AN ABSTRACT OF THE THESIS

Elizabeth A. Stamm for the degree of Master of Science in Botany and Plant Pathology presented on March 16, 2012
Title: The Effects of *Phytophthora ramorum* Stem Inoculation on Aspects of Tanoak Physiology and Xylem Function in Saplings and Seedlings

Abstract approved

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Phytophthora ramorum, an oomycete plant pathogen, is the causal agent of sudden oak death, a serious disease of Fagaceous trees in California and Oregon over the last decade. Tanoak (*Notholithocarpus densiflorus*) is one of the most susceptible host species, but the cause of host mortality is poorly understood. Previous research has implicated disruption in stem water transport, phloem girdling, and activity of a class of secreted proteins known as elicitins as possible mechanisms of pathogenesis.

In this study I investigated certain physiological impacts of *P. ramorum* infection on tanoak saplings and tanoak seedlings. In growth chamber experiments, stems of plants were inoculated with isolates that differed in the amount of elicitin secreted *in vitro*. Stem-wounded, non-inoculated plants served as controls. Parameters measured included net photosynthetic rate, stomatal conductance, whole plant water usage, stem specific hydraulic conductivity, tylosis production, starch partitioning, and mortality.

Inoculated saplings exhibited a reduction in whole plant water usage, followed by a reduction in stem specific hydraulic conductivity implicating an interruption in stem water transport as the primary symptom. A reduction in net photosynthetic rate and stomatal conductance occurred one week later. Experiments conducted on inoculated tanoak seedlings supported the hypothesis that a reduction in stem water transport is the primary disease symptom. Stem specific hydraulic conductivity was the only parameter that appeared to be significantly impacted when treatments were compared during each measurement period. There was, however, a significant

difference between treatments over the course of the entire experiment. Due to differences in isolate growth rates and similar levels of elicitin secretion, symptom expression could not be tied to elicitin production. To determine where elicitins are produced *in planta*, an immunolabeling technique was tested utilizing an elicitin-specific fluorescent antibody. The elicitin proteinwas most apparent in paratracheal parenchyma cells, although nonspecific staining in control samples confounded interpretation.

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The Effects of *Phytophthora ramorum* Stem Inoculation on Aspects of Tanoak Physiology and Xylem Function in Saplings and Seedlings

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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.

Elizabeth A. Stamm

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Chapter 1: Introduction

Phytophthora ramorum is an aggressive plant pathogen capable of causing disease on over 100 taxa (APHIS 2012). While it is obvious that the pathogen has devastating implications for affected species, the cause of host mortality is still poorly understood. *P. ramorum* produces three types of disease on host species: dieback, leaf blight, and stem cankers (Hansen et al., 2002). The type of disease depends on the host species that is infected. Stem cankers are generally limited to hosts in the *Fagaceae* family, while twig dieback and leaf blight can be found on most hosts.

Initially it was assumed that host mortality in species that exhibit stem cankers was caused by phloem girdling, but since then evidence has surfaced that *P. ramorum* is capable of penetrating xylem tissue (Brown and Brasier 2007; Parke et al., 2007). Infection of the xylem has been associated with reduced hydraulic conductivity (Parke et al., 2007) and increased tylosis production in tanoak (*Notholithocarpus densiflorus*) (Collins et al., 2009), a major fagaceous host.

In *Rhododendron macrophyllum*, a host that exhibits shoot dieback and leaf blight, *P. ramorum* infection has been associated with reduced photosynthetic efficiency (Manter et al., 2007). The same study implicated a class of 10kD proteins known as elicitins that are secreted by all *Phytophthora* and *Pythium* species. Elicitins have been named for their ability to elicit a hypersensitive response in non-host species (Ricci et al., 1989). It has been hypothesized that disease symptoms may be partially the result of host species showing slow or incomplete hypersensitive response. Specific recognition of *Phytophthora* elicitins has also been shown to induce systemic acquired resistance (SAR) in non-host species (Keller et al., 1996).

Two *P. ramorum* elicitins were identified: RAM1 and RAM2. Rhododendron leaf disks exposed to a purified elicitin solution showed reduced chlorophyll functionality and reactions indicative of a hypersensitive response such as increased

ethylene production and H^+ uptake, but to lesser extent than leaf disks of a non-host species, tobacco, that were also exposed to the elicitin solution.

While *P. ramorum* affects various hosts in different ways, it is possible that elicitins act as pathogenesis related proteins in all cases. A better understanding of the role elicitins play could be an important step in understanding how resistance can be attained.

In this study we aimed to determine what physiological factors contribute to tanoak mortality after trees are infected with *P. ramorum* and to investigate the relationship between elicitin secretion and disease symptoms. We investigated how photosynthesis, stomatal conductance, water usage, stem specific hydraulic conductivity, and starch partitioning were affected following inoculation of tanoak trees with *P. ramorum* isolates that differed in elicitin secretion.

Experimental Objectives

- Determine the physiological effects of infection by *P. ramorum* in tanoak (*Notholithocarpus densiflorus*) for parameters including: whole plant water usage, net photosynthetic rate, stomatal conductance, and starch content.
- Examine the temporal aspects of symptom development for previously mentioned physiological parameters.
- Explore whether isolates of *P. ramorum* with higher levels of elicitin secretion produce more severe physiological symptoms for previously mentioned parameters.

Literature Review

Phytophthora ramorum and Sudden Oak Death

In 1995, homeowners in Marin County observed dead and dying tanoak (*Notholithocarpus densiflorus*)(Svihra 1999). The primary symptoms reported by homeowners were dead or dying crowns and bleeding cankers on trunks. Two years later, similar symptoms were observed on coast live oaks (*Quercus agrifolia*) and California black oaks (*Quercus kelloggii*). On initial examination, the mortality was associated with *Hypoxylon thouarsianum* fruiting bodies and bark and ambrosia beetle infestation (Swiecki 2010). Although these two organisms were frequently found on dead and dying trees, they were believed to be secondary pathogens colonizing stressed trees.

After several years of speculation about the underlying cause of oak and tanoak mortality, an organism from the genus *Phytophthora* was isolated from several bleeding cankers. Completion of Koch's postulates then confirmed it as the causal agent of oak and tanoak mortality in both mature and juvenile trees (Rizzo et al., 2002).

In Europe, a new species known as *Phytophthora ramorum* was identified as the causal agent of a leaf blight and stem dieback disease on *Rhododendron* (Werres et al., 2001). On closer examination, the *Phytophthora* species causing disease on *Rhododendron* in Europe appeared to be morphologically identical to the species causing disease on oak and tanoak. A comparison of the internal transcribed spacer (ITS) sequence confirmed that the two isolates were the same species. *P. ramorum* is now known to be a pathogen capable of causing disease on over 100 plant taxa, including several species native to California and Oregon forests (Davidson et al., 2003). Since it was first observed in Marin County, confirmed *P. ramorum* infections in forests have been reported in 14 California counties and one Oregon county.

P. ramorum was first observed in American nurseries in 2001 when it was isolated from infested rhododendrons. Since then, *P. ramorum* has been reported in nurseries within 11 states, including several states on the east coast (Kliejunas

2010). Due to the rapid spread of sudden oak death and related diseases caused by *P. ramorum,* it has become increasingly important to understand the pathogen's biology and the mechanisms that lead to host mortality.

Organisms in the genus *Phytophthora* are Oomycetes in the kingdom Straminopila. They are characterized by a fungal-like mycelium, coenocytic hyphae, and an absorptive mode of nutrition. They reproduce asexually through sporangia, which can germinate directly, or release motile, biflagellate zoospores. Asexual survival spores, known as chlamydospores, are present in some *Phytophthora* species. Sexual reproduction occurs through the fusion of an oogonium and an antheridium to produce the sexual spore known as an oospore. Oospores can be produced through both homothallic and heterothallic means (Alexopolous et al., 1996).

While *Phytophthora* species look and behave similarly to organisms in the kingdom Fungi, they are more closely related to organisms such as diatoms and brown algae. Unlike organisms in kingdom Fungi that are haploid with cell walls made of chitin, *Phytophthora* species are diploid and have cell walls made of cellulose and β-glucans (Erwin 1996).

The genus *Phytophthora* is host to many formidable plant pathogens, most notably, *Phytophthora infestans,* the causal agent responsible for the Irish potato famine (Agrios 2004). This pathogen provides a striking example of how a single plant pathogen can, in addition to the obvious impact on host plants, have broad economic and social implications.

Like *P. ramorum*, there are several other *Phytophthora* species that cause stem cankers on woody hosts. *P. cambivora* causes stem cankers and root rot, and collar rot on chestnut and several *Quercus* species, and has long been associated with oak decline in the Mediterranean region (Brasier et al., 1993). Shortly after *P. ramorum* was associated with sudden oak death, a new species, *P. kernoviae,* was identified as a canker-causing pathogen of trees and shrubs in Europe (Brasier et al., 2005).

Pathogenesis of Tanoak

Three general hypotheses have been proposed to explain pathogenesis of *P. ramorum* on oaks and tanoaks. The first hypothesis proposes that the phloem of infected trees is girdled by necrotic cankers. Trees infected with *P. ramorum* typically exhibit large cankers that can encompass the entire circumference of a tree, effectively girdling phloem tissue (Rizzo et al., 2002). Girdling inhibits transfer of sugars, which can ultimately lead to carbohydrate accumulations in leaves and a reduction in photosynthesis and starvation of root tissue (Stitt and Schulze 1994),. Evidence for phloem girdling has been seen in alder infected with *P. alni* and beech infected with *P. citricola*. When infected, both tree species exhibit reduced net photosynthetic rate without any evidence of reduced stem water transport (Fleischmann et al., 2005; Clemenz et al., 2008). Accumulation of starch in leaf tissue has been linked with a reduction in the net photosynthetic rate (Azcón-Bieto, 1983). The lack of impact on stem water transport suggests that the reduction in net photosynthetic rate is likely the result of starch accumulation in the leaves caused by damage to the phloem.

Initially it was thought that canker causing *Phytophthoras,* such as *P. ramorum,* could only survive in nutrient-rich phloem (Erwin 1996), however, some symptoms of sudden oak death, such as flagging and wilting, are indicative of reduced water transport. Because of this, a second hypothesis was proposed, suggesting that *P. ramorum* interferes with the conductive properties of xylem tissue (Brown and Brasier 2007; Parke et al., 2007). While death of phloem tissue may play an important role in tree death, Brown and Brasier (2007) and Parke et al. (2007) showed that *P. ramorum* could consistently be isolated from discolored xylem tissue directly under cankers. Moreover, tanoaks infected with *P. ramorum* had reduced sap flow when compared to uninfected tanoak trees. Closer examination of xylem tissue of infected trees revealed vessels occluded with hyphae and tyloses (Parke et al., 2007). Tylosis production is also known to be directly associated with *P. ramorum* infection in inoculated trees and was correlated with a reduction in stem specific conductivity (Collins et al., 2009). Despite an abundance

of evidence that impairment of xylem function contributes to tree mortality, leaf water potential measured on naturally infected trees revealed that dying trees were not suffering from water stress (Swiecki and Bernhardt 2002)

Tyloses are outgrowths of ray and paratracheal parenchyma (Esau 1977). The cell wall of a tylosis originates from the secondary cell wall of adjacent parenchyma cells. The vessels have simple pits through which the tyloses protrude (Murmanis 1975). Tylosis formation often occurs in conjunction with the build up of phenolic compounds in the surrounding tissue (Nečesaný 1973).

Tylosis formation has been associated with many environmental stimuli, but the trigger mechanisms are poorly understood. While tyloses do impair xylem function, they are thought to serve a role in certain environmental conditions that may cause long-term damage or mortality (Biggs 1987). Tylosis formation has been seen in conjunction with mechanical wounding (Shain 1979), freeze cycles (Cochard and Tyree 1990), and pathogen infection (Robb et al., 1979). It has long been thought that the underlying cause of tyloses is the formation of embolisms (Zimmermann 1978), which could potentially occur with all environmental triggers, but recently it has been shown that tyloses still form in the absence of embolisms if high levels of ethylene are present (Sun et al., 2007).

Shigo (1984) proposed that tyloses may actually be the product of a more specific defense response involving recognition of an offending pathogen. Specific defense responses are typically triggered by recognition of a pathogen associated molecular pattern (PAMP). PAMPs are generally molecules that come in direct contact with host cells, such as secreted proteins and cell wall components (Chisholm et al., 2006). Plants that have coevolved with a particular pathogen tend to respond quickly when a PAMP is recognized, while a plant exposed to a foreign pathogen may respond slowly even if a PAMP is recognized, leading to an incomplete defense response that can manifest as disease symptoms (Heckman et al., 2001).

Evidence that PAMPS are capable of eliciting disease symptoms when recognition occurs slowly suggested the third hypothesis, namely that a class of secreted proteins, known as elicitins, could cause the physiological symptoms, such

as development of necrotic lesions, a reduction in net photosynthetic rate, and reduced stem specific hydraulic conductivity in diseases caused by *Phytophthora ramorum*. Elicitins are a class of 10 kD proteins secreted by all *Phytophthora* and *Pythium* species (Ricci et al., 1989). Elicitins may have a role in transporting sterols from plant cells to the pathogen (Boissy et al., 1999). *Phytophthora* and *Pythium* species do not possess the biosynthetic pathway necessary to produce sterols, so it is essential that they are obtained from the plants they infect. Elicitins are divided into two categories, alpha elicitins and beta elicitins, based on their isoelectric point. Alpha elicitins have an acidic isoelectric point and have been seen to have greater sterol loading capacity and cause a greater degree of necrosis than beta elicitins having a basic isoelectric point (Pernollet et al., 1993).

In experiments conducted with tobacco cell suspensions, the elicitin secreted by the plant pathogen *Phytophthora cryptogea,* known as cryptogein, exhibited a high binding affinity with transmembrane receptor proteins. This kind of relationship indicates a specific, gene-for-gene type interaction in incompatible hosts (Zhang et al., 1998). They are named for their ability to elicit a hypersensitive response in tobacco plants as well as certain *Brassica* species (Ricci et al., 1989). Leaf disks of host species treated with a purified elicitin solution exhibit increased H^+ uptake and ethylene production, reactions that are commonly associated with a hypersensitive reaction (Manter et al., 2007).

Elicitins can also trigger more general defense responses (Yu 1995). The *Phytophthora infestans* elicitin, INF1, has been shown to trigger the production of jasmonic acid and activation of ethylene signaling pathways in tomatoes. Stimulating the production of these two compounds with INF1 was shown to lead to resistance to bacterial wilt disease in tomatoes (Kamoun et al., 1998).

When infecting potato, a compatible host, *Phytophthora infestans* down regulates production of INF1, providing further evidence that elicitins can be recognized by their plant hosts (Kamoun et al., 1997).

Because elicitins appear to act as avirulence factors in incompatible host species, and are also capable of triggering more general defense in susceptible host

species, it has been hypothesized that they could act as virulence factors in compatible host species.

In this research, I investigated the whether primary symptoms of *P. ramorum* infection on tanoak were the direct result of damage to xylem or phloem. This was achieved in a series of growth chamber experiments where the net photosynthetic rate, stomatal conductance, water usage, stem specific hydraulic conductivity, tylosis production, and starch content in leaves and roots were measured on artificially inoculated tanoak trees. If the primary symptom is caused by damage to the phloem, one would expect to see a decline in the net photosynthetic rate accompanied by accumulation of starch in the leaves as the first observable symptoms. If the primary symptom is the result of damage to the xylem, one would expect to see a reduction in water usage and stem specific hydraulic conductivity as the first observable symptoms. The first experiment was conducted with tanoak saplings, but because additional saplings were not available the experiment was repeated with tanoak seedlings. The seedling experiment was conducted twice and will be referred to as trial 1 and trial 2.

To determine if the elicitin contributes to the development of certain physiological symptoms, trees were inoculated with each of two isolates, with one isolate producing significantly more elicitin than the other. If the elicitin affects certain physiological symptoms, the expectation would be that trees inoculated with the isolate that produces a larger amount of elicitin would exhibit earlier and more severe symptoms.

Chapter 2: Materials and Methods

Growth chamber experiment: Sapling inoculation

Plant material

Potted two-year-old tanoak (*Notholithocarpus densiflorus*) saplings from the Garbelotto and Dodd laboratories, University of California, Berkeley greenhouses, were used in this artificial inoculation experiment. Plants were transported from Berkeley to Corvallis on May 22, 2009. Saplings were grown from acorns collected from a variety of different family groups collected from a variety of locations in California, and were randomly distributed among treatments. Plants were grown in pots in soil-free potting medium. Plants had a mean height of 47 cm ± 6 cm (std. dev.) and a mean stem caliper of 0.92 cm ± 0.15 cm (std. dev.). Plants were kept in a growth chamber (Conviron PGV, Winnipeg, Manitoba) at 18°C with a 12-hour photoperiod and a relative humidity of 60% for 2 weeks before use in experiments. The level of photosynthetically active radiation at the average tree height was 50 μmol m^{-2} s^{-1}. The level of photosynthetically active radiation in the growth chamber was much lower than that found in tanoak habitat (~250 μmol m^{-2} s^{-1}).

Inoculum

P. ramorum isolates PR-07-058 and 4353 were selected for this experiment because of their differences in elicitin production *in vitro* as reported by Manter et al. (2010). Isolate PR-07-058 produces more elicitin than isolate 4353. Isolates were started from stock cultures in water storage and grown on pimaricin ampicillin rifampicin (PAR) semi-selective growth medium for one week (Jeffers & Martin, 1986). Mycelial plugs taken from colony margins were then placed on 1/3 V8 medium made with clarified V8 juice and 23.4 g bacto-agar. 4353 originates from the NA1 clonal lineage, a lineage associated with California and Oregon forests (Grunwald et al., 2008). In liquid culture, it was shown to produce 4.52 μg/ml of elicitin (Manter et al., 2010). PR-07-058 originates from the NA2 clonal lineage, a

lineage associated with nurseries in California and Washington nurseries (Grunwald et al., 2008). In liquid culture, it was shown to produce 7.23 μg/ml of elicitin (Manter et al., 2010).

Artificial inoculation

On September 20, 2009, trees were inoculated with one of three treatments: *P. ramorum* isolate PR-07-058 (high elicitin-expressing isolate, n=20 trees), *P. ramorum* isolate 4353 (low elicitin-expressing isolate, n=20 trees), or a sterile 1/3 V8 agar plug (control, n=20 trees). Isolates within the NA clonal lineages were chosen for different levels of elicitin production as reported by Manter et al. (2010). Inoculations were made by making a small incision in the bark with a scalpel going no deeper than the cambium, and then placing either a mycelial plug or sterile agar plug on the wound. Plugs were then covered with moist cheesecloth and wrapped in Parafilm. Inoculations were made 20 cm down from the apical bud.

Mortality

A count of dead trees was taken weekly. A tree with no living foliage was considered dead.

Water usage

On day one of the experiment, all potting medium was watered to saturation and allowed to drain for three hours. The tops and bottoms of plant containers were then sealed with plastic plates with a central opening to accommodate the stems. Plates were secured with duct tape and the remaining space between the stem and plate was sealed with modeling clay. The weight of each potted plant was recorded. Once per week, for eight weeks, the weight of each potted plant was recorded. Because the tops and bottoms of the pots were sealed, I assumed that any loss in weight was due to transpiration, allowing total water usage to be calculated.

