

Magnetic Resonance Imaging (MRI) of Oak Trees Infected With *Phytophthora ramorum* to Determine Potential Avenues of Infection in Bark¹

Edwin R. Florance²

Abstract

Non-destructive magnetic resonance imaging (MRI) revealed pathological anatomical features of coast live oak trees (*Quercus agrifolia*) that were naturally infected with *Phytophthora ramorum*. Fresh excised whole slices showing typical macroscopic cankers and bleeding were examined. Infected areas (i.e. cankers) were compared to presumed healthy sections. Various infected tissues were revealed and the depth of infection into the xylem could be estimated. Discontinuous distribution of water in the outer layer of sapwood was observed and high water concentrations appeared in the cankers. MRI also revealed channels in the periderm (bark) with high water concentration. Microscopic examination revealed the channels to be rays continuous with the rays extending into the xylem. The rays function in the radial conduction of water, and it is suggested that they may serve as an avenue of infection for *P. ramorum*.

Key words: magnetic resonance imaging, microscopy, periderm, *Phytophthora ramorum*,

Introduction

A major question regarding the biology of *Phytophthora ramorum* is what plant structures does it utilize to establish infection? Recently hyphae of *P. ramorum* have been identified growing in lenticels of naturally field-infected material and in stomata of both field- and experimentally infected plant species (Florance 2002) (figs. 1 & 2). Lenticels are present in young growth, i.e. 1- to 5-year-old twigs. In deciduous plant species stomata are only present during certain parts of the growing season. However, it is known that infections occur in older stems and in very old tree trunks where *P. ramorum* causes cankers and bleeding. As a result, it is not known whether other avenues that can be utilized by *P. ramorum* to establish infections. The following questions need to be answered: 1) What structures are available as avenues for infection during the periods of the year when stomata and/or lenticels are not available? and 2) What structures could be utilized

¹ A version of this paper was presented at the Sudden Oak Death Second Science Symposium: The State of Our Knowledge, January 18-21, 2005, Monterey, California.

² Lewis & Clark College, 0615 SW Palatine Hill Rd., Portland, OR. 97219; florance@lclark.edu

to establish infections in older trees, e.g., trees more than 10 years old or older? Earlier attempts to investigate the bark (periderm) of *Lithocarpus densiflorus* and *Quercus spp.* infected with *P. ramorum* using the techniques of histopathology combined with scanning electron microscopy (SEM) provided limited data (Florance 2002). Therefore, other techniques were evaluated. It was determined that high resolution Magnetic Resonance Imaging (MRI) would be a valuable technology that could expand understanding of diseased oak trees (MacFall and others 1994). This recently developed technology is utilized mostly in areas related to human medicine. It has not been generally applied in areas of plant pathology (Kramer and others 1990). However, it is highly effective, and lately has allowed for the analysis of internal wood and bark structure (Hall and others 1986; Wang and Chang 1986; Hailey and Swanson 1987; Chang and others 1989; Olson and others 1990; Chang and others 1991; Coates 1998; Contreras and others 2002). The major advantage of using MRI is that it is non-destructive. It can also provide both anatomical and functional data about the infected host (MacFall and others 1994). However, a limitation is that resolutions are in the range of approximately 100 μ m to 200 μ m. Since the anatomical structures produced by *P. ramorum* are smaller (e.g., hyphae are 5 μ m to 8 μ m in diameter), individual structures cannot be resolved. Therefore, SEM, which achieves resolutions of approximately 200 to 300Å, combined with MRI, can provide a more complete understanding of *P. ramorum* in infected oak trees.

MRI is an interaction between an external magnetic field, radio waves, and hydrogen nuclei in the tissues being examined. Because it detects hydrogen nuclei, and water is the most abundant molecule in biological material, image contrast is achieved by comparing areas high in water concentration to areas of lower concentration. The brightest areas in an MRI are areas of greatest water concentration. Preliminary data indicate: (1) high water concentration in the areas where cankers occur, (2) disruption of the outer most water conducting xylem, and (3) channels in the bark through which fungi and other organisms may infect the tree.

