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

Introduction

Pierce's disease (PD) of grapevine is caused by the bacterial pathogen *Xylella fastidiosa* (*Xf*) and can eventually kill infected vines. A few studies have indicated that development of the external PD symptoms depends largely on the ability of the pathogen to spread via the vessel system in the xylem of infected grapevines. When the pathogen is introduced into the vessel system, we believe that the following three factors from grapevine should affect *Xf*'s systemic spread and, thus, are relevant to the PD resistance of the grape plant. First, the distribution pattern of vessels and vessel-associated parenchyma cells might affect the size of the *Xf* population initially entering the vessel system. Second, for the *Xf* entering vessels, pit membranes (PM) separating neighboring vessels should function as barriers that the pathogen must digest to facilitate its systemic spread. Third, production of tyloses and pectin-rich gels that develop in vessels in response to *Xf*'s presence may also be related to disease symptom development or the grapevine's resistance. This project is aimed at understanding the possible roles of these factors in the grapevine's resistance to PD. This information also may be essential for identifying an efficient approach for control of the disease.

Our previous reports have covered these three aspects. In the first aspect, our data have shown that differences in certain structural and quantitative characteristics related to the xylem's distribution of water-conducting cells among the five grapevine genotypes/varieties with different PD resistances may affect the entrance of pathogens to the vessel system and their subsequent spread, thus contributing to their differences in PD resistance. In the second aspect, we have also clarified the differences in polysaccharide composition of intervessel PMs among our test grape genotypes/varieties. We found that the more resistant genotypes/varieties had no or much lower concentrations of the polysaccharides that are the potential substrates of *Xf*'s cell wall degrading enzymes (polygalacturonase and endo-ß-1,4-glucanase enzymes). This may explain why *Xf* cells in resistant grapevine genotypes have a restricted, localized distribution in vessels long after inoculation. In the third aspect, our research has revealed the temporal

development and spatial distribution of vascular occlusions (tyloses, gels and crystals) and their relation to the *Xf* spread via the vessel system in the PD-susceptible Chardonnay vines. The data have indicated that tylose development occurred in most vessels of secondary xylem everywhere in the vines with severe PD symptoms, but *Xf* cells were present in only a few vessels and were not numerous enough to block the vessels by themselves. We also found that the intensive tylose development did not stop the *Xf*'s systemic spread; rather it made the symptom development worse by reducing the water supply to the vines.

During this period we have investigated the integrity of intervessel and vessel-parenchyma PMs in infected grapevines. Our previous investigations and other research have suggested that *Xf* must break down the intervessel PM barriers to make their way for a systemic spread. However, direct evidence showing the breakdown of PMs has been lacking. Since this is closely related to the understanding of the PD resistance mechanism and the development of a possible approach for disease control, we recently used a PD-susceptible genotype, Chardonnay, to examine if *Xf* infection can lead to modification of intervessel PMs, which are related to *Xf* spread across vessels, and vessel-parenchyma PMs, which are involved in tylose development and gel secretion. If so, then how does the modification on the two types of PMs occur? Here we report our results on these two aspects.

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 *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.

Results

We investigated the impact of *Xf* on intervessel PM integrity by using PD-susceptible Chardonnay vines. Each vine was trained with only two shoots branching at the base of the main stem. Needle inoculation of *Xf* for each vine was conducted to only one shoot at the 12th internode from the shoot's base when the shoot was about 6 weeks old. Control vines were inoculated with a solution of phosphate buffered saline in the same way. All the control and infected shoots were maintained at 25 nodes in length by pruning the tops off. Samples were collected from both shoots of each vine weekly after the inoculation. The data reported here are

from both infected vines and control vines at Week 12 after treatment when the infected vines had shown severe PD symptoms.

Comparison of intervessel PM integrity between control vines and Xf-infected vines

In secondary xylem of grapevines, pits between adjacent vessels are horizontally elongated bordered pits which are arranged in a scalariform pattern along the long axis of the

