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

**Project Leader:**
John Labavitch
Dept. of Plant Sciences
University of California
Davis, CA 95616
jmlabavitch@ucdavis.edu

**Cooperators:**

| | | | |
|---|---|---|---|
| Mark Matthews | L. Carl Greve | Qiang Sun | Tom Rost |
| Dept. of Vitic. and Enol. | Dept. of Plant Sciences | Dept. of Plant Sciences | Section of Plant Biology |
| University of California | University of California | University of California | University of California |
| Davis, CA 95616 | Davis, CA 95616 | Davis, CA 95616 | Davis, CA 95616 |


**Reporting Period**: The results reported here are from work conducted July 1, 2006 to September 30, 2006.

## ABSTRACT
Studies planned for this proposal will (1) examine further the impacts of cell wall-degrading proteins on pit membrane integrity, (2) describe what our uses of the *Xylella fastidiosa* (*Xf*) cell wall-degrading enzymes tell us about the pit membrane polysaccharide network, and (3) specifically examine the relationship between pit membrane disruption, grapevine ethylene production, and xylem water conduit obstruction. Of particular interest because of its potential for identifying a new mechanism for a vine's resistance to PD, will be tests of the role of *Xf* cell wall xyloglucan-degrading endo-β-1,4-glucanases (EGases) in increasing the pit membrane's porosity and efforts to identify natural pant proteins that are inhibitors of those EGases.

This is a new project, approved in Spring 2006, with funding beginning July 1. Dr. Alonso Pérez-Donoso, who had recently finished his Ph.D. work in our laboratory was to have been the primary bench scientist in the project. However, he was offered a faculty position in Santiago, Chile and left to assume that position in early Spring 2006. Therefore, progress toward meeting our objectives has been slow. We are fortunate in that Dr. Quang Sun will be taking a position as a postdoctoral researcher in the project, beginning October 1, 2006. We anticipate rapid progress on Objectives 2 and 3 once Dr. Sun has become comfortable with his new laboratory environment. We have been able to begin testing of xyloglucanase-inhibiting proteins (XGIPs) on *Xf* EGase activity. Unfortunately, no inhibition was detected.

## 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 two PD Symposia strongly suggest that enzymes, likely produced by *Xylella fastidiosa (Xf)* resident in xylem water-conducting cells (also Roper et al. 2004) 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 moved, into leaves where disease symptoms then become evident. Work planned for 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. 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. Examine the full range of effects on grapevine pit membrane porosity that result from introduction of cell wall-degrading polygalacturonase (PG) and EGase.
3. 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 have reported that the introduction of PG and EGase to the xylem of explanted grapevine stems causes breakdown of pit membrane structure (see the report for the project "The contribution of the pectin-degrading enzyme polygalacturonase (PG) in transmission of *Xf* to grape

and the use of PG-inhibiting proteins for transgenic resistance to Pierce's disease" in these *Proceedings*) while increasing pit membrane porosity (Labavitch et al. 2005). Our colleagues Cecila Aguero and Abhaya Dandekar have reported that the expression of the gene encoding the pear fruit PG-inhibiting protein (PGIP) in transgenic grapevines slows the development of PD in the modified genes. We have also shown that the *Xf* EGase is active in digesting xyloglucan, a primary cell wall polysaccharide that is likely to be the pit membrane target of the EGase. Thus the *Xf* EGase can be considered to be a xyloglucanase (XGase). Therefore, if the tomato protein that has been identified as an XGase-inhibiting protein (XGase-IP) is able to inhibit the *Xf*-XGase, then expressing it in combination with the pear PGIP in transgenic grapevines could provide substantially enhanced PD tolerance.

The tomato XGase-IP was provided by our colleague, Dr. Will York, at the Complex Carbohydrate Research Center at the University of Georgia. *Xf* EGase/XGase was isolated from *E. coli* transformed by Dr. Caroline Roper to express one of the pathogen's □-1,4-glucanase-encoding genes (Labavitch and Matthews, 2003). We also tested the ability of the tomato XGase-IP to block the activity of a purified GWSS β-1,4-glucanase and a fungal XGase provided by colleagues at Novozymes (positive control) (Figure 1).