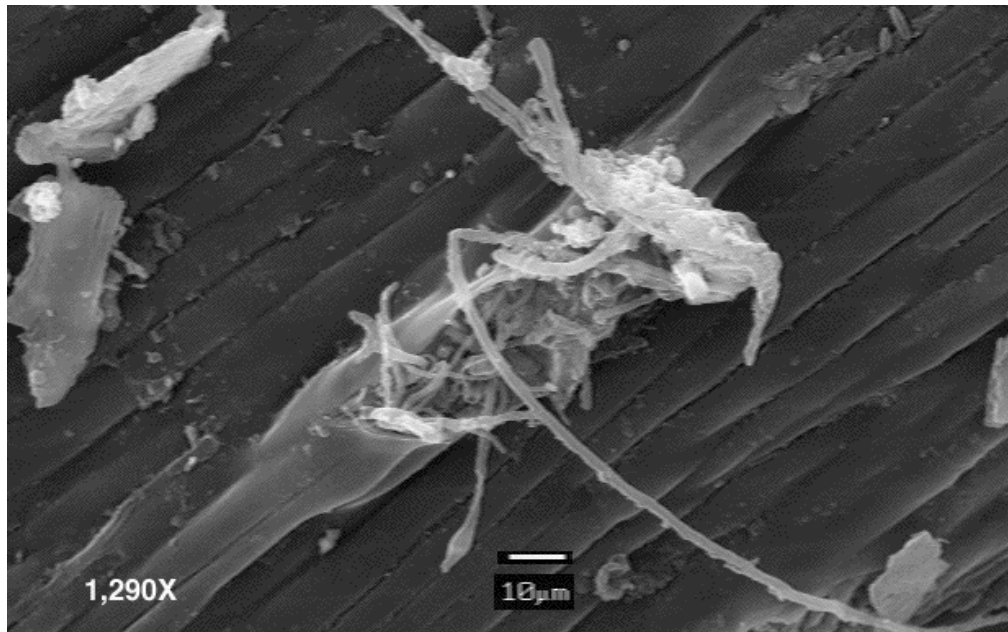


Figure 1– Hyphae of *Phytophthora ramorum* growing in a lenticel of tanoak, *Lithocarpus densiflorus*. Infection was positively identified via PCR.

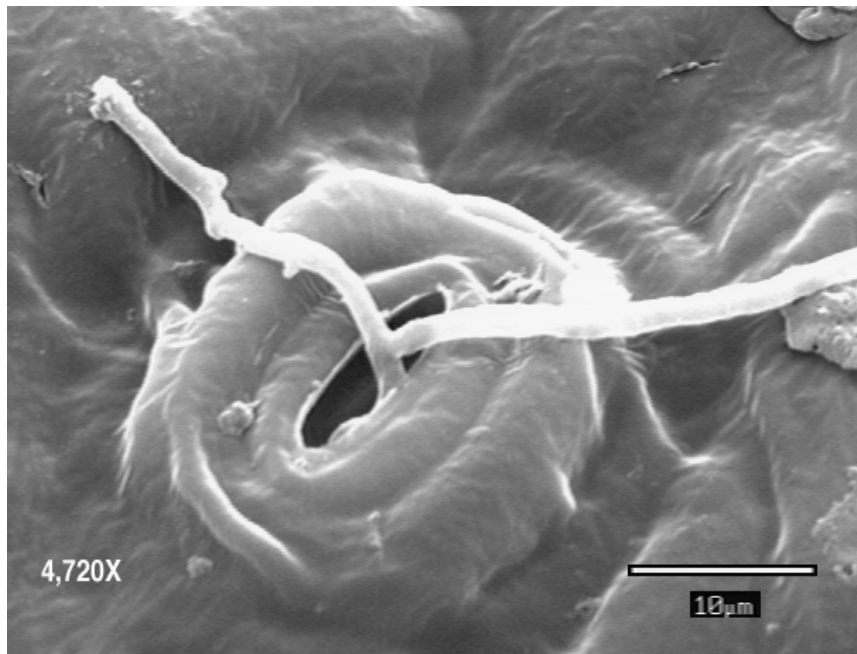


Figure 2– Hyphae growing in a stoma of infected *Umbellularia californica* (California bay laurel). The tree was naturally infected but demonstrated to be positive for *P. ramorum* via PCR.

