THE PIT MEMBRANE BARRIER TO *XYLELLA FASTIDIOSA* MOVEMENT IN GRAPEVINES: BIOCHEMICAL AND PHYSIOLOGICAL ANALYSIS

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ABSTRACT
The overall goal of the work in this project is to characterize the role of the pit membranes of grapevine xylem vessels in limiting the systemic movement of *Xylella fastidiosa*. Work carried out in the project in the last year has made use of monoclonal antibodies that recognize specific cell wall polysaccharides (pectins with varying degrees of methyl esterification and xyloglucan [XyG]) to identify polysaccharides in grapevine pit membranes. The demonstration that these polysaccharides are present is consistent with earlier observations indicating that polygalacturonase (PG) and endo-ß-1,4-glucanase (EGase) are used by the pathogen to digest pit membranes as its population expands and spreads systemically because these enzymes would be expected to digest pectins and XyG. Another study in this project year was carried out to see if we could increase the information content of the MRI analyses we have been doing on Pierce's Disease- (PD) infected grapevines. Our earlier work in a now concluded CDFA-supported project had shown that MRI could non-destructively identify xylem vessels that are cavitated (i.e., air-filled) and non-functional. However, our studies and those of other PD researchers have shown that more permanent obstructions (tyloses and pectin-rich gels) also occur in the vessels of PD-infected vines. Work in this project year has shown that adjustments in the instrument protocols used to obtain MRI images of grapevine stems may allow discrimination between these different potential vessel obstructions

INTRODUCTION
For five years, Labavitch and the listed collaborators have been testing a model proposed to describe the development of Pierce's Disease (PD) in grapevines (Labavitch et al., 2001, 2002; Labavitch and Matthews, 2003; Labavitch et al., 2004, 2005; Pérez-Donoso, 2006; Pérez-Donoso et al., 2006). Findings reported in the last three PD Symposia indicate that PG and EGase enzymes, likely produced by *Xf* resident in xylem water-conducting cells (also Roper et al. 2007) are important contributors to the escape of the pathogen from the vessels into which it has been introduced by GWSS, thus initiating its systemic spread through the vine and the subsequent development of PD symptoms. However, observations made only in the past year have suggested that seasonal changes in normal grapevine development may also contribute to the systemic spread of *Xf*, beginning in late Spring. These observations may be linked to those made by collaborators Rost, Matthews et al. (Thorne et al., 2006) suggesting that relatively long xylem conduits, likely to be of primary xylem origin, may allow relatively long distance passage (i.e., the length of 2-3 internodes) of *Xf* into grape leaves. While this pathway is not likely to facilitate long distance systemic spread of the pathogen through stems, it may facilitate rapid movement from stems into which *Xf* has been introduced, into leaves where disease symptoms then become evident. Work in this project will examine aspects of these reports, with a strong focus on factors that might affect the integrity of the pit membranes in grapevine xylem water conduits.

OBJECTIVES
1. To characterize the biochemical action of *Xf* EGase, *in vitro* and *in planta* and determine if it is inhibited by plant proteins that have been identified as xyloglucan-specific endoglucanase (EGase)-inhibiting proteins.
2. To examine the full range of effects on grapevine pit membrane porosity that result from introduction of cell wall-degrading polygalacturonase (PG) and EGase.
3. To repeat our 2005 observations of a late Spring, dramatic increase in the porosity of grapevine pit membranes.

RESULTS
Objective 1. Characterization of the biochemical action of *Xf* EGase, *in vitro* and *in planta* and determine if it is inhibited by plant proteins that have been identified as xyloglucan-specific endoglucanase (EGase)-inhibiting proteins.
We reported on our work on this Objective in last year's symposium proceedings (Labavitch, 2006). The one EGase-inhibiting protein reported thus far is from tomato. It specifically inhibits EGase enzymes that digest XyG. It was provided to us by a colleague, Dr. Will York, at the Complex Carbohydrate Research Center at the University of Georgia. However, while the *Xf* EGase cloned and expressed by Dr. Caroline Roper does digest XyG polysaccharides, presumably targeting XyGs in pit membranes (see Objective 2, below), it is not inhibited by the tomato EGase-inhibiting protein. Further work on this aspect of the work must await the identification of new EGase-inhibiting proteins from other plants.

