

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

The introduction of *Xylella fastidiosa* (*Xf*) to grapevine xylem tissues often results in Pierce's disease (PD) and, ultimately, to vine death. Several studies have indicated that the development and progression of external PD symptoms depends largely on the ability of the pathogen to spread via the xylem, more specifically, the vessel system, in the infected grapevine. When the pathogen is introduced into the vessel system of a host plant, we believe that the following three factors from the host plant should affect *Xf*'s systemic spread and, thus, are relevant to the PD resistance of the grape plant. First, distribution pattern of vessels and vessel-associated parenchyma cells in xylem 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, blocks in vessels that develop in response to *Xf*'s presence may also be related to disease symptom development or the host plant'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.

In our previous reports, we have presented data showing that there are differences in structural and quantitative characteristics of the secondary xylem in five grapevine genotypes/varieties that differ in their susceptibility to PD. These differences may affect the entrance of pathogens to the vessel system and subsequent pathogen spread, thus contributing to the differences in PD resistance shown by these genotypes. More recently, we have also reported that there are differences in PM polysaccharide composition among these grape genotypes/varieties differing in PD resistance. We found that intervessel PMs of resistant genotypes lacked (or contained much lower concentrations of) polysaccharides that are the potential substrates of *Xf*'s cell wall degrading polygalacturonase and endo- β -1,4-glucanase enzymes. This may explain why *Xf* cells in resistant grape genotypes have a restricted,

localized distribution in vessels long after inoculation. Our research during this period has focused on the third factor outlined above: development of vascular occlusions in response to *Xf* infection. Although it is well known that tyloses, as one type of vascular occlusion, may develop in infected grapevines, the tylose functional role (either contributing to the host's PD resistance by preventing pathogen spread through the host plant's xylem or hastening disease symptom development by blocking or reducing water supply) is still unclear. To understand the tylose role in PD susceptibility/resistance, detailed information about the temporal progress, spatial distribution and quantitative characteristics of tylose development in host plant is essential. Recently, a delicate anatomical analysis on internal abnormal xylem structures after inoculation has been conducted. Here we report on the location and intensity of vascular inclusions that develop in infected vines. Our study also includes information about the spatial distribution and morphology of *Xf* in vines with severe external PD symptoms, which cannot be revealed via molecular approaches.

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

Comparison of vascular occlusion formation between control vines and sick vines

PD susceptible Chardonnay vines were used in our experiment. Each scion was pruned back with only two buds left at the base. Thus two branches developed. When the branches were 6 weeks-old, one branch of each treatment vine was needle-inoculated with *Xf* at the 12th internode from the base. Control vines were inoculated with PO₄ buffer at the corresponding internode on one of each vines' branches. Control and inoculated branches were kept at 25 nodes in length by pruning. Samples were collected from both branches of each vine at different times after the inoculation. This report only includes data from vines 12 weeks after treatment, when inoculated vines had developed severe external PD symptoms.

