AN ABSTRACT OF THE THESIS OF

Bradley R. Collins for the degree of Master of Science in Botany and Plant Pathology presented on June 3, 2008.
Title: The Effects of *Phytophthora ramorum* Infection on Hydraulic Conductivity and Tylosis Formation in Tanoak Sapwood

Abstract approved

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Jennifer L. Parke

Tanoak (*Lithocarpus densiflorus*), along with some other members of Fagaceae, are susceptible to sudden oak death caused by the oomycete *Phytophthora ramorum.* Symptoms of the disease include dying crowns, bleeding cankers, and eventually death of infected trees. The cause of mortality is not well understood, but recent research indicates that stem water transport is reduced in infected trees as compared to healthy trees. Possible mechanisms for reduced hydraulic conductivity include the presence of fungal structures in xylem vessels or tyloses. In this study, the spatial and temporal development of tyloses was studied in relation to water conductivity of *P. ramorum*-infected sapwood. A log inoculation experiment and a field inoculation experiment using live tanoak trees were conducted. In the log experiment, tylosis development in xylem was compared at several sites in non-inoculated and *P. ramorum*- inoculated logs at two, four, and seven weeks. Inoculated logs showed no significant difference in tylosis frequency at two weeks, but had a significantly higher frequency of tyloses than the non-inoculated logs after four weeks. At seven weeks, the frequency of tyloses was lower than non-inoculated logs. In the field inoculation experiment, trees inoculated with *P. ramorum* and non-inoculated trees were harvested after five and 14 months. Sapwood tissue was excised from specific sections of each harvested tree and specific conductivity and tylosis

development were measured. Inoculated trees with xylem infections had a significantly higher frequency of tyloses as compared to wounded, non-inoculated trees. An increase in tyloses in tanoak sapwood was associated with a decrease in specific conductivity, suggesting that tyloses induced by the infection of *P. ramorum* may interfere with sapflow in the tree stem. At 14 months, tylosis development was affected in tissues further away from the inoculation site as compared to five months, paralleling the vertical spread of infection. This pattern suggests that large, mature cankers might contain numerous tyloses and could significantly impede the transport of water in diseased trees.

The Effects of *Phytophthora ramorum* Infection on Hydraulic Conductivity and Tylosis Formation in Tanoak Sapwood

by
Bradley R. Collins

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Bradley R. Collins, Author

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CHAPTER 1: INTRODUCTION

History and Biology of *Phytophthora*

In 1994, homeowners in Marin County, California noticed scattered occurrences of dead and dying tanoaks (*Lithocarpus densiflorus* [Hook. and Arn] Rehder) around the Mill Valley area, and the situation was documented in April 1995 (Svihra, 1999). These trees were described as having dying crowns and bleeding basal cankers. By 1999, the disease, called sudden oak death, was described as epidemic on tanoaks and coast live oaks (*Quercus agrifolia* Née) within a 300 km long area along the central coast of California (McPherson *et al*, 2000, Rizzo *et al,* 2002).

Through completion of Koch's postulates, the pathogen causing the tanoak and coast live oak mortality was shown to be a *Phytophthora* (Rizzo *et al*, 2002). Culture morphology and internal transcribed spacer (ITS) sequences of ribosomal RNA supported the species identification as *Phytophthora ramorum* Werres, de Cock, & In't Veld, which had previously been recovered only from ornamental plants in Germany and the Netherlands (Werres *et al*, 2001). The origin of *P. ramorum* is unknown but several hypotheses have been proposed. It is possible, for example, that this organism has been in California for many years, but a change in environmental conditions led to an increase in virulence (Rizzo *et al*, 2002). Brasier *et al* (2002) proposed that this pathogen is the result of a change in host specificity by a local *Phytophthora* species through a hybridization event, but this hypotheis is not well supported. Based on ITS sequence data, experts infer that *P. ramorum* is closely related to another Oregon forest pathogen, *P. lateralis* (Cooke, *et al*, 2000). The most widely accepted hypothesis, however, is that *P. ramorum* is an exotic species that was introduced into California by the international nursery trade and escaped into native forest ecosystems (Rizzo *et al*, 2005).

Phytophthora is a genus of fungus-like organisms known as oomycetes, more commonly known as water molds, and is placed in the kingdom Stramenopila (Alexopoulos *et al*, 1996). *Phytophthora* mycelia and mycelia of other members of Pythiaceae are characterized by diploid, coenocytic hyphae and production of flagellated zoospores within zoosporangia (Erwin & Ribeiro, 1996). Sexual reproduction results in oospores which function as survival spores. Asexual survival spores called chlamydospores are also produced and are morphologically similar to oopsores.

Species of *Phytophthora* are distinguished from species of *Pythium*, a closely related genus, by comparing morphologies of hyphae and sporangia, as well as comparing the nature of zoospore differentiation within the sporangia (Erwin & Ribeiro, 1996).

There are over 60 described species of *Phytophthora* worldwide. Most of these are plant pathogens and some have caused widespread devastation and severe economic loss. The infamous Irish potato famine that occurred in the1840s was caused by *Phytophthora infestans*, a pathogen that still causes economic losses in potato crops (Agrios, 2004).

Many species of *Phytophthora* including *P. cinnamomi* and *P. cambivora* (Peace, 1962), and *P. lateralis* (Hansen, *et al*, 2000), have long been known to cause diffuse cankers and root rots in trees. Diffuse cankers are those in which the developing lesion grows faster than the host can build wound periderm or callus tissue to stop the infection. These cankers are usually lethal (Tainter & Baker, 1996). More recently, *P. kernoviae* has been described as a canker-causing *Phytophthora* on tree species in the UK (Brasier, *et al*, 2005). Symptoms of the diseases caused by these *Phytophthoras* are similar, and usually involve diffuse cankers containing discolored tissue, and dying crowns. Interruption of water transport has been associated with some root-infecting *Phytophthora* species. When *P. cinnamoni* colonizes root tissue of *Eucalyptus marginata* Donn ex Smith in the jarrah forests of Western Australia, the pathogen can be recovered from root xylem tissue, and Davidson *et al* (1993) showed

evidence of xylem dysfunction and suggested that an interruption of water movement may be a part of the disease cycle.

Currently, over 100 plant species are known to be hosts for *P. ramorum* (USDA APHIS, 2006), many of which are important plants in the nursery industry. In addition to causing the sudden oak death symptoms on tanoaks and some other members of Fagaceae, *P. ramorum* causes foliar blight and tip dieback on many of the forest and nursery plants included on the host list. The pathogen has spread throughout 14 counties along the coast of California and in Curry County in southwest Oregon. In these regions it has caused extensive tree mortality and has the potential to cause some devastating ecological effects (Rizzo *et al*, 2005). In addition, eradication efforts in forests and nursery systems have had some profound economic impacts. Currently, a restriction on the interstate movement of all nursery stock from California, Oregon, and Washington is in effect (USDA APHIS, 2006).

Central to the success of disease management strategies and control of this pathogen is a solid understanding of the biology of the disease. Although research continues and much is learned each year, some fundamental questions remain. One important question still unanswered is how *P. ramorum* causes mortality in host plants.

Infection in Tanoak

Tanoak is highly susceptible to *P. ramorum* and is the host that experiences the highest mortality in mixed evergreen and redwood-tanoak forests of the West Coast forests (Rizzo *et al*, 2005). Symptoms include bole cankers that exude or "bleed" a deep red liquid and rapid death of the crown. Necrotic lesions, characterized by dark staining in the phloem tissue, can be observed by removing the outer bark around the infected area. Disease symptoms may be a result of the canker girdling the phloem and killing the tree (Davidson *et al*, 2003), but the exact mechanism of how the pathogen causes mortality is poorly understood.

Recently, Parke and Lewis (2007) demonstrated that *P. ramorum* can colonize the primary and secondary xylem tussue of rhododendron when leaves are inoculated

with the pathogen. Brown & Brasier (2007) demonstrated that *P. ramorum*, *P. kernoviae*, and other *Phytophthora* species could be recovered from the sapwood of some tree species in the UK. Parke *et al* (2007) demonstrated that *P. ramorum* could not only colonize the sapwood of tanoak trees, but also could spread through xylem vessels, produce chlamydospores, and reduce sap flow. It is not known, however, by what mechanism sap flow is reduced when infected with *P. ramorum*, but Parke *et al* (2007) reported that vessels occluded by hyphae and tyloses were readily observed in infected tissue.

Tyloses and Xylem Architecture

It is well known that the physiological condition of trees depends on the water status of their conductive tissue and that hydraulic conductivity is a very important factor in tree health (Kozlowski & Pallardy, 1997). Water stressed trees tend to become infected by pathogens more quickly than non-water stressed trees (Manion & Griffin, 1992). In fact, mortality of trees infected with wilt pathogens, such as those causing oak wilt or Dutch elm disease, can be predicted by the extent of xylem dysfunction (Kuroda, 2001).

Trees have long been known to actively occlude their conductive tissue in response to wounding or infection. There is more than one type of structure that creates this occlusion, depending on tree species, but all are induced by the tree's defense system (Boddy, 1992). The two most common and best studied are gums (or gels) and tyloses (Tyree & Zimmerman, 2002). Both structures are produced by either ray parenchyma or axial parenchyma cells that are adjacent to the xylem vessel. Gums are amorphous, pectin-based compounds secreted by the parenchyma cells. They accumulate in the pits or junctions between vessels and may prevent fungal hyphae or spores from progressing through the xylem (Beckman, 1987).

Tyloses are actually outgrowths of parenchyma cells themselves that have increased in volume, perhaps in response to growth hormones like IAA (Beckman, 1987), and grown into the lumen of the vessel through the vessel wall pits. As tyloses increase in size and number, the lumens of the vessels become increasingly occluded.

The exact mechanism of tylosis formation is still not completely understood (Rioux, *et al*, 1998) but it is generally accepted that their growth is triggered by the introduction of air into a vessel, known as an embolism. Embolisms are caused by an air bubble entering into the xylem sap either by physical damage to the vessel walls or the introduction of volatiles such as alcohols, turpenes, or phenolics (Sperry & Tyree, 1988 & Beckman, 1987). There is also some debate regarding whether the defense response is an active mechanism for resistance (Shigo, 1984), a non-specific response to cavitation to maintain sapwood function (Rayner, *et al*, 1986), or a combination of the two (Shain, 1979). Although tyloses are very common and occur naturally in angiosperm vessels, they are readily observed in conifer wood tracheids as well (Hessburg & Hansen, 1987).

Tanoak, like several other species in Fagaceae, has radial-porous wood (Hirose *et al*, 2005), where the vessels appear in cross-section to be arranged in radial lines. Unlike ring-porous wood, the vessel diameters remain relatively uniform regardless of their distance from the cambium. The distribution of vessel lumen diameters in sapwood and the degree to which the vessels are aggregated have considerable affect on the water conductivity of the stem (Tyree & Zimmerman, 2002) and must be taken into account when conducting comparative studies on sap flow.

In ring-porous species such as *Quercus alba* L., tyloses often fill the vessel lumens within a few years so that sap flow is limited to the outer few years' growth. In radial-porous species like tanoak, however, vessels remain conductive for as much as 20 years (Hirose *et al*, 2005). Consequently, the relative importance of phloem girdling vs. reduced sap flow as mechanisms of pathogenesis is unknown.

Objectives

This research is an attempt to elucidate the mechanism by which *P. ramorum* causes disease and mortality in tanoak trees by specifically addressing the following objectives:

1. Determine the spatial and temporal development of tylosis formation in tanoak inoculated with *P. ramorum;*

2. Determine the relationship between tylosis frequency and xylem specific conductivity.

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CHAPTER 2: MATERIALS AND METHODS

Log Inoculation Study

Disease free tanoak trees were selected from a tanoak stand near Upper Bean Creek, northeast of Brookings, OR and on October 3, 2005, 30 logs, each measuring approximately 45 cm long and 20 cm in diameter, were cut from the trees for this study. Log ends were sealed with wax and were transported to Oregon State University. On October 5, each log was prepared for inoculation by removing a plug of bark to the depth of the cambium with a 6mm cork borer at the midpoint along its length. Fifteen logs were inoculated at cambium depth with an agar plug containing *P. ramorum* while 15 were inoculated with a sterile agar plug as wounded controls. The bark plug was reinserted into the inoculation hole and the wound was covered with gauze and aluminum foil and sealed with duct tape. All logs were enclosed in 3 mil plastic sleeves and incubated in a growth chamber at 19-21°C. After two weeks of incubation, five inoculated and five wounded control logs were cut into successive 2cm-thick disks with an electric miter saw. Five inoculated logs and five wounded control logs were processed at two weeks, four weeks, and again at seven weeks after inoculation as follows.

From each inoculation site, a block of wood tissue measuring 1cm per side and located directly beneath the cambium was removed from the appropriate disks with a wood chisel. Another block sample was taken from 4cm away from the inoculation site. Blocks were taken from the same locations on non-inoculated, wounded controls. Furthermore, an identically sized block was removed at the same depth but on the opposite side of the log from the inoculation site (Figure 1). These samples served as non-inoculated, non-wounded controls.

From each of these blocks a smaller block measuring 2-3mm per side was excised with a straight-edge razor blade and quickly put into FAA (50% ethanol, 5% formalin 10% acetic acid) for fixing. All samples were subsequently placed under vacuum for 12 hours to replace the water with FAA.

Tissue was prepared for light microscopy by slicing a thin section (approximately 400μm) from each of the fixed blocks with a single-edged razor blade. Each slice was placed on a depression slide with a drop of FAA and a cover slip. Tissue was examined using a 10X objective. The frequency of tyloses in the vessels was recorded as was the maximum degree (%) to which each vessel was occluded, determined subjectively and rounded to the nearest 10% while focusing through the length of the vessel. Any observable structures of *P. ramorum* were also recorded. All vessels in the sample were viewed. Vessel frequency is relatively low in this species; the average number of vessels viewed per sample was 48.

