MAGNETIC RESONANCE IMAGING: A NON-DESTRUCTIVE APPROACH FOR DETECTION OF XYLEM BLOCKAGES IN *XYLELLA.FASTIDIOSA*-INFECTED GRAPEVINES

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ABSTRACT

Our report in the 2005 Pierce's Disease Research Symposium (Shackel and Labavitch, 2005) demonstrated the value of using MRI to follow the development of cavitations in grapevine xylem following introduction of *Xylella fastidiosa* (*Xf*) via needle inoculation. Strong correlations between increasing proportions of stem xylem conduits that were cavitated, visualized using MRI, and decreased water conductance. Similar observations were made on stems after treatment of vines with ethylene, supporting, but not proving, the suggestion of our Pierce's disease (PD) development model (Labavitch et al., 2005) that vine ethylene production was an important factor in the development of *Xf* infection. This year's effort has been devoted primarily to the replication of the observations of the first two years of this project. In addition, we have continued with the development of conventional, destructive anatomical approaches that we will use to define the nature of the more permanent xylem obstructions, gels and tyloses, whose presence in the water conduits of infected vines may be associated with the occurrence of cavitations. Finally, after considerable delay, we can report that our collaboration with colleagues in the University of California, Davis (UCD) NMR Facility to develop an NMR probe designed for ease of use in grapevine imaging and greater resolution appears to be on course.

INTRODUCTION

Results from several Pierce's Disease (PD) research programs reported in the 2001 to 2005 PD research symposia in San Diego have supported the idea that obstructions in the grapevine's water-transporting xylem tissue develop rapidly post-inoculation, before an appreciable bacterial population has been established. Thus, careful analysis of the timing of changes in xylem element anatomy and function relative to *Xylella fastidiosa (Xf)* introduction and the appearance of the external symptoms of disease development, is important for establishing reliable indicators of the "stage" of PD development. Because the more conventional destructive analyses of xylem function made it impossible to fully understand the progression of internal symptoms and loss of grapevine water-conducting capacity with symptom appearance, we began testing the possibility of using MRI to follow xylem function in individual vines over time. In the course of this study we have developed imaging techniques for obtaining quantitative information about xylem function in individual vine internodes over time, defined the limitations of these techniques, and demonstrated that both PD infection and ethylene treatment trigger decreases in vine water-moving capacity (Shackel et al., 2005; Pérez-Donoso, 2006; Pérez-Donoso et al., 2006). The techniques we have developed also have been used in our tests of a model for PD development (Figure 1; Labavitch et al., 2005). We are currently attempting to define the relationship of the tracheid and vessel cavitations revealed by MRI and other, less transient occlusions that develop in the xylem of *Xf*-infected grapevines.

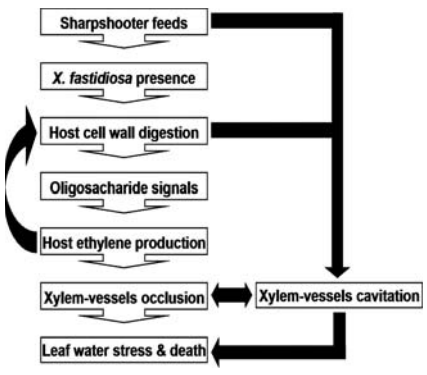

Figure 1. Hypothetical model for PD development. PD starts with infection caused by the glassy-winged sharpshooter's introduction of *Xf* locally (i.e., into one or a few vessels). Once *Xf* is in the xylem the bacteria become systemic, which implies that *Xf* must be able to cross (digest away?) the cell wall in the pit membranes that separate two neighboring vessels. The digestion of the cell wall by bacterial enzymes would generate transient oligosaccharides with biological activity. The presence of these oligosaccharides is detected by the plant triggering a series of defensive responses, including an increase in ethylene production. Ethylene has been shown to induce tylose formation. Cavitation of vessels may be also important for the disruption of water transport in the plant. Cavitations may happen during insect feeding or during PD progression. The "bottom line" of our thinking is that PD is primarily caused by the grapevine's responses (local and systemic) to *Xf* presence.

