Progress Report for CDFA Contract 08-0174

Project Title Do cell wall structures limit *X. fastidiosa* distribution in inoculated, Pierce's disease (PD)-susceptible and –resistant grapevines?

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Objectives & Progress

Objectives (Note: Only Objectives 1 and 2 of the proposal were approved for the funding)

Objective 1: Determine if the development of xylem obstructions (tyloses and pectin-rich gels) and the polysaccharide structure and integrity of pit membranes are affected by *Xylella fastidiosa* (*Xf*) inoculation of grapevines transformed to express the PGIP from pear and other plant species in rootstocks and in scions.

Objective 2: Determine whether there are differences in pit membrane porosity or polysaccharide structure between resistant and susceptible grapevines. To what extent are these PM characteristics and the production of tyloses and gels modified by introduction of *Xf* to PD-resistant and -susceptible genotypes?

Objective 3: Determine the extent to which changes in pathogen virulence resulting from altered production of diffusible signal factor (DSF) correlate with the appearance of tyloses, gels and damaged PMs in inoculated vines.

Objective 4: Determine whether the impacts of inoculation on PM integrity and the production of vascular system occlusions identified in tested greenhouse-cultured vines also occur in infected vines growing in the field.

Progress

Grapevine Pierce's disease (PD) is caused by the xylem-limited bacterium *Xf*. Its symptom development depends largely on the pathogen's spread via the vessel system in infected vines and the subsequent vessel blockage caused by the vascular occlusions that are produced in response to the pathogen presence. Thus, we believe that the xylem structure should affect the grapevine's resistance to PD by affecting the entry and spread of the pathogen and the development of vascular occlusions. Our previous study has led to discovery of the differences in vessel arrangement patterns among some grape genotypes. Recently, we have

further investigated secondary xylem of a few grape genotypes with differential PD resistance to analyze any possible relationship between xylem structural features and PD resistance.