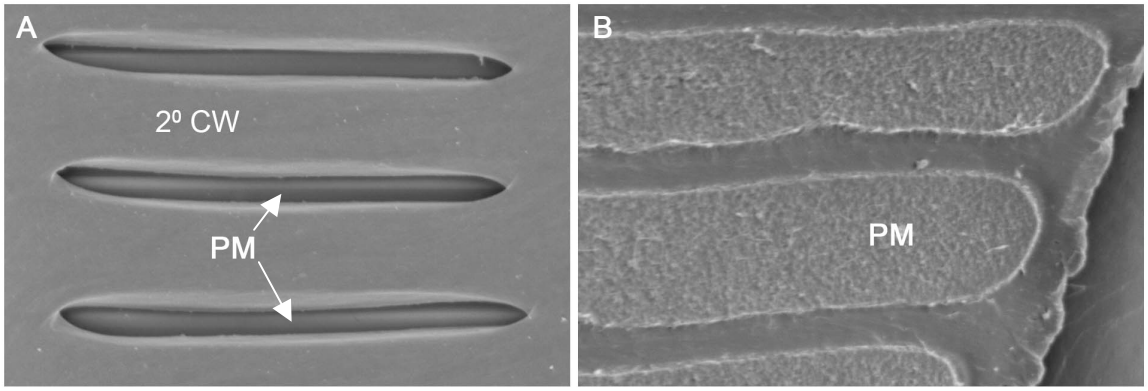

Figure 1.Scanning electron micrographs of intervessel pits and PMs in secondary xylem of a control Chardonnay vine. A. Bordered pits, showing secondary cell wall borders (2° CW) and PMs viewed through narrow pit apertures. B. Intact PMs are exposed after the secondary cell wall borders are peeled off.

vessel elements. For each pit, the borders of secondary cell wall are arched over the PM (including the primary cell walls of the two adjacent vessel elements and the middle lamella). Only a very small portion of the PM can be seen through the slit-like opening in the secondary wall in the surface view of the vessel lateral wall (Fig. 1A). However, the entire PM can be clearly examined for its integrity with a scanning electron microscope (SEM) after the removal of the secondary wall borders (Fig. 1B).

Our data have indicated that control vines have intact intervessel PMs whose surfaces are smooth, without distinguishable pores (Fig. 1). These intact PMs were also common in the vessels not associated with *Xf cells* in both inoculated and uninoculated shoots of the diseased vines. However, in the vessels inhabited with *Xf* cells, at least some PM barriers may be eventually degraded (Fig. 2). The degradation usually occurred initially as small separate patches in the central region of a PM (Fig. 2A). The PM surface in those patches became rough, probably because some wall materials have been removed and the remaining wall materials are loosely arranged and extend away from the PM surface. As more wall materials are removed, the rough region of the PM may expand to cover a central band region throughout most of the PM's width (Fig. 2B) and tiny pores in the PM region are visible under the loosely arranged surface wall materials (Fig. 2C). The peripheral region of the PM at this stage is intact and remains relatively smooth (Fig. 2C). Following this is usually the removal of most of the loosely arranged wall materials from the PM and the exposure of obvious small pores in the central region of the PM (Fig. 2D). Further degradation of the PM includes the enlargement of the pores in the PM's central region and the appearance of new pores in its peripheral region (Fig. 2F). Some pores are large enough for *Xf* cells to pass through. The increased porosity also weakens the PM itself and consequently, results in a crack along the central region of the PM throughout

its width (Fig. 2E). It seems that the two primary surfaces of a PM (i.e., the primary cell wall of