OBJECTIVES

1. Optimize the use of MRI (Magnetic Resonance Imaging) and to spatially visualize altered water movement in grapevines.
2. Test correlations of observed vascular system obstructions (based on grapevine dissection and microscopy techniques) with predictions based on MRI data.
3. Use MRI to follow the development of grapevine obstructions over time in vines infected with *Xf* or treated with ethylene, bacterial wall-degrading enzymes or plant cell wall oligosaccharides, all of which may be important intermediates in regulating the vine's response to infection and the eventual development of PD symptoms.
4. Use NMR imaging to determine whether localized xylem cavitation occurs at the site and time of *Xf* inoculation or introduction by the glassy-winged sharpshooter.

RESULTS

Objective 1. Optimization of MRI for visualizing water transport deficiencies in PD-infected grapevines.
Some progress has been made on this objective in the last few months. In fact, this objective has been a problem for two years. We had planned to have a prototype of an MRI coil optimized for use in imaging water in grapevine xylem at the end of year one of this project. This part of the work was developed with cooperator Dr. Jeffrey Walton of the UCD NMR Facility. Jeff developed the plans for the coil and then a series of losses of suppliers of key parts and the failure of the outside group that was to do the fabrication to deliver have stalled things. We are now nearing the end of the project's final year and there is no coil thus far. However, Dr. Walton recently redrew the detailed plans for the new coil and they are now being examined by the group that will do the fabrication. We are hopeful that the "advanced" coil will be available before much more delay.

Alonso Pérez has now finished his Ph.D. program (Pérez-Donoso, 2006) and returned to a faculty position in Santiago, Chile. We will have funds left in the project budget and will ask for a no-cost extension. Dr. Qiang Sun, who has been working with Professors Tom Rost and Mark Matthews in their PD research projects, will join our group on October 1, 2006. Qiang has some experience with MRI and will take the MRI training program offered by the UCD NMR Facility in Fall quarter. He will then continue with the remaining work in this project and work in a new project ("The pit membrane barrier to *Xf* movement in grapevines: Biochemical and physiological analysis") that was approved this year.

Objective 2. Test for correlations of observed vascular system obstructions with predictions based on MRI data and **Objective 3. Use of MRI to follow the development of grapevine obstructions over time in vines infected with *Xf* or treated with ethylene.**
A series of greenhouse-grown 'Chardonnay' grapevines was inoculated with *Xf*, enclosed in chambers and treated with ethylene for 48 h, or left untreated (controls). MRI was used to follow the development of cavitations over time. At intervals, imaged stems were marked to indicate where cavitations had been observed, excised fixed and sectioned. The intent is to develop a thorough histochemical analysis sections taken along the lengths of these stems to determine whether there is a correlation between the positions of tyloses and vascular system gels and points along the stems where a great deal of cavitation has been seen. This effort was begun as a collaboration involving Pérez, Dr. Katy Pinney in Prof. Vito Polito's lab (Plant Sciences Department), and undergraduate researcher Joshua Lenhof. Following Pérez' departure, Pinney and Lenhof have continued the work. The final correlations of cavitations, tyloses and gels have not been developed because that requires completion of the full histochemical analysis. However, the histochemical work has made clear that PD and ethylene treatment also influences the formation of readily distinguishable xylem obstructions (Figure 2).

A manuscript describing much of the work done in the three years of this project (Pérez-Donoso et al., 2006) is now being reviewed by the journal *Plant Physiology*.