Objective 2. To examine the full range of effects on grapevine pit membrane porosity that result from introduction of cell wall-degrading polygalacturonase (PG) and EGase

Before joining our research program, postdoctoral researcher Qiang Sun had collaborated with us to show that the introduction of PG and EGase to the xylem of healthy grapevine stems resulted in the digestion of pit membranes resulting in perforations that would likely permit unhindered passage of *Xf* cells. Dr. Sun's work in the past year made use of antibodies that bind specifically to epitopes in pectin and XyG polysaccharides to provide the first demonstration that pit membranes contain polysaccharides typically found in the primary cell walls of dicots. While this has been a long-standing presumption, Dr. Sun's use of immuno-localization techniques has confirmed it. Because the *Xf* PG and EGase are likely to digest these polymers, Sun's work explains why these enzymes had digested grapevine pit membranes.

These studies were carried out using monoclonal antibodies that recognize (1) homogalacturonan (HG) with a low level of methyl-esterification (antibody JIM 5), (2) HG with a high level of methyl-esterification (antibody JIM 7), (3) HGs that are cross-linked via Ca^{2+}-cross bridges (antibody 2F4) and (4) fucosylated xyloglucan (antibody CCRC-M1). After reaction with the primary antibody (i.e., the antibody that recognizes specific wall polysaccharide structures), the bound primary antibody is revealed by using a secondary antibody labeled with green fluorescent FTIC that can be observed using a confocal or fluorescence microscope (Figures 1, 2 & 3). In some of the images we have also used these antibodies to ask what the cell walls surrounding tyloses are composed of (Figures 1 & 2, right images). This is an important question related to vascular system obstructions in PD-infected grapevines. Tyloses form early in inoculated vines and numerous tyloses have been reported to accumulate on grape vessels, often completely obstructing them. Tyloses develop from parenchyma cells that are adjacent to vessels and the primary walls of these parenchyma cells share the vessel-parenchyma pit membranes illustrated in some of the images above (Figure 1) and below (Figures 2 & 3).

The MRI analysis of grape stems developed by Dr. Alonso Pérez-Donoso proved to be very useful for detecting cavitated vessels but could not distinguish between water- and pectin-filled vessels. Drs. Greve and Sun first prepared glass capillary tubes filled with either water or water solutions of pectins with a high degree of methyl esterification (71-72%; this is **Pectin #1**)) or a low degree of esterification (33-40%; this is **Pectin #2**). An additional variable in the experiment (Figure 4) was the concentration of each pectin that was tested. Capillaries with 1%, 2% and 4% solutions of pectins #1 and #2 were prepared. Figure 4 shows the array of the capillaries that was placed in the core of the NMR instrument. The pattern of the array identified in Figure 4A, with water in the larger, central capillary is the same in panels B & C.

The images in Figure 4A used the NMR settings routinely used by Alonso in his work with grape stems (Pérez-Donoso et al., 2007). For panel B, the NMR operational parameters were adjusted. An additional adjustment was made in panel C (see figure legend). In panels B and C, note that as TI (the time of inversion delay) is increased the image of the water-filled capillary fades and the images of the pectin-filled capillaries begin to be discriminated from one another, based on pectin concentration and type. The images in panel C demonstrate that the signal is stronger for the more highly Me-esterified pectin #1.

The encouraging results demonstrated by the images in Figure 4 led to an additional experiment in which the grapevine xylem perfusion system used by Perez-Donoso and Greve was used to fill the xylem systems of different 'Chardonnay' grape vines with air, water, a 4% water solution of the low Me-esterified pectin (pectin #2 in Figure 4), and then a 4% gel of that pectin (made by adding $CaCl_2$, once the 4% pectin solution had been perfused into the xylem). The distinctions revealed in the capillary experiments were then shown to work with grape stems (Figure 5).

Objective 3. To repeat our 2005 observations of a late Spring, dramatic increase in the porosity of grapevine pit membranes.