Field Inoculation Study

Field Plot Establishment

Field plots were established in a Douglas-fir-tanoak stand at El Corte de Madera Preserve in the Midpeninsula Regional Open Space District in San Mateo Co., California (N37.41 W122.33, elev. 1720-1897 ft.). Boles of understory tanoak trees (10-15 cm DBH) were inoculated May 23, 2006 with mycelial plugs of *P. ramorum* placed at the cambium. The boles of control trees received agar plugs without the pathogen. The field plot consisted of 12 pairs, each containing one inoculated tree and one control tree. The trees in six of the pairs were harvested on September 20, 2006 by removing a log section approximately five feet long, with the inoculation point in the center. Log ends were sealed with wax and the logs were wrapped in 3 mil plastic sleeves for transport to Oregon State University where they were stored at 19-21°C until processing. The remaining trees were harvested on July 10, 2007, about 13 ½ months after the experiment was begun.

Sample processing

Log sections were brought back to the laboratory within two days of harvest and processed quickly thereafter. Each log was examined for symptoms of infection and each was photographed with a digital camera. Outer bark was removed and the length and width of inner bark lesions were measured. Logs were cut with an electric

miter saw into 12-cm-long subsections (Figure 1a). Log subsections A, B, and D were used for conductivity assays and microscopy while subsection C was used to confirm the presence of *P. ramorum* using culture and diagnostic PCR. The extent of sapwood discoloration, including the depth of discoloration at the inoculation point, was also noted in subsection C. Two sticks each were chiseled from subsections A, B, and D (Figure 1b) and used for the conductivity assays and microscopy.

After excision, each stick was labeled with the sample number and promptly submerged in a 0.01M solution of HCl (pH adjusted to 2) to slow microbial growth. Sticks were then placed under vacuum to remove air bubbles as required for the conductivity assay and stored at 3°C.

Conductivity Assay

Specific conductivity (k_s) describes the permeability of xylem tissue to water for a given length and cross sectional surface area. Values for k_s are calculated according to Darcy's Law as

$$k_s = \frac{Ql}{A\Delta P}$$

where Q is the volume flow rate (kg $m^{-2}s$), l is the length of the stick (m), A is the cross-sectional area of the stick (m^2), and ΔP is the pressure gradient through the stick (MPa). The units for k_s are kg $m^{-1}s^{-1}MPa^{-1}$ (Reid *et al*, 2005).

Prior to measuring conductivity, the ends of each stick were trimmed under water with a razor blade and the dimensions were measured and recorded. Conductivity measurements were made similar to the methods described in Spicer and Gartner (1998) and Parke *et al* (2007). The ends of the sticks were fitted tightly with vinyl tubing to prevent water loss and the sticks and tubing were sealed with Parafilm. The conductivity apparatus consisted of a 2L Erlenmeyer flask containing a dilute HCl solution (pH 2) filtered to 22 μm fitted with a vinyl siphon tube. The flask was

elevated to create a pressure difference and the height of the top of the solution relative to the bench top on which the conductivity measurements were made was recorded for each measurement. Since gravity exerts a force of 0.01MPa per meter on a column of water, the height of the top of the solution in meters was multiplied by 0.01 MPa per meter to convert the height measurement to units of pressure.

The siphon tube was fitted with a control valve and similar vinyl tube continued from the valve to the sample. The end of this tube was inserted into the vinyl tubing attached to the stick and the system was purged of air bubbles. The narrow end of a 1ml pipette was inserted into the vinyl tubing on the opposite end of the stick (Figure 3).

The flow rate (Q) was measured by opening the control valve and recording the time required for the solution to reach regular volume increments within the pipette. By using lap mode on the stopwatch, up to ten measurements could be recorded as the solution filled the pipette and the average of these measurements was used to calculate k_s. After the conductivity measurements were made, each stick was returned to a dilute HCl (pH 2) storage solution.

Since the measured specific conductivity (k_s) is dependent on the conductive area of the vessels in the tissue sample, the k_s values were normalized by dividing the value by the conductive area (%) of each sample and the normalized k_s values were used in comparisons of conductivity.

Vessel Quantification

Since differences in vessel diameters and distributions within the xylem tissue could affect conductivity comparisons, it was necessary to examine each stick microscopically and quantify both variables. After conductivity measurements were completed, each stick was fixed in FAA. After fixing, a sliding microtome was used to cut thin sections (approx. 30 μm) at each end of the stick as well as 1/3 and 2/3 of the way along the length of the stick so that a total of four increments were represented. Unstained sections were mounted on glass slides with Polymount, (Polysciences, Warrington, PA; cat. no. 18606) and covered with a glass cover slip.

Three representative digital microscopic images were made of each section using a Zeiss compound microscope (60X magnification) fitted with a digital camera and imaging software (QCapture Pro 5.0.1, QImaging Corp., Surrey, BC). Individual vessel areas and the total percent conductive tissue of each section were measured using Assess image analysis software (American Phytopathological Society, St. Paul, MN). Total percent conductive tissue of inoculated and non-inoculated sections was compared with a single-factor ANOVA. Individual vessel diameters were calculated from the vessel area measurements, and the frequency distributions of inoculated and non-inoculated samples were compared by using pair-wise t-tests on vessel diameters within each size class.

According to the Hagen-Poiseuille Law, the contribution of a single vessel to the total hydraulic conductivity of a sample is proportional to the diameter of that vessel raised to the fourth power (Tyree & Zimmerman, 2002). Consequently, it is necessary to quantify a weighted hydraulic diameter of the conductive tissue since simply using observed diameters does not reflect the exponential effect of vessel diameters to conductivity. The hydraulic mean radius (HMR) of the tissue sample is $\Sigma r^5 / \Sigma r^4$ where r is the vessel radius (Kolb & Sperry, 1999). The HMR of inoculated and non-inoculated samples were compared using single-factor ANOVA.

The frequency of tyloses within the vessels was quantified by observing the sample with a compound microscope under 100X magnification, randomly selecting 100 vessels from each section, and recording the number containing tyloses.

Statistical Analysis

All statistical analyses were performed using S-plus 6.1 Professional (Insightful Corp., Seattle, WA). Tables and graphs were constructed using S-plus or Microsoft Excel (Microsoft, Corp., Redmond, WA). Analysis of variance models involving tylosis frequency data was performed using tylosis frequency data that was transformed using the arcsine-square root function since the values were percentages. Analysis of variance models involving specific conductivity measurements were performed on untransformed normalized k_s data. Regression models were performed

on un-transformed tylosis frequency and normalized k_s data. The total number of replicates in tylosis and conductivity experiments was 120. This number reflects data taken from 21 trees, 3 bole subsections per tree, and 2 sticks from each subsection, minus 6 sticks that were lost, damaged, or incorrectly numbered and consequently unidentifiable.

Chapter 2 Figures

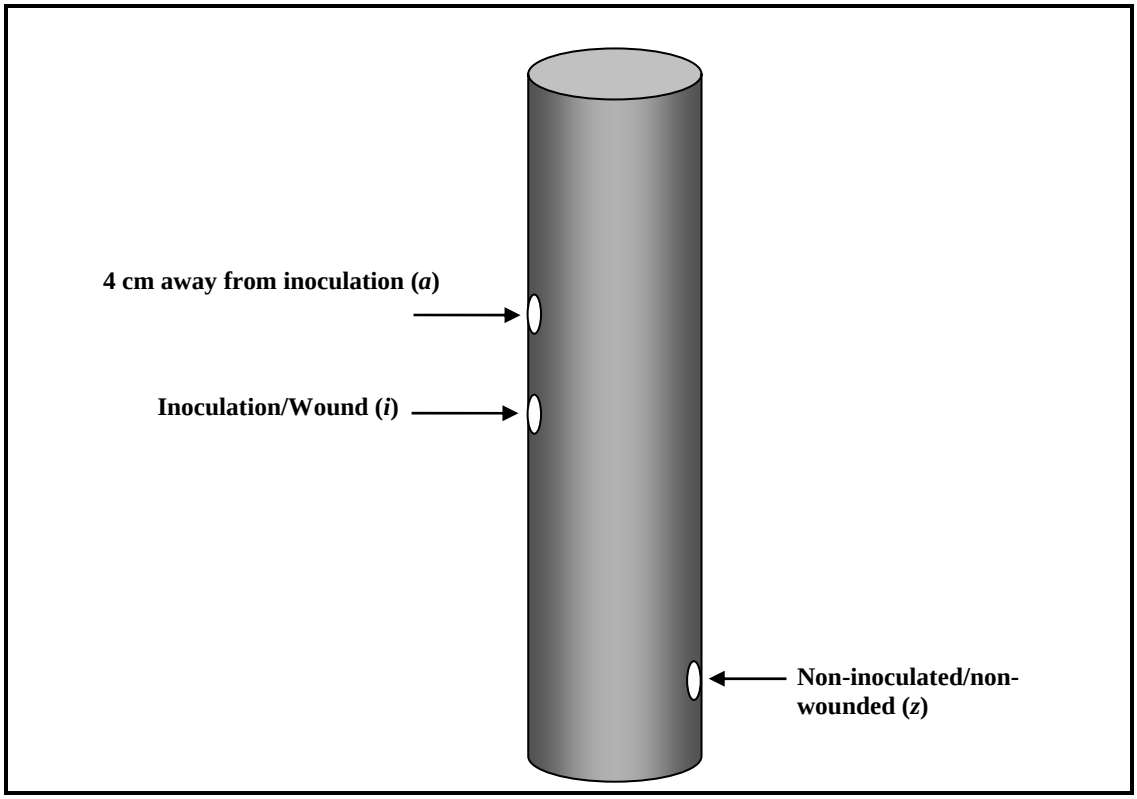

Figure 1. Locations on tanoak logs from which tissue samples were taken. Four cm away from the inoculation/wound was referred to as site *a*, the inoculation/wound was referred to as site *i*, and the non-inoculated, non-wounded site was referred to as site *z*.

Chapter 2 Figures (Continued)

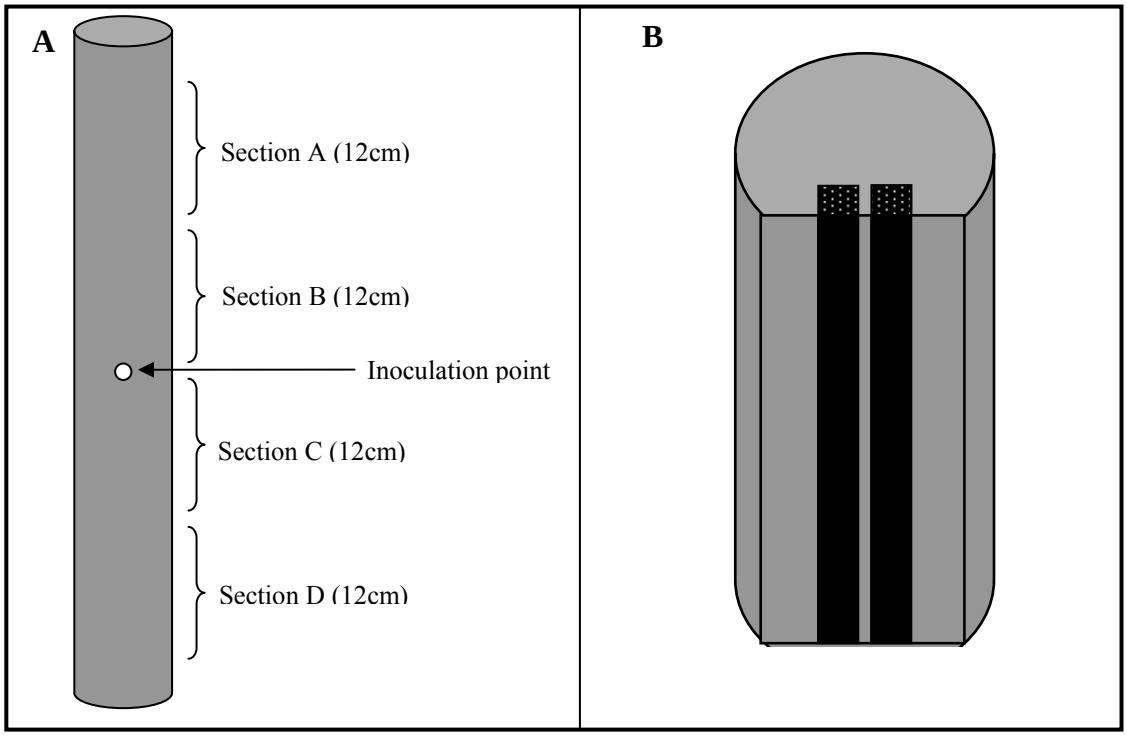

Figure 2. Diagram of subsections and sticks taken from tanoak logs. A) Locations of subsections in relation to the inoculation point. B) Shaded areas represent the origin of the 1cm x 1cm x 12cm sticks that were removed from log subsections.