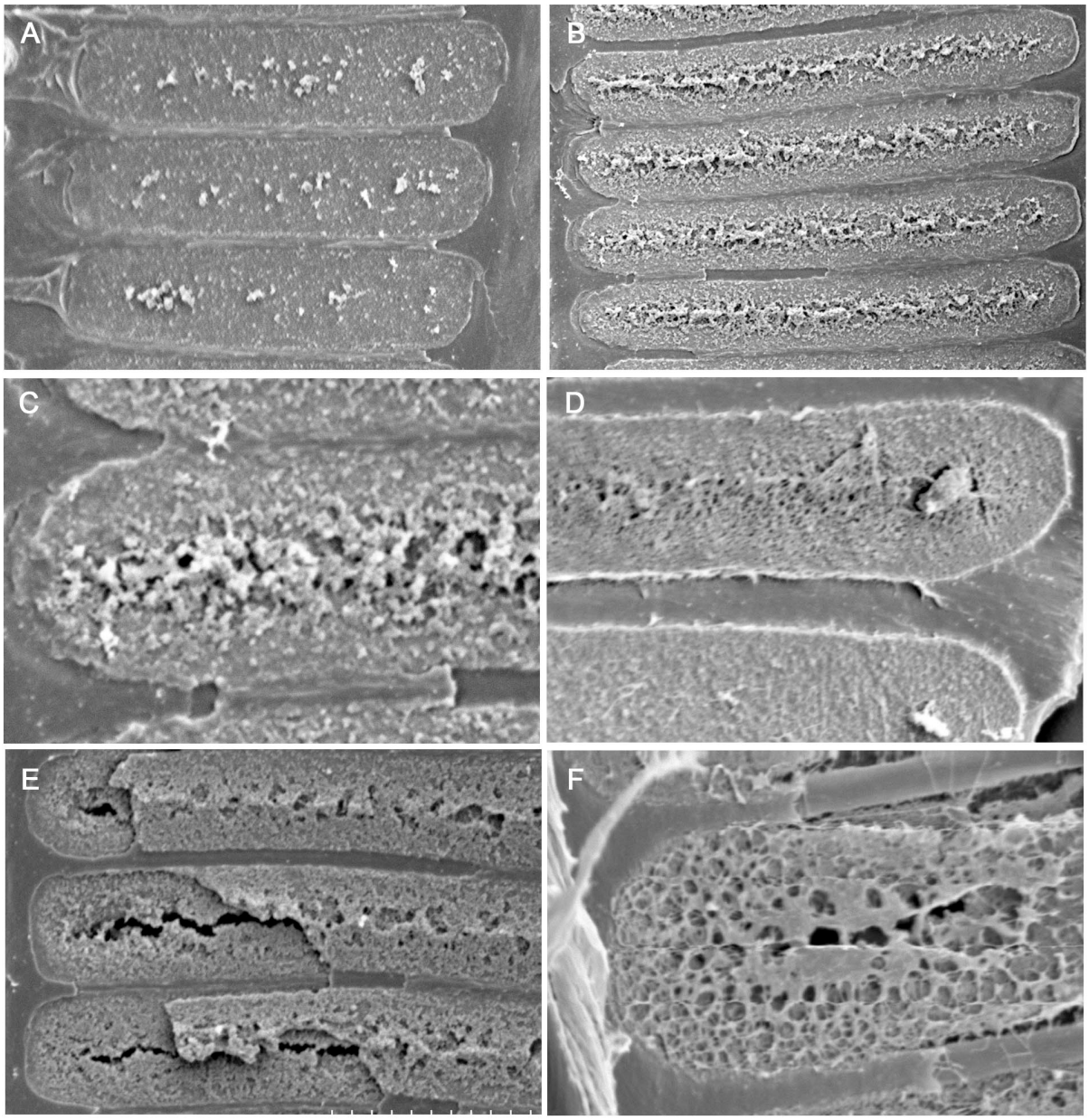

Figure 2. Scanning electron micrographs of intervessel PMs in secondary xylem of a *Xf*-infected Chardonnay vine, showing the PM degradation process. A. Few rough patchy regions with loosely arranged wall material in PMs. B. A central band of PM surface becomes rough with loose wall material. C. An enlargement of B. D. An enlargement of part of panel B showing tiny pores under the wall material. E. Two primary cell walls of each PM. The facing primary wall is highly porous and part of it is gone. The primary wall beneath forms a crack across its width. F. Large pores are present throughout the facing primary wall of the PM.

each of the neighboring vessels that "shares" a given PM) are no longer tightly attached at this stage, because the separation of the two primary walls of the damaged PM is common at this stage. The highly porous PMs may be partly or completely detached from their original positions and the PM barriers between adjacent vessels may disappear.

The degradation process may not always occur simultaneously among the different PMs of the same vessel element. Similarly, the two primary cell walls of a given PM may also differ in the progression of their degradation. We have seen that one primary wall of a PM has partially disappeared while the other primary wall has only a few small pores in its central region.

Xf cells were frequently observed inside pit chambers. They are mostly present in the central region of a PM (Fig. 3A), probably because the secondary wall borders of a pit arch over

