AN ABSTRACT OF THE THESIS OF

Sunny L. Lucas for the degree of Master of Science in Botany and Plant Pathology
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Title: Susceptibility of Coast Redwood Seedlings to *Phytophthora ramorum*

Abstract approved

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Jennifer L. Parke

Coast redwood *(Sequoia sempervirens)* (D. Don) Endl. is a host for *Phytophthora ramorum* but it was unclear if the pathogen represents a significant disease risk to this tree species. In this study the susceptibility of coast redwood seedlings and the likelihood of sporulation on redwood were examined. Two methods were used to assess susceptibility. Seedling stems were artificially inoculated and pathogen colonization distance was measured in both main stems and side branches after 8 and 16 weeks. Pathogen colonization distance was usually minimal, rarely exceeding 15 mm from the inoculation site. In two seedlings the pathogen colonized tissue greater than 40 mm from the inoculation site. No seedling mortality was observed. However, small side branches (2.4 mm mean diameter) were often killed. A dose response experiment was conducted to determine the minimum zoospore concentration necessary to cause infection. Seedlings exposed to $\geq 5 \times 10^3$ zoospores/mL became symptomatic. Nonetheless, symptom expression was variable unless seedlings were inoculated with $> 1 \times 10^5$ zoospores/mL. Sporulation was quantified by determining sporangia density (sporangia/cm^2) on detached immature and mature foliage of redwood seedlings, and leaves of California bay (*Umbellularia californica*). In the first trial, significantly more sporangia/cm^2 were observed on California bay compared to redwood, but in the second trial, the results were reversed. Sporulation density on attached redwood foliage was also determined. Comparisons were again made between young and mature redwood foliage. In the attached branch experiment, sporangia density was higher than previously observed and

no significant difference was found between sporangia/cm^2 necrotic tissue on young vs. mature redwood branches. Sporulation results and implications of these findings are discussed in relation to sporulation levels reported on other hosts. This study highlights the importance of determining both host susceptibility and the risk of *P. ramorum* spread from hosts to other uninfected plants.

Susceptibility of Coast Redwood Seedlings to *Phytophthora ramorum*

by
Sunny L. Lucas

A THESIS

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<u>Master of Science</u> thesis of <u>Sunny L. Lucas</u> presented on <u>March 18, 2008</u>.

APPROVED:

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Major Professor, representing Botany and Plant Pathology

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Chair of the Department of Botany and Plant Pathology

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Dean of the Graduate School

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

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Sunny L. Lucas, Author

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Introduction

Sudden oak death was first reported in the United States in the 1990s in the San Francisco Bay area (Rizzo et al. 2002). In 2002, the pathogen causing sudden oak death was identified as *Phytophthora ramorum*, a species first described in 2001 in Europe (Werres et al. 2001). *P. ramorum* has caused significant mortality of *Lithocarpus densiflorus* (tanoak) and several oak species including *Quercus agrifolia* (coast live oak), *Quercus kellogii* (California black oak), and *Quercus parvula* var. *shrevei* (Shreve's oak), affecting 14 central coastal counties in California and one county in Oregon (Rizzo et al. 2002; Davidson et al. 2003; California Oak Mortality Task Force 2008). The pathogen responsible for Sudden Oak Death also causes Ramorum blight on many hosts. Currently, *P. ramorum* has the largest host range of any quarantined pathogen in the United States (Rizzo et al. 2005). According to the United States Department of Agriculture, Animal & Plant Health Inspection Service (APHIS), over 100 plant species have been designated as hosts or associated hosts (U. S. Department of Agriculture 2008). Koch's postulates have not been completed for those species listed on the associated host list. Being a recently described species and disease, the impact that *P. ramorum* could have in both the forest and nursery is unknown. As new pieces of the *P. ramorum* puzzle are learned almost daily, this story evolves each year.

Phytophthora ramorum exhibits a variety of symptoms on a wide range of hosts. Specific symptoms and extent of infection vary by host. Much work has been done since the initial characterization of *P. ramorum* to define the host range and describe disease symptoms (Davidson and Shaw 2003; Davidson et al. 2003; Parke et al. 2004; Tooley et al. 2004; Hansen et al. 2005; Shishkoff 2006; Tooley and Kyde 2007).

On tanoak and coast live oak, the most susceptible hosts to *P. ramorum*, the bleeding cankers are the most diagnostic symptom of sudden oak death. Though cankers are a rare symptom over the broad host range of *P. ramorum*, they are a consistent and dramatic part of this west coast epidemic. Initially, individual bleeding spots through intact bark occur on the main trunk of the host. As the disease progresses, the bark may crack and the red, clear exudates continue through both cracked and intact bark. Cankers

are reddish brown to black. In general, discoloration is more extensive in the bark and cambium than in the xylem tissue. Cankers may range in size from a few centimeters to 3 meters in length. On tanoak these cankers may be surrounded by water-soaked tissue. Cankers have only been found on mature coast live oak trees, but may appear on tanoak at any stage of development (Davidson et al. 2003).

In addition to the bleeding cankers typical of sudden oak death, *P. ramorum* also causes Ramorum blight on many hosts. Foliar symptoms are much more common across hosts than are cankers. On *Rhododendron spp.* necrotic lesions on leaves may appear anywhere on leaf surfaces. They are easily confused with lesions caused by other *Phytophthoras*. Leaf infection may occur from stem infection via the petiole. In this case, the necrotic region remains close to the midvein. Foliar symptoms on other hosts vary, appearing like sun scorch, necrosis with a chlorotic ring, bull's eyes, or water-soaked tissue (Davidson et al. 2003). Many hosts suffer from necrotic cankers, discoloration of stems, and/or shoot dieback. Ramorum blight, on some hosts, can also lead to an overall reduction in vigor, leaf flagging or wilting. On both tanoak and coast live oak, the crown may suddenly become brown, or gradual leaf loss may occur (Davidson et al. 2003). In response to main stem girdling, elicitin production, or reduced stem hydraulic conductivity, sudden oak death and Ramorum blight are lethal to some hosts (Davidson et al. 2003, Manter et al. 2007a, Parke et al. 2007).

Many conifers have been tested for susceptibility and several have been determined susceptible (Denman et al. 2005; Hansen et al. 2005; Chastagner et al. 2006). Seedlings or sprouts of several conifers have been found naturally infected in California and Oregon forests: *Sequoia sempervirens* (coast redwood), *Pseudotsuga menziesii* (Douglas-fir), *Taxus brevifolia* (Pacific yew), *Abies grandis* (grand fir), *Abies magnifica* (red fir) and *Abies concolor* (white fir) (Davidson et al. 2002; Maloney et al. 2002; Garbelotto et al. 2003; Bienapfl et al. 2006; Chastagner et al. 2006; Goheen et al. 2006; U. S. Department of Agriculture 2008). Currently, Koch's postulates have only been completed for coast redwood and Douglas-fir; therefore these are regulated hosts (U. S. Department of Agriculture 2008).

Symptoms on Douglas-fir include cankers on small branches, wilting of new shoots, dieback of branches, and loss of leaves. On small saplings, death of the leader and

top whorls of branches is also possible (Davidson et al. 2002; Garbelotto et al. 2003; Goheen et al. 2006). Key factors in symptom development are the level of inoculum and growth stage of the plant. Seedlings are most susceptible immediately following bud break (Hansen et al. 2005; Chastagner et al. 2006). Symptoms on coast redwood seedlings and saplings include discolored needles and cankers on small branches. Symptoms have been found on sprouts of mature trees, and it appears that sprouts can be killed by the pathogen (Maloney et al. 2002; Garbelotto et al. 2003). Coast redwood symptoms are similar to those found on Douglas-fir, though thus far redwood symptoms appear to be less extensive (Davidson et al. 2002; Maloney et al. 2002; Hansen et al. 2005).

It has been suggested that *P. ramorum* is exotic to both North America and Europe (Werres et al. 2001; Rizzo et al. 2002; Brasier 2003; Kluza et al. 2007). *P. ramorum* has been found in the nursery, the forest, and the urban-wildland interface in the United States. Ramorum blight occurs discontinuously in coastal mixed-evergreen forests in California and Oregon. As of 2007, 14 California counties and part of one Oregon county were federally quarantined (U. S. Department of Agriculture 2008). One of the factors influencing spread of the disease is the abundance of hosts. Host species are found on 4.5 million acres of forested public and private land in the 14 quarantined California counties (reported acreage excluding state and national parks). Coast redwood is found on 1.5 million and is the dominant species on 877,000 of those forested acres (Barrett et al. 2006). In a study looking at disease prevalence in coast redwood forests, researchers found disease incidence levels on overstory tree hosts to be highest on *Umbellularia californica* (California bay) with a frequency of 42-69%, followed by tanoak with a frequency of 32-45%, and redwood with a frequency of .3-1% (Maloney et al. 2005). While redwood was not the most frequently infected host, disease does occur and this host is present through much of the infested forests.

P. ramorum* propagules have been recovered from rainwater, in topsoil, litter, and in streams (Davidson et al. 2005; Fichtner et al. 2007). Experimental data collected from an infected coast live oak/bay laurel forest in California showed that inoculum could not be recovered from these sources during the dry, hot summer (Davidson et al. 2005).

Inoculum was recovered throughout the winter and spring. Inoculum recovery was highest during the spring when rain was continuous and temperatures mild. These results are consistent with findings that *Phytophthora spp.* in general require very high moisture levels for production of sporangia (Ribeiro 1983; Davidson et al. 2005). Currently, little is known about over-summering inoculum reservoirs, either in hosts or in the environment. There is evidence for summer survival of propagules in soil, suggesting that this may be one source of inoculum for fall disease development (Fichtner et al. 2005; Fichtner et al. 2007). In a redwood-tanoak forest, *P. ramorum* was recovered from the soil during a summer proceeded by late spring rain. Soil moisture, level of organic matter, and recovery frequency of *P. ramorum* were measured under California bay, tanoak, and redwood. Soil moisture and level of organic matter were highest under redwood. Yet recovery of *P. ramorum* was highest under California bay, followed by tanoak, and occasionally under redwood (Fichtner et al. 2007).

Spores are the main mechanism of dispersal, therefore understanding and preventing their spread is crucial to management of this disease. *P. ramorum* dispersal follows a classic dispersal gradient. Most inoculum remains close to the host, and number of propagules decreases with distance from site of formation (Davidson et al. 2005). Yet, there is the possibility of a few propagules being dispersed long distances. This long distance infection can occur in several ways. A dehiscent sporangium blowing above the canopy during a windy rainstorm could initiate an infection far from its origin. There is also the risk of human-involved long distance dispersal. In the forest setting, a viable propagule can get lodged on hikers' shoes or bicycle tires (Davidson et al. 2005; Cushman and Meentemeyer 2006). This is especially troublesome in areas of heavy human traffic, such as recreational areas around the San Francisco Bay and the Avenue of the Giants in Humboldt County.

Like other aerial *Phytophthoras*, *P. ramorum* is transmitted by water splash and windborne rain (Fitt et al. 1989). In the forest, it has been shown that propagules in the soil or leaf litter can infect plant tissue through rain splash (Davidson et al. 2005). It is thought that propagules in both the soil and water are viable for long periods, perhaps six months to over a year (Fichtner et al. 2005).

P. ramorum has not been observed reproducing on all hosts. Reproductive propagules have not been recovered from the bark of the species most devastated by sudden oak death, coast live oak and tanoak. A low number of sporangia have recently been recovered from symptomatic coast live oak leaves (Vettraino et al. 2008). On the other hand, sporangia are produced readily on California bay leaves. This species is often found in association with coast live oak and tanoak in infected forests (Davidson et al. 2005; Davidson in press). Presence of this species is positively correlated with incidence of Ramorum blight and sudden oak death (Maloney et al. 2005). Ramorum blight is not lethal on California bay. It is thought that dehiscent sporangia produced on California bay can readily infect coast live oak and tanoak in the forest ecosystem (Davidson et al. 2005). Therefore, California bay acts as a recurring source of inoculum (Davidson et al. 2005).

Several studies have documented sporulation on other hosts (Davidson and Shaw 2003; Denman et al. 2007; Shishkoff 2007; Vettraino et al. 2007; Davidson in press). Sporulation has been observed on redwood at low frequencies compared to California bay leaves and tanoak branches (Davidson in press). Any level of sporulation can be important though, because while these hosts may not be playing a large role in terms of levels of disease, they may be helping the disease spread through the forest and nurseries. A few studies suggest that sporulation may even be occurring on hosts without any correlation to the extent of symptoms (Denman et al. 2007; Vettraino et al. 2007). The implications of this pose obvious challenges for management.

Sequoia sempervirens (D. Don) Endl. (coast redwood) was one of the first non-oak species to be identified as a host for *P. ramorum* (Maloney et al. 2002; Garbelotto et al. 2003). Though redwood is not affected as intensely as tanoak or coast live oak, it is still a species found through much of the forest infested with sudden oak death. Inoculum levels are high in some of these areas, and an understanding of the relationship between the pathogen and this tree species is crucial for management of the disease. Because of this, as well as because of its economic and cultural value, coast redwood is a worthwhile target of study. Coast redwood has been included in several previous *P. ramorum* studies, but an in-depth look at the risk *P. ramorum* poses to coast redwood has not yet been

carried out (Maloney et al. 2002; Garbelotto et al. 2003; Denman et al. 2005; Hansen et al. 2005; Maloney et al. 2005; Chastagner et al. 2006; Fichtner et al. 2007; Davidson in press).

The first report of *P. ramorum* being isolated from coast redwood leaves in the forest appeared in 2002 (Maloney et al. 2002). The researchers inoculated leaves and stems of seedlings and saplings. Discoloration of leaves adjacent to those inoculated was noted in seedlings. In saplings, discoloration in the xylem above the inoculation point on stems was observed. They also observed narrow streaks in the xylem extending from the discolored areas upward. This is interesting in light of recent evidence that *P. ramorum* is capable of invading and moving through the vascular system of some hosts. The presence of hyphae in xylem was observed in the roots and stems of Rhododendron using fluorescence and scanning laser confocal microscopy (Parke and Lewis 2007). The pathogen has also been recovered from sapwood above and below the inoculation point on mature tanoak logs, as well as from the sapwood of naturally infected tanoak trees (Parke et al. 2007). Histological samples from tanoak logs have revealed extensive mycelial colonization of xylem vessels (Parke et al. 2007). In Europe, researchers have isolated *P. ramorum* from the xylem of several naturally infected tree species, including *Quercus cerris* and *Fagus sylvatica* (Brown and Brasier 2007). Studies have found a strong correlation between plant tissue discoloration and recovery of *P. ramorum* (Brown and Brasier 2007; Parke et al. 2007). No work of this kind has yet been attempted on coast redwood.

Another important aspect to consider is the level of inoculum detrimental to coast redwood. By exposing seedlings to various levels of inoculum, and establishing a dose response curve for coast redwood, we can determine redwood's susceptibility relative to other conifers. Previous research conducted on Douglas-fir suggests at least 10^4 zoospores/mL are necessary to cause symptoms (Chastagner et al. 2006). This finding could be related back to levels of inoculum found in the natural ecosystem to determine if the level of inoculum necessary to cause infection is present in coast redwood forests.

