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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Time Period Covered: December, 2008 ~ February, 2009

Objectives & Progress:

Introduction: The initial introduction of *Xf* to grapevines by the glassy-winged sharpshooter (GWSS) involves one or a few water-conducting vessels. For *Xf* to spread systemically and cause PD, *Xf* move successively from one vessel to the next, thus the bacterium must move through the pit membranes (PMs) that separate one vessel from its neighbors. PMs are primary cell wall polysaccharide "meshworks" that act as filters to prevent long-distance movement of particles through the xylem. The porosity of the PMs (i.e., the size of the spaces between the PM polysaccharides) is too small to permit *Xf* passage. As a consequence, *Xf* uses polysaccharide-degrading enzymes (e.g., polygalacturonase [PG] and endo-β-1,4-glucanase [EGase]) to digest a path through PMs and, eventually, establish a systemic population.

While *vinifera* varieties are quite susceptible to PD. However, greenhouse and field evaluations of wild *Vitis* species and some of their hybrids with *vinifera* varieties have identified PD tolerance or resistance. Quantitative analyses of the concentration and distribution of the pathogen in inoculated vines have indicated that spread of *Xf* from the inoculation site in resistant genotypes *Xf* is more limited than in susceptible *vinifera* varieties, suggesting that PMs of resistant genotypes differ from those of susceptible ones in susceptibility to *Xf*'s enzymes, suggesting differences in polysaccharide composition. Therefore, characterization of genotype-associated differences in PMs should (1) add to our understanding of susceptibility *vs.* resistance-determining interactions of the pathogen with PMs and (2) provide a feature that may prove useful in screening germplasm for its ability to limit PD development.

Objectives (**Note**: Objectives 1 and 2 were to be addressed in year 1 of the proposal)

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 *X. fastidiosa* 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 *X. fastidiosa* 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.

Because of delays in the establishment of the sub-contract at the University of Wisconsin-Stevens Point, PI Sun did not have a great deal of "summer" research time to devote to the project before his busy teaching schedule began. However, substantial progress has been made in the work using cell wall polysaccharide-targeting antibodies to describe the kinds of polysaccharides in the PMs of susceptible and resistant grape germplasm. The observations that have been made support work under all objectives, but are directly relevant to the goals described for Objective 2.

We have used four grape genotypes/varieties with different PD susceptibility: *Vitis vinifera* var. Chardonnay (susceptible), *V. vinifera* var. Riesling (less susceptible), *Muscadinia rotundifolia* (highly tolerant) and 89-0908 (resistant, a hybrid of *V. arizonica* x *rupestris*). The immunohistochemical techniques and confocal laser scanning microscopy that were used here had been developed in our initial studies of susceptible *V. vinifera* var. Chardonnay and identified the cell wall polysaccharide substrates for *Xf*'s PG (homogalacturonan, HG) and EGase (xyloglucan, XyG) in Chardonnay PMs. Because other studies in our group had shown that a combination of PG and EGase would digest the PM polymer meshwork, our initial immunohistochemical work on the polymer compositions of the PMs of the four test grape lines has used anti-HG and -XyG antibodies. Three kinds of monoclonal cell wall antibodies (JIM5, JIM7 and CCRC-M1) were used to identify the polysaccharide compositions of PMs. JIM5, JIM7 and CCRC-M1 can recognize weakly methyl-esterified HGs (Me-esterified HGs), heavily Me-esterified HGs and fucosylated XyGs, respectively.

The investigation examined both intervessel PMs (IV-PMs) and vessel-parenchyma PMs (VP-PMs). IV-PMs affect *Xf*'s systemic spread because these are the PMs that separate one vessel from the "next" vessel. VP-PMs separate a vessel from neighboring xylem parenchyma cells. These living parenchyma cells are the source of gels and tyloses that cause vascular occlusions which may contribute either to disease resistance (limiting pathogen movement) or PD symptoms (closing down water movement). Thus examination of both IV-PMs and VP-PMs may identify features that are useful in identifying the susceptibility or resistance of grape germplasm.

Our results have shown that the vessels of the four test genotypes/varieties have both IV-PMs and VP-PMs in their lateral walls, thus comparisons between these lines can be made on the basis of both PM types. Individual IV-PMs are transversely elongated and across the whole cell wall surface that is shared with neighboring vessels; these are arranged in a tight scalariform (i.e., ladder-like) pattern perpendicular to the vessel long axis (Fig. 2A). VP-PMs are round, oval or slightly transversely elongated (Fig. 2D).