After plant weight was recorded, water was added through a port in the sealed container lid to return potted plants to approximately the same weight as on day one. The port also prevented conditions within the sealed pot from becoming completely anoxic. Plant growth did occur over the course of the experiment, but because the starting weight was reestablished each week, and measurements were based on the weight difference over the course of a week additional weight as the result of growth had a negligible impact on results. At the conclusion of the experiment, leaves were removed, scanned with a flat bed scanner, and one-sided leaf area was determined using image analysis software (Assess, 2002, American Phytopathological Society, St. Paul, MN) enabling water usage values to be normalized

Photosynthesis and stomatal conductance

Once a week, the net rate of photosynthesis (μmol CO_2 $m^{-2}s^{-1}$), and stomatal conductance (μmol H_2O $m^{-2}s^{-1}$) were measured between the hours of 10:00 am and 4:00 pm. Measurements were taken on three apical leaves of each plant using a portable photosynthesis system (LI-6400, Li-Cor Biosciences, Lincoln, NE). Prior to measuring, the flow meter and infrared gas analyzer were zeroed and allowed to stabilize. Leaves of interest were placed in the cuvette and exposed to blue and red light with a photon flux density of 1000 μmol $m^{-2}s^{-1}$. The starting CO_2 concentration in the sample chamber as well as the constant CO_2 concentration in the reference chamber were set at 400 μmol m^{-2} s^{-1}. Net photosynthetic rate and stomatal conductance data for each plant were based on the average for the three leaves.

Specific hydraulic conductivity

Over the course of the experiment, trees (n=4) from each treatment were destructively sampled at weeks two, four, and six after inoculation to measure stem specific hydraulic conductivity. At the time of harvest, a 5-cm stem section (17.5 cm from apical bud) was taken at the point of inoculation from each tree, and a 2-cm

stem section (15.5 cm from apical bud) was taken just above the point of inoculation (Fig. 1). Excised stem sections were then placed in acidified water (pH adjusted with HCl to pH 2) and placed under vacuum pressure to remove any native embolisms.

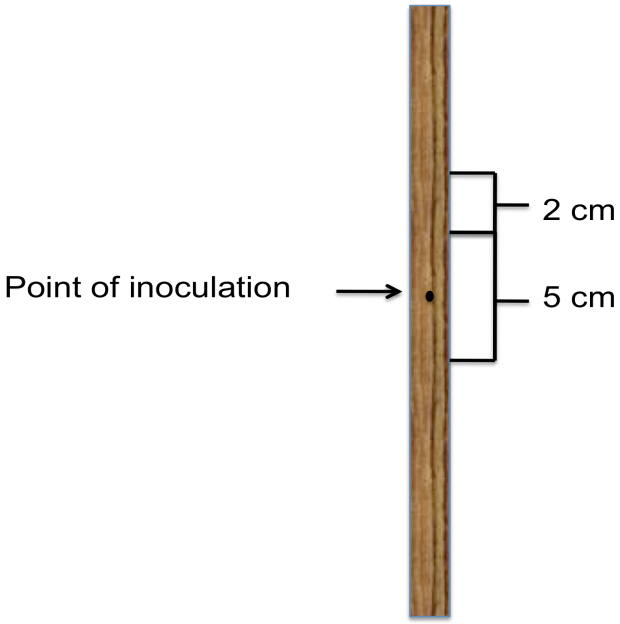


Fig. 1 Location of stem sections for measurement of stem-specific hydraulic conductivity in sapling growth chamber experiment.

Hydraulic conductivity was measured on both 2-cm and 5-cm stem sections with the apparatus shown (Fig. 2) by placing pH 2 water in an Erlenmeyer flask and placing a piece of rubber tubing into the water. Prior to each measurement, the ends of stem sections were trimmed under water with a razor blade. The distal end of each stem section was then placed approximately 5 mm into the end of the tubing and secured with Parafilm. Another piece of tubing attached to a volumetric pipette was attached to the proximal end of the stem section. The knob on the stopcock was then turned to allow the water to flow through the stem sample and the amount of time required for a specific volume of water to flow through the sample was recorded.

Stem specific hydraulic conductivity (K_s) was calculated utilizing Darcy's law:

$$K_s = (Ql)/(A\Delta P)$$

In this equation, Q is equal to flow rate (volume per unit time), l is equal to the length of

each stem, ***A*** is the cross-sectional conductive area of each stem, and ΔP is equal to the difference in pressure at the two ends of the stem section.

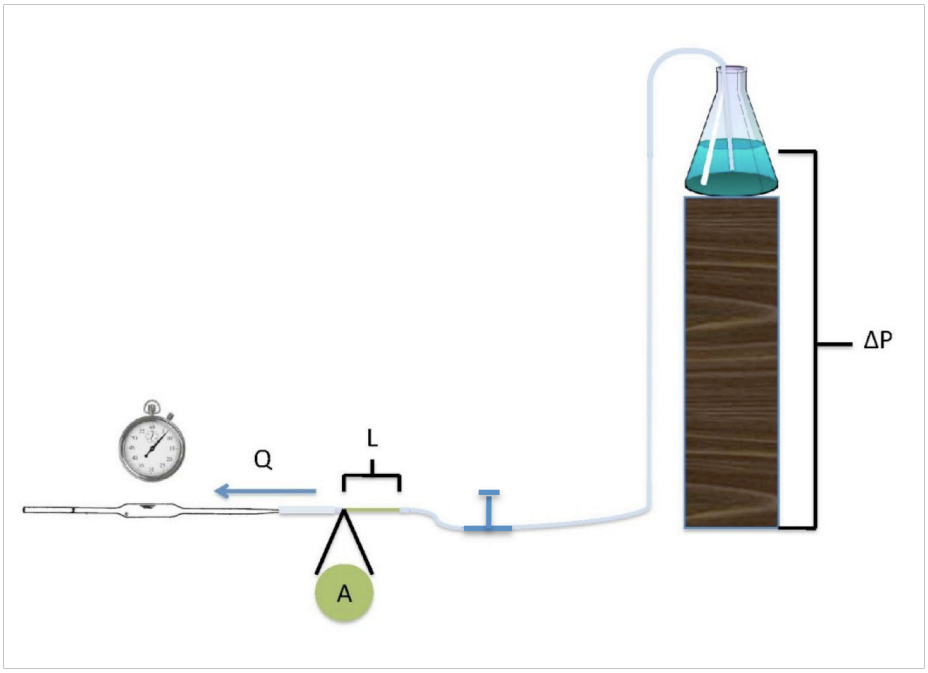


Fig. 2 Apparatus used to measure stem specific hydraulic conductivity.

Total cross-sectional area was measured for each stem section. Bark was removed prior to measurement. Total vessel area was measured in cross-section in four places for the 5-cm sections, and 2 sections for the 2-cm sections. Total vessel areas were measured in four 40 μm-thick cross-sectional sections made using a sliding microtome (one from each end, and two from the center). Sections were permanently mounted using Polymount (Polysciences, Inc., Warrington, PA) and photographed at 10x magnification using a compound microscope fitted with a digital camera. Photographs of stem sections were imported into image analysis software (Assess, 2002, American Phytopathological Society, St. Paul, MN) where vessels were traced and total areas measured. Ks values were normalized by average vessel area for each stem section.

Abundance of tyloses

Tyloses were quantified in the four 40 μm-thick stem sections for each 5-cm stem section used in the stem specific hydraulic conductivity assays. Immediately after hydraulic conductivity assays were conducted, stem samples were fixed in a formalin acetic acid (FAA) solution. Stem cross-sections were then cut with a sliding microtome. Samples were placed in a 2.5% analine blue solution and allowed to soak for one minute. Samples were then cleared in a 95% ethanol solution and permanently mounted using Polymount. Slides were viewed at 10x magnification and the entire section was scanned for tyloses. The percentage of vessels with tyloses present was determined for each section. Values for each of the four stem sections were averaged for each tree.

Reisolation

Lesions developed on 19 out of 20 inoculated trees and none of the 20 control trees. Lesion development was assessed visually at the time of harvest. To confirm the presence of the pathogen in the inoculated trees, *P. ramorum* was isolated from two trees per inoculated treatment at each harvest by placing five approximately 2mm x 4mm lesion-adjacent bark and cambium pieces on PAR medium.

Statistical Analysis

Photosynthesis, stomatal conductance, and water usage data were analyzed by performing a repeated measures ANOVA to determine the overall treatment effect, the overall effect over time, and the treatment effect over time.
Because of tree mortality and bi-weekly destructive sampling, the number of trees per treatment was not constant throughout the experiment. To accurately determine the treatment effect, a separate one-way ANOVA with weighted sum of squares was performed for each measurement period. For measurement periods that showed a significant treatment effect, a Tukey's HSD analysis was performed to

find individual differences between treatments.

Data for stem specific hydraulic conductivity and tylosis frequency was measured by performing a one-way ANOVA for each sampling period. Even though these two parameters were measured over time, measurements were taken on different experimental units for each sampling period making a repeated measures ANOVA inappropriate. Statistical tables are provided in Appendix A.

Isolate growth rates

Isolate growth rates were measured to determine whether differences in growth rate may have contributed to the observed differences in physiological effects of inoculation with isolates PR-07-058 and 4353. Growth rates of other isolates were also measured to determine if a more appropriately matched pair of isolates could be selected for subsequent experiments. All of the isolates tested by Manter et al. (2010) previously tested for elicitin production were included in the growth rate experiments. *P. ramorum* isolates 4313, 4353, 9650, CSL-2065, CSL2066, CSL-1727, CSL-2097, CSL-2026, PR-05-002, PR-07-031, PR-07-057, PR-07-058, and PR-07-166 (Table 1) were started from water culture and plated on corn meal agar amended with PAR (n= 3 plates per isolate). Plates were stored in the dark at 18°C. After one week, agar plugs were taken from the margin of each colony and placed on corn meal agar β-sitosterol plates to maximize growth rates. Plates were incubated in the dark at 18°C. Colony size was measured for each isolate at 3, 5, 7, and 14 days (n=3 colonies per isolate). Measurements were taken by tracing the outline of each colony with a permanent marker and then scanning each plate. The areas of each outlined colony were then measured using image analysis software (Assess, 2002, American Phytopathological Society, St. Paul, MN). The areas of each colony were then used to calculate radii lengths.

Table 1. *P. ramorum* isolates used in sapling and seedling growth chamber experiments and isolate growth rate study.

Isolate	Clonal lineage	Original host	Geographic source
4313	NA1	*Rhododendron*	OR
4353	NA1	*Notholithocarpus*	OR
9650	NA1	*Notholithocarpus*	OR
PR-05-002	NA2	*Rhododendron*	CA
PR-07-031	NA2	soil	WA
PR-07-057	NA2	*Rhododendron*	WA
PR-07-058	NA2	*Rhododendron*	WA
PR-07-166	NA2	*Rhododendron*	WA

Statistical analysis

Isolate growth rate data were analyzed by performing repeated measures ANOVAs. The first repeated measures ANOVA determined overall isolate effect, the overall time effect, and the effect of isolate over time. Differences between individual isolates were determined by performing a pair-wise T-test with a pooled standard deviation. The second repeated measures ANOVA examined the overall clonal lineage effect, the overall time effect, and the effect of clonal lineage over time. Differences between clonal lineages were determined by performing a pair-wise T-test with a pooled standard deviation. See appendix A for statistical tables.

Growth chamber experiment: Seedling inoculation (trial 1 & 2)

Plant material

Because additional potted tanoak saplings were not available for further inoculation experiments, two trials (1 and 2) were conducted with potted tanoak seedlings. The two trials were identical except for the age of the plant material. In Trial 1, the tanoak seedlings were two months old, with an average height of 19 cm ± 3 cm (std. dev.) and average stem caliper of 0.33 cm ± 0.09 cm (std. dev.). In Trial 2, plants were three months old, with an average height of 21 ± 4 cm (std. dev.) and average stem caliper of 0.35 cm ± 0.11 cm (std. dev.) . Plants were grown from acorns collected from Curry Co., Oregon by the Oregon Department of Forestry. Acorns came from several different parent trees, and were randomized using the R statistical computing random number generator (R Development Core Team, n.d.). Plants were kept in a growth chamber (Percival Advanced Intellect, Perry, IA) at 18°C with a 12-hour photoperiod and a relative humidity of 60%. The level of photosynthetically active radiation at average tree height was 25 μmol $m^{-2}s^{-1}$.

Inoculum

Two different *P. ramorum* isolates, PR-06-002 and PR-06-166, found to have similar growth rates *in vitro* (Table 2), were retrieved from water storage and grown on PAR selective growth medium for one week. These isolates belong to the NA2 lineage, and although no NA2 *P. ramorum* isolate has ever been isolated from tanoak in nature, these were the only two isolates that differed significantly in elicitin secretion yet had similar growth rates. Mycelial plugs taken from colony margins were then placed on 1/3 V8 medium. *In vitro* elicitin expression for each of the isolates was determined previously by Manter et al. (2010).

Artificial inoculation

For this experiment, trees were inoculated with one of three treatments: *P. ramorum* isolate PR-06-002 (high elicitin-expressing isolate, n= 37 trees), *P.*

ramorum isolate PR-06-166 (low elicitin-expressing isolate, n= 37 trees), selected for their similarity of growth rate *in vitro*, and a sterile V8 agar plug (control, n= 37 trees). Because the seedlings for this experiment were smaller than the saplings used in the first experiment, inoculations were made by making a small scratch in the bark with a dissection needle and then placing either a mycelial plug or sterile agar plug on the wound. Plugs were then covered with moist cheese cloth and wrapped in Parafilm. Inoculated trees were arranged in a completely randomized design.

Photosynthesis and stomatal conductance

Twice a week, for two weeks, the net rate of photosynthesis and stomatal conductance were measured on 21 trees (n = 7 trees per treatment). Measurements were made on three apical leaves and plants were measured in a random order determined using the R statistical computing random number generator (R Development Core Team, n.d.).

Water usage

Water usage was determined as described above (p. 10) twice per week for 2 weeks (n = 7 trees per treatment). At the end of the experiment, leaves were removed and total leaf area per plant was determined as described previously.

Stem specific hydraulic conductivity

Trees were destructively sampled and stem sections excised twice a week for two weeks (n=4 trees per sampling per treatment). Stem specific hydraulic

conductivity was measured on 5-cm stem sections taken from directly above the point of inoculation (Fig. 3).

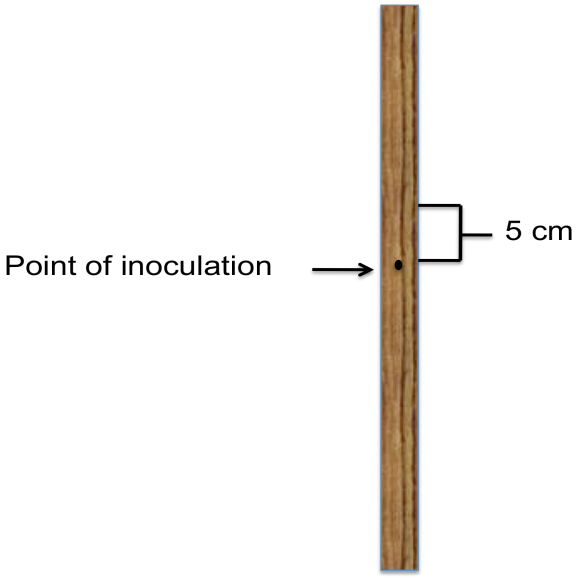


Fig. 3 Location of stem section taken for stem-specific hydraulic conductivity in the seedling inoculation experiment.

Starch content

Four to six leaves were randomly selected from the 4 destructively sampled plants per sampling and used to measure starch content in leaves and roots (American Association Cereal Chemists 1995). Leaves were collected from the top, middle, and bottom whorls. Root systems were also removed from each plant and rinsed with deionized water to remove potting media. Leaves and roots were then placed separately in a 50°C oven and dried to prevent starch degradation. Dried leaves and roots were then separately ground into a fine powder using a Cyclotec 1093 sample mill and stored in glass bottles. Prior to analysis, ground tissue was re-dried, and allowed to cool in a desiccator. Once cool, 50-100 mg of each sample was measured out to use in the starch analysis assay. Glucose and maltodextrins were removed by adding 5 ml of 80% ethanol to each sample and incubating at 80°C for five minutes. Samples were then centrifuged for ten minutes at 1000 x g and the supernatant was discarded. Pellets were resuspended in 10 ml of 80% ethanol and centrifuged for ten minutes at 1000 x g (Sigma-Aldrich, n.d.). Starch was then

measured using a starch assay kit (amylase/amyloglucosidase method) (Sigma-Aldrich,St . Louis, MO).

Statistical analysis

Photosynthesis, stomatal conductance, and water usage data were analyzed by performing a repeated measures ANOVA to determine the overall treatment effect, the overall efftect over time, and the treatment effect over time. To determine differences between treatments, differences between sampling periods, and differences between treatments for each sampling period, a Tukey's HSD analysis was performed.

Data for stem specific hydraulic conductivity and tylosis frequency was measured by performing a one-way ANOVA for each sampling period. Even though these two parameters were measured over time, measurements were taken on different experimental units for each sampling period making a repeated measures ANOVA inappropriate. See appendix A for statistical tables.

Immunolabeling assay

In an attempt to visualize the location of *P. ramorum* elicitins *in planta*, a fluorescent antibody prepared by D. Manter (USDA-ARS Laboratory, Ft. Collins, CO) was tested on *P. ramorum* infected tanoak stem tissue and *P. ramorum* as follows.

Necrotic tissue was excised from infected tanoak stems and infected rhododendron leaves and placed in formalin acetic acid (FAA) solution (47.5% ethanol, 5% acetic acid, 3.7% formalin, 53.8% water). Samples were then placed under vacuum to insure that the FAA solution penetrated the entire sample. Cross sections 40 μm thick were made using a sliding microtome and placed in phosphate buffered saline (PBS) solution (pH 7.2) overnight. Samples were then rinsed twice more in PBS solution.

After samples were rinsed, each sample was placed in the well of a 20-well microwell plate. A 0.05% bovine serum albumin (BSA) and 0.75% glycine blocking

solution was made in PBS plus Tween (PBST). The blocking solution was then added to each well and samples were incubated at 32°C for one hour. Samples were then washed four times in PBST. A 0.001% rabbit anti-elicitin antibody and 0.05 % BSA solution was made in PBST and added to each sample well. Samples were then incubated at 32°C for one hour. Samples were then rinsed four times in PBST. A 0.0001% goat anti-rabbit with FITC fluorophore and 0.05% solution in PBST was made and added to each sample well. Sampled were then incubated at 32°C for two hours. Samples were then rinsed four times in PBST and three times in PBS. Rinsed sampled were then mounted on slides with a small drop of PBS solution and covered with a cover slip. Samples were viewed under a compound microscope at 10x with a FITC filter.

Chapter 3: Results

Sapling inoculation

Saplings inoculated with either of the isolates began to die three weeks after inoculation (Fig. 4). The highest percent mortality occurred in the group inoculated with the high elicitin isolate, with 45% of the trees dead at the end of the experiment five weeks after inoculation. Percent mortality was relatively low for the group inoculated with the low elicitin isolate (10%), and none of the wounded control trees died.