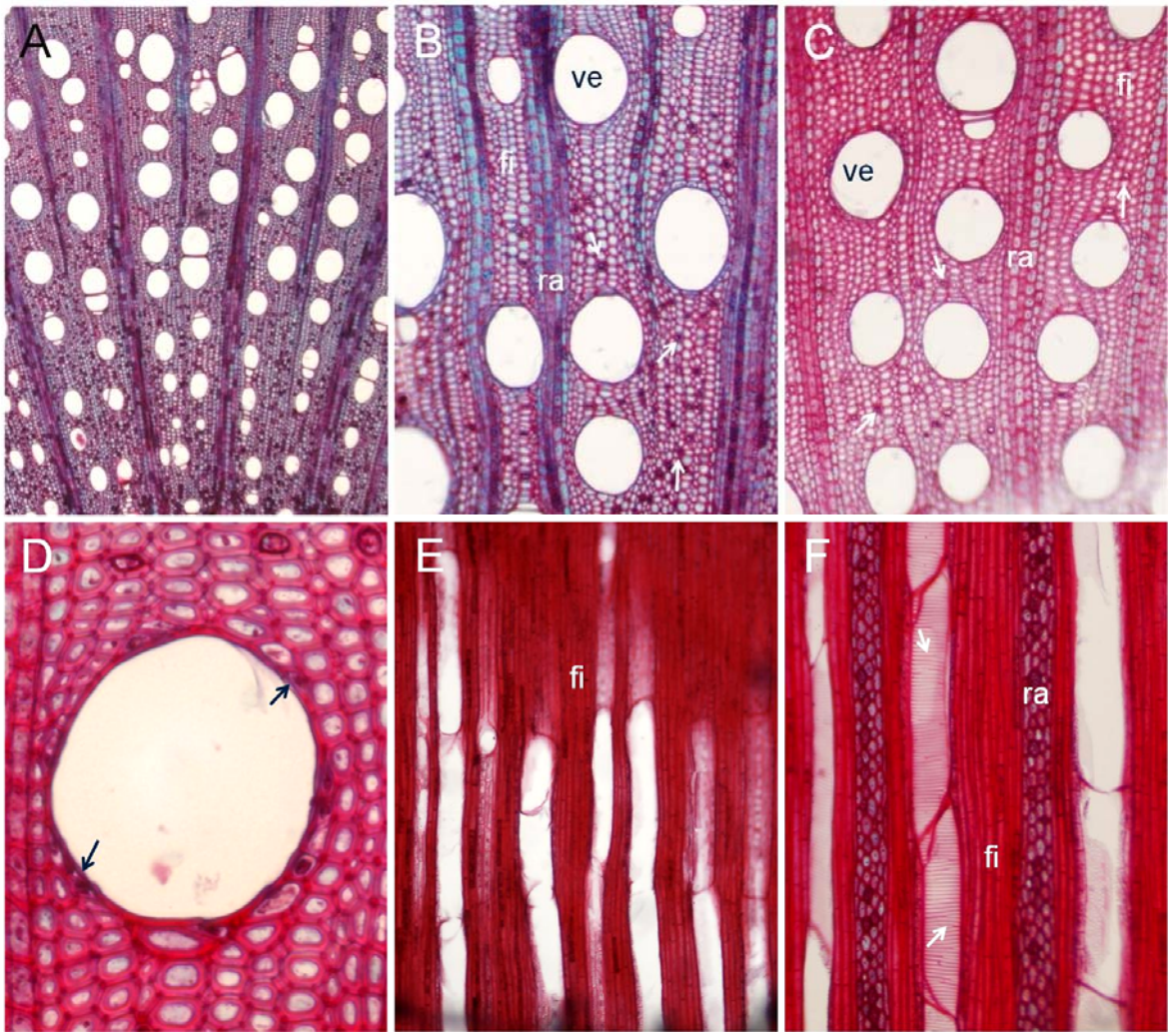

Figure 1. Secondary xylem structures of two susceptible grape genotypes: Chardonnay (A & B) and Riesling (C-F). A-D, Transverse sections. A. Relatively uniformly arranged vessels. B &C. Large, solitary vessels (ve), abundant fibers (fi), multiseriate rays (ra) and a few tannin containing fibers (arrows). D. Axial parenchyma cells (arrows) are adjacent to a vessel. E. A radial section, showing septate fibers (fi). F. A tangential section, showing scalariform intervessel pits (arrows), septate fibers (fi), and multiseriate rays (ra).

Five grape genotypes with different PD resistance were used in the current study: Chardonnay (susceptible), Riesling (less susceptible), Rotundifolia (highly tolerant), 89-0908 (resistant) and 89-0917 (resistant). For each genotype, several 4 cm long stem segments were collected from the internodes of about 3 months old shoots and fixed in formalin-acetic acid-alcohol for over 48 hours. Before tissue sectioning, three 1 cm long stem samples were cut from each fixed stem segment, rehydrated to water via an ethanol series, and then softened in boiling water for 2-4 hrs. Transverse, tangential and radial sections with a thickness of 25 µm were obtained from the three samples, respectively, using a sliding microtome. The sections were then dehydrated through an ethanol series, stained with Safranin O and Fast Green FCF, and

