MAGNETIC RESONANCE IMAGING: A NON-DESTRUCTIVE APPROACH FOR DETECTION OFXYLEM BLOCKAGES IN *X. FASTIDIOSA*-INFECTED GRAPEVINES

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INTRODUCTION

Results from PD research programs led by Matthews, Rost and Labavitch (reported in 2001 and 2002 in San Diego) have provided substantial support for the idea that obstructions in the vine's water-transporting xylem tissue develop rapidly post-inoculation, before an appreciable bacterial population has been established. The results also strongly suggest that these obstructions, and likely other aspects of the Pierce's disease (PD) "syndrome", result from the grapevine's active responses to the presence of *X. fastidiosa* (*Xf*), rather than to direct "action" by the bacterium. Thus, careful analysis of the timing of changes in xylem element anatomy and function relative to *Xf* introduction, as well as to external symptoms of disease development, is important for establishing reliable indicators of the "stage" of PD development. The analyses done thus far have been based on destructive tissue sampling. While the Matthews and Labavitch project continues to test a model of PD development, sampling of infected vines is essentially blind and must, therefore, be destructive. This is particularly so since it has become clear that important internal responses to the bacterium are correlated with the presence of rather few bacteria in the tissues, at a time, post-inoculation, that is well before external PD symptoms are in evidence.

Perez and Walton have has carried out a number of pilot studies aimed at "observing" the development of xylem vessel obstructions in infected vines without damaging the plants. The value of the work is that periodic examinations can be done on each of several vines over a number of weeks (reported in 2002). The NMR images provided clear evidence of the progressive, localized deterioration of water movement capacity in treated vines. The data provide spatial information about where, around the circumference of a vine as well as along its length, obstructions are present. This information should provide specific guidance for eventual destructive sampling to assess the presence and nature of xylem obstructions. Optimization of the analytical approach (our Objective 1) in order to maximize the signal to noise ratio by reducing the noise component will add considerably to the value of the technique. This will lead to studies aimed at addressing whether the vessels cavitate (i.e., air "embolisms" develop) when the glassy-winged sharpshooter feeds on vines and non-destructive testing of the impacts of *Xf* cell wall-degrading enzymes, pectin-derived oligosaccharides, and ethylene on vine water transport, as discussed in the Matthews and Labavitch PD development model (Figure 2, below).

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 *X. fastidiosa* 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 *X. fastidiosa* inoculation or introduction by the glassy-winged sharpshooter.

RESULTS

Optimization of the use of MRI for visualizing water transport deficiencies in PD-infected grapevines

Progress on this Objective has been delayed because a supplier for a key electronic element of the new MRI probe that has been designed for use with grapevines no longer provides the key part. We are proceeding with the testing of aspects of the PD model using the NMR instrument in its more conventional configuration.

MRI will show non-functional sections in the xylem of a PD-infected grapevine stem

Usually the techniques to evaluate xylem function are destructive. Magnetic Resonance Imaging (MRI) allows us to visualize vessels that are functional and full of movable water. Functional vessels appear as bright spots in an MRI view of the stem cross-section, non-functional vessels lack water and appear as dark spots in the area of the stem where water-conducting cells are found. Figures 1a and 1b show the difference in the distributions of functional vessels in an infected vine at a point where leaf symptoms of PD are apparent (Figure 1a) and nearer to the stem apex at a point where the leaves show no sign of PD symptoms (Figure 1b). Compare these images with that for a healthy vine (Figure 3a). Because the MRI analysis is non-destructive, this technique allows periodic examinations on each of several vines over a number of weeks. These features make MRI an excellent tool to study the time course progression of xylem disruption in PD-infected vines.

We can use MRI to visualize air embolisms in grapevines

The PD model (Figure 2) predicts that air embolisms may occur when the glassy-winged sharpshooter feeds and/or as pit membranes between xylem vessels are digested. Cavitations can be easily observed with MRI (Figure 2). When there are dark spots in the center region of the stem where vessels are located, the dark spots indicate that vessels are non-functional. The loss of vessel function can result from cavitation or obstruction with bacteria or polysaccharide gels that have been observed in infected vines. If dark spots in an image are the result of air embolism, then we should be able to refill the vessels by forcing pressurized water through the stem. That this approach is valid was demonstrated in an experiment in which the image of a shoot in a healthy, well hydrated vine was made (Figure 3a) and compared to another image that was taken after a section of stem through ca. 80% of the stem's cross section was removed, allowing air to embolize the vessels above the cut (where the Figure 3b images were taken). Later the stem segment that contained the embolisms was excised and refilled with pressurized water. A new image that confirmed the refilling of the vessels was taken (Figure 3c). This experiment also demonstrated the more general principle that the dark spots seen in the MRIs of xylem correspond to non-functional vessels.

