THE DEVELOPMENT OF PIERCE'S DISEASE IN XYLEM: THE ROLES OF VESSEL CAVITATION, CELL WALL METABOLISM AND VESSEL OCCLUSION

Project Leaders:

John Labavitch
Department of Pomology
University of California
Davis, CA 95616

Mark Matthews
Department of Viticulture and Enology
University of California
Davis, CA 95616

Cooperators:

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

Susan Lurie
The Volcani Institute
Bet-Dagan, Israel

Eleanor Thorne
Department of Viticulture and Enology
University of California
Davis, CA 95616

Alonso Perez
Department of Pomology
University of California
Davis, CA 95616

Tom Rost
Section of Plant Biology
University of California
Davis, CA 95616

Josh Stevenson
Section of Plant Biology
University of California
Davis, CA 95616

Caroline Roper
Department of Plant Pathology
University of California
Davis, CA 95616

Reporting Period: The results reported here are from work conducted from October 1, 2002 to October 1, 2003.

INTRODUCTION

This proposal is directed toward discovering the plant responses to infection that are fundamental to the progression of Pierce's disease (PD) in grapevine. The disease is caused by the growth of the bacterium *Xylella fastidiosa* (*X.f.*) in the xylem vessels of stems, petioles and leaf blades. The disease progresses rapidly, causing severe water deficits in infected shoots (Goodwin et al., 1988) and vine death-often within two years. However the progression of the disease and the mechanism(s) by which the disease produces water deficits and death in infected tissues have not been well established.

The prevailing notion has been that vessels become occluded with bacteria or products of metabolism. However, we have shown that PD symptoms appear in grapevines prior to the development of a large *Xf* population. However, how the bacterium moves through and between vessels, whether vessels cavitate upon introduction of the bacterium by the insect vector or artificial inoculation, the nature and origin of the materials that occlude the vessels of infected vines, and the nature of the plant regulators that influence the vine's response to *Xf* are not known. We have continued our work testing the model (below) that proposes answers to these important questions about the development and progression of PD symptoms in grapevines.

X.f. introduction to vessels—>vessel cavitation—> initial water deficit—> X.f. population increase—> production of enzymes by X.f. (signals ?) —> cell wall digestion —> oligosaccharide signals —> ethylene synthesis rise—> a "wave" of vessel occlusion beyond the infection site —> global collapse of vine water transport—> leaf abscission—>vine death

OBJECTIVES

For this research period we have addressed a number of the elements of the PD development model.

1. What is the nature of the occlusions found in vessels of infected vines?
2. What is the impact of a vine's "water status" on the development of disease symptoms?
3. What is the evidence that *Xf* produces cell wall-degrading enzymes to facilitate the systemic spread of the bacterial population?
4. What is the role of ethylene in the development of vine responses to *Xf* presence?

RESULTS AND CONCLUSIONS

What is the nature of the xylem occlusions found in infected vines?

In prior reports we have reported that "gels" isolated from infected stems have sugar compositions suggesting that they are products of both *Xf* and plant metabolism. We have continued these studies, but it is difficult to isolate these occluding materials without co-isolation of bacterial and plant polysaccharides. Therefore, we have begun testing of immunohistochemical approaches for biochemical characterization of the occlusions. We have used anti-pectin antibodies linked to a fluorescent tag to "light up" the cell walls of tyloses in infected stems (Figure 1a). We have previously reported that tyloses form relatively early following vine inoculations with *Xf*. It is not a surprise that the tylose wall reacts; pectin is known to be an important polysaccharide in higher plant cell walls. Figure 1b shows that the same tagged antibody reacts with gels that occlude vessels and that the pectin gels co-localize with bacteria that are revealed by the use of an additional tagged antibody (Figure 1c).

The sequence of the *Xf* genome "predicts" that the bacterium can produce an extra-cellular polysaccharide with a structure very much like that of the bacterium *Xanthomonas campestris* (Figure 2a). Our analysis of polysaccharides extracted from infected grape stems suggests the presence of material containing the sugars of the predicted "fastidian gum" (Figure 2b). We are now using specific chemical and biochemical procedures to modify commercially available xanthan gum so that it has a structure like that predicted for the *Xf* gum. This will be used for the generation of antibodies that can be tagged (as above) and used to determine whether the occlusions in vessels of infected vines contain polysaccharides of bacterial origin.

What is the impact of a vine's "water status" on the development of disease symptoms?

In order to test the effect of a vine's relative water status on the development of PD symptoms, we subjected 'Chardonnay' (PD-susceptible) vines to three levels of water status (well-watered, moderately-stressed, and severely-stressed) by manipulating the watering schedules in the greenhouse. Vines were inoculated with *Xf* and standard physiological measurements of water stress were taken (to verify that the watering regimes had led to differing degrees of water stress) at intervals along with making visual assessments of PD leaf scorch symptoms. (At the end of the experiment we took stem and petiole samples of all of the vines. These will be tested for *Xf* presence soon.) The first indications of leaf scorch were seen 48 d after inoculation (Figure 3). Evaluations of symptoms were also made 77d and 91d after inoculation. The data indicate that the greater the water stress a vine experiences the quicker PD symptoms develop. Even after 91d, one-third of the well-watered vines showed no leaf symptoms. A poster at the 2003 PD Symposium will show additional data describing standard physiological measurements describing the vine stress caused by our watering regimes.