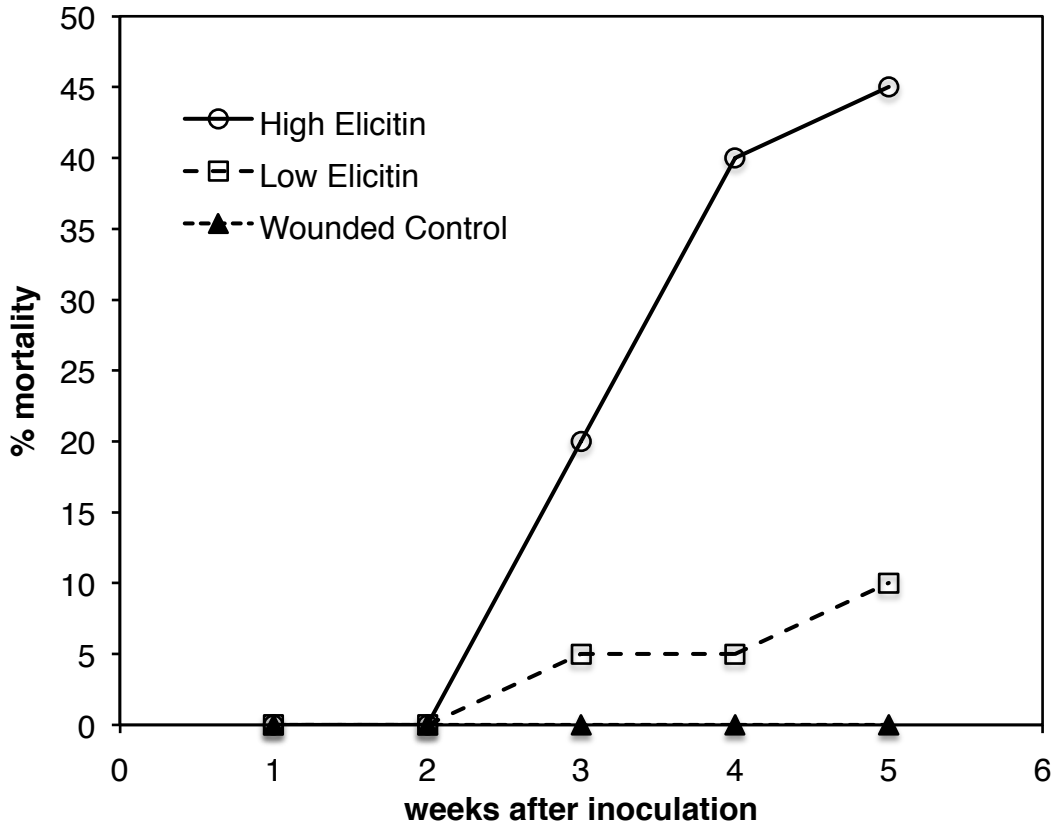


Fig. 4. Percent mortality over the course of five weeks for tanoak saplings inoculated with a high elicitin-producing isolate PR-07-058, a low elicitin-producing isolate 4353, or a wounded only treatment. N=20 trees per treatment.

In this experiment, the physiological effects monitored weekly included net photosynthetic rate (Fig. 5A), stomatal conductance (Fig. 5B), and whole plant water usage (Fig. 5C). Of the parameters studied, water usage (Fig. 5C) was the first to be affected. Water usage was reduced in the high elicitin treatment as compared to the wounded control ($p=0.09$) and it remained low for the duration of the experiment. By the end of week two, stem specific hydraulic conductivity in the 2-cm stem sections (Fig. 6A) was reduced in trees infected with the high elicitin treatment as compared to the wounded control ($p=0.02$) and it also remained low for the rest of the experiment. Stem specific hydraulic conductivity in the 5-cm sections taken at the point of inoculation exhibited reduced hydraulic conductivity across all treatments indicating a non-specific wound response, so these results were not included in the analysis. The 2-cm sections were from 2.5-4.5 cm above the point of inoculation, and did not exhibit a non-specific wound response.

Three weeks post inoculation there was a decline in the net rate of photosynthesis (Fig. 5A) ($p=0.02$) and stomatal conductance (Fig. 5B) ($p=0.01$) in trees infected with the high elicitin isolate, but trees inoculated with the low elicitin isolate did not differ from control trees for either parameter. Four weeks post inoculation there was an increase ($p=0.06$) in tylosis frequency in cross-section in trees inoculated with high elicitin isolate relative to the other treatments (Fig. 6B). By six weeks post inoculation, tylosis frequency in trees inoculated with the low elicitin isolate was also greater ($p=0.09$) than the wounded control.

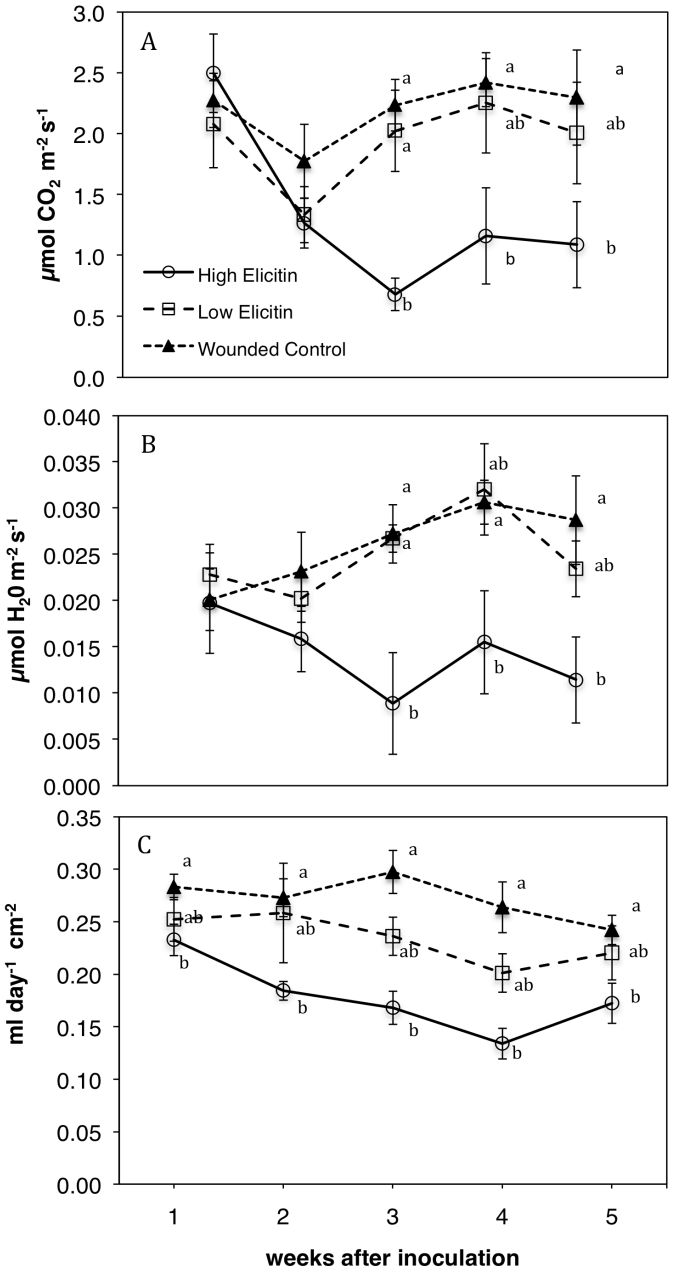


Fig. 5. A) Mean net rate of photosynthesis, B) stomatal conductance, and C) water usage in tanoaks saplings artificially inoculated with a high elicitin-producing *P. ramorum* isolate (PR-07-058), and low elicitin-producing *P. ramorum* isolate (4353) or a wounded but not inoculated control. For water usage, units are mL water lost from sealed pots per day per cm^2 leaf area. Bars indicate standard error of the mean. Different lowercase letters within each sampling date represent values that differ significantly ($p \leq 0.05$) based on Tukey's HSD.

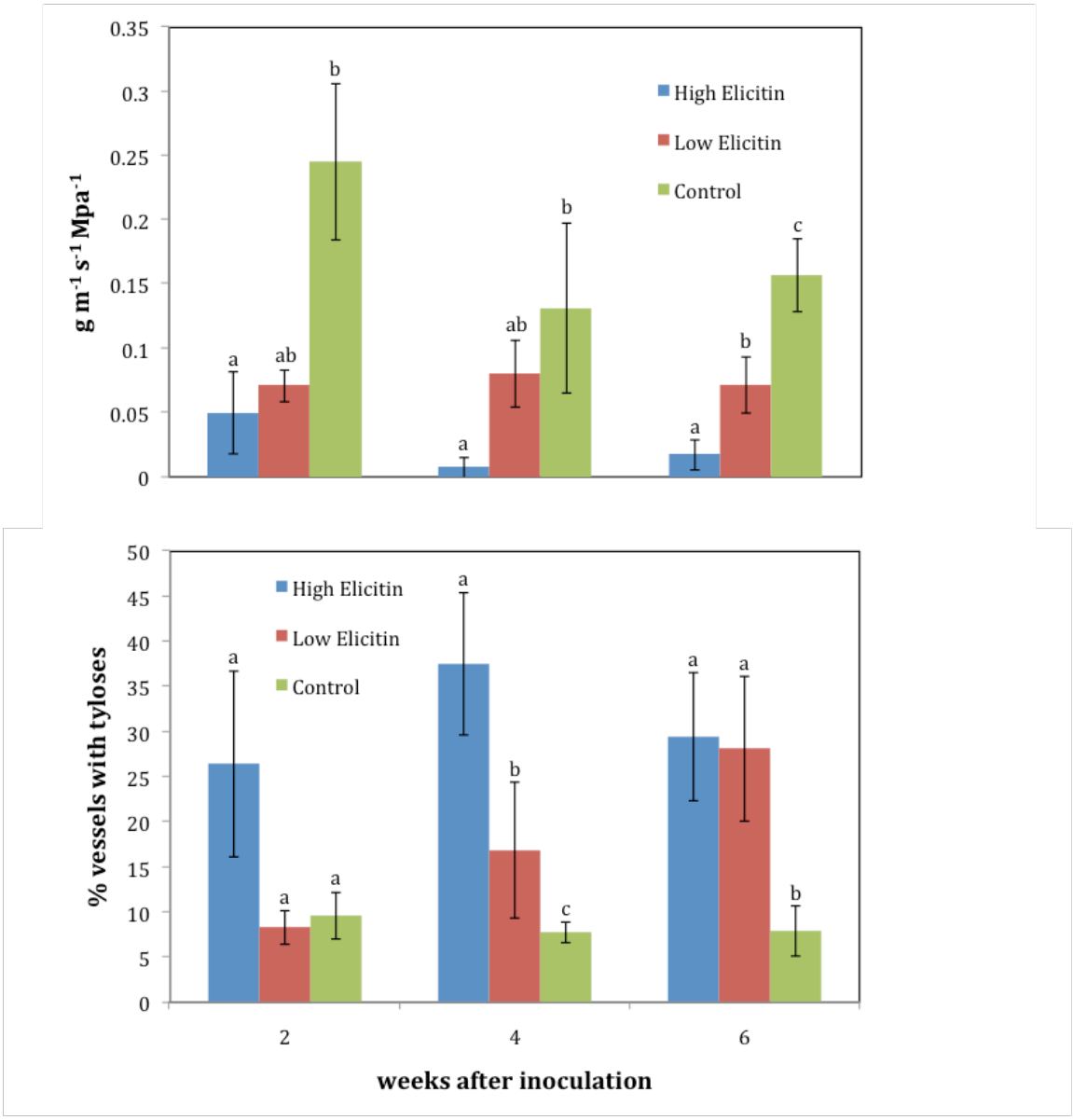


Fig. 6. A) Stem specific hydraulic conductivity and B) tylosis frequency in tanoaks saplings artificially inoculated with a high elicitin-producing *P. ramorum* isolate (PR-07-058), and low elicitin-producing *P. ramorum* isolate (4353) or wounded but not inoculated (control). Bars indicate standard error of the mean. Different lowercase letters within each sampling date represent values that differ significantly ($p \leq 0.05$) based on Tukey's HSD.

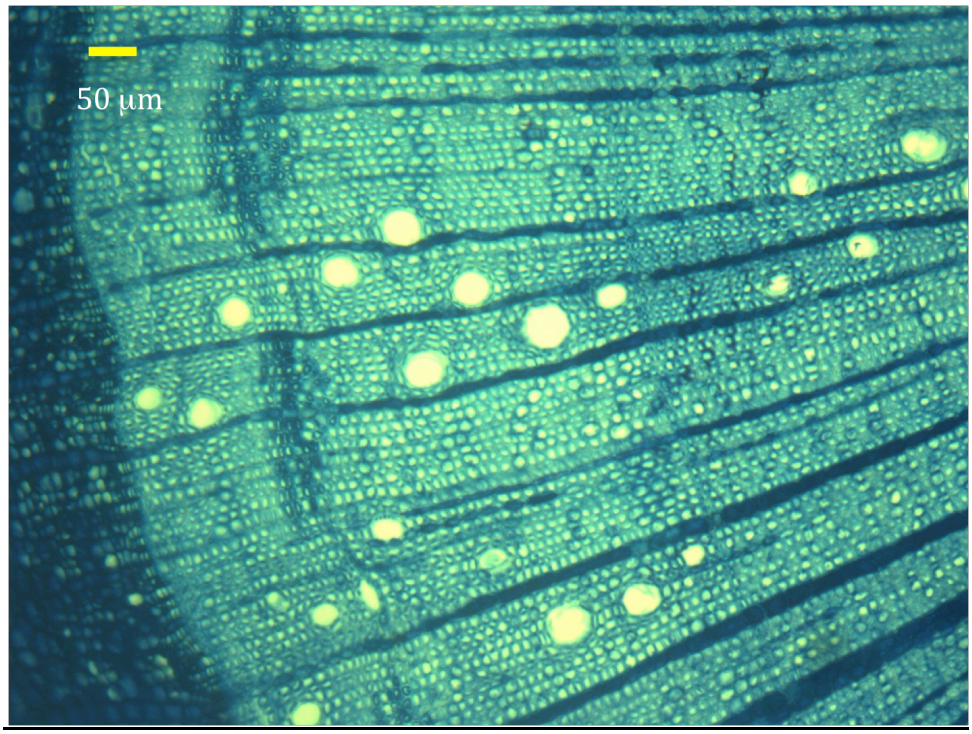


Fig. 7. Cross section of noninoculated tanoak stem showing vessels without tyloses at 10x magnification.

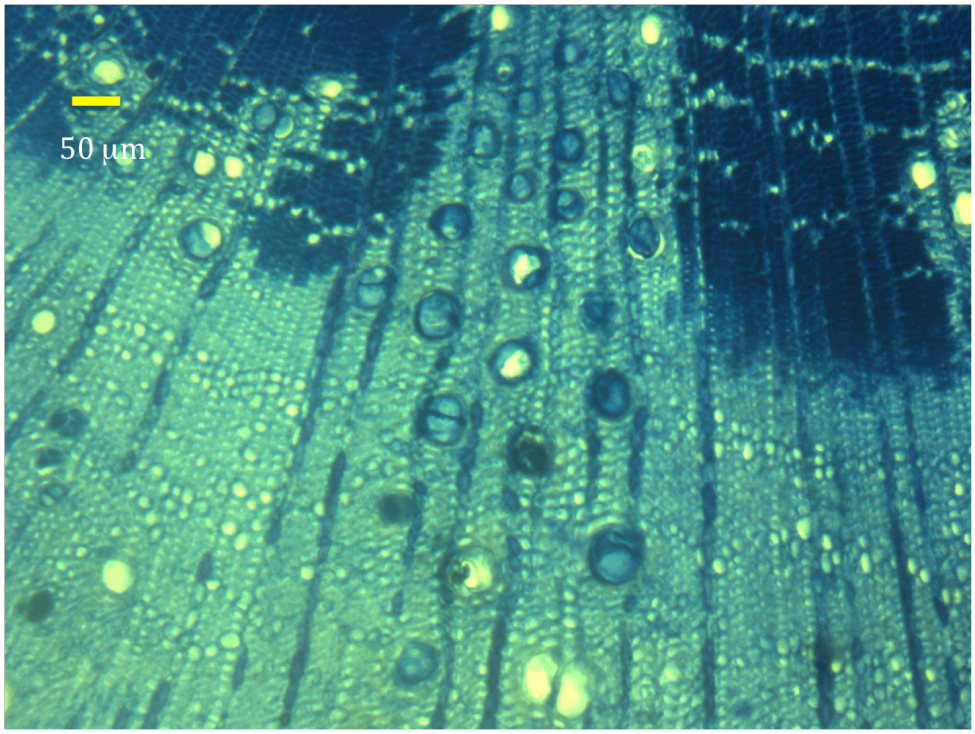


Fig. 8. Cross section of *P. ramorum* infected tanoak stem exhibiting abundant tyloses at 10x magnification.

Isolate growth rates

Isolates grown on CMA amended with β-sitosterol exhibited significantly different growth rates, from 0.68 to 1.31 mm day^{-1} (Table 2) ($p=<0.0001$). For the sapling experiment, trees were inoculated with either PR-07-058, a high elicitin producing isolate, or 4353, a low elicitin producing isolate. However, a pairwise t-test with pooled standard deviation indicated that the mean growth rate for PR-07-058 (1.29 mm day^{-1}) and 4353 (0.68 mm day^{-1}) are significantly different ($p = 0.05$). Because the greater physiological impact of the high-elicitin isolate relative to the low elicitin isolate might be attributable its faster growth rate, two different isolates were selected for subsequent experiments.

For the seedling inoculation trials, the high elicitin producing isolate, PR-05-002, and the low elicitin producing isolate PR-05-166 were selected. These isolates had previously been shown to produce different amounts of elicitin in vitro (Manter et al., 2010), but a pairwise t-test with pooled standard deviation showed that their mean growth rates did not differ significantly ($p = 0.43$).

Table 2. Mean growth rates, and elicitin production for *P. ramorum* isolates representing three clonal lineages. Isolates 4353 and PR-07-057 were used in the sapling inoculation experiment, and isolates PR-06-002 and PR-07-166 were used in the seedling inoculation experiments. Different lowercase letters within each column represent values that differ significantly ($p \leq 0.05$) based on Tukey's HSD.

Isolate	**Clonal lineage**	**Elicitin production (μg/ml)[1]**	**Growth rate (mm/day)**
4313	NA1	5.12 a	0.91 b
4353[2]	NA1	4.52 a	0.68 a
9650	NA1	4.52 a	1.08 b
PR-05-002[3]	NA2	7.92 c	1.07 b
PR-07-031	NA2	7.55 bc	1.32 c
PR-07-057	NA2	6.34 b	0.99 b
PR-07-058[2]	NA2	7.23 bc	1.29 bc
PR-07-166[3]	NA2	4.95 a	0.92 b

[1] (Manter, Kolodny, Hansen, & Parke, 2010)
[2] Isolate used in sapling experiment.
[3] Isolate used in seedling experiments.

Seedling inoculation (Trials 1 and 2)

Two seedling trials were conducted, one month apart. Data from both trials 1 and 2 were initially analyzed as a single, pooled dataset for each measured parameter. A repeated measures ANOVA with the variable "trial" in the model revealed significant differences between trials for net rate of photosynthesis, water usage, and stem specific hydraulic conductivity differences (see Appendix A, Tables 98-103). This indicates that conditions for the two trials may not have been identical. A possible contributing factor to the differences between trials could have been seedling age; seedlings in the second trial were one month older than the seedlings in the first trial. Therefore, data were analyzed for each trial separately.

Seedling inoculation Trial 1

For trial 1, inoculation treatment significantly affected photosynthesis ($p=0.009$) (Appendix A, Table 43). The low elicitin treatment had reduced net photosynthetic rate compared to the wounded control ($p=0.006$) (Fig. 9A). Although there appeared to be a general decline in photosynthesis in both inoculated treatments relative to the control beginning on day 9, none of the treatments differed significantly within any sampling period.

Inoculation treatment also affected stomatal conductance ($p=0.024$) (Appendix A, Table 47) with significantly less stomatal conductance in seedlings inoculated with the low elicitin treatment as compared to the wounded control ($p=0.018$) (Fig. 9B). Twelve days after inoculation, stomatal conductance in the low elicitin treatment was significantly ($p=0.029$) reduced as compared to the control.

Whole plant water usage was not affected by inoculation treatment ($p=0.071$) (Fig. 9C) over the course of the experiment (Appendix A, Tables 43-44).