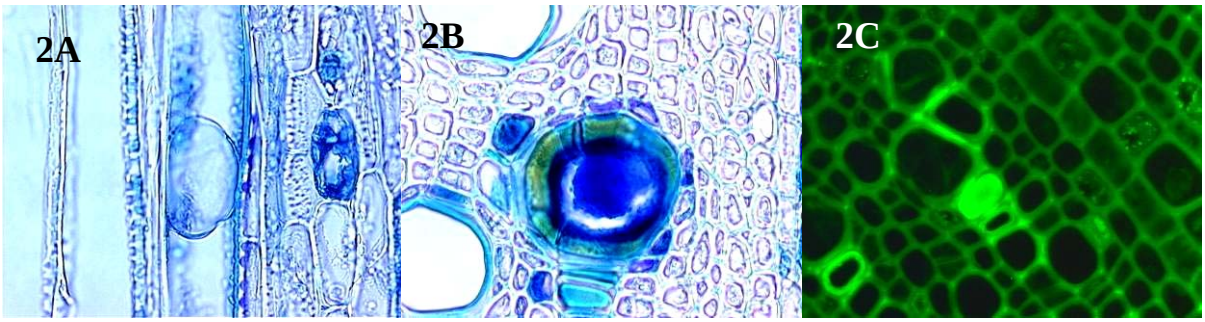

Figure 2. Sections were taken from a region of a PD-infected stem in which MRI analysis had shown a relatively high amount of xylem cavitation. **A.** A longitudinal section of the stem showing an open vessel adjacent to a vessel blocked with a tylose. **B.** A cross-section of the stem showing a xylem conduit blocked with what appears to be a non-cellular, vascular system gel. Panels **A** & **B** are stained with the non-specific stain toluidine blue. **C.** A cross section through stained with coryphosphine-O. The bright green fluorescence indicates the presence of pectin. The conduit in the center of the panel is filled with a pectin-rich gel.

Objective 4. Use of MRI to determine whether localized xylem cavitation occurs at the site and time of *Xf* inoculation or introduction by the glassy-winged sharpshooter.

The work to determine whether GWSS feeding on grapevines is accompanied by xylem cavitation has begun (see the report for the project "Linking the model of the development of PD in grapevines to an understanding of the dynamics of GWSS transmission of *Xf* to grapevines and grapevine gene expression markers of PD" in these *Proceedings*). The work has not progressed to the point where MRI analysis is needed.

CONCLUSIONS

We expect that our combined approach (use of non-destructive and destructive methods) to study xylem function will determine which kinds of disruption (tyloses, pectin gels, or air embolisms) predominate in PD-infected stems and describe the developmental progression of vascular system occlusions that occur during the different stages of the disease.

REFERENCES

Labavitch J. M., E. Backus, M. A. Matthews, and K. A. Shackel. 2005. Linking the model of the development of Pierce's disease 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. 2005 Pierce's Disease Research Symposium, p. 34-38.

Pérez-Donoso, A.G. 2006. Studies on the physiological consequences of *Xylella fastidiosa* infection in grapevines. A proposed model for pathogenicity mechanisms. Ph.D. Dissertation.

Pérez-Donoso, A.G., L.C. Greve, J.H. Walton, K.A, Shackel and J.M. Labavitch. 2006. *Xylella fastidiosa* infection and ethylene exposure result in xylem and water movement disruption in grapevine shoots. Plant Physiology (submitted for publication).

Shackel K. A., and J. M. Labavitch. 2005. Magnetic resonance imaging: A non-destructive approach for detecting xylem blockages in *Xylella fastidiosa*-infected grapevines. 2005 Pierce's Disease Research Symposium. p. 66-71.

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MAP-BASED IDENTIFICATION AND POSITIONAL CLONING OF *XYLELLA FASTIDIOSA* RESISTANCE GENES FROM KNOWN SOURCES OF PIERCE'S DISEASE RESISTANCE IN GRAPE