Previous work has been done to quantify levels of inoculum in a mixed-evergreen forest by counting colony forming units (CFU) from collected rainwater (Davidson et al. 2005). The Davidson et al. study (2005), done in Sonoma County, CA, found that

inoculum was detectable seasonally: present during the winter rains and absent during the hot, dry summers (Davidson et al. 2005). It is unknown if the pattern of seasonal recovery found in Sonoma County, or the levels of inoculum detected, hold true for the slightly cooler and wetter northern infested forests in Humboldt County, CA.

In infested redwood forests, no unusual mortality or disease has been reported on overstory redwoods (Maloney et al. 2002; Maloney et al. 2005). However, the pathogen appears to be able to kill sprouts (Maloney et al. 2002). *P. ramorum* has been detected from a mature coast redwood stump using diagnostic PCR. The presence of other pathogens and abiotic factors, as well as the possibility that *P. ramorum* propagules landed on the stump after tree removal, made cause of death unascertainable (Garbelotto et al. 2003).

The goal of this study was to assess the risk that *P. ramorum* poses to coast redwood. My objective was approached in two ways. Field experiments were set up in infested areas of the redwood forest in southern Humboldt County, CA. For the duration of one rainy season, I attempted to quantify inoculum levels in rainwater collections at three different field sites using the protocol set forth in Davidson, et al. (2005). I placed redwood seedlings out at each of these field sites to try and determine disease incidence at various times during the year, as well as disease severity in the presence vs. absence of overstory California bay. (See Appendix for more information on field experiments.)

The second part of this study involved laboratory experiments carried out at Oregon State University. I assessed susceptibility of coast redwood seedlings by determining pathogen colonization distance in the stem upon artificial inoculation. I also conducted a dose response experiment to determine the minimum inoculum load necessary to cause visible symptoms. Finally, I conducted sporulation trials on both detached and attached redwood branches to determine if sporulation is occurring on this host, as well as estimate sporulation potential on redwood.

Materials & Methods

Redwood seedlings used for the first trial of the pathogen colonization distance experiment were obtained from Althouse Nursery in Cave Junction, OR in May 2006.

All other redwood seedlings, used for both lab and field studies, were acquired from Plum Creek Nursery in Cottage Grove, OR in November 2006. Seedlings were grown from seed source Coastal 090-0.5, native to Humboldt County, California. Seedlings were received at approximately one year old. All seedlings used for laboratory experiments were potted in gallon pots in a mixture of 90% peat moss and 10% perlite. Seedlings were kept under controlled greenhouse conditions, or outside of the greenhouse during the summer, until used in experiments. Seedlings were watered weekly and fertilized at least two weeks before sporulation experiments.

Phytophthora ramorum isolate 4581 was used for all controlled experiments. Isolate 4581 is a North American genotype (NA1 lineage), A2 mating type. Originally this isolate was recovered from the forest in Curry County, Oregon on *Rhododendron macrophyllum*. Cultures of this isolate were maintained on one-third-strength V8 agar (100 mL clarified V8 juice broth, 1200 mL water, 23.4 g bacto-agar) at room temperature (19°-20°C).

Pathogen Colonization Distance

Coast redwood seedling stems were artificially inoculated in order to determine pathogen colonization distance and assess susceptibility. Both main stems and small side branches were wound inoculated. Pathogen recovery measurements were taken at two different harvest times.

Either the main stem or a side branch was inoculated on each seedling. Main stems were inoculated approximately three-quarters of the way up the stem from the soil line. Side branches were chosen from about this height, as well. On the side branches, the

incision was initiated 3 cm away from the main stem. Initial height and stem caliper were measured for each seedling.

Seedlings were inoculated with colonized agar plugs removed from the growing margin of two-week-old colonies growing on one-third-strength clarified V8 juice agar. Controls were inoculated with clean agar plugs from plates of one-third-strength V8. Plugs were 6 mm in diameter. Using a scalpel, the stem was cut to the cambium. Attempts were made to make the slice only slightly longer than the diameter of the plug in order to minimize the wound. Plugs were removed from the plate, placed sideways on an empty plate, and then cut in half with a scalpel. The surface of the thin plug was placed against the inner exposed cambium, and secured against the stem with the external flap of tissue. A piece of wet cheesecloth was placed over the plug, followed by a secure wrapping with parafilm.

This experiment was set up as a 2x2x2 factorial with 2 inoculation locations (branch vs. stem) x 2 harvest times (8 vs. 16 week) x 2 trials. This experiment was conducted twice, beginning in June 2006 and again in July 2007. For both trials 12 seedlings were used for each main stem or side branch inoculation. For the first trial 10 seedlings were used for each of two control treatments, main stem and side branch inoculations with non-colonized agar plugs. For the second trial only six seedlings were used for each control treatment. Seedlings were kept in a growth chamber at 18° C and on a 12 h daylight schedule for the duration of the experiments. Seedlings were randomly assigned places in the growth chamber. Half of the seedlings were harvested at 8 weeks and half were harvested at 16 weeks.

Evidence of any symptoms and heights of seedlings were recorded. The inoculation point, a stem section of about 15 mm in length, was first removed from the seedling. This chunk was cut into pieces for microscopy, isolation and diagnostic PCR. Samples, 15 mm in length, were taken above and below the inoculation point. For the first trial, the end nearest the inoculation point was cut off for microscopy (approximately 1 mm). The remaining chunk was cut into 4 pieces. Two pieces were used for microscopy, one for isolation, and one for diagnostic PCR. In the second trial, 5 mm samples were taken every 10 mm. These chunks were divided for microscopy, isolation and PCR (see Figure 1).

For main stem inoculations in the first trial, 5 samples were taken above and 5 below the inoculation site (75 mm from the inoculation site in each direction). For side branch inoculations in the first trial, 3 samples were taken out towards the tip of the branch beyond the inoculation site (45 mm beyond the inoculation site), and the main stem was sampled beyond the location of inoculated branch attachment (3 sections up and 3 down the main stem). During the first trial, main stem samples were *P. ramorum* positive up to 3 sections away from the inoculation point (45 mm). Therefore, for the second trial main stem inoculations, four 15 mm sections were taken above and below each inoculation point (up to 60 mm from the inoculation site). In the first trial side branch inoculations, only one section beyond the inoculation point was ever *P. ramorum* positive, and in one case the last main stem section sampled was positive. Therefore, for the second trial side branch inoculations, two 15 mm sections were taken out towards the tip of the branch, 1 towards the main stem, 4 up the main stem, and 4 down the main stem.

Isolations were carried out on modified PAR, a Phytophthora-selective medium (17 g cornmeal agar, 10 mg pimaricin, 200 mg ampicillin, and 10 mg rifampicin per 1 L distilled water) (Kennwischer and Mitchell 1978). Plates were examined at 3 days, and every other day up to 2 weeks. *P. ramorum* colonies were confirmed at 3 weeks by the distinctive hyphal growth pattern, as well as presence of characteristic amber chlamydospores. Tissue samples collected for diagnostic PCR analysis were stored in 2 mL microfuge tubes at -20ºC until processed. DNA was analyzed using a multiplex polymerase chain reaction (amplification of more than one DNA target), and amplification was conducted using the internal transcribed spacer (ITS 4 and 5) region of rDNA. The diagnostic primers and methods used were designed for *Phytophthora lateralis* and are effective in amplifying *P. ramorum* (Winton and Hansen 2001; Hansen et al. 2005).
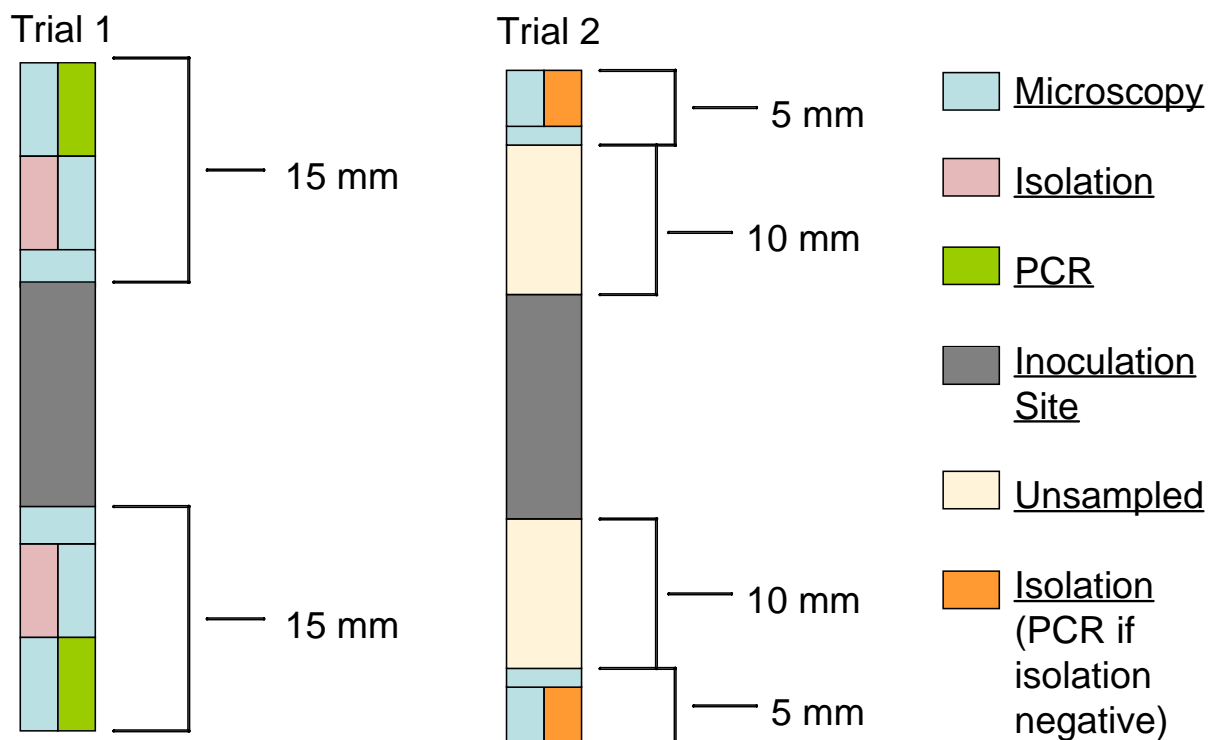
Figure 1. Diagram of sampling methods used in each trial of the pathogen colonization distance experiment. Each column represents an inoculated stem and one section above and below the inoculation point. Colors illustrate how sections were partitioned. Sampling was repeated in sections moving away from the inoculation site.

Inoculum Density in Relation to Disease Incidence (Dose Response)

Ten-day-old cultures, grown out on one-third-strength clarified V8 juice agar, were used to generate zoospore suspensions for inoculations. Plates were flooded with 5 mL of reverse-osmosis (RO) water and scraped with a rubber policeman. This solution was poured into a clean deep Petri dish. Another 5 mL of RO water was added to the original culture, the plate scraped, and solution poured off. Liquid from five plates was combined in one deep Petri dish, for a total of 50 mL of *P. ramorum* suspension per dish (two dishes total). The two Petri dishes were chilled at 4° C for one hour to shock sporangia. After an hour dishes were placed at room temperature (18-20° C) for 45 minutes. Plates were examined under the dissecting microscope to ensure that most

sporangia had released zoospores. Solutions from the two plates were combined when filtered through a 20 μm nylon mesh screening (Aquatic Eco-systems, Inc. Apopka, FL) filter to remove chlamydospores, hyphal fragments, and most sporangia.

Zoospores were quantified from the resultant suspension using a Coulter counter. Preliminary tests were conducted to determine zoospore size range so that all particles within the optimum range (6-14 μm) would be counted. Three counts were taken and averaged from each sample. The counts from the two samples were then averaged to determine final zoospores per mL.

To determine the effect of inoculum dose on disease incidence, unwounded branches of redwood seedlings were dipped in a tenfold dilution series of zoospore concentrations, and disease incidence measured. In each of two trials, six seedlings were randomly chosen for each treatment, and one branch from each seedling was dipped in the appropriate concentration of zoospores. Branches were held in the suspension for approximately five seconds. After dipping, branches were bagged and the bags secured around the main stem.

For the first trial, conducted in August 2007, the treatments were zoospore concentrations of 4.9×10^5, 4.9×10^4, 4.9×10^3, and 0. To detect the lowest zoospore concentration necessary to cause symptoms, an additional treatment was added to the second trial, conducted in September 2007. The zoospore concentrations used for that trial were 3.3×10^5, 3.3×10^4, 3.3×10^3, 3.3×10^2, and 0. To confirm viability of propagules in each treatment, detached leaves from rhododendron variety 'Nova Zembla' were dipped in each treatment after all of the redwood branches. These leaves were kept in crispers on moist paper towels, and monitored for symptom development.

Seedlings were kept in the growth chamber for three weeks. Seedlings were watered weekly until time of harvest. Upon harvest, inoculated branches were cut off of seedlings.

For the first trial, branches were rinsed in a small amount of distilled (DI) water and water was examined for presence of sporangia under the dissecting scope. Presence or absence of sporangia was noted for each seedling in all treatments. Branches were then

washed in DI water to remove any surface particles for one minute. Each branch was placed on a clean paper towel for further processing.

In the second trial, all branches from each treatment (6 branches per treatment) were vortexed in a 50 mL Falcon tube with DI water for approximately 15 seconds. Branch washings were examined for sporangia using the same procedure outlined for sporulation experiments. The branches were then rinsed with DI water for one minute. Branches were placed on a flatbed scanner and branches scanned. The scanner was sterilized with 70% EtOH between samples. Care was taken to ensure that tissue from different samples did not come into contact.

Both the first and second trials were identically harvested from this point forward. Individual samples were photographed and symptoms noted. For each branch, up to 5 symptomatic needles and up to 5 pieces of symptomatic stem tissue were chosen and plated on PAR. Three asymptomatic needles and 2 asymptomatic stem tissue pieces were chosen for plating as well. If no symptomatic tissue was present, 5 asymptomatic needles and 5 stem pieces were chosen and plated. Plates were examined at 5, 7, and 14 days. *P. ramorum* colonies were confirmed by the distinctive growth pattern as well as presence of amber chlamydospores. Samples that were negative for *P. ramorum* were then transferred to microtubes and frozen for further testing using diagnostic PCR.

The relationship between inoculum concentration and disease incidence was examined using a logistic model. Results from the two trials were analyzed using PROCLOGISTIC in the SAS statistical package, v. 9.1 (SAS Institute, Cary, NC).

Sporulation – Detached Tissue

Detached redwood branches and leaves of California bay (*Umbellularia californica*) were inoculated by dipping in a zoospore suspension. Suspension was generated from 14-day-old colonies grown out on one-third strength V8. Zoospores were generated following the protocol previously described. The concentration of zoospores/mL was measured using a hemacytometer.