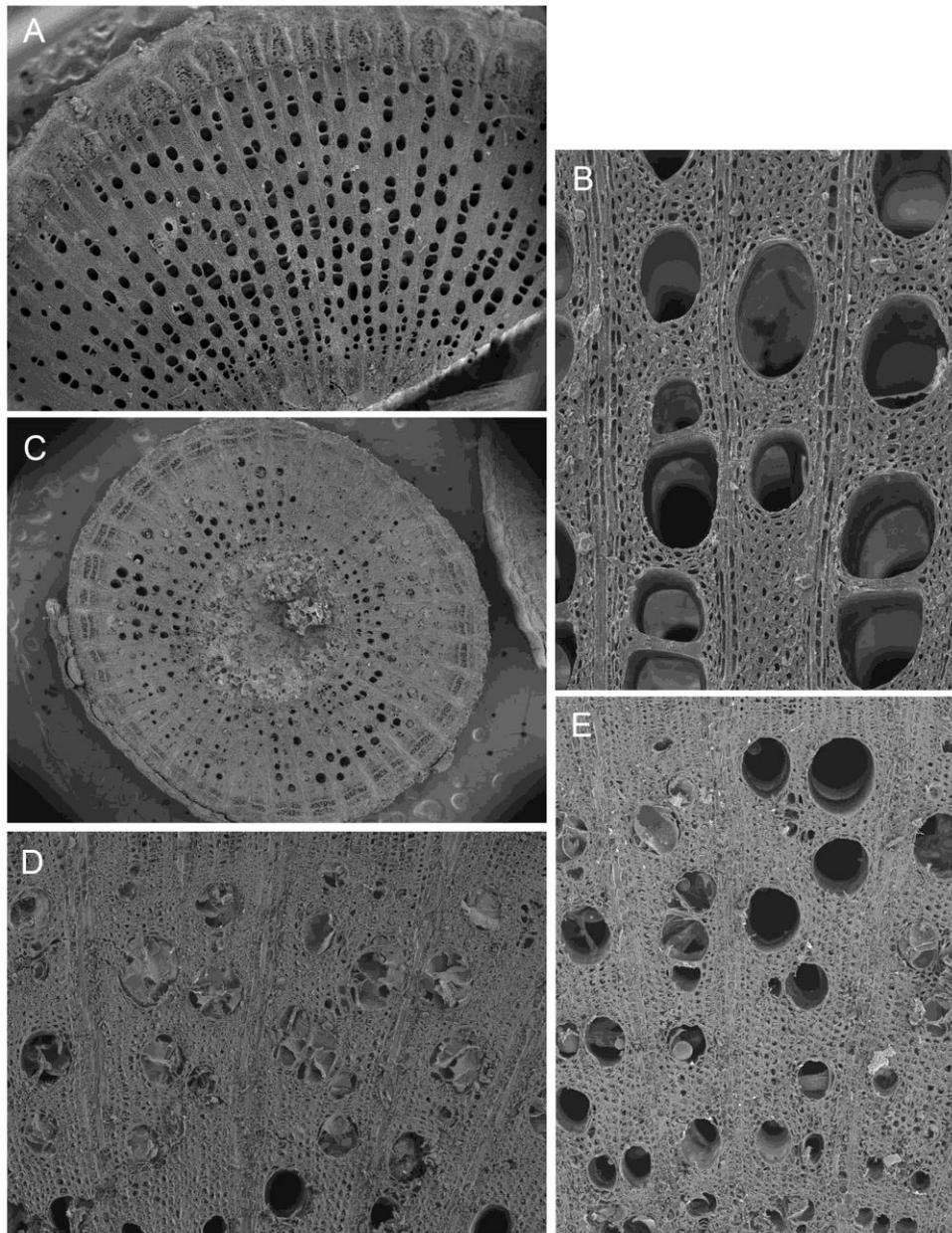


Figure 1. Xylem structure of control (A and B) and inoculated (C-E) vines. A-B. No vascular occlusions occurred in secondary xylem vessels (A); a closer image shows that vessel lumens are empty (B). C. Vascular occlusions developed in secondary xylem of inoculated branches and showed uneven distribution. D. Xylem region with extensive vascular occlusions, showing most vessels blocked by vascular occlusions. E. Xylem region with fewer vascular occlusions and some empty vessels.

There were obvious differences in secondary xylem structure of vines inoculated with *Xf* versus buffer (Fig. 1). No vascular occlusions were observed in secondary xylem of control vines, even in the internode where buffer inoculation was carried out (Fig. 1A and B). In vines inoculated with *Xf* there was extensive formation of vascular occlusions in secondary xylem vessels (Fig.

1C). Vascular occlusions in infected vines did not occur evenly in vessels across the transverse section. Instead, in some regions of xylem, they were present in most of the vessels (Fig. 1D), while in other regions, most vessels were free of occlusions (Fig. 1E). The cause for this patchy occurrence of vascular occlusions in secondary xylem is not known.

Our investigation of the spatial distribution of occlusions indicated that they occurred in the internodes of both branches of each infected vine, no matter the distance of the examined internodes from the inoculation site. Quantitative analysis of vascular occlusions revealed that the percentage of the vessels with vascular occlusions was usually around 60% for all examined internodes, with no big differences between the two branches of each vine or among different internodes of each branch (Fig. 2).