Materials and Methods

Slices from *P. ramorum*-infected *Quercus agrifolia* were provided by Jennifer Davidson (Pacific Southwest Research Station, USDA Forest Service and the University of California, Davis) for cytological and MRI data acquisition. *Figure 3* shows an example of an infected tree cross section used as one of the study samples.

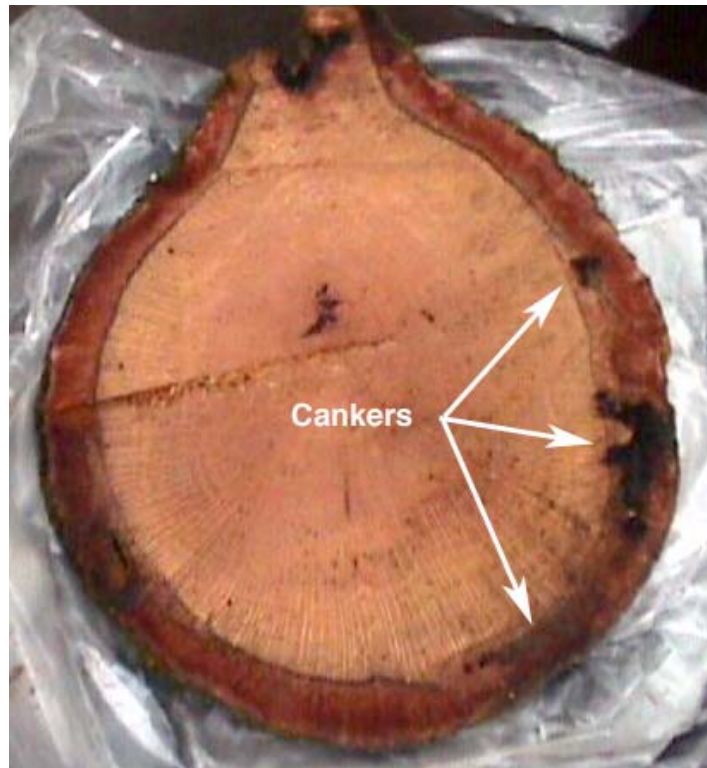


Figure 3– Slice from a *P. ramorum*-infected coast live oak tree (*Quercus agrifolia*). Note the infected areas in the bark (cankers).

The dark colored areas in the bark are the infected sites referred to as cankers. Collected material was prepared immediately for cytological examination. Bark cubes, 5 mm per side, were removed and fixed directly in 4 percent phosphate buffered glutaraldehyde (pH 7.0 at 4°C) for 2 hours. After fixation, all samples were washed thoroughly with the buffer and post-fixed in 1 percent osmium tetroxide. Dehydration was achieved through an ascending series of ethanol solutions (EtOH/Distilled H₂O), 30 percent, 50 percent, 70 percent, 90 percent, and 100 percent ethanol. Samples were soaked three times for 10 minutes in each solution. After dehydration the samples were prepared for critical point drying by passing them through an ascending series of ethanol/amyl acetate solutions, 30 percent, 50 percent, 70 percent, 90 percent, and 100 percent

amyl acetate. Again, the samples were given three 10-minute soaks in each solution. Samples were transferred immediately to a critical point drier using liquid CO₂ as the transition fluid. Analysis was done using either an AMRAY 1810T scanning electron microscope or a Zeiss Axiostar light microscope. Data were captured digitally and measurements were made using NIH image software. Because MRI does not require fixation procedures, cookies were maintained in a fresh condition, placed in the requisite magnetic coil, and inserted into the MRI chamber for data acquisition.

Results

MRI Data

The outer layer of xylem cells (i.e., the youngest cells, bright band) are filled with water (*fig. 4*). The water concentration in the xylem is disrupted around the upper left circumference of the tree where the cankers exist (*fig. 4*). At the site of a canker, water concentration increases significantly. Water channels in the bark that are continuous with the water conducting xylem are revealed (*figs. 4 & 5*). The channels correspond to rays observed with scanning electron microscopy (*fig. 7*).