Chapter 2 Figures (Continued)

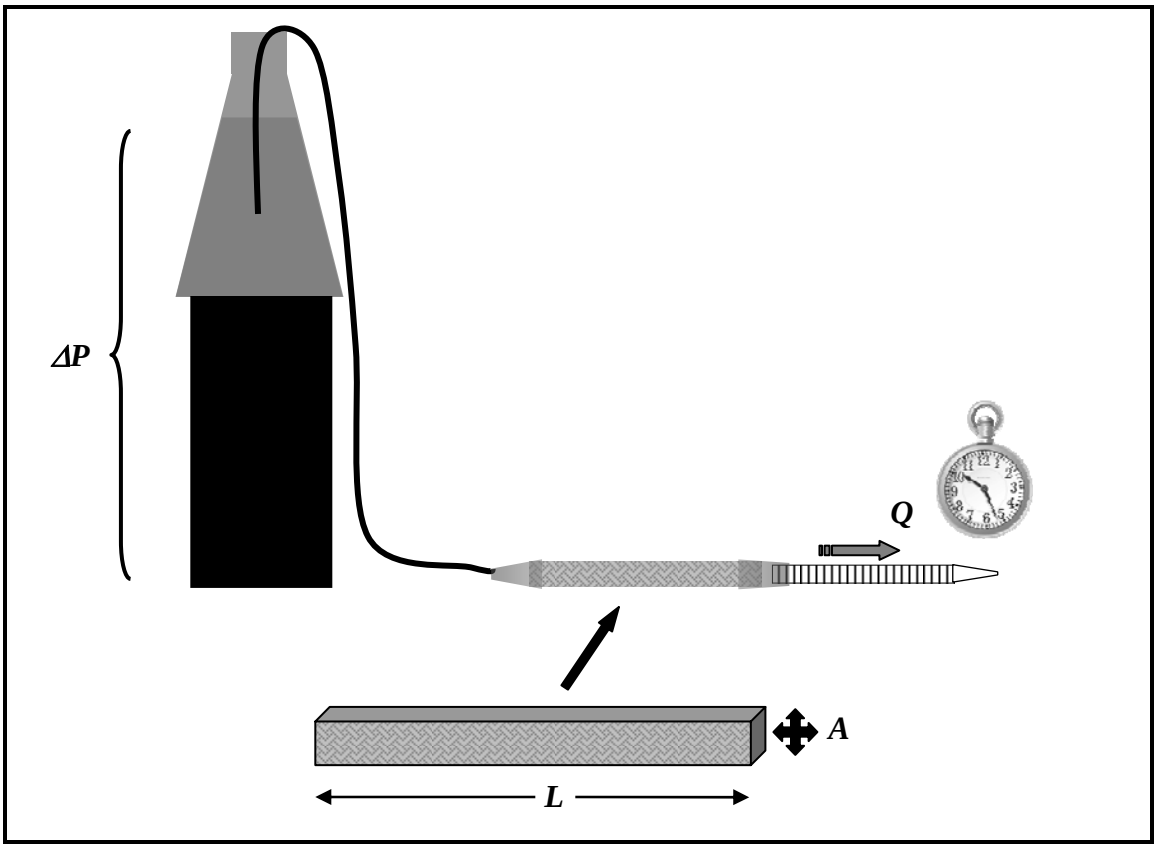

Figure 3. Schematic diagram of apparatus used to perform specific conductivity measurements and to determine components of Darcy's Law. ***Q*** is the volume flow rate (kg $m^{-2}s$), ***L*** is the length of the stick (m), ***A*** is the cross-sectional area of the stick (m^2), and ***ΔP*** is the pressure gradient through the stick (MPa).

Chapter 2 References

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CHAPTER 3: RESULTS

Log Inoculation Study

Tylosis Development

The frequency of tyloses in a cross-sectional view of xylem vessels (referred to subsequently as "tylosis frequency") was compared between inoculated and non-inoculated logs over the seven-week time period. At two weeks, there was no significant difference among any of the samples (Figure 4). At four weeks, the number of vessels with tyloses was significantly greater in inoculated tissue at 4cm away from the inoculation point (*a*) than the other sites. Throughout the seven week time period, the frequency of tyloses increased steadily in non-inoculated, non-wounded tissue (*z*) and in non-inoculated tissue at the wound point (*w*). At seven weeks, the tylosis frequency decreased significantly in inoculated tissue at both sample sites *(a* and *i*) and was significantly lower in inoculated tissue at the point of inoculation (*i*) than any other site.

Extent of Vessel Occlusion

The extent to which the cross-sectional view of vessels was occluded by tyloses followed much the same pattern as the frequency of tyloses. Occlusion increased over time in non-inoculated tissue (*w* and *z*) (Figure 5). The extent of occlusion was significantly greater at four weeks in inoculated tissue 4 cm away from the inoculation point than at the other sites. In both sites (*i* and *a*) from inoculated tissue the extent of occlusion decreased significantly after four weeks and vessel occlusion at the site of inoculation (*i*) was significantly less than any other site.

Presence of P. ramorum Structures in Xylem Vessels

P. ramorum hyphae could be observed in xylem vessels and the frequency of vessels containing visible hyphae was recorded (Figure 6). When cross-sections of sapwood samples were viewed microscopically, hyphae were observed in 10% of the

vessels from inoculated tissue, but not in tissue taken from non-inoculated logs. The frequency of hyphae decreased between two and four weeks in both tissue samples taken from inoculated logs (*i* and *a*), then remained fairly constant from four to seven weeks.

Chlamydospores were observed only in samples taken from inoculated logs, and most frequently from tissue taken at the point of inoculation at the initial sampling date (Table 1).

Field Inoculation Study

Symptom Development and Tree Condition

Of the 24 trees in the study, 8 trees were excluded (Table 2). Two non-inoculated control trees were removed from the harvest 1 data. Tree 10068 died before it could be harvested for the study. This tree showed evidence of beetle damage and had fruiting bodies of a species of *Hypoxylon* growing on the outer bark. Tree 10118 showed symptoms of being infected by a fungal pathogen, which diagnostic PCR showed was not *P. ramorum*. Two inoculated trees were removed from the harvest 1 data. Tree 10136 developed extensive lesions but *P. ramorum* was not isolated from the tissue. Tree 10206 was removed because no disease developed after inoculation.

One non-inoculated control tree was removed from the harvest 2 data. Tree 487 was dead at harvest time, apparently as a result of damage from a nearby fallen tree. Four inoculated trees, 8735, 10129, 10211, and 10212, were removed from the harvest 2 data because very little disease developed (bark canker ≤ 7.5 cm after 14 months).

Among trees that were kept for the study, the lengths of external bark lesions were variable (Table 2). Lesion lengths averaged 9.9(±0.8) cm on inoculated trees from harvest 1 and 0.9(±0.3) cm on non-inoculated controls. Lesion lengths averaged 75.0(±10.5) cm on inoculated trees from harvest 2 and 0.5(±0.2) cm on non-inoculated controls.

Depth of sapwood discoloration at the point of inoculation averaged 0.6 cm for harvest 1 and 1.3 cm for harvest 2. None of the non-inoculated, wounded controls exhibited sapwood discoloration.

Effect of Variables in the Experimental Design

To determine whether any variables were associated with either a change in conductivity or a change in tylosis frequency, the data on tylosis frequency were transformed using an arcsine-square root function and an analysis of variance was performed with position, harvest date, treatment, and pair in the statistical model. The statistical model did not include trees that were removed from the study.

There was a significant difference in tylosis frequency between treatments (ANOVA, $p<0.001$, $n=89$) (Table 3) and positions on the bole ($p=0.05$) but not between harvest dates ($p=0.12$). There was a significant interaction between position on the bole and treatment ($p=0.003$) suggesting that tylosis frequency varied. There were also significant interactions between position on the bole and harvest ($p=0.017$) and between treatment and harvest dates ($p=0.023$). There was a significant effect of tylosis frequency ($p=0.003$) (Table 4) and a significant difference between harvest dates ($p=0.008$) on specific conductivity, but no significant difference among positions on the bole or treatments ($p>0.05$). There were no significant interactions among any of the variables.

Frequency of Tyloses in Inoculated vs. Non-Inoculated Trees

Tyloses were much more abundant in inoculated trees than from non-inoculated control trees in both harvest 1 and harvest 2 (Figure 7 & 8). The frequency of tyloses in all bole sections of harvest 1 ranged from 0% to 82% (16% mean ± 4.2 SE) in inoculated trees, as compared to a range of 0-3% (0.35% mean ± 0.2 SE) in non-inoculated control trees. The frequency of tyloses in all bole sections of harvest 2 ranged from 0% to 58% (29% mean ± 6.5 SE) in inoculated trees, as compared to a range of 0-5% (0.4% mean ± 0.2 SE) in non-inoculated control trees.

Spatial and Temporal Aspects of Tylosis Development

Vessels within samples taken from each of the three subsections of inoculated trees in harvest 1 had significantly more tyloses than corresponding subsections from control trees (ANOVA, n=89) (Table 5) (Figures 9). There was also a significant difference in tylosis frequency between corresponding subsections of inoculated and control trees from harvest 2 (Table 6) (Figure 10). For the inoculated trees at harvest 1, the greatest frequency of tyloses occurred in the B subsection, the 12-cm section immediately above the point of inoculation. At harvest 2, the greatest frequency of tyloses was observed in the A subsection, 12-24 cm above the inoculation point.

A comparison was made between harvest 1 and harvest 2 for average tylosis frequency of bole subsections of inoculated trees. There was a significant increase in average tylosis frequency from harvest 1 to harvest 2 in subsection A only (ANOVA, $p=0.018$, n=24) (Figure 11). There was no significant difference in average tylosis frequency from harvest 1 to harvest 2 in subsections B or D ($p>0.05$). Subsection A of inoculated trees from harvest 1 had significantly lower tylosis frequency than subsections B or D of inoculated trees (Figure 12). Subsection A of inoculated trees from harvest 2 had significantly more tyloses that subsection D but not subsection B (Figure 13).

Lesion Development as a Predictor of Tylosis Development

The size of the external bark lesion on the tanoak trees was a good predictor of tylosis development in the xylem vessels (Figure 14). Regression analysis of the lesion length and tylosis frequency suggested a significant correlation (linear regression, ($p<0.001$, $R^2=0.66$, n=15). There was a significant difference in tylosis frequency between trees that had discoloration in the xylem and those that did not (t-test, $p=0.006$, n=21).

Effect of Inoculation on Ks

Four months after inoculation, there was a significant difference in normalized k_s between inoculated trees and non-inoculated control trees for all bole sections

combined (p=0.03) (Table 5). When Ks was compared among individual bole subsections, a significant difference was observed between inoculated trees and control trees in subsection D only (ANOVA< p=0.08, n=47) (Table 5) (Figure 15). By harvest 2, 14 months after inoculation, normalized k_s for all bole sections combined was significantly greater in non-inoculated trees than in non-inoculated trees at harvest 2 (ANOVA, p= 0.006, n= 42) (Table 6). There were significant differences in normalized k_s from inoculated vs. control trees for each subsection (subsection A p=0.08, subsection B p=0.04, subsection D p=0.02) (Table 6) (Figure 16). A comparison of normalized k_s from harvest 1 to harvest 2 was not performed due to differences in how the samples from the two harvest dates were handled and processed. Two different vacuum chambers were used when removing air from sapwood samples before the conductivity assay was performed, and the difference in the proportion of air that was successfully removed using the two chambers from the sapwood is unknown.

The Effect of Xylem Architecture on Xylem Conductivity

To eliminate the possibility that differences in conductivity were due to anatomical differences rather than host response to infection, two methods were used: a comparison of vessel size distributions and a comparison of hydraulic mean radii (HMR). Vessels size distributions were compared by creating a histogram of the vessel sizes from inoculated and non-inoculated tissue (Figure 17). When the frequency of vessel within size classes were compared, there were no significant differences (ANOVA, p>0.05, n=89).

The HMR of the vessels in the three bole sections of inoculated trees were compared to those of the non-inoculated controls. No significant difference existed between any of the treatments (ANOVA, p>0.05, n=89) (Figure 18).

Specific Conductivity and Tylosis Development within Tanoak Trees

Sapwood subsections that developed tyloses within xylem vessels generally showed a decrease in normalized k_s compared to sapwood from non-inoculated control tree sapwood (Figures 19 & 20).

Regression analysis indicates that an increase in tylosis frequency is associated with a decrease in specific conductivity in both harvest dates (Harvest 1, $p=0.04$, $R^2=0.08$, $n=47$) (Harvest 2, $p=0.02$, $R^2=0.12$, $n=42$).

Chapter 3 Tables

	Time Period		
Location of Tissue	Week 2	Week 4	Week 7
4 cm from inoculation (a)	0.6±0.7	0	0
at inoculation (i)	10.1±0.7	0	1.3±0.7
at wound (w)	0	0	0
non-wounded (z)	0	0	0

Table 1. The frequency of tanoak xylem vessels containing chlamydospores (mean ± SE).

Chapter 3 Tables (Continued)

Harvest 1											
Inoculated						Non-Inoculated					
Tree Number	External Lesion Length (cm)	Depth of Sapwood Discoloration (cm)	Length of Sapwood Discoloration (cm)	Isolation of *P. ramorum*	dbh (in.)	Tree Number	External Lesion Length (cm)	Depth of Sapwood Discoloration (cm)	Length of Sapwood Discoloration (cm)	Isolation of *P. ramorum*	dbh (in.)
10128	10.5	0.7	6.6	+	2.7	10086	0.0	0	0	-	4.0
10133	10.5	0.8	6.4	+	3.7	10087	1.0	0	0	-	3.3
10136	171.0	0.5	0.0	-	3.8	10102	1.2	0	0	-	4.0
10205	9.0	0.6	7.6	+	4.0	10295	1.2	0	0	-	2.5
10206	0.0	0.0	0.0	+	4.0	10068	*dead*	*dead*	*nd*	*nd*	*3.9*
10208	9.5	1.0	6.0	+	3.2	10118	*other infection*	*other infection*	*nd*	-	*2.5*
Harvest 2											
Inoculated						Non-Inoculated					
Tree Number	External Lesion Length (cm)	Depth of Sapwood Discoloration (cm)	Length of Sapwood Discoloration (cm)	Isolation of *P. ramorum*	dbh (in.)	Tree Number	External Lesion Length (cm)	Depth of Sapwood Discoloration (cm)	Length of Sapwood Discoloration (cm)	Isolation of *P. ramorum*	dbh (in.)
8735	4.8	0.6	9.6	+	4.6	490	0.0	0	0	-	3.0
10129	4.5	0.9	6.8	+	3.7	10016	0.0	0	0	-	2.6
10201	68.3	2.3	9.0	+	2.7	10104	1.0	0	0	-	4.0
10202	71.3	2.5	90.0*	+	4.8	10281	0.0	0	0	-	2.6
10211	7.5	0.6	7.0	+	3.3	10296	1.3	0	0	-	2.9
10212	5.6	1.0	0.0	+	2.7	487	*dead*	*dead*	*nd*	*nd*	*2.5*

Table 2. Lengths of phloem lesions and depths of sapwood discoloration from inoculated and non-inoculated tanoak logs measured after outer bark was removed. Asterisk (*) indicates that the lesion extended the full length of the log but was not continuous. Values designated *nd* were not determined.

Chapter 3 Tables (Continued)

	Df	Sum of Squares	Mean Square	F	Pr(F)
Position	2	0.2	0.1	3.1	0.050
Treatment	1	3.2	3.2	125.5	<0.001
Harvest	1	0.1	0.1	2.4	0.122
Position:Treatment	2	0.3	0.2	6.4	0.003
Position:Harvest	2	0.2	0.1	4.3	0.017
Treatment:Harvest	1	0.1	0.1	5.4	0.023
Position:Treatment:Harvest	2	0.3	0.2	6.2	0.003
Residuals	77	1.9	0.0		

Table 3. ANOVA table showing effects and interactions of experimental variables on tylosis frequency in tanoak sapwood. There was a significant difference in tylosis frequency between treatments and positions on the bole but not between harvest dates.