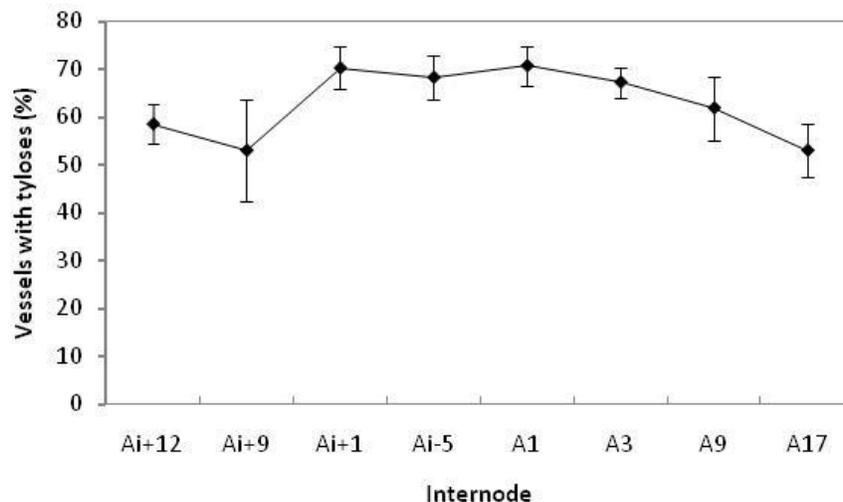


Figure 2. Comparison of vascular occlusion occurrence among different internodes of two shoots. “Ai” and “A” are the shoots with *Xf* inoculation and without inoculation, respectively. The number following “Ai” indicates a specific internode with the positive or negative number showing that the counting of internode started from the inoculated internode and moved upward (positive) or downward (negative), respectively. The number following “A” shows the internode in the non-inoculated shoot, counted from its base.

When tracking along the length of vessels, we found that tyloses did not always continuously block a whole vessel; i.e., there were gaps where no vascular occlusions developed (Fig. 3D). Considering this, the actual percentage of vessels affected by vascular occlusions is likely higher than the value measured at any transverse section. The effect of vascular occlusion on hydraulic conductivity of xylem has been evaluated (Pérez-Donoso et al., 2007).

Our investigation also makes clear that three types of vascular occlusions excluding masses of *Xf* cells, formed in secondary xylem. Tyloses are the predominant type and accounted for over 95% of the occlusions in vessels (Fig. 1C and D). Pectin-rich gels were another type of occlusion observed; these were found in less than 3% of the total vessels (Fig. 4A and B). Occasionally, crystals were also found in the vessels of infected vines and may partially or completely block the vessels where they are found (Fig. 4C and D).

Tyloses at different stages of development were observed in infected vines. Besides size differences, differences were also found in the appearance of their surface structure. At the beginning of tylose development, the cell surface was always rough with many short filamentous