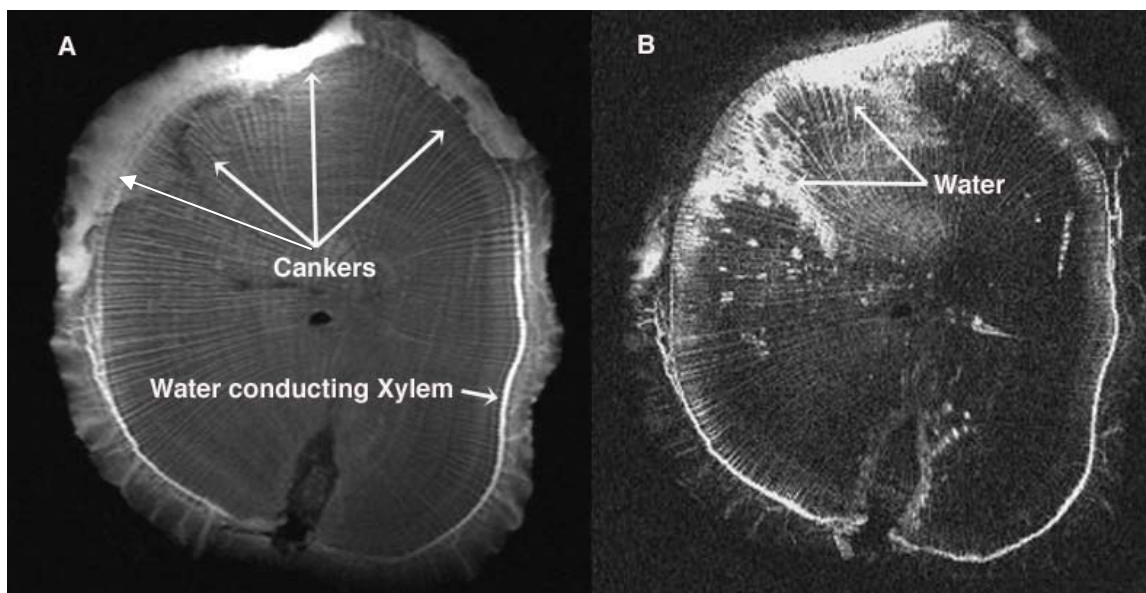


Figure 4 - MRIs of infected *Quercus agrifolia* showing the cankers (i.e. infected) areas. Also note the outer water conducting xylem and the white bands in the bark containing water. The images were captured at two different strengths of the magnetic field.

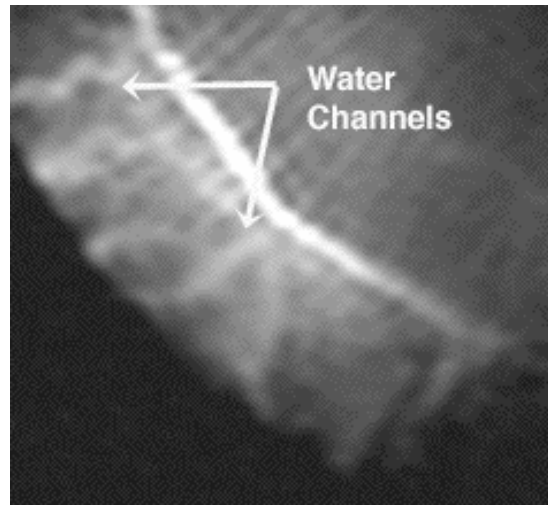


Figure 5 - Small section of the image in *fig. 4* to further demonstrate the water-filled channels in the bark.

Microscopy Data

Since MRI does not have the capability of resolving individual cell structure, the water channels were further investigated using light and scanning electron microscopy. At low magnifications, using a dissecting microscope, bands that traverse radially across the periderm can be identified (*fig. 6*).

