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 Pomology
University of California
Davis, CA 95616

Elaine Backus
Exotic Pests & Diseases Lab
USDA, ARS
Parlier, CA 93648

Mark Matthews
Dept. of Vitic. & Enology
University of California
Davis, CA 95616

Ken A. Shackel
Dept. of Pomology
University of California
Davis, CA 95616

Cooperators:

L. Carl Greve
Dept. of Pomology
University of California
Davis, CA 95616

Bruce Kirkpatrick
Dept. of Plant Pathology
University of California
Davis, CA 95616

David Morgan
PD Control Program
CDFA
Riverside, CA 92504

Alonso Pérez,
Dept. of Pomology
University of California
Davis, CA 95616

Caroline Roper
Dept. of Plant Pathology
University of California
Davis, CA 95616

Reporting Period: The results reported here are from work conducted from April 1, 2004 to October 1, 2004. (**Note**: This includes work on an earlier "Pierce's Disease Model" research proposal, and work begun on this new proposal.)

INTRODUCTION

For three years, our group has been testing the "steps" in PD development that were proposed in a model.

***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**

In the course of that research, we have shown that xylem vessel obstruction (tyloses, plant cell wall component-derived gels, and, perhaps, bacterial extracellular polysaccharides) and consequent reductions in stem water transport capacity are early consequences of infection with *Xylella fastidiosa* (*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 and, more recently, at disciplinary scientific society meetings and in refereed reports (Stevenson et al., 2004).

Work to retest aspects of our model, those parts relating specifically to the involvement of cell wall breakdown caused by the action of *Xf* enzymes, remain and will be tested in this new proposal (see Objectives). Also to be tested are ideas based on the reports of the studies of others involved in unraveling problems associated with the transmission and spread of PD, within and between grapevines. We will link the anatomical, biochemical and physiological findings from our "model testing" to the work of Cook et al. (), describing genes that are expressed in vines relatively soon after *Xf* infection. We have nothing to report on this aspect of the new proposal. We will also address a question that entomologists and plant biologists generally have differing opinions about. Do vessels cavitate (i.e., become air-filled and, hence, non-functional when the glassy-winged sharpshooter (GWSS) starts or finishes its feeding on a vine? The answer to this question may have important implications regarding *Xf* transmission, GWSS' feeding strategy and spread of the bacteria in an infected vine. Below and in the report from Shackel and Labavitch in these proceedings, we report on the start we have made in addressing this question.

OBJECTIVES

1. To complete testing of our model of PD development in grapevines.
2. To determine whether GWSS feeding on grapevines is accompanied by xylem vessel cavitation.
3. To 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 *X. fastidiosa* presence/PD infection.

RESULTS

The Path of Xf Movement in the Grapevine Xylem.

In previous reports, we have described tests that indicate the porosity (i.e., the space between the polysaccharides) of vessel pit membranes is between less than 29 nm, much too small to permit passage of *Xf*. We have refined those tests by using colloidal gold particles having diameters of 20 and 5 nm. While the particles are very difficult to see under the microscope, their presence can be readily detected chemically by reacting samples containing the particles with Sigma Chemical Company's "silver enhancer". A segment of grapevine stem is fitted into a tube attached to a valving device that permits introduction of a small volume containing colloidal gold particles to the stem while maintaining pressure on a water line that drives water through the segment. Introduction of food coloring, whose movement through the stem is not impeded by pit membranes, to the system and collection of the water + dye exiting the stem at the distal end indicates that the volume of water needed to move from one end of a 50 cm stem segment is less than 200 µl. Colloidal gold particles with a 5 nm can move through healthy stem segments, particles of 20 nm diameter cannot (Figures 1 & 2). However, when we used a vine that was showing the initial visible symptoms of PD **at its base** (i.e., its older internodes) and tested the movement of colloidal gold particles through a stem segment cut from the younger portion of the stem that had not yet begun to show PD symptoms, particles of 20 nm diameter moved through the xylem and were collected at the distal end. These results suggest that a decreased pit membrane polymer integrity, hence increased porosity, occurs in healthy appearing stems on infected vines. These results must be confirmed and expanded on (for instance, how much larger are the pores in infected vines?), but they suggest that pit membranes are being opened up in infected vines, perhaps to permit the systemic movement of *Xf*).

The Importance of Xf's Cell Wall-degrading Enzymes to PD Infection.