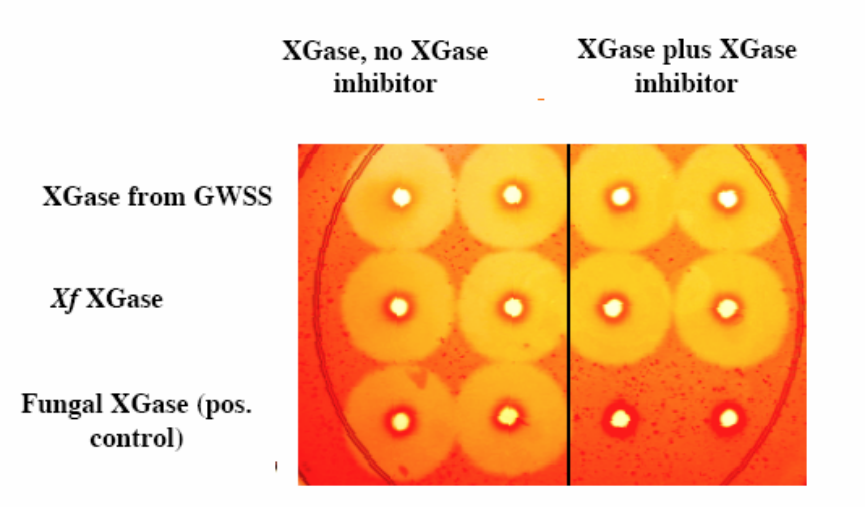


**Figure 1.** Shown is a radial diffusion assay of XGase activity. The xyloglucan (XG) substrate is dissolved in buffer and then mixed with melted agar. The agar is poured into a Petri dish and hardens. Wells are cut in the agar and then samples of the GWSS, *Xf* or fungal XGases are placed in the wells (left half). As the enzyme diffuses into the substrate-containing agar it digests it. The agar is stained with the dye Congo red to reveal the presence of undigested XG. The bigger the clear zones (shown above in yellow) the greater the XGase activity. The XGases were mixed with the tomato XGase-IP (right hand half) and the XGase activity was determined as described above. If the addition of the XGase-IP has caused inhibition of the XGase (i.e., reduces the size of the clear zone, as for the positive control) then it is an effective inhibitor. However, neither the GWSS nor the *Xf* XGase was inhibited (i.e., the clear zones are the same size whether the XGase-IP is present or not).

While the absence of inhibition of the *Xf* BGase/XGase indicates that the tomato XGase-IP will likely not be useful for enhancing tolerance of PD, this result does not eliminate the idea from consideration. We have studied the PG-inhibiting proteins (PGIPs) of plants for many years. They are very selective in the PGs that they inhibit (Stotz et al., 2000). Some PGs are strongly inhibited by a given PGIP while other PGs are not inhibited at all. It is reasonable to think that XGase-IPs display the same selectivity. We are not engaged in studies to discover new sources of XGase-IPs. However, as additional inhibitors are reported we will attempt to obtain them in order test their action against the *Xf* XGase.

**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.

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

Work on these objectives has not begun. Dr. Sun will be joining the lab soon and work will begin on these objectives at the start of Spring, 2007.

**CONCLUSIONS**

The only concrete conclusion that we can report for this new project at this time is that tomato XGase-IP does not inhibit the *Xf* and GWSS β-1,4-glucanases that we have previously purified.

**REFERENCES**

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. 2006. *Xylella fastidiosa* infection and ethylene exposure result in xylem and water movement disruption in grapevine shoots. Plant Physiology (submitted for publication).

Stotz H.U., J.G. Bishop, C.W. Bergmann, M. Koch, P. Albersheim, A.G. Darvill and J.M. Labavitch. 2000. Identification of target amino acids that affect interactions of fungal polygalacturonases and their plant inhibitors. Physiological and Molecular Plant Pathology 56:117-130.