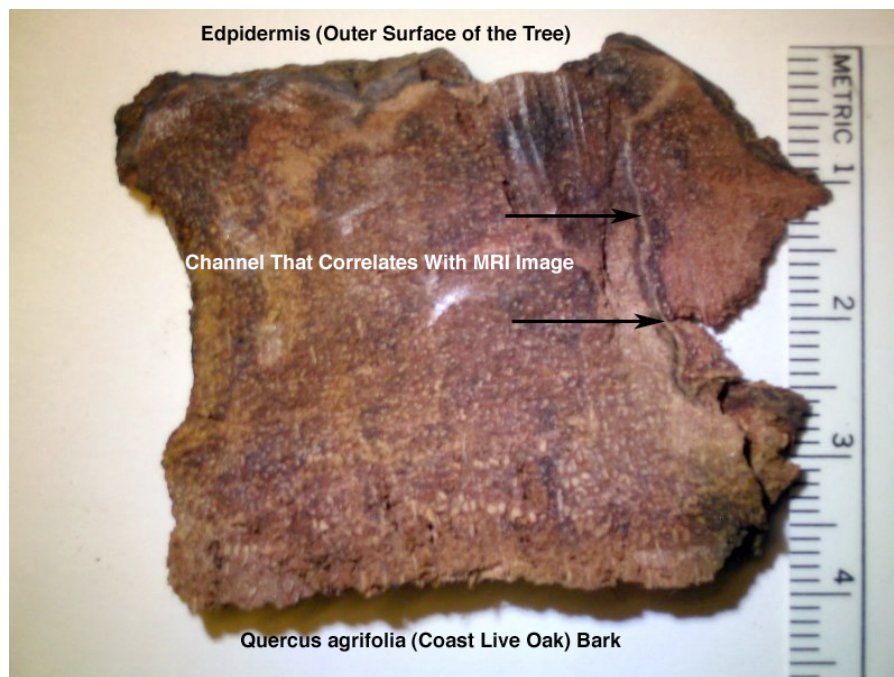


Figure 6– Low magnification photo showing a line of cells that traverse the bark. This line of cells correlates with the channels indicated in *fig. 4*.

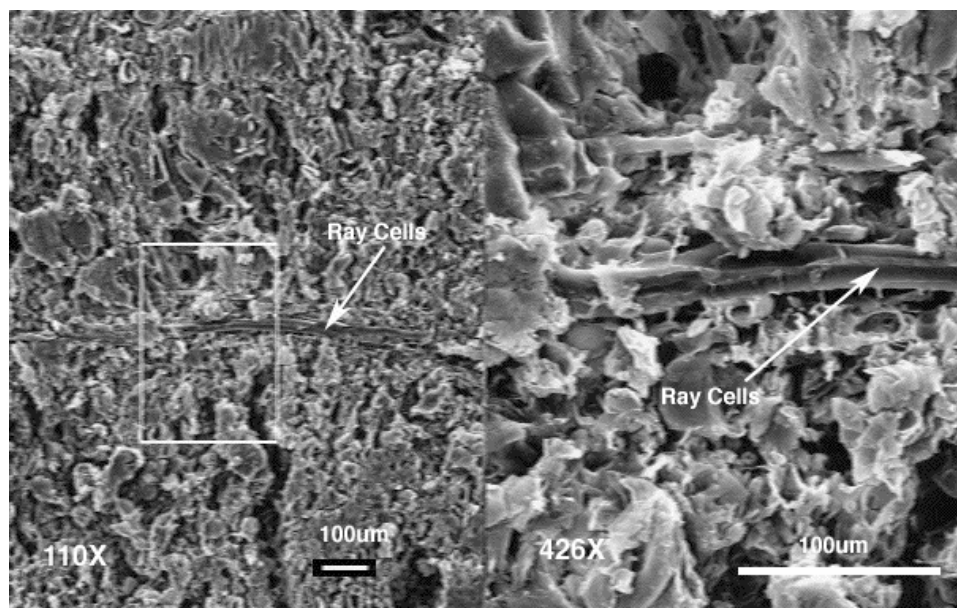


Figure 7– Scanning electron micrograph of ray cells. They are the cells that form the white, water-filled bands in the bark revealed by MRI.

Scanning electron microscopy revealed the bands to be ray cells elongated radially toward the outside of the tree (*fig. 7*). These cells correlate with the water-filled bands in the periderm shown in *fig. 5*.