Chapter 3 Tables (Continued)

	Df	Sum of Squares	Mean Square	F	Pr(F)
Tylosis Freq	1	17.6	17.6	9.4	0.003
Position	2	2.2	1.1	0.6	0.568
Treatment	1	2.9	2.9	1.5	0.222
Harvest	1	14.2	14.2	7.5	0.008
Tylosis Freq:Position	2	1.4	0.7	0.4	0.693
Tylosis Freq:Treatment	1	4.9	4.9	2.6	0.110
Position:Treatment	2	4.0	2.0	1.1	0.350
Tylosis Freq:Harvest	1	0.2	0.2	0.1	0.748
Position:Harvest	2	5.3	2.6	1.4	0.254
Treatment:Harvest	1	0.1	0.1	0.0	0.841
Tylosis Freq:Position:Treatment	2	1.3	0.6	0.3	0.716
Tylosis Freq:Position:Harvest	2	9.0	4.5	2.4	0.100
Tylosis Freq:Treatment:Harvest	1	0.3	0.3	0.1	0.709
Position:Treatment:Harvest	2	0.0	0.0	0.0	0.996
Residuals	66	124.4	1.9		

Table 4. ANOVA table showing effects of and interactions between experimental variables on specific conductivity of tanoak sapwood. There was a significant effect of tylosis frequency and a significant difference between harvest dates, but no significant difference among positions on the bole, treatments, or pairs.

Chapter 3 Tables (Continued)

	Inoculated Trees	Non-Inoculated Trees	p-value
	External lesion length (cm)		
	9.9(26.9)	0.9(0.3)	<0.001
Bole Subsection	**Tylosis Frequency (%)**		
Subsection A	6.3(2.1)	0.38(0.38)	0.03
Subsection B	34.6(8.6)	0.5(0.27)	0.005
Subsection D	7.9(2.7)	0.29(0.29)	0.03
Combined	*16.3(4.2)*	*0.39(0.17)*	*0.003*
Bole Subsection	***Ks* (kg $m^{-1}s^{-1}MPa^{-1}$)**		
Subsection A	2.44(0.63)	2.65(0.43)	0.78
Subsection B	1.09(0.19)	2.58(0.90)	0.15
Subsection D	1.24(0.12)	2.44(0.57)	0.08
Combined	*1.59(0.02)*	*2.57(0.03)*	*0.03*
	Hydraulic Mean Radius (HMR)(μm)		
	47.2(1.41)	48.8(1.0)	0.37

Table 5. Summary data for inoculated an non-inoculated tanoak trees (mean(SE)) from harvest 1 and p-values from t-test.

Chapter 3 Tables (Continued)

	Inoculated Trees	Non-Inoculated Trees	p-value
	External lesion length (cm)		
	75.0(15.1)	0.75(0.3)	<0.001
Bole Subsection	**Tylosis frequency (%)**		
Subsection A	43.3(13.1)	0(0)	0.05
Subsection B	26.5(11.1)	0(0)	0.01
Subsection D	17.5(7.5)	1.1(0.6)	0.04
Combined	*29.1(0.65)*	*0.36(0.21)*	*0.001*
Bole Subsection	***Ks* (kg $m^{-1}s^{-1}MPa^{-1}$)**		
Subsection A	0.28(0.07)	1.42(0.45)	0.08
Subsection B	0.56(0.23)	2.12(0.53)	0.04
Subsection D	0.70(0.25)	1.71(0.37)	0.02
Combined	*0.51(0.11)*	*1.75(0.26)*	*0.006*
	Hydraulic Mean Radius (HMR)(µm)		
	50.6(1.7)	49.6(1.5)	0.64

Table 6. Summary data for inoculated an non-inoculated tanoak trees (mean(SE)) from harvest 2 and p-values from t-test.

Chapter 3 Figures

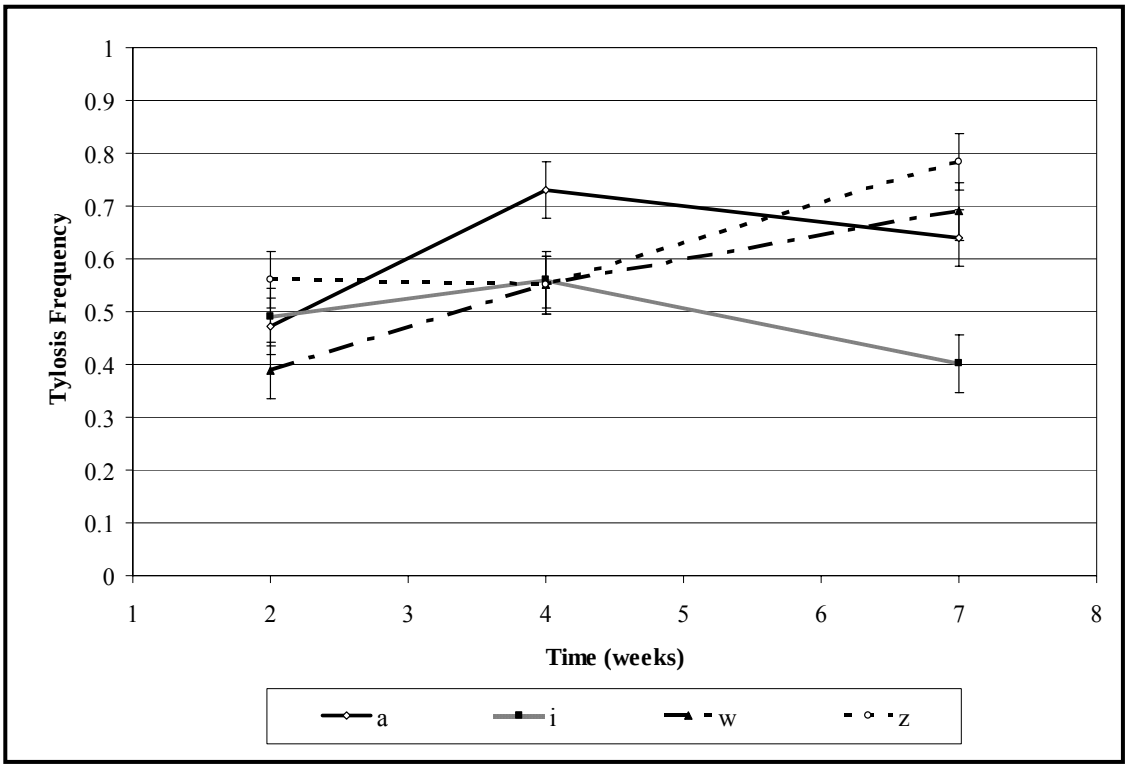

Figure 4. The frequency of tyloses (±SE) in a single cross-sectional view of tanoak xylem vessels over time from log inoculations. (i) represents tissue taken at the point of inoculation, (w) represents tissue taken at the point of wounding in non-inoculated control logs, (a) represents tissue taken 4 cm away from point of inoculation, and (z) represents non- wounded,

Chapter 3 Figures (Continued)

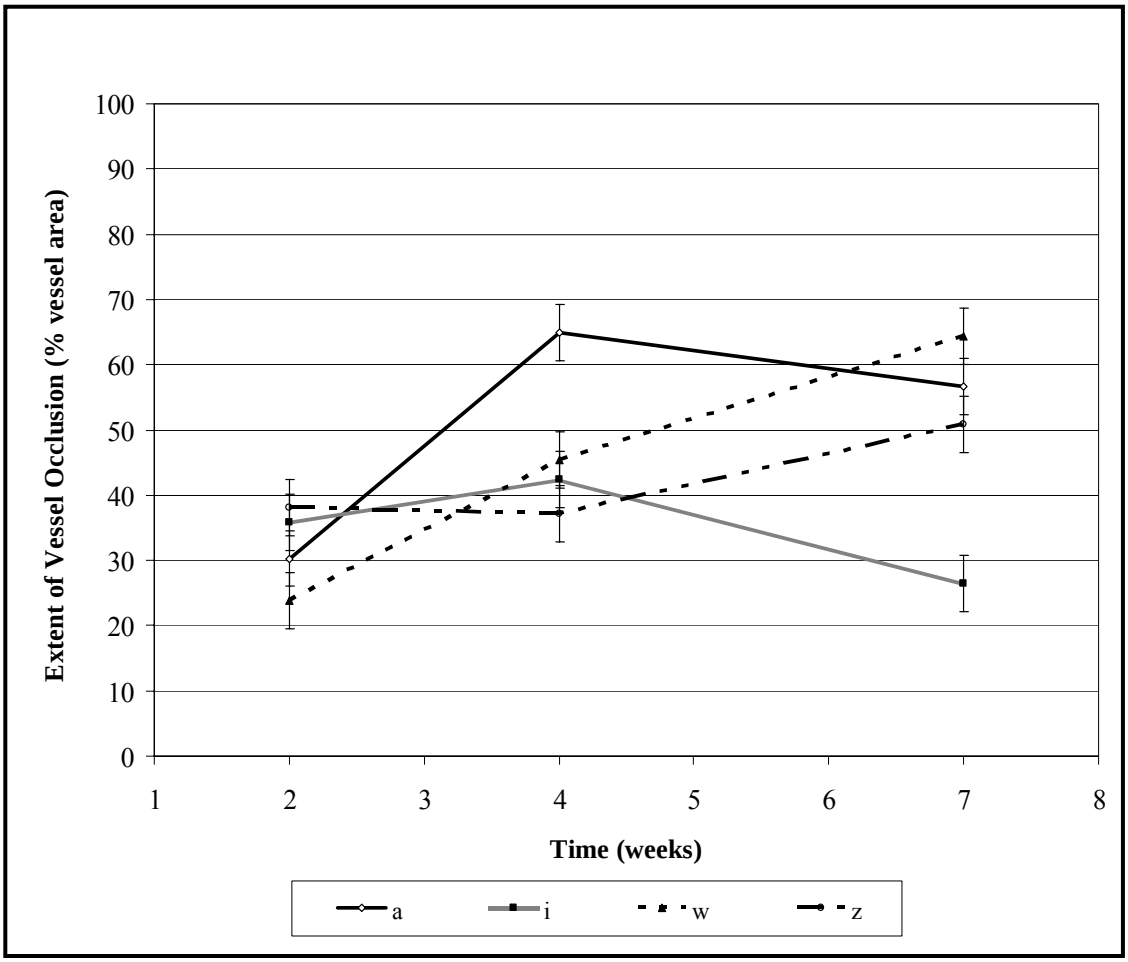

Figure 5. The extent to which tanoak xylem vessels were occluded expressed as a percent of vessel cross-sectional area (±SE). (i) represents tissue taken at the point of inoculation, (w) represents tissue taken at the point of wounding in non-inoculated control logs, (a) represents tissue taken 4 cm away from point of inoculation, and (z) represents non- wounded, non-inoculated tissue taken on the opposite side of the log from the point of inoculation.

Chapter 3 Figures (Continued)

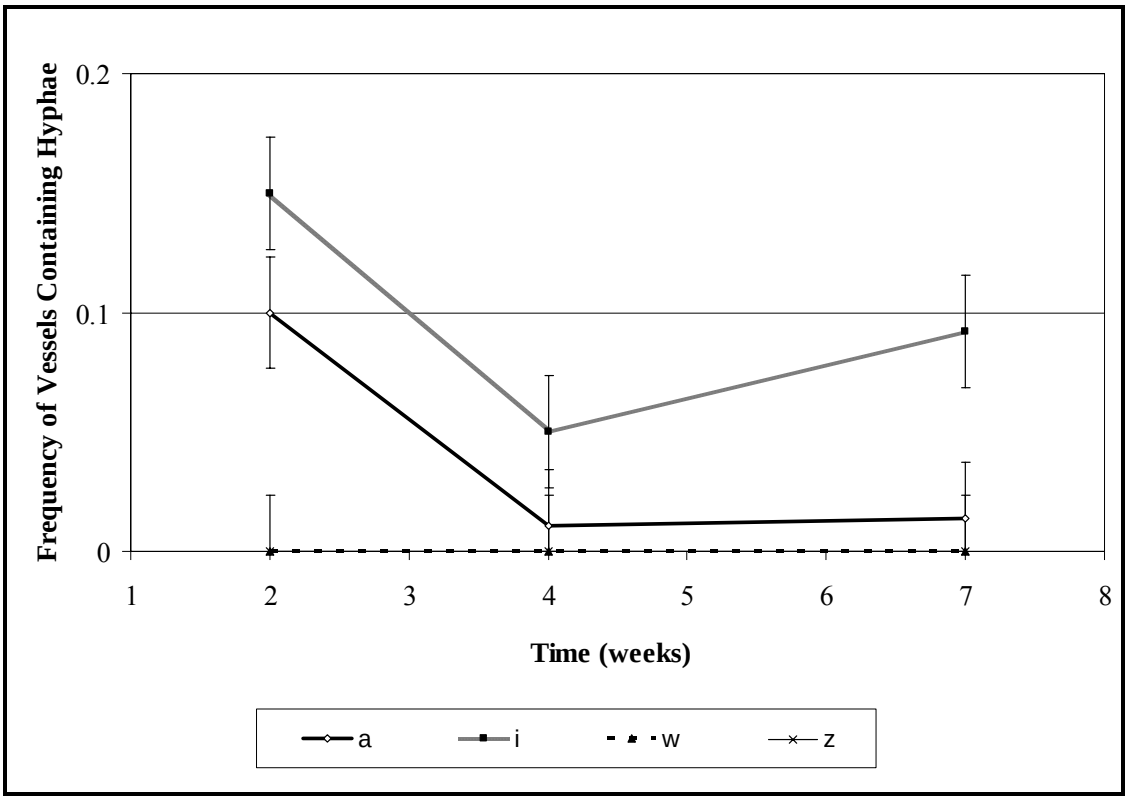

Figure 6. The frequency of tanoak vessels (±SE) in a single cross-sectional view containing *P. ramorum* hyphae. (i) represents tissue taken at the point of inoculation, (w) represents tissue taken at the point of wounding in non-inoculated control logs, (a) represents tissue taken 4 cm away from point of inoculation, and (z) represents non-wounded, non-inoculated tissue taken on the opposite side of the log from the point of inoculation.

