole remained attached to the stem. Lamina broke off from the petioles consistently in a fracture zone where xylem from the petiole anastomoses into the five major veins of the leaf. No separation layer was found to explain this pseudoabscission.

INTRODUCTION

Xylella inoculation of stem xylem precedes a relatively rapid movement of bacteria through the hydraulic network (system of xylem) to the leaves. Once bacteria moving in the transpiration stream enter regions of the hydraulic network that contain narrow tracheary elements and terminal tracheary elements (i.e. shorter vessels in petioles and leaves), bacteria may be 'filtered out', accumulate, and become embedded in a gel which effectively blocks water flow in that conduit. Tyloses are cell wall extensions of xylem parenchyma cells into tracheary elements. Tylose formation in the stem coincides with bacterial infection, but at least initially, is not present to such a degree that bacterial movement is apparently prevented or that the water supply to distal tissues is restricted to levels causing visual symptoms. Additionally, bacteria can move relatively quickly from an inoculated shoot to another shoot via the subtending trunk.

A similar understanding of the progression of events is needed for resistant varieties and species in order to localize investigations into the mechanism(s) of resistance. The anatomical symptoms of PD, xylem occlusions of gums and tyloses, are well documented in both susceptible (Esau 1948) and resistant plants (Mollenhauer and Hopkins 1976). However, it is not clear whether these occlusions are related to susceptibility or resistance. Only the susceptible plants express leaf scorch and eventual death, and these disease symptoms are widely understood to be water stress (Hopkins, 1989). Sufficient occlusions would produce water deficits downstream. Plants resistant to PD may remain healthy despite systemic populations of *Xylella* present in the vascular tissue because tylose and gum formation are not induced compared to susceptible varieties. Alternatively, the occlusions may prevent the movement of the bacteria, and comparative studies report that the frequency of occlusions is greater in resistant than in susceptible varieties (Fry and Milholland, 1990). Thus, resistant varieties or species may restrict *Xf* to regions of the hydraulic network proximal to the point of inoculation, either by occlusions or other mechanisms described below. In the reported experiments, we have initiated those studies. Regardless of whether resistance is dependent upon controlling the movement of *Xf*, Pierce's Disease is fatal because *Xf* becomes systemic. Host species in which *Xf* is confined to specific tissues, or is otherwise prevented from becoming systemic, do not display symptoms of PD (Hill and Purcell, 1995).

It is generally accepted that the fatal nature of Pierce's Disease is a result of the bacteria becoming systemic and water stress becoming increasingly severe until the plant is no longer able to function (Goodwin et al., 1988). However, the classic PD symptoms: patchy leaf chlorosis, persistent "green islands" on stems, and "matchsticks" (leaf abscission at the petiole/blade junction) are not generally observed in vines exposed to water stress alone. If the symptoms of PD are not, in fact, a result of water deficit, then studies relying on the assumption that water stress is the ultimate killer of plants suffering from PD, may result in misleading information and add years to finding solutions to the PD problem. Our second annual report addresses these concerns.

OBJECTIVES

1. Study the progression of anatomical symptoms created by *Xf* over a time-course in a PD resistant grapevine species, *Muscadinia rotundifolia* cv Cowart.
2. Determine the hydraulic architecture of a PD resistant species, *M. rotundifolia*.
3. Study the integrity of pit membranes of both PD susceptible *Vitis vinifera* cv Chardonnay and resistant *M. rotundifolia* by following the in situ movement of fluorescently tagged beads.
4. Determine the rate of tylose development from wounding in both PD *V. vinifera* and *M. rotundifolia*.
5. Study the developmental anatomy of green island and matchsticks in *V. vinifera*.

RESULTS

1. PROGRESSION OF PD SYMPTOMS IN RESISTANT SPECIES

The progression of anatomical symptoms created by infection by *Xf* was studied along a time-course as was previously conducted with *V. vinifera* (Stevenson, Matthews and Rost, 2004). Similar experiments were conducted with PD resistant *M. rotundifolia* in an attempt to discern quantitative or qualitative anatomical differences in a six-month post-inoculation period. The development of symptoms in the resistant species was qualitatively similar to that in resistant species (development of tyloses in stems, development of gums in petioles), however the rate of development and overall occlusion created by these symptoms was dramatically lower. In the resistant species overall occlusion was minimal (<5% of vessels) after nearly four months (Figure 1), whereas in susceptible species overall occlusion was great (~50% of vessels).

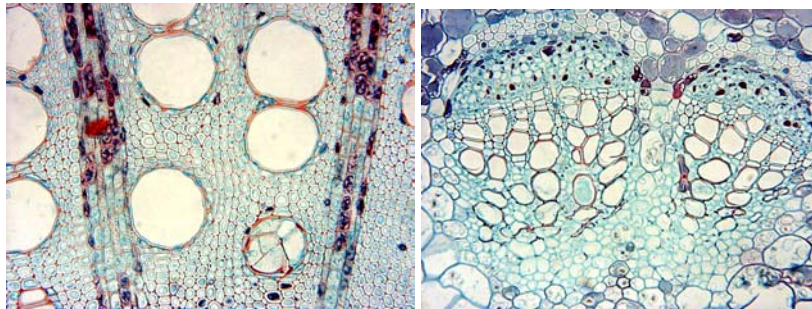


Figure 1. Minimal vessel occlusion in stem (left) and petiole (right) xylem of *M. rotundifolia* 122 days post-inoculation with *Xf*.