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ABSTRACT
Development of a framework simple sequence repeats (SSR) genetic linkage map based on the 181 genotypes of 9621 family, which segregates for Pierce's disease (PD) resistance is complete. The current genetic linkage map consists of 236 non-AFLP markers (SSR, EST-SSR and ESTP-RFLP) in 19 linkage groups. The PD resistance locus, *PdR1*, maps to linkage group 14 (LG - essentially a chromosome) of the male parent (F8909-17), which now consists of 30 markers, nine of which are localized within 10 cM (very closely) of *PdR1*. The 9621 mapping population was expanded from 181 to 457 genotypes. A total of 13 markers polymorphic for F8909-17 mapped to LG 14 and were added to 276 segregants (core population set is 457). We also screened an additional 400 seedlings with two markers (one on either side of *PdR1*) and a total of 50 unique recombinant plants were planted in the field. To avoid confounding affects of resistance inherited from D8909-15 (which is also highly resistant, but with a very different form of resistance) the 04-190 population was selected and a map of LG 14 with 220 genotypes was completed. 04-190 is a cross of *V. vinifera* F2-7 (Cabernet Sauvignon x Carignane) x F8909-08 (sibling of F8909-17). We have used F8909-08 extensively in PD resistant wine and table grapes, therefore it is necessary to validate that *PdR1* gene segregates 1:1 in progeny from its crosses. We completed greenhouse screening of 160 genotypes from the 04-190 population to verify the molecular marker results. The *PdR1* resistance locus segregates 1:1 and mapped to the same position with surrounding markers ctg1025882 and VMCNg2b7.2. We also increased the core population of 04-190 from 220 to 395 seedling plants. Leaf tissue for DNA extraction and green cuttings for greenhouse testing and ELISA screening from the additional 175 plants were collected in late summer, and results are expected in early spring 2007.

Efforts to construct a bacterial artificial chromosome (BAC) library from b43-17 (the basis of the *PdR1*) were initiated. A total of 200 green cuttings were collected that resulted in 160 plants that are being cultivated for young etiolated shoot tips that provide an excellent source of DNA for the BAC library. This BAC library is being developed to provide markers from BAC end sequencing for LG 14, so that we can create a physical map of the *PdR1* gene family, which will lead to genetic engineering efforts. We are also working to add resistance gene analogs (RGA) markers, which are generalized genetic sequences involved in a wide range of pest and disease defense responses in plants, to our genetic maps. The addition of these markers may identify common regions of disease resistance and possible functions of the *PdR1* gene family.

In order to understand the stability and segregation of PD resistance from different sources, work on six different mapping populations was completed. We are also continuing mapping efforts in the 0023 population, a cross of D8909-15 x *V. vinifera* B90-116, to identify quantitative trait loci (QTL) and then saturate linkage groups with these QTLs with more markers. This population is important because we have extensive data for cluster and berry traits, and *Xylella fastidiosa* (*Xf*) resistance data for about 200 plants. We completed the characterization of Mexico collection, the source of the exceptional resistance to *Xf* and collected by Dr. Olmo in 1960. We are using these unique selections in our genetic and molecular breeding to produce PD resistant table and wine grape cultivars.

INTRODUCTION
We have been mapping resistance to *Xylella fastidiosa (Xf)* in three (9621, 0023, and 04-190) populations, and to *Xiphinema index*, the dagger nematode in two (9621 and 0023) populations. The preliminary AFLP-based 9621 genetic map has been published (Doucleff et al. 2004). The 9621 population was then mapped with the more informative microsatellites or SSR markers, which provide a more reliable and repeatable framework for initial mapping of candidate genes and QTLs. In addition, tightly linked SSR markers are ideal for marker-assisted selection (MAS) due to their applicability across different genetic backgrounds and ease of use. This year, mapping efforts within the 9621 and 04-190 populations have concentrated on linkage group 14 that harbor the *PdR1* resistance locus (Krivanek et al. 2006; Riaz et al. 2006). The addition of SSR markers to this linkage group was greatly aided by the existence of other SSR-based genetic maps of grape that have been developed within *V. vinifera* populations and by the availability of expressed sequence tag polymorphism (ESTP) markers developed by other grape researchers and available on various genetic databases. e are now initiating construction of a BAC library. A high quality BAC library with good coverage is essential for the isolation of the BAC clones that harbor *PdR1* resistant genes. BAC end sequencing of these clones will allow us to develop a physical map in conjunction to genetic map, develop more markers around the *PdR1* region, and lead genetic engineering of susceptible *V. vinifera* grapes with the *PdR1* gene.