Differences in HG and/or XyG compositions of IV- and VP-PMs were identified among the four comparison grape lines. In 89-0908, both IV-PMs (Fig. 1A) and VP-PMs (Fig. 1B) do not contain fucosylated XyGs. The two anti-HG antibodies also failed to identify cross-reactive pectins in 89-0908's IV-PMs (Figs. 1C, weakly Me-esterified HG, and 1E, heavily Me-esterified HG). Whether the lack of antibody reactions with these PMs indicates the absence of the targeted

polysaccharides or the presence of additional PM components that prevent access of the antibodies to their targets is not clear at this time. However, 89-0908's VP-PMs contain both weakly Me-esterified HGs (Fig. 1D) and heavily Me-esterified HGs (Fig. 1F).

In *Muscadinia rotundifolia,* strong fluorescence signals were detected from both IV-PMs (Fig. 2A) and VP-PMs (Fig. 2B) when samples were incubated with CCRC-M1, indicating the abundant presence of fucosylated XyGs in both types of PMs. A small amount of heavily Me-esterified HGs is also present in both types of PMs (Fig. 2E and 2F). Weakly Me-esterified HGs were identified in VP-PMs (Fig. 2D), but were not detected in IV-PMs (Fig. 2C).

In *Vitis vinifera* var. Riesling, both types of PMs contain fucosylated XyGs (Figs. 3A, 3B) and heavily Me-esterified HGs (Fig. 3E and 3F). VP-PMs also have weakly Me-esterified HGs (Fig. 3D), but IV-PMs lack them (Fig. 3C).

In *Vitis vinifera* var. Chardonnay, fucosylated XyGs (Fig. 4A) and weakly Me-esterified HGs (Fig. 4B) are abundantly present in both PM types. Heavily Me-esterified HGs occur in a large quantity in VP-PMs (Fig. 4D), but are undetectable in IV-PMs (Fig. 4C).

In summary: The IV-PMs of resistant genotypes appear to lack fucosylated XyGs and weakly Me-esterified HGs (or contain additional components or structural features that interfere with antibody access), and contain relatively little heavily Me-esterified HGs. In contrast, the IV-PMs of the less resistant genotypes/varieties all have fucosylated XyGs and substantial amounts of either heavily or weakly Me-esterified HGs. The absence of the HGs and XyGs that are the primary substrates *Xf*'s PG and EGase or the presence of additional, potentially interfering PM components in the IV-PMs of resistant genotypes may contribute to the restricted, local distribution of *Xf* in their vessels following bacteria introduction by GWSS or needle inoculation, potentially contributing to the host plant's PD resistance. On the other hand, VP-PMs do not show obvious differences in the three groups of polysaccharides among the genotypes/varieties, with the exception of 89-0908. In 89-0908, VP-PMs lack fucosylated xyloglucans, but contain both weakly and heavily Me-esterified HGs. In the other genotypes/varieties, all the three groups of polysaccharides are common in their vessel-parenchyma PMs. The VP-PM difference between 89-0908 and the others may indicate some variation in the potential for vessel obstruction development following infection because pathogen-triggered modification walls of parenchyma cells that neighbor vessels likely affects gel and/or tylose formation.

Intellectual Property:

As far as we can tell, there is no intellectual property that will be directly tied to this project. However, the data presented above suggest that a useful basis for early screening of germplasm for PD susceptibility/resistance might be characterization of germplasm PM contents of XyG and HG polymers. Of course, the relationship of PM polysaccharide content to PD-resistant phenotypes must be examined more thoroughly. If the relationship proves reliable then our results will be an important contributor to the development of intellectual property/patentable germplasm.

Appropriate References:

A draft of a manuscript describing our early studies on PMs, their porosity and their susceptibility to PG and EGase is nearing completion. PI Sun will begin drafting a manuscript describing the results from this project when his academic teaching program at Wisconsin-Stevens Point ends this May.

