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The disease ecology, epidemiology and population genetics, of the emergent forest pathogen Phytophthora ramorum in a coastal California watershed.

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by

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Professor Matteo Garbelotto, Chair
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Abstract

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Sudden Oak Death (SOD), caused by the oomycete pathogen, *Phytophthora ramorum,* is an introduced forest disease causing large-scale tree mortality in the oak woodlands of California. Pathogen dynamics are bound to be affected by site ecology, forest structure and composition and by interactions among sympatric pathogens. In the four chapters included in this dissertation I address various aspects of the both ecology and epidemiology of the Sudden Oak Death disease, including the competition between three sympatric species of *Phytophthora,* the population structure of the *P.ramorum* on its three main hosts, California bay laurel (*Umbellularia californica*), tanoak (*Notholithocarpus densiflorus*) and coast live oak (*Quercus agrifolia)*, and at sites with different levels of disease incidence as well as the population structure of isolates found in two different substrates, soil and bay laurel leaves, in various weather conditions.

Using molecular tools coupled with an intensive multi-year field sampling approach, of a plot network within a single coastal California watershed with a relatively long and uniform history of disease presence, I determined the following:

When in competition with other *Phytophthora species,* specifically *P. nemorosa, P. ramorum* prevalence increases to levels higher than those of the competing species when abundant rainfall triggers its sporulation. Despite *P. nemorsa* having a broader geographic range, it exhibits a narrower ecological amplitude and, in any given region, occupies fewer sites than *P. ramorum*. Results additionally suggest that, perhaps due to priority effects, *P. nemorosa* can persist at levels comparable to those of *P. ramorum* in ecologically suitable plots when climate favors *P. ramorum* dormancy.

Shifts in weather, primarily levels of rainfall are accurate predictors in the likelihood of both bay laurel and oak infection as well as transitions between dormant and active infection of the pathogen itself. Additionally site specific factors such as aspect, bay laurel density and bay laurel basal area drive differences in levels of both disease incidence and prevalence on both bay laurel and oak hosts. For oaks specifically, infection rates are greater among larger trees, yet levels of mortality are greater among smaller trees. Neighborhood effects such as proximity to

infected bay laurel foliage, and surrounding bay laurel density and basal area are important factors in predicting oak infection.

Ten microsatellites were used for genetic analyses on cultures from successful isolations to determine differences in population structure between different substrates, namely populations isolated from soil versus aerial populations isolated from bay laurel leaves in various weather conditions. Migration of genotypes among sites was low and spatially limited during dry periods, but intensity and range of migration of genotypes significantly increased for leaf populations during wet periods. Only leaf genotypes persisted significantly between years, and genotypes present in different substrates were distributed differently in soil and leaves. It was concluded that epidemics start rapidly at the onset of favorable climatic conditions through highly transmissible leaf genotypes, and that soil populations are transient and may be less epidemiologically relevant than previously thought.

Population structure of the pathogen in each of the three main hosts was examined to provide evidence of contagion pathways among hosts, as well as differences in population structure in wet vs. dry years and at sites with various levels of disease incidence and prevalence. The relationships among *P. ramorum* populations in bay laurels, oaks and tanoaks were analyzed and structure was found to exist among hosts. It was determined that bay laurel is the source population for both tanoak and oak infection, and that tanoak contributes minimally to oak infection. In spite of their common source of inoculum, oaks and tanoaks were found to be sinks that select for different pathogen genotypes, due to the variance in selection pressure in each host type. Additionally, different sites supported a dominance of different genotypes, more genotypes overall and more persistent genotypes, when compared to other sites, and these 'hotspots' are likely to play a more significant epidemiological and evolutionary role for the pathogen.

Together these results help to advance the state of knowledge surrounding the ecology, epidemiology and population genetics of the Sudden Oak Death pathogen in California, add to the growing body of research on invasive plant pathogens, support theories of invasion biology and most importantly can be applied to regulatory and land management practices in attempt to mitigate the spread of this disease.