Sporulation was measured on 100 young, succulent redwood branches, 100 mature redwood branches, and 100 California bay leaves. After dipping, samples were placed, abaxial side up, in crispers on damp paper towels. Each crisper contained 10 samples. Young redwood, mature redwood, and bay leaves were kept in separate crispers to prevent contamination between treatments. Half the samples were measured within 24 hours to determine initial levels of sporangia on redwood and bay leaves inoculated with this method. The other half of the samples was measured 10 days after inoculation. The branches and leaves that were sampled at 10 days were misted daily with DI water until harvest. This experiment was conducted in November 2007 and January 2008.

Each sample was rinsed in a 50 mL Falcon tube containing 10 mL DI water. The tube was vortexed for 15 seconds. All 10 samples from an individual crisper were rinsed in the same tube. A small amount of $CuSO_4$ was added to each Falcon tube to stop biological activity (30 μL of 160 mM $CuSO_4$). Wash solutions were placed at 4° C until filtering. Within 24 hours, the suspension was resuspended and a vacuum pulled through a 20 μm nylon mesh screening. This filter pore size is small enough to catch most sporangia. A few drops of 0.01% Calcofluor White M2R (Tinopal) (Sigma Chemical, St. Louis, MO) were added to the filter before counting sporangia with a Zeiss Axiostar epifluorescence compound microscope with a Calcofluor filter specific for excitation of 425 nm. Images of the filters were collected using a Micropublisher 3.3 RTV digital camera and Q-Capture Pro imaging software (see Figure 2). Four random fields of view were chosen per filter for counting sporangia. These sporangial counts were averaged, the field of view measured, and the number of sporangia/mm^2 determined. By determining the area of the entire filter, total number of sporangia per filter was extrapolated. The 10 samples, from a particular crisper, were then scanned and this image was used to determine total leaf area in ASSESS image analysis software (see Figure 3) (APS Press, St. Paul, MN). The number of sporangia per cm^2 plant tissue or lesion area was used for comparison between treatments.
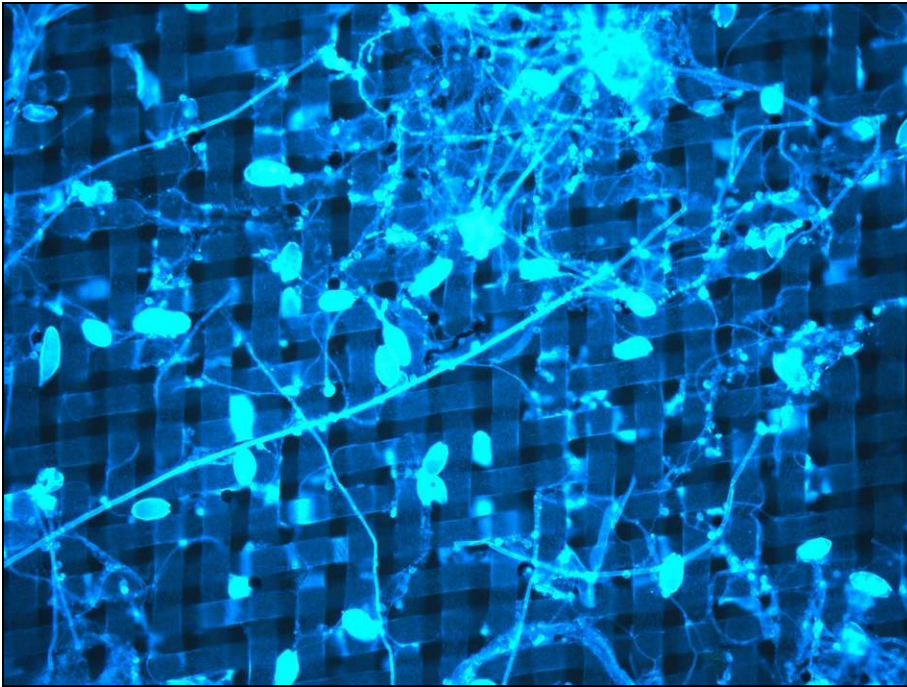
Figure 2. An example of fluorescing sporangia on a filter after Calcofluor was added.
Sporangia were averaged from 4 fields of view per filter in order to calculate sporangia
densities in both detached and attached leaf experiments.
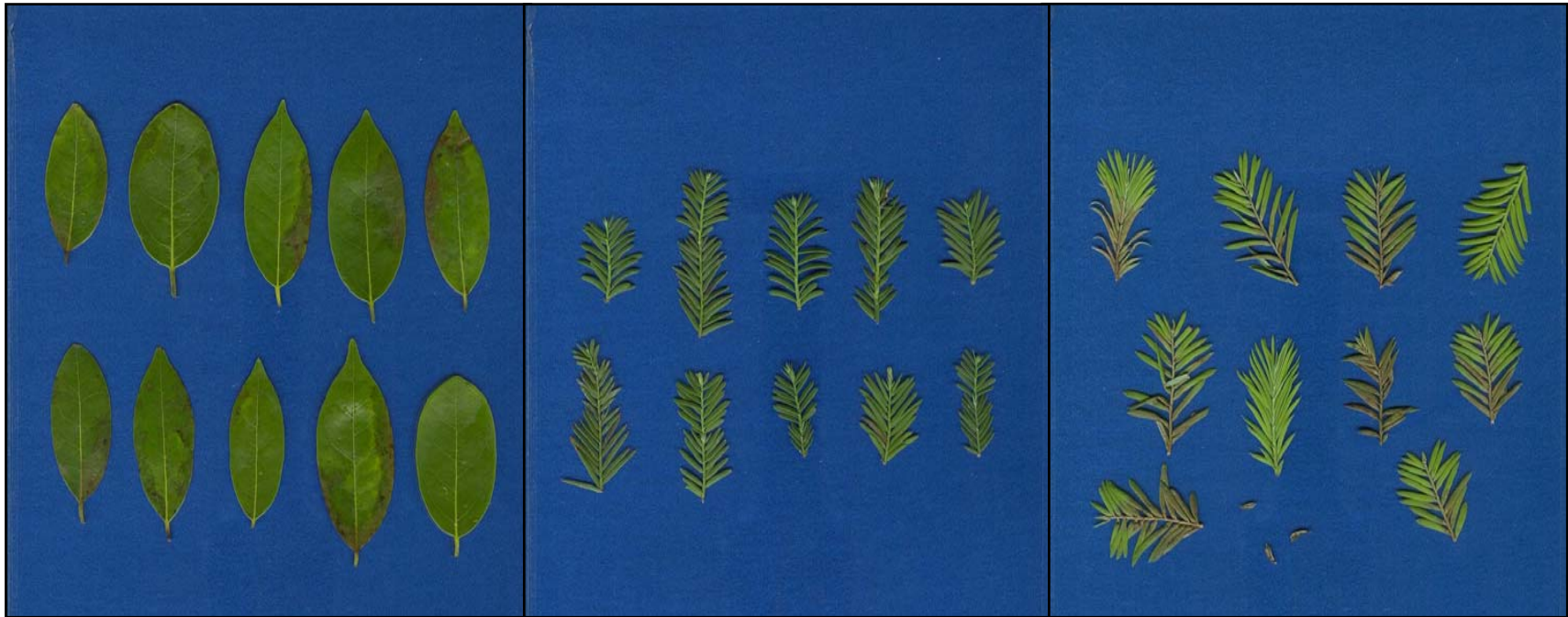
Figure 3. Sporulation on detached leaves and branches. 10 samples, from a particular crisper, were scanned and this image was used to determine total leaf area and lesion area in ASSESS image analysis software. From left to right: California bay, mature redwood, and young redwood.

Sporulation – Attached Tissue

Redwood branches were inoculated by submergence in a zoospore suspension for 12 h. The suspension was generated from 14-day-old colonies grown out on one-third strength V8. Zoospores were generated following the protocol previously described. Zoospores/ml was measured using a hemacytometer.

The tips of branches, containing succulent new growth, as well as an approximately equal amount of mature tissue adjacent to the new growth were marked with a Sharpie and placed in 50 ml Falcon tubes attached to the seedling. Tubes were filled with 20 mL of zoospore suspension (see Figure 4). After 12 h, the tubes were removed, plastic bags were gently placed over the branch and taped closed around the main stem (see Figure 4). Bags contained approximately 1 ml of DI water to ensure high humidity. 48 hours before harvest a small cut was made in the plastic bag, and branches were gently misted. Nine seedlings were used in each of two trials. Five branches were chosen per seedling, 4 were submerged in zoospore suspension, and the fifth was submerged in DI water for the control. Both trials were conducted in January 2008.

Figure 4. Sporulation on attached branches: inoculation and incubation methods. Branches were submerged in 20 mL of zoospore suspension for 12 hours (on the left). After 12 hours, plastic bags were gently placed over the branch and taped closed around the main stem (on the right).

Branches were harvested 10 days after inoculation. For each of the 4 inoculated branches on a seedling, new growth and mature growth were carefully separated. All new growth from each seedling was rinsed in one 50 ml Falcon tube, while all the mature growth was rinsed in another 50 ml Falcon tube. The control branch was rinsed in a third 50 ml Falcon tube.

Sporangia were removed from branches and counted following the protocol previously described for the detached branch assay. Sporangia densities (sporangia/cm^2) over total plant tissue and lesion area were compared between young and mature redwood growth.

Results

Pathogen Colonization Distance

Although the experiment was set up as a 2 x 2 x 2 factorial, an analysis of variance could not be used because of a discrete response variable (presence or absence of *P. ramorum*), data with a heavily skewed distribution, unequal sample sizes that are small, and many zeroes as responses. Therefore, a nonparametric statistical analysis, the Wilcoxon Rank-Sum Test, was used to test for differences between trials, colonization distance and sampling time. *P*-values are reported from the Monte Carlo Exact Estimate Test. If no significant difference was detected between trials, they were then lumped together and tested for a significant difference between sampling times.

For main stem inoculations, no significant difference was found between trials or sampling time. Therefore, all main stem inoculation data were pooled together for frequency of inoculations that resulted in different colonization distances. Five inoculated seedlings were both isolation and PCR negative at the inoculation site; therefore they were not included in the results. Lesion lengths were not measured due to the difficulty in determining the difference between lesions and typical redwood growth. Discoloration in the xylem was observed in both inoculated and control seedlings. No tissue from any of the 16 main stem controls was *P. ramorum* positive by isolation or PCR.

Generally, *P. ramorum* did not colonize tissue very far from the inoculation site in the main stem (see Table 1). The median colonization distance was 0 mm. In 58% of seedlings, the pathogen was not recovered beyond the inoculation site and in only 3 of 19 replicates did the pathogen colonize tissue beyond 15 mm. The furthest distance from which the pathogen was recovered was 60 mm; this occurred in 2 of 19 seedlings. In one of these two seedlings the pathogen colonized tissue 30 mm above and 30 mm below the inoculation site for a total distance of 60 mm. In the other seedling the pathogen colonized tissue below the inoculation site to a distance of 60 mm.

Table 1. The total distance of *Phytophthora ramorum* colonization within stems, isolation and PCR results reported separately, from artificially inoculated seedlings. Results from 8 and 16 week sampling times combined.

Maximum Colonization Distance (mm)	Main Stem (Trials combined)[a]		Side Branch (Trial 1)[b]		Side Branch (Trial 2)[b]	
	Isolation[c]	PCR[c]	Isolation[c]	PCR[c]	Isolation[c]	PCR[c]
0	10/19	1/19	0/12	1/12	6/12	0/12
15	4/19	1/19	1/12	2/12	3/12	1/12
30	0/19	0/19	1/12	6/12	1/12	0/12
45	2/19	0/19	0/12	0/12	1/12	0/12
60	1/19	1/19	0/12	0/12	0/12	0/12
75	0/19	0/19	0/12	0/12	0/12	0/12
90	0/19	0/19	0/12	1/12	0/12	0/12

[a] The frequency of main stem inoculated seedlings, out of the total 19, at each maximum colonization distance. No significant difference was found in recovery distance between trials or sampling times (8 vs. 16 weeks) for main stem inoculated seedlings, therefore these are all reported together

[b] The frequency of side branch inoculated seedlings at each maximum colonization distance, out of 12 for each trial. A significant difference was found between trials 1 and 2, therefore these results are reported separately.

[c] Maximum colonization distance determined by either isolation or diagnostic PCR. In cases where isolation and PCR were both positive at the farthest distance, then the results are reported as isolation positive. Isolation + PCR = total number of seedlings positive at each maximum colonization distance.

For side branch inoculations, differences between trials within each sampling time were not significantly different, nor was there a significant difference between sampling times within trials. When data from the two sampling times were combined, and trials compared, there was a significant difference (two-sided p-value = .0092). Therefore, results for farthest pathogen colonization distance were kept separate for the two trials (see Table 1 and Figure 5). Lesion length was not measured on branches due to the difficulty in determining the difference between lesions and typical redwood growth or the entire branch became necrotic (see Figure 6). No tissue from any of the 16 side branch controls was *P. ramorum* positive by isolation or PCR.
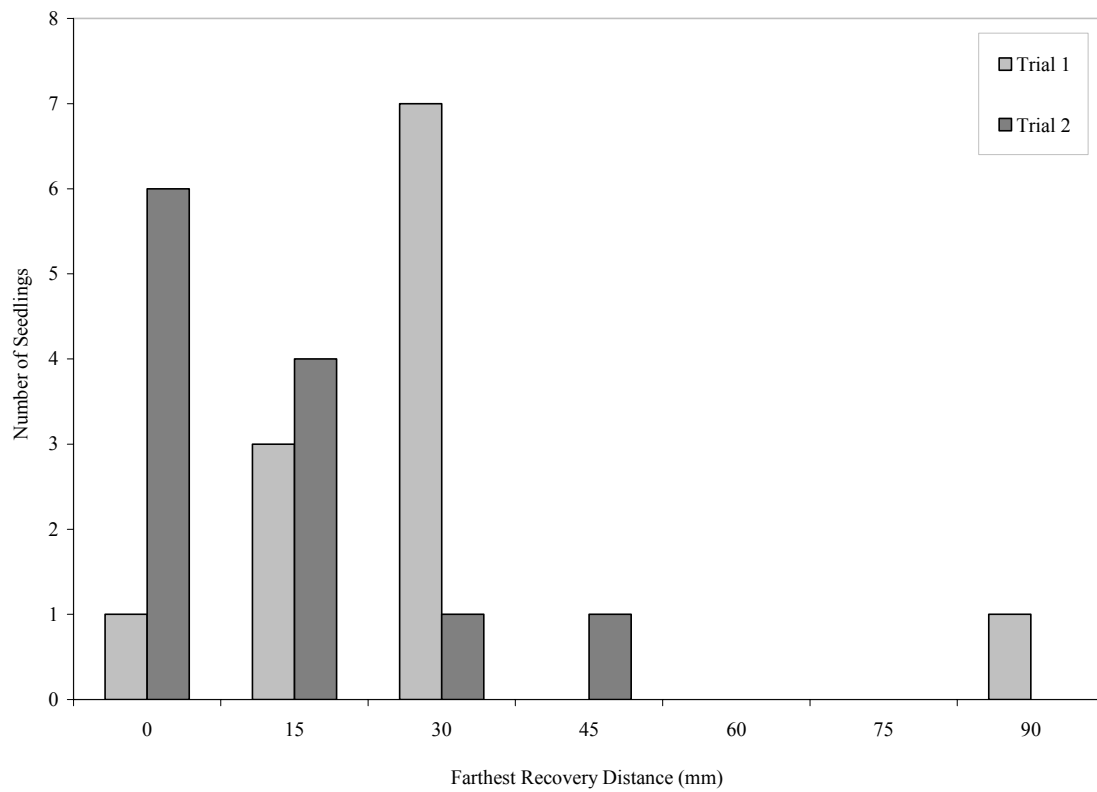
Figure 5. Maximum distance colonized by *P. ramorum* in inoculated side branches: trial 1 vs. trial 2.

