Viability of *Phytophthora ramorum* on California Bay Laurel After Exposure to Temperature Extremes

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ABSTRACT

The California Bay Laurel (*Umbellularia* californica) is one of the primary hosts and vectors for *Phytophthora ramorum,* the pathogen responsible for sudden oak death (SOD) in California and Oregon's coastal woodlands. This study focused on the effects of temperature and host-pathogen interactions on the viability and growth rates of *P. ramorum*. Laboratory infected Bay Laurel cuttings from trees with observed high and low susceptibility, were subjected to hot or cold temperature extremes for seven weeks and *P. ramorum* growth was determined. The highest *P. ramorum* growth rates occurred in the cold treatment and the lowest occurred in the hot treatment. Significant negative effects on growth were found to be associated with the hot and control treatments. Tree susceptibility and plot also were variables that had significant effects on growth. Highly susceptible, or "hotspot" trees and plots 8 and 10 all had significant positive effects on *P. ramorum* growth across all treatments. When the interaction between the variables "tree susceptibility" and "plot" were modeled, I found that "hotspot" trees within plots 8 and 10 had a significant negative effect on pathogen growth. These results suggest that temperature, tree susceptibility, and the local environment all play a role in pathogen growth. With the uncertain future of California's climate, it is essential to understand how climatic variables affect the viability of such a destructive pathogen as *Phytophthora ramorum.* With a better understanding of these disease dynamics, we can more accurately predict the effects of climate change on the spread and distribution of sudden oak death.

KEYWORDS

Sudden Oak Death (SOD), *Umbellularia californica*, forest pathology, climate change, plant diseases

INTRODUCTION

Climate change is expected to have varying effects on precipitation levels, temperatures, and ecosystems throughout California. Estimates project the future average temperature change in California over this century to be +1.5ºC under low green house gas (GHG) emission scenarios and +4.5ºC under high GHG emission scenarios within the next 70 years (Cayan et al. 2008). In addition to more extreme high and low temperature changes, climate change is predicted to bring about much more variability in precipitation and extreme weather events (Cayan et al. 2008). Drastic changes in temperature pose a threat to forest ecosystems because temperature and drought stressors have been shown to increase the vulnerability of trees to insect attacks and infections from plant pathogens (Dale et al. 2001, Garrett et al. 2006). Currently, little is known about the effects of climate change on the plant pathogen *Phytophthora ramorum,* also known as sudden oak death, which is of particular concern for California oak woodland ecosystems (Werres et al. 2001). The introduction in the mid 1990's and subsequent spread of *P. ramorum* throughout half of California's coastal counties has resulted in the deaths of hundreds of thousands of oaks and tanoaks (Rizzo and Garbelotto 2003, Guo et al. 2005).

With more than 109 known woodland and nursery host species (Denman et al. 2005, Hansen et al. 2005, Tooley and Kyde 2007, and Tooley et al. 2004), and human-mediated transport (Rizzo and Garbelotto 2003, Grünwald et al. 2012), *P. ramorum* has the potential to spread throughout regions of California with suitable climates. It is possible that with changes in temperature, precipitation, and host distribution brought about by climate change, the distribution of *P. ramorum* could expand. *P. ramorum's* peak sporulation occurs under cool, moist conditions of roughly 18-22ºC (Rizzo and Garbelotto 2003), and temperature changes associated with seasonality play a role in *P. ramorum's* ability to infect plant material. During the hot, dry summer months, *P. ramorum* enters a dormant phase; at the beginning of the rainy season, there is an observed lag-time between precipitation and pathogen sporulation, which is potentially associated with colder winter temperatures (Davidson et al. 2005). If unfavorable temperatures prolong dormancy, it is possible that the severity and spread of infection would decrease because of a shorter climatic window for pathogen sporulation.

Currently, however, little is known about the effects of extreme temperatures inducing *P. ramorum* dormancy on bay laurel (*Umbellularia californica*), one of its primary hosts and propagators in California coastal woodlands.

The pathogen-host interactions that occur between bay laurel and *P. ramorum* are also thought to contribute to the pathogen's ability to persist in the environment. Current research being conducted in the Crystal Springs Reservoir watershed in San Mateo County, CA has identified bay laurel trees with seemingly higher and lower susceptibilities to infection. When trees with visible signs of infection were sampled three seasons out of the year, *P. ramorum* was isolated consistently from some trees rarely or never isolated from others. Trees with observed high susceptibility were dubbed "hotspot" trees and those with low susceptibility were called "coldspot" trees. Variation has been found in the susceptibility to *P. ramorum* within and among populations of California bay laurel (Dodd et al. 2005,2008; Hüberli et al. 2012, Meshriy et al. 2006), but there is little to no research examining the effects of bay laurel susceptibility on rates of pathogen dormancy.