2. HYDRAULIC ARCHITECTURE OF RESISTANT SPECIES

The general hydraulic architecture of PD susceptible *V. vinifera* has been presented (Stevenson et al. 2004). Similar studies were conducted with PD resistant *M. rotundifolia* in an attempt to elucidate anatomical differences that may explain PD susceptibility or resistance. Regions of grapevine stem were serially sections to follow xylem arrangement in the node and internode. No significant differences were observed in the organization of stem xylem or in the divergence of xylem to lateral organs between resistant and susceptible species. The only difference found between the species was that *M. rotundifolia* possessed significantly narrower vessels than were found in *V. vinifera*. The difference may be contribute to restricting bacterial movement. Narrow vessels may cause bacterial conglomeration closer to the point of inoculation and prevent long distance bacterial seeding. Additionally, narrower vessels have less overall pit surface, which may further reduce the number of alternative pathways available to bacteria. Both of these proposals require further investigation.

3. PIT PROPERTIES OF SUSCEPTIBLE AND RESISTANT SPECIES

Preliminary investigations were conducted towards the study of the characteristics and integrity of pit membranes in susceptible and resistant grapevine species. The movement of *Xf* bacteria in the host is potentially facilitated by damaged pit membranes of grapevine, compromised either in development, or as a result of frequent cavitation/refilling cycles (Hacke et al. 2001, Sperry et al. 1987).

A. Movement of Fluorescent Beads

Fluorescent beads of similar size to *Xf* bacterial cells were injected into stem xylem of *V. vinifera* and *M. rotundifolia* (Figure 2). The distance of bead travel from the inoculation point was recorded as an indicator of vessel length and pit membrane integrity. Beads were observed to travel similar distances in both species (*V. vinifera* 1.6 ± 0.5 nodes, *M. rotundifolia* 1.8 ± 0.4 nodes). The relatively short distance that these beads traveled indicates a general integrity within the vessel pits and is evidence against pit damage commonly occurring. Beads were never observed to pass into petiole xylem, which suggests

that some pit membrane disruption, is required for bacteria to colonize petiole and leaf tissue (Stevenson, Matthews and Rost, 2004a).

B. Resin-casting and Macerations

Resin casts were made of the internal spaces of vessel lumina and pit surface morphology in both *V. vinifera* and *M. rotundifolia* (Figure 2). Superficially, no differences were seen in pit patterns, pit integrity, or relative pit surface area between the species. Further study is required to investigate subtle characters of pit membranes (ex. total pit membrane area, dimensions of pit apertures) that may facilitate pit membrane disruption by bacteria.

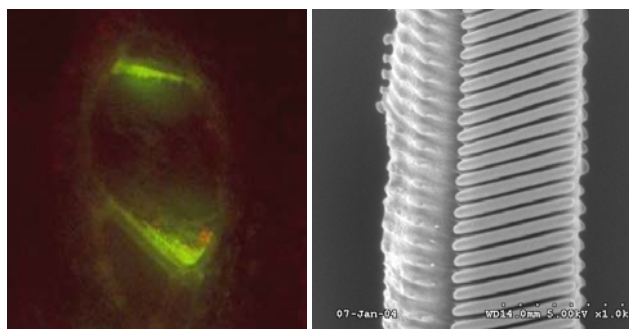


Figure 2. Fluorescent beads within stem xylem of *V. vinifera* used to mimic movement of passive bacterial cells (left), and resin casts of xylem vessels from *M. rotundifolia* to show fine detail of pit surfaces (right).

4. TYLOSE DEVELOPMENT

A. Rate of Tylose Development

A working hypothesis was developed that differential susceptibility to PD among grapevine species may involve differences in the rate of tylose development. The rate of tylose development was studied in both resistant and susceptible grapevines following wounding (pruning) injury. Tylose development was then observed allowing one, four, and eight days for tyloses to develop. Initial tylose development was found within a day, about half of the vessels were occluded by day four, and at day eight, most vessels of the stems were observed to be significantly blocked by tyloses (Figure 3). No superficial difference was seen between the rate of tylosis in PD susceptible *V. vinifera* and resistant *M. rotundifolia* at any of the time intervals, however, further quantitative analysis is necessary.