There were no significant differences between the high elicitin treatment and the control for the previously mentioned parameters. While patterns of means suggest that there were declines in the net rate of photosynthesis, stomatal conductance, and water usage, large standard error precludes these differences from being significant when considered for each measurement period.

Seven days post inoculation there was a reduction in stem specific hydraulic conductivity (Fig. 10) in trees infected with the low elicitin isolate ($p=0.09$) (Appendix A, Table 55). By ten days after inoculation, stem specific hydraulic conductivity was reduced in trees infected with both isolates. Stem specific hydraulic conductivity was reduced to zero by thirteen days after inoculation. Ten days post inoculation there was a reduction in stem specific hydraulic conductivity in trees infected with the high elicitin isolate ($p=0.03$) (Appendix A, Table 57). Stem specific hydraulic conductivity in trees infected with the high elicitin isolate remained reduced for the duration of the experiment.

There were no differences between treatments in terms leaf starch content over the course of the experiment (Fig. 11A), however, seven days post inoculation there was a reduction in root starch content (Fig. 11B) for trees infected with the low elicitin isolate ($p=0.007$) as well as trees inoculated with the high elicitin isolate ($p=0.003$) (Appendix A, Table 66). Root starch content returned to levels comparable to the control treatment the following week.

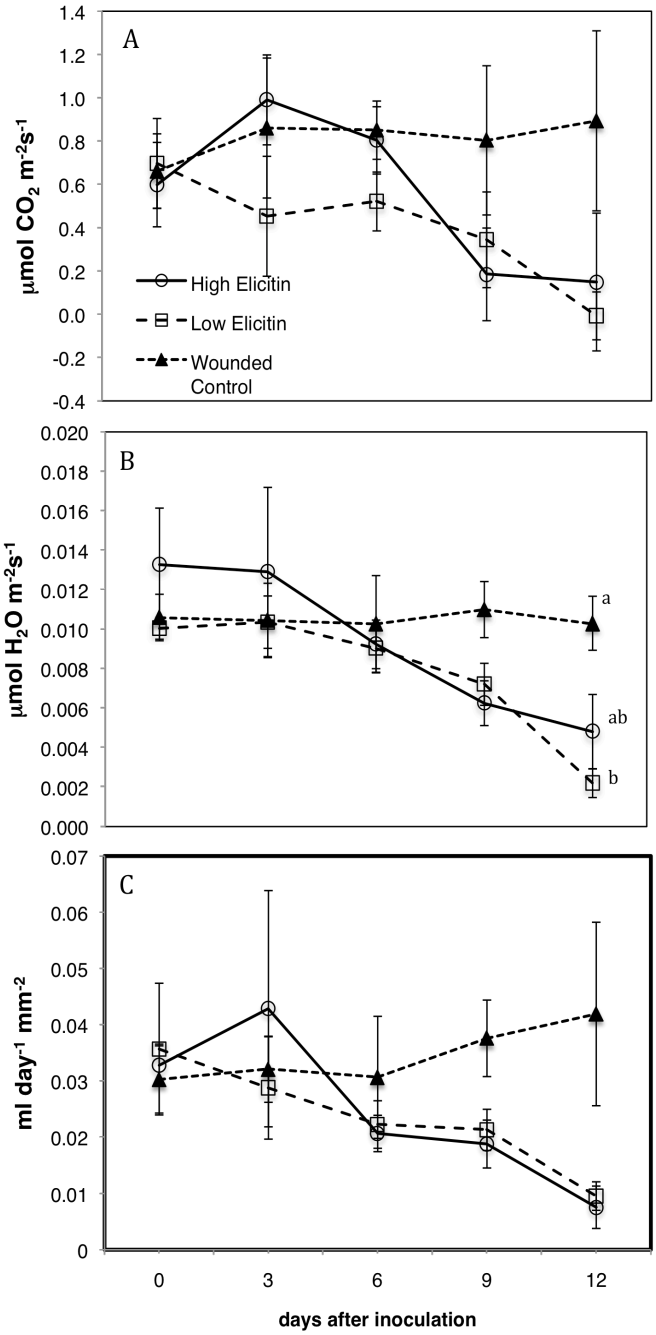


Fig. 9. A) Mean net rate of photosynthesis B) stomatal conductance and C) water usage in tanoak seedlings (Trial 1) artificially inoculated with a high elicitin-producing *P. ramorum* isolate (PR-05-002), and low elicitin-producing *P. ramorum* isolate (PR-07-166) and a non-inoculated wounded control. Bars indicate standard error of the mean. Different lowercase letters within each sampling date represent values that differ significantly ($p \leq 0.05$) based on Tukey's HSD.

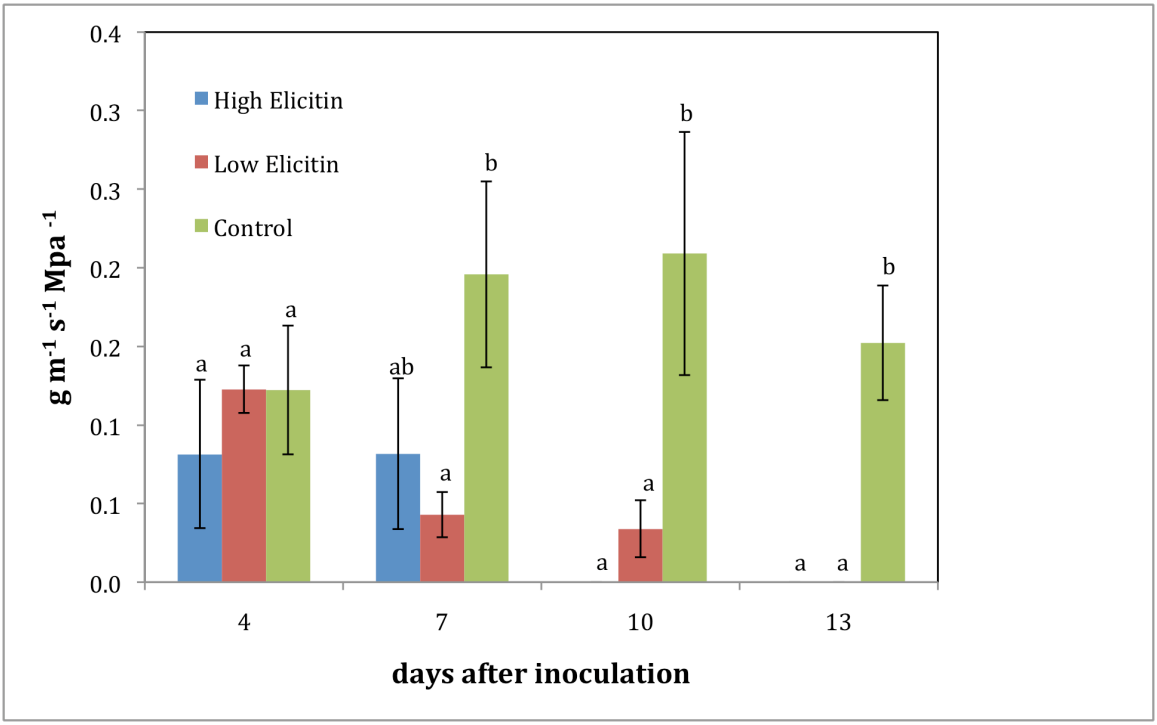


Fig 10. Stem specific hydraulic conductivity in tanoak seedlings (Trial 1) artificially inoculated with a high elicitin-producing *P. ramorum* isolate (PR-05-002), low elicitin-producing *P. ramorum* isolate (PR-07-166) or a noninoculated wounded control. Bars indicate standard error of the mean. Different lowercase letters within each sampling date represent values that differ significantly ($p \leq 0.05$) based on Tukey's HSD.

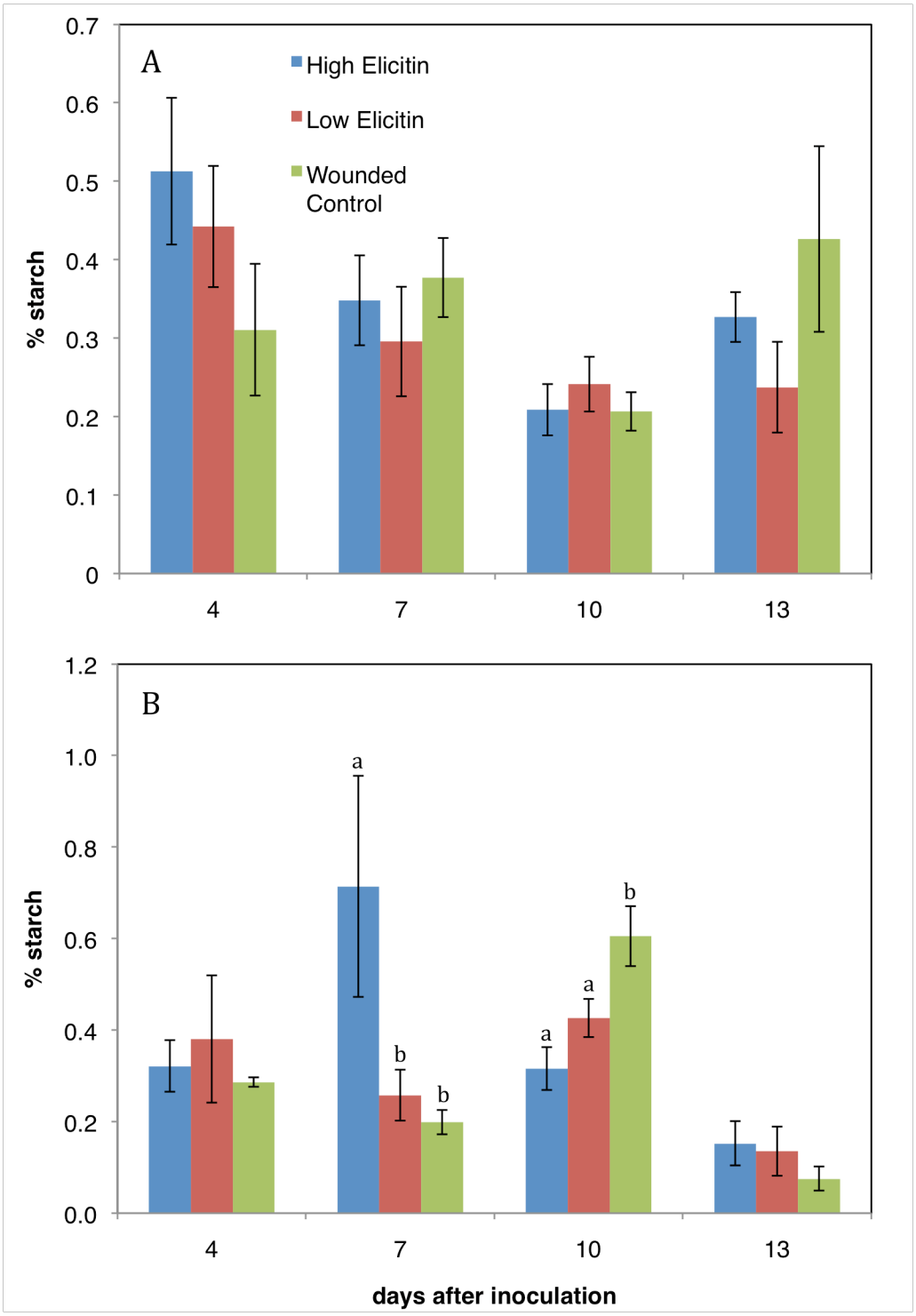


Fig. 11. A) Leaf starch content and B) root starch content in tanoak seedlings (trial 1) artificially inoculated with a high elicitin-producing *P. ramorum* isolate (PR-05-002), low elicitin-producing *P. ramorum* isolate (PR-07-166) and a noninoculated wounded control. Bars indicate standard error of the mean. Different lowercase letters within each sampling date represent values that differ significantly ($p \leq 0.05$) based on Tukey's HSD.

Seedling inoculation Trial 2

For trial 2, inoculation treatment significantly affected photosynthesis (Fig. 12A) ($p=0.005$) (Appendix A, Table 70). The high elicitin treatment and low elicitin treatment had reduced net photosynthetic rate compared to the wounded control (high elicitin vs. control $p=0.07$, low elicitin vs. control $p=0.004$) (Appendix A, Table 71). Although there appeared to be a general decline in photosynthesis in both inoculated treatments relative to the control beginning on day 9, none of the treatments differed significantly within any sampling period.

Inoculation treatment also affected stomatal conductance ($p=0.0006$) (Appendix A, table 74) with significantly less stomatal conductance in inoculated seedlings as compared to the wounded control (high elicitin vs. control $p=0.007$, low elicitin vs. control $p=0.001$) (Appendix A, Table 75) (Fig. 12B). Although there appeared to be a general decline in stomatal conductance in both inoculated treatments relative to the control beginning on day 9, none of the treatments differed significantly within any sampling period.

Whole plant water was also impacted by inoculation treatments ($p=0.01$) (Fig. 12C) over the course of the experiment (Appendix A, Table 78). The high elicitin treatment and low elicitin treatment had reduced whole plant water usage compared to the wounded control (high elicitin vs. control $p=0.04$, low elicitin vs. control $p=0.02$) (Appendix A, Table 79). Although there appeared to be a general decline in stomatal conductance in both inoculated treatments relative to the control beginning on day 9, none of the treatments differed significantly within any sampling period.

There were no significant differences between the high elicitin treatment and the control for the previously mentioned parameters. While patterns of means suggest that there were declines in the net rate of photosynthesis, stomatal conductance, and water usage, large standard error precludes these differences from being significant when considered for each measurement period.

Seven days post inoculation there was a reduction in stem specific hydraulic conductivity (Fig. 13) in trees infected with the high elicitin isolate ($p=0.01$)

(Appendix A, Table 83). By ten days after inoculation, stem specific hydraulic conductivity was reduced in trees infected with both isolates. Stem specific hydraulic conductivity was reduced to zero by thirteen days after inoculation. Ten days post inoculation there was a reduction in stem specific hydraulic conductivity in trees infected with the low elicitin isolate ($p=0.0007$) (Appendix A, Table 87). Stem specific hydraulic conductivity in trees infected with the high elicitin isolate remained reduced for the duration of the experiment.

There were no differences between treatments in terms leaf starch content.

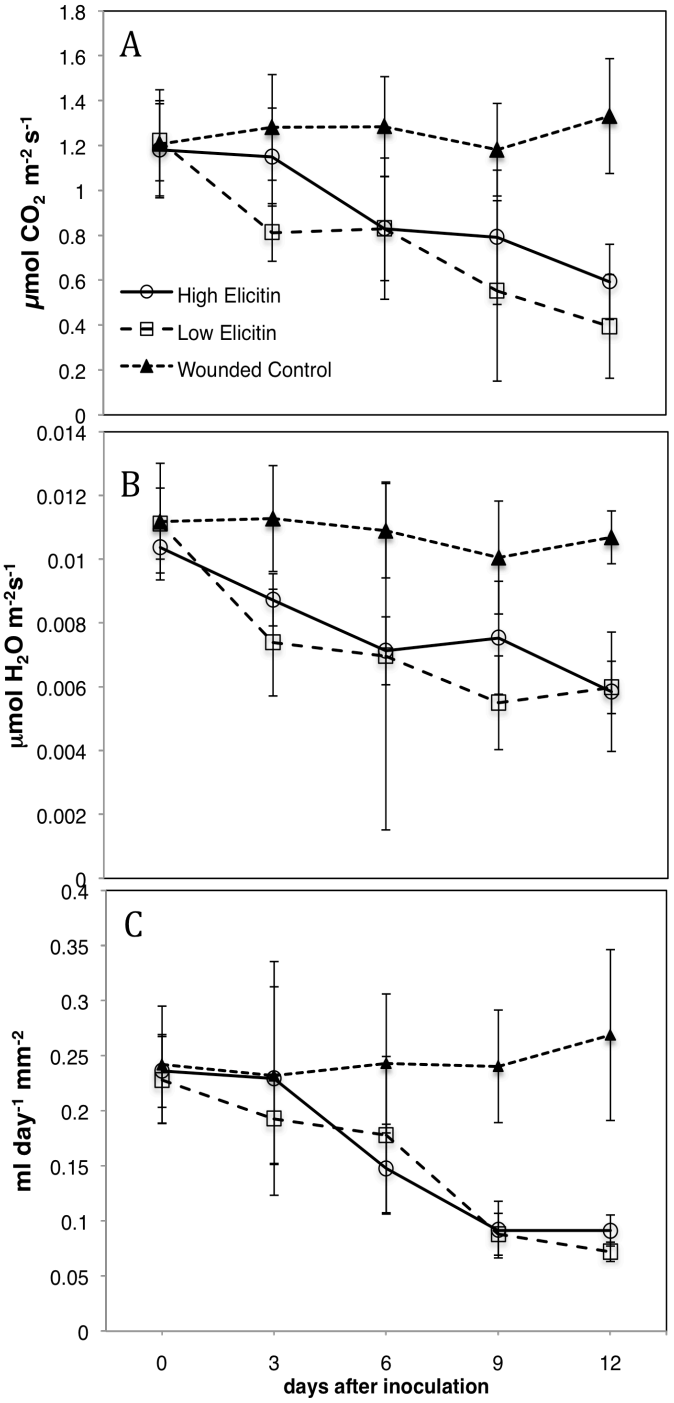


Fig. 12. A) Mean net rate of photosynthesis B) stomatal conductance, and C) water usage in tanoaks seedlings (Trial 2) artificially inoculated with a high elicitin-producing *P. ramorum* isolate (PR-05-002), a low elicitin-producing *P. ramorum* isolate (PR-07-166) and a noninoculated wounded control. Bars indicate standard error of the mean. Within each sampling period, there were no statistically significant differences between treatments.

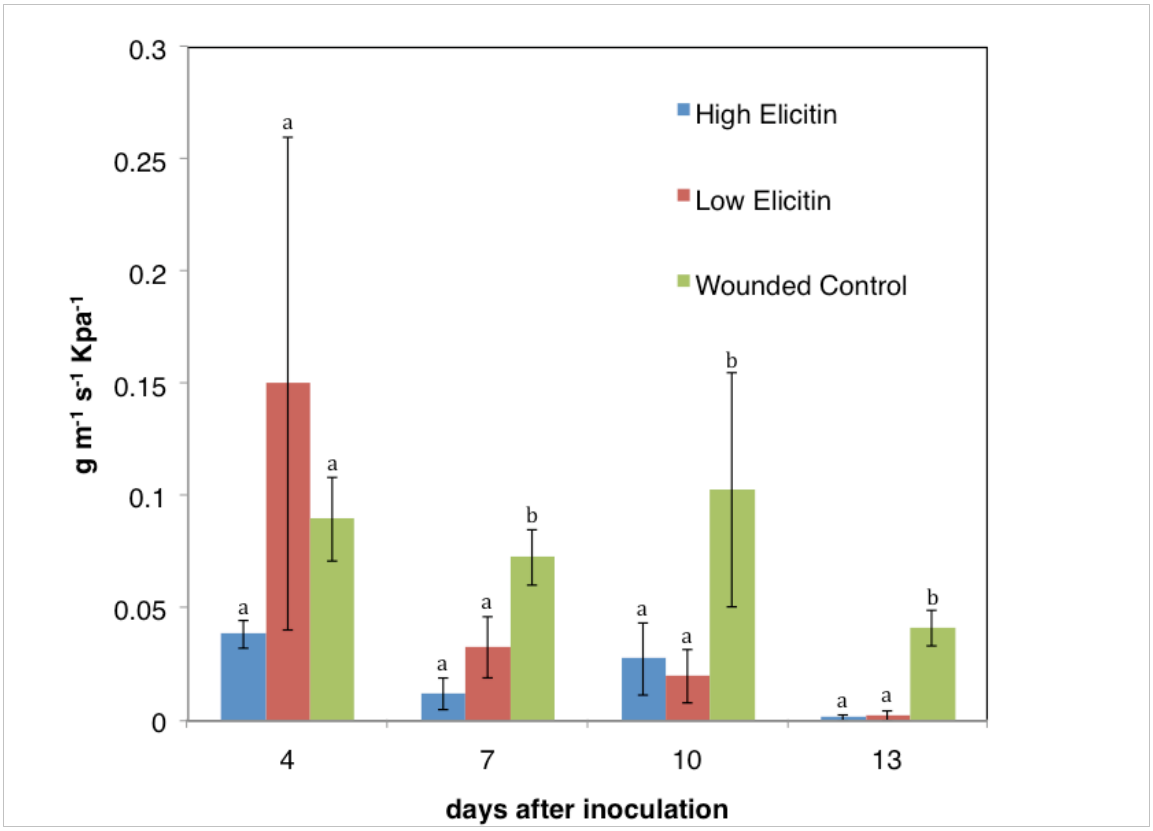


Fig. 13. Stem specific hydraulic conductivity in tanoak seedlings (Trial 2) artificially inoculated with a high elicitin-producing *P. ramorum* isolate (PR-05-002), a low elicitin-producing *P. ramorum* isolate (PR-07-166) and a noninoculated wounded control. Bars indicate standard error of the mean. Different lowercase letters within each sampling date represent values that differ significantly ($p \leq 0.05$) based on Tukey's HSD.