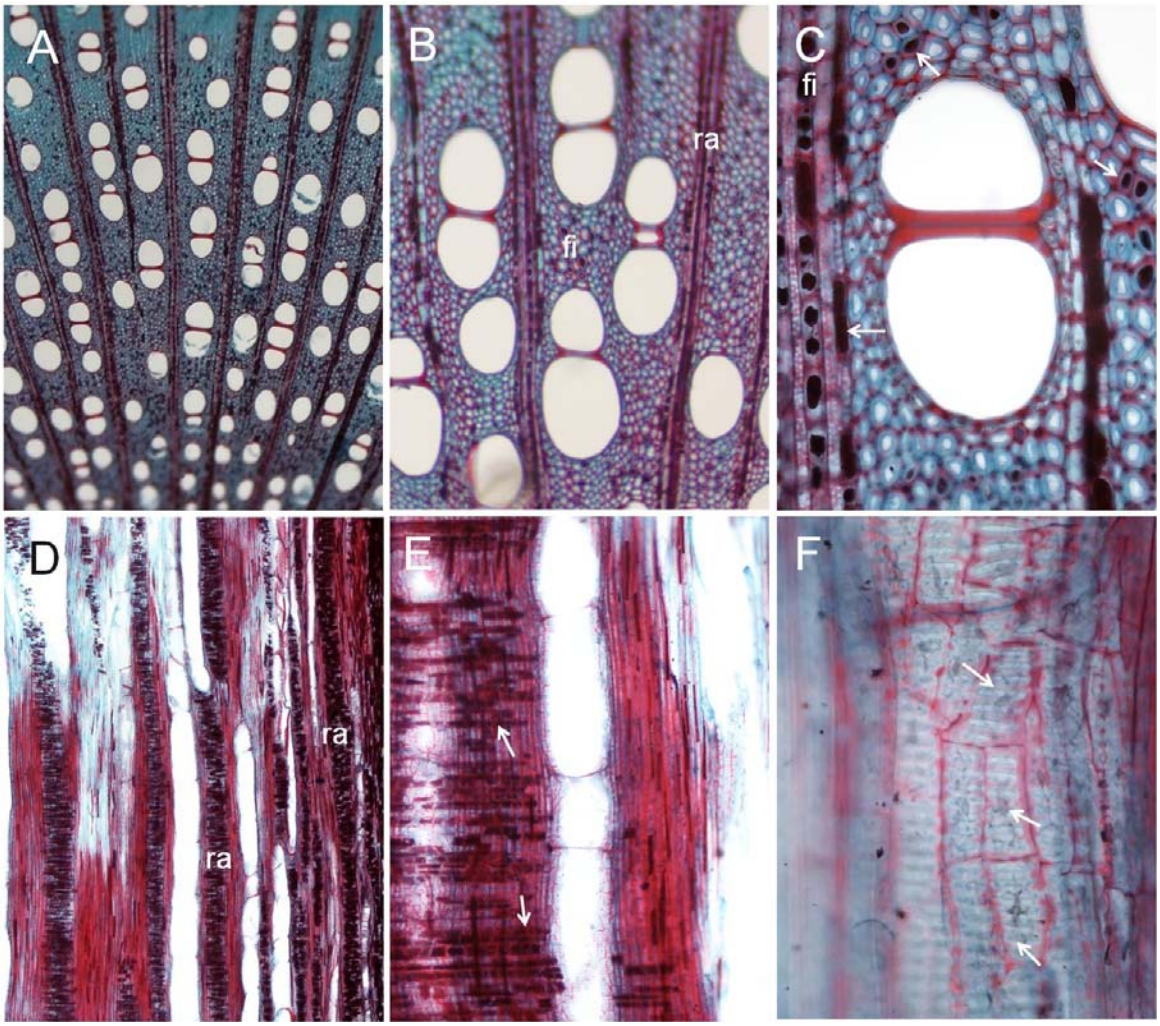

Figure 2. Secondary xylem of a tolerant grape genotype, Rotundifolia. A-C. A transverse section with increasing magnification. A. Vessels occur mostly in radial chains of 2-3 cells and solitary vessels are very few. B. Abundant fibers (fi) and multiseriate rays (ra). C. Tannin-containing fibers and ray parenchyma cells (arrows). D. A tangential section, showing multiseriate rays (ra). E. A radial section, showing that most ray parenchyma cells contain tannin (arrows). F. A tangential section, showing vessel-axial parenchyma pits (arrows) arranged in vertical rows parallel to the long axis of the vessel and parenchyma cells.

cleared with xylene. The cleared sections were used to prepare permanent slides, and observed and photographed under a light microscope.

Our results indicate that the five grape genotypes show some similarities as well as some differences in their secondary xylem structure. In terms of the structural similarities, all the genotypes included four types of xylem elements: vessel elements, fibers, axial parenchyma cells and ray parenchyma cells. Vessel elements had an exclusively simple perforation on each transverse or slightly oblique end wall. Intervessel pits were scalariformly arranged bordered pits (Figs. 1Fand 3D), while vessel-axial parenchyma pits formed half-bordered pit pairs with a bordered pit on the vessel side and a simple pit on the parenchyma cell side. Vessel-axial parenchyma pits were round, oval or transversely elongated and were arranged in more or less vertical rows along the axis of the vessel and parenchyma cell (Fig. 2F). Fibers were abundantly present in the secondary xylem of the five genotypes (Figs. 1B-C, 2B-C and 3B-C). They were

all septate with 2-4 septa (Figs. 1F and 3D-E), indicating their longevity. Axial parenchyma cells, the cellular sources for tyloses and gels, were very few and paratracheal (only adjacent to vessels) (Figs. 1D, 2F and 3E), sometimes forming a continuous layer surrounding a vessel. Rays were multiseriate, mostly 3-6 cells at their greatest width in the tangential sections in all the genotypes (Figs. 1F, 2D and 3D). Very few rays were adjacent to vessels. This indicates that ray parenchyma cells should make little direct contribution to the tylose development and gel secretion in vessels that may occur in response to *Xf* infection.