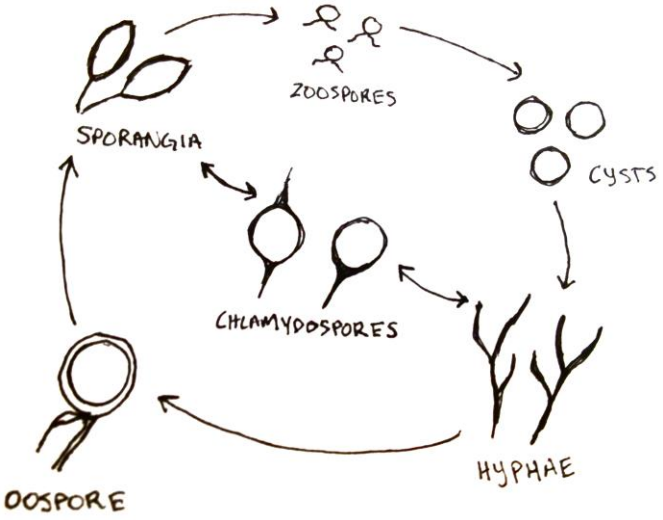
The objective of this study was to determine the significance of extreme temperatures and tree susceptibility on the dormancy rates of the pathogen *Phytophthora ramorum.* I determined the rate of *P. ramorum* dormancy over time under extreme temperatures based on the current annual maximum and minimum day and night temperatures in San Mateo County. I predicted that the majority of branches infected with *P. ramorum* would show signs of pathogen activity for seven or more weeks under extreme temperatures given consistent and ideal humidity levels. This would likely allow for a quick transition to sporulation given a return to ideal climatic conditions (Davidson et al. 2005). I also predicted that exposure to extreme temperatures would have more of an effect on pathogen growth than host tree susceptibility.

BACKGROUND INFORMATION

Study organism

The pathogen's life cycle (Fig. 1) and host interactions are critical to understanding the study design and methods employed throughout this experiment. *P. ramorum* can sexually reproduce with a different mating type to form oospores; however, very few have been produced in lab cultures and no evidence of oospores has been documented in nurseries (Grünwald et al. 2008a). In California woodlands the pathogens reproduction is strictly asexual. *P. ramorum* sporangia are produced on infected twigs and leaves, and can germinate under favorable conditions to produce short-lived swimming zoospores capable of infecting new plant material (Davidson et al. 2005). Following foliar infection, chlamydospores are produced, which are capable of withstanding a range of unfavorable environmental conditions (e.g. hot and dry climatic conditions) until they are able to start the life cycle again (Tooley et al. 2008). In these unfavorable conditions the pathogen will go dormant until a precipitation event renews its activity (Davidson et al. 2005, Fichtner et al. 2007).

Figure 1. *Phytophthora ramorum* life cycle. All stages except for the oospore form are observed in the forests of Northern California. This is because the oospore is formed through the sexual recombination of two mating types, A1 and A2, only one of which is currently in the wild. The primary agent of infection is the zoospore, which serves to perpetuate the asexual portion of the life-cycle shown below.



P. ramorum is also characterized by different clonal lineages and mating types specific to Europe and North America (Mascheretti et al. 2008, Goss et al. 2009). Out of the three existing clonal lineages, NA1, NA2, and EU1, I used isolate 1461 (from the Garbelotto labs culture collection) from the NA1 lineage (Grünwald et al. 2009). I chose an isolate from NA1 because clones from only this lineage are responsible for the entire CA infestation.

Host organism

I used *Umbellularia californica,* also known as California bay laurel, as the host study organism. *U. californica* is a native hardwood of California that grows in moist coastal forests that also are important habitat to the California coast live oak *(Quercus agrifolia).* The pathogen, *P. ramorum,* has different interactions with host species in these coastal woodlands. Coast live oaks are considered to be dead-end hosts: the spores encyst, germinate and produce hyphae that kill the phloem, effectively girdling the trees, but no instances of *P. ramorum* sporulation have been noted on these hosts (Davidson 2002c). In the case of tanoaks (*Notholithocarpus densiflorus*), the pathogen is both lethal and transmittable. In contrast, *U. californica* are not adversely affected by the pathogen, and incite high levels of *P. ramorum* sporulation (Davidson et al. 2005). Since the pathogen isn't lethal to *U. californica,* they are thought to be large contributors to the survival and spread of *P. ramorum* in California's coastal woodlands (Davidson et al. 2005).

Host collection site

I collected *U. californica* branches from the Crystal Springs Reservoir watershed in San Mateo County, CA. The watershed is a coastal oak woodland ecosystem with an average annual temperature of 48-71ºF or 8.8-21.5ºC, and average annual precipitation of 24.5 inches with the most precipitation from November to March. The elevation of the watershed is 85 meters. I selected *U. californica* trees showing signs of infection with *P. ramorum* from three of sixteen pre-existing plots. The locations of these plots were as follows: [plot 2: lat 37.57741445, long -122.41225672; plot 8: lat 37.55628292, long -122.39539166; plot 10: lat 37.56582571, long -122.4038863].