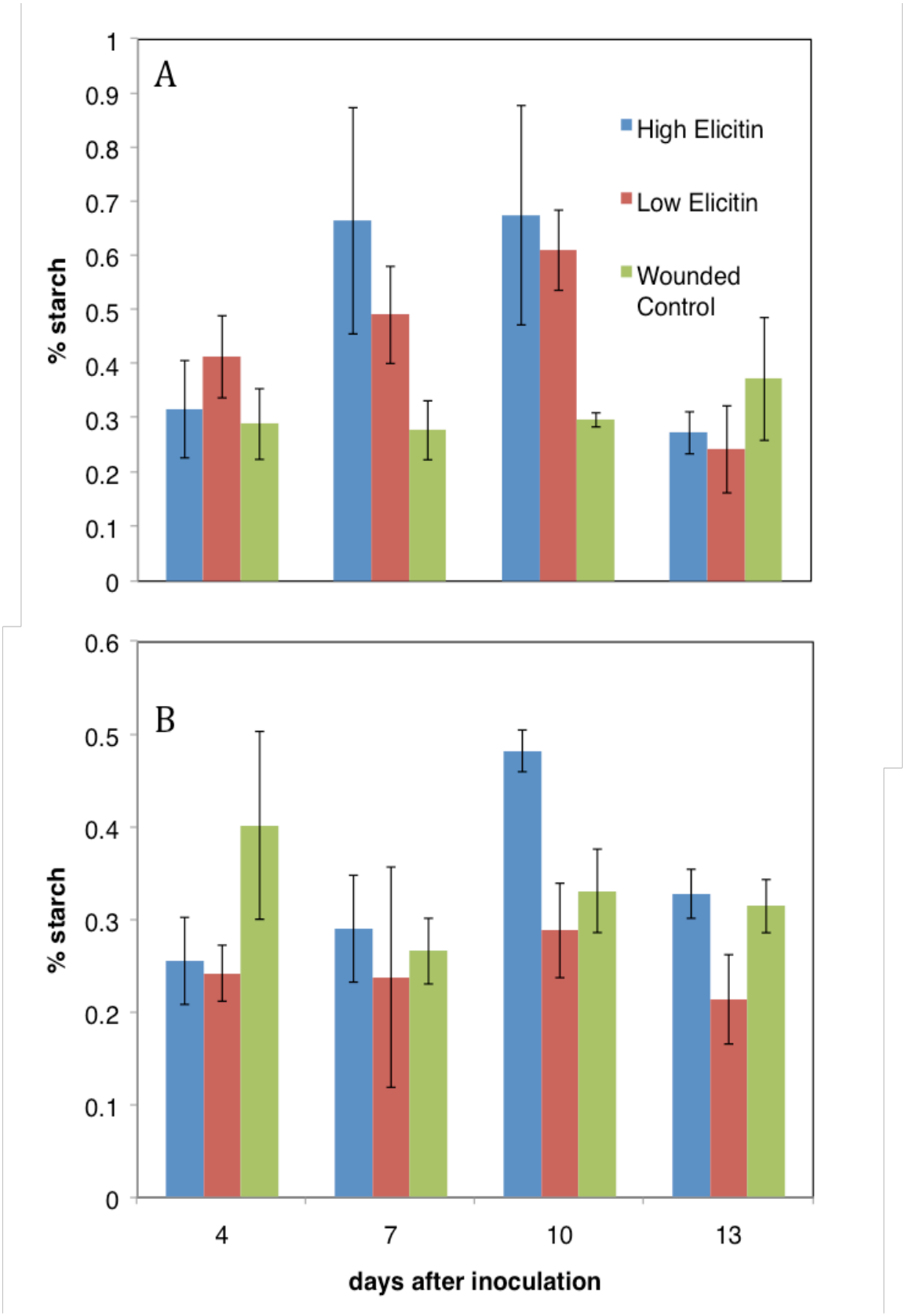


Fig. 14. A) Leaf starch content and B) root starch content in tanoak seedlings (Trial 2) artificially inoculated with a high elicitin-producing *P. ramorum* isolate (PR-05-002), and low elicitin-producing *P. ramorum* isolate (PR-07-166) or a noninoculated wounded control. Bars indicate standard error of the mean. Within each sampling date there were no statistically significant differences between treatments.

Immunolabeling assay

Most uninfected tanoak stem cross-section samples showed little staining when treated with the elicitin-specific fluorescent antibody (Fig. 15). Cross-sections from inoculated tanoak stems treated with elicitin-specific fluorescent antibody exhibited fluorescence in xylem tissue directly adjacent to vessels (Fig. 16 and Fig. 17). There were also noticeable amounts of staining within the pith (Fig. 18). Non-specific staining in the pith was also present in some uninfected tanoak stem samples (Fig. 19).

Most samples of uninfected rhododendron leaf tissue exhibited strong background staining (Fig. 20). In contrast, fluorescence microscopy of infected leaf disks revealed strong fluorescence associated with *P. ramorum* hyphae present on the leaf surface (Fig. 21).

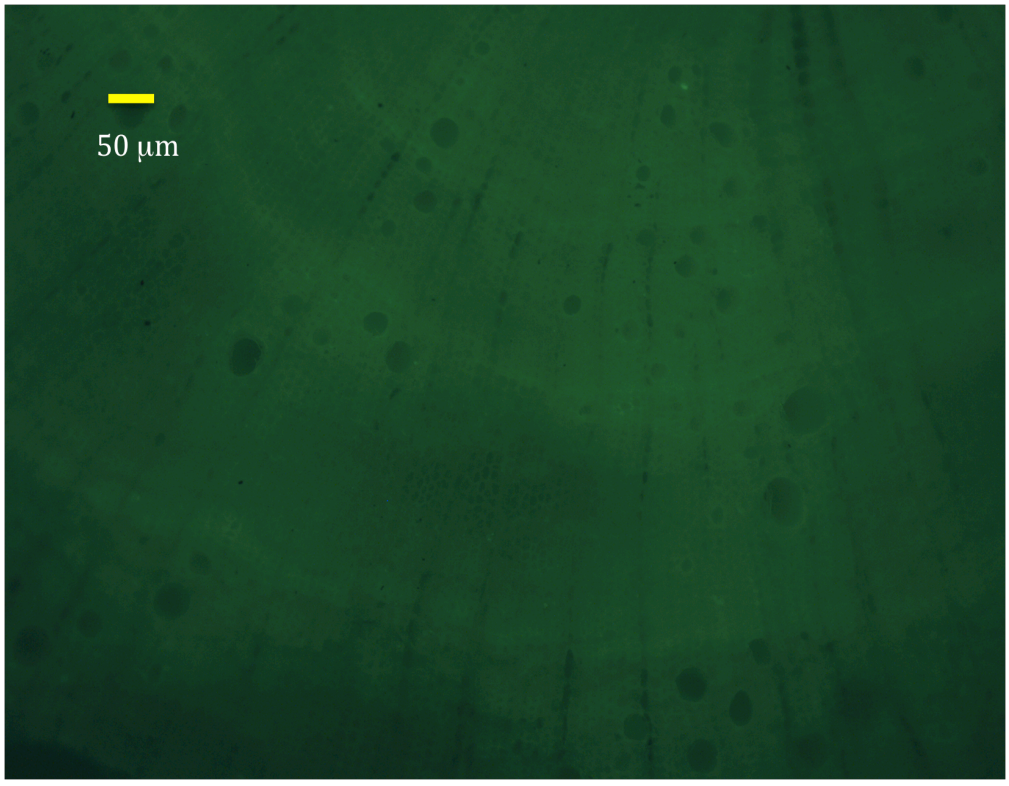


Fig. 15 Cross-section of uninfected tanoak stem treated with elicitin specific fluorescent antibody exhibiting no fluorescence.

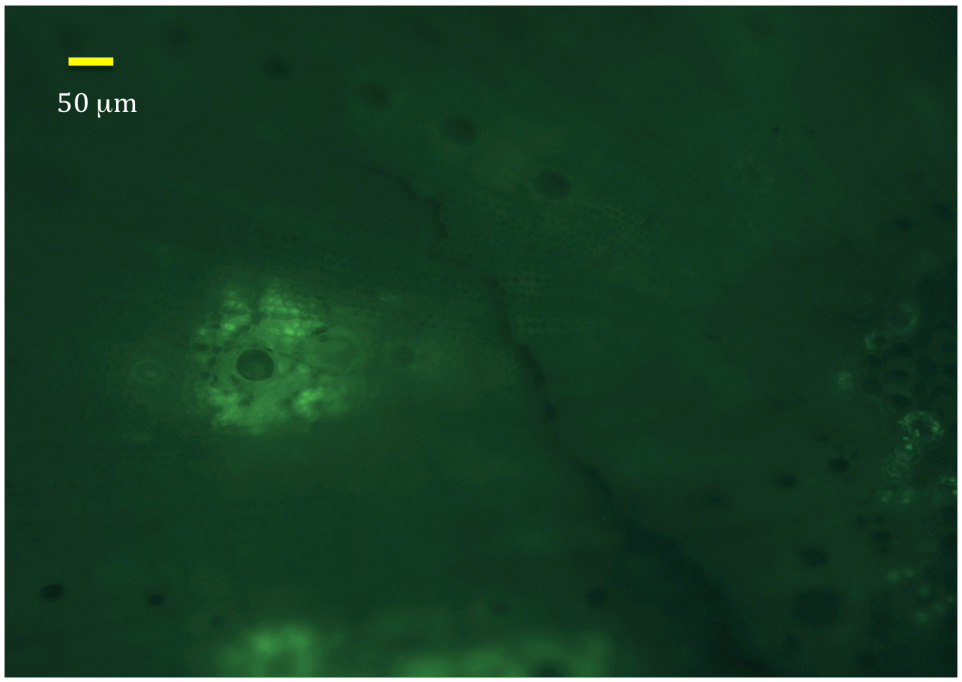


Fig. 16. Cross-section of *P. ramorum* infected tanoak stem treated with elicitin-specific fluorescent antibody exhibiting fluorescence in paratracheal parenchyma cells.

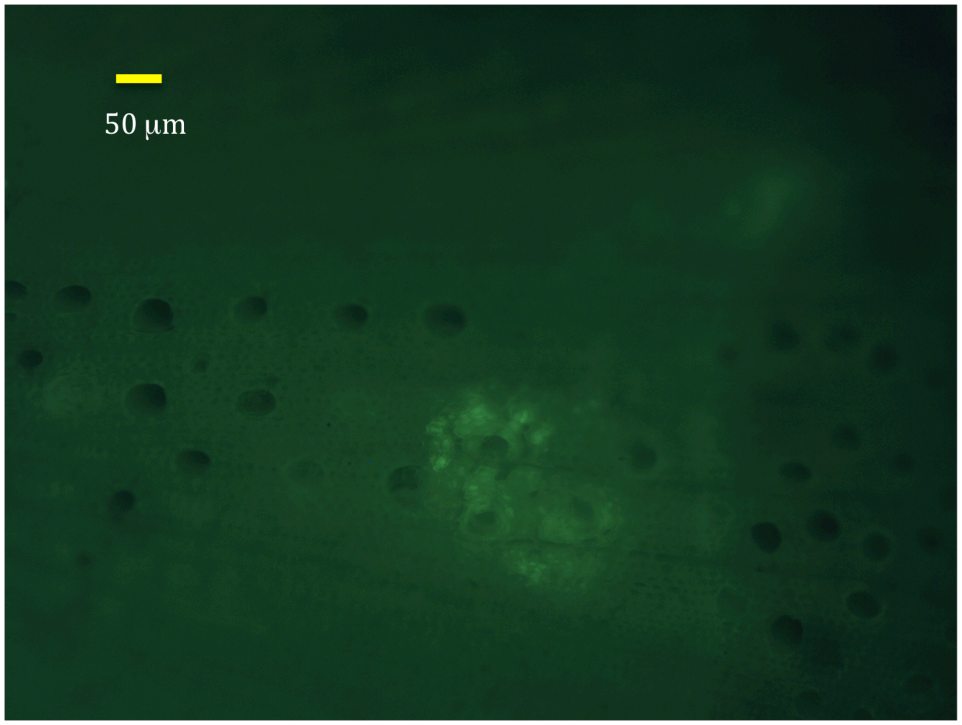


Fig. 17. Cross-section of *P. ramorum* infected tanoak stem treated with elicitin-specific fluorescent antibody exhibiting fluorescence in paratracheal parenchyma cells.

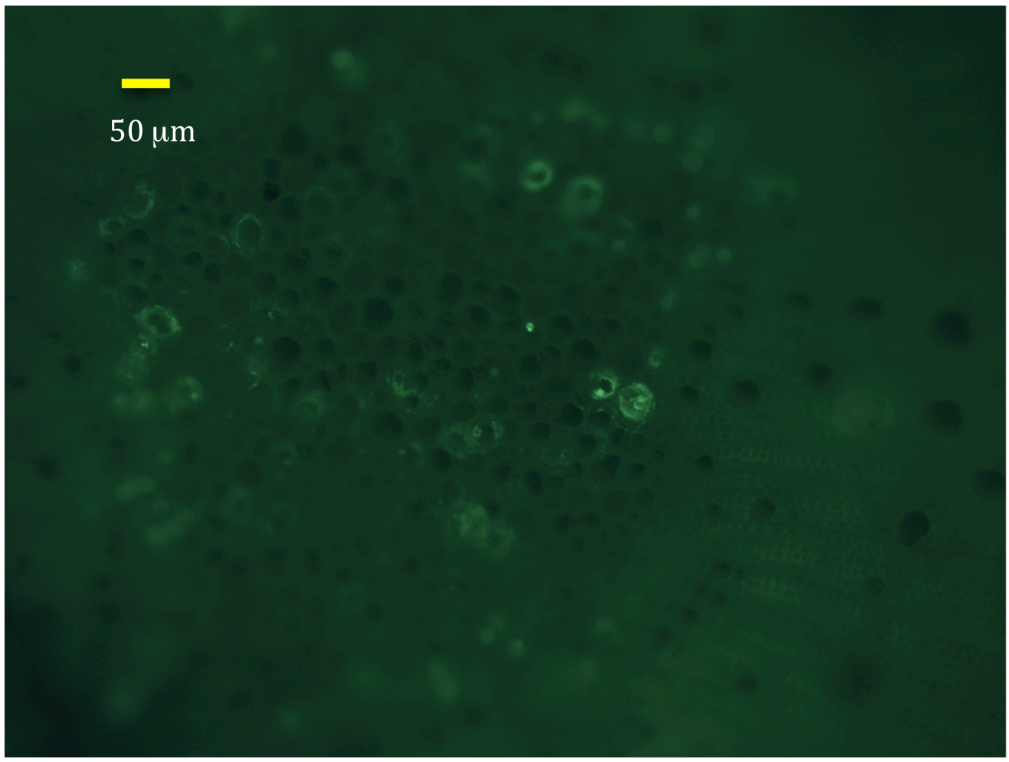


Fig. 18. *P. ramorum* infected tanoak stem treated with eilicitin-specific fluorescent antibody showing fluorescence in the pith.

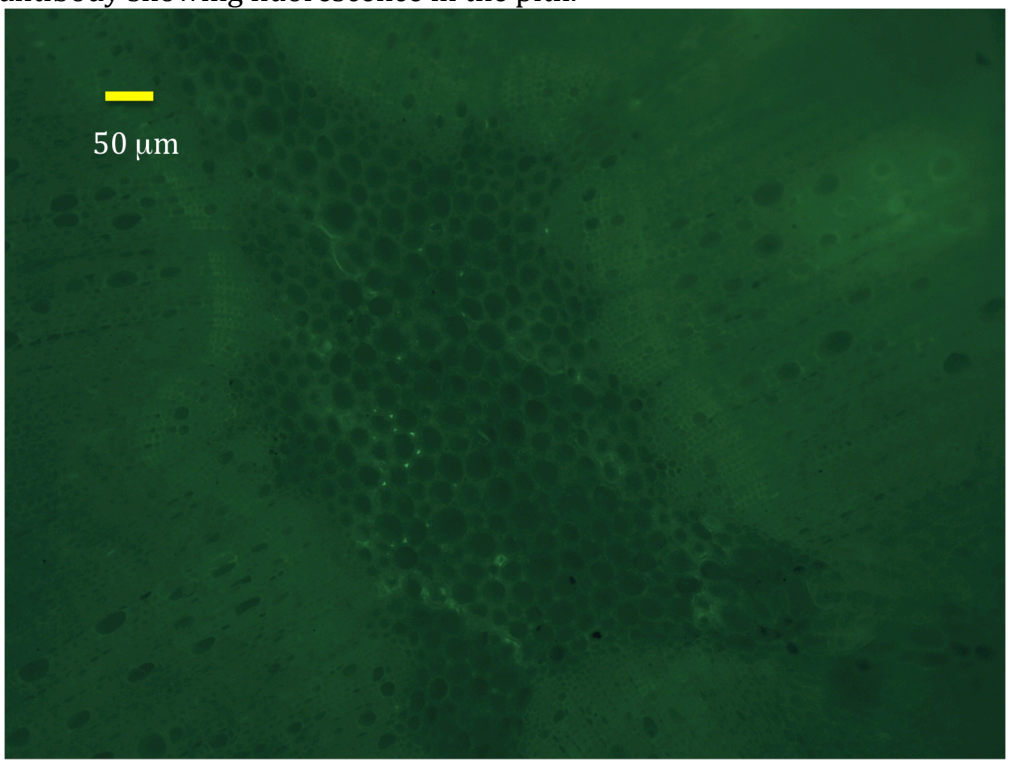


Fig. 19. Uninoculated tanoak stem treated with elicitin-specific fluorescent antibody exhibiting nonspecific staining in pith.