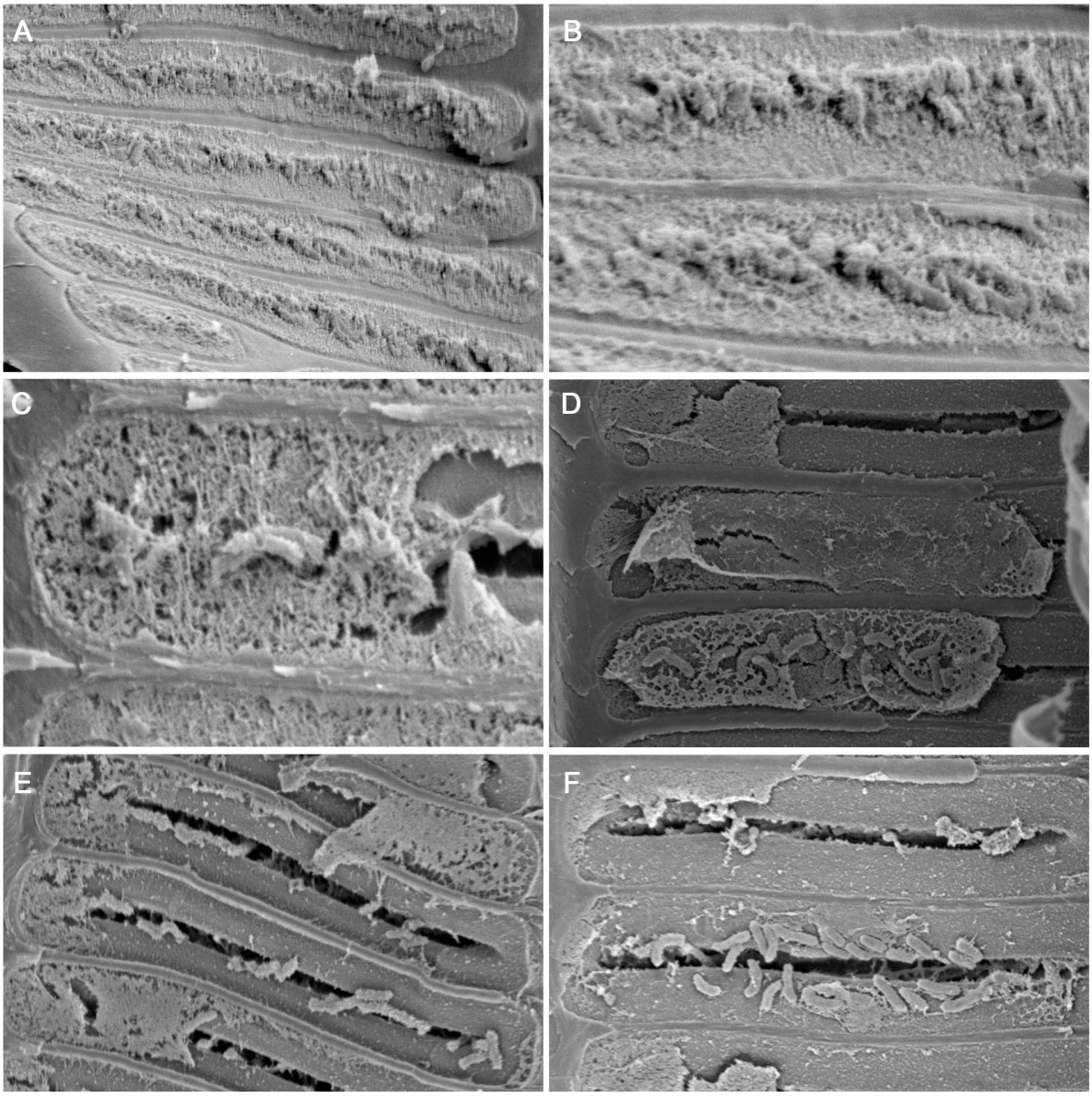

Figure 3. Scanning electron micrographs of intervessel PMs associated with *Xf* cells in Chardonnay vines, showing the different stages of PM degradation. A. *Xf* cells are accumulated in the central region of each PM. B. The enlargement of part of panel A, showing tiny pores rough PM surface. C. Porous PM after the removal of more wall materials. D. Degradation does not occur simultaneously among different PMs. The facing primary wall has disappeared in the upper two PMs but is highly porous in the lowest one. E-F. PMs have partly or completely disappeared and the remaining parts of the PMs are highly porous.

the PM, leaving too small spaces for *Xf* cells to accumulate in the peripheral regions of the PM.

The degradation of the PMs associated with *Xf* cells occurred in the same way as described above. At the beginning, the central region of PMs is commonly rough (Fig. 3B) and has tiny pores which can be seen wherever bacteria do not exist. As more wall materials have been removed from the PM, the size and number of the pores in the PM increase (Fig. 3C-D). Further degradation of this PM will greatly reduce its integrity as well as its strength and leads to its complete or partial removal from its original site (Fig. 3E-F).