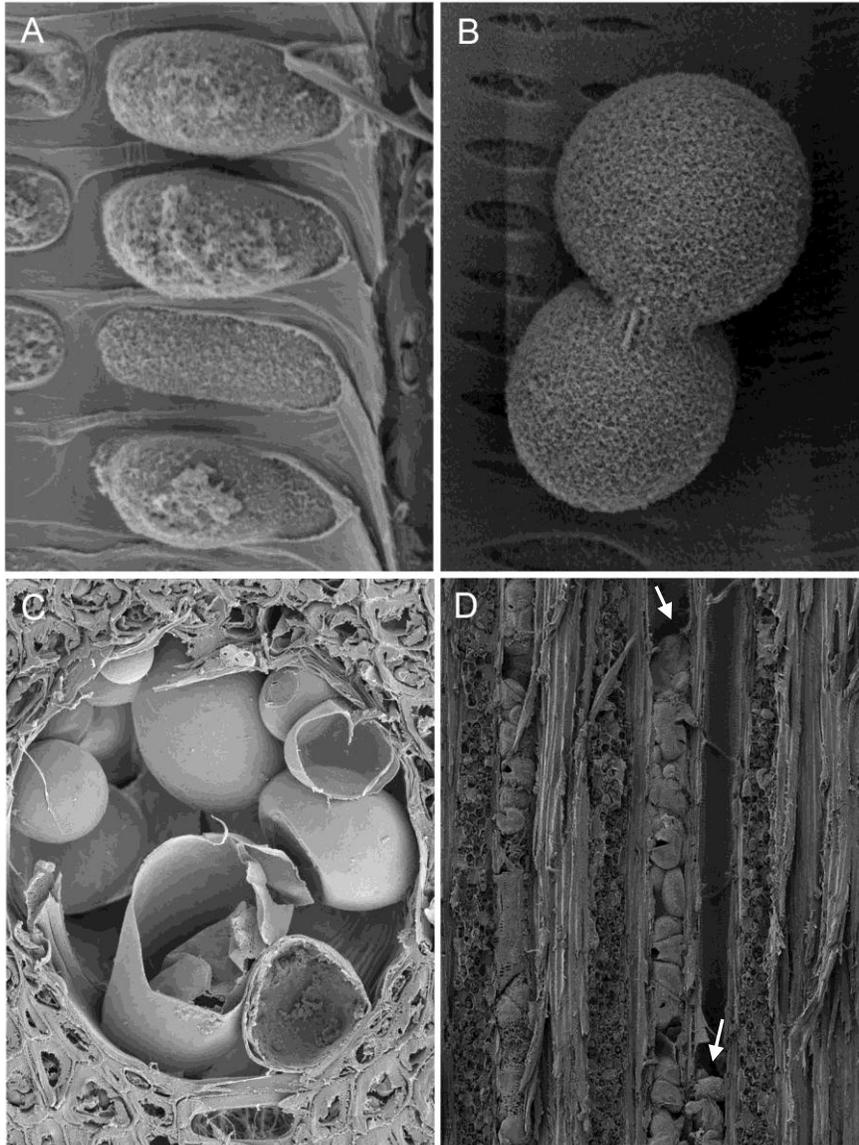


Figure 3. Stages of tylose development. A. Early stage of tylose development, showing rough surfaces with filamentous substances and tiny holes. B. Enlarging tyloses whose rough surfaces lacked the tiny holes. C. Tyloses reaching stable sizes were packed tightly together, thus blocking the vessel. These tyloses had smooth surfaces. D. Blockage of a vessel by tyloses was discontinuous along the vessel's long axis (arrows).

structures and pores of less than 100 nm were also common (Fig. 3A). As tyloses enlarged, their surfaces were still rough but lacked the short filamentous structures and the pores disappeared (Fig. 3B). When tyloses had reached their possible maximal sizes in a vessel, their surfaces became smooth usually without distinguishable pores and filamentous structures (Fig.

3C). It seems reasonable for us to infer that the changes in tylose surface during different developmental stages may indicate changes in the formation of its cell wall and, perhaps, also the wall's polymer composition. Further investigation is needed to clarify this process. Investigation of the expanding tylose wall may reveal important things about the cell wall changes associated with plant cell wall growth in general.

No correlation was found between the size of a vessel and the number of vascular occlusions it contained, although larger vessels were usually occupied by more tyloses when