B. Vitality of Tyloses and Paratracheal Parenchyma

The presence of living cells surrounding the vessels during tylose formation following pruning was studied using the vital stain fluorescein diacetate. This technique was used to discern a correlation between the amount of tylose occlusion found in the vessel and the number of vital paratracheal cells surrounding that vessel, and whether the number of vital paratracheal cells was significantly greater in PD susceptible grapevine species. Both resistant and susceptible grapevines were observed in this manner over the eight-day time course described in 4A. No superficial differences were seen in the vitality of paratracheal parenchyma surrounding vessels in the two species, however greater quantitative analysis is required. Overall, tyloses fluoresced greatly, indicating vital development, whereas paratracheal cells fluoresced only occasionally (Figure 3). These results suggest that very few active paratracheal cells are required to result in significant tylose development.

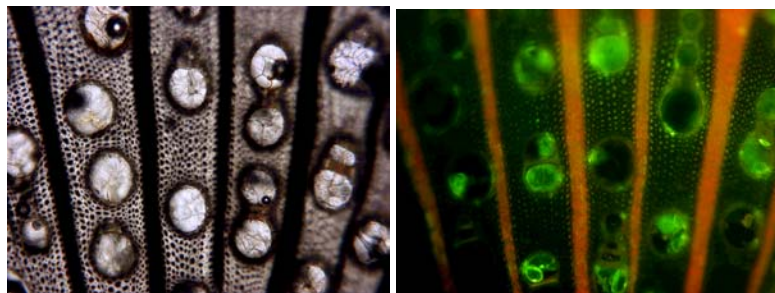


Figure 3. Micrographs of similar grapevine stems eight days following pruning. A bright field light micrograph (left) shows significant occlusion by tyloses at this interval. A fluorescence micrograph (right) shows fluorescent green vital staining predominantly by tyloses, but occasionally by paratracheal

5. DEVELOPMENTAL ANATOMY OF MATCHSTICKS AND GREEN ISLANDS

The development of the external visual PD symptoms of matchsticks and green islands was studied from an anatomical perspective (Stevenson, Matthews and Rost 2004b).

A. Matchsticks

Matchsticks result from pseudoabscission of the leaf lamina from the petiole. Following significant leaf scorching, the lamina breaks from the petiole at a predictable fracture zone. No separation zone develops as is common with typical leaf abscission, and hence this process is described as pseudo-abscission. Following pseudoabscission, exposed petiole tissues dehydrate and blacken to take on the appearance of a burnt matchstick. Occasionally, a wound periderm will form near the fracture zone following pseudoabscission. When this periderm forms, dehydration of the petiole is minimal. The process of matchsticking has never before been described anatomically.

B. Green Islands

Green islands arise from the incomplete development of the deep-seated phellogen (cork cambium) in *V. vinifera*. In regions of the stem where the phellogen arises and produces subsequent phellem (cork), external tissues (phloem, cortex, epidermis) are cut off from their nutrient sources and begin to die and brown. The juxtaposition of stem regions with active phellogen, and the juvenile character of no phellogen, creates green islands. It is unknown whether green regions are delayed in their development, or whether brown regions display advanced development. No obvious correlation was seen in the level of vessel occlusion proximal to green or brown regions. Additionally, periderm formation was observed in *M. rotundifolia*. Periderm formation in this species is subepidermal (vs. deep-seated) and consequently green islands may not form in this species (Stevenson et al. 200x). This is important point for researchers using green islands as an indicator of PD resistance.

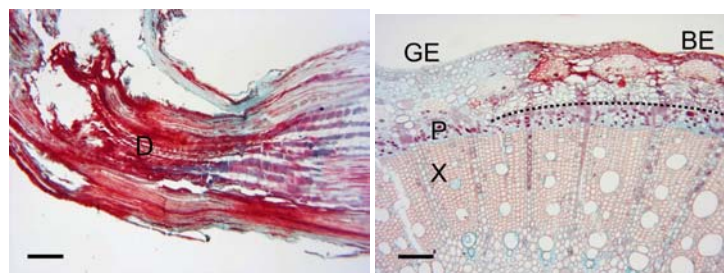


Figure 4. Longitudinal section through a matchsticked petiole (left) displaying basipetal dehydration (D) following pseudoabscission. Transverse section through a stem with green island (right) showing regions of green epidermis (GE) and brown epidermis (BE) created by presence of absence of phellogen initiation.

CONCLUSIONS

1. The development of tyloses and gums in response to *Xf* infection were qualitatively similar in the resistant *M. rotundifolia* cv Cowart and the susceptible *V. Vinifera* cv Chardonnay, although the resistant species tended to form fewer tyloses.
2. The only observable difference in hydraulic architecture was that the resistant species had narrower vessels.
3. Fluorescent beads were loaded into stems of both species. Beads moved approximately the same distance (~1.6-1.8 nodes) and in both cases did not enter into petioles.
4. Tyloses were first seen about 24 hours after pruning in both species. After four days about 50% of vessels were blocked. By eight days most vessels were blocked in both species.
5. Matchsticks formed in *V. vinifera* leaves after several days of *Xf* infection. This symptom consisted of the pseudoabscission of the petiole from the leaf blade. Green islands are green areas of the stem created by incomplete formation of periderm in infected plants.

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