Figure 6. The branch on the left was inoculated with *P. ramorum* vs. the branch on the right was a wounded control. Both were from the second trial, 16 week sampling time. Lesion length was not measured on branches due to the difficulty in determining the difference between lesions and typical redwood growth. Discoloration may also have been caused by a wound response irrespective of the presence of *P. ramorum*.

In the first trial, the maximum distance colonized by *P. ramorum* was 30 mm beyond the inoculation site in a majority of inoculated side branches (58 %). Overall, the maximum colonization distance was 90 mm; this occurred in 1 of 12 seedlings. In this seedling, 15 mm of tissue was colonized above the inoculation site (isolation negative, but PCR positive) and 75 mm of tissue was colonized below the inoculation site (isolation positive), 45 mm down the main stem. No visible symptoms were observed on the outside of the stem, but discoloration in the wood was present in tissue beyond pathogen recovery. Discoloration was observed in wood beyond pathogen recovery in several samples (see Figure 7). The pathogen was limited to the inoculation site in only 1 replicate of the first trial. Median maximum colonization distance in the first trial was greater than in the second trial, 30 mm vs. 7.5 mm, respectively. In the second trial, the

pathogen was limited to the inoculation site in 50% of seedlings. In this trial, the maximum distance from which the pathogen was recovered was 45 mm, from 1 of 12 seedlings. Overall, the pathogen spread beyond 45 mm in one of the 24 inoculated side branches.

Figure 7. Discoloration in wood from a sample colonized by *P. ramorum* (on the left) and beyond pathogen recovery (on the right). Both samples are main stem cross-sections from the same side branch inoculated seedling. The sample on the left was located a total of 30 mm below the inoculation site, and 15 mm below the attachment of the inoculated branch to the stem. The sample on the right was located 45 mm below the attachment of the inoculated branch to the stem.

Severity of symptoms on inoculated side branches differed between trials. In the first trial, 10 of the 12 inoculated side branches were dead by harvest time. In the second trial, only 1 branch died by harvest time. Stem caliper at the inoculation point of each seedling was recorded. The mean stem diameter for trial 1 was 2.5 mm (SE = .15). When the experiment was repeated, 54 weeks later, the mean stem diameter was 3.0 mm (SE = .12) for the second trial. This difference was significant (t-test, two-sided p-value = .013). Because the second trial was conducted a year after the first trial, seedlings in the second trial were larger and in general more vigorous.

Inoculum Density in Relation to Disease Incidence (Dose Response)

Symptoms first appeared on redwood seedlings 7 days after inoculation. Seedlings were destructively sampled 3 weeks after inoculation. Symptoms varied from a few necrotic needles to dramatic shoot dieback. New growth was more susceptible than more mature growth. Symptoms were more extensive on the new succulent growth of the branches, though *P. ramorum* was isolated from mature needles and stem tissue. Overall, symptoms were more extensive on infected seedlings in the second trial (see Figure 8).

All seedlings inoculated with the highest doses, 4.9×10^5 and 3.3×10^5 zoospores/mL, were isolation positive, though results from lower doses were inconsistent within and between trials. In the first trial, *P. ramorum* was isolated from all 6 seedlings inoculated with the highest dose, 4.9×10^5 zoospores/mL. None of the seedlings inoculated with 4.9×10^4 zoospores/mL were isolation positive, though PCR analysis of symptomatic stem tissue from one seedling was positive. Two seedlings inoculated with 4.9×10^3 zoospores/mL were isolation positive, and one additional seedling was PCR positive from symptomatic needles. In the second trial, *P. ramorum* was isolated from all 6 seedlings inoculated with 3.3×10^5 zoospores/mL. Four of 6 seedlings inoculated with 3.3×10^4 zoospores/mL were isolation positive. This was the lowest dose yielding isolation or PCR positive results (see Table 2). Necrotic needles were isolation positive from 14 of the 22 infected seedlings. Necrotic stem tissue was isolation positive from 12 of the 22 seedlings. Asymptomatic stem tissue was isolation positive from 4 of the 20 seedlings (see Table 2). None of the asymptomatic needles sampled were positive.

Figure 8. Symptomatic branches from dose response. Top row: The three most symptomatic branches from the first trial at dosages: 4.9×10^5, 4.9×10^3, 4.9×10^3 zoospores/mL (left to right). Bottom row: The three most symptomatic branches from the second trial at dosages: 3.3×10^5, 3.3×10^5, 3.3×10^4 zoospores/mL (left to right).

Table 2. Relationship between inoculum dose and disease incidence.

Inoculum Dose[a]	Log (Dose)[b]	Disease Incidence[c]	Symptomatic Needle +[d]	Symptomatic Stem +[d]	Asymptomatic Stem +[d]	PCR +[e]
4.9E+05	13.1	6/6	5/6	2/6	0/6	
3.3E+05	12.7	6/6	4/6	6/6	3/6	
4.9E+04	10.8	1/6	0/6	0/6	0/6	1/6
3.3E+04	10.4	4/6	3/6	2/6	1/6	
4.9E+03	8.5	3/6	2/6	2/6	0/6	1/6
3.3E+03	8.1	0/6				
3.3E+02	5.8	0/6				
0.0E+00	-3.0	0/12				

[a]zoospores/mL.
[b]The explanatory variable in Figure 9. Used log (.05) for the controls.
[c]Proportion of seedlings that was positive for *P. ramorum* at each dosage, isolation and PCR results combined.
[d]Proportion of seedlings tested positive for *P. ramorum* from specific tissue isolations.
[e]Proportion of seedlings tested positive for *P. ramorum* by diagnostic PCR, though all isolation samples from the particular seedlings were negative.

These results were fit to a logistical regression model, combining isolation and PCR positives. Inoculum dose was used as the sole explanatory variable. Because this experiment was conducted twice, incidence of disease may be affected by trial. Although trial may be another parameter in this model, it is impossible to separate this from dose because the dosages were different between trials. Therefore, any effect trial may have had is lumped together with dosage effect. In this model, inoculum dose was log transformed.

Including all data points, the log (dose) parameter coefficient was significantly correlated with disease incidence (two-sided p-value = .0006, see Figure 9). With a doubling in inoculum, the odds of infection increase by a factor of 1.86 (approximate 95% confidence interval: 1.38 to 2.86). Although log (dose) is significant, the Pearson's goodness of fit statistic is rather large relative to the degrees of freedom (12.3 to 7, respectively), and the two-sided p-value is relatively small (.09).
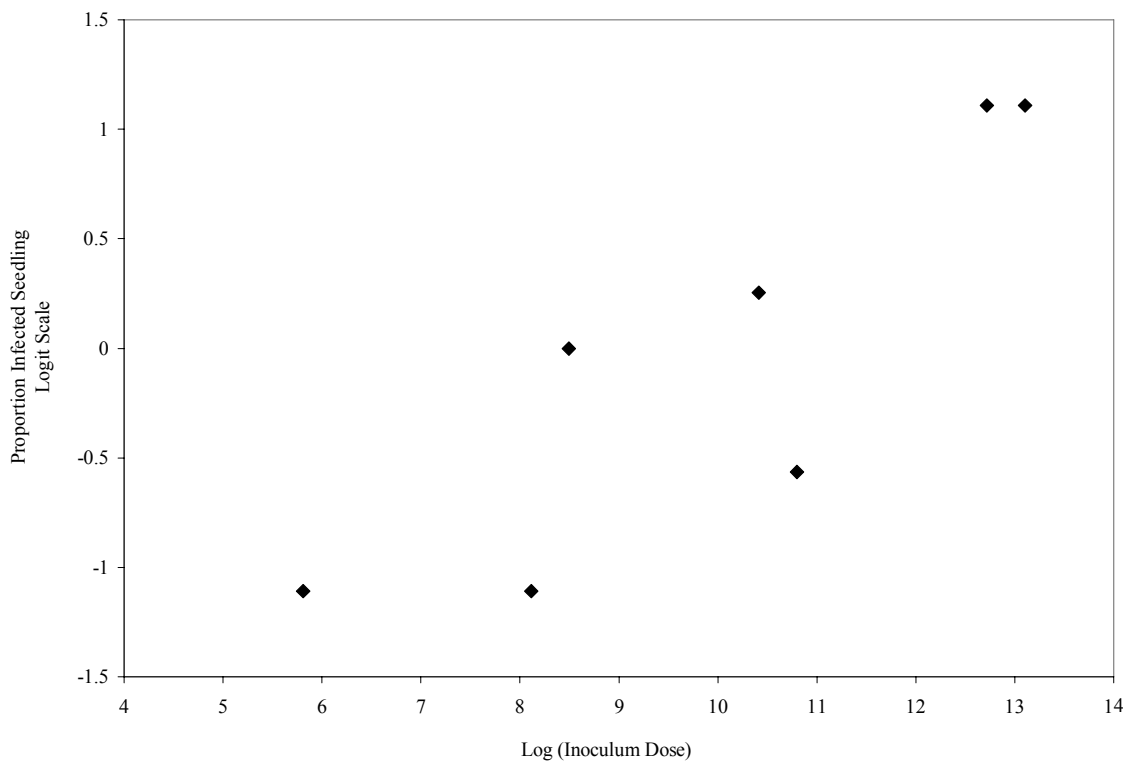
Figure 9. Proportion of seedlings infected (Logit) vs. log (Inoculum Dose).

Sporulation – Detached Tissue

To determine if sporulation occurred on bay, mature redwood, and young redwood, sporangia density (sporangia/total tissue area [cm^2]) was compared between samples measured immediately after inoculation and samples measured 10 days after inoculation. Sporangia density at 10 days vs. 0 days significantly differed for individual treatments in both trials, confirming that sporulation occurred on all treatments (see Table 3). In the first trial, sporulation on bay, mature redwood, and young redwood were 1090, 69, and 92 sporangia/cm^2 respectively (reporting the difference between means at 10 days vs. 0 days). In the second trial, levels of sporulation on bay, mature redwood and young redwood were 173, 71, and 449 sporangia/cm^2 respectively.

To determine if the amount of sporulation differed on the various plant tissues, it was first established that there was not a significant difference between sporangia densities at the beginning of each trial (total leaf area sporangia/cm^2 at 0 days). An analysis of variance (ANOVA) indicated significantly different sporangia densities between trials (F-test *p*-value = .018) but not a significant difference between plant tissues (F-test *p*-value = .077) at 0 days. Therefore, sporangia left on the leaves from inoculations were randomly distributed across treatments.

Trials were analyzed separately for differences between treatments. I compared sporangia densities between treatments over total tissue area and lesion area. In the first trial, sporangia densities over total leaf area differed between treatments (one way ANOVA, F-test *p*-value = .0002). Bay had an average of 999 more sporangia/cm^2 than young redwood tissue (95% confidence interval 596 to 1400) according to a least significant difference (LSD) test. Bay had an average of 1010 more sporangia/cm^2 than mature redwood tissue (95% confidence interval 608 to 1410) according to a LSD test. There was not a significant difference between young and mature redwood sporangia densities. In the second trial there was also evidence for a difference between treatments (F-test *p*-value = .028). Young redwood had an average 343 more sporangia/cm^2 than mature redwood (LSD test, 95% confidence interval 99 to 587). Bay was not found to be significantly different than either mature or young redwood (see Figure 10).

Table 3. Level of sporulation on detached California bay leaves (Bay), branches of mature redwood tissue (MR), and branches of young redwood tissue (YR) for two different trials.

Treatment	Trial	Initial Sporangia Density [a] (sporangia/ cm^2)	Final Sporangia Density [b] (sporangia/ cm^2)	Net Sporangia Density [c] (sporangia/ cm^2)	SE [d]	P-value [e]	Mean Lesion Area (cm^2) [f]	SE [g]	Sporangia Denisty over Lesion Area [h] (sporangia/ cm^2)	SE [i]
Bay	1	5	1095	1090	222	0.004	33.7	6.8	5469	2446
Bay	2	154	327	173	86	0.046	23.0	5.1	2242	1003
MR	1	14	83	69	29	0.039	16.9	2.9	498	223
MR	2	41	113	72	39	0.050	1.3	0.3	5207	2329
YR	1	3	95	92	35	0.028	19.0	2.3	512	229
YR	2	6	455	449	134	0.022	21.2	2.4	1477	738

[a] Mean sporangia density over total leaf area (sporangia/cm^2) at 0 days, immediately following inoculation.
[b] Mean sporangia density over total leaf area (sporangia/cm^2) at 10 days after inoculation.
[c] Values are differences between means of sporangia density (sporangia/cm^2) over total leaf area at 10 days after inoculation and at 0 days, immediately following inoculation.
[d] Standard errors of the difference between means.
[e] One-way p-values generated from two-sample t-tests assuming unequal variances.
[f] Mean lesion area (cm^2) for leaves/branches in each treatment.
[g] Standard errors of mean lesion area.
[h] Mean sporangia density over lesion area (sporangia/cm^2) area for each treatment.
[i] Standard errors of mean sporangia/lesion area.

Figure 10. Mean sporangia densities per total leaf area (sporangia/cm^2) on inoculated California bay leaves (Bay), mature redwood branches (MR), and young redwood branches (YR) separated by trial with standard error bars. Analyses of variance were conducted on trials separately. Significant differences were found between sporangia/cm^2 on California bay and mature redwood branches, and between California bay and young redwood branches in the first trial (multiple comparisons with LSD test). Significant differences between treatments in trial 1 are indicated with different capitol letters above the bars. Significant differences between treatments in trial 2 are indicated with different lower case letters above bars.