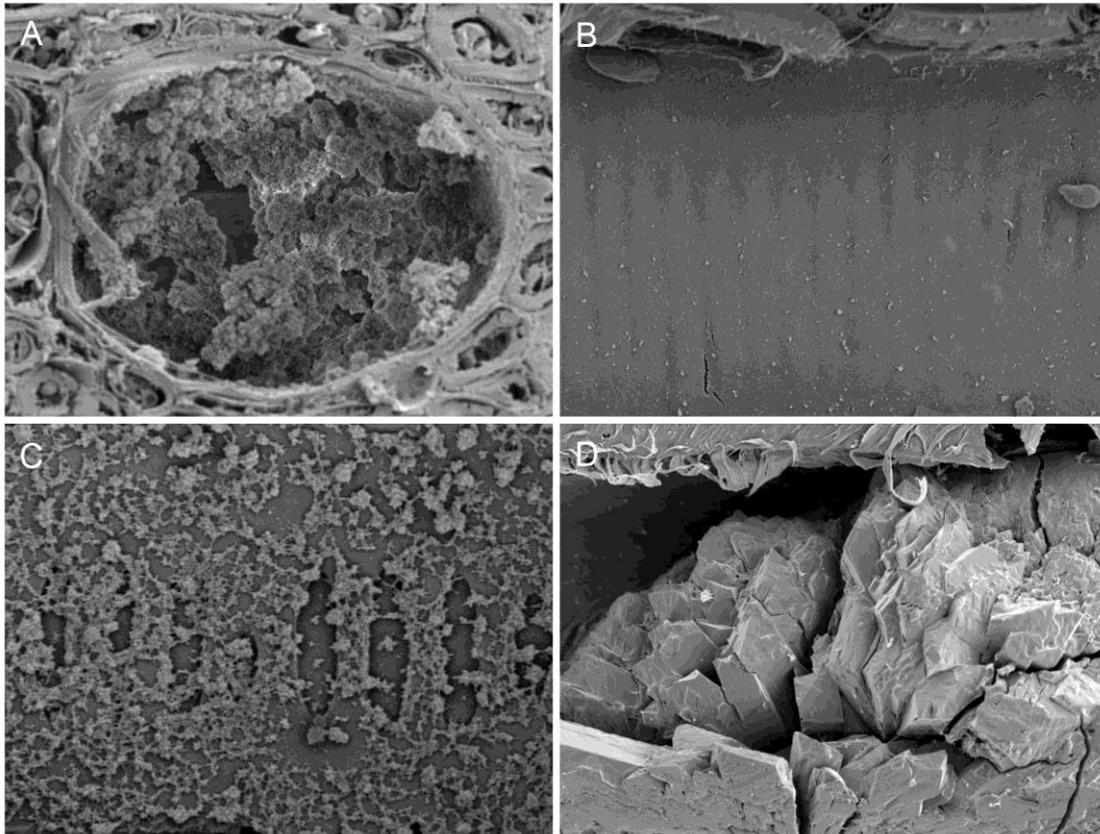


Figure 4. Other types of vascular occlusions in infected grapevines. A. Gels in a vessel lumen. B. Gels covering the lateral wall of a vessel. C. Gels sparsely attached to the vessel lateral walls. D. Crystals filling a vessel lumen.

viewed in the transverse section, probably because more adjacent xylem parenchyma cells were available to contribute to tylose formation.

Distribution of Xf after inoculation

In the vines with severe external PD symptoms, *Xf* cells were observed in all the examined internodes of the two branches (Fig. 5). This indicated that the bacteria could move upward from the inoculation site in a shoot, and also downward from the inoculation site to enter the main trunk and move from the trunk into the uninoculated branch and then upward in that, towards the top internodes.

Our observations also indicated that vines with severe symptoms contained *Xf* cells in only very few vessels. Vessels with *Xf* usually represented less than 10% and 3% of the vessels