Work on these objectives was started in Spring-Summer, 2007 but was not completed. Dr. Sun was made an offer he could not refuse. At the start of August, 2007 he and his family moved to Steven's Point, WI where Qiang began work as an Asst. Professor. The full season series of measurements will be made in Spring-Summer, 2008.

CONCLUSIONS

1. The cell wall substrates for the *Xf* PG and EGase have been demonstrated to be structural components of grapevine pit membranes.
2. Preliminary tests have shown that modifications of the way grapevine stem MRIs are obtained should be able to distinguish between air-embolised (i.e., cavitated) vessels and vessels that are filled with water or pectin.

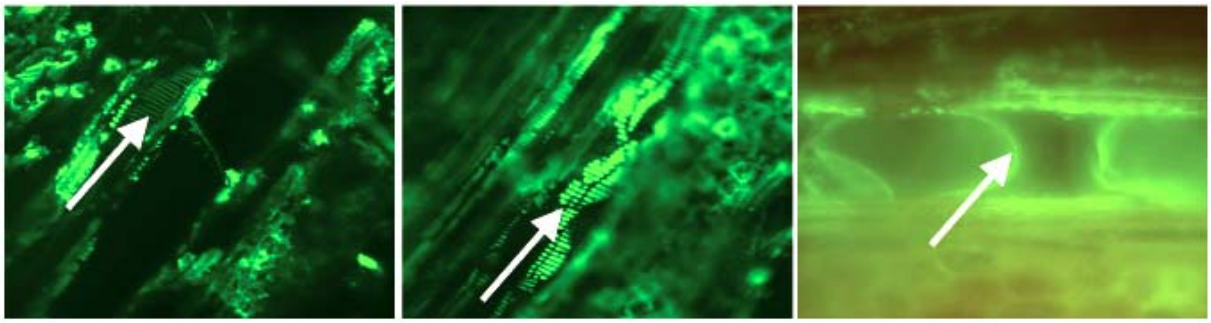

Figure 1. The use of JIM 5 reveals the presence of weakly Me-esterified HGs in the walls of grapevine intervessel pit membranes (left image), in vessel-parenchyma pit membranes (middle image) and in developing tyloses (right image). The arrows in the left and middle images point to the rows of pit membranes that are aligned in the vertical orientation along the length of a vessel. In the right image, the arrow points to the wall of one tylose in a vessel that contains at least two tyloses. The left and center images were made using the confocal microscope. The right image is from the fluorescence microscope.

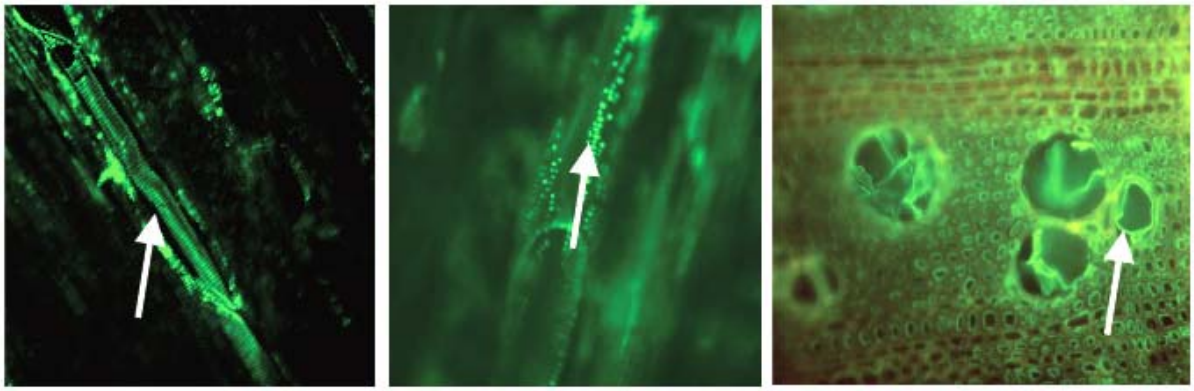

Figure 2. The use of CCRC-M1 reveals the presence of xyloglucan in the walls of grapevine intervessel pit parenchyma pit membranes (middle image) and in developing tyloses (right image). The arrows in the left and middle images point to the rows of pit membranes that are aligned in the vertical orientation along the length of a vessel. In the right image, the arrow points to the wall of one tylose in a vessel that contains at least two tyloses. The left and center images were made using the confocal microscope. The right image is from the fluorescence microscope.