A more applicable comparison may be between treatment sporangia densities using lesion area instead of total leaf area. In both trials significant differences were found between treatments (trial 1: F-test p-value = .0018; trial 2: F-test p-value = .013). In the first trial, sporulation on bay was found to be significantly greater than on both mature redwood (average 4970 sporangia/cm^2 greater, LSD test 95% confidence interval 2320 to 7620) and young redwood (average 4960 sporangia/cm^2 greater, LSD test 95% confidence interval 2310 to 7600). No difference was found between mature and young redwood. In the second trial, sporulation on mature redwood was significantly greater

than on both bay (average 2970 sporangia/cm^2 greater, LSD test 95% confidence interval 655 to 5280) and young redwood (average 3730 sporangia/cm^2 greater, LSD test 95% confidence interval 1280 to 6180). No difference was found between bay and young redwood (see Figure 11).
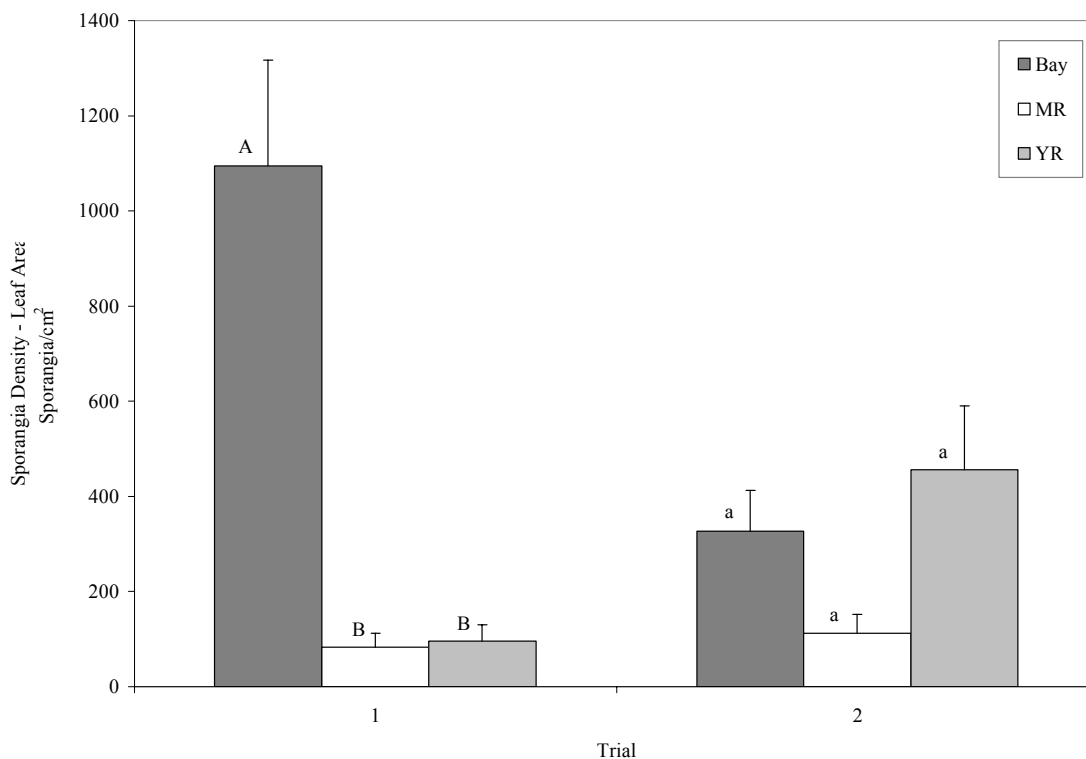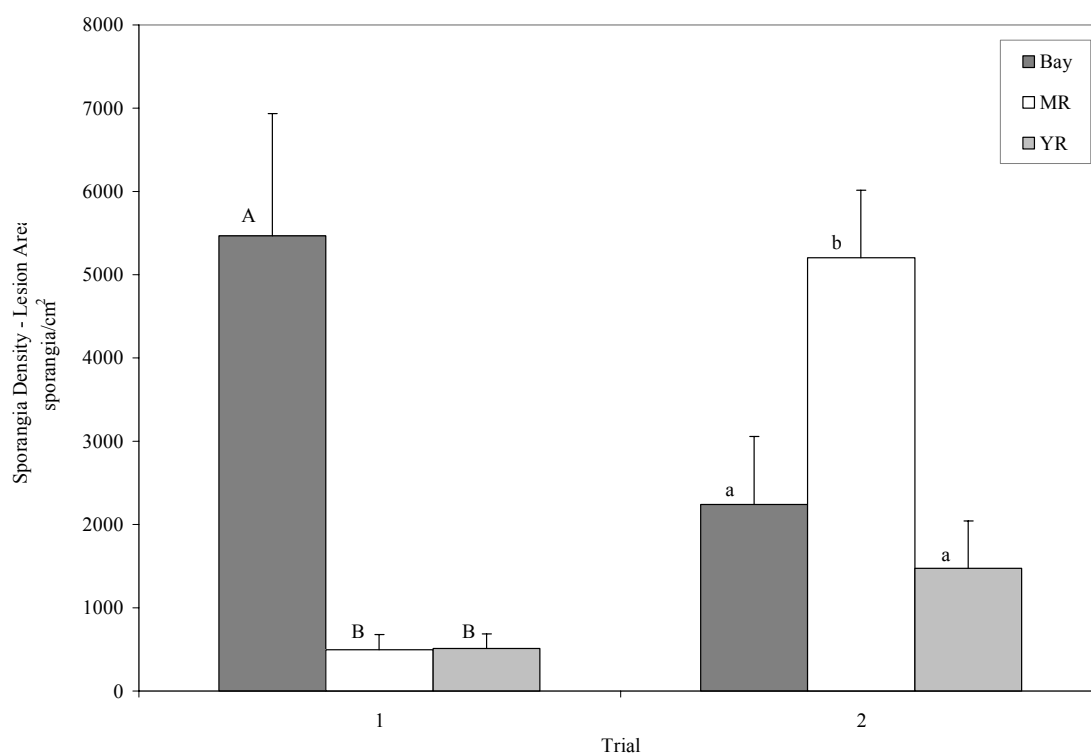

Figure 11. Mean sporangia densities per lesion area (sporangia/cm^2) on inoculated California bay leaves (Bay), mature redwood branches (MR), and young redwood branches (YR) separated by trial, with standard error bars. Significant differences between treatments were found in each trial (trial 1 F-test *p*-value = .0018; trial 2 F-test *p*-value = .013). In trial 1, bay was significantly different than both mature and young redwood (at a 95% confidence interval, LSD test). In trial 2, mature redwood was significantly different than both bay and young redwood (at a 95% confidence interval, LSD test). Significant differences between treatments in trial 1 are indicated with different capitol letters above the bars. Significant differences between treatments in trial 2 are indicated with different lower case letters above bars.

I examined the relationship between necrotic area (cm^2) and total sporangia for each plant tissue. To determine if data from the two trials could be combined, I first looked for differences between trials' sporangia densities for each treatment at 0 days. There were not significant differences between sample means for bay (t-test, two-sided *p*-value = .11) or young redwood (t-test, two-sided *p*-value = .48) at 0 days. There was however a significant difference between trials' sample means for mature redwood (t-test two-sided *p*-value = .039). For uniformity, treatments were analyzed separately for each trial. Simple linear regression analyses were done using necrotic area as the explanatory variable and total number sporangia as the response variable (see Figure 12). After examining scatter plots, F-test *p*-values, and R^2 values, it was clear that no linear relationship existed between total number sporangia and necrotic area for any treatment in the first trial. From the second trial, there was a significant linear relationship for mature redwood. The regression equation, y = 9365x – 4470, estimates 4895 sporangia are present per necrotic cm^2 of mature redwood needles and/or branches (necrotic area *p*-value = .0013, intercept *p*-value = .0282; F-test *p*-value = .0013, R^2 = .98). This estimate assumes that there are no sporangia on asymptomatic tissue. A nearly significant linear relationship was found for young redwood (F-test p-value = .065, R^2 = .87). The equation, y = -3023x + 91460, shows an inverse relationship between necrotic area and number of sporangia (necrotic area *p*-value = .065, intercept *p*-value = .035).

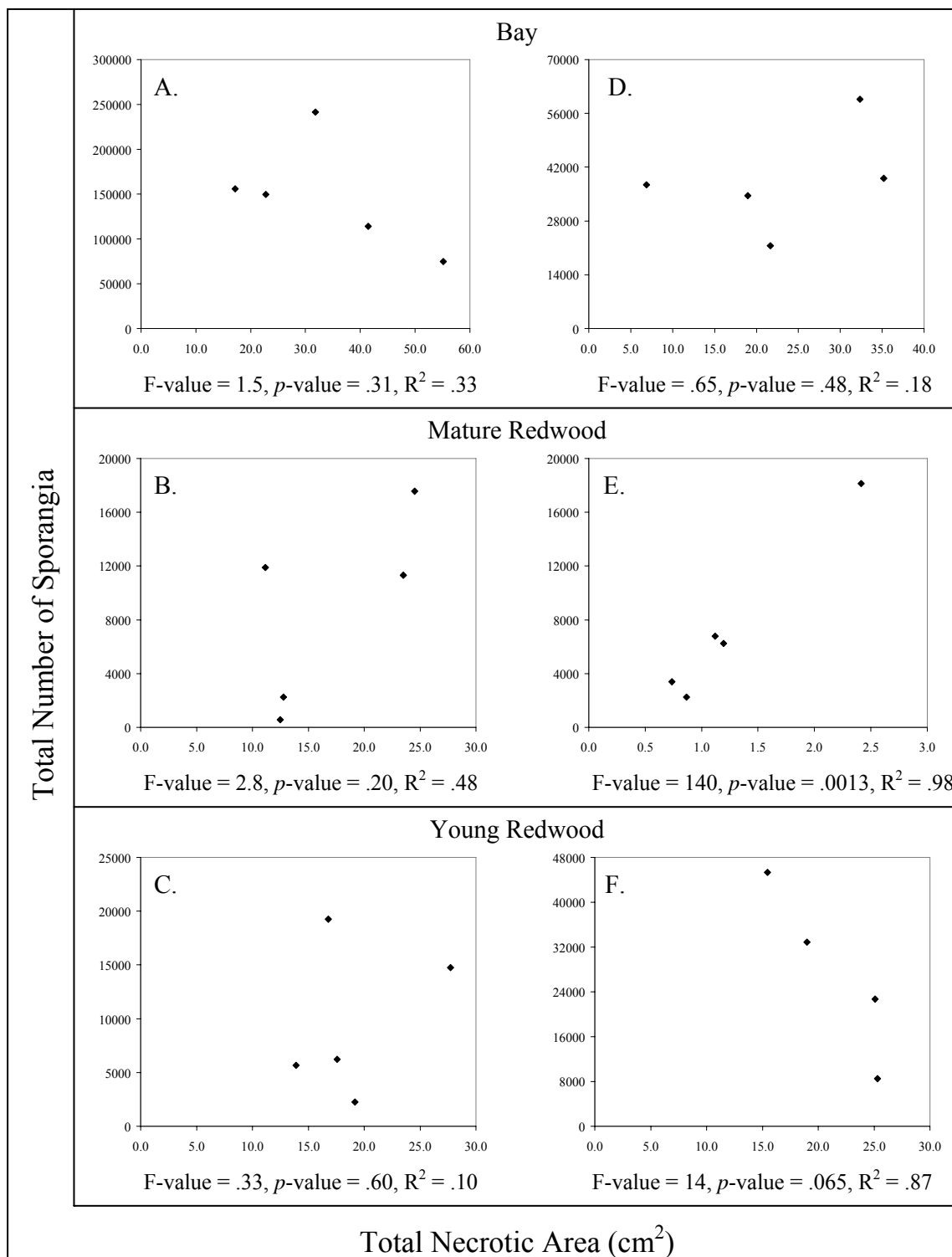Figure 12. Total number of sporangia vs. the total necrotic area (cm^2) for each treatment, separated by trial (A-C: Trial 1; D-F: Trial 2). F-values, *p*-values, and R^2 are the results of simple linear regression analysis for each treatment. A significant linear relationship was found for the mature redwood treatment in the second trial (E), y = 9365x – 4470 (necrotic area *p*-value = .0013, intercept *p*-value = .028).

Sporulation – Attached Tissue

Redwood branches were inoculated and sporulation measured on young and mature tissue. Sporangia density (sporangia/cm^2 per total leaf area and lesion area) was compared between young and mature growth. Examination of scatter plots revealed no correlation between total number of sporangia and total tissue area. No sporangia counts were made immediately after inoculation, unlike in the detached sporulation experiment. The number of sporangia on branches from inoculation was assumed not to differ between treatments. Because number of sporangia in inoculating suspensions may have differed between trials, data from the two trials were analyzed separately. Upon harvest, inoculated branches were dissected into young and mature growth and data was collected for each. Paired t-tests were done to determine how likely a difference between treatment means was by examining the mean of the differences between pairs. No sporangia were detected from any of the control branches; therefore they were excluded from analysis.

Sporangia densities for each treatment were first calculated and compared as sporangia per total leaf area (see Figure 13). Density means for the first trial were 652 sporangia/cm^2 (SE = 181) for mature redwood and 1140 sporangia/cm^2 (SE = 361) for young redwood. Evidence suggests the mean difference between sporangia densities on young vs. mature tissue was not significantly different (two-sided p-value = .30 from a paired t-test). Mean sporangia densities for the second trial were 742 sporangia/cm^2 (SE = 150) for mature redwood and 839 sporangia/cm^2 (SE = 178) for young redwood. No mean difference in sporangia density between young and mature tissue was found (two-sided p-value = .17 from a paired t-test).
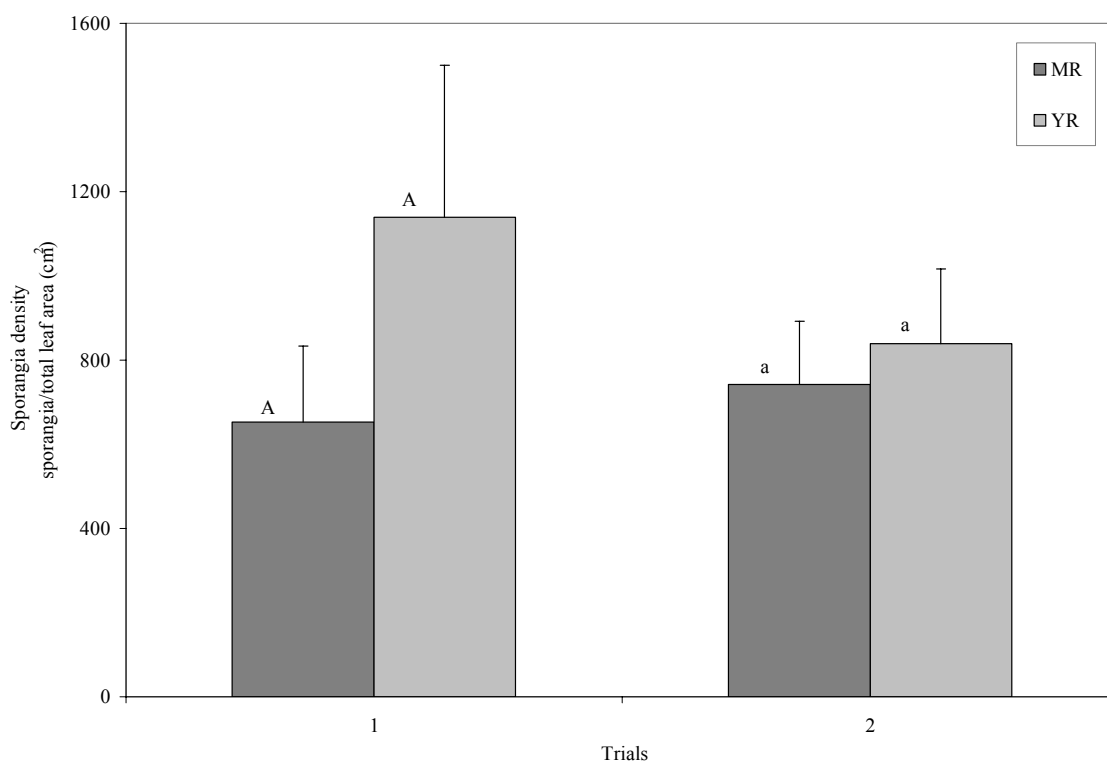
Figure 13. Sporangia density (sporangia/total tissue area [cm^2]) for mature and young branch pairs for trials 1 and 2. The mean difference between young and mature growth for trial 1 was not significantly different (two-sided *p*-value = .30), suggesting no difference in population means. The mean difference between young and mature growth for trial 2 was not significantly different (two-sided *p*-value = .17). Bars represent means +/- standard errors. Significant differences in trial 1 are indicated with different capitol letters above the bars. Significant differences in trial 2 are indicated with different lower case letters above bars.