The Relationship of the Potential Results from this Project and Solutions to the PD Problem in CA:

This point has been addressed earlier in our narrative. The proposed 2-year project was designed to indicate more clearly how projects aimed at developing more resitant grapevine germplasm by conventional genetic means (Walker et al. 2008) and by transgenic manipulations (Lindow et al. 2008and Labavitch et al. 2008) affect the ability of *Xf* cells in grapevine xylem to eliminate the PM "barrier" to systemic movement. In 2008, the proposed project was supported for only one year. In one year a full description of the *Xf*-PM biochemical and structural interactions will not be possible. However, the initial PM polysaccharide comparisons for resistant and susceptible grape genotypes/varieties has suggested what may be a very useful (correlative) basis to rapidly screen for PD resistant or tolerant germplasm. (Note: References cited in this paragraph are reports in the 2008 Symposium Proceedings)

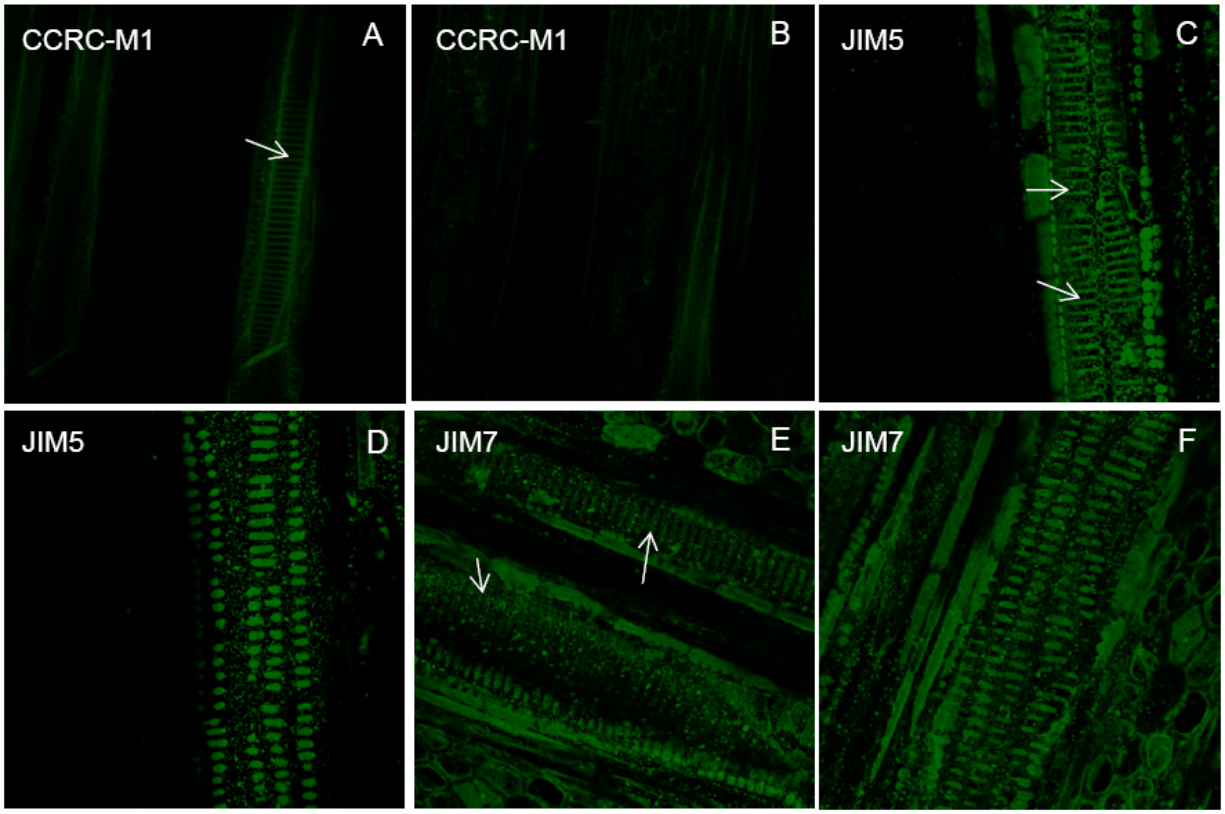

Figure 1. Polysaccharide compositions in intervessel pit membranes (A, C, E) and vessel-parenchyma PMs (B, D, F) in 89-0908, a PD-resistant *Vitis* genotype. A & B, No green fluorescence from IV-PMs (A) and VP-PMs (B) in xylem tissue treated with CCRC-M1, indicating that fucosylated XyGs in the two PM types are below the detectable level. C & D. PM polysaccharide composition revealed by JIM 5. Weakly Me-esterified HGs are detected in VP-PMs (arrow, D) but not in IV-PMs (arrow, C). E & F. PM polysaccharide composition revealed by JIM7. Very weak fluorescence and relatively strong fluorescence are detected from IV-PMs and VP-PMs, respectively, indicating that heavily Me-esterified HGs are present in only low amounts in IV-PMs PMs, but in larger amount in VP-PMs.