Discussion and Conclusions

Technically, the outer most tissues of secondary origin in an oak tree should be referred to as the periderm. The word bark, on the other hand, is a non-technical term that refers to everything outside the vascular cambium. Each new growing season the periderm is composed of many living cells, e.g. cork cambiums, cortical cells, etc. An abundant supply of water must be radially translocated into these tissues to maintain cell division and growth. As demonstrated by MRI, the ray system functions in that capacity. And by correlating the MRI data with the structural data obtained with SEM, it has been demonstrated that the water-filled rays traverse from the xylem through the phloem and into the periderm. Regarding the various portals that may serve as infection entry points for *P. ramorum*, the bark, or periderm, appears to be a likely point of access. Bark is generally considered to be protective and more or less impenetrable by many pathogens. However, data obtained by MRI suggests otherwise. These water filled channels, or rays, could serve as natural avenues for the hyphae of *P. ramorum* to penetrate into the deeper tissues of and older oak tree.

It is important to point out that the samples studied were naturally infected in the field. Even though the presence of *P. ramorum* was demonstrated by culture and PCR, other microorganisms may have contributed to canker size and tissue disruption as demonstrated by MRI.

What has been presented above are preliminary data obtained from MRI observations correlated with considerable cytological work. MRI is a powerful technology, and when combined with scanning electron microscopy answers to the previously stated questions can be obtained. In addition, significant functional data can be acquired. For example, answers to questions about the depth and breadth of an infection may be determined, and specific tissues that have been disrupted can be identified.

Acknowledgements

The reported research has been supported, in part, by the Pacific Southwest Research Station, USDA Forest Service, through agreement No. 01-CR-11272138-174. Dr. Jennifer Davidson graciously collected and provided “cookies” of *Quercus agrifolia*. Dr. Matteo Garbelotto, University of California, Berkeley provided infected plant material. Mr. Paul Trusty, Lewis & Clark College, assisted in my laboratory.

References

- Chang, S.J.; Cohen, M.; and Wang, P.C. 1991. **Ultrafast scanning of hardwood logs with NMR scanner.** In: Proceedings of the 4th International Conference on Scanning Technology in the Wood Industry.. Miller-Freeman Publ. San Francisco, CA. pp. Chang-1-3.
- Chang, S.J.; Olson, J.R.; and Wang, P.C. 1989. **NMR imaging of internal features in wood.** Forest Products Journal 39(6): 43-9.
- Coates, E.R. 1998. **Internal defect detection in hardwood logs with fast magnetic resonance imaging.** Baton Rouge (LA), Louisiana State University: 321. Available from: UMI, Ann Arbor, MI; 9922067.
- Contreras, I.; Guesalga, A.; Paulina Fernandez, M.; Guarini, M.; and Irarrasaval, P. 2002. **MRI fast tree log scanning with helical undersampled projection acquisitions.** Magnetic Resonance Imaging 20 (10): 781-87.
- Florance, E.R. 2002. **Plant Structures Through Which *Phytophthora ramorum* Establishes Infections.** Sudden Oak Death, a Science Symposium The State of Our Knowledge, Monterey, CA., USDA Forest Service, Pacific Southwest Research Station.
- Hailey, J.R.T. and Swanson, J.S. 1987. **Imaging wood using magnetic resonance.** In: Proceedings of 2nd International Conference on Scanning Technology in Sawmilling. Miller- Freeman Publ. San Francisco, CA. pp. VIII-1-11.

- Hall, L.D.; Rajanayagam, V.; Stewart, W.A.; and Steiner, P.R. 1986. **Magnetic resonance imaging of wood.** Canadian Journal of Forest Research 2: 423-26.
- Kramer, P.J.; Siedow, J.N.; and MacFall, J.S. 1990. **Nuclear magnetic resonance research on plants.** New York: Academic Press. pp. 403-442.
- MacFall, J.S.; Spaine, P.; Doudrick, R.; and Johnson, G.A. 1994. **Alterations in growth and water-transport processes in fusiform rust galls of pine determined by magnetic resonance microscopy.** Phytopathology 84(3): 288-93.
- Olson, J.R.; Chang, S.J.; and Wang, P.C. 1990. **Nuclear magnetic resonance imaging: a noninvasive analysis of moisture distributions in white oak lumber.** Canadian Journal of Forest Research 20(5): 586-91.
- Wang, P.C. and Chang, S.J. 1986. **Nuclear magnetic resonance imaging of wood.** Wood and Fiber Science 18: 308-14.