METHODS

Host collection methods

Two *U. californica* "hotspot" trees from which *P. ramorum* was isolated year round, and two "coldspot" trees from which *P. ramorum* was never isolated were selected from each of the three plots. I collected fifteen *U. californica* roughly ¼ inch diameter branches with little or no observed signs of infection from each selected "hotspot" and "coldspot" tree using pole pruning shears. The branches were selected with some woody material at the base of the stem, as it was easier to propagate branches of that size and maturity. Leaves with any sign of infection were removed from each branch. I sterilized and filled pots with a 2:1 sand and perlite mixture for planting.

Zoospore inoculum preparation

I used isolate 1461 (from the Garbelotto labs culture collection) from the NA1 lineage (Grünwald et al. 2009) to infect all branches. To prepare zoospore inoculum, I used pre-existing protocol adapted from Harnik 2005, Linzer 2008, and Linzer 2009. With a sterile scalpel I cut 0.5 cm x 0.5 cm plugs from the edges of a 7-10 day old *P. ramorum* culture growing on V8 medium (Werres et al. 2001). I prepared 2% soil tea extract with 20g of soil and 1L of diH2O, then autoclaved and filtered the tea (Linzer 2008). I then placed 15-30 plugs in sterile Petri dishes and filled them with 2% soil extract to the top of the plugs.

I incubated plugs at 18ºC for 3-5 days (Linzer 2009) allowing for sporangial growth, sporangia were then shocked into releasing zoospores using the following approach. Zoospores will encyst on any plastic surface and not reach the intended target of inoculation: to avoid this I acid-washed all of the equipment that would be directly touching zoospores in a 5M HCl solution for 24 hours (Harnik 2005). I then poured the soil tea and V8 plugs from all of the Petri dishes into a Nalgene bottle, which was placed into an ice water bath for 30 minutes. I removed the bottle from the ice bath and left it to recover at room temperature for 45 minutes. I removed 15µl from the inoculum and observed it under a microscope to ensure that zoospores had been released from the sporangia (Linzer 2009). I counted zoospores using a hemocytometer. Once I confirmed

the presence of zoospores, and noted the quantity, I diluted the inoculum to form 16L of 10000 zoospores/ml.

Branch inoculation

To prepare the 180 branches I collected for inoculation I first sterilized the surface of each leaf on each branch with a 70% ethanol solution. I then removed any leaves with obvious signs of a pre-existing foliar infection or disease. I poured 1L of zoospore inoculum into each of 16 acid-washed trays to cover the bottom of the tray and then placed twelve branches in each tray with the undersides of the leaves soaking in the inoculum. I left branches soaking for 24 hours in their inoculum trays in a growth chamber set to 18ºC. I then removed branches from the inoculum and placed them in their respective pots. I left all 180 branches at 18ºC for 3 days to ensure foliar infection, and then transferred them to their respective growth chambers (Hüberli et al. 2012).

Growth Chamber Settings

I used three growth chambers for this experiment, each containing 60 branches, five from each of the selected trees. I set chambers to 12 hours of daylight and 12 hours of darkness. A mister added humidity four times a day in each chamber. I selected three temperature ranges for each chamber that fluctuated daily to mimic day and night temperature flux. One chamber was used as a control and ideal temperatures for the pathogen were maintained (16-20ºC). I set the cold chamber to fluctuate between 4 - 10ºC, and the warm chamber between 12-26ºC. See Table 1 for growth chamber specifics.

Table 1. Growth chamber settings used throughout the growth chamber experiment. Only temperature differed between treatments. The hot and cold temperatures were chosen to reflect maximum and minimum temperatures that would be seen in the San Mateo County watershed in a given year. The control temperature is the commonly cited ideal growth temperature for *P. ramorum*.

Growth Chamber	Trees per Chamber	Branches per Tree	Total Branches per Chamber	Day/Night (hrs)	Humidity	Temperature (Day – Night)
Cold	15	5	60	12/12	4 times/day	10ºC – 4ºC
Hot	15	5	60	12/12	4 times/day	26ºC – 12ºC
Control	15	5	60	12/12	4 times/day	20ºC – 16ºC

Sampling methods

After the branches had spent three days under ideal conditions, I sampled them to ensure that inoculation had been successful, and then I sampled leaves every 5 days following the first sampling date for a duration of 50 days, or roughly 7 weeks. I sampled four infected leaves from each branch in each growth chamber. I took samples with an ethanol-flame sterilized hole-punch and plated them with a sterile scalpel onto a selective medium containing four antibiotics, pimaricin-ampicillin-rifampicin-PCNB (PARP), which allow only the growth of *P. ramorum* (Vettraino et al. 2009). Once I plated all 720 samples onto PARP, I placed the Petri dishes in cupboards (antibiotics are light-sensitive) at room temperature for 5 days and then observed and recorded growth or inactivity from the samples.