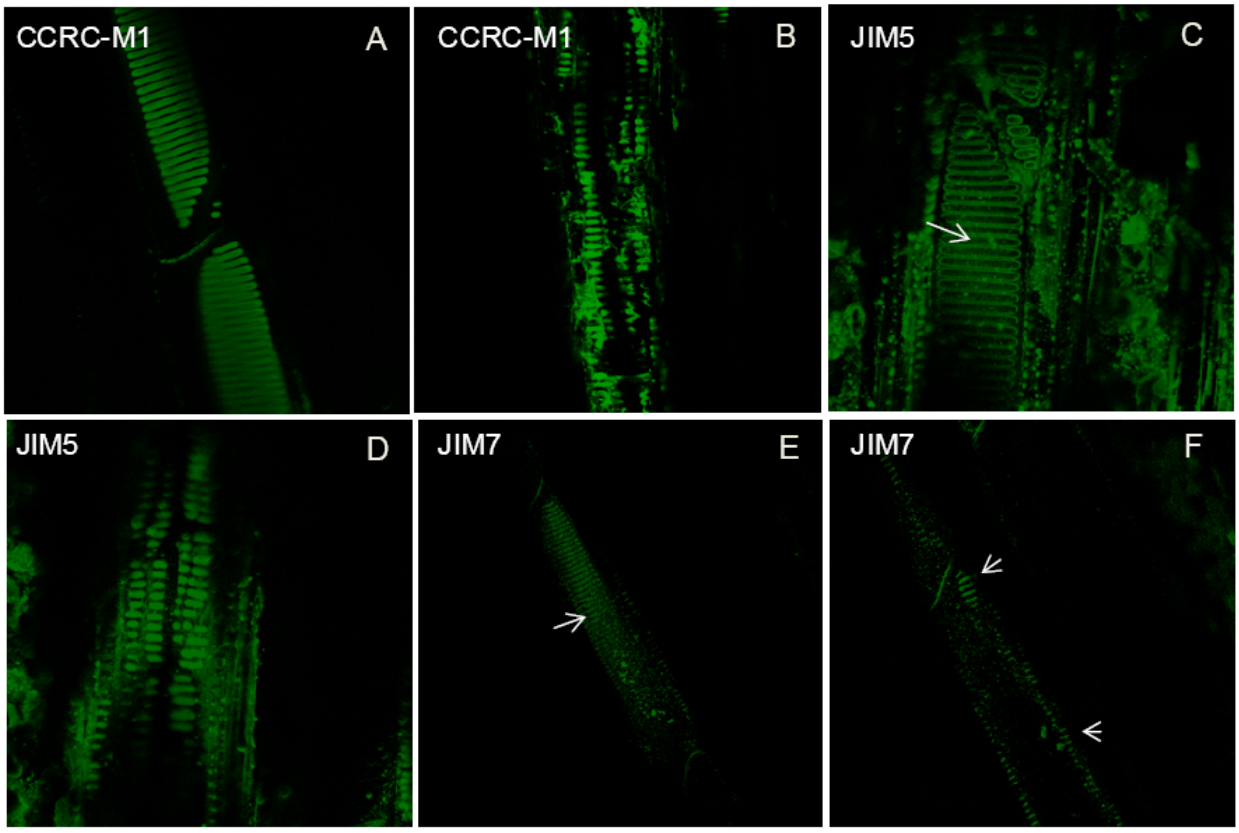

Figure 2. Polysaccharide compositions in IV-PMs (A,C,E) and VP-PMs (B,D,F) in *Muscadinia rotundifolia*, a highly PD-tolerant grape genotype. A & B, Cell wall composition revealed by CCRC-M1, showing the presence of fucosylated XyGs in both IV-PMs (A) and VP-PMs (B). C & D. Cell wall composition revealed by JIM5. The presence of weakly Me-esterified HGs is **not** detected in IV-PMs (C), but they are present abundantly in VP-PMs (D). E & F. Cell wall composition revealed by JIM7. The fluorescence signal is relatively weak from both IV- and VP-PMs, indicating a limited amount of heavily Me-esterified HGs in both PM types.