Comparison of vessel-parenchyma PM integrity between control vines and Xf-infected vines

Certain significant morphological changes also occur in vessel-parenchyma PMs after *Xf* infection. In control vines, vessel-parenchyma PMs have an intact and smooth surface without distinguishable pores under SEM (Fig. 4A). Although the vessel-parenchyma PMs with these

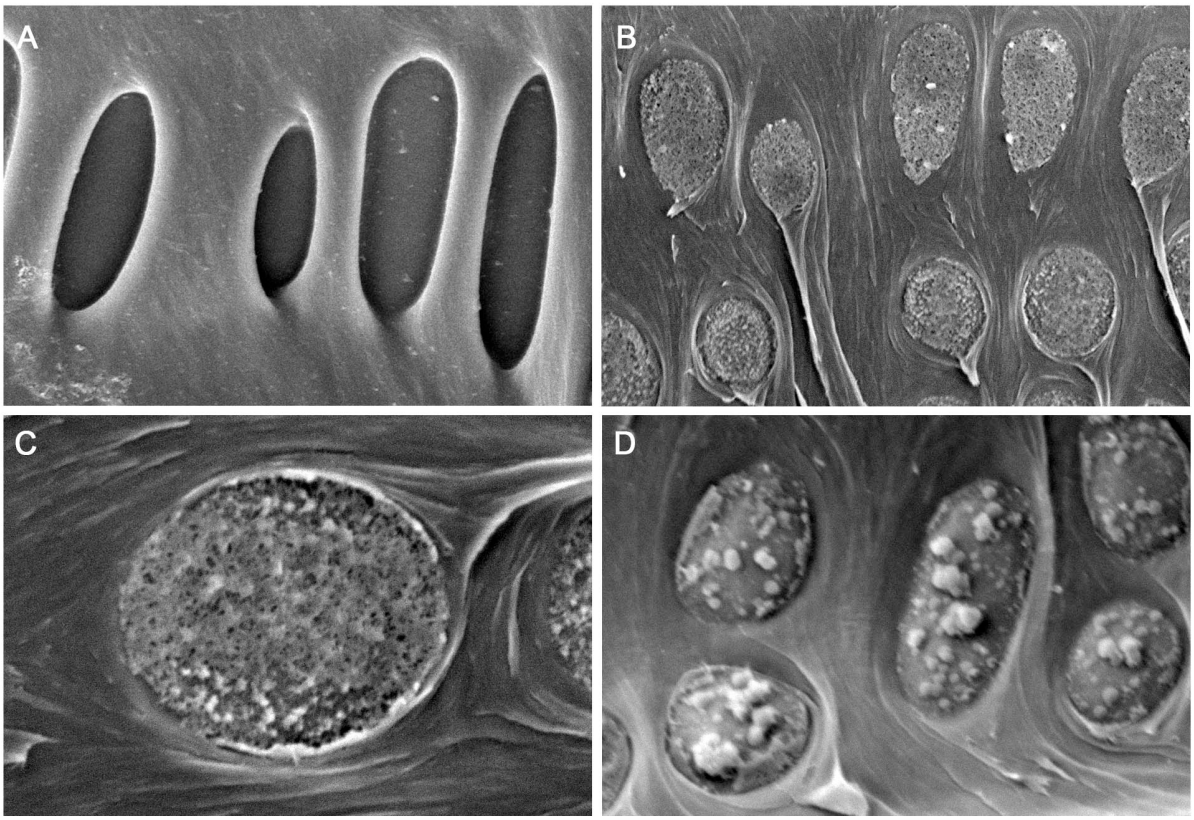

Figure 4. Scanning electron micrographs of vessel-parenchyma PMs in control vines (A) and *Xf*-infected vines (B-D, secondary wall borders have been removed). A. Intact and smooth vessel-parenchyma PMs in control Chardonnay vines. B. More or less rough vessel-parenchyma PMs contain many tiny pores. C. The enlargement of part of panel B, showing tiny pores in the PM. D. Substance accumulation on the surface of vessel-parenchyma PMs.

features have been observed also in the vessels with and without *Xf* cells in infected vines, a majority of vessel-parenchyma PMs, especially in the vessels associated with *Xf* cells, may be modified in two different ways. The predominant way is the formation of more or less rough surface and the appearance of tiny pores with a diameter of usually less than 0.2 μm. Some PMs with this modification seem to remain unchanged, but some other PMs just appear to be at

the early stage of tylose development. The other version of morphological change is accumulation of substance(s) on the vessel-parenchyma PM surface. This occurs with only a few PMs. Although the origin and composition of the accumulation still needs to be identified, it is highly likely to have its origin as a secretion from the parenchyma cells and may be the first sign of gel formation.

Intellectual Property

The research results for this period will not lead to any direct intellectual property. The data about the change in PM integrity are essential for understanding the PD resistance mechanism of grapevines. These results along with those in the previous reports about the internal symptoms (vascular occlusions) and the spread and amount of *Xf* in the grapevine xylem system may be used as indicators to evaluate the PD resistance of grape genotypes/varieties developed by either a traditional breeding program or transgenic techniques.

Appropriate References

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