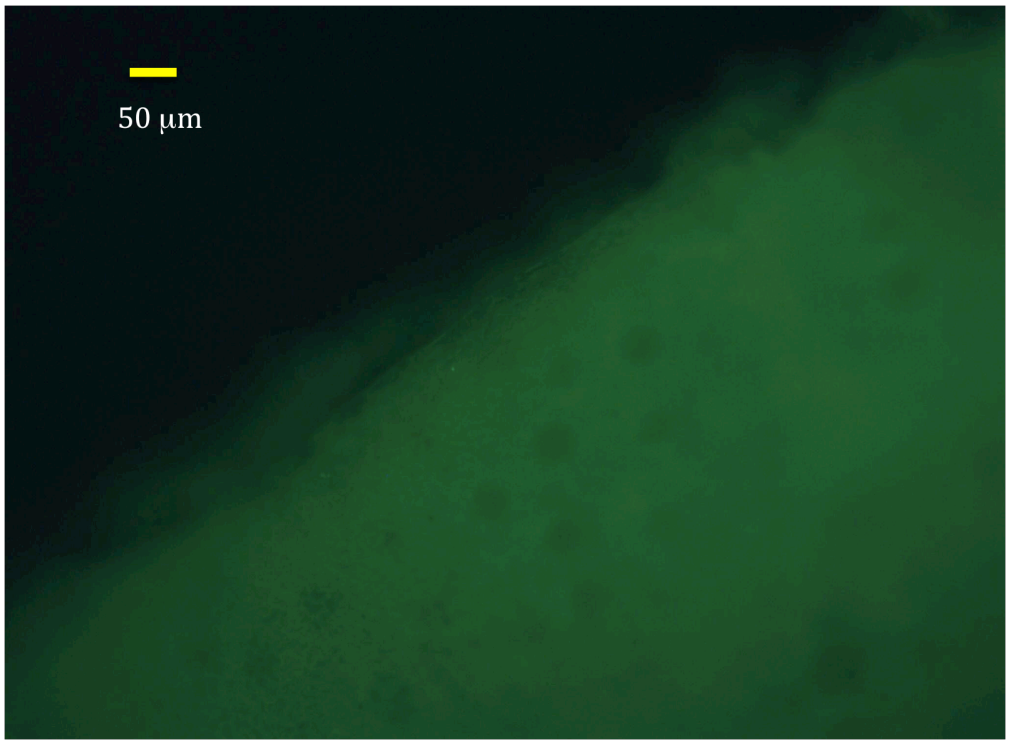


Fig. 20. Uninfected rhododendron leaf disk treated with elicitin-specific fluorescent antibody exhibiting high levels of background staining.

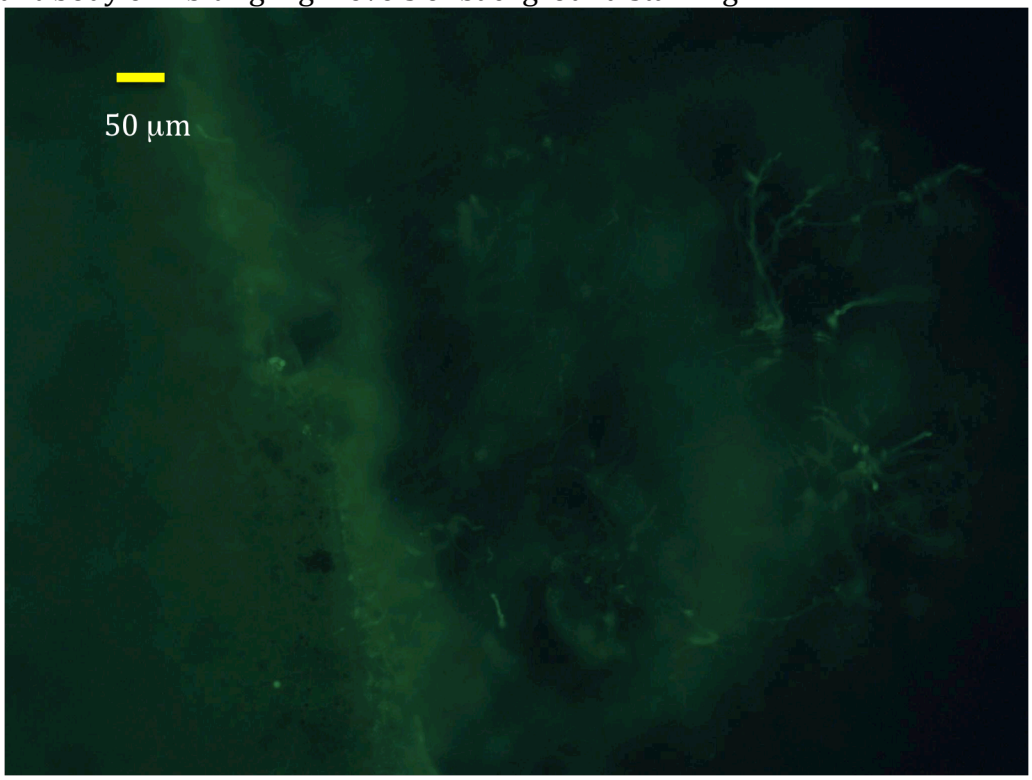


Fig. 21. Margin of *P. ramorum* infected rhododendron leaf disk treated with elicitin-specific fluorescent antibody. *P. ramorum* hyphae exhibiting fluorescence.

Chapter 4: Discussion

This research shows that, of the physiological parameters measured, a reduction in stem specific hydraulic conductivity is the first observable symptom after stem inoculation of young tanoak trees with *P. ramorum.* In all three physiology experiments, stem specific hydraulic conductivity declined before there was any noticeable impact on photosynthesis, stomatal conductance, or water usage. In the sapling inoculation experiment, increased tylosis frequency may have contributed to reduced stem specific hydraulic conductivity. The trees used in the seedling inoculation experiment were too young to form tyloses; however, a reduction in conductivity could have resulted from the production of gums and gels and/or the result of vessel collapse. This suggests that an interruption in water transport is the initial symptom of disease resulting from stem infections, consistent with the hypothesis that water stress contributes to tree death.

The decrease in stem specific hydraulic conductivity likely led to a decrease in the net rate of photosynthesis, and reduced stomatal conductance and water usage. The impact of reduced stem water transport was reflected in the other physiological responses within a few days. Possible reasons for the lag time include drought resistance properties of tanoak, such as abundant trichomes and thick cuticle which reduce transpirational water loss from leaves (Taiz & Zeiger, 2006). So long as guard cells remained hydrated, a reduction in root to shoot water transport would not immediately lead to reduced stomatal conductance and a lower net rate of photosynthesis.

P. ramorum has previously been found to colonize xylem tissue of mature trees (Brown and Brasier, 2007; Parke et al., 2007) and infection has been associated with reduced hydraulic conductivity and tylosis production in mature tanoak (Parke et al., 2007; Collins et al., 2009). In this study, increased tylosis production was observed in tanoak saplings infected with *P. ramorum*; however, tyloses were not seen in experiments performed on seedlings. Tylosis formation is thought to be age-dependent for xylem tissue (Sun et al., 2006). Therefore, it could be that the xylem tissue found in the seedlings was not yet mature enough to

produce tyloses. Reduction in hydraulic conductivity measurements may be the product of gums and gels often associated with pathogen infection (Clerivet et al., 2000). It could also be attributed to the overall damaged state of the xylem tissue, but wounded control trees did not exhibit the same reduction in hydraulic conductivity.

While stem specific hydraulic conductivity was clearly reduced in *P. ramorum* infected tanoak seedlings when compared to the wounded control treatment in bothseedling inoculation trials, there was an overall reduction in stem specific hydraulic conductivity in the second trial as compared to the first trial. The seedlings used for the second trial were approximately one month older than those used in the first, and exhibited a greater extent of secondary growth. As a result of wounding in all treatments, trees may have initiated a general defense response. Trees with a slightly more developed secondary xylem may have been able to respond to wounding more quickly and effectively by producing gums and gels.

In contrast to the current study, where a reduction in stem water transport appears to be the initial symptom in *P. ramorum* infection of stems, previous studies have shown that a reduction in the net rate of photosynthesis was the first observable symptom after the initiation of a *Phytophthora* infection, as follows.

Alder saplings artificially inoculated at the stem base with *P. alni* showed a reduction in carbon assimilation rates, but a less negative leaf water potential as compared to wounded/non-wounded or non-inoculated/non-infected controls, indicating that the pathogen did not reduce stem water transport. Leaves on infected trees also exhibited increased starch concentrations relative to non-infected controls, suggesting that photosynthate accumulation due to phloem damage triggered stomatal closure. The stomatal closure then resulted in a decline in the net rate of photosynthesis (Clemenz et al., 2008).

In a study on rhododendrons artificially inoculated with *P. ramorum,* photosynthesis was believed to be affected before plant water stress symptoms occurred (Manter et al., 2007).The efficiency of rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase), a key enzyme in carbon fixation, was reduced first,

followed by a reduction in stomatal conductance and hydraulic conductivity. However, since leaf starch content was not measured in this study, it is also possible that carbohydrate accumulation in leaves contributed to stomatal closure in this instance. Additional experiments conducted on leaf disks led to the hypothesis that the reduction in the net rate of photosynthesis is due to the *P. ramorum* elicitins, RAM1 and RAM2, traveling systemically through infected plants and reducing chlorophyll functionality (Manter et al., 2007).

Reported differences in certain physiological responses to *Phytophthora* infection among researchers could result from several different factors related to the host species, the method of inoculation, the age of the hosts, and the biology of the particular *Phytophthora* species involved. Tanoak, like many species of true oak, is known to have large rays with several tangential rows of ray parenchyma, providing a nutrient-filled pathway to xylem colonization. Both rhododendron and alder have narrow, uniseriate rays (Hoadley, 1990), which may make it more difficult for *Phytophthora* species to colonize the xylem. However, xylem colonization occurred in several tree species, including *Acer nikoens and Acer pseudoplatanus* (Brown and Brasier 2007) , both of which have narrow rays.Tanoak, unlike rhododendron and alder, also forms tyloses readily. *P. ramorum* infection is associated with tylosis production in tanoak, and tylosis frequency has been correlated with reduced hydraulic conductivity (Collins et al., 2009). The inoculation methods used for physiological tests also differed between researchers. Manter et al. (2007) inoculated plants with a mycelial plug below the lowest leaf, and alder studied by Clemenz et al. (2008) were inoculated at the stem base in contrast to the stem inoculations placed midway up the stem used in the present study. *P. ramorum* characteristically causes bole cankers (as well as twig infections) on tanoak, whereas *P. alni* is primarily a root and collar pathogen of alder.

While interruption in water transport is the first of the observed symptoms in this study to occur in tanoak seedlings and saplings, it is quite possible that the sequence of events might be different in older trees with more mature tissue. There are many structural and mechanical differences between mature and juvenile wood. Juvenile wood tends to be less dense with shorter and weaker fiber tracheids and

less frequent vessels and parenchyma cells (Bao et al., 2001). The stronger material of mature wood could prevent some of the xylem damage observed in juvenile tissue. More mature trees may also require a greater capacity to create and store carbohydrates, whereas juvenile trees can deplete carbohydrates overnight (Taiz & Zeiger, 2006). However, nonstructural carbon storage in plants is still poorly understood. Certain tree species may actively store sugars to regulate osmotic potential in leaf tissue, whereas carbon storage may be a solely passive process in other tree species (Sala et al., 2012). As a result, older trees may exhibit initial symptoms more related to phloem damage than to xylem obstruction. However, previous studies on mature, naturally infected (Parke et al., 2007) or mature, inoculated (Collins et al., 2009) tanoak demonstrate that stem hydraulic conductivity and sap flow are restricted relative to non-infected trees.

The impact of elicitin secretion on symptom development remains poorly understood and requires further investigation. While elicitins have been shown to trigger both specific and general defense responses in non-host species when recognized by specialized kinase-like receptors (Zhang et al., 1998), it is unclear whether host plants possess the necessary molecular equipment to recognize elicitins.

Due to the hypersensitive-like responses elicitins can initiate in non-host species (Ricci et al., 1989) they will most likely remain a molecule of interest when examining the cause of host mortality. Currently there is conflicting evidence regarding the involvement of elicitins in pathogenesis. Host leaf disk assays examining the impact of purified *P. ramorum* elicitins showed that leaves exposed to the elicitin had reduced chlorophyll functionality and exhibited symptoms indicative of a hypersensitive response including increased ethylene production and H^+ uptake (Manter et al., 2007). Further experiments conducted on detached rhododendron leaves infected with several different *P. ramorum* isolates showed a strong relationship between elicitin production in vitro and pathogen virulence. Isolates with higher levels of elicitin production in vitro consistently produced larger, necrotic lesions than isolates with lower levels of elicitin production in vitro (Manter et al., 2010). Conversely, European beech trees that were artificially

inoculated with *P. citricola* exhibited no physiological changes when elicitin production occurred in planta (Fleischmann et al., 2005). Due to the many differences between the composition of leaf tissue and stem tissue, the elicitin could play more or less of a role in pathogenesis depending on the location of infection.

Isolates used in the physiological experiments failed to elucidate the role of elicitins in pathogenesis. Results from the experiment with tanoak saplings were confounded by the significantly different growth rates as well as several genotypic differences of the chosen isolates. Isolates chosen for physiological experiments on tanoak seedlings, while belonging to the same clonal lineage and having no drastic difference in growth rate, may not have had a great enough difference in elicitin secretion to create differences in symptom production. Because of these confounding factors, it would be impossible to attribute any difference in symptom production to elicitin secretion alone. It seems that the only way to truly understand the role of elicitins in pathogenesis would be to create an elicitin knockout strain of *P. ramorum*.

Attempts to visualize elicitin localization using a fluorescent immunolabeling technique were only partially successful. Fluorescence observed in association with paratracheal parenchyma, as found in the current study, is thought to indicate elicitin presence. Roots of oak trees infected with *P. quercina* also revealed the *P. quercina* elicitin, quercinin, present in the intercellular space surrounding the cortical parenchyma (Brummer et al., 2002). However, in the present study, nonspecific staining was also found in control samples.

Immunolabeling techniques used to examine the presence and localization of *P. ramorum* elicitins in infected tanoak bark showed elicitins in association with hyphae (Giesbrecht et al., 2011). Fluorescence was most prominent in hyphal tips, which is where one would expect to see the greatest concentration of a secreted protein in *Phytophthora* species (Alexopolous et al., 1996). It has been suggested that elicitins travel systemically through the xylem of host plants (Manter et al., 2007). It is possible that the selected stem cross-sections may have either not contained actively growing hyphal tips or been the target of elicitin secretion at the time of sampling.

This research contributes to a growing body of knowledge about how *Phytophthora* species kill trees. In a recent review, Davison (2011) described four different main mechanisms of pathogenesis: 1) extensive fine-root necrosis leading to reduced water uptake, decline and death; 2) root and stem cankers resulting from phloem colonization and death of the cambium, leading to carbon starvation of the roots; 3) xylem invasion, reducing hydraulic conductivity, leading to death by water deficit; and 4) hormonal imbalance and/or damage from toxins *sensu lato* (including elicitins). Because *P. ramorum* does not colonize tanoak roots, fine root necrosis can be ruled out as a cause of death, as can hormonal imbalances resulting from root necrosis. Clearly, *P. ramorum* does infect xylem and can reduce hydraulic conductivity of stems, in young trees as well as mature trees. The research presented here suggests that these symptoms occur before effects on photosynthesis and stomatal conductance in young tanoak trees. However, it is possible that *P. ramorum*, and other canker-causing *Phytophthora* species that invade the xylem, may kill trees through a combination of mechanisms resulting from both xylem and phloem colonization. Toxins or elicitins may also contribute to pathogenesis. Further comprehensive studies of host physiology are needed to resolve this issue.

Concluding remarks

This research provides important insights regarding the physiological impact of *P. ramorum* infection on young tanoak. Xylem damage appears to play a significant early role in the development of disease symptoms in stem-inoculated tanoak seedlings and saplings, but the importance of elicitins and the involvement of phloem damage and starch accumulation in pathogenesis are still unclear.

Several limitations were made evident over the course of these experiments, and should be taken into account when planning future experiments. It was important to work with uniform experimental units, and quarantine restrictions required that these experiments be conducted in a growth chamber on potted

plants. However, potted tanoak trees are difficult to procure. Their limited availability led to a sample size that was smaller than ideal for experiments. Because of the variable nature of the physiological parameters measured, a larger sample size would have increased the power of the statistical analysis. The trees that were available were far younger and smaller than most affected trees found in nature. While valuable insight was gained through these experiments, there are limitations in applying the results to infections found in nature. Similarly, although wound inoculation of the stem is a common practice in studies with forest pathogens such as *P. ramorum*, another inoculation method such as zoospore inoculum, which does not requiring wounding, might have been preferable.

Future experiments should be performed on larger trees with much mature tissue to more accurately simulate infection on the most commonly afflicted tree size. This cannot be accomplished in a growth chamber. Additionally, the Conviron nd Percival growth chambers provided several challenges. While they are each equipped with a full spectrum light source, it was impossible to replicate the levels of photosynthetically active radiation (PAR) found in nature. Measurements of the net rate of photosynthesis taken on trees acclimated to natural light were significantly higher than those taken on trees acclimated to artificial light (data not shown). Ideally, future experiments should be conducted at an outdoor facility such as the National Ornamentals Research Site at Dominican University of California (NORSDUC) located within a quarantined county.

The isolates used during the physiological experiments also posed a series of challenges. The isolates used in the first physiology experiment (PR-07-058 and 4353) were chosen for their drastically different levels of elicitin secretion. However, their significantly different growth rates and overall genotypic differences disallow comparisons based on elicitin secretion alone. The isolates used in the second and third physiology experiments (PR-05-002 and PR-07-166) had similar growth rates, and were of the same clonal lineage, but while the difference in elicitin secretion was statistically significant, it is difficult to say whether the relatively small difference was biologically significant.

To truly elucidate the role of elicitins in pathogenesis, an elicitin knockout strain or strain with upregulated elicitin production must be made. Finally, immunolabeling techniques offer potential for observing the site of elicitin production in planta, but greater consideration should be given to choosing an appropriate sampling scheme, taking into account both the spatial and temporal characteristics of elicitin secretion and transport.

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Appendix A: Statistical tables

Contents

Tables 1-39: Sapling experiment

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Table 1: Repeated measures ANOVA comparing differences in the net rate of photosynthesis over time.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	2.290	1.145	0.7570	0.47049
Week	4	7.877	1.969	1.3019	0.27085
Treatment*Week	8	24.110	3.014	1.9924	0.04937
residuals	190	287.392	1.513		

Table 2: ANOVA comparing differences in the net rate of photosynthesis between treatments for week one of tanoak sapling experiment.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	1.712	0.856	0.4745	0.6246
Residuals	57	102.842	1.804		

Table 3: ANOVA comparing differences in the net rate of photosynthesis between treatments for week two of tanoak sapling experiment.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	3.131	1.565	1.2641	0.2903
Residuals	57	70.580	1.238		

Table 4: ANOVA comparing differences in the net rate of photosynthesis between treatments for week three of tanoak sapling experiment.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	13.58	6.79	6.9094	0.002957
Residuals	35	34.394	0.983		

Table 5: Tukey's HSD table comparing differences in the net rate of photosynthesis between treatments during week 3 of tanoak sapling experiment..

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	-1.5536639	-2.6157573	-0.4915705	0.0028937
L-C	-0.2109533	-1.0968004	0.6748938	0.8301731
L-H	1.3427106	0.2806172	2.4048039	0.0105414

Table 6: ANOVA comparing differences in the net rate of photosynthesis between treatments for week four of tanoak sapling experiment.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	4.9495	2.4748	2.6965	0.09072
Residuals	21	19.2735	0.9178		

Table 7: Tukey's HSD table comparing differences in the net rate of photosynthesis between treatments during week 4 of tanoak sapling experiment.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	-1.13910533	-2.4617088	0.1834981	0.0998242
L-C	-0.04654815	-1.1560411	1.0629448	0.9938555
L-H	1.09255719	-0.2543162	2.4394306	0.1262539

Table 8: ANOVA comparing differences in the net rate of photosynthesis between treatments for week five of tanoak sapling experiment.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	5.3234	2.6617	1.8985	0.1758
Residuals	20	28.0402	1.4020		

Table 9: Repeated measures ANOVA comparing differences in stomatal conductance over time for tanoak sapling experiment.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.003329	0.001664	6.7183	0.001513
Week	4	0.001018	0.000254	1.0271	0.394495
Treatment*Week	8	8 0.001938	0.000242	0.9780	0.454462
residuals	192	0.047566	0.000248		

Table 10: ANOVA comparing differences in stomatal conductance between treatments for week one of tanoak sapling experiment.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.0001087	0.0000544	0.1598	0.8527
Residuals	57	0.0193903	0.0003402		

Table 11: ANOVA comparing differences in stomatal conductance between treatments for week two of tanoak sapling experiment.