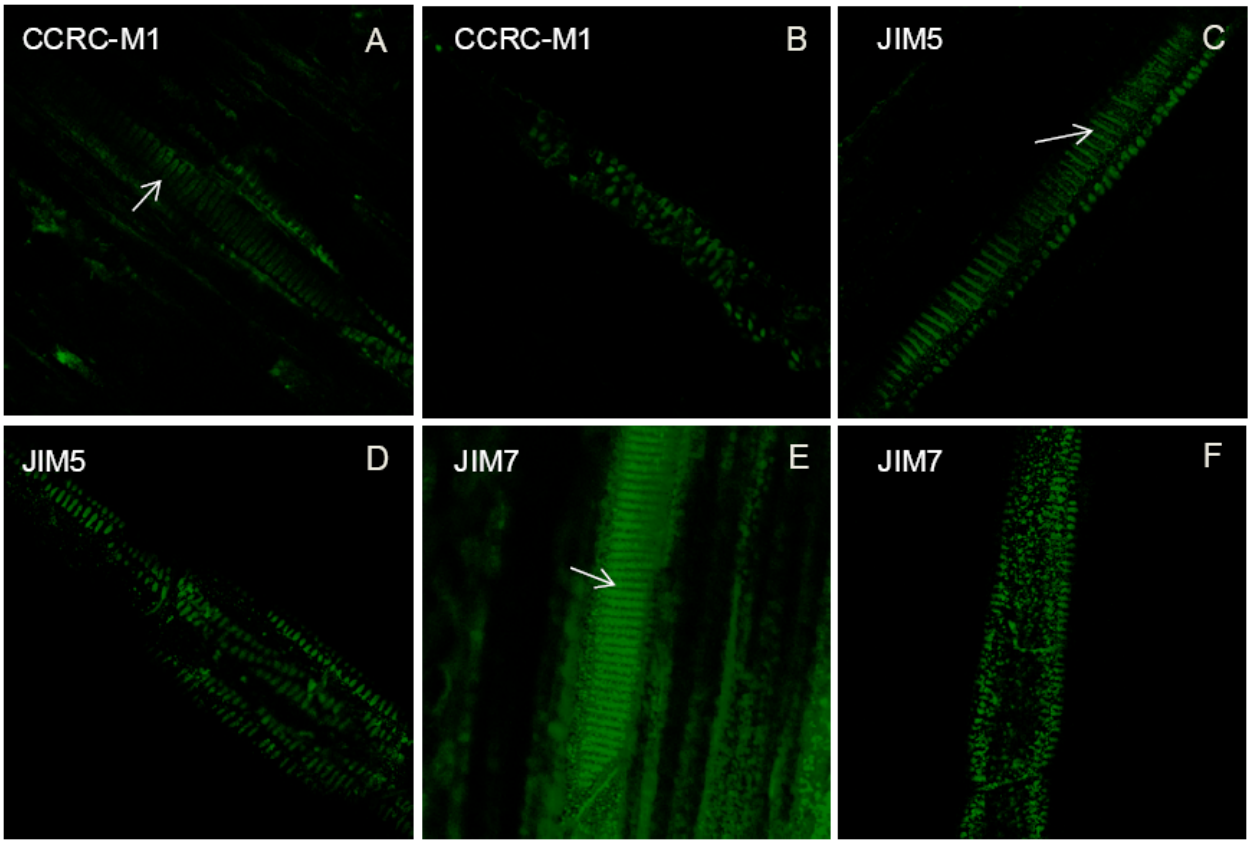

Figure 3 (previous page). Polysaccharide compositions in intervessel PMs (A,C,E) and vessel-parenchyma PMs (B,D,F) in *Vitis vinifera* var. Riesling, a less PD-susceptible genotype. A-B, Green fluorescence is detected from both intervessel PMs (A) and vessel-parenchyma PMs (B) incubated with CCRC-M1, indicating they contain fucosylated xyloglucans. C-D. In the xylem incubated with JIM5, no or very weak fluorescence from intervessel PMs (C) but strong fluorescence from vessel-parenchyma PMs (D) indicates that weakly Me-esterified HGs are present in a limited amount in intervessel PMs and abundantly in a large amount in vessel-parenchyma PMs. E-F. Xylem tissue incubated with JIM7, showing strong fluorescence from intervessel PMs (E) and vessel-parenchyma PMs (F). Heavily Me-esterified HGs are abundantly present in both types of PMs.

