Progress report for CDFA contract number 06-0225

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

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Time period covered by the report: March, 2009 through July, 2009

Objectives and Progress:

Introduction: This project focuses on the pit membrane (PM), the primary plant cell wall meshwork that separates one vessel from its neighbors and serves as a filter to assure that particles (e.g., bacterial cells) are not moved throughout the plant in the plant's water-conducting system. Research prior to the initiation of this project in 2006 had suggested that grapevine PMs provided an important barrier to the systemic movement of *X. fastidiosa* (*Xf*) cells in the xylem system and, therefore, suggested that PM integrity was a key to successful vine defense against Pierce's Disease (PD) development. Earlier reports for this project have supported the idea of an important PM role in a grapevine's PD defense and this information is a central tenet to current projects led by Lindow and Chatterjee (2008), Lindow et al. (2008) and Labavitch et al. (2008) that involve genetic manipulations of grapevine rootstocks to limit the spread of *Xf* in scions. A few questions that are related to factors affecting *Xf* spread in grapevines remained at the end of the project's original funding period.

Objectives: This project is now in a no-cost extension phase. The two objectives listed are restated from the original project. They are pieces of the original 2006 project's goals.

Objective 1. To repeat our 2005 observations of a late Spring, dramatic increase in the porosity of grapevine pit membranes in uninfected vines.

Objective 2. To determine whether an infected grapevine's production of and response to ethylene impacts its reaction to *Xf* and its relative PD susceptibility or resistance.

Progress: See the "note" at the end of this report. Because of an unanticipated drain on our research time, we have only addressed Objective 1 in this reporting period. At present, we have not analyzed the full data set because we are still collecting data. The important question being addressed is whether water flow rates through grape stems increase over the course of a growing season and whether changes in growth rate and, potentially, water flow rate are correlated with increases in PM porosity. If so, this could mean that with increasing stem growth rate the ability of PMs to prevent systemic movement of *X. fastidiosa* decreases.

In early June, stems on fifty 2-shooted grapevines were trimmed to 10 cm in length, with each shoot having at least three potential growing buds. In mid-July we began monitoring water flux through stems that were explanted from the trained plants. Water flux rates (water moved/time) are measured continuously over 4 hours with explants in our flow-testing system (Labavitch, 2006). When we have the full data set, data analysis will look for relationships between rates and several explant parameters (shoot explant age and length, diameter at the explant base and apex, the length of the longest vessel in the explant).

Intellectual Property:

No new intellectual property will be developed by this work. It is conceivable that the manipulation of the grapevine's ethylene response sensitivity will have a beneficial impact on its response to *Xf* introduction. Thus our results could shape studies "grapevine manipulation" efforts that eventually lead to the development of intellectual property.

Appropriate References:

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

Labavitch JM, ALT Powell, AB Bennett, D King and R Booth (2008) Optimizing grape rootstock production and export of inhibitors of *Xylella fastidiosa* polygalacturonase activity. Proceedings of the 2008 Pierce's Disease Research Symposium. pp 214-219.

Lindow S and S Chatterjee (2008) Control of Pierce's Disease by methods involving pathogen confusion. Proceedings of the 2008 Pierce's Disease Research Symposium. pp 180-186.

Lindow S, D Trauner and E Beaulieu (2008) Exploiting pathogen signal molecules for control of Pierce's Disease. Proceedings of the 2008 Pierce's Disease Research Symposium. pp 187-192.

The Relationship of the Potential Results from this Project and Solutions to the PD Problem in CA:

The ability of pit membranes to withstand the impacts of *Xf* and its cell wall-degrading enzymes and prevent the systemic spread of the pathogen appears to be a key to grapevine resistance to PD. Several studies based on this idea are already underway. Whether the continuing work in this project identifies additional opportunities for grapevine protection is not certain at this time.

Note:

In late Winter/early Spring, 2009 we encountered a greenhouse management problem that directly affected progress in our Pierce's Disease (PD) research contract 08-0171 and, because it demanded time from personnel involved in my entire research program, delayed progress in our other two PD research contracts (06-0225 and 08-0174). In the greenhouse that we shared with another UC Davis research colleague, we were propagating a series of transgenic 'Thompson Seedless' and 'Chardonnay' grapevines that expressed the pear fruit gene encoding a polygalacturonase-inhibiting protein (PGIP). These PGIP-expressing vines had been used to demonstrate (Agüero et al., 2005) that PGIPs could provide partial protection of vines against PD. Our plan for project 08-0171 was to graft non-transgenic scions onto transgenic roots and test the scions for PD susceptibility. Work was progressing well until a serious problem arose.

Our greenhouse was also being used to grow Miscanthus (*Miscanthus giganteus*), a sub-tropical perennial grass that has the potential to be a useful "energy" crop, a producer of "lignocellulosic" residues from which biofuels could be produced. The "panicle rice mite" (*Steneotarsonemus spinki*) was identified in a neighboring greenhouse used by a rice research program. Subsequently, although no rice was in our shared greenhouse, the mite was found on the Miscanthus. The panicle mite is not a problem in CA rice fields but it can cause a great deal of loss in rice-growing areas where it is prevalent. Therefore the campus and CDFA took action to eliminate the

problem in the UC Davis greenhouse facility. That action was coordinated through the UCD panicle rice mite task force led by Prof. Richard Bostock. The original action suggested was that the grapevines should be destroyed. This led to considerable follow-up discussion and a decision to (1) cut the transgenic vines way back, (2) completely wrap them in plastic, and (3) transport them to a clean isolation greenhouse away from the facility where the mite was originally identified. The vines in isolation now are growing well and the greenhouse contains clean "sentinel" rice plants to determine that facility has no mite contamination.

This rescue operation has, for the moment, saved the considerable investment that went into the generation of the pear PGIP-expressing vines and their maintenance and propagation over several years. However it did prevent some of the work in project 08-0171 that was planned for this summer and took a great deal of scientist time (notably that of Dr. L.C. Greve and Ph.D. candidate Z. Chestnut) away from the work planned for other PD projects (including 06-0225) and other research in the lab.