Data analysis

To interpret the general trends in my data I created a simple line graph showing growth rates for different treatments and tree susceptibilities over time. Due to the nested nature of my data (Fig. 2), I used statistical models that would track the data for an individual over time. I used R, version 2.15.1 GUI 1.52, for all statistical analyses (Gentleman and Ihaka 1997). I used a generalized estimating equation (GEE) and a survival analysis to determine the effects of temperature, time, plot, and tree type on *P. ramorum* growth. The dependent variable for my models was growth and the independent variables were plot, tree susceptibility, and temperature. The GEE accounted for a change in autocorrelation in the data over time and determined which variables have a positive or negative effect on *P. ramorum* growth. The finalized GEE model equation was *growth ~ temperature + plot + tree susceptibility + plot*tree susceptibility*, with trees held as clusters over time. The term "*plot*tree susceptibility*" was added in to represent a potential biological interaction between those two variables because of the observed variation in infection between plots, which might have an effect on the observed "hotspot" and "coldspot" trees. The survival analysis determined the probability of survival of the pathogen over time.

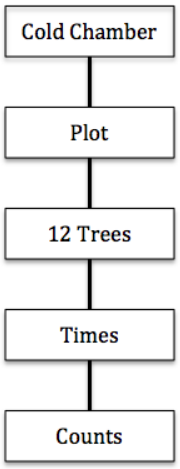


Figure 2. Data hierarchy/nesting example for cold chamber. The nested nature of the data comes from the fact that each sample belongs to a specific branch from one of the twelve trees selected from three different plots. That branch was sampled over time in one of three treatments, so it is important to take into account all of the variables that a particular sample may represent.

RESULTS

Effects of Temperature on *P. ramorum* Growth

I observed a negative trend in pathogen growth among all treatments and individuals over a 50-day sampling period (Fig. 3). Significantly lower rates of pathogen growth were found in the hot and control treatments (Table 2; $P < 2e\text{-}16$, $P = .0007$). Over time, the cold treatment had the highest proportion of growth and the hot treatment had the lowest (Fig. 3). This was also seen in the survival analysis, where the probability of survival for *P. ramorum* decreased the most in the hot treatment and the least in the cold treatment (Fig. 4). In the hot and cold treatments, *P. ramorum* on "hotspot" trees had generally higher growth rates than on "coldspot" trees and it was determined that "hotspot" trees had a significant positive effect on growth (Table 2; $P = 0.017$).

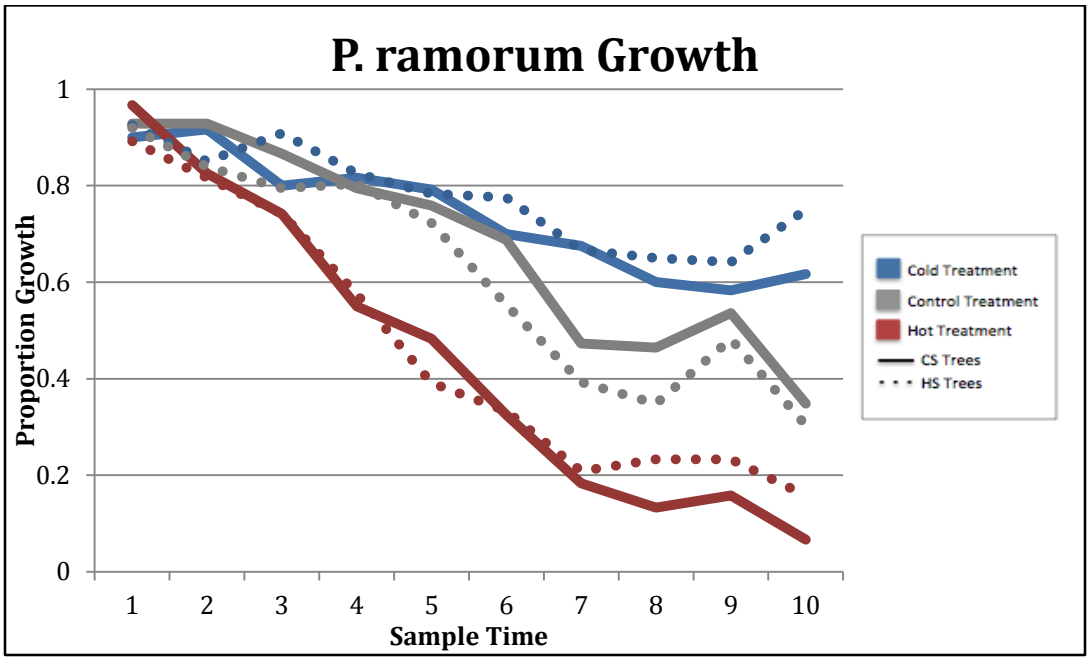


Figure 3. **Proportion of *P. ramorum* growth over time among treatments.** The proportion of samples that exhibited *P. ramorum* growth was highest in the cold treatment and lowest in the hot treatment. The control treatment had growth rates roughly in between the cold and hot treatments. The "hotspot" and "coldspot" trees within each treatment seem to follow a similar trend overall as portrayed by this figure.