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.

**FUNDING AGENCIES**

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.

# LINKING THE MODEL OF THE DEVELOPMENT OF PIERCE'S DISEASE IN GRAPEVINES 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

**Project Leaders:**

John Labavitch
Dept. of Plant Sciences
University of California
Davis, CA 95616
jmlabavitch@ucdavis.edu

Elaine Backus
USDA ARS PWA
Crop Dis., Pests and Gen.
Parlier, CA 93648
ebackus@fresno.srs.usda.gov

Mark Matthews
Dept. of Vit. & Enology
University of California
Davis, CA 95616
mamatthews@ucdavis.edu

Ken A. Shackel
Dept. of Plant Sci.
University of California
Davis, CA 95616
kashackel@ucdavis.edu

**Cooperators:**

L. Carl Greve
Plant Sciences, UC Davis

Alonso Pérez
Plant Sci, UC Davis

Holly Shugart
Crop Dis., Pests, ARS Parlier

Bruce Kirkpatrick
Plant Pathol., UC Davis

David Morgan
CDFA
Riverside, CA 92501

Rex Cocroft
Div. of Biological Sciences
University of Missouri-Columbia

**Reporting Period**: The results reported here are from work conducted October 1, 2005 to Sept. 30, 2006.

## ABSTRACT

For several years we have been studying the development of Pierce's disease (PD) in grapevines. Our studies have been guided by a model of PD development proposed with our initial application for funding. The Model proposed several "steps" in disease development following introduction of the PD causal agent, the bacterium *Xylella fastidiosa* (*Xf*):

***Xf* introduction to vessels =>vessel cavitation =>initial water deficit => *Xf* population increase => production of enzymes by *Xf* =>cell wall digestion => oligosaccharide signals => ethylene synthesis rise => a "wave" of vessel occlusion beyond the infection site => collapse of vine water transport => leaf abscission => vine death**

Our hypotheses have proven quite accurate, although aspects of the model are still being tested. We have shown that xylem vessel obstruction (tyloses, plant cell wall component-derived gels, and bacterial extracellular polysaccharides) and consequent reductions in stem water transport capacity are early consequences of infection with *Xf*, before bacterial populations are substantial and have spread far from the inoculation point. We have shown that ethylene treatment of vines also triggers vessel obstruction development and reduced water movement and that ethylene emanation from vines may increase following infection. We have also developed data for xylem vessel length distributions in grapevines and shown that *Xf* must pass through vessel pit membranes if the bacterial population is to develop systemically, thus suggesting that digestion of cell wall polymers in the pit membranes is likely to be important to disease spread. These findings are reported in several reports at the annual PD Symposium (Labavitch et al., 2001, 2002, 2004, 2005; Labavitch and Matthews, 2003) and, more recently, at disciplinary scientific society meetings (Perez et al., 2004; Roper et al., 2004) and in refereed reports (Stevenson et al., 2004). We describe herein the continuing studies that have made clear that the *Xf* genome contains genes that encode cell wall-degrading polygalacturonase (PG) and endo-β-1,4-glucanase (BGase) and that these two enzymes are sufficient to open the pit membrane network, suggesting that this is the mechanism used by the pathogen to permit systemic development in infected grapevines.

## INTRODUCTION

Overall, many of the investigators listed above are involved in three CDFA-supported projects that are centered in the Labavitch lab. Two of these projects are outgrowths of our earlier project that was designed to test our proposed model for Pierce's disease (PD) development. Thus, it is difficult to avoid discussing some of the work in our other two projects in this report for the third project, which is an expansion of the primary model to link it to the studies of other PD researchers.

## OBJECTIVES

1. Complete testing of our model of PD development in grapevines.
2. Determine whether glassy-winged sharpshooter (GWSS) feeding on grapevines is accompanied by xylem vessel cavitation.
3. Determine whether the grapevine "regulators" that we have identified as important to development of PD affect the expression of grapevine genes that have been shown to be important markers of *Xf* presence/PD infection.