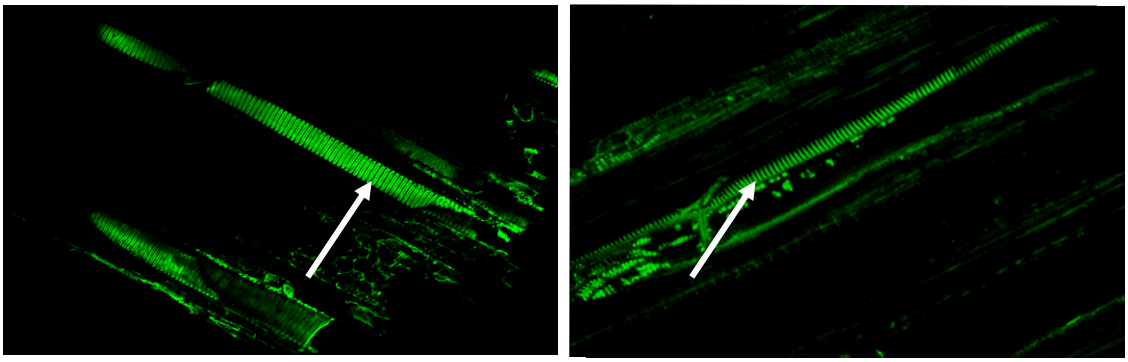

Figure 3. These images of intervessel pit membranes make clear the ladder-like arrangement of pits and pit membranes in the vessel walls. The left image shows the fluorescent fucosylated xyloglucan revealed by the CCRC-M1 antibody. The right image shows the fluorescent Ca-cross linked HG network revealed by the 2F4 antibody. The images were made using the confocal microscope.

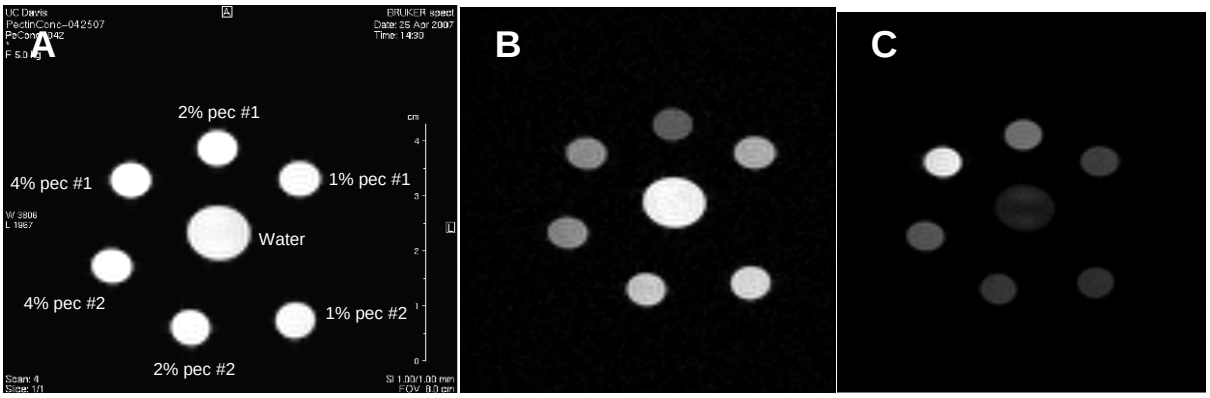

Figure 4. In panel A, the NMR TR (time of repetition) was set at 1906 msec and TE (time of echo) at 15 msec. In panel B the TR and TE were 3005 msec and 10 msec, respectively and TI (time of inversion delay) was set at 800 msec. In panel C, the TR and TE were as in panel B and the TI was set at 1000 msec.