What is the evidence that Xf produces cell wall-degrading enzymes to facilitate the systemic spread of the bacterial population?

We have already reported that the "porosity" of the primary cell walls of the pit membranes that "separate" one vessel from its neighbors is much too small to permit passage of *Xf* from one vessel to the next, unless the pit membrane is damaged. Thus, systemic spread of bacteria introduced to a few vessels requires pit membrane degradation. Our model suggests that this is caused by cell wall-degrading enzymes, presumably produced by *Xf*. This idea is supported by the observation that the genome of the Brazilian and Temecula strains contain sequences that are similar to sequences known to encode the cell wall polysaccharide-degrading enzymes polygalacturonase (PG) and ß-1,4-glucanase. The idea is also supported by the report from the Meredith, Dandekar and Aguero PD project, that transgenic introduction of a PG Inhibitor Protein (PGIP) from pear fruits into grapevines reduced their susceptibility to PD.

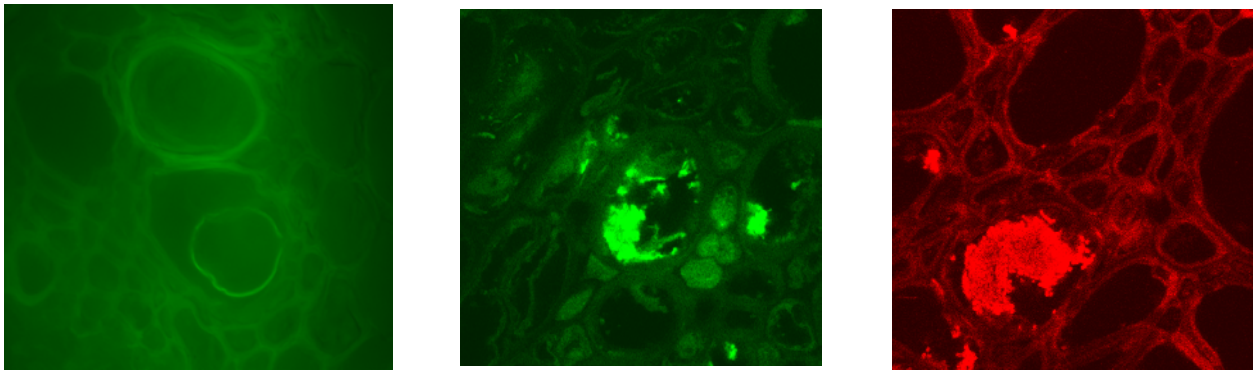
Accordingly, these *Xf* DNA sequences have been cloned. We are currently attempting to express these sequences in the bacterium *E. coli*. When we have expressed the glucanase and/or PG this will confirm that the bacterial sequences encode these enzymes and provide a source (the *Xf* gene-expressing *E. coli* cultures) of the enzymes for testing of other aspects of the cell wall model. Figure 4 describes the cloning and gene expression strategies we are testing.

What is the role of ethylene in the development of vine responses to Xf presence?

We have previously reported that treatment of grapevines with ethylene will trigger the occlusion of vessels with tyloses, just as occurs in PD-infected vines. Additional data supporting this point is provided in a poster presented at the 2003 PD Symposium from the Shackel and Labavitch, CDFA-funded project. We have developed chambers that will be used to monitor emanation of ethylene from healthy and infected vines. We will first test these chambers by measuring ethylene production by vines manipulated to force them to produce ethylene. We will then test them with inoculated vines. Our PD model places ethylene at a key point for the regulation of PD symptom development. If the model is accurate on this point, we will then examine the development of symptoms on inoculated vines that have been treated so that they are not able to respond to ethylene.

CONCLUSION

Continuing testing supports the proposed model of PD development in grapevines. Some steps predicted by the model have not yet been tested and confirmatory experiments are still necessary. Even without that work, however, it seems clear that a great deal of the problems caused in vines by introduction of *Xf* is caused by a vine response to the bacterium, rather than something specifically done to the vine by the bacterium.



a b c

Figure 1. Specific fluorescent antibodies that "light up" cell wall pectins were used to demonstrate the presence of pectin at the surface (e.g., cell wall) of expanding tyloses (1a) and in the gels that accumulate in vessels of PD-infected grapevines (1b). A second antibody (recognizing *Xf*) lights up an accumulation of bacteria in a vessel of an infected vine.