To my family and friends,
whom without I wouldn't have had the mountain of support and endless love necessary to complete this dissertation,
and to my many colleagues in the scientific community,
for their constant words of encouragement, selfless dedication and ever inspiring work.

Table of Contents

Abstract 1
Dedication i
Table of Contents ii
Introduction iv

Chapter 1: Interspecific interactions between the Sudden Oak Death pathogen *Phytophthora ramorum* and two sympatric *Phytophthora* species in varying ecological conditions.

Abstract 1
Introduction 2
Methods 4
Results 8
Discussion 10
References 16
Tables and Figures 22

Chapter 2: Disease ecology and epidemiology of the Sudden Oak Death pathogen *Phytophthora ramorum* in a mixed bay laurel-oak woodland.

Abstract 38
Introduction 39
Methods 42
Results 45
Discussion 48
References 52
Tables and Figures 57

Chapter 3: Population dynamics of aerial and terrestrial populations of *Phytophthora ramorum* in a California forest under different climatic conditions.

Abstract ……66
Introduction ……67
Methods ……69
Results ……72
Discussion ……75
References ……80
Tables and Figures ……87

Chapter 4: Population genetics clarify the epidemiological relationships among the three main California hosts of the pathogen *Phytophthora ramorum*, causal agent of Sudden Oak Death

Abstract ……100
Introduction ……101
Methods ……104
Results ……107
Discussion ……109
References ……113
Tables and Figures ……116

Introduction

The field of invasion biology, more specifically the examination of factors that promote successful invasions and the impacts of invasive organisms outside of their native range, is still a relatively new area of study, particularly when the invasive organisms are plant pathogens. It is fair to state the impacts of invasions on biodiversity and ecosystem function caused by invasive organisms and invasive pathogens in particular still remain to be fully understood (Pimentel et al. 2005; Lockwood et al. 2007, Simberloff et al. 2013). Since the late 1990's, research on invasive organisms has received a significant impetus, and an increasing body of empirical data and experimentation has been published to test theoretical hypotheses (Pyšek and Hulme 2009; Moles et al. 2012). Just recently, the results of longer-term studies (Jeschke et al. 2012) have been published, better informing our understanding of how to predict microbial invasions and mitigate the impacts of invaders on biodiversity (Milgroom et al. 1996: Milgroom and Cortesi 2004, Jeschke et al. 2012), ecosystem function (Garbelotto et al. 2019), and species evolution (Garbelotto and Gonthier 2003).

While the vast majority of the theory and research on biological invasions has been focused on the introduction of exotic plant and animal species, microbes can be extremely dangerous invaders, specifically in the form of plant pathogens (Wingfield et al. 2017; Desprez-Loustau et al. 2007). The number of emergent and infectious diseases being detected worldwide is on the rise due to continually increasing human activity, specifically the global trading of plant material (Santini 2013). Plant pathogens make up a large subset of the exotic invaders that have proven to be some of the most detrimental both ecologically and economically speaking (Pimentel et al. 2005). Historically plant pathogens in particular have wreaked havoc not only on ecosystems but have caused major societal impacts as well. Some of the most well-known examples of these include *Cryphonectria parasitica*, an introduced ascomycete fungus from Japan and the causal agent of Chestnut blight (Milgroom et al. 1996: Milgroom and Cortesi 2004), a disease that dramatically transformed the hardwood forests of eastern North America by essentially eliminating the once dominant American chestnut (*Castanea dentata*) from the overstory (Anagnostakis 1987); and the "Great Hunger", also known as the Irish potato famine of the 1840's, caused by the introduced *Phytophthora infestans* from the Americas (Ristaino, 2000), which led to the deaths of an estimated 1 million people and the emigration of another million people out of Ireland (Ristaino, 2000). Despite their long lasting impacts, our understanding of invasions by pathogens is still limited.