Chapter 3 Figures (Continued)

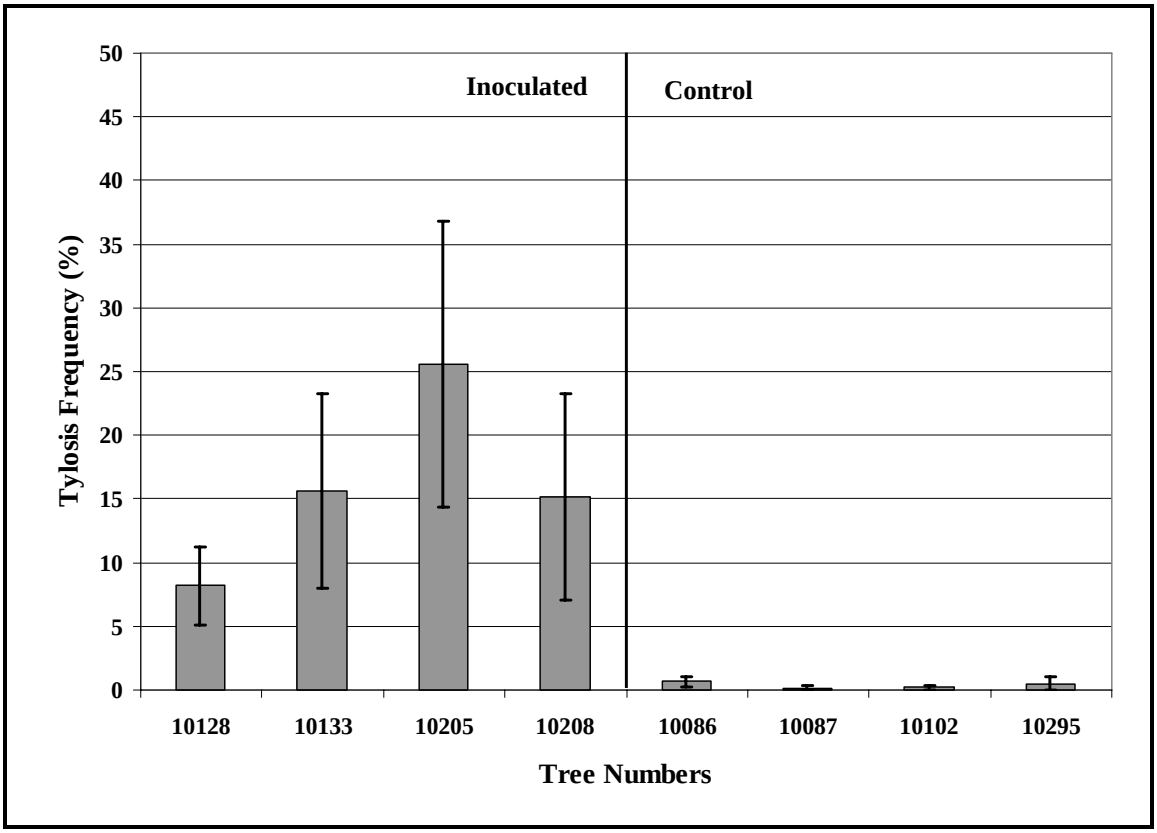

Figure 7. Tylosis frequency (%) in inoculated and non-inoculated tanoaks from harvest 1 (mean ± SE). Tylosis frequency is the number of vessels out of 100 viewed in cross-section where tyloses were visible.

Chapter 3 Figures (Continued)

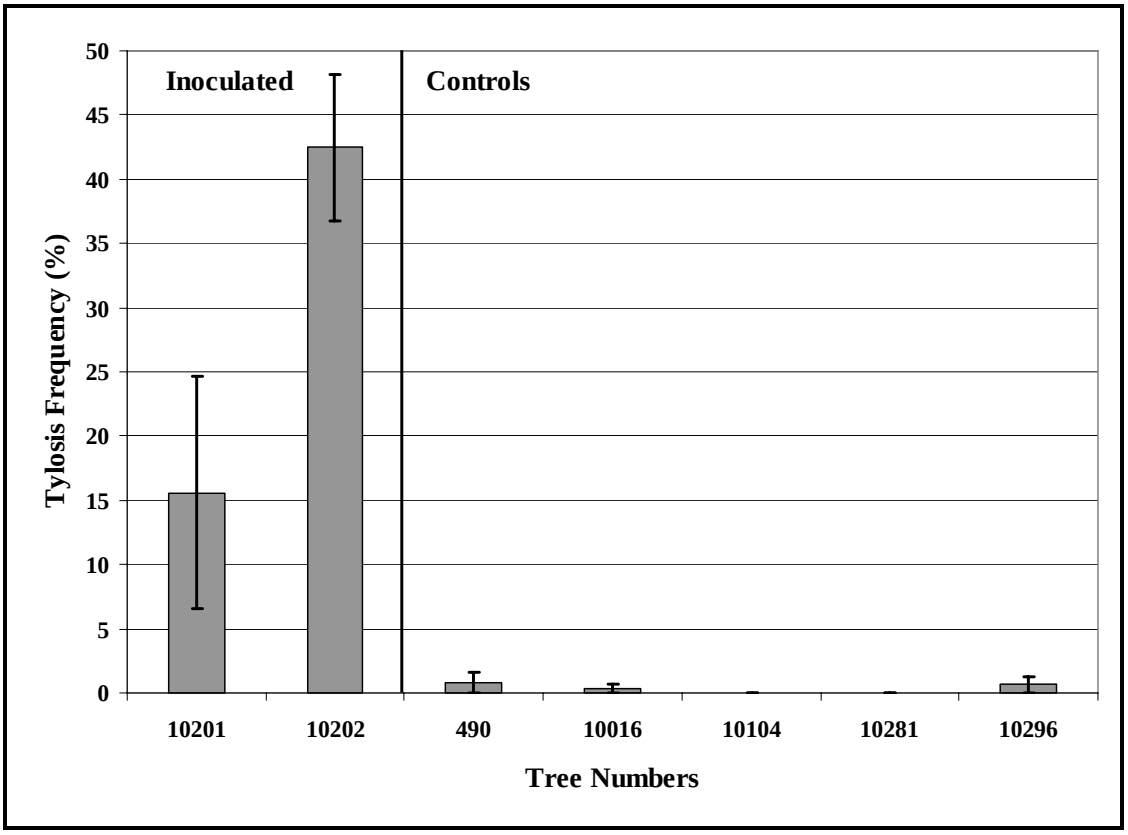

Figure 8. Tylosis frequency (%) in inoculated and non-inoculated tanoaks from harvest 2 (mean ± SE). Tylosis frequency is the number of vessels out of 100 viewed in cross-section where tyloses were visible.

Chapter 3 Figures (Continued)

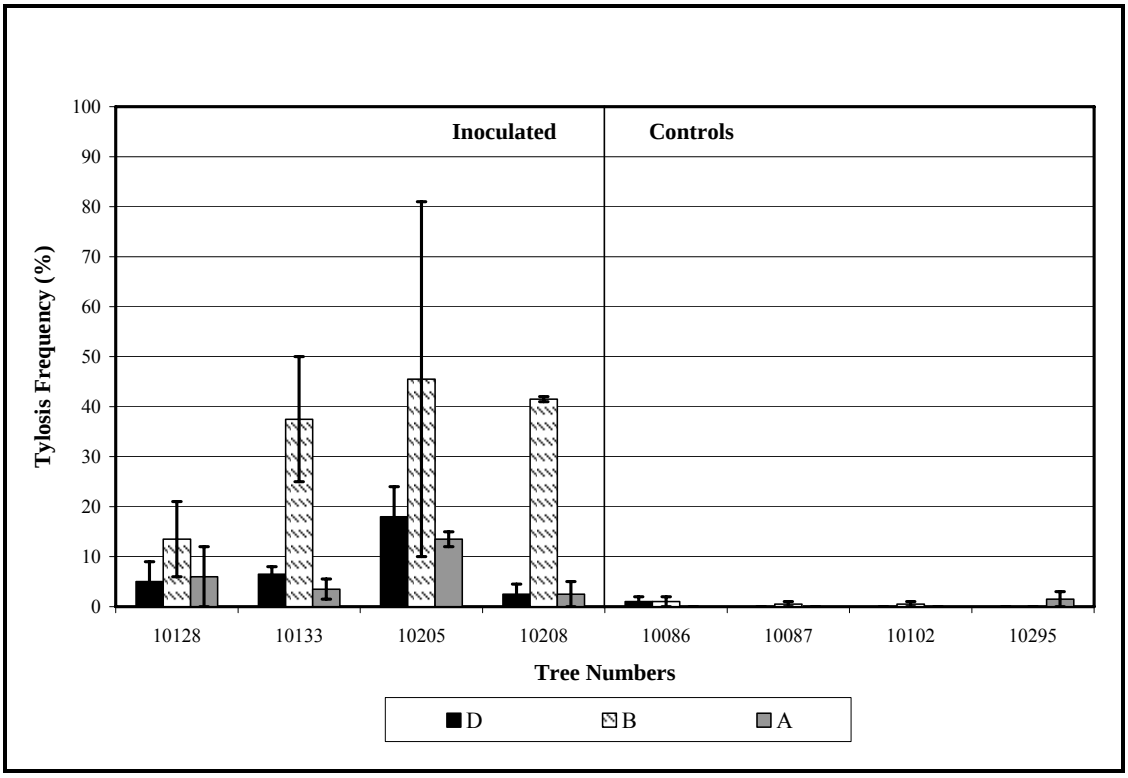

Figure 9. Comparison of tylosis frequency in individual bole subsections between inoculated and non-inoculated tanoak trees in harvest 1 (mean ± SE).

Chapter 3 Figures (Continued)

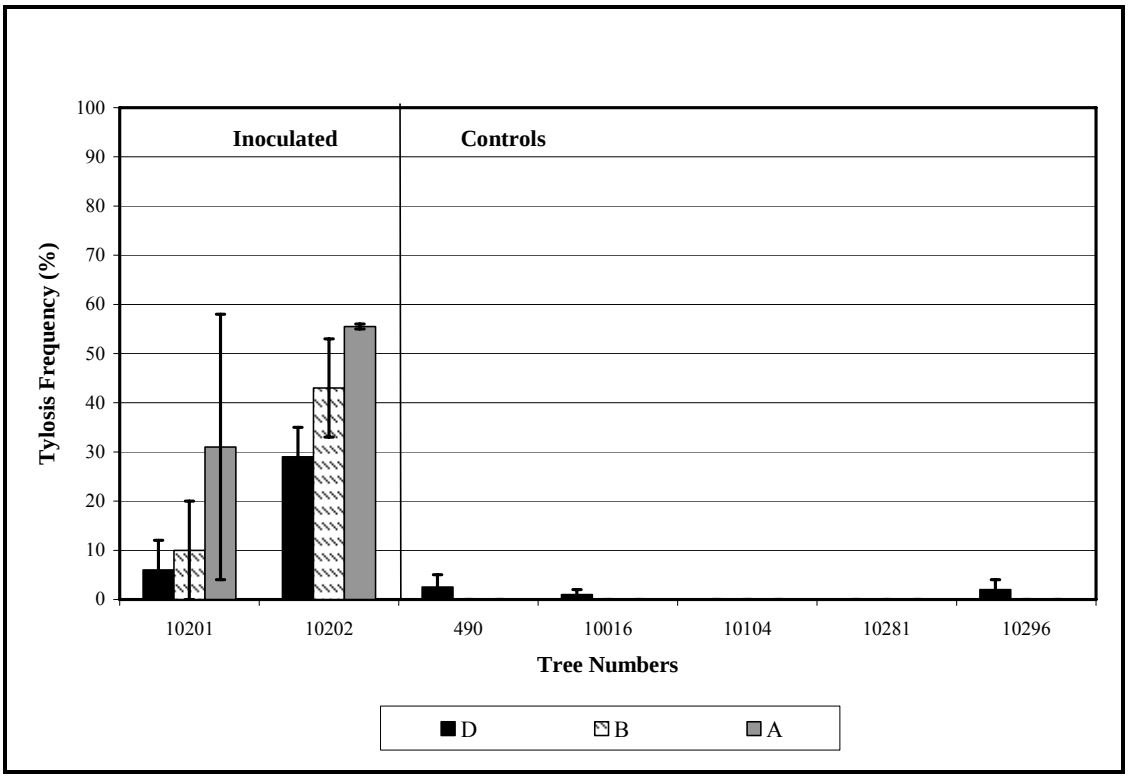

Figure 10. Comparison of tylosis frequency in individual bole subsections between inoculated and non-inoculated tanoak trees in harvest 2 (mean ± SE).