Sporangia densities were also calculated as sporangia per lesion area (see Figure 14). Density means for the first trial were 5650 sporangia/cm^2 (SE = 1330) for mature redwood and 5660 sporangia/cm^2 (SE = 4530) for young redwood. The mean difference between treatments was not significantly different (two-sided *p*-value = 1.0 from a paired t-test). Density means for the second trial were 11,100 sporangia/cm^2 (SE = 3340) for mature redwood and 13,000 sporangia/cm^2 (SE = 6930) for young redwood. There was no significant difference between the mean differences for this trial either (two-sided *p*-value = .81).
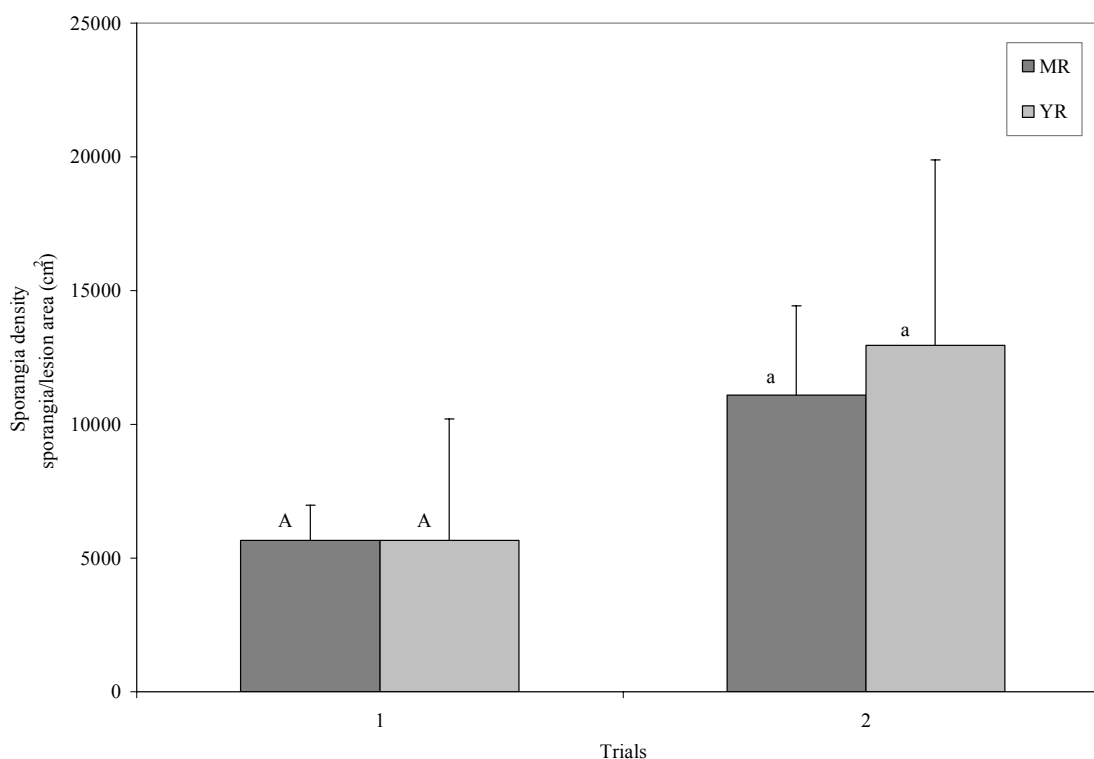
Figure 14. Sporangia density (sporangia/lesion area [cm^2]) for mature and young branch pairs for trials 1 and 2. For the first trial, the mean difference between young and mature growth was not significantly different (two-sided *p*-value = 1.0). The mean difference between young and mature growth for the second trial was not significantly different either (two-sided *p*-value = .81). Bars represent means +/- standard errors. Significant differences in trial 1 are indicated with different capitol letters above the bars. Significant differences in trial 2 are indicated with different lower case letters above bars.

I investigated whether the amount of necrotic area differed in young vs. mature growth in each trial. The mean difference between young and mature necrotic area (cm^2) was significant for the first trial (two-sided *p*-value = .00015 from a paired t-test). There was also a significant mean difference between young and mature necrotic area (cm^2) in the second trial, though the evidence for this difference was not as strong (two-sided *p*-value = .047 from a paired t-test). It is interesting to note that 100% of young growth in the first trial became necrotic. This was not the case in the second trial.

I examined the relationship between total number of sporangia and necrotic area for each treatment using simple linear regression analyses (see Figure 15). Trial 1 young

redwood was analyzed with and without the outlier. No significant relationships between these variables were found in the first trial. In the second trial, total sporangia vs. necrotic area for young growth did fit a linear model (F-value = 21, two-sided p-value = .0024, R^2 = .75). The necrotic area coefficient was 3440 (two-sided p-value = .0024). Mature growth from this trial did not fit a linear model.
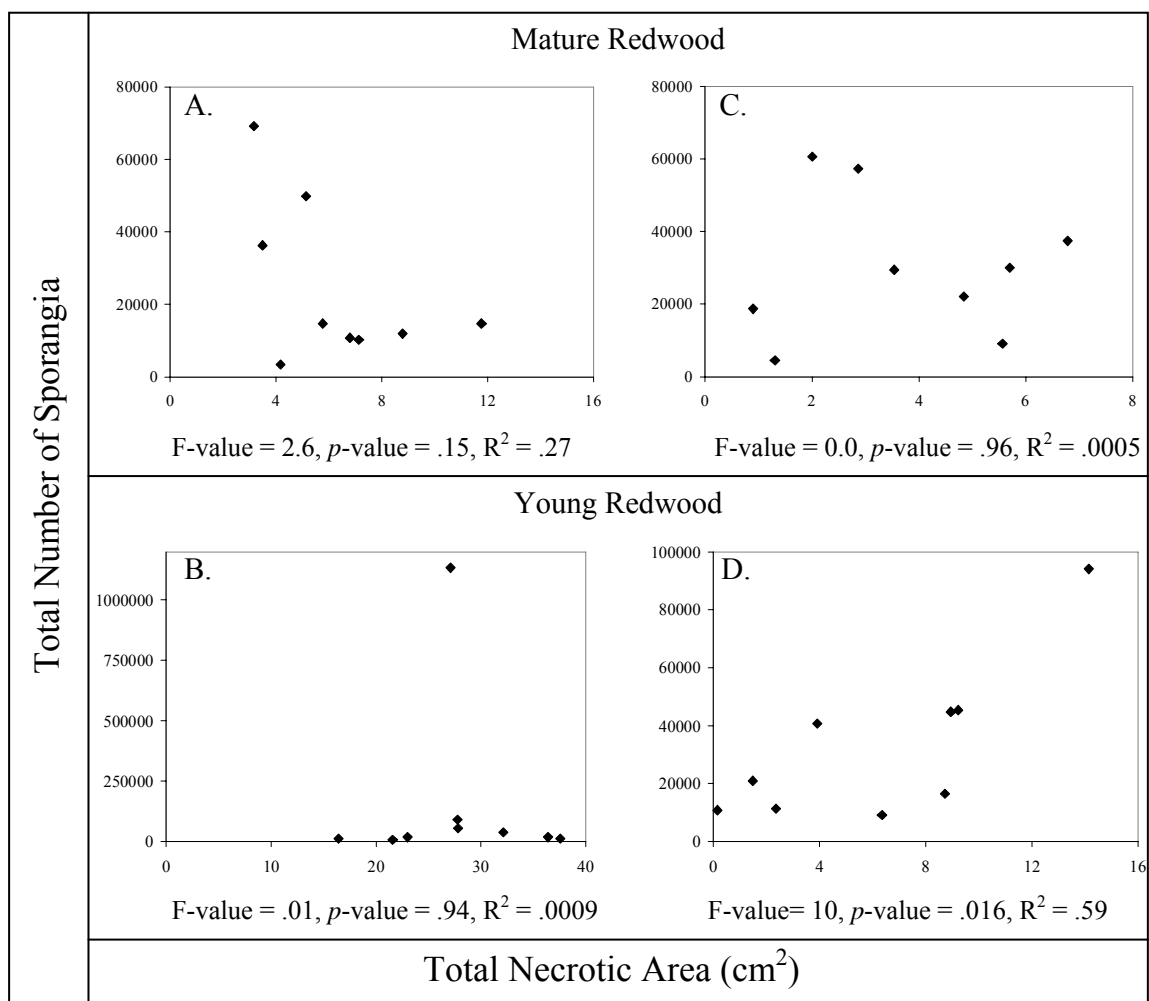
Figure 15. Total number of sporangia vs. the total necrotic area (cm^2) for mature and young redwood, separated by trial (A-B: Trial 1; C-D: Trial 2). F-values, p-values, and R^2 are the results of simple linear regression analysis for each treatment. Trial 1 young redwood was also analyzed without the outlier (F-value = .07, p-value = .81, R^2 = .011). A significant linear relationship was found for the young redwood treatment in the second trial (D), y = 4639x + 4088 (necrotic area p-value = .016, intercept p-value = .72).

Discussion

Experiments conducted under controlled conditions indicate that coast redwood seedlings are not very susceptible to *Phytophthora ramorum.* However, this study affirms the need to consider the potential for disease spread in addition to the damage a disease may cause to this host.

To assess the within host susceptibility to infection by *P. ramorum*, researchers in other studies have measured lesion length in artificial stem inoculations of potted redwood plants. Inoculation of stems, approximately the same diameter as those in my study (5 mm), resulted in 2 mm lesions (Hansen et al. 2005). Seedling stems, approximately 1 cm in diameter, had mean lesion lengths of 6.2 mm, and saplings, approximately 2.5 – 4.5 cm in diameter had mean lesion lengths of 20 mm (Maloney et al. 2002). In my study, lesion length was not measured, due to the difficulty in determining the margins of infected tissue.

Lesion size on coast redwood seedlings appears to be significantly lower than on many other inoculated conifers and hardwoods. Average lesions on known conifer hosts of *P. ramorum*, Douglas-fir, Pacific yew, and grand fir seedlings, were 8.0, 2.6, and 1.1 cm, respectively (Hansen et al. 2005). Even some non-hosts developed stem lesions that were greater than for inoculated coast redwood: western white pine (6.8 cm), western larch (6.5 cm) and western hemlock (6.5 cm). Tooley and Kyde (2007) measured lesion area on inoculated hardwoods, and found that lesion area was not always correlated with susceptibility. For example, lesion area for white oak, a non-host, was greater than for coast live oak seedlings, a species highly susceptible to sudden oak death. Lesion size from seedling stem wound inoculations must be interpreted carefully, as this may not accurately reflect the susceptibility of species in the forest. A plant dip in a zoospore suspension may result in a better approximation of natural infection, especially for assessing susceptibility to foliar blight (Hansen et al. 2005). Artificial stem inoculation is appropriate for determining if the pathogen is spreading through the wood and if it is capable of spreading from sprouts back into the mother tree.

My goal was to determine the distance colonized by *P. ramorum* within the host. My results show that it is unlikely that *P. ramorum* will move from infected basal sprouts back into the mother tree. However, in a few replicates *P. ramorum* successfully colonized tissue several centimeters from the inoculation site. In 58% of main stem inoculations, the pathogen was not detectable beyond the inoculation site, and in only 3 of 19 replicates was it detected beyond 15 mm. In branches, the pathogen was recovered beyond the inoculation site more frequently, in 92% and 50% of seedlings in the first and second trials, respectively. The colonization of tissue 4.5 cm down the main stem from a branch inoculation indicates that *P. ramorum* could potentially move from a young sprout back into the mother tree, albeit rarely. The variation in maximum colonization distance between replicates likely indicates differences in susceptibility between individuals.

No main stem inoculations caused seedling mortality. Small side branches were often killed, apparently girdled, by the time of harvest. Significantly different mean diameters between branches that were killed and those that were not indicates *P. ramorum* is only capable of killing relatively small branches (mean diameter 2.4 mm) in redwood. In contrast, on Douglas-fir, stems 1 cm in diameter were occasionally girdled by *P. ramorum* (Davidson et al. 2002). Nurseries with young coast redwood seedlings should be concerned about branch dieback. Seedling mortality in natural settings is not likely to be a problem as year old seedlings did not die in my experiments, even with wound inoculation and ideal conditions for disease development.

Most coast redwood seedlings effectively localized the pathogen by the eighth week after inoculation. I observed xylem discoloration beyond the pathogen recovery distance in several samples. Discoloration is likely the host's response to the presence of the pathogen and/or wounding (Shigo 1984). In canker causing diseases, a host's ability to quickly compartmentalize the pathogen allows it to quickly recover after initial infection (Shigo and Tippett 1981; Shigo 1984; Smith et al. 1997). The lack of spread and lack of girdling of main stems shows that coast redwood quickly compartmentalized the pathogen and may recover from the infection.

While I have shown that host tissue is generally resistant to infection by *P. ramorum*, determining what tissues are being colonized will still be informative. Recent studies have shown xylem colonization by *P. ramorum* of other hardwood hosts (Pogoda

and Werres 2004; Brown and Brasier 2007; Parke and Lewis 2007; Parke et al. 2007). Microscopy is currently underway to determine which tissues of coast redwood are colonized by *P. ramorum.*

Host susceptibility was also assessed by examining the relationship of zoospore inoculum dose to disease incidence. In this study, the lowest dosage to cause at least 50% of exposed branches to become infected was 4.4 x 10^3 zoospores/mL. A high inoculum dose (10^5 zoospores/mL) was required for consistent disease incidence and severity. *P. ramorum* was only detected from one seedling inoculated with 4.4 x 10^4 zoospores/mL; while disease incidence was higher on seedlings inoculated with lower dosages. Detached rhododendron leaves dipped in the 4.4 x 10^4 zoospores/mL suspension were infected, confirming the viability of the inoculum. Differences in host susceptibility between individuals could explain the low incidence of disease observed at this dose compared to other inoculum doses.

Qualitative observations indicate that dose was not necessarily correlated with symptom severity. More extensive symptom development in the second trial could be correlated with a greater amount of succulent host tissue. The time from fertilization to inoculation was similar between trials, yet their stage of growth may not have been uniform.

In the context of dose response experiments on other hosts, the most relevant comparison to this study would be to other susceptible conifers. In previous work done on Douglas-fir, researchers determined that inoculum levels of at least 10^4 zoospores/mL were necessary to cause symptoms and that disease incidence was dramatically influenced by host phenology (Hansen et al. 2005; Chastagner et al. 2006). Similar inoculum levels cause symptoms on coast redwood.