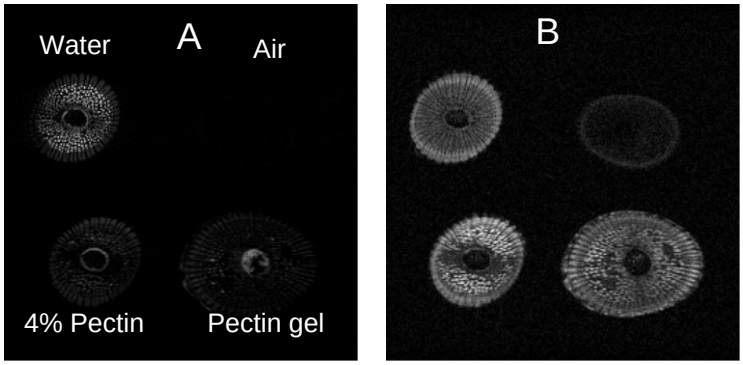

Figure 5. Stems segments (ca. 5 cm in length) were perfused with the indicated solutions. The images in panel A were obtained with the NMR settings shown for Figure 4B. The images in panel B were obtained with the settings used for Figure 4C.

REFERENCES

Labavitch J.M. 2006. The pit membrane barrier to *Xylella fastidiosa* movement in grapevines: Biochemical and physiological analysis. 2006 Pierce's Disease Research Symposium. p. 280-282.

Labavitch J.M., M.A. Matthews and T.A. Rost. 2001. The development of Pierce's Disease in xylem: the roles of vessel cavitation, cell wall metabolism and vessel occlusion. 2001 Pierce's Disease Research Symposium. p. 58-61.

Labavitch J.M., M.A. Matthews and L.C. Greve. 2002. The development of Pierce's Disease in xylem: the roles of vessel cavitation, cell wall metabolism and vessel occlusion. 2003 Pierce's Disease Research Symposium. p. 61-64.

Labavitch J.M. and M.A. Matthews. 2003. The development of Pierce's Disease in xylem: the roles of vessel cavitation, cell wall metabolism and vessel occlusion. 2003 Pierce's Disease Research Symposium. p. 150-153.

Labavitch J.M., E.A. Backus, M.A. Matthews and K.A. Shackel. 2004. Linking the model of the development of Pierce's disease to an understanding of the dynamics of glassy-winged sharpshooter transmission of *Xylella fastidiosa* to grapevines and grapevine gene expression markers of Pierce's disease. 2004 Pierce's Disease Research Symposium, p. 19-21.

Labavitch J.M., E. Backus, M.A. Matthews, and K.A. Shackel. 2005. Linking the model of the development of Pierce's disease to an understanding of the dynamics of glassy-winged sharpshooter transmission of *Xylella fastidiosa* to grapevines and grapevine gene expression markers of Pierce's disease. 2005 Pierce's Disease Research Symposium, p. 34-38.

Pérez-Donoso, A.G. 2006. Studies on the physiological consequences of *Xylella fastidiosa* infection in grapevines. A proposed model for pathogenicity mechanisms. Ph.D. Dissertation.

Pérez-Donoso, A.G., L.C. Greve, J.H. Walton, K.A, Shackel, and J.M. Labavitch. 2007. *Xylella fastidiosa* infection and ethylene exposure result in xylem and water movement disruption in grapevine shoots. Plant Physiology 143:1024-1036.

Roper, M.C., L.C. Greve, J.G. Warren, J.M. Labavitch and B.C. Kirkpatrick. 2007. *Xylella fastidiosa* requires polygalacturonase for colonization and pathogenicity in *Vitis vinifera* grapevines. Mol. Plant-Microbe Interactions 20:411-419.

Thorne, E.T., B.M. Young, G.M. Young, J.F. Stevenson, J.M. Labavitch, M.A. Matthews and T.L. Rost. 2006. The structure of xylem vessels in grapevine (Vitaceae) and a possible passive mechanism for the systemic spread of bacterial disease. American Journal of Botany 93:497-504.

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