Table 2. Results of a best-fit generalized estimating equation (GEE) determining the effects of temperature, tree susceptibility, and plot on pathogen growth over time. This table shows the variables that were found to have significant positive or negative effects on *P. ramorum* growth. Plot 8, plot 10, and "hotspot" trees were found to have significant positive effects on growth. Both the control and hot treatments had significant negative effects on *P. ramorum* growth. Additionally, "hotspot" trees within plots 8 and 10 had significant negative effects on growth.

Variable	Estimate	S.E.	Wald	P
Plot 8	0.26330	0.07603	11.992	0.000534 ***
Plot 10	0.25890	0.11390	5.167	0.023019 *
Control	-0.15791	0.04651	11.526	0.000686 ***
Hot	-0.52614	0.06143	73.353	< 2e-16 ***
Hotspot	0.26589	0.11182	5.654	0.017417 *
Plot 8: Hotspot	-0.37091	0.12752	8.461	0.003629 **
Plot 10: Hotspot	-0.38638	0.14092	7.518	0.006108 **

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

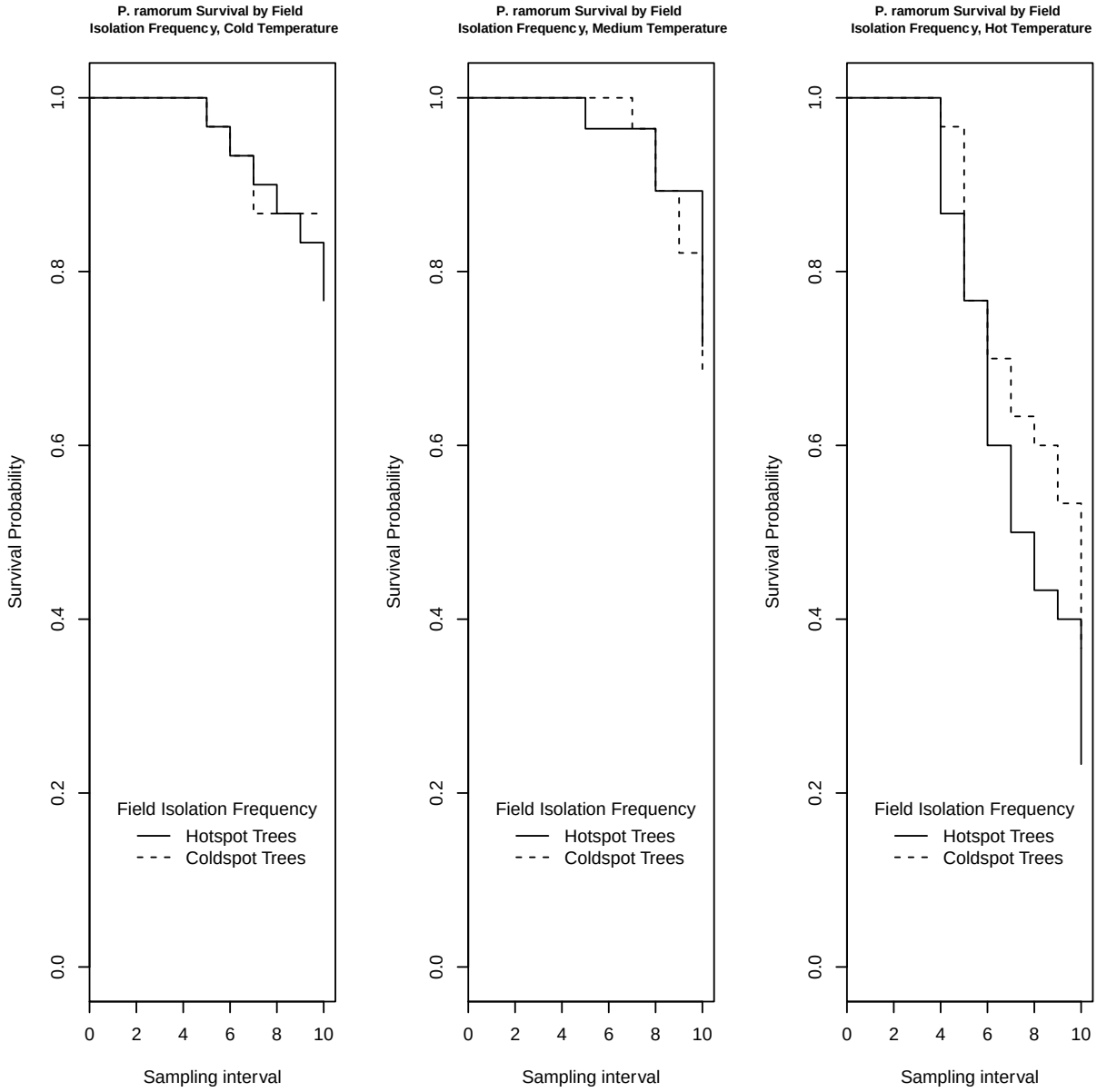


Figure 4. Survival analysis of *P. ramorum* across temperature treatments. These figures show the probability of survival of *P, ramorum* on "hotspot" and "coldspot" trees in each treatment. Probability of survival was lowest over time in the hot treatment, dropping down to a probability close to P= 0.2 at sample time 10. The highest probability of survival over time was found in the cold treatment with roughly P= 0.75 at sample time 10. The control temperature treatment had a probability of survival somewhere in between the other two treatments with roughly P= .7 at sample time 10. In the hot treatment, a difference in the probability of survival between "hotspot" and "coldspot" trees can also be observed between sample times 6-10, with "coldspot" trees having a higher probability of survival.

Effects of Tree Susceptibility and Plot on *P. ramorum* Growth

Tree susceptibility and plot both have independent and interactive effects on *P. ramorum* growth. I found that across all treatments and all plots, "hotspot" trees had a significant positive effect on *P. ramorum* growth (Table 2; P= 0.017). Similarly, across all treatments and tree susceptibilities, plots 8 and 10 had significant positive effects on *P. ramorum* growth (Table 2; P= 0.0005, P= 0.023). When an interaction between the variables "plot" and "tree susceptibility" was modeled however, the combined effect of "hotspot" trees and both plots 8 and 10 were shown to have a significantly negative effect on growth (Table 2). This means that "hotspot" trees within plot 8 across all treatments had a significantly negative effect on growth (Table 2; P= 0.0036). Similarly, "hotspot" trees within plot 10 across all treatments had a significantly negative effect on growth (Table 2; P= 0.0061). This can also be seen in the survival analysis of *P. ramorum* on "hotspot and "coldspot" trees by plot (Fig. 5). The lowest probabilities of *P. ramorum* survival are on "hotspot" trees from plots 8 and 10 (Fig. 5). There is also a sharp decline in the survival probability of *P. ramorum* on "coldspot" trees from plot 2, where the probability of survival on "hotspot" trees from the same plot was noticeably better.