Chapter 3 Figures (Continued)

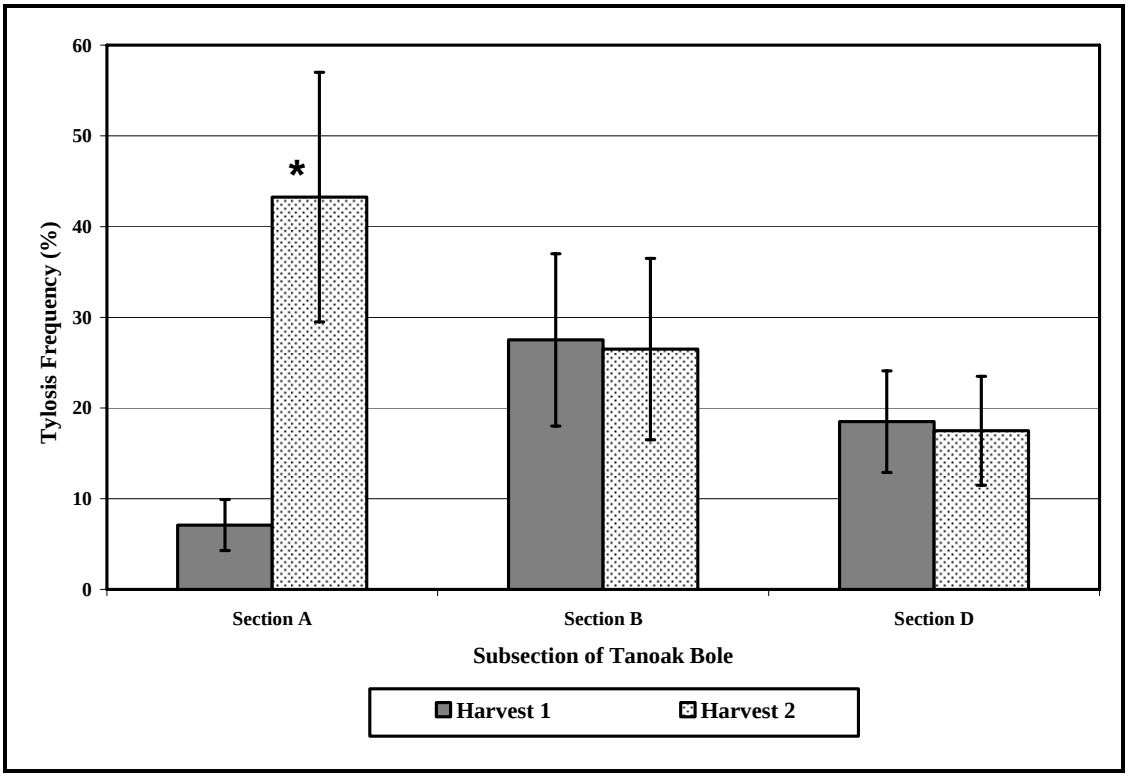

Figure 11. Tylosis frequency (%) in subsections of inoculated tanoak trees from harvest 1 and 2. There is a significant increase in tyloses in subsection A (*) between harvest 1 and 2 (ANOVA, $p=0.018$, $n=24$) but not in subsections B or D ($p>0.05$).

Chapter 3 Figures (Continued)

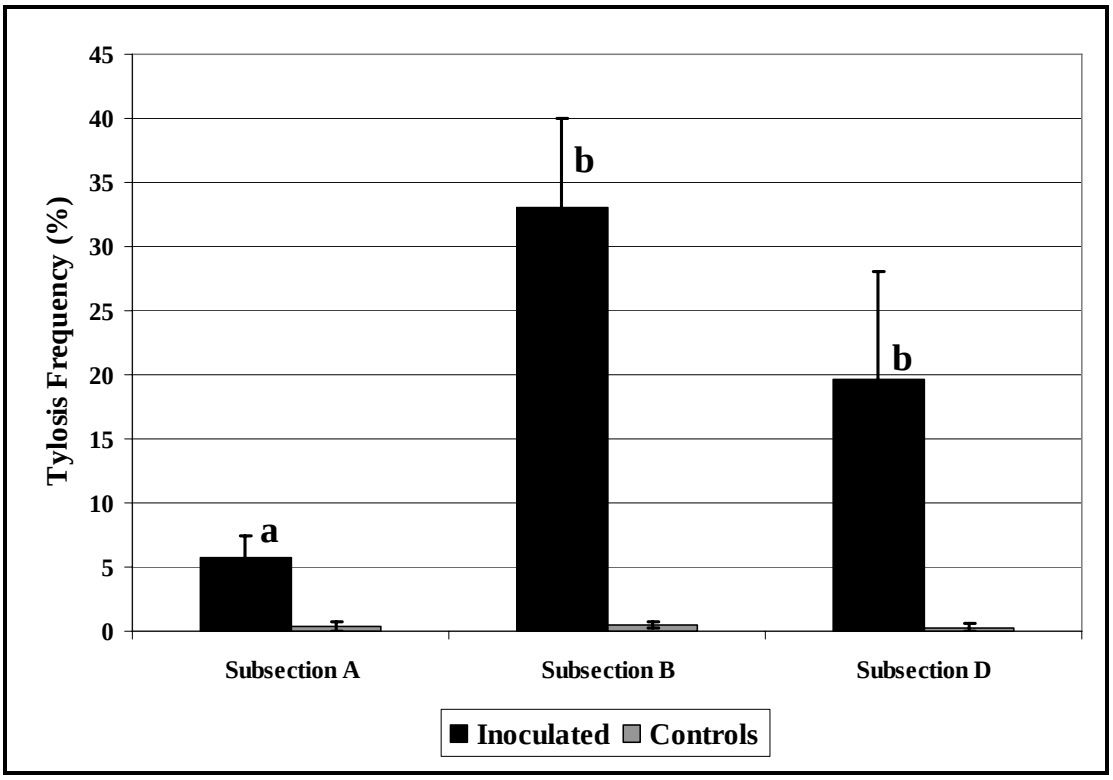

Figure 12. Tylosis frequency (mean ±SE) in subsections of inoculated and non-inoculated control tanoak trees from harvest 1. Vessels within subsections from inoculated trees have significantly more tyloses than vessels of the corresponding subsections from non-inoculated control trees (ANOVA, $p>0.05$, $n=47$). Lowercase letters indicate significantly different groups.

Chapter 3 Figures (Continued)

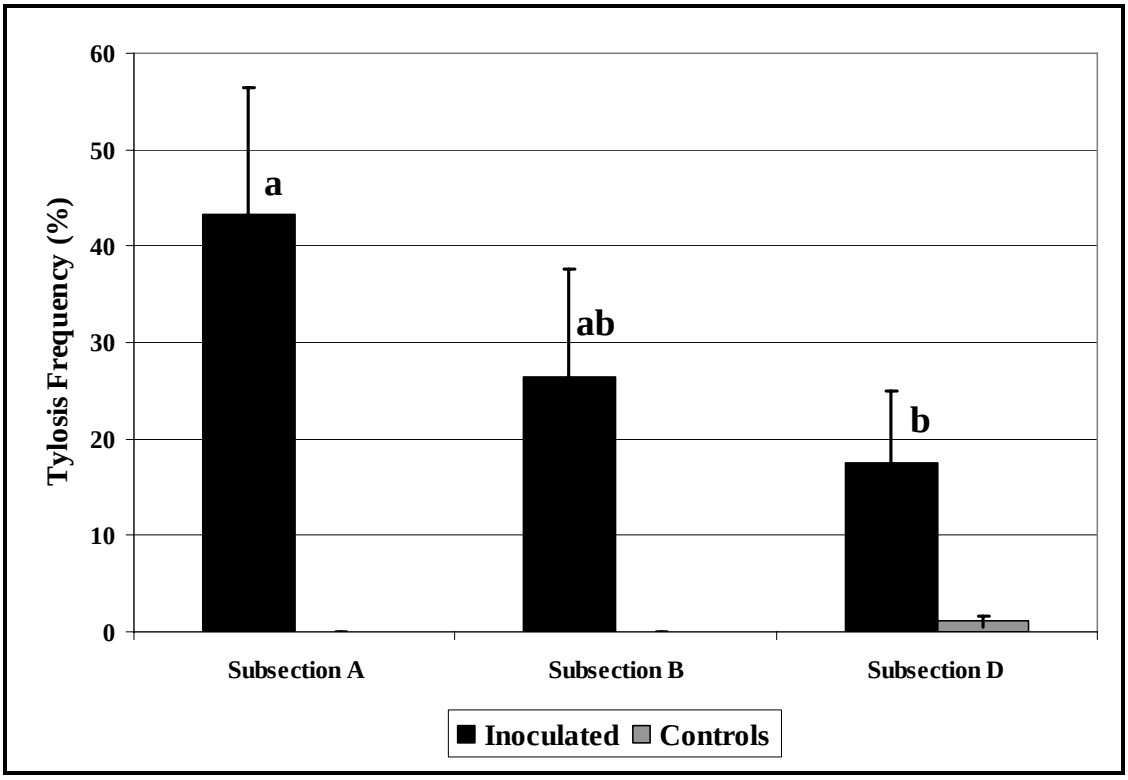

Figure 13. Tylosis frequency (mean ±SE) in subsections of inoculated and non-inoculated control tanoak trees from harvest 2. Vessels within subsections from inoculated trees have significantly more tyloses than vessels of the corresponding subsections from non-inoculated control trees. Lowercase letters indicate significantly different groups.

Chapter 3 Figures (Continued)

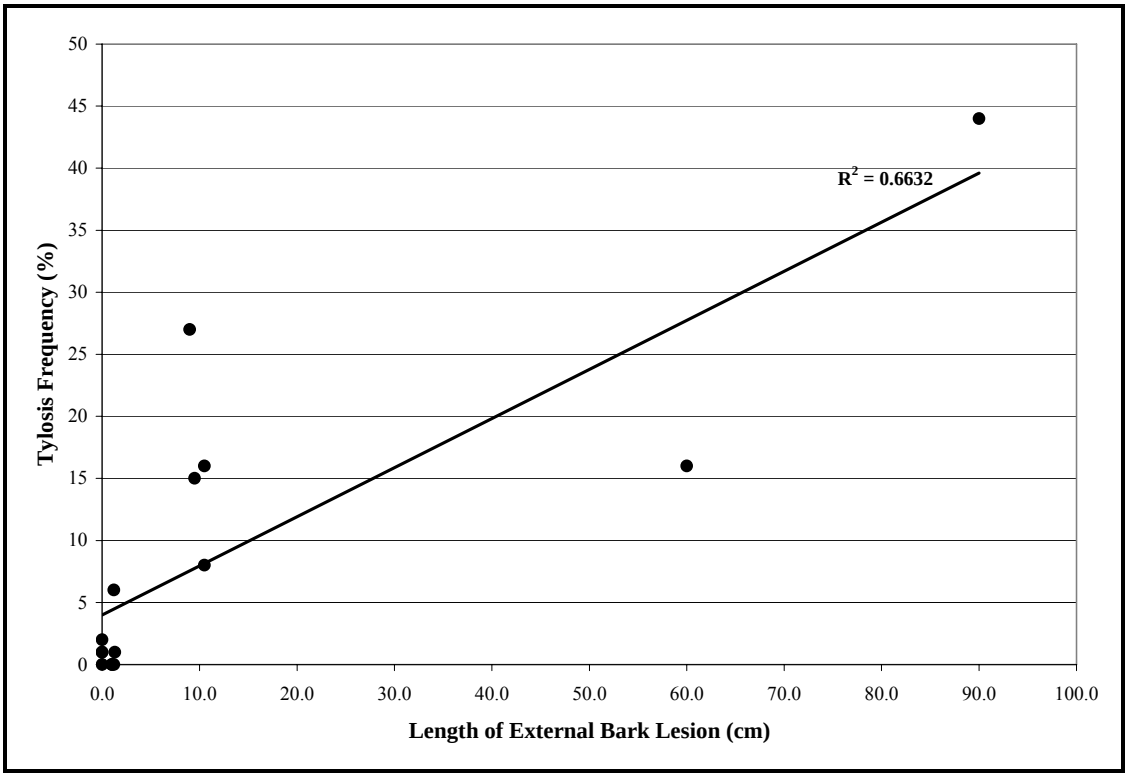

Figure 14. Scatterplot of the length of the visible external lesion on tanoak trees and the average frequency of tyloses within the xylem vessels. Linear regression suggests a strong correlation ($p<0.001$, $R^2=0.66$ $n=15$).

Chapter 3 Figures (Continued)

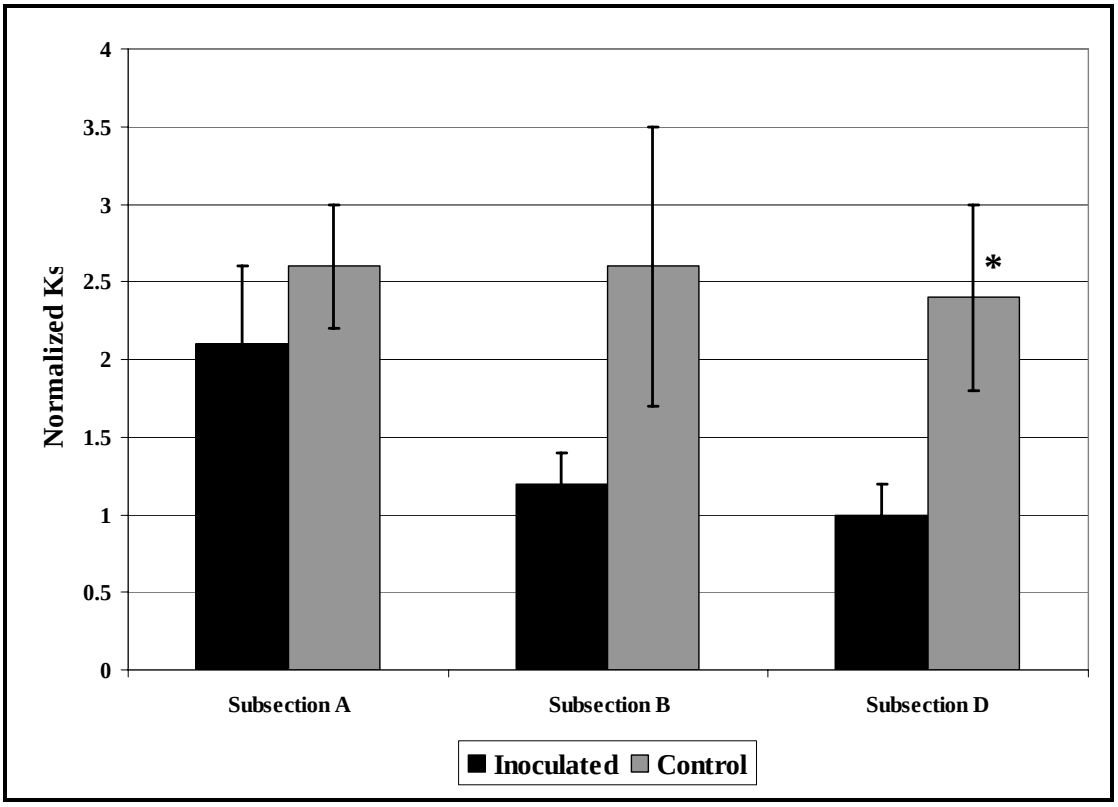

Figure 15. Comparison of normalized k_s from individual bole subsections between inoculated and non-inoculated tanoak trees in harvest 1 (mean ± SE). Black bars represent non-inoculated control trees and grey bars represent inoculated trees. There was a significant difference between corresponding subsections in subsection D only (*).