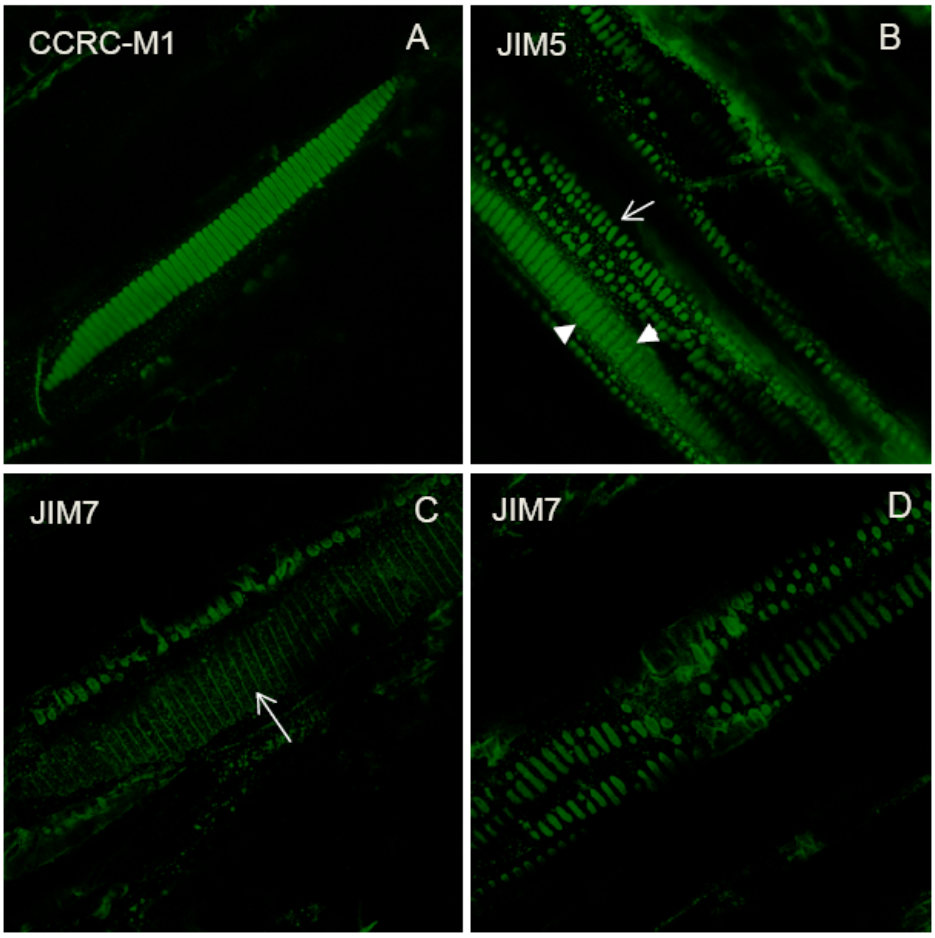

Figure 4. Polysaccharide compositions of intervessel PMs (A,C,E) and vessel-parenchyma PMs (B,D,F) in *Vitis vinifera* cv. Chardonnay, a PD-susceptible genotype. A. Intervessel PMs have strong fluorescence when incubated with CCRC-M1, indicating the abundant presence of fucosylated xyloglucans. B. Xylem tissue incubated with JIM5, showing that weakly Me-esterified HGs are the common components in both intervessel PMs (arrow heads) and vessel-parenchyma PMs (arrow). C-D. Xylem tissue incubated with JIM7. Fluorescence is below the detectable level in intervessel PMs (arrow, C) and is strong from vessel-parenchyma PMs (D), indicating heavily Me-esterified HGs are in very little amount in intervessel PMs (C) and abundantly present in vessel-parenchyma PMs (D).