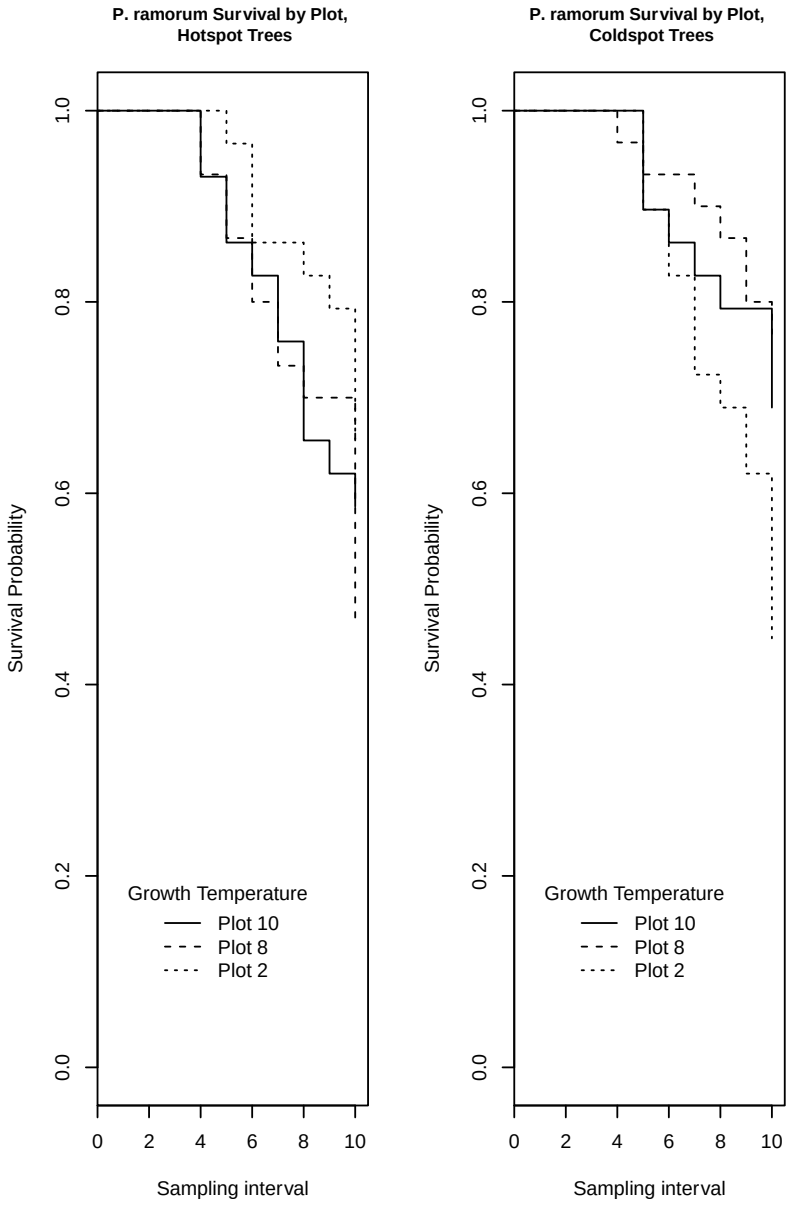


Figure 5. Survival analysis of *P. ramorum* on "hotspot" and "coldspot" trees by plot. The first figure depicting the survival of "hotspot" trees by plot shows that overall, the lowest probability of survival was found on "hotspot" trees from plots 8 and 10. When looking at the probability of survival of *P. ramorum* on "coldspot" trees, the lowest probability of survival was found on those trees from plot 2. Overall, the probability of survival was lower for *P. ramorum* on "hotspot" trees than on "coldspot" trees across plots when comparing the two figures.

DISCUSSION

The severity of *P. ramorum* infection can be attributed to both climatic factors and host susceptibility to infection (Anacker et al. 2008, Davidson et al. 2005, 2008; Hayden et al. 2011, and Hüberli et al. 2012). I initially thought that a shift in temperature due to climate change is more likely to determine the persistence of the pathogen in the environment rather than the susceptibility of its host. When subjected to temperature extremes, I found that *P. ramorum* had significantly lower growth rates in the hot and control temperature treatments. In addition, I found that "hotspot" trees, on which isolates of *P. ramorum* could be found year round, had a significantly positive effect on *P. ramorum* growth rates. Plots 8 and 10 also had significant positive effects on pathogen growth, but when looking at "hotspot" trees within those plots, the effects on growth were significantly negative. Furthermore, my data suggest that the influence of temperature and microclimate may play a larger role in pathogen growth than differences in host susceptibility.

Effects of Temperature on *Phytophthora ramorum* Growth

An established relationship between *P. ramorum* sporulation and temperature exists in both field and laboratory settings (Davidson et al. 2005, 2008; Englander et al. 2006). Because the hot and control treatments in my study had significantly lower growth rates than the cold treatment, my findings indicate that higher daily temperatures are less favorable for pathogen activity. These findings agree with Hüberli et al. (2012) and Tooley et al. (2009) who showed that *P. ramorum* infection decreases at temperatures up to 26ºC, above which it is practically unviable. The highest growth rates occurred in my 4-10ºC treatment, which conflicted with the determined optimal temperature range of 18-22ºC for *P. ramorum* growth, which was a similar temperature range used for the control treatment (Hüberli et al. 2012, Tooley et al. 2009). This unusual growth pattern may be attributable to the *U. californica* cuttings exhibiting higher rates of stress and desiccation when subjected to higher temperatures, thus leading to the potential inhibition of *P. ramorum* growth (Judelson and Blanco 2005). It is also possible that the relationship between temperature and humidity could have an effect on the observed growth rates.

The moisture content of air at warmer temperatures is higher than air at colder temperatures; thus, there was likely more water condensation in the colder chamber than in the warmer chamber. Higher levels of condensation could provide an ideal medium for zoospores to continue their cycle of infection, potentially explaining the higher growth rates observed in the cold chamber.

Effects of Tree Susceptibility on *Phytophthora ramorum* Growth

Genetic variability within host populations can influence pathogen-host interactions, particularly the host's susceptibility to a disease (Dodd et al. 2005, 2008; Hüberli et al. 2012, Meshriy et al. 2006). This study found a positive relationship between *P. ramorum* growth and tree susceptibility, with the "hotspot" trees having a significant positive effect on *P. ramorum* growth. These findings are consistent with disease expression in the field where "hotspot" and "coldspot" trees showed differing levels of infection within plots in the same microclimate. These data suggest that potential genetic variability among host individuals may also have an effect on the growth of the pathogen. Findings from Anacker et al. (2008) show evidence of genetic variability in susceptibility to *P. ramorum* infection between individuals of *U. californica* and they claim that environmental factors could override tree-to-tree genetic variation in susceptibility. My findings seem to reflect those of Anacker et al. (2008)'s because the effect of temperature in my data is shown to have much more of a significant effect on pathogen growth than tree susceptibility (Table 2). While the effects of temperature seem to have a greater effect on pathogen growth, further research on the relationship between tree susceptibility and *P. ramorum* activity is needed, with an emphasis on using multiple *P. ramorum* isolates to best measure this effect and apply it to disease patterns in the field.

