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 (PMs) of grapevine xylem vessels in limiting the systemic movement of *Xylella fastidiosa (Xf)*. Work carried out in the project in previous years has made use of monoclonal antibodies that recognize specific cell wall polysaccharides (pectins with varying degrees of methyl esterification and xyloglucan [XyG]) and this work has revealed the presence of these polysaccharides in grapevine pit membranes. The demonstration that these polysaccharides are present is consistent with earlier observations indicating that polygalacturonase (PG) and endo-b-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. We now report on tests showing that PG and EGase introduction into grape stem explants is sufficient to open the PMs so that *Xf* can freely traverse the stem explants containing several internodes. In addition, several groups, including our own, have reported that tyloses, induced barriers to pathogen movement in the grapevine xylem system, also are important in limiting Pierce's disease (PD) spread but are also an important cause of the breakdown in water movement through infected vines. Other studies have suggested that ethylene synthesis by grapevines may be an important factor in PD development and tylose formation. In this report we describe an experiment that may pave the way for testing the extent to which ethylene produced by an infected vine is responsible for the full development of PD symptoms.

INTRODUCTION
For several 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 four 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 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 two-three 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. In this report, we report on work that may have identified a way to test the role of ethylene produced by *Xf*-infected vines in the development of tyloses, vessel blockages that are likely to be more permanent barriers of *Xf* movement in grapevines which also are barriers that reduce nine water movement and, thus, may play an important role in the vine decline that accompanies PD.

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 AND DISCUSSION

Objective 2. In previous PD Research Symposia (Labavitch et al., 2006, 2007), we reported observations suggesting that that the *Xf* 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 *Xf* 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 *Xf* '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 *Xf* 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.

A continuing objective. Remaining from another project that has ended and also to the overall interest in barriers that serve to limit pathogen spread that is a theme of this current project was an experiment to determine whether the production of ethylene plays a role in PD symptom development in *Xf*-inoculated grapevines. A key to performing that experiment has been the need to have a way to block the grapevine's responses to ethylene. Earlier, inconclusive tests were based on spray applications of the ethylene receptor-blocking compound 1-methylcyclopropene (1-MCP). These did not suppress the vine's ethylene response. However, earlier this year we obtained a new, sprayable formulation of 1-MCP. Postdoctoral researcher (now Asst. Prof.) Qiang Sun had reported that grapevines respond to Winter pruning by producing tyloses in vessels near to the pruning cuts (within two-three 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 six vines (the test vines) was sprayed with a solution of the new 1-MCP formulation at a concentration 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, seven days after pruning, the terminal three 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 five 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 had dramatically reduced tylose formation.

This result *per se* demonstrates that grapevine responses to ethylene that affect vessel function can be inhibited. The pruning-induced tylose formation is a response to ethylene produced by wounded grapevines. However, we presume that the inhibitor will also influence a vine's response to infection-promoted ethylene. Thus, we are now in a position to test the possible role of inoculation/infection-induced ethylene production in PD symptom development. This test will be carried out in the Spring/Summer, 2009.

CONCLUSIONS

1. The introduction of pure PG and EGase, two enzymes produced by *Xf* within the grapevine xylem system, into grapevine stem explants will introduce a pathway through the vessels (presumably *via* their pit membranes) that permits free passage of the pathogen.
2. 1-MCP sprays can block grapevine responses to vine-produced ethylene, paving the way to studies that test the role of ethylene in PD symptom development.

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