Chapter 3 Figures (Continued)

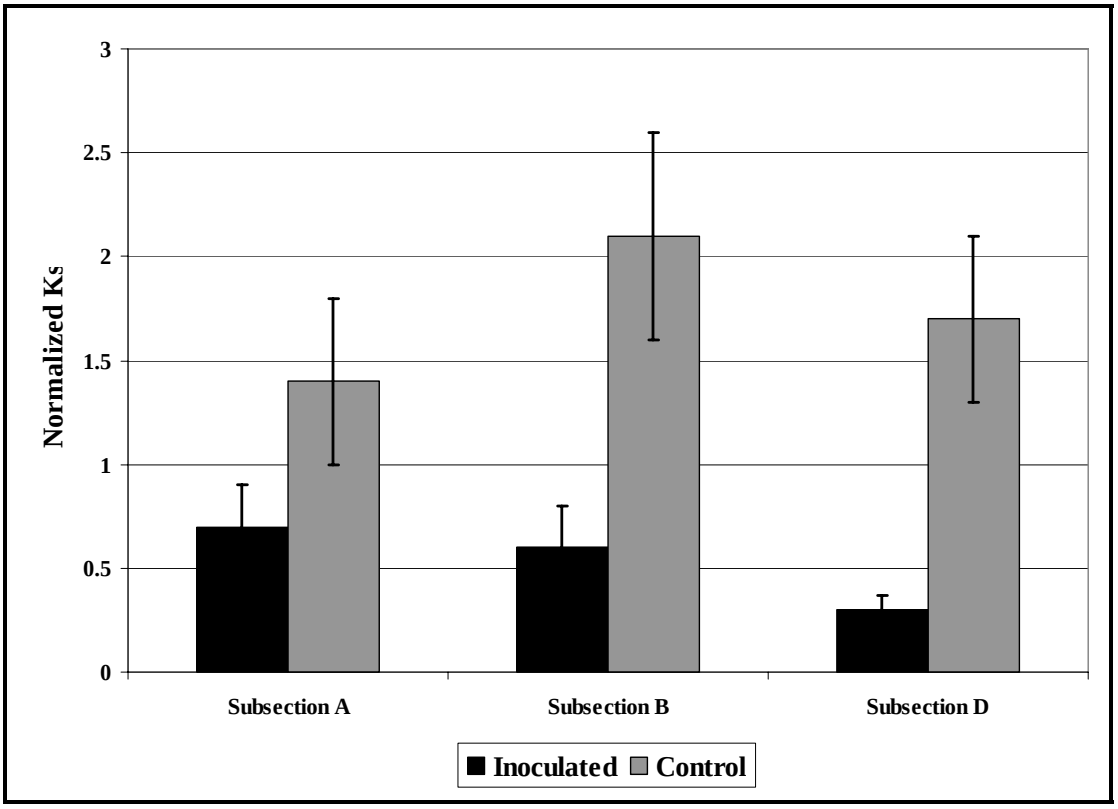

Figure 16. Comparison of normalized k_s from individual bole subsections between inoculated and non-inoculated tanoak trees in harvest 2 (mean ± SE). Black bars represent non-inoculated control trees and grey bars represent inoculated trees. There was a significant decrease in normalized k_s between inoculated and control trees (ANOVA, p=0.005, n=42) but no significant difference among subsections of inoculated trees.

Chapter 3 Figures (Continued)

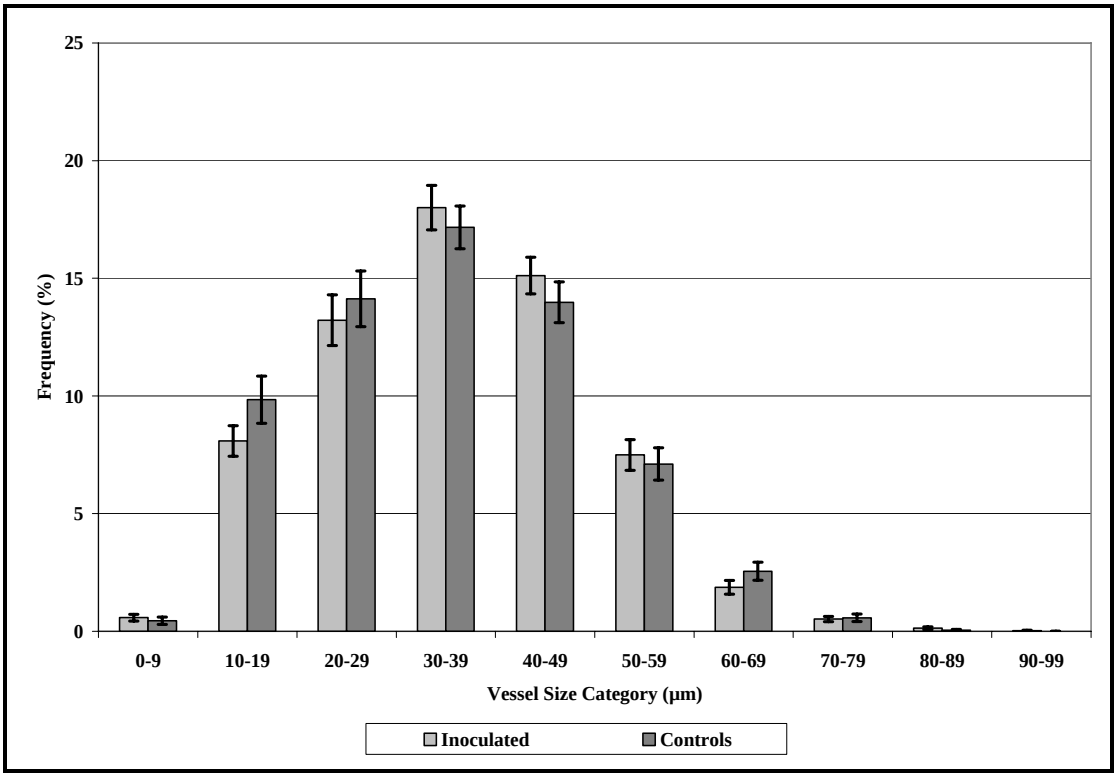

Figure 17. Comparison of tanoak vessel size distributions between tissues taken from inoculated trees versus non-inoculated controls (mean ± SE). No significant difference exists between inoculated tanoaks and controls (ANOVA, $p>0.05$, $n=89$).

Chapter 3 Figures (Continued)

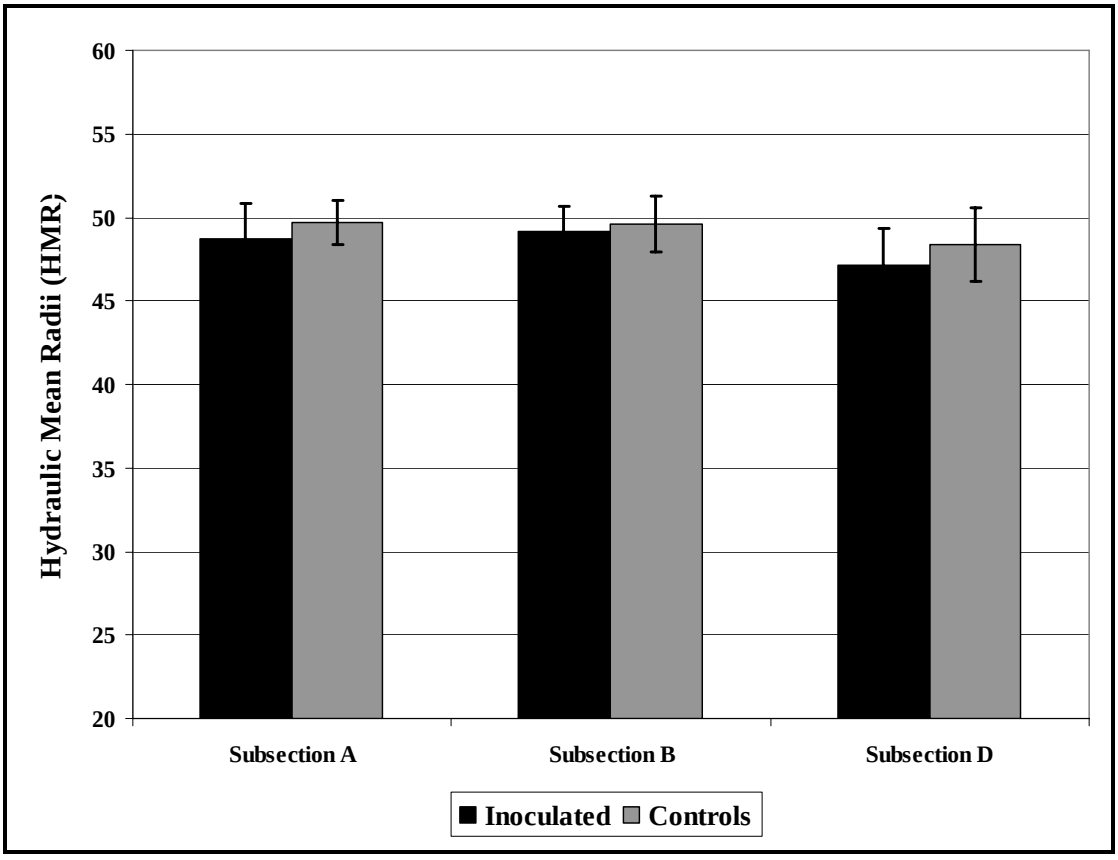

Figure 18. Average(±SE) Hydraulic Mean Radii of bole subsections of inoculated and non-inoculated control trees from both harvests. No significant difference exists between inoculated and controls (ANOVA, p=0.94, n=89).

Chapter 3 Figures (Continued)

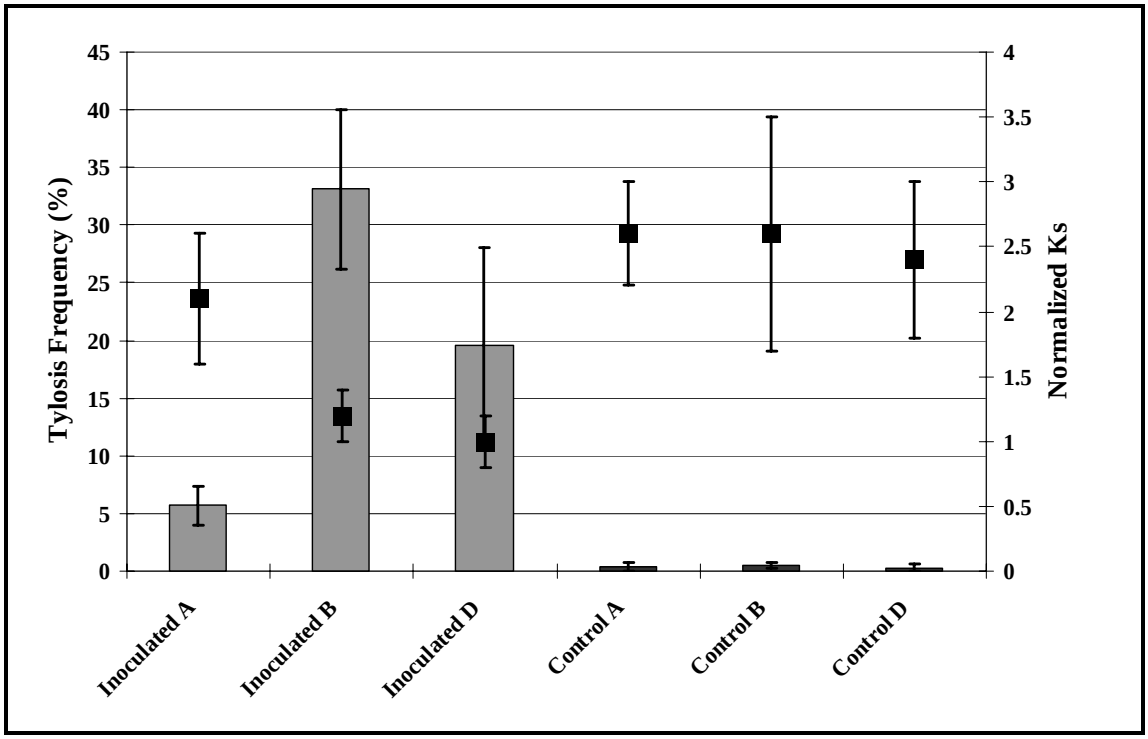

Figure 19. Extent of tylosis development and normalized k_s in tanoak tree sapwood subsections taken during harvest 1. Grey bars represent mean (±SE) tylosis frequency of samples subsections of inoculated trees and black bars represent non-inoculated control trees. Black squares represent mean (±SE) normalized k_s.

Chapter 3 Figures (Continued)

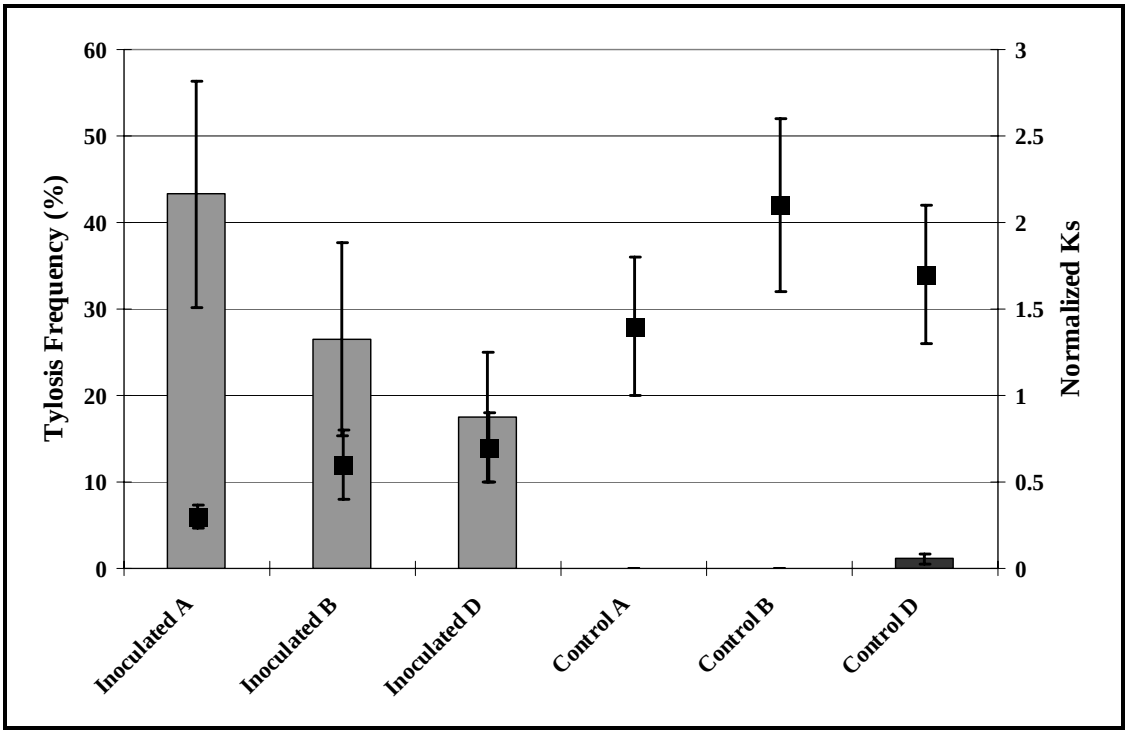

Figure 20. Extent of tylosis development and normalized k_s in tanoak tree sapwood subsections taken during harvest 2. Grey bars represent mean (±SE) tylosis frequency of samples subsections of inoculated trees and black bars represent non-inoculated control trees. Black squares represent mean (±SE) normalized k_s.