UC Davis Plant Pathology Ph.D. candidate Caroline Roper and Carl Greve have been working to characterize the gene products of the putative polygalacturonase- (PG) and β-1,4-glucanase- (BGase) encoding sequences identified in the *Xf* genome. In a report at last year's PD Symposium (Labavitch and Matthews, 2003) we reported on Caroline's work with cloning of bacterial "PG" and "BGase" sequences and expression of the cloned genes in *E. coli*. Apparently the *E. coli*-produced proteins are accumulating in inclusion bodies. This is not an uncommon result with this sort of approach, but it does increase the problems with isolating and characterizing the enzymes produced. The work with BGase has proceeded more rapidly. We have shown that the *E. coli* lines expressing the cloned sequences have been induced to express the genes and proteins showing BGase and PG activity have been isolated from them. We are using a combination of protocols to enhance expression and isolation (extraction, solubilization and proper refolding of the expressed proteins) of the two enzymes for use in testing the ability of these enzymes to facilitate *Xf*, polystyrene bead and colloidal gold particle movement through healthy vines. In the meantime, we have initiated an interaction with Novozymes (a Danish biotech enzyme company with a research operation in Davis) to obtain pure microbial PG and BGase for preliminary tests of the impact of these enzymes on pit membrane porosity. The role of PG is particularly important with regard to understanding the reported control of PD development in grapevines that is provided by transgenic expression of a PG-inhibiting protein (PGIP) in *V. vinifera* (The work of Dr. Cecilia Aguero, reported in Meredith and Dandekar, 2002 and 2003; also Aguero et al., 2004 *in press*).

While we are still working to isolate and characterize the *Xf* PG and glucanase, we have developed a strong case for the importance of PG in PD development. Roper has generated an *Xf* mutant with its PG gene knocked out by homologous recombination insertion of a defective PG sequence. Pathogenicity tests with the wild type and PG-deficient *Xf* strains have shown that while the PG-deficient bacteria are able to persist in grapevines they are much less virulent (Figure 3, Table 1) (Roper et al., 2004). We continue to test the relative pathogenicity of these strains and hope to identify specific differences in the gene expression responses of grapevines to inoculation with them.

Is Vessel Cavitation Associated with GWSS Feeding on Grapevines?

In a separate report in the proceedings for this symposium, Shackel and Labavitch report on the work of Plant Biology Ph.D. candidate Alonso Perez indicating that the cavitation of vessels can be readily seen in MRIs of grapevine stems (also in Perez et al., 2004). Elaine Backus and her colleagues at the USDA research facility in Parlier are now set up to perform EPG monitoring of sharpshooter feeding in their new lab. Our groups have been interacting to combine MRI and EPG monitoring with testing for acoustical emissions from grapevines (an indicator of vessel cavitation events) to ask whether vessels cavitate during insect feeding. These tests will probably be made in the first half of 2005.

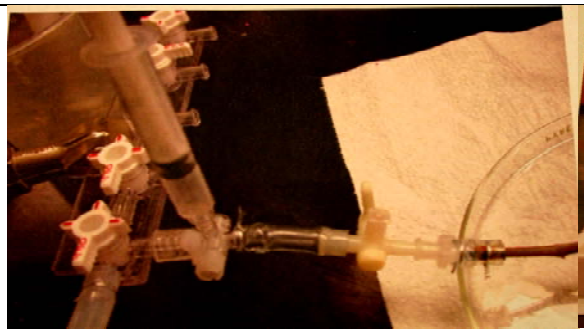

Figure 1. Proximal end of a cut grapevine stem fitted into the valving system that allows (1) pressure driven passage of water through the stem and (2) introduction of colloidal gold particles.

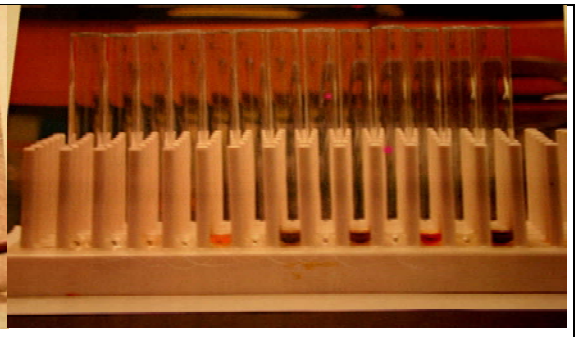

Figure 2. A series of tubes, each containing a 100 ul volume of the water forced through the stem shown in Fig. 1 and collected (left to right, in sequence) as it flowed from the distal end. The presence of gold particles is revealed by the use of silver enhancer.

Table 1. Disease severity of greenhouse-grown grapevines inoculated with wild-type *Xf* (Fetzer isolate), the Fetzer isolate with mutated (non-functional PG sequence) and water. Plants were rated for visual symptoms from 0 to 5, with 0=healthy (no symptoms) and 5=dead. 10 plants evaluated per treatment.

Time post-inoculation	Vines inoculated with:		
	WT *Xf*	PG-*Xf*	Water
12 weeks	0.56	0	0
13 weeks	1.22	0	0

Figure 3. 'Chardonnay' grapevines inoculated 13 weeks previously with, left to right, the Fetzer *Xf* isolate, the Fetzer isolate with its PG gene knocked out, and water. Note the differences in disease symptoms. See Table 1.

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FUNDING AGENCIES

Funding for this project was provided by the USDA Animal and Plant Health Inspection Service and the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.