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

B. CDFA Contract number: 06-0225 (this project is now in a 1-year, no-cost extension, ending 06/30/09)

C. Time period covered by this progress report: November, 2007 to June 30, 2008

D. Personnel:

Principal Investigator: John Labavitch, Plant Sciences Department, University of California, Davis, Davis, CA 95616

Cooperators:

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E. Objectives

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.

In previous PD Research Symposia (Labavitch et al., 2006, 2007) we reported observations suggesting that that the *X. fastidiosa* PG and EGase play important roles in digestion of PMs so that the pathogen can spread through infected grapevines *via* the xylem. However, we had not shown that the combined actions of the two pathogen enzymes did, in fact, open a pathway that *X. fastidiosa* could use to move through PMs. We have now used our grapevine xylem flushing system (Labavitch, 2006) to introduce PG and EGase to the lower (proximal) end of explanted stems and then followed enzyme introduction with cells of the *X. fastidiosa* 'Fetzer' strain. Then the stem was continuously flushed with water and fractions of the water eluted from the distal stem end were collected. These fractions were then assayed for *X. fastidiosa* presence by PCR. This experiment was replicated and in each case, PCR revealed the pathogen's presence in collected fractions. Thus, the PG and EGase open up PMs so that they no longer block pathogen movement.

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

In progress. There are no results to report at this time. The focus of this study is to determine whether PM porosity changes over the course of a growing season.

A "continuation"objective

Remaining from another project that has ended is an experiment to determine whether the production of ethylene plays a role in PD symptom development in *X. fastidiosa*-inoculated grapevines. A key to performing the experiment has been to have a way to block the grapevine's response to responses to ethylene. Prior tests were based on spray applications of the ethylene receptor-blocking compound 1-methylcyclopropene (1-MCP) and we were unable to suppress the vine's ethylene response. However, earlier this year we obtained a new, sprayable formulation of 1-MCP. Postdoc (now Asst. Prof.) Qiang Sun had reported that grapevines respond to Winter pruning by producing tyloses in vessels near to the pruning

cuts (within 2-3 cm of the cuts), thereby blocking the vessels and showed that this was a response to the ethylene made by the cut grapevine stem tissues (Sun et al., 2006, 2007). We therefore carried out a test of the ability of the new 1-MCP spray to block pruning-induced tylose formation. Sets of 'Chardonnay' vines were used for the test. One set of 6 vines (the test vines) was sprayed with a solution of the 1-MCP formulation that was calculated to provide a 1-MCP concentration of 200 ppm). The other set of vines (control vines) was not treated. On the following day, the test vines were again sprayed with 1-MCP and all control and test vines were pruned. Dr. Sun's study (Sun et al., 2006) had reported extensive tylose formation in pruned vines within one week of pruning. Therefore, 7 days after pruning, the terminal 3 cm of each of the pruned stems in the control and test vines was removed and fixed for histochemical examination of tylose development. Sections from the distal 5 mm of these stem explants were cut, stained with toluidine blue, and examined with the light microscope (Shackel and Labavitch, 2006). These observations indicated that there was extensive tylose development near the tips of pruned stems that had not been treated with 1-MCP and that the 1-MCP treatment dramatically reduced tylose formation.

This result *per se* is of little importance in relationship to PD development. However, because pruning-induced tylose formation is a response to ethylene produced by wounded grapevines, the results show that the new 1-MCP treatment can block the vine's response to ethylene. Thus, we are now in a position to test the possible role of inoculation/infection-induced ethylene production in PD symptom development. This will be done in the summer.

F. Research accomplishments

We have shown that the combined action of two cell wall-degrading enzymes produced by *X. fastidiosa* (EGase and PG) digest PMs and permit the pathogen to move from one vessel to the next.

We have identified a protocol for 1-MCP treatment of grapevines that suppresses the vine's response to ethylene, at least in terms of suppressing pruning-induced tylose formation. This protocol should be useful in determining the extent to which PD symptom development in infected vines involves response(s) to infection-induced ethylene production.

G. Publications etc.

A draft of a manuscript describing the impacts of EGase and PG on grapevine pit membrane integrity is now being written. At present, no other publications describing results presented above are being written. Some of these results will be presented at the 2008 Pierce's Disease Research Symposium.

H. Research Relevance Statement (How this research will contribute towards solving the PD/GWSS problem in California)

The post-inoculation events preceding the development of visible PD symptoms undoubtedly include changes that determine whether and how quickly *X. fastidiosa* infection will cause PD and kill the vine. Therefore, understanding these events is likely to be important in work to identify plant-based factors that can be exploited to establish true grapevine resistance to Pierce's Disease. The research that our collaborating team has been doing for several years, including the results described herein, are focused on understanding these early events.

Our program has already developed information supporting the idea that plant PG-inhibiting proteins (PGIPs) might be manipulated to provide biologically based grapevine protection and a proposal to develop these is now supported by the CDFA (funded for 3 years in the 2008 proposal round). It is not clear whether the work on ethylene's potential role in PD development could lead to improved management of PD. This is, in part, because whether and how ethylene is involved in PD development is not clear. However, there are many ways (genetic and biochemical) to modify a plant's ability to produce and respond to ethylene. Therefore, depending on what we learn about a role for ethylene, some utilization of the information for PD control could be possible.

I. Summary in lay terms of the specific accomplishments of the research project

The development of visible grapevine Pierce's Disease symptoms proceeds slowly after the natural or laboratory introduction of *Xylella fastidiosa* cells into the vine's xylem water-conducting system. However, while visible signs of the disease can take 2-3 months to appear, many internal events that determine whether PD will develop and kill the vine are underway soon after infection. Studies have already shown that the pathogen's spread throughout the vine is supported by pathogen enzymes that digest the natural filters (called pit membranes) that initially confine *X. fastidiosa* to a few directly inoculated water-conducting cells. The results reported under Objective 2 confirm that two enzymes thought to be involved in assisting the spread of the pathogen are, in fact, important for PD development.

Ethylene is an important plant hormone. It plays many roles in plants and, when healthy grapevines are treated with ethylene, the vine's water-conducting xylem becomes obstructed, just as it does when a vine is infected with *X. fastidiosa* (Perez-Donoso et al., 2007). For this and other reasons, it is important to determine if ethylene plays an important role in natural PD development. The results described for our continuation objective indicate that a new 1-MCP spray formulation can be used to block a vine's response to ethylene and, therefore, make it possible to test the role played by ethylene in PD development.

J. Summary and status of intellectual property produced during this research project

PIPRA is involved in an analysis of PGIP-related IP questions. They will be relevant for the project that starts this summer. It is conceivable that the ethylene work could lead to patentable strategies that would make use of genes or chemicals already covered by IP claims.

K. References Cited

Labavitch JM (2006) The pit membrane barrier to *Xylella fastidiosa* movement in grapevines: Biochemical and physiological analyses. Proceedings of the 2006 Pierce's Disease Symposium, p. 280-282.

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Pérez-Donoso AG, Greve LC, Walton JH, Shackel KA, and Labavitch JM (2007) *Xylella fastidisa* infection and ethylene exposure result in xylem and water movement disruption in grapevine shoots. Plant Physiology. 143:1024-1036.

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