	DF	Sum Sq	Mean Sq	F-value	p-value

Treatment	2	0.0005478	0.0002739	1.0663	0.3510
Residuals	57	0.01464	0.0002568		

Table 12: ANOVA comparing differences in stomatal conductance between treatments for week three of the first physiology experiment.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.0020665	0.0010333	5.6198	0.006646
Residuals	35	0.0064352	0.0001839		

Table 13: Tukey's HSD table comparing differences in stomatal conductance between treatments during week 3 of tanoak sapling experiment.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	-0.018331777	-0.032859654	-0.003803902	0.0106997
L-C	-0.000502666	-0.012619753	0.011614420	0.9943347
L-H	0.0178291113	0.003301235	0.032356988	0.0132713

Table 14: ANOVA comparing differences in stomatal conductance between treatments for week four of the first physiology experiment.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.0007894	0.0003947	3.8259	0.0383
Residuals	21	0.00216645	0.00010316		

Table 15: Tukey's HSD table comparing differences in stomatal conductance between treatments during week four of tanoak sapling experiment.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	-0.015143152	-0.028951510	-0.001334793	0.0299867
L-C	-0.004272235	-0.016168178	0.007623708	0.6430414
L-H	0.010870917	-0.003724116	0.025465950	0.1699694

Table 16: ANOVA comparing differences in stomatal conductance between treatments for week five of tanoak sapling experiment.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.001077	0.0005385	3.0076	0.07103
Residuals	21	0.00357599	0.000179		

Table 17: Tukey's HSD table comparing differences in stomatal conductance between treatments during week five of tanoak sapling experiment.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	-0.017283454	-0.035474333	0.000907424	0.0645169
L-C	-0.002335871	-0.018007368	0.013335626	0.9254206
L-H	0.014947583	-0.004279646	0.034174813	0.1471815

Table 18: Repeated measures ANOVA comparing differences in water usage over

timefor tanoak sapling experiment.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.22241	0.11121	12.4735	7.409e-06
Week	4	0.11834	0.02959	3.3185	0.01154
Treatment*Week	8	0.05145	0.00643	0.7213	0.67261
residuals	219	1.95245	0.00892		

Table 19: ANOVA comparing differences in water usage between treatments for week one of tanoak sapling experiment.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.025885	0.012942	2.4068	0.0992
Residuals	57	0.306513	0.005377		

Table 20: Tukey's HSD table comparing differences in water usage between treatments during week oneof tanoak sapling experiment.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	-0.05045729	-0.10626040	0.005345827	0.0840633
L-C	-0.03087530	-0.08667841	0.024927813	0.3838431
L-H	0.01958199	-0.03622113	0.075385098	0.6771489

Table 21: ANOVA comparing differences in water usage between treatments for week two of tanoak sapling experiment.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.09058	0.04529	2.5604	0.08614 .
Residuals	57	1.00825	0.01769		

Table 22: Tukey's HSD table comparing differences in water usage between treatments during week two of tanoak sapling experiment.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	-0.08872417	-0.18993299	0.01248466	0.0968570
L-C	-0.01453855	-0.11574737	0.08667027	0.9363297
L-H	0.07418562	-0.02702321	0.17539444	0.1909505

Table 23: ANOVA comparing differences in water usage between treatments for week three of tanoak sapling experiment.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.043604	0.021802	2.9428	0.06394
Residuals	41	0.303750	0.007409		

Table 24: Tukey's HSD table comparing differences in water usage between

treatments during week three of tanoak sapling experiment.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	-0.06167341	-0.13191735	0.008570536	0.0952295
L-C	0.03976025	-0.08738755	0.166908055	0.7291063
L-H	0.10143366	-0.03262514	0.235492466	0.1694955

Table 25: ANOVA comparing differences in water usage between treatments for week four of tanoak sapling experiment.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.120328	0.060164	10.209	0.0002523
Residuals	41	0.241621	0.005893		

Table 26: Tukey's HSD table comparing differences in water usage between treatments during week four of tanoak sapling experiment.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	-0.13143167	-0.202167344	-0.060695993	0.0001514
L-C	-0.06258648	-0.129675589	0.004502628	0.0718094
L-H	0.06884519	-0.000856633	0.138547010	0.0534986

Table 27: ANOVA comparing differences in water usage between treatments for week five of tanoak sapling experiment.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.017293	0.008647	2.1542	0.1388
Residuals	23	0.092317	0.004014		

Table 28: Tukey's HSD table comparing differences in water usage between treatments during week fiveof tanoak sapling experiment.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	-0.06775966	-0.14969153	0.01417220	0.1181822
L-C	-0.02190716	-0.09286224	0.04904791	0.7228516
L-H	0.04585250	-0.03607937	0.12778436	0.3566528

Table 29: ANOVA comparing differences between treatments in stem specific hydraulic conductivity for week two of tanoak sapling experiment.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	1.2273e-07	6.1366e-08	5.1588	0.02417
Residuals	12	1.4275e-07	1.1895e-08		

Table 30: Tukey's HSD comparing differences between treatments in stem specific

hydraulic conductivity for week two of tanoak sapling experiment.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	-1.954727e-04	-0.0003634667	-2.747865e-05	0.0229989
L-C	-1.558423e-04	-0.0003615921	4.990748e-05	0.1495340
L-H	3.963033e-05	-0.0001661195	2.453801e-04	L-C L-H 0.8660989

Table 31: ANOVA comparing differences between treatments in stem specific hydraulic conductivity for week four of tanoak sapling experiment.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	4.2617e-08	2.1308e-08	4.042	0.03943
Residuals	15	7.9075e-08	5.2720e-09		

Table 32: Tukey's HSD comparing differences between treatments in stem specific hydraulic conductivity for week four of tanoak sapling experiment.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	-1.242269e-04	-2.424338e-04	-6.019955e-06	0.0388641
L-C	-5.113414e-05	-1.693410e-04	6.707276e-05	0.5148394
L-H	7.309271e-05	-2.771447e-05	1.738999e-04	0.1776810

Table 33: ANOVA comparing differences between treatments in stem specific hydraulic conductivity for week six of tanoak sapling experiment.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	4.9774e-08	2.4887e-08	9.4534	0.003426
Residuals	12	3.1591e-08	2.6330e-09		

Table 34: Tukey's HSD comparing differences between treatments in stem specific hydraulic conductivity for week six of tanoak sapling experiment.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	-0.0001399546	-2.265283e-04	-5.338089e-05	0.0026789
L-C	-0.0000855258	-1.720995e-04	1.047912e-06	0.0529167
L-H	0.0000544288	-3.214491e-05	1.410025e-04	0.2533443

Table 35: ANOVA comparing differences between treatments in tylosis frequency conductivity for week two of tanoak sapling experiment.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	1075.6	537.8	1.8973	0.1923
Residuals	12	3401.7	283.5		

Table 36: ANOVA comparing differences between treatments in tylosis frequency for week four of tanoak sapling experiment.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	2672.0	1336.0	4.7262	0.02701
Residuals	14	3957.4	282.7		

Table 37: Tukey's HSD comparing differences between treatments in tylosis frequency for week four of tanoak sapling experiment.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	29.772361	2.191343	57.353378	0.0338881
L-C	8.598621	-19.805891	37.003133	0.7136673
L-H	-21.173740	-45.655350	3.307871	0.0947592

Table 38: ANOVA comparing differences between treatments in tylosis frequency for week six of tanoak sapling experiment.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	1413.89	706.94	4.0168	0.04901
Residuals	11	1935.95	176.00		

Table 39: Tukey's HSD comparing differences between treatments in tylosis frequency for week six of tanoak sapling experiment.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	21.539579	-1.121593	44.20075	0.0627080
L-C	20.198578	-3.837224	44.23438	0.1028188
L-H	-1.341000	-25.376802	22.69480	0.9875742

Table 40: ANOVA comparing growth rates of *P. ramorum* isolates from three clonal lineages over time

	DF	Sum Sq	Mean Sq	F-value	p-value
Isolate	13	4025704	309670	19.3936	< 2.2e-16
Time	3	17011218	5670406	355.1198	< 2.2e-16
Isolate * time	39	3927869	100715	6.3074	3.048e-14
residuals	104	1660629	15968		

Table 41: ANOVA comparing growth rates of *P. ramorum* isolates from three clonal lineages over time

	DF	Sum Sq	Mean Sq	F-value	p-value
Isolate	13	4025704	309670	19.3936	< 2.2e-16
Time	3	17011218	5670406	355.1198	< 2.2e-16
Isolate * time	39	3927869	100715	6.3074	3.048e-14
residuals	104	1660629	15968		

Table 42: Repeated measures ANOVA comparing growth rates of three *P. ramorum* clonal lineages over time.

	DF	Sum Sq	Mean Sq	F-value	p-value
Clonal lineage	2	3054822	1527411	65.298	< 2.2e-16
Time	3	17011218	5670406	242.413	< 2.2e-16
Clonal lineage * time	6	3097433	516239	22.070	3.048e-14
residuals	148	3461947	23392		

Table 43: Repeated measures ANOVA comparing the net rate of photosynthesis over time for seedling inoculation experiment - trial 1.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	2.9765	1.4882	4.9407	0.009207
Time	4	2.0614	0.5153	1.7109	0.154495
Treatment * time	8	2.8933	0.3617	1.2007	0.307676
residuals	90	27.1096	0.3012		

Table 44: Tukey's HSD table comparing differences in the net rate of photosynthesis between treatments for seedling inoculation experiment - trial 1.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	-0.2149976	-0.5276516	0.09765636	0.2348101
L-C	-0.4122850	-0.7249390	-0.09963102	0.0063593
L-H	-0.1972874	-0.5099414	0.11536660	0.2940061

Table 45: Tukey"s HSD table comparing differences in the net rate of photosynthesis over time for seedling inoculation experiment - trial 1.

Comparison	Difference	Lower limit	Upper limit	p-value
1-0	0.11533373	-0.3561777	0.5868452	0.9600957
2-0	0.07244802	-0.3990634	0.5439594	0.9929036
3-0	-0.20850833	-0.6800198	0.2630031	0.7333297
4-0	-0.21802579	-0.6895372	0.2534856	0.6996821
2-1	-0.04288571	-0.5143971	0.4286257	0.9990773
3-1	-0.32384206	-0.7953535	0.1476694	0.3185454
4-1	-0.33335952	-0.8048709	0.1381519	0.2899238
3-2	-0.28095635	-0.7524678	0.1905551	0.4644817
4-2	-0.29047381	-0.7619852	0.1810376	0.4300811
4-3	-0.00951746	-0.4810289	0.4619940	0.9999977

Table 46: Tukey's HSD table comparing differences in the net rate of photosynthesis between treatments for seedling inoculation experiment - trial 1.

Comparison	Difference	Lower limit	Upper limit	p-value
H:0-C:0	-0.0623190476	-1.0854414	0.96080334	1.0000000
L:0-C:0	0.0358142857	-0.9873081	1.05893667	1.0000000
L:0-H:0	0.0981333333	-0.9249891	1.12125572	1.0000000
H:1-C:1	0.1300035714	-0.8931188	1.15312596	1.0000000
L:1-C:1	-0.4076035714	-1.4307260	0.61551881	0.9863029
L:1-H:1	-0.5376071429	-1.5607295	0.48551524	0.8782572
H:2-C:2	-0.0471428571	-1.0702652	0.97597953	1.0000000
L:2-C:2	-0.3293392857	-1.3524617	0.69378310	0.9983407
L:2-H:2	-0.2821964286	-1.3053188	0.74092596	0.9996982
H:3-C:3	-0.6189869047	-1.6421093	0.40413548	0.7247838
L:3-C:3	-0.4594000000	-1.4825224	0.56372238	0.9615872
L:3-H:3	0.1595869047	-0.8635355	1.18270929	0.9999997
H:4-C:4	-0.4765428571	-1.4996652	0.54657953	0.9486462
L:4-C:4	-0.9008964286	-1.9240188	0.12222596	0.1477534
L:4-H:4	-0.4243535714	-1.4474760	0.59876881	0.9803524

Table 47: Repeated measures ANOVA comparing differences in stomatal conductance between treatments, over time for seedling inoculation experiment - trial 1.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.00013315	0.00006657	2 3.9019	0.0237093
Time	4	0.00045205	0.00011301	6.6238	0.0001018
Treatment*time	8	0.00027169	0.00003396	1.9905	0.0564876
residuals	90	0.00153556	0.00001706		

Table 48: Tukey's HSD table comparing differences in stomatal conductance between treatments for seedling inoculation experiment - trial 1.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	-0.001227986	-0.003581059	0.0011250878	0.4308217
L-C	-0.002752981	-0.005106054	-0.0003999074	0.0176078
L-H	-0.001524995	-0.003878069	0.0008280783	0.2752557

Table 49: Tukey's HSD table comparing differences in stomatal conductance over time for the seedling inoculation experiment - trial 1.

Comparison	Difference	Lower limit	Upper limit	p-value
1-0	-0.0000702381	-0.003618893	0.0034784167	0.9999979
2-0	-0.0017957143	-0.005344369	0.0017529405	0.6237221
3-0	-0.0031527778	-0.006701433	0.0003958770	0.1059660
4-0	-0.0055345476	-0.009083202	-0.0019858928	0.0003500
2-1	-0.0017254762	-0.005274131	0.0018231786	0.6586038
3-1	-0.0030825397	-0.006631194	0.0004661151	0.1198590
4-1	-0.0054643095	-0.009012964	-0.0019156547	0.0004288
3-2	-0.0013570635	-0.004905718	0.0021915913	0.8240669
4-2	-0.0037388333	-0.007287488	-0.0001901785	0.0337404
4-3	-0.0023817699	-0.005930425	0.0011668849	0.3417728

Table 50: Tukey's HSD table comparing differences in stomatal conductance between treatments, over time for seedling inoculation experiment - trial 1.

Comparison	Difference	Lower limit	Upper limit	p-value
H:0-C:0	2.680714e-03	-0.005019435	0.0103808632	0.9962620
L:0-C:0	-5.478571e-04	-0.008248006	0.0071522918	1.0000000
L:0-H:0	-3.228571e-03	-0.010928720	0.0044715775	0.9783971
H:1-C:1	2.457500e-03	-0.005242649	0.0101576489	0.9984852
L:1-C:1	-8.535714e-05	-0.007785506	0.0076147918	1.0000000
L:1-H:1	2.542857e-03	-0.010243006	0.0051572918	0.9978280
H:2-C:2	-1.036071e-03	-0.008736220	0.0066640775	1.0000000
L:2-C:2	-1.242143e-03	-0.008942292	0.0064580061	0.9999996
L:2-H:2	-2.060714e-04	-0.007906220	0.0074940775	1.0000000
H:3-C:3	-4.744286e-03	-0.012444435	0.0029558632	0.6990070
L:3-C:3	-3.787619e-03	-0.011487768	0.0039125299	0.9227288
L:3-H:3	9.566667e-04	-0.006743482	0.0086568156	1.0000000
H:4-C:4	-5.497786e-03	-0.013197935	0.0022023632	0.4579849
L:4-C:4	-8.101929e-03	-0.015802078	-0.0004017796	0.0293578
L:4-H:4	-2.604143e-03	-0.010304292	0.0050960061	0.9972191

Table 51: Repeated measures ANOVA comparing differences in water usage over time for seedling inoculation experiment - trial 1.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.003078	0.001539	2.7237	0.07105
Time	4	0.002823	0.000706	1.2491	0.29603
Treatment*time	8	0.006107	0.000763	1.3511	0.22907
residuals	90	0.050852	0.000565		

Table 52:Tukey's HSD table comparing differences in water usage between treatments for seedling inoculation experiment - trial 1.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	-0.010937576	-0.02447881	0.002603658	0.1375863
L-C	-0.011963888	-0.02550512	0.001577346	0.0944043
L-H	-0.001026312	-0.01456755	0.012514922	0.9821794

Table 53: Tukey's HSD table comparing differences in water usage over time for seedling inoculation experiment - trial 1.

Comparison	Difference	Lower limit	Upper limit	p-value
3-0	0.001649527	-0.06502160	0.06832066	0.9999948

6-0	0.083569633	0.01689850	0.15024076	0.0065844
9-0	-0.007022946	-0.07369407	0.05964818	0.9983575
12-0	-0.013250485	-0.07992161	0.05342064	0.9812687
6-3	0.081920107	0.01524898	0.14859124	0.0081687
9-3	-0.008672473	-0.07534360	0.05799866	0.9962629
12-3	-0.014900011	-0.08157114	0.05177112	0.9711748
9-6	-0.090592579	-0.15726371	-0.02392145	0.0025306
12-6	-0.096820118	-0.16349125	-0.03014899	0.0010326
12-9	-0.006227539	-0.07289867	0.06044359	0.9989754

Table 54: Tukey's HSD table comparing differences in water usage between treatments, over time for seedling inoculation experiment - trial 1.