Other dose response studies have focused on disease severity, as opposed to disease incidence. Symptom expression on California bay was limited to 6 x 10^4 zoospores/mL (Hansen et al. 2004). Other foliar hosts, *Rhododendron macrophyllum* (Pacific rhododendron), *Vaccinium ovatum* (evergreen huckleberry), *Arbutus menziesii* (madrone), tanoak, *Arctostaphylos uva-ursi* (bearberry) and *Kalmia latifolia* 'Minuet' (Minuet mountain laurel) all exhibited some level of necrosis when inoculated with 2 x 10^3 zoospores/mL (Tooley et al. 2004; Hansen et al. 2005). Although percent necrosis

was not measured in my study, a few seedlings showed symptom development at a dose approximate to the lowest dose in the above experiments.

While disease severity is informative, it alone does not translate to host susceptibility. For example, large lesions do not typically develop on California bay and tanoak leaves, yet they are both extremely susceptible. Disease incidence measurements are also necessary. When zoospore suspensions were applied as soil drenches, mortality of Rhododendron cv. Nova Zembla seedlings was high at 4.5 x 10^4 zoospores/mL (Parke and Lewis 2007). In my study, rhododendron leaves were used as a positive control. One hundred percent of rhododendron leaves exposed to all doses, as low as 3.4 x 10^2 zoospores/mL, expressed symptoms. In terms of disease incidence, rhododendron appears to be a more susceptible host. Overall, coast redwood develops symptoms when exposed to inoculum levels similar to levels that cause symptoms on other hosts.

While knowledge of host susceptibility helps to elucidate the effect *P. ramorum* may have on coast redwood seedlings in the field or nursery, knowledge of the pathogen's ability to disperse from coast redwood helps us to understand the threat posed to other hosts in the environment.

I quantified sporulation on young and mature coast redwood tissue using a new method, and compared this to sporulation on California bay leaves, a foliar host known to support abundant sporulation of *P. ramorum* (Davidson et al. 2005). In my detached leaf assay, sporangia density was significantly higher on California bay than on coast redwood in the first trial. This is not surprising, as other research has shown that extensive sporulation on this host is driving the epidemic in California (Davidson et al. 2005). A more interesting outcome is the significantly higher sporulation density on mature redwood tissue in the second trial, and comparable levels of sporulation between young redwood and California bay.

Davidson et al. (2005) quantified zoospores from artificially inoculated bay leaf disks. The researchers observed a maximum of approximately 800 sporangia/cm^2 lesion area. After adjusting for different incubation periods between studies, they measured up to 4000 sporangia/cm^2; I measured 2000-5000 sporangia/cm^2 lesion area for California bay. While this estimate is rough, it does show that my findings are in a similar range.

Number of sporangia/cm^2 lesion area on California bay leaves was also estimated in a project report published on-line by DEFRA in the UK (2004). They estimated *P. ramorum* produced 216 sporangia/cm^2 lesion area on bay leaves after a 7 day incubation period. One conifer species, *Taxus baccata* (European yew), was included in the study. Sporangia density on this species was one of the lowest measured, at 8 sporangia/cm^2. Experimental design may contribute to these low numbers. Researchers cut out lesions and counted sporangia rinsed from this tissue only. Also, there is no mention of keeping the samples moist during incubation, which may have limited sporulation (U. K. Department for Environment, Food and Rural Affairs 2004).

Vettraino et al. (2007) quantified sporangia/cm^2 lesion area on 51 species in detached leaf assays. They reported sporangia densities up to 6500 sporangia/cm^2 lesion area on *Ruscus aculeatus* (Butcher's broom), the species with the highest level. The mean sporulation density for this species was approximately 5500 sporangia/cm^2, approximately what I observed on California bay in the first trial and on mature redwood in the second trial. The lowest mean sporangia density calculated in my study still places coast redwood higher than half of the species studied by Vettraino et al. (2007). It is unknown what inoculation or quantification methods were used in this study.

Sporangia production on mature and young redwood in the second trial is high, perhaps alarmingly so. The surprising result of significantly higher sporangia density on mature redwood was due to minimal symptom development, even though total number of sporangia produced was relatively similar between trials. It is clear that extensive symptom development is unnecessary for high sporulation. In other systems, it has been shown that ideal environmental conditions for symptom development are not necessarily ideal for sporulation (Rotem and Cohen 1970).

Differences in temperature and light intensity have been shown to affect *P. ramorum* in vitro and in vivo (U. K. Department for Environment, Food and Rural Affairs 2004; Davidson et al. 2005; Englander et al. 2006). Other variables that influence sporulation in vivo are humidity, duration of leaf wetness, and aeration (Rotem et al. 1978). In this study incubation method may have influenced treatment differences. Ten leaves/branches of the same treatment were maintained in their own separate crisper. While this method prevented contamination between treatments, differences in

microclimates between crispers could have contributed to differences in sporangia densities. Further research is necessary to determine the influence of environmental factors on sporulation in vivo.

In trying to simulate more natural conditions, sporulation on attached branches was also examined. Young and mature branches were sampled as pairs to try to control some of the environmental variability between treatments. All mean sporangia densities in this experiment were greater than the highest density in the detached experiment. No significant differences were found between mature and young redwood sporangia densities.

In each trial, sporulation on one replication was dramatically higher than the rest. In the first trial, sporulation on one seedling was 13 times greater than on the next highest replication. In the second trial, the highest sporangia density was 5 times greater than the next highest replication. This highlights the potential for extremely high levels of sporulation and the variability in sporulation between individuals. Variability in inoculum production on different individuals of California bay has been documented (Arnold 2007). The sporangia densities reported here are higher than those reported elsewhere for *P. ramorum*, yet they are not uncharacteristic for a *Phytophthora* species. For example, it has been reported that *P. infestans* produces 10^5-10^6 sporangia/cm^2 of leaf tissue within a wetting period of one night (Rotem et al. 1978).

I also examined the relationship between lesion area and total number of sporangia produced. In species comparisons, researchers have reported no correlation between lesion area and total number of sporangia (U. K. Department for Environment, Food and Rural Affairs 2004), and an inverse relationship between lesion area and total number of sporangia (Vettraino et al. 2007). No clear trend emerged in my study. Lesion area and total number of sporangia did exhibit a positive linear relationship in two cases. One treatment showed a trend towards an inverse relationship. For redwood, lesion area

is not a good predictor of sporulation level. The specific site of sporulation was not examined in these experiments. If we hypothesize that sporulation is occurring along the lesion margin on twigs or needles, it is not surprising that sporulation did not necessarily increase with lesion area. Future studies should help elucidate where sporulation is occurring and reveal if sporangia density is correlated with lesion perimeter measurements.

My results show that sporulation on redwood does occur under certain environmental conditions, and the potential for sporulation may be high. There is only preliminary evidence for sporulation on coast redwood under natural conditions, however. Davidson et al. (in press), observed sporangia on 25% of naturally infected redwood branches collected in February but not on any branches collected in October 2003.

Results from sporulation studies conducted in a controlled environment may differ substantially from sporulation under natural conditions. Davidson et al. (in press) quantified zoospores from naturally infected California bay leaves and tanoak branches. They found approximately 13 sporangia/leaf for one season and 51 sporangia/leaf for the second season. These numbers are much lower than laboratory studies done on sporulation. Because of host and environmental variability, seasonality of sporulation, and discrepancies between laboratory and field results, it is crucial to do further research on quantifying sporulation on naturally infected redwood. In order to truly assess sources and quantities of inoculum, studies on the viability, dispersal, and infectivity of inoculum are also necessary.

Conclusion

Understanding the threat posed by *P. ramorum* to coast redwood is important. Coast redwood is a dominant species in much of the forest currently infested with sudden oak death. For this reason, many people are looking to use redwood in reforestation projects in infested areas (Yana Valachovic, University of California Cooperative Extension, Humboldt and Del Norte counties, personal communication). It appears that redwood vitality is not drastically reduced by *P. ramorum*, though this should be confirmed through further field observations. Governmental agencies have created a natural barrier in California in an attempt to limit the northern spread of the pathogen. To my knowledge, there are no plans to remove or limit presence of redwood in this barrier. From an epidemiological standpoint, the effectiveness of this strategy could be compromised by the possibility of abundant sporulation from infected redwood tissue, should infection occur. In addition, nonlethal infections on non-oak hosts may allow the pathogen to persist for a long time in the forest (Rizzo et al. 2005).

Land managers should plant nonhost species when possible, at least until there is a better understanding of disease spread. Applying this recommendation is of course difficult in infested areas, where many natives are susceptible. Where redwoods are near infested areas, careful monitoring is essential. Removal of symptomatic tissue is probably not an effective strategy, as authors of a recent review article note findings of *P. ramorum* propagules in rainwater collected up to 25 m in the crowns of emergent redwood trees (Rizzo et al. 2005). A useful strategy for those interested in planting coast redwood in infested areas may be screening for more resistant seedlings, and clonal propagation of these, as there appears to be considerable variation in host susceptibility. This is evidenced by the range in pathogen colonization distances in inoculated stems and the inconsistencies in disease incidence observed in branches exposed to inoculum concentrations below 10^5 zoospores/mL.

Redwood is valued in the timber industry when decay resistance is important. Studies are underway to determine if sapwood can become infected if inoculated (Jennifer Parke, personal communication). This could have major implications for

regulations regarding transport of wood. Researchers have found that *P. ramorum* propagules increase more in redwood wood chips under California bay than in western redcedar wood chips due to redwood lacking a specific antimicrobial compound (Manter et al. 2007b). Bark chips have not been compared between species. This may be important, as redwood bark chips are currently used as garden mulch.

Coast redwood is important culturally, as well. Much effort continues to be put into saving old-growth redwood forests. If a pathogen were threatening these forests, many organizations would likely get involved. An economically valuable tourist industry has been built around coast redwood. Risk of disease spread from moving redwood plants or products (like burls) out of quarantined areas seems low. Though sporulation on redwood was observed, a high inoculum level is required to get initial infection and pathogen colonization is usually minimal.

Coast redwood is a resilient species. No tree-killing diseases have been described, other than seedling diseases caused by damping off and *Botrytis cinerea* on seedlings less than a year old (Olson Jr. et al. 1990). Though redwood is not resistant to *P. ramorum*, symptom development and disease progress are limited. The real threat lies in the ability of *P. ramorum* to sporulate on coast redwood tissue. In attempting to manage this disease, it is crucial to know the sporulation potential on various hosts, in addition to host susceptibility. While sporulation levels under artificial and natural conditions likely differ, controlled studies can point out a direction for further research. Inconsistent presence and quantity of sporangia on infected tissue offers a research challenge. Regardless, controlled experiments addressing sporulation requirements on redwood, as well as other hosts, may help guide field studies.

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Appendix: Fieldwork

Appendix: Fieldwork

Introduction

The objective of this study was to evaluate the risk *P. ramorum* poses to coast redwood seedlings in the field. To do this, I attempted to determine disease incidence in relation to time of year, precipitation and inoculum levels. I also wanted to examine disease development on redwood seedlings in association with a specific overstory species, California bay (*Umbellularia californica*).

Experimental Sites

Experimental sites were located in southern Humboldt County, the site of the northernmost California sudden oak death infestation. Conditions in this area are conducive for disease spread because of mild temperatures, heavy precipitation, "hot spots" of infestation and prevalence of hosts. Infestation around the Avenue of the Giants is unsettling due to the high amount of tourist traffic in the area.

Fieldwork for this study was carried out at three sites located along the south fork of the Eel River on alluvial flats (see Table 1). All sites were at an elevation of approximately 100 m. Two of the sites were on California State Parks land. The first was on the John B. Dewitt State Reserve. Raintraps were set up on approximately 1.5 ha of the 234 ha reserve. The area chosen for raintraps was part of the Margaret Whelan O'Meara Grove. This area was one of the first places where sudden oak death was detected in Humboldt County. The overstory was dominated by coast redwood and a few Douglas-fir. Overstory tanoak were also abundant. There was a dense understory of small, shade-suppressed redwood and tanoak. Bole cankers were observed on tanoak. The infestation level on tanoak appeared to be very high. *P. ramorum* was consistently isolated from tanoak through the duration of this study. At the start of the study, *P. ramorum* was also isolated from redwood needles. The grove was heavily shaded, receiving little direct sunlight even during the middle of the day.

The second site located on State Parks land was located 8 km north of Myers Flat, immediately adjacent to the Avenue of the Giants, a scenic thoroughfare paralleling Hwy 101. This site was chosen because of its proximity to Jay Smith Road. The University of

California Forestry Extension in Humboldt County conducted eradication trials at the Jay Smith site. Symptomatic bay and tanoak were present at this site before the prescribed burn of 20 ha in November 2006. The Harrison Chandler Grove is just south of the burned area. Raintraps were set up on approximately 3 ha in the Harrison Chandler Grove. Overall, the forest here appeared to be much healthier than that at the O'Meara Grove. The overstory was dominated by coast redwood. On half of this site, a south facing slope and draw beneath, tanoak and California bay were approximately equal in abundance. Symptoms were minimal, though present, on both tanoak and California bay. No bole cankers were noted on tanoak. On the other half of the site, a north facing slope, California bay was absent from the overstory. Foliar lesions on tanoak were the only visible symptoms. *P. ramorum* was isolated from tanoak and California bay at this site.

The third site was located in Tooby Memorial Park, a part of the Southern Humboldt Community Park in Garberville. Raintraps were set up on 3 ha of the 192 ha park. The overstory was again dominated by coast redwood with a few Douglas-fir. Symptomatic tanoak and California bay were present at this site. Bole cankers on tanoak were observed. Many small redwood saplings were also present. Disease incidence and severity on tanoak and California bay appeared to be high. *P. ramorum* was isolated from all three hosts at the initiation of the study, and throughout the study from tanoak and California bay.

Table 1. Field sites in southern Humboldt County. All sites had an overstory of coast redwood and tanoak. California bay was not present at all sites. The stand density of symptomatic tanoak differed between sites.

Site	Location	Prevalence of disease symptoms on tanoak	Presence of California bay
O'Meara Grove	Redway, CA Humboldt Redwood State Park (HRSP)	High	No
Tooby Park	Garberville, CA Garberville Community Park	High	Yes
Chandler Grove	5 miles north of Meyers Flat, CA HRSP	Low	No
Chandler Grove	5 miles north of Meyers Flat, CA HRSP	Low	Yes

Detecting Inoculum

Rainwater was collected and attempts made to quantify inoculum. Raintraps consisted of a 5 gallon bucket, partially buried in the duff, with a mesh net secured over the top of the bucket. Buckets were placed around the sites in groups of three. When collecting, water from the three buckets was poured together, and the sample taken from the pooled collection. Buckets were placed under symptomatic foliage of bay or tanoak. Often, foliage from redwood was further above in the canopy over the buckets. Fifteen buckets were placed out at the O'Meara Grove and another fifteen buckets were placed at Tooby Park. Thirty buckets were placed at the Harrison Chandler Grove, half on the side with California bay and half on the side without California bay. Five 1 L water samples from O'Meara and Tooby and 10 samples from Harrison Chandler were collected weekly from December 2006 through April 2007.