Effects of Plot on *Phytophthora ramorum* Growth

While plot was not a variable that I focused on in this study, I found that it significantly affected *P. ramorum* growth. Both plots 8 and 10 had significant positive effects on *P. ramorum* growth. When modeled as an interaction variable between plot and tree susceptibility, I found that "hotspot" trees within plots 8 and 10 had significantly

negative effects on pathogen growth. These data suggest that the influence of site and potentially microclimate may play a role in determining the growth of *P. ramorum* under adverse conditions. The positive effect of plots 8 and 10 on pathogen growth may be explained by the fact that these two plots are both very infected in the field and have a much higher severity of infection than plot 2. The positive effect on pathogen growth for these two plots, coupled with the observed positive effect of "hotspot" trees on growth, resulted in an overall negative effect on *P. ramorum* growth. It is very likely that these "hotspot" tree cuttings from plots 8 and 10 were predisposed to higher levels of infection, leading to the potential physiological stressing of the branch. If these cuttings were severely infected at the beginning of the experiment, they would have had less living leaf tissue to photosynthesize and perform all of the necessary functions that the plant would need to survive. The lower fitness level of these branches most likely would have resulted in the decreased growth rates of *P. ramorum* due to the decrease in healthy leaf tissue over time. To determine if plot location plays a larger role in the growth of the pathogen, future studies could expand the number of plots from which cuttings were collected in order to have a larger sample size. It is well established that climatic differences have a significant influence on the activity of many pathogens, and in an environment suited for *P. ramorum* growth and sporulation, it is possible that differences in microclimate and infection severity of a site may effect *P. ramorum* growth.

Desiccation Effects on *Phytophthora ramorum* Growth

The prolonged length of this study coupled with the different temperature treatments resulted in increased branch and leaf desiccation over time. I observed a relationship between *P. ramorum* growth, the degree of desiccation of the branch, and the temperature treatment over the 50-day study. Because the genus *Phytophthora* has limited survival as a saprophyte (an organism that lives off of dead organic matter), if a host's health degenerates, the pathogen must rely on the spread of spores to infect new hosts or healthy plant tissue (Judelson and Blanco 2005). It is likely that, with the *P. ramorum* infection in the 12-26ºC chamber, both the temperature and the declining health of *U. californica* cuttings due to heat stress negatively affected the growth rates of the pathogen. While the use of seedlings rather than cuttings might have reduced the effect of

desiccation, insect pollination and out-crossing between individuals also increases the genetic diversity of *U. californica* offspring (Kasapligil 1951). This potential increase in genetic diversity might then have negated the differences in *P. ramorum* activity observed on individual "hotspot" and "coldspot" trees in the field.

Future Directions

The findings in this study suggest that there are more complex relationships between climate, host susceptibility, site, and pathogen activity that must be taken into account in order to mitigate the spread and severity of this destructive pathogen. With the understanding that temperature and microclimate may play a larger role in an ecosystem's infection cycle than host susceptibility, further research is needed to determine the effects of other climatic factors, such as precipitation, on *P. ramorum* growth. While this study was unable to examine both of these variables, the effects of temperature and precipitation on *P. ramorum's* activity is especially important for being able to more accurately predict changes in pathogen dynamics under climate change. With our understanding of the relationship of pathogen growth and temperature, it might be possible to more accurately identify areas at high risk of infection based on current and future climatic parameters.

Conclusions

This study shows that climatic changes, localized patterns of infection, and genetic variability among hosts all play a part in the activity and viability of *P. ramorum*. My results showed that even after fifty days of exposure to extreme temperatures, *P. ramorum* was still present on its host *U. californica* in all treatments. Additionally, I found that warmer temperatures had significantly negative effects on pathogen growth. However, the desiccation of leaves under heat stress likely influenced the lower pathogen growth rates observed in the control and hot chambers. Highly susceptible "hotspot" trees were found to have a significantly positive effect on *P. ramorum* growth across all temperature treatments. Two of the three plots used in this study were found to have significantly positive effects on pathogen growth. Further studies using seedlings might reveal different pathogen growth rates under warmer temperature conditions that this

study was not able to detect. We are facing a future of uncertain climate in California that will affect disease spread and geographic distribution. The observed resilience of this pathogen to temperature extremes shows how important it is that we continue to study the effects of host genetics and climate on pathogen activity to better predict and prevent the spread of this disease in the future.

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