Comparison	Difference	Lower limit	Upper limit	p-value
H:0-C:0	0.0025402384	-0.04177181	0.046852292	1.0000000
L:0-C:0	0.0053984746	-0.03891358	0.049710528	1.0000000
L:0-H:0	0.0028582361	-0.04145382	0.047170289	1.0000000
H:1-C:1	0.0107677609	-0.03354429	0.055079814	0.9999315
L:1-C:1	-0.0033211223	-0.04763318	0.040990931	1.0000000
L:1-H:1	-0.0140888831	-0.05840094	0.030223170	0.9985450
H:2-C:2	-0.0148355303	-0.05914758	0.029476523	0.9974946
L:2-C:2	-0.0132420776	-0.05755413	0.031069976	0.9992576
L:2-H:2	0.0015934527	-0.04271860	0.045905506	1.0000000
H:3-C:3	-0.0153804792	-0.05828544	0.027524482	0.9949894
L:3-C:3	-0.0128638254	-0.05576879	0.030041136	0.9992302
L:3-H:3	0.0025166539	-0.04179540	0.046828707	1.0000000
H:4-C:4	-0.0396393830	-0.08576083	0.006482064	0.1750223
L:4-C:4	-0.0376504037	-0.08377185	0.008471044	0.2419930
L:4-H:4	0.0019889793	-0.04232307	0.046301032	1.0000000

Table 53: ANOVA comparing differences in stem specific hydraulic conductivity between treatments four days after inoculation for seedling inoculation experiment - trial 1.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	4.2590e-09	2.1300e-09	0.353	0.713

Residuals	8	4.8258e-08	6.0320e-09		

Table 54: ANOVA comparing differences in stem specific hydraulic conductivity between treatments seven days after inoculation for seedling inoculation experiment - trial 1.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	5.0522e-08	2.5261e-08	3.1547	0.09158
Residuals	9	7.2067e-08	8.0070e-09		

Table 55: Tukey's HSD comparing differences in stem specific hydraulic conductivity between treatments seven days after inoculation for seedling inoculation experiment - trial 1.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	-0.0001140963	-0.0002907606	6.256813e-05	0.2228058
L-C	-0.0001528722	-0.0003295366	2.379213e-05	0.0896216
L-H	-0.0000387760	-0.0002154404	1.378884e-04	0.8168958

Table 56: ANOVA comparing differences in stem specific hydraulic conductivity between treatments ten days after inoculation for seedling inoculation experiment - trial 1.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	1.0074e-07	5.0369e-08	5.9846	0.02223
Residuals	9	7.5748e-08	8.4160e-09		

Table 57: Tukey's HSD comparing differences in stem specific hydraulic conductivity between treatments ten days after inoculation for seedling inoculation experiment - trial 1.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	-0.00020912	-0.0003902397	-2.800029e-05	0.0255338
L-C	-0.00017512	-0.0003562397	5.999709e-06	0.0577539
L-H	0.00003400	-0.0001471197	2.151197e-04	0.8616921

Table 58: ANOVA comparing differences in stem specific hydraulic conductivity between treatments thirteen days after inoculation for seedling inoculation experiment - trial 1.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	6.1857e-08	3.0929e-08	17.448	0.0008002
Residuals	9	1.5954e-08	1.7730e-09		

Table 59: Tukey's HSD comparing differences in stem specific hydraulic conductivity between treatments thirteen days after inoculation for seedling inoculation experiment - trial 1.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	-1.523038e-04	-2.354253e-04	-6.918223e-05	0.0016368
L-C	-1.523037e-04	-2.354253e-04	-6.918223e-05	0.0016368
L-H	2.710505e-20	-8.312152e-05	8.312152e-05	1.0000000

Table 60: ANOVA comparing differences in leaf starch content between treatments four days after inoculation for seedling inoculation experiment - trial 1.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.083730	0.041865	1.4857	0.2827
Residuals	8	0.225434	0.028179		

Table 61: ANOVA comparing differences in leaf starch content between treatments seve days after inoculation for seedling inoculation experiment - trial 1.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.013681	0.006840	0.4795	0.634
Residuals	9	0.128387	0.014265		

Table 62: ANOVA comparing differences in leaf starch content between treatments tne days after inoculation for seedling inoculation experiment - trial 1.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.003041	0.001521	0.3951	0.6848
Residuals	9	0.034642	0.003849		

Table 63: ANOVA comparing differences in leaf starch content between treatments thirteen days after inoculation for seedling inoculation experiment - trial 1.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.003041	0.001521	0.3951	0.6848
Residuals	9	0.034642	0.003849		

Table 64: ANOVA comparing differences in root starch content between treatments four days after inoculation for seedling inoculation experiment - trial 1.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.018084	0.009042	0.2996	0.7482
Residuals	9	0.271593	0.030177		

Table 65: ANOVA comparing differences in root starch content between treatments seve days after inoculation for seedling inoculation experiment - trial 1.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.63655	0.31828	12.973	0.002233
Residuals	9	0.22081	0.02453		

Table 66: Tukey's HSD comparing differences in root starch content between treatments seven days after inoculation for seedling inoculation experiment - trial 1.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	0.51540536	0.2061731	0.8246376	0.0030741
L-C	0.05901973	-0.2502125	0.3682520	0.8574546
L-H	-0.45638563	-0.7656179	-0.1471534	0.0065839

Table 67: ANOVA comparing differences in root starch content between treatments ten days after inoculation for seedling inoculation experiment - trial 1.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.25342	0.12671	11.551	0.003272
Residuals	9	0.09873	0.01097		

Table 68: Tukey's HSD comparing differences in root starch content between treatments ten days after inoculation for seedling inoculation experiment - trial 1.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	-0.3482851	-0.5550632	-0.14150693	0.0028708
L-C	-0.2378262	-0.4446044	-0.03104809	0.0260295
L-H	0.1104588	-0.0963193	0.31723697	0.3394838

Table 69: ANOVA comparing differences in root starch content between treatments thirteen days after inoculation for seedling inoculation experiment - trial 1.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.013091	0.006546	0.9093	0.4368
Residuals	9	0.064784	0.007198		

Table 70: Repeated measures ANOVA comparing differences in the net rate of photosynthesis between treatments, over time for seedling inoculation experiment - trial 2.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	4.697	2.348	5.6257	0.004987
Time	4	2.567	0.642	1.5376	0.198062
Treatment*time	8	2.050	0.256	0.6139	0.764020
residuals	90	37.570	0.417		

Table 71: Tukey's HSD table comparing differences in the net rate of photosynthesis between treatments for seedling inoculation experiment - trial 2.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	-0.3487771	-0.7168431	-0.8742074	0.0671063
L-C	-0.5061414	-0.8742074	-0.13807550	0.0042171
L-H	-0.1573643	-0.5254302	0.21070164	0.5669412

Table 72:Tukey's HSD table comparing differences in the net rate of photosynthesis over time for seedling inoculation experiment - trial 2.

Comparison	Difference	Lower limit	Upper limit	p-value
1-0	-0.12269048	-0.6777683	0.4323873	0.9723094
2-0	-0.24004524	-0.7951231	0.3150326	0.7491309
3-0	-0.36185952	-0.9169373	0.1932183	0.3715638
4-0	-0.43090000	-0.9859778	0.1241778	0.2040962
2-1	-0.11735476	-0.6724326	0.4377231	0.9764661
3-1	-0.23916905	-0.7942469	0.3159088	0.7516549
4-1	-0.30820952	-0.8632873	0.2468683	0.5358530
3-2	-0.12181429	-0.6768921	0.4332635	0.9730235
4-2	-0.19085476	-0.7459326	0.3642231	0.8733775

4-3	-0.06904048	-0.6241183	0.4860373	0.9968590

Table 73:Tukey's HSD table comparing differences in the net rate of photosynthesis over time for seedling inoculation experiment - trial 2.

Comparison	Difference	Lower limit	Upper limit	p-value
H:0-C:0	-0.026928571	-1.2313799	1.1775227	1.0000000
L:0-C:0	0.013571429	-1.1908799	1.2180227	1.0000000
L:0-H:0	0.040500000	-1.1639513	1.2449513	1.0000000
H:1-C:1	-0.132214286	-1.3366656	1.0722370	1.0000000
L:1-C:1	-0.468214286	-1.6726656	0.7362370	0.9890755
L:1-H:1	-0.336000000	-1.5404513	0.8684513	0.9996565
H:2-C:2	-0.455285714	-1.6597370	0.7491656	0.9916089
L:2-C:2	-0.509314286	-1.7137656	0.6951370	0.9767489
L:2-H:2	-0.054028571	-1.2584799	1.1504227	1.0000000
H:3-C:3	-0.390428571	-1.5948799	0.8140227	0.9982133
L:3-C:3	-0.629221429	-1.8336727	0.5752299	0.8828204
L:3-H:3	-0.238792857	-1.4432441	0.9656584	0.9999944
H:4-C:4	-0.739028571	-1.9434799	0.4654227	0.7049743
L:4-C:4	-0.937528571	-2.1419799	0.2669227	0.3134653
L:4-H:4	-0.198500000	-1.4029513	1.0059513	0.9999995

Table 74: Repeated measures ANOVA comparing differences in stomatal conductance between treatments, over time for seedling inoculation experiment - trial 2.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.00023822	0.00011911	7.9010	0.0006897
Time	4	0.00015844	0.00003961	2.6275	0.0396111
Treatment*time	8	0.00006761	0.00000845	0.5606	0.8073445
residuals	90	0.00135676	0.00001508		

Table 75: Tukey's HSD table comparing differences in stomatal conductance between treatments for seedling inoculation experiment - trial 2.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	-0.0028962286	-0.005108070	-0.0006843876	0.0067931
L-C	-0.0034275571	-0.005639398	-0.0012157162	0.0010969
L-H	-0.0005313286	-0.002743170	0.0016805124	0.8351085

Table 76:Tukey's HSD table comparing differences in stomatal conductance over time for seedling inoculation experiment - trial 2.

Comparison	Difference	Lower limit	Upper limit	p-value
1-0	-0.0017586667	-0.005094330	1.576996e-03	0.5859212
2-0	-0.0025581667	-0.005893830	7.774962e-04	0.2145539
3-0	-0.0031910952	-0.006526758	1.445676e-04	0.0676933
4-0	-0.0033839048	-0.006719568	-4.824191e-05	0.0450549
2-1	-0.0007995000	-0.004135163	2.536163e-03	0.9628924
3-1	-0.0014324286	-0.004768091	1.903234e-03	0.7539594
4-1	-0.0016252381	-0.004960901	1.710425e-03	0.6568605
3-2	-0.0006329286	-0.003968591	2.702734e-03	0.9842359
4-2	-0.0008257381	-0.004161401	2.509925e-03	0.9583587
4-3	-0.0001928095	-0.003528472	3.142853e-03	0.9998467

Table 77:Tukey's HSD table comparing differences in stomatal conductance over time for seedling inoculation experiment - trial 2.

Comparison	Difference	Lower limit	Upper limit	p-value
H:0-C:0	-8.114286e-04	-0.008049411	0.006426554	1.0000000
L:0-C:0	-6.300000e-05	-0.007300982	0.007174982	1.0000000
L:0-H:0	7.484286e-04	-0.006489554	0.007986411	1.0000000
H:1-C:1	-2.548571e-03	-0.009786554	0.004689411	0.9958094
L:1-C:1	-3.889000e-03	-0.011126982	0.003348982	0.8594979
L:1-H:1	-1.340429e-03	-0.008578411	0.005897554	0.9999976
H:2-C:2	-3.765357e-03	-0.011003339	0.003472625	0.8860401
L:2-C:2	-3.927500e-03	-0.011165482	0.003310482	0.8505650
L:2-H:2	-1.621429e-04	-0.007400125	0.007075839	1.0000000
H:3-C:3	-2.515143e-03	-0.009753125	0.004722839	0.9963316
L:3-C:3	-4.553714e-03	-0.011791697	0.002684268	0.6679936
L:3-H:3	-2.038571e-03	-0.009276554	0.005199411	0.9996171
H:4-C:4	-4.840643e-03	-0.012078625	0.002397339	0.5699394
L:4-C:4	-4.704571e-03	-0.011942554	0.002533411	0.6168796
L:4-H:4	1.360714e-04	-0.007101911	0.007374054	1.0000000

Table 78: Repeated measures ANOVA comparing differences in water usage between treatments, over time for seedling inoculation experiment - trial 2.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.18873	0.09436	4.4436	0.01444
Time	4	0.15403	0.03851	1.8133	0.13315
Treatment*time	8	0.12214	0.01527	0.7190	0.67425
residuals	90	1.91126	0.02124		

Table 79: Tukey's HSD table comparing differences in water usage between treatments for seedling inoculation experiment - trial 2.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	-0.085852325	-0.16886836	-0.002836289	0.0409778
L-C	-0.093526847	-0.17654288	-0.010510811	0.0232682
L-H	-0.007674522	-0.09069056	0.075341514	0.9736060

Table 80:Tukey's HSD table comparing differences in water usage over time for seedling inoculation experiment - trial 2.

Comparison	Difference	Lower limit	Upper limit	p-value
1-0	-0.017329707	-0.1425256	0.10786623	0.9952478
2-0	-0.045880469	-0.1710764	0.07931547	0.8454340
3-0	-0.095198042	-0.2203940	0.02999790	0.2220439
4-0	-0.091322811	-0.2165188	0.03387313	0.2599851
2-1	-0.028550762	-0.1537467	0.09664518	0.9689813
3-1	-0.077868336	-0.2030643	0.04732761	0.4201680
4-1	-0.073993104	-0.1991890	0.05120284	0.4728654
3-2	-0.049317573	-0.1745135	0.07587837	0.8078411
4-2	-0.045442342	-0.1706383	0.07975360	0.8499412
4-3	0.003875231	-0.1213207	0.12907117	0.9999873

Table 81:Tukey's HSD table comparing differences in water usage over time for seedling inoculation experiment - trial 2.

Comparison	Difference	Lower limit	Upper limit	p-value
H:0-C:0	-0.0057669570	-0.27742691	0.26589299	1.0000000

L:0-C:0	-0.0140242140	-0.28568416	0.25763574	1.0000000
L:0-H:0	-0.0082572570	-0.27991721	0.26340269	1.0000000
H:1-C:1	-0.0025082663	-0.27416822	0.26915168	1.0000000
L:1-C:1	-0.0391859716	-0.31084592	0.23247398	0.9999999
L:1-H:1	-0.0366777053	-0.30833766	0.23498225	1.0000000
H:2-C:2	-0.0953667119	-0.36702666	0.17629324	0.9959343
L:2-C:2	-0.0652129736	-0.33687292	0.20644698	0.9999408
L:2-H:2	-0.0301537383	-0.24150621	0.30181369	1.0000000
H:3-C:3	-0.1482093234	-0.41986927	0.12345063	0.8454843
L:3-C:3	-0.1524448783	-0.42410483	0.11921507	0.8170504
L:3-H:3	-0.0042355549	-0.27589551	0.26742440	1.0000000
H:4-C:4	0.1774103681	-0.44907032	0.09424958	0.6092317
L:4-C:4	-0.1967661991	-0.46842615	0.07489375	0.4332468
L:4-H:4	-0.0193558310	-0.29101578	0.25230412	1.0000000

Table 82: ANOVA comparing differences in stem specific hydraulic conductivity between treatments four days after inoculation for seedling inoculation experiment - trial 2.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	1.8772e-08	9.3860e-09	0.8547	0.4655
Residuals	7	7.6869e-08	1.0981e-08		

Table 83: ANOVA comparing differences in stem specific hydraulic conductivity between treatments seven days after inoculation for seedling inoculation experiment - trial 2.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	7.6307e-09	3.8154e-09	7.3874	0.01263
Residuals	9	4.6482e-09	5.1650e-10		

Table 84: Tukey's HSD comparing differences in stem specific hydraulic conductivity between treatments seven days after inoculation for seedling inoculation experiment - trial 2.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	-6.075e-05	-1.056165e-04	-1.588346e-05	0.0109049
L-C	-4.005e-05	-8.491654e-05	4.816537e-06	0.0796740
L-H	2.070e-05	-2.416654e-05	6.556654e-05	0.4359871

Table 85: ANOVA comparing differences in stem specific hydraulic conductivity between treatments ten days after inoculation for seedling inoculation experiment - trial 2.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	1.6857e-08	8.4290e-09	2.0262	0.1877
Residuals	9	3.7437e-08	4.1600e-09		

Table 86: ANOVA comparing differences in stem specific hydraulic conductivity between treatments thirteen days after inoculation for seedling inoculation experiment - trial 2.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	4.1723e-09	2.0862e-09	22.681	0.0003057
Residuals	9 8.2780e-10	9.2000e-11			

Table 87: Tukey's HSD comparing differences in stem specific hydraulic conductivity between treatments thirteen days after inoculation for seedling inoculation experiment - trial 2.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	-4.0000e-05	-5.893407e-05	-2.106593e-05	0.0006010
L-C	-3.9095e-05	-5.802907e-05	-2.016093e-05	0.0007090
L-H	9.0500e-07	-1.802907e-05	1.983907e-05	0.9902404

Table 88: ANOVA comparing differences in leaf starch content between treatments four days after inoculation for seedling inoculation experiment - trial 2.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.034087	0.017044	0.7074	0.5184
Residuals	9	0.216840	0.024093		

Table 89: ANOVA comparing differences in leaf starch content between treatments seven days after inoculation for seedling inoculation experiment - trial 2.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.30016	0.15008	2.0624	0.1831
Residuals	9	0.65491	0.07277		

Table 90: ANOVA comparing differences in leaf starch content between treatments tne days after inoculation for seedling inoculation experiment - trial 2.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.19930	0.09965	1.2504	0.3433
Residuals	7	0.55787	0.07970		

Table 91: ANOVA comparing differences in leaf starch content between treatments thirteen days after inoculation for seedling inoculation experiment - trial 2.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.036937	0.018469	0.6661	0.5373
Residuals	9	0.249521	0.027725		

Table 92: ANOVA comparing differences in root starch content between treatments four days after inoculation for seedling inoculation experiment - trial 2.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.062810	0.031405	1.7551	0.2272
Residuals	9	0.161041	0.017893		

Table 93: ANOVA comparing differences in root starch content between treatments seven days after inoculation for seedling inoculation experiment - trial 2.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.005647	0.002824	0.3601	0.7084
Residuals	8	0.062735	0.007842		

Table 94: ANOVA comparing differences in root starch content between treatments ten days after inoculation for seedling inoculation experiment - trial 2.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.083087	0.041543	6.0658	0.02147
Residuals	9	0.061639	0.006849		

Table 95: Tukey's HSD comparing differences in root starch content between treatments ten days after inoculation for seedling inoculation experiment - trial 2.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	0.15115894	-0.01222407	0.31454194	0.0692154
L-C	-0.04283009	-0.20621309	0.12055291	0.7514887
L-H	-0.19398903	-0.35737203	-0.03060602	0.0221687

Table 96: ANOVA comparing differences in root starch content between treatments thirteen days after inoculation for seedling inoculation experiment - trial 2.

	DF	Sum Sq	Mean Sq	F-value	p-value
Treatment	2	0.031070	0.015535	3.0247	0.09891
Residuals	9	0.046225	0.005136		

Table 97: Tukey's HSD comparing differences in root starch content between treatments thirteen days after inoculation for seedling inoculation experiment - trial 2.

Comparison	Difference	Lower limit	Upper limit	p-value
H-C	0.01329985	-0.1281873	0.15478702	0.9628964
L-C	-0.10067483	-0.2421620	0.04081235	0.1711297
L-H	-0.11397468	-0.2554619	0.02751250	0.1156664

Table 98: Repeated measures ANOVA comparing differences in the net rate of photosynthesis for both trials in seedling inoculation experiment.

	DF	p-value
Treatment	2	5.097e-05
Time	4	0.02663
Trial	1	1.519e-05
Treatment*time	8	0.16692
Treatment*trial	2	0.7950
Time*trial	4	0.8069
Treatment*time*trial	8	0.98300
residuals	180	

Table 99: Repeated measures ANOVA comparing differences in stomatal conductance for both trials in seedling inoculation experiment.

	DF	p-value
Treatment	2	3.75e-05
Time	4	4.36e-06

Trial	1	0.38803
Treatment*time	8	0.08742
Treatment*trial	2	0.46592
Time*trial	4	0.26694
Treatment*time*trial	8	0.53597
residuals	180	

Table 100: Repeated measures ANOVA comparing differences in water usage for both trials in seedling inoculation experiment.

	DF	p-value
Treatment	2	0.004783
Time	4	0.066
Trial	1	2.2e-16
Treatment*time	7	0.413843
Treatment*trial	2	0.0415
Time*trial	4	0.241952
Treatment*time*trial	8	0.892472
residuals	180	

Table 101: Repeated measures ANOVA comparing differences in stem specific hydraulic conductivity for both trials in seedling inoculation experiment.

	DF	p-value
Treatment	2	1.356e-06
Time	3	0.037131
Trial	1	0.069573
Treatment*time	6	0.023660
Treatment*trial	2	0.008606
Time*trial	3	0.750902
Treatment*time*trial	5	0.916089
residuals	70	

Table 102: Repeated measures ANOVA comparing differences leaf starch content for both trials in seedling inoculation experiment.

	DF	p-value
Treatment	2	0.132946
Time	3	0.378906
Trial	1	0.024357
Treatment*time	6	0.152845
Treatment*trial	2	0.120736
Time*trial	3	0.00178

Treatment*time*trial	5	0.318101
residuals	70	

Table 103: Repeated measures ANOVA comparing differences root starch content for both trials in seedling inoculation experiment.

	DF	p-value
Treatment	2	0.0176062
Time	3	5.296e-07
Trial	1	0.3256381
Treatment*time	6	0.0002018
Treatment*trial	2	0.4931533
Time*trial	3	0.0002710
Treatment*time*trial	5	1.198e-05
residuals	70	