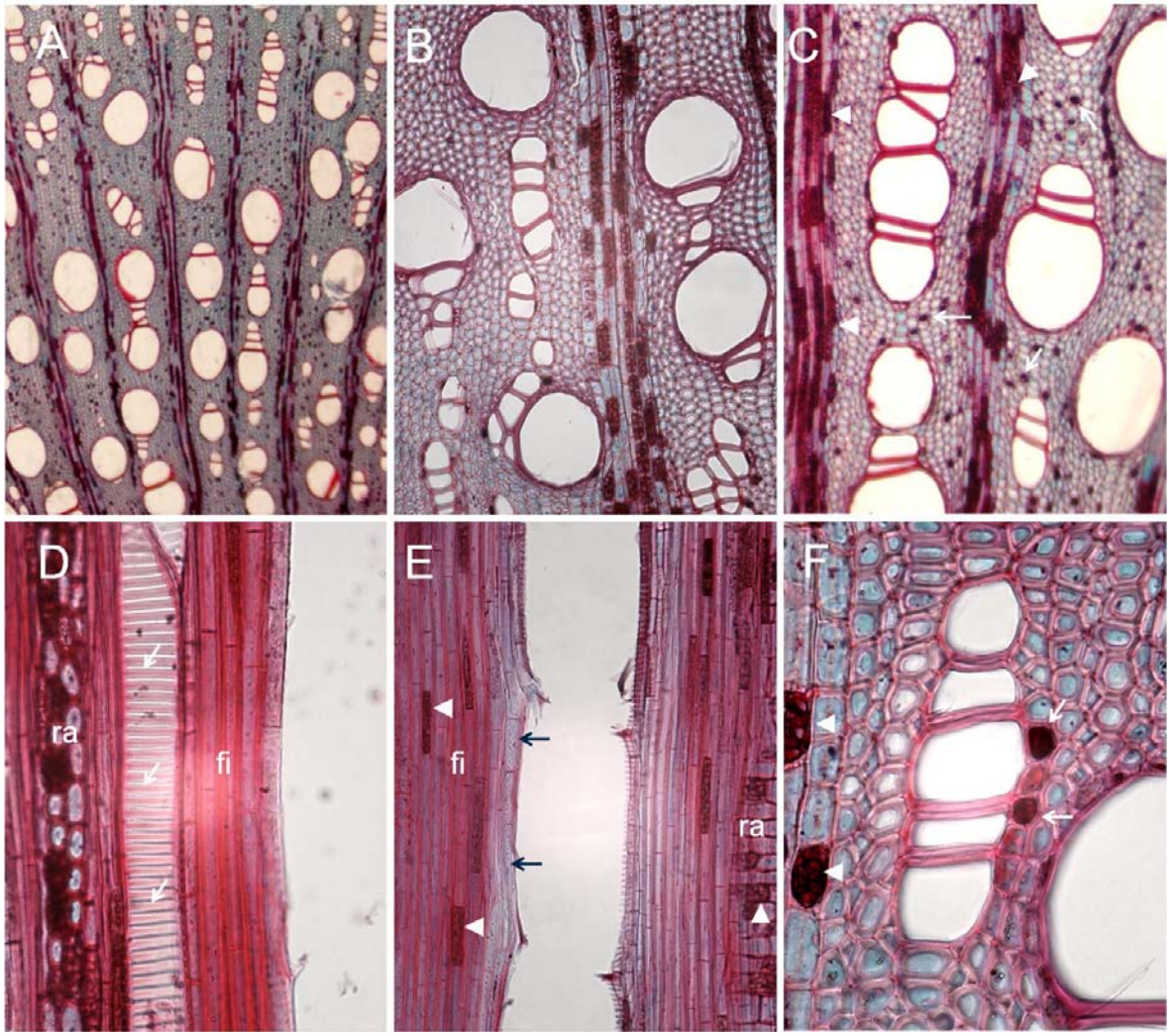

Figure 3. Secondary xylem of two resistant grape genotypes: 89-0908 (A, C & E) and 89-0917 (B, D & F). A & B. Transverse sections showing that vessels are unevenly distributed and vary greatly in size. C & F. Transverse sections. Vessels form radial chains of over 4 cells, fibers are abundantly present with tannin in many of them (arrows); many cells in multiseriate rays contain tannin (arrowheads). D. A tangential section showing a tannin-containing multiseriate ray (ra), scalariform intervessel pits (arrows), and septate fibers (fi). E. A radial section, showing a vessel (ve) and its adjacent axial parenchyma cells (arrows), septate fibers (fi), and ray tissue (ra). Some fibers and rays contain tannin (arrowheads).

The grape genotypes with differential PD resistance also differed in some structural characteristics of their secondary xylem. In vessel arrangement, the susceptible genotypes (i.e., Chardonnay and Riesling) had vessels that were the most evenly distributed in their secondary xylem (Fig. 1A-C), when distribution patterns were compared with those of the other genotypes

examined. The vessels in the susceptible genotypes were mostly solitary, relatively size-uniform, and the largest in diameter (Fig.1A-C). The resistant genotypes (i.e., 89-0908 and 89-0917) had secondary xylem with the least evenly distributed vessels (Fig. 3A), which were mostly in radial chains of 3-8 cells and the most varied in size (Fig. 3A-C and 3F). Vessels in the highly tolerant genotype (i.e., Rotundifolia) were less evenly distributed and smaller in diameter than those in the susceptible genotypes (Fig. 2A-B). But they were mostly in radial chains of 2-3 cells and more uniform in size than those in the resistant genotypes (Fig. 2A-C). These differences in the arrangement and quantitative characters of vessels are likely to be related to the differential PD resistance of the examined grape genotypes. According to Zimmermann (2001), large, solitary vessels are more efficient in conducting water but are less safe. When vascular occlusions, such as tyloses and gels, block a vessel at one or few locations, the solitary vessel may become completely dysfunctional. But if the vessel is in grouping that includes multiple vessels, the water can bypass the blocked regions