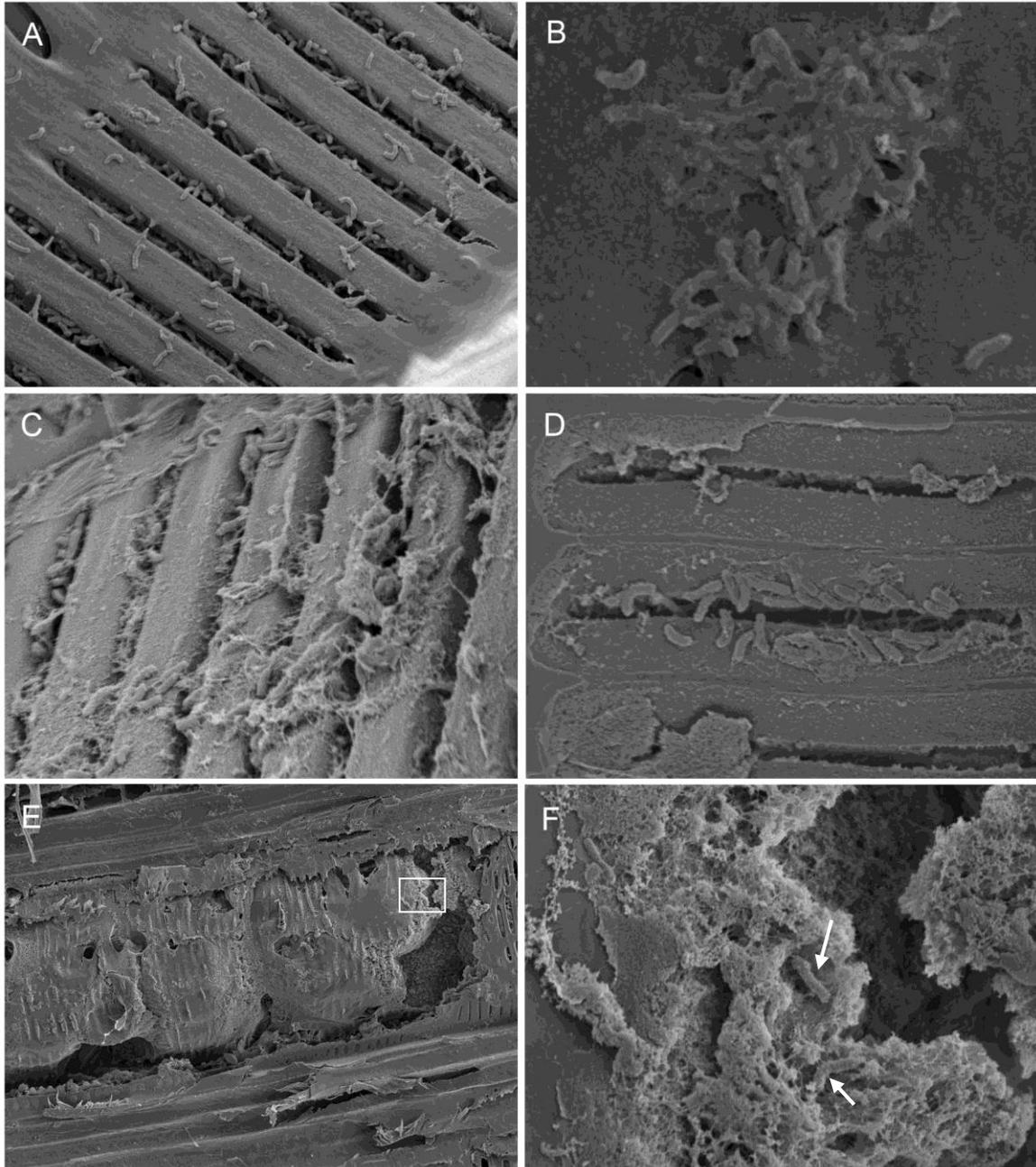


Figure 5. Distribution of *Xf* in infected vines. A. Bacteria are mostly present freely in the internode just above the internode with the inoculation site. B. Most bacteria in aggregate in the 9th internode (the counting started from the inoculated internode with it as zero). C. Some free bacteria and some bacteria in aggregate in the 9th internode of the non-inoculated shoot (the counting started from the shoot base with the lowest internode as one). D. Free bacteria in the 17th internode of the non-inoculated shoot (the counting started from the shoot base with the lowest internode as one). E. A vessel filled with tyloses in the lowest internode of the non-inoculated shoot. Gels were present between tyloses. F. The enlargement of the rectangle region in E, showing bacteria embedded in the gels.

in the inoculated and non-inoculated shoots, respectively. The number of bacteria in an "occupied" vessel was larger in the internodes of an inoculated shoot than in those of a non-

inoculated shoot. However, no vessels have been observed to contain a large enough *Xf* population to completely block vessels, as suggested in earlier reports. Since *Xf* cells are only present in few vessels in limited amount, a direct influence of the bacterial population on water transport through the vessel system is likely to be very limited.

Xf cells in vessel lumens took several different forms. Most commonly, they occurred as free individuals (Fig. 5A and D). Bacteria in this form were observed in the internodes of both inoculated and non-inoculated shoots. Aggregates of 2-6 cells were also common, in which bacteria were sparsely bound together through a filamentous network (Fig. 5C). Occasionally, aggregates of tens or hundreds of bacteria were observed in some vessel lumens (Fig. 5B). Bacteria were also observed between loosely or compactly arranged tyloses (Fig. 5E and F). In this case, bacteria were always embedded in gels whose origin (tylose or bacterium) is not clear.

Intellectual Property

The research results completed in this period project will not lead to any direct intellectual property. The data about development of 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 are preparing for a manuscript describing an immuno-fluorescence method to describe the cell wall composition of pit membranes, based on the data in our previous progress reports.