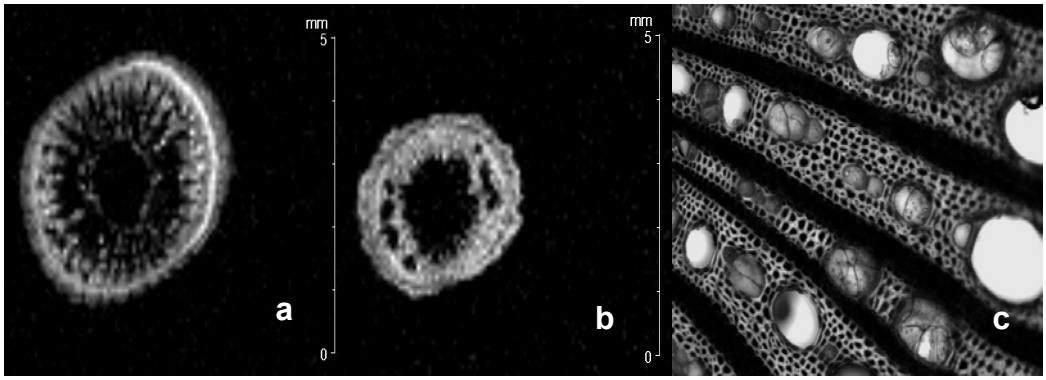

Figure 1. MRI of a PD-infected stem in a basal internode (a), and closer to the apex (b). Bright spots between the central pith (dark) and the ring of vascular cambium show functional vessels (compare to a healthy stem in Fig. 3a). Tyloses (cellular-physical blockages of the vessels) are often associated with dark spots in MRIs of infected xylem, as shown by optic microscopy in (c).

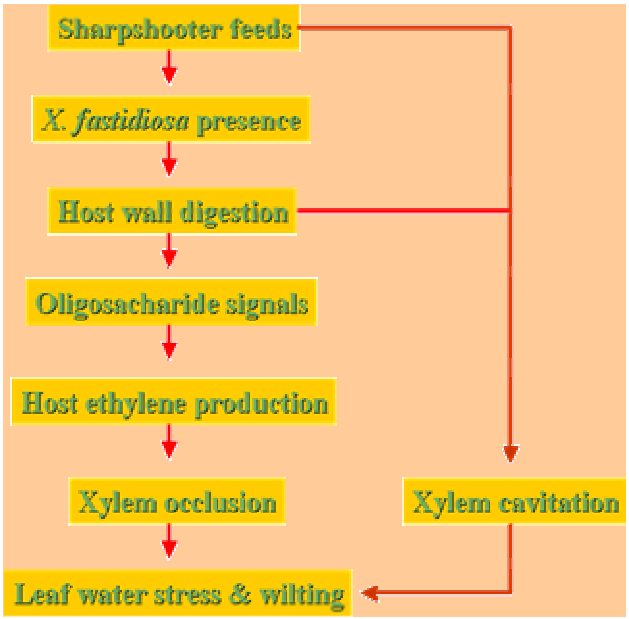

Figure 2. Hypothetical model for PD development. PD starts with a local infection caused by the glassy-winged sharpshooter's introduction of *Xf* locally (i.e., into one or a few vessels). Once *Xf* is in the xylem the bacteria become systemic, which implies that *Xf* must be able to cross (digest away?) 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 a raise 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.

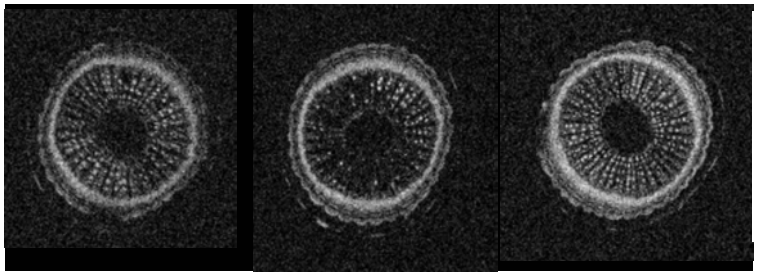

Figure 3. (a) MRI of an intact stem segment in a healthy shoot. (b) Image of the same stem portion after an important part of the cross section has been removed below it, thus causing cavitation of many vessels. (c) The same stem segment after it has been refilled with water.

CONCLUSIONS

MRI will be a powerful adjunct to other, more conventional approaches for characterizing the changes that occur in grapevine xylem following introduction of *Xf*.

REFERENCES

Clearwater, M.J., and C.J. Clark. 2003. *In vivo* magnetic resonance imaging of vessel contents in woody lianas. Plant, Cell and Environment 26: 1205-1214.

FUNDING AGENCIES

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