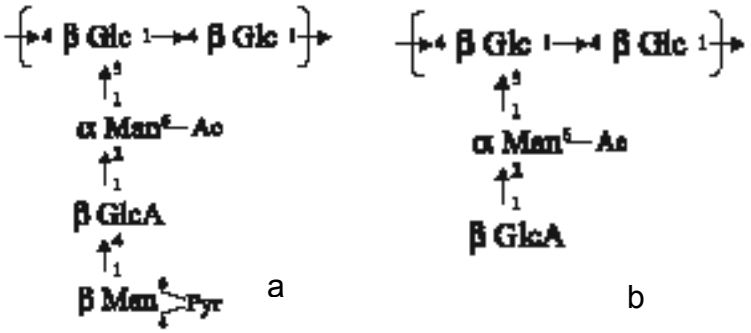


Figure 2. Chemical analysis of xanthan gum shows it to have a polymer backbone made up of ß-1,4-linked glucosyl residues and a "unit" structure consisting of 2 backbone residues, one of which bears a side chain of a terminal, pyruvylated mannosyll residue linked to a galacturonosyl residue linled to and acetylated mannosyl residue (2a). Analysis of the genes in the *Xf* genome suggests that the PD pathogen will make a polysaccharide (dubbed "fastidian" gum) lacking the terminal modified mannosyl residue. Our intent is to remove the terminal residue of xanthan gum and then raise antibodies to the modified polymer.

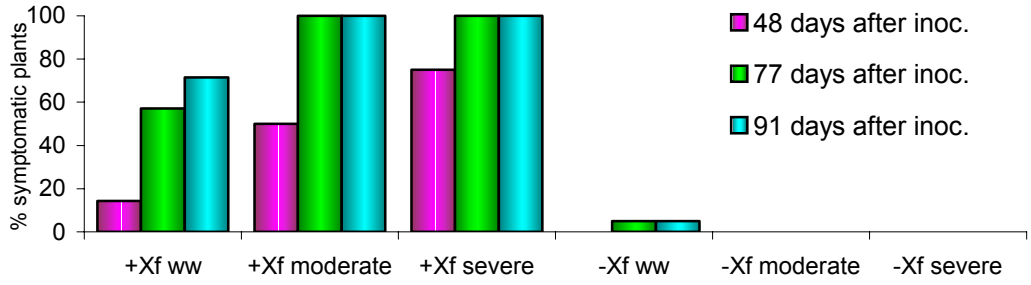


Figure 3. 'Chardonnay' and 'Cowart' grapevines in pots were watered according to three regimes, two of which were designed to cause the vines to experience water stress. Well-watered (ww) plants had an average leaf water potential of –0.6 MPa, moderately water-stressed vines (moderate) averaged –1.0 MPa, and the most water-stressed (severe) plants were –1.4 MPa. Vines were needle-inoculated with *Xf* and monitored at intervals (48, 77 and 91 d after inoculation) to determine the percent of vines showing leaf scorching and matchsticks. 'Cowart' vines showed no symptoms.

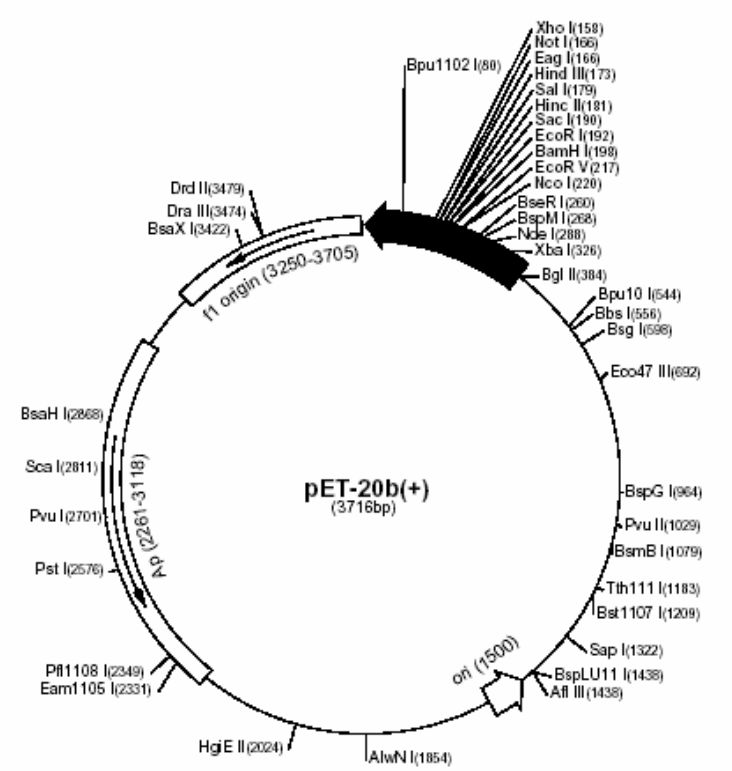


Figure 4. The pET-20b (+) vector contains an N-terminal pelB signal sequence that directs the expressed protein to the periplasmic space, which increases the chances of proper folding. The pET-20b (+) vector also contains a C-terminal histidine tag that can be used in purifying the recombinant protein. This expression system is driven by the strong T7 promoter and controlled by IPTG (isopropyl-β-D-thiogalactopyranoside) induction.

REFERENCES

Goodwin, P.H., J.E. DeVay, and C.P. Meredith. 1988. Physiological responses of *Vitis vinifera* cv. 'Chardonnay" to infection by the Pierce's disease bacterium. Physiol Mol Plant Pathol 32: 17-32.

FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program, the California Department of Food and Agriculture, and the USDA Animal and Plant Health Inspection Service.