through the adjacent vessels and then move back to the unblocked portion of the blocked vessel and, thus, the vessel can still be functional. Therefore, vascular occlusions should have less effect on the water conduction in the stems with many vessel multiples. In this way, the arrangement and quantitative characters of vessels may affect the PD symptom progression caused by inefficient water supply, and, consequently, be related to the PD resistance of a given grapevine genotype.

The grape genotypes with different PD resistance also differed in some structural features of fibers and ray cells. Tannins were observed in both fibers and ray parenchyma cells (Figs. 1B-C, 2C, 2E and 3C-F). However, the quantities of the fibers and ray parenchyma cells containing tannin were much fewer in the susceptible genotypes (Fig. 1B-C) than in the tolerant and resistant genotypes (Figs. 2C, 2E and 3C-F). One of the well-known physiological roles of tannin is to help a host plant to defend itself against diseases by a general (i.e., non-specific) deactivation of the enzymes secreted by pathogens. Therefore, the quantitative differences in tannin distribution among the genotypes may also be related to their differential PD resistance. Further investigation of this characteristic is still needed. The cell wall thickness of fibers decreased in the order of tolerant, susceptible and resistant genotypes.

Conclusions

1. Grape genotypes with different PD resistances differ in some structural features of their secondary xylem.

2. Differences in some of these xylem structural features among the susceptible, tolerant and resistant genotypes reflect their varietal differences in the hydraulic conductivity of infected vines, possibly contributing to their differential PD resistance.

3. The amount of tannin varied among the genotypes with different PD resistance.

Intellectual Property

The research results for this period may be used as morphological indicators to assess the PD resistance of grape genotypes/varieties generated through either a traditional breeding program or transgenic techniques.

Appropriate References

We now are preparing a manuscript describing our immuno-fluorescence method to identify the cell wall polysaccharide compositions of pit membranes, based on the data in previous progress reports.