After collection, the water was prefiltered through Miracloth (VWR 80058-394) to remove any coarse debris. Then the sample was filtered through a 5 μm Millipore filter, a pore size small enough to catch any *P. ramorum* propagules. The filter was placed onto pimaricin-ampicillin-rifampicin selective media (PAR), and the plates examined for colony forming units (CFU) at OSU. This method was initially used for quantification of *P. ramorum* CFU by researchers in the Rizzo lab at UC Davis (Davidson, Wickland et al. 2005).

P. ramorum positive samples were recovered on two occasions using this first method. On February 11, 2006 5 positive samples were collected from O'Meara and Tooby. On February 17, 2006 2 positive samples were collected from O'Meara.

Due to erratic detection, methodology was altered at the end of April (see Figure 1). A baiting method, like those used to detect other *Phytophthoras* in water, was used (Erwin and Ribeiro 1996). A detection protocol similar to that used in Curry County, OR was followed. Rhododendron leaf baits, with a small amount of water, were placed in each of the 60 raintraps, collected biweekly, and plated onto PAR to determine presence or absence of inoculum. This method was used until June 2007.

On April 28, 2007 1 leaf bait, collected from a raintrap in Harrison Chandler, yielded *P. ramorum*. On May 13, 2007 positives were detected at all three sites from a

total of 6 traps. Daily rainfall data was collected near Miranda (approximately 5 km north of the Harrison Chandler Grove) by the Department of Water Resources and accessed at the California Data Exchange Center (http://cdec.water.ca.gov/cgi-progs/staMeta?station_id=MRD).
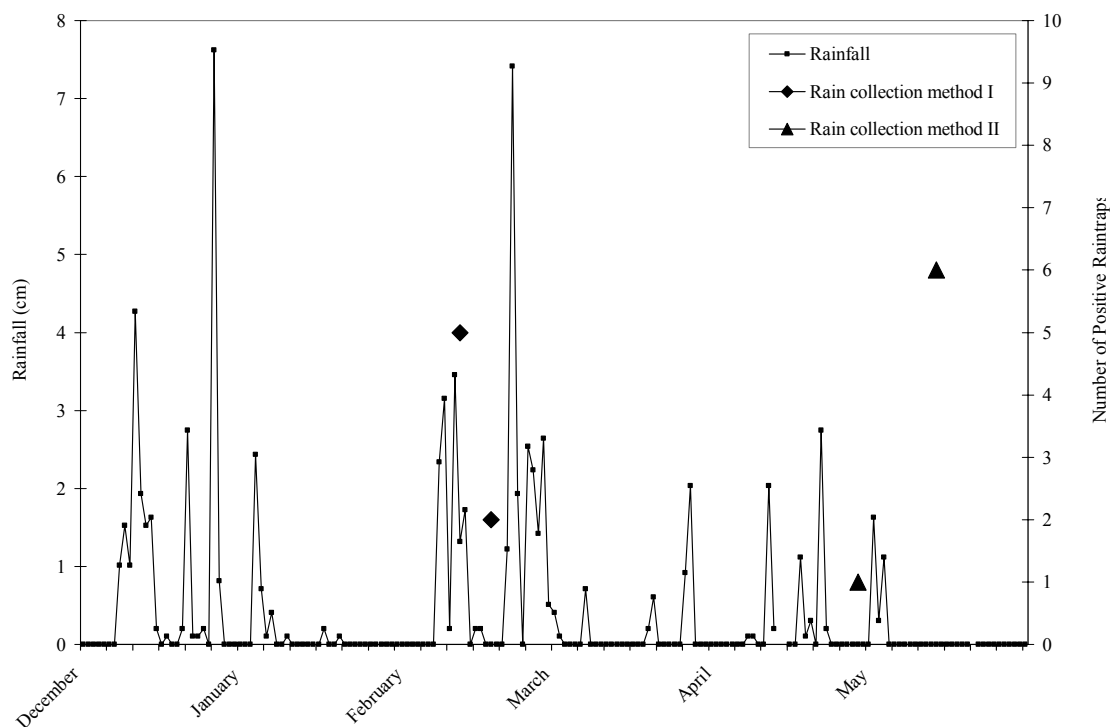

Figure 1. Daily rainfall vs. number of raintraps positive for *P. ramorum*. Rainfall was measured along the Eel River in Miranda, CA (approx. 5 km from the Harrison Chandler field site). Rain collection method I (♦) was discontinued at the end of April. Rain collection method II (▲) was begun at this time.

Seasonality of Disease Incidence

Using a complete random design, 20 healthy, potted seedlings were placed at Tooby Park for a two-month period beginning in December 2006. A new group of seedlings was placed at Tooby Park every two months through June 2007.

Presence of *P. ramorum* was determined by isolation and PCR from selected stems, needles, and buds of each seedling. No seedlings were found positive for *P.*

ramorum at any time period. Therefore, we were unable to determine when redwood seedlings are most susceptible to infection.

Symptom Development

Two healthy seedlings were planted by each of the 60 raintraps (120 seedlings total) in December 2006. Symptom development was monitored monthly through June 2007. A count of necrotic needles, cankers and length of stem dieback per seedling was measured monthly. Growth of seedlings over time was used as an indicator of overall vigor.

A small amount of branch dieback occurred on 1 of the 120 seedlings placed in the field. Approximately 3 cm of tip dieback was noticed on a seedling in Tooby Park in April 2007. Attempts were not made to isolate *P. ramorum* from this tissue because of the desire to avoid destructive sampling during the course of this study and watch symptom development over time. Seedlings at O'Meara suffered extensive insect damage. Disease symptoms did not develop on any of these seedlings. No symptoms were observed on seedlings at the Harrison Chandler Grove. Because of the low number of seedlings developing symptoms, no relationship between symptom development and inoculum levels or presence of California bay could be determined.

Discussion

P. ramorum was rarely detected from rainwater for the duration of this study, December 2006 to June 2007. Other researchers in the area baiting rainwater at known infested sites had few positive detections throughout the season, as well (Chris Lee, University of California Cooperative Extension, Humboldt and Del Norte counties, personal communication). In comparison, Curry County, OR had consistent detections from rainwater during this same period (Everett Hansen, Oregon State University, personal communication), but also received considerably more rainfall at 179.7 cm vs. 76.5 cm at my sites (see Figure 2). Curry County also received more consistent rainfall during this period (see Figure 3). This may be important because of the necessity of moisture for sporulation. More days of rain could lead to more sporulation events, and therefore higher detection. Not only was rainfall in Humboldt County less than in Curry

County, it was also considerably less than usual. Rainfall in southern Humboldt County was less than half its normal level from December 2006 to May 2007 at 76.5 cm vs. 132.6 cm average (see Figure 4). Rainfall data for both Humboldt County, CA and Curry County, OR are from the National Climatic Data Center website (http://www.ncdc.noaa.gov/oa/ncdc.html).

Lack of symptoms on redwood seedlings may be due to absence of inoculum because of unfavorable environmental conditions. Given the lack of data collected it is impossible to say from this study alone. In future studies on incidence and symptom development, a more susceptible host, such as rhododendron, should be included as a positive control to help ascertain if viable inoculum is present. Supplementary experiments conducted on redwood seedlings under controlled environmental conditions will also help elucidate the relationship between host and pathogen. At least one additional season would be necessary to assess the susceptibility of coast redwood to *P. ramorum* in the field.
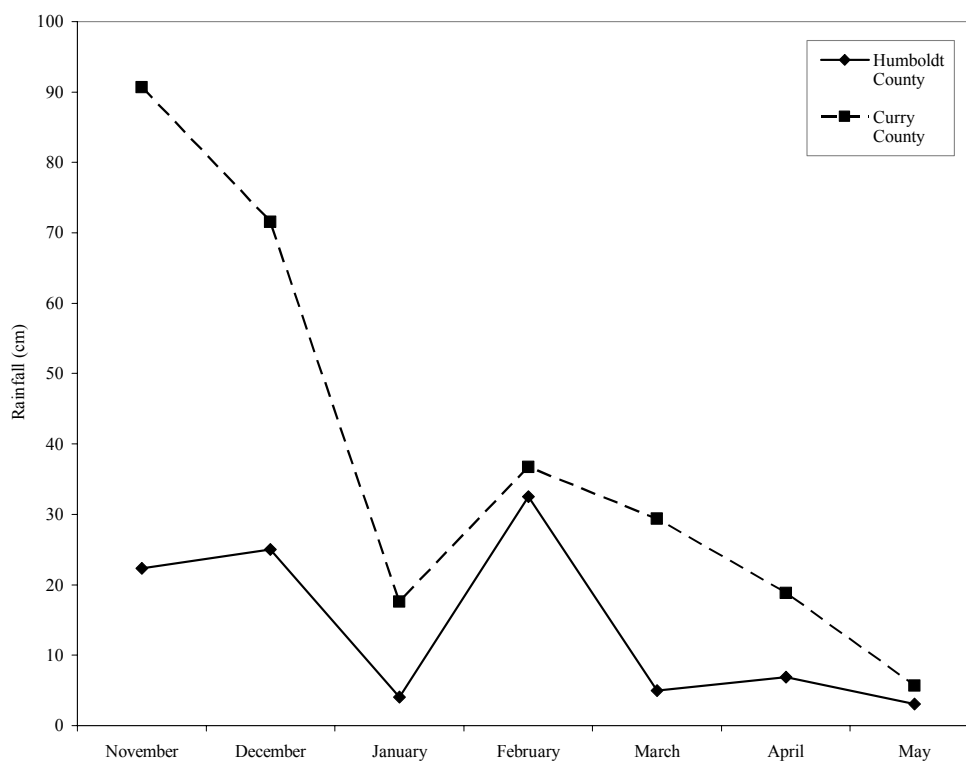

Figure 2. Monthly rainfall: Humboldt County (Miranda, CA) [◆] vs. Curry County (Brookings, OR) [- ■ -] from November 2006 through May 2007.
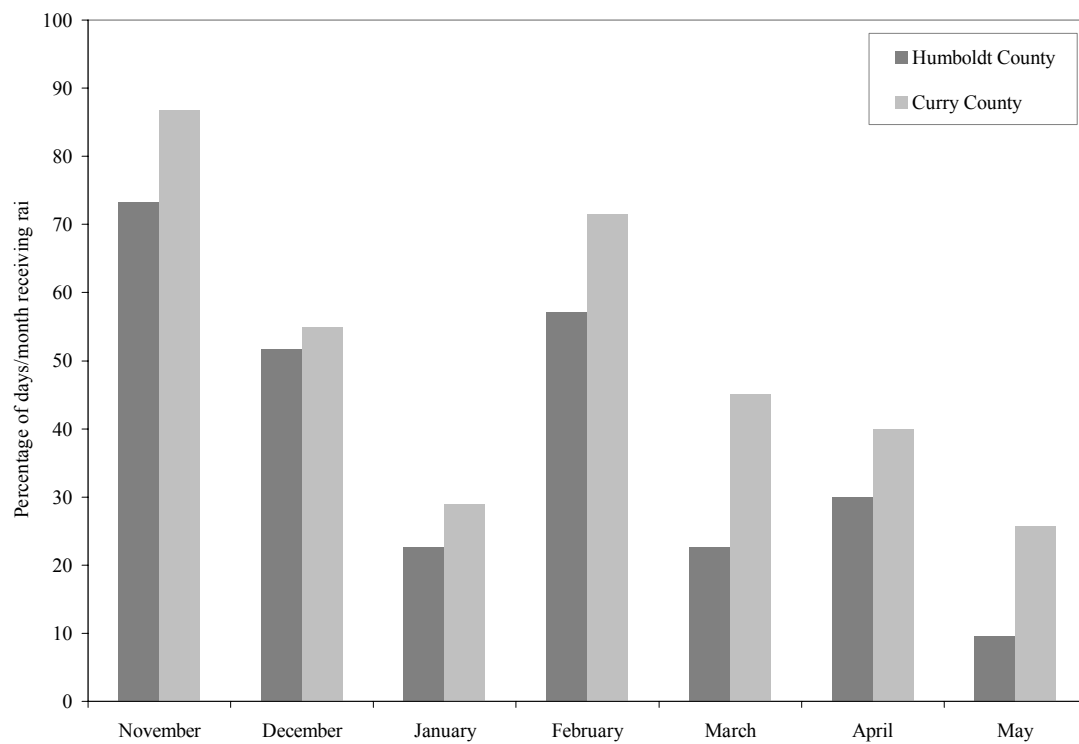
Figure 3. Percentage of days/month receiving at least .254 cm of rain in Humboldt County (Miranda, CA) vs. Curry County (Brookings, OR) November 2006 – May 2007.
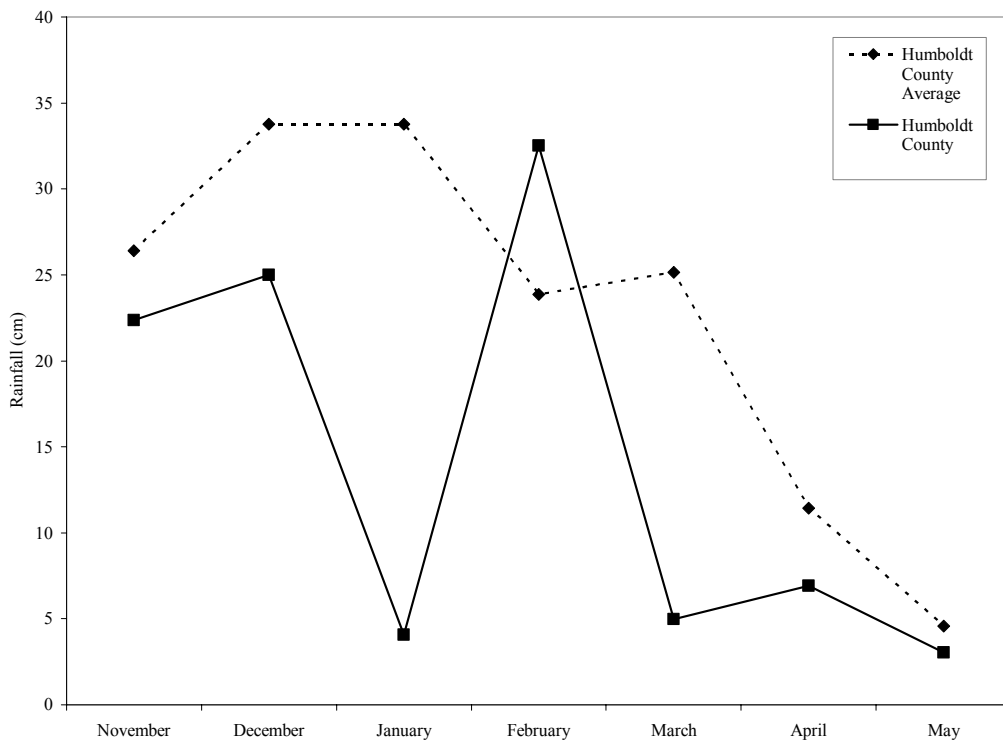
Figure 4. Humboldt County (Miranda, CA) monthly rainfall November 2006 – May 2007 [■] vs. average monthly rainfall November – May [◆].