Chapter 3 Figures (Continued)

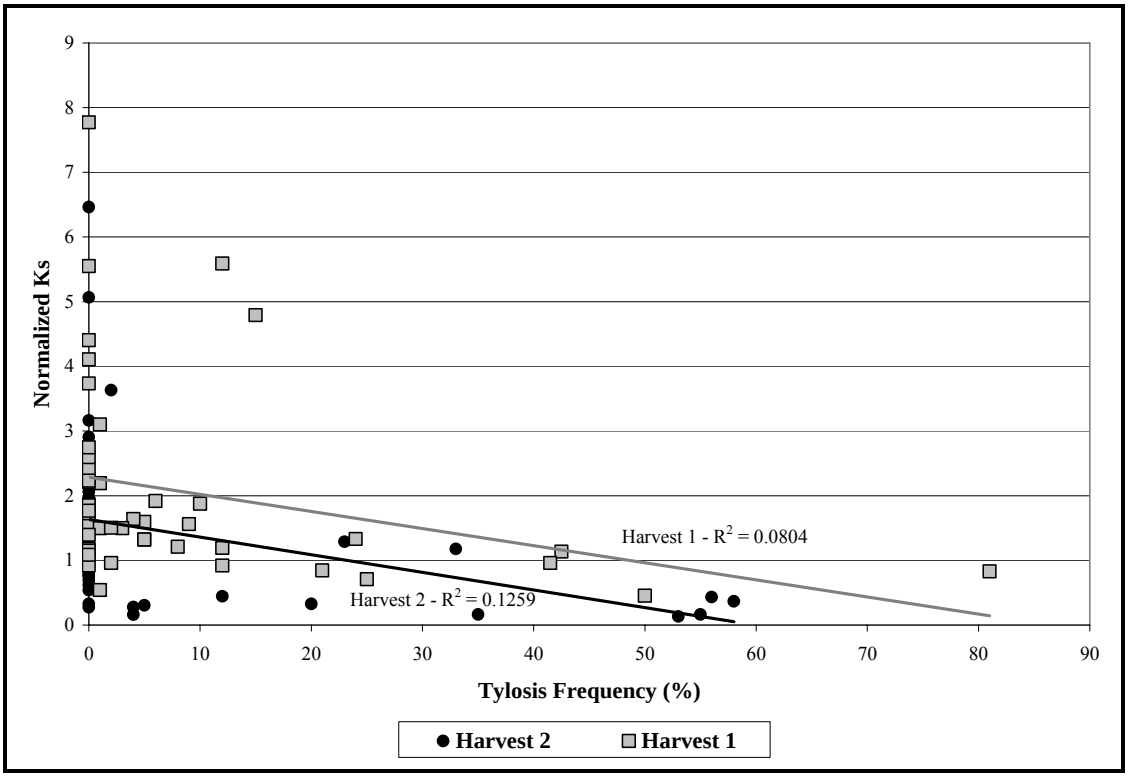

Figure 21. Scatterplot with regression lines of tylosis frequency (%) in tanoak vessels and normalized k_s. Black circles represent samples taken at harvest 1 and grey squares represent samples taken at harvest 2. Regression analysis indicates that an increase in tylosis frequency is associated with a decrease in specific conductivity in both harvest dates (Harvest 1, p=0.04, R^2=0.08, n=47) (Harvest 2, p=0.02, R^2=0.12, n=42).

CHAPTER 4: DISCUSSION

This research shows that infection by *P. ramorum* induces tyloses in the xylem vessels of tanoak and that the resulting occlusion of the conductive tissue reduces the conductivity of the sapwood. The development of tyloses in the xylem of plants has been associated with other host-pathogen systems such as Dutch elm disease and oak wilt (Beckman, 1987), and the resulting loss in conductivity has been reported (Tyree & Zimmerman, 2002). These data support the hypothesis presented by Parke *et al* (2007) that *P. ramorum* infection reduces conductivity of tanoak sapwood and offers a mechanism for this reduction.

The specific mechanism by which the tyloses are induced was not studied and is not well understood. Early hypotheses suggested that a reduction in the internal pressure of the xylem sap pulled the axial parenchyma into the vessel lumen in a ballooning fashion (Gerry, 1914). Contemporary hypotheses implicate the presence of air in the vessel lumen as the trigger for the growth of tyloses (Rioux, 1998). Another hypothesis suggests that tyloses form as the result of a need for a cell to reduce the concentration of toxic metabolic by-products that have accumulated in the parenchyma cell by increasing the volume of the cell (Stewart, 1966) but this theory does not appear to hold in all situations (Meyer, 1967).

The exact mechanism by which a pathogen causes the embolism required for tylosis formation is not well understood either. Beckman (1987) maintains that the physical damage inflicted on the vessel wall during the infection process or the ability of a pathogen to fill and physically block the vessel can both allow air to enter the vessel lumen. Parke *et al* (2007) reported *P. ramorum* hyphae growing extensively into the vessel lumen but it is not known whether cell wall disruption was sufficient to allow air-seeding.

The ability of volatile molecules such as turpenes and phenolics to reduce the surface tension of water in the xylem sap and induce embolism has also been reported (Kuroda, 1991 & 2001, Sperry & Tyree, 1988). It is not known whether *P. ramorum* produces any of these volatile molecules, but the necrotrophic action of this pathogen

certainly could stimulate the production or release of these chemicals by the host (Frankel et al, 2008).

It has been shown that *P. ramorum* can produce toxins (elicitins) that damage host plant cells (Manter *et al*, 2007). Since tyloses are essentially part of a living parenchyma cell, the presence of such a toxin could have the same effect on a tylosis as it does on other host cells. Furthermore, other species of *Phytophthora* have the ability to degrade cell wall pectin and disrupt the integrity of the cell (Benhamou & Côté, 1992). The host-pathogen interactions of *P. ramorum* and host tissue have not been well studied, but it is possible that *P. ramorum* has similar mechanism since it is clearly a necrotrophic pathogen.

One disadvantage of the log inoculation experiment was that the destructive process of cutting the tree into logs facilitated embolisms resulting in the formation of tyloses. Although the inoculated logs showed a significantly higher frequency of tyloses as compared to the controls after four weeks, the controls became increasingly occluded over time as vessels became dysfunctional from the cutting process. Consequently, a long-term investigation of tylosis development with this experiment was not possible.

One surprising result of the log inoculation experiment was the apparent reduction in the number of tyloses over time in the presence of *P. ramorum*. Behind the advancing margin of the infection in inoculated logs, there seemed to be a decrease in tylosis frequency. Non-inoculated tissue continued to steadily produce tyloses over time in response to wounding. One possible explanation is that within the confines of the infected tissue, *P. ramorum* damages the cell walls of the newly formed tyloses enough for them to rupture. *P. ramorum*, like other *Phytophthora* species, requires sterols for growth and sporulation. Manter, *et al* (2007) postulated that elicitins may function in disrupting sterol-rich host cell membranes to enable the pathogen to better scavenge sterols. The reduction in tyloses over time in colonized tissue may result from their breakdown by *P. ramorum* either physically or by the production of elicitins. Tyloses typically appeared thin-walled and hyaline, although a small number of vessels also contained dark-staining, lignified tyloses.

The field inoculation study showed that *P. ramorum* can induce the formation of tyloses in living tanoak trees and showed that tylosis frequency is related to a reduction in specific conductivity. The symptoms of sudden oak death on tanoak resemble the effects of water stress and this could be at least partially due to the occlusion of the vessels by tyloses. Although a more comprehensive study involving the effect of *P. ramorum* on the conductive ability of the entire stem is needed, these data show that such a reduction in conductivity is likely to be involved.

Based on results from this experiment, the size of the external bark lesion is a good predictor of tylosis formation in the sapwood, more so than the presence of xylem discoloration. It appears that external bark lesions expand at a rate faster than the discoloration extends into the xylem. It has also been shown that *P. ramorum* hyphae can colonize sapwood vertically via xylem vessels (Parke *et al*, 2007, Brown & Brasier, 2007). This discrepancy between bark lesion size and xylem lesion area probably reflects the vertical movement of *P. ramorum* within xylem tissue. *P. ramorum* may require a significant amount of time to penetrate deeper into the xylem and cause extensive losses in conductivity. *P. ramorum* hyphae can spread vertically fairly quickly without penetrating the sapwood enough to significantly disrupt sapflow.

The vessels of many ring-porous tree species such as *Quercus alba* fill with tyloses after just a few years and so the volume of water that is transported up the stem is confined to the vessels of the outer few annual rings (Saitoh, 1992). Consequently an infection by a pathogen such as *P. ramorum* that can induce tyloses and reduce conductivity in the xylem vessels in the outer sapwood is likely to have a significant impact on the health of the tree. Conversely, radial-porous species are not known to fill their xylem vessels with tyloses in older sapwood. In these species, vessels have been shown to be capable of transporting water after more than 20 years and the volume of water in the stem was shown to be consistent across the radius of the stem (Hirose, 2005). It is likely, therefore, that a significant decrease in conductivity across the entire stem of a tanoak tree would require colonization of a large cross-sectional area of the stem.

One complication of this experiment was the highly variable nature of specific conductivity measurements. Some sapwood samples had relatively large conductive areas and few tyloses but had relatively low specific conductivities. Other samples had a high frequency of tyloses but relatively high specific conductivity. One explanation is that quantifying tylosis frequency does not necessarily describe the degree to which the vessels were occluded and in some cases, tyloses were small enough to have little effect on conductivity but were still counted. Additionally, field data did not include physiologic or physical assessments of each tree. An accumulation of tension wood in leaning trees, for example, might contribute to deviations of specific conductivity from expected values. It is clear, however, that when a sapwood infection occurs and tylosis development increases, conductivity is reduced.

Based on field examination of infected tanoak, a larger difference in tylosis frequency between harvest 1 and harvest 2 was expected. Rizzo (2002) reports large canker formation and mortality within two years after inoculation. Trees selected in this study were understory trees that were slow-growing. In addition, weather data indicates that the summer of 2006 was dry, perhaps not adequately moist for extensive lesion development. Despite the small increase in tylosis frequency, the results from this experiment show that tylosis development increases vertically with the advancing infection, suggesting that advanced lesions may be associated with extensive tylosis development and a significant reduction in conductivity throughout the sapwood.

This research provides evidence that *P. ramorum* induces the formation of tyloses within the xylem vessels of tanoak sapwood. It is based on a confined area adjacent to the point of inoculation and in proximity to the discolored area of the developing infection. Nonetheless, it has raised further questions about the spatial and temporal effects of *Phytophthora* infections on the water relations of host trees. The implications of this research suggest that the mortality of tanoak trees by *P. ramorum* may involve mechanisms similar to the wilting mechanisms observed in Dutch elm disease or oak wilt. Further investigations should examine sapflow of whole stems to elucidate the extent of reduced conductivity. There is now a need to resolve these questions at the whole tree level. Understanding the mechanism by which *P. ramorum*

causes mortality in tanoak and other host trees is important for the ability to more accurately predict under what circumstances an infected tree will die. Accurate predictions of this nature can influence control or eradication measures in both forest ecosystems and residential properties.

Conclusions

This research provides important evidence that *P. ramorum* infection in tanoak results in extensive tylosis development. Further, the data suggest that the abundance of tyloses is correlated with reduced specific conductivity of xylem tissue. Before further generalizations can be made, however, additional research is needed in several areas. First, the relationship between the formation of tyloses and the resulting loss in conductivity and tree mortality has not been made. It is critical that further investigations involving tylosis development and losses in conductivity be performed on more vigorous trees and over a longer period of time. Investigations that begin with inoculation and end with tree death would provide valuable information regarding the ability of tanoak to limit the spread of infection and determine if a loss in conductivity can be extensive enough to cause tree death. Second, it is unknown whether other host species exhibit the same reaction to *P. ramorum*. Coast live oak is another host species that develops bleeding cankers from a *P. ramorum* infection and has experienced mortality as well. However, investigations involving tylosis development and losses in conductivity have not been performed on this species. Third, the role that elicitins play in tylosis formation has not been studied. The exact mechanism of tylosis formation in tanoak is poorly understood and it may involve embolism. It is possible that tylosis formation involves elicitins.

There were some important limitations to this study that confounded results and prevented further generalizations. First, the trees selected were slow-growing understory trees. Some of these trees did not develop adequate lesions that contributed to tylosis formation whereas healthier, faster growing trees may have provided better results. Second, the experimental design included only 12 pairs of trees (6 trees per treatment for each harvest). When damaged trees and trees developing insufficient

disease symptoms were removed, the result was a small sample size. Further studies should include a larger tree sample size to accommodate the loss from damage or trees that do not develop extensive infections. Fourth, the presence of a species of *Armillaria* in the field site may have affected tree growth or lesion development. For further investigations, it would be necessary to evaluate the presence of other pathogens before plot establishment.

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