The tens rule (Williamson and Fitter 1996) states that only 10% of invaders are able to successfully themselves established in a new geographic region as conditions must be just right in order for them to take hold in their new environment, and the factors that lead to an invasive plant pathogen becoming established in a new system are not well understood. The enemy release hypothesis (Keane and Crawley 2002) attributes the success of invaders to the escape from their natural enemies, predators and competitors (De Roy et al. 2013), but the organism still must still be able to survive in the new environment. Ecologically speaking, the invader will be more likely to survive and become established if the new environment is similar climatically to native range (Guernier et al. 2004; Santini et al. 2013; Vacher et al. 2008) and for plant pathogens, appropriate host species must also be available to colonize (Gilbert, 2007). The pathogen itself must also have a high reproductive potential and be highly infectious, in order to compete with native microbes, as well as a short life cycle and the ability to wait out unfavorable conditions in a dormant phase (Gilbert, 2007). These factors are summed up by a leading tenet of

plant pathology known as the disease triangle which states that environmental factors, along with host susceptibility and pathogen virulence, play a strong role in potential outbreaks (Francl, 2001).

Along the west coast of North America, specifically in California and Oregon, Sudden Oak Death has quickly become a familiar issue among both residents and researchers. The disease is caused by the etiological agent *Phytophthora ramorum*, (Kingdom Stramenopila, Order Peronosporales), a heterothallic (i.e sexually outcrossing) oomycete that reproduces clonally when only one mating type is present, as is the case in the California infestation. Other members of the genus *Phytophthora,* a name that translates to 'plant destroyer' in Greek (Fry 2008)*,* are responsible for a suit of other destructive forest and agricultural diseases, including *P. lateralis*, the closest relative of *P. ramorum*, affecting of Port Ordford Cedar in northern California and Oregon (Hansen et al. 2000), *P. cinnamomi* threatening Manzanita species in California (Swiecki et al. 2003) and causing devastating mortality occurring in the Jarrah forests of Australia (Hardham 2005) and the infamous *P. infestans* responsible for the *Solanum* blight that caused the great Irish potato famine mentioned above (Ristaino, 2000). The emergent pathogen *P. ramorum* is responsible for killing over 50 million tanoaks (*Notholithocarpus densiflorus*) and hundreds of thousands of coast live oaks (*Quercus agrifolia*) in coastal California forests, and while effects of the disease are most dramatically noticed with increasing mortality of these two species, disease incidence and transmission is most prevalent on the primary transmissive host, the ubiquitous California bay laurel (*Umbellularia californica*), likely to be infected in the tens of millions. Incidentally, bay laurel provides an excellent avenue to study this pathogen as trees can be visually assessed for symptoms quickly, and the pathogen can be isolated from leaves relatively easily. In this system, bay laurel is strictly a foliar host, meaning this species is not killed by the pathogen, but rather it provides a persistent substrate for the proliferation, sporulation and even survival of the pathogen. The pathogen undergoes rapid population expansion on bay leaves in ideal weather conditions, but the same leaves serve as a substrate for dormant spores to wait out unfavorable conditions. This is in sheer contrast with the biology of the pathogen on oaks, hosts that are simply a dead end for the pathogen as no sporulation occurs oak boles infected by the pathogen. Tanoaks act as both foliar and dead end hosts, meaning sporulation occurs on leaves and twigs, which can in turn potentially infect a trees own trunk or trunks of neighboring tanoaks causing bole cankers where sporulation does not occur.

Not only have the number of studies on plant pathogens been increasing as more and more emergent diseases arise, but the application of molecular tools to answer ecological questions about forest disease have been used more frequently to elucidate the epidemiology of these emergent and novel diseases. To date, much of the work on the Sudden Oak Death pathogen has focused on a large scale, such as the entire infestation within a state, or in North America, or has attempted to make comparisons between sites with a different history of invasion, namely time since establishment. What differentiates the approach I have taken in this dissertation is the focus on a single watershed in San Mateo County Ca, which along with Marin county and Santa Cruz county is one of the original introduction and establishment sites of the pathogen (Mascheretti et al. 2008, 2009; Croucher et al. 2013). The San Francisco Public Utility Commissions (SFPUC) land is a 9300 ha stretch of uninterrupted coastal woodland that has been infested with SOD since the mid 1990's, with the earliest reports of disease in this area coming before *P. ramorum* had been identified as the causal agent (David Rizzo, personal communication). This site with a relatively old and established population of *P. ramorum*

provides the ideal arena to investigate specific questions about the ecology, epidemiology and population genetics of this disease system, and to contribute to the growing body of knowledge on invasions by pathogens.

In my dissertation, I have addressed the following components of the Sudden Oak Death epidemic by conducting extensive, multi-year field surveys which spanned the end of a drought period and extend into a very wet period within a single watershed with a uniform infestation history:

1. Interspecific competition in different weather conditions between *Phytophthora ramorum* and native sympatric species of *Phytophthora* that occupies the same host range but potentially different climatic niche.
2. Climatic, topographic, forest structure and composition factors as predictors of disease prevalence and incidence, both spatially and temporally, at heterogeneous sites, and both within year and between years, on both foliar transmissive and bole dead end hosts.
3. Differences in population structure in various substrates, (aerial vs soil populations) in different seasons, and years with varying weather conditions. This was a necessary study for *Phytophthora ramorum*, the first aerial forest *Phytophthora* specis ever discovered in temperate forests. All *Phytophthora* species discovered prior to *P. ramorum* are soilborne and/or waterborne
4. Understand the complex epidemiology of the disease in presence of its three main California hosts. Population genetics approaches were employed to study inter host population differentiation, contagion among hosts in different years with varying weather conditions and across a heterogenous landscape at sites with different levels of disease incidence. This is the first study that provides direct evidence on which hosts act as a source of infection and which hosts act instead as a sink.

In the first chapter I combined field surveys with inoculation experiments to understand disease prevalence dynamics and competitive interactions among three species of sympatric *Phytophthora* species, *P. ramorum*, *P. nemorosa* and *P. pseudosyringae,* the latter two causing little to no mortality in oak yet often colonizing the leaves of bay laurel in the same sites, and even on the same leaves of bay laurel. Despite the broader geographic distribution of *P. nemorosa* with respect to that of *P. ramorum*, our results suggested that *P. nemorosa* exhibits a narrower ecological amplitude and, in any given region, occupies fewer sites than *P. ramorum*. Our results additionally suggested that, perhaps due to priority effects, *P. nemorosa* can persist at levels comparable to those of *P. ramorum* in ecologically suitable plots when climate favors *P. ramorum* dormancy. However, *P. ramorum* prevalence increases to levels higher than those of the competing species when abundant rainfall triggers its sporulation. Understanding the determinants and outcomes of competition between these species has important implications for understanding the epidemiology and possible control strategies for Sudden Oak Death.

In the second chapter I modeled the likelihood of bay laurel infection in different forest sites across a heterogenous landscape, in different seasons throughout the year and across several years spanning a dry to wet period. I also modeled the seasonal transitions between dormant and active infection stages of the pathogen, as well as infection and mortality in oaks. The results of these models affirm the importance of seasonal and annual rainfall shifts in driving the prevalence of active infection, and reveal the importance of plot-level heterogeneity, particularly in bay laurel density, basal area and slope aspect. For oaks specifically, infection rates were found to be greater among larger trees, yet levels of mortality were found to be greater among

smaller trees. Proximity to infected bay laurel foliage was an important factor in predicting oak infection, (as oaks with shorter distance to nearest infected bay laurel foliage exhibited greater rates of infection). For the first time, this work provides solid evidence that oaks are infected only when precipitation is exceptionally high, and not on a yearly basis, These results help to suggest avenues for control efforts and aid in obtaining a better understanding of this pathogen's disease ecology and are invaluable to land owners and land managers attempting to mitigate the spread of disease.

In the third chapter, I employ population genetic tools to examine the structure of *P. ramorum* populations in various substrates. Specifically in the soil population versus the aerial populations isolated from the leaves of bay laurels. Ten microsatellites were used to genotype isolates collects from both substrates in different seasons across two years, a dry year and a wet year. Results demonstrated that incidence of leaf infection tripled at the onset of the first wet period in 3 years in the spring of 2010, while that of soil populations remained unchanged. Migration of genotypes among sites was low and spatially limited under dry periods, but intensity and range of migration of genotypes significantly increased for leaf populations during wet periods. Only leaf genotypes persisted significantly between years, and genotypes present in different substrates distributed differently in soil and leaves. We concluded that epidemic periods of disease start rapidly at the onset of favorable climatic conditions through highly transmissible leaf genotypes, and that soil populations are transient and may be less epidemiologically relevant than previously thought.

In the fourth chapter I examine the relationship that exists between the three major hosts in this disease system, on a genetic level, to clarify the contribution that each host may have on the epidemiology of SOD and on the microevolution of its causal agent. Additionally, I explore differences in population structure in wet vs. dry years and at sites with various levels of disease incidence and prevalence. We conclude that structure does exists between hosts, that bay laurel is the source population for both tanoak and oak infection, and that tanoak contributes only minimally to oak infection. We also conclude that in spite of their common source of inoculum, oaks and tanoaks are sinks that select for different pathogen genotypes due to the variance in selection pressure in each host type. Finally we conclude that different sites supported a dominance of different genotypes, more genotypes overall and more persistent genotypes, when compared to other sites, and that these 'hotspots' are likely to play a more significant epidemiological and evolutionary role for the pathogen.

I designed both the ecological and population genetic studies in such a way that the component of 'time since invasion' was relatively even and with the knowledge that since the pathogen was relatively established within the watershed it would be in an endemic phase and not in an acute phase where things were changing rapidly otherwise the conclusions from this work would not be as significant.

Once again the tens rule (Williamson and Fitter 1996) states that approximately 10% of introduced species become established and that 10% of those have the potential to become invasive or "pests". However this can be misleading to those outside of the scientific community, understating the risks posed by the few invaders that do become established (Jarić and Cvijanović 2012) and leading policy makers to not take the threat of new invasions seriously enough. A pathogen like that causing Sudden Oak Death, once established, cannot be eradicated (Cunniffe et al. 2016), we can only attempt to use our understanding of the diseases' dynamics to curb any further spread to new and potentially sensitive habitats, and to view this knowledge as a cautionary tale. The only way to prevent the future establishment of emergent forest pathogens

is to heavily regulate the trade of living plant material and work diligently to prevent future introductions from occurring.

The results of this work can help to advance the state of knowledge surrounding invasive plant pathogens, support the theories of invasion biology and can be applied to the regulatory practices and land management in attempts to prevent future outbreaks of forest disease, and mitigate the outbreaks that will inevitably arise.

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Chapter 1

Interspecific interactions between the Sudden Oak Death pathogen *Phytophthora ramorum* and two sympatric *Phytophthora* species in varying ecological conditions

Abstract

Even when introduced invasive pathogens lack their natural predators or competitors, they must still interact with other organisms in their introduced range. Sudden Oak Death (SOD), caused by *Phytophthora ramorum* (Oomycota), is an introduced disease causing large-scale tree mortality. Two additional *Phytophthora* species, *P. nemorosa* and *P. pseudosyringae*, cause significantly lower oak mortality, yet they also commonly colonize leaves of *Umbellularia californica*, the major transmissive host of SOD in California. We combined field surveys and inoculation experiments to understand disease prevalence dynamics and competitive interactions among these pathogen species. Despite the broader geographic distribution of *P. nemorosa* with respect to that of *P. ramorum*, our results suggest that *P. nemorosa* exhibits a narrower ecological amplitude and, in any given region, occupies fewer sites than *P. ramorum*. Our results additionally suggest that, perhaps due to priority effects, *P. nemorosa* can persist at levels comparable to those of *P. ramorum* in ecologically suitable plots when climate favors *P. ramorum* dormancy. However, *P. ramorum* prevalence increases to levels higher than those of the competing species when abundant rainfall triggers its sporulation. Understanding the determinants and outcomes of competition between these species has important implications for understanding the epidemiology and possible control strategies for Sudden Oak Death.

Introduction

Although introduced invasive pathogens can have drastic effects on a host species, potentially accentuated by release from natural predators and/or competitors ("enemy release"; Keane and Crawley 2002), interactions between a pathogen and a host do not occur in isolation. Instead, a host is likely to interact – either simultaneously or asynchronously – with numerous native or introduced organisms, including other pathogens and mutualists. These interactions can result in coexistence of multiple pathogens or in the exclusion of some due to competition via resource capture or direct antagonism ("exploitation" or "interference" competition; Dobson 1985). As in other ecological systems, species coexistence may be influenced by priority effects; i.e., earlier-arriving species may facilitate or inhibit the establishment of later-arriving species (Connell and Slatyer 1977).

Manipulative field and greenhouse experiments have shown that the outcome of plant infection by multiple fungal or oomycete pathogens can be shaped by a number of factors including comparative levels of virulence, differences in antagonistic competitive ability, and priority effects. Multiple infections may trigger selection for more virulent and/or faster growing genotypes that can capture resources and/or reproduce more effectively (Lopez-Villavicencio et al. 2007, 2011). In these cases, competitive displacement of a weaker competitor by a stronger one may occur (Siou et al. 2015); however, priority effects may alter the outcome, allowing an earlier-arriving pathogen to persist in the presence of a later-arriving one, even if it is a weaker competitor when inoculated simultaneously (Hood 2003; Simpson et al. 2004; Al-Naimi et al. 2005; Laine 2011). Although resource capture is likely to play a role in such effects, in at least some cases an important mechanism appears to be stimulation of host systemic resistance by the earlier-arriving pathogen, resulting in the plant mounting a defensive response against the later-arriving pathogen (Harman 2006; Ditmore et al. 2008; Laine 2011). The outcome of a multiple infection event may also differ based on the specific genotypes of the pathogens (Clément et al. 2012). Due to this multitude of potential outcomes, predicting the results of an invasive pathogen requires specific knowledge of the disease system including the other organisms present and the nature of their interaction with the pathogen of interest.

Sudden Oak Death (SOD) is an introduced forest disease with the capacity to cause large-scale transformations of forested landscapes (Rizzo and Garbelotto 2003; Garbelotto and Hayden 2012; Cobb et al. 2013). The causal agent of SOD, the oomycete *Phytophthora ramorum* (Rizzo et al. 2002), appears to have been introduced multiple times in proximity to the San Francisco Bay area through contaminated nursery stock in the late 20th century (Mascheretti et al. 2008, 2009; Grunwald et al. 2012; Croucher et al. 2013). Other *Phytophthora* species are well known as agents of destructive plant diseases, including late blight of potato caused by *P. infestans* (Nowicki et al. 2011), Port Orford Cedar root disease caused by *P. lateralis* (Hansen et al. 2000), and Jarrah dieback of *Eucalyptus* caused by. *P. cinnamomi* (Jung et al. 2013). *Phytophthora ramorum* is rather unusual among *Phytophthora* species affecting forest trees by being aerially transmitted rather than being soil- and waterborne (Garbelotto and Hayden 2012). In recent years, field surveys throughout California and Oregon have resulted in the description of two additional *Phytophthora* species, *P. nemorosa* and *P. pseudosyringae*. These species produce symptoms indistinguishable from those of *P. ramorum* on several hosts including the transmissive host California bay laurel (*Umbellularia californica*), and appear to also be aerially transmitted (Martin and Tooley 2003; Davidson et al. 2005; Murphy and Rizzo 2006; Wickland et al. 2006; Wickland et al. 2008; Hüberli et al. 2011). Although all three species have been

identified as agents of oak and tanoak mortality (Hansen et al. 2003; Jung et al. 2003; Wickland et al. 2008; Scanu et al. 2010; Scanu et al. 2012), they are not phylogenetically closest relatives (Martin and Tooley 2003), and there are significant biological differences among them: most notably, *P. nemorosa* and *P. pseudosyrinagae* can self-cross and easily produce thick walled oospores in nature, while *P. ramorum* does not. On the west coast of the United States, *P. nemorosa* and *P. pseudosyringae* have a broader geographic range and appear to be associated with much lower levels of host mortality than *P. ramorum* (Wickland et al. 2008), suggesting important differences in ecology, virulence, and possibly coevolutionary history with the host plants (Linzer et al. 2009).

Although the smaller geographic range currently occupied by *P. ramorum* (Wickland et al. 2008) suggests a narrower ecological amplitude than that of the other two species, its full amplitude may be in part masked by its short time since establishment (Mascheretti et al. 2009; Croucher et al. 2013). The pathogen reproduces prodigiously during the short periods of high rainfall and mild temperatures occurring in the wet season of California's Mediterranean climate (Davidson et al. 2005; Hüberli et al. 2011). Even after a drought lasting several years, at the onset of such favorable conditions, population sizes can triple or quadruple in a few days (Eyre et al. 2013). The impressive reproductive potential of this pathogen makes it likely that *P. ramorum* will spread to forests where environmental conditions and host distribution allow, unless establishment is limited by competition with other organisms.

The Sudden Oak Death epidemic has been extensively studied in regard to its likely origin and pathways of spread; host range and pathogenicity; reproductive mode; and autecology including environmental triggers for dormancy and reproduction, persistence in soil and water, and colonization of California bay laurel as a major transmissive host (Grünwald et al. 2012, Hayden and Garbelotto 2012). Our aim in the present study is to gain a deeper understanding of the SOD epidemic by examining the spread and seasonal dynamics in prevalence of *P. ramorum* on California bay laurel relative to that of the other two sympatric *Phytophthora* species.

Based on previous reports of both *P. nemorosa* and *P. ramorum* on the San Francisco peninsula, and the location of the peninsula within the range of *P. pseudosyringae* (Wickland et al. 2008), we considered it likely that all three may be established in our field sites located in the San Francisco Public Utilities Commission watershed within this area. Between 2009 and 2011, we undertook an extensive monitoring effort including repeated surveys (3 per year) focusing on the occurrence of *Phytophthora* spp. on California bay laurel leaves. The survey targeted 388 trees in 15 sampling plots, with the objective of investigating potential niche differentiation and temporal dynamics of these pathogens in nature. The occurrence of a transition from a period of drought (2009) to a period of above-average rainfall (2010-2011) provided an additional opportunity for a 'natural experiment' allowing the effect of larger climatic shifts to be examined by comparing sampling periods by season between years.

In addition to the field survey, we performed a controlled growth chamber co-inoculation experiment on bay laurel leaves to determine whether interactions between the three *Phytophthora* species may be antagonistic, neutral, or synergistic. Results from this experiment are important to correctly interpret results from the field surveys.

Our combined approach allowed the following hypotheses to be tested:

H1: If dominance of a pathogen species occurs at the plot (rather than tree or leaf) level, distribution patterns can be correlated to discernable environmental differences between plots (i.e., niche differentiation).

H2: Alternative hypotheses regarding distribution of the three pathogens:

H2a: Consistent with previously-reported geographic trends suggesting rapid spread of *P. ramorum*, plots dominated by *P. nemorosa* or *P. pseudosyringae* will shift to *P. ramorum* dominance in time. This shift in dominance will be accompanied by a