California Department of Food and Agriculture
CDFA PD/GWSS Board
March, 2008 Progress Report

A. Project Title: The pit membrane barrier to *X. fastidiosa* movement in grapevines: Biochemical and physiological analysis

B. CDFA Contract Number: 06-0225

C. Time period coverd by the progress report: The results reported here are for work conducted from 11.01.07 to 02.29.08.

D. Principal Investigator and Cooperators:

PI: John M. Labavitch (Professor, Plant Sciences Department, University of California, Davis

Cooperators: Dr. L. Carl Greve (Staff Research Associate IV), Dr. Qiang Sun (Postdoctoral Researcher, now Asst. Professor, University of Wisconsin at Stevens Point, WI) and Mr. Joshua Lenhof (Junior Research Specialist), Plant Sciences Department, UC Davis

E. List of Objectives and description of activities conducted to accomplish each objective

Objective 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.

This objective was proposed, based on the observations that (1) *Xf* PG and EGase work together to digest the pectins and xyloglucans in grapevine pit membranes (Labavitch et al., 2006) and (2) the inhibition of the pathogen's PG by pear fruit PGIP reduces the development of PD symptoms in transgenic pear PGIP-expressing grapevines (Agüero et al., 2005). Only one xyloglucanase-inhibiting protein (from tomatoes) is currently available and it does not inhibit the *Xf* EGase (Labavitch, 2006). Thus, work on this objective is not active at this time. However, should other inhibitors become available they will be tested.

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.

Antibodies that specifically recognize cell wall pectin and xyloglucan polysaccharides were used to identify these polymers in pit membranes using fluorescence microscopy (Labavitch, 2007). This is important because it makes clear why PG and EGase introduction to explanted grape stem segments increases pit membrane porosity; i.e., because these wall components are digested by PG and EGase. Our technique of monitoring the passage of gold particles through stem explants (Labavitch et al., 2005) will be expanded to include particles larger than 20 nm and *Xf* cells as we test the impact of enzyme addition on pit membrane porosity further.

Some relevant studies that were proposed in CDFA-supported projects that are now terminated are relevant to factors in grapevine xylem that influence the spread of *X. fastidiosa* through infected vines. These will be reported under Objective 2 in this currently active project because they were not completed in the original projects. These include:

1. Studies combining (1) MRI examinations of internodes of PD-infected grapevines (to

reveal non-functional, air-embolised xylem vessels) with (2) histological examination of sections from these same internodes to identify whether vessels are obstructed by tyloses or pectin-rich gels. The intent of this work is to determine if the positions of embolised vessels correspond with the vessels in infected stems where gels and tyloses have developed. These studies were started by a collaborative study involving former grad student, now Ph.D. and Asst.Professor Alonso Pérez-Donoso and Mr. Josh Lenhof, an undergraduate researcher. Lenhof, with input from Pérez-Donoso is now completing the study.

2. Our original model for PD development included a role for vine-produced ethylene in the development of vessel occlusions in infected vines. It is clear that ethylene treatment of vines can cause the blockage of grapevine vessels (Pérez-Donoso et al., 2007; Sun et al., 2007). Thus, an important issue to address is whether infected vines develop occlusions in response to ethylene. One way to ask this question experimentally is to block the vine's ability to respond to any ethylene it may produce by treating vines with the ethylene receptor-blocking compound 1-methyl cyclopropene (MCP). We are now working with a formulation of this compound that can be sprayed onto test vines. In early studies using this formulation of MCP we had not identified the appropriate concentrations of the inhibitor to use. This may explain why the MCP treatment, followed by needle inoculaton of treated vines, resulted in no impact on PD symptom development. After the original tests, the company that produces MCP did trials to identify the appropriate concentrations to use with grapevines. We are now in the process of determining an appropriate concentration of MCP to use in the inoculation tests. The published work of our former postdoctoral researcher, Dr. Qiang Sun, showed that the pruning of grapevines in late Winter/early Spring causes the development of tyloses in pruned grapevine stems and that this pruning effect was a response to ethylene produced by pruned vines. Thus, vines were treated with concentrations of the MCP formulation that should provide 100-200 times more MCP than was used in our original tests. Vines treated with different MCP concentrations on the first day of the trial were given a second MCP treatment after 7 days (same concentration as the first) and pruned. Samples of the terminal 2 cm of pruned stems were collected 7 days after pruning and fixed for histochemical analysis. Josh Lenhof, who has developed considerable skill with microscopy procedures, will examine these fixed tissues by sectioning and staining them and then looking for tylose development. Untreated control vines should show considerable tylose development (Sun et al., 2007). If the MCP concentrations used are sufficient to block vine response to ethylene then tylose development should be greatly reduced in the treated tissues. If the MCP concentrations used are effective, we will then carry out an experiment to determine the extent to which ethylene produced by vines is important in the development of PD symptoms such as vessel obstruction and impaired water transport.

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

These studies will involve the use of our tests of pit membrane porosity (Labavitch et al., 2005) on stems explanted from grapevines at regular intervals (Spring through Summer) during a growing season.

F. Summary of Major Research Accomplishments and Results (by Objective)

Objective 1: Completed, findings presented in earlier reports

Objective 2: A major aspect of this Objective is to confirm the presence in grapevine pit membranes of pectins and xyloglucans, the presumed cell wall polysaccharide targets that are digested by *Xf* PG and EGase, thus opening the pit membrane barrier and permitting the pathogen's spread. Work utilizing antibodies for immunolocalization of these polysaccharides were presented at the 2007 Research Symposium (Labavitch, 2007). This work confirmed pectin and xyloglucan presence in grapevine pit membranes.

Included in the present progress report are two studies, now underway, that were first described in previous CDFA-supported PD research projects. Work to determine the positional relationship of cavitated vessels in PD-infected vines (MRI analysis) and physical vessel obstructions (histochemical analysis) has shown that cavitations and more permanent obstructions are, to the extent this can be proven, coincident in infected vines (Figures 1 and 2). The co-aligned MRI image and histochemical image of an internode cros-section from an infected vine reveals non-functional vessels (i.e., vessels with no water in them) clustered in the same regions of the stem where substantial tylose development is observed. This suggests, but does not prove, that cavitations develop in vessels where resistance to water flow is greatly increased because of constriction or complete blockage of the cross-sectional area of the vessel "pipes". This result is of particular importance in relationship to the second continuing study, because ethylene treatment of vines can cause the formation of tyloses (Sun et al., 2007) and also vessel cavitations and increased xylem resistance to water flow (Pérez-Donoso et al., 2007). We will begin this study as soon as we have defined the MCP concentrations needed to block grapevine responses to ethylene.

Objective 3: Because our talented postdoctoral researcher was hired into a faculty position at the University of Wisconsin at Steven's Point we were not able to complete this aspect of our study, which was planned for Summer, 2007. We will request a no-cost extension of this project and complete the work in Summer, 2008.

G. Publications, reports and presentations where the information developed from the research was presented. The results of the work on this proposal were presented by Postdoctoral Scholar Qiang Sun in a poster at the joint meeting of the Botanical Society of America and the Amrican Society of Plant Biologists in Chicago in July, 2007. The poster won a "best poster" prize! Some of the work presented in this report was also presented (a talk and a poster) at the 2007 Pierce's Disease Research Symposium. Papers to be submitted for publication are now being written. The delay is because the two major researchers, Asst. Professors Alonso Pérez-Donoso and Qiang Sun. have been very busy with their teaching duties.

H. Research Relevance Statement

The studies described in this proposal were designed to confirm and extend earlier observations indicating that the *X. fastidiosa* PG and EGase enzymes play important roles in breaking down grapevine pit membranes. This function of these enzymes would, therefore, support the systemic spread of the pathogen away from the xylem cells into which it has been introduced by the insect vector and facilitate PD spread. The inhibition of *Xf* PG by PG-inhibiting Protein (PGIP) limits PD spread in grapevines and so PGIP manipulation in grapevines is a PD defense strategy that will be developed in additional research. Theoretically, if a factor that inhibits the *Xf* EGase were identified, this too could be exploited for defense of grapevines against PD

I. Lay summary of current year's results

Major factors affecting the spread of the *X. fastidiosa* pathogen in grapevines and the subsequent killing of vines are the spreading of *Xf* from the vessels into which the GWSS vector introduces it and the resulting shut-down of water transport. The work of this proposal extends earlier work aimed at understanding (1) the destruction of pit membranes during PD development (pit membranes are the barriers that keep the pathogen in the originally infected vessel) and (2) factors that limit water movement (such as tyloses and embolisms). We are trying to understand how these things occur and use that information to identify strategies to prevent them, thus preventing PD development in vines that have been inoculated with *Xf*.

J. Intellectual property status

We do not anticipate that any intellectual property will be developed directly from the results of our studies. However, work based on our findings that will guide modification of grapevines to provide enhanced PD defense (follow-up proposals) likely would lead to Intellectual Property.

REFERENCES CITED

Aguero, C.B., S.L. Uratsu, L.C. Greve, A.L.T. Powell, J.M. Labavitch, C.P. Meredith, and A.M. Dandekar. (2005) Evaluation of tolerance to Pierce's Disease and *Botrytis* in transgenic plants of *Vitis vinifera* L. expressing the pear PGIP gene. Molecular Plant Pathology 6:43-51.

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

Labavitch J.M., E. Backus and D. Morgan D (2006) The contribution of the pectin-degrading enzyme polygalacturonase (PG) in transmission of *Xylella fastidiosa* to grape and the use of PG-inhibitor proteins for transgenic resistance to Pierce's disease. 2006 Pierce's Disease Research Symposium, p. 287-289.

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

Sun Q., T.L. Rost, M.S. Reid and M.A. Matthews. 2007. Ethylene and not embolism is required for wound-induced tylose development in stems of grapevines. Plant Physiology 145:1629-1636.

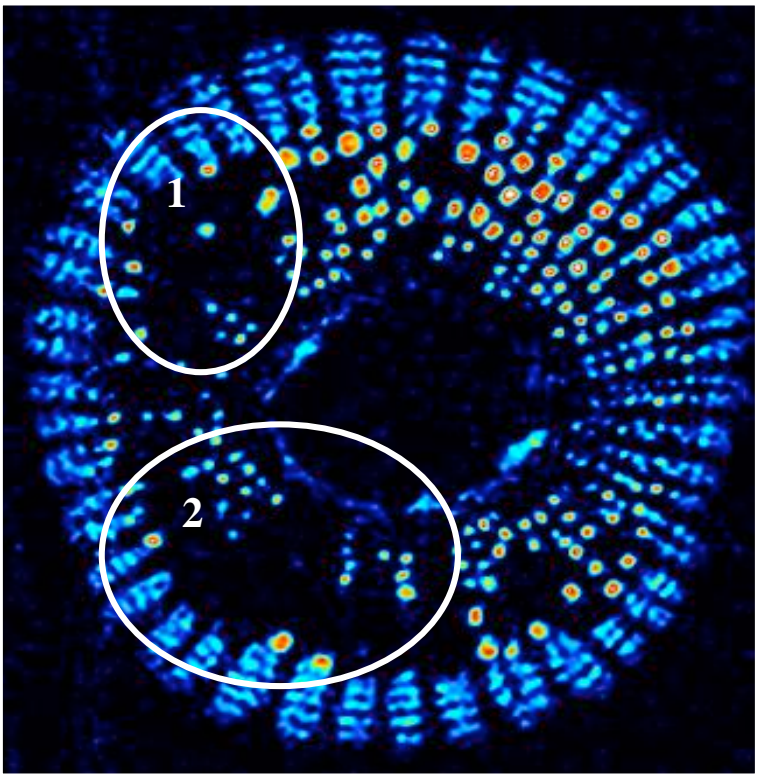

Figure 1. False color image of an MRI of a grapevine stem that had been needle inoculated with *X. fastidiosa*. The image was taken from the second internode above the inoculation point 8 weeks after inoculation. The vine showed no external PD symptoms. Bright spots in the image indicate water-filled (functional) vessels. The red spots indicate the larger water-filled vessels in the stem. Some sectors (within ovals 1 and 2) show relatively few functional vessels. When no water is present the MRI signal is weak or absent. These dark sectors contain cavitated (air-embolised) vessels. The MRI was obtained from an intact grapevine.

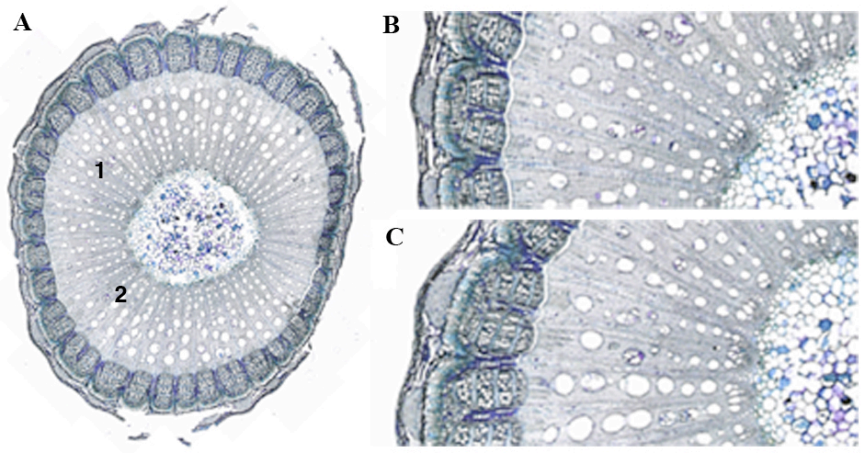

Figure 2. The stem shown in Figure 1 was collected, fixed for histochemical analysis, and sections were cut at the point where the MRI of Figure 1 was taken. Sections were stained and examined with the microscope. Because of the size (diameter) of the section, several separate images were taken in order to cover the entire cross-sectional area of the internode. These separate images were then assembled into a single image of the cross-section (A) using the microscope software package. Using Photoshop, sectors overlapping oval 1, Figure 1 (B) and oval 2 (C) were taken and enlarged. These sector images show that many of the vessels in these areas each contain several tyloses. Because the images shown in Figs. 1 and 2 represent less than a millimeter of the vessels' length and the total length of these grapevine stem vessels is ca. 10 cm, it is likely that most of the vessels in the dark regions (ovals) in Fig. 1 will contain tyloses. There is some uncertainty in this conclusion because we did not perform the histological examination of sections cut at intervals 5-10 cm above and below the point where the MRI (Fig. 1) was taken. However, the idea that tyloses and cavitations occur in the same vessels in PD-infected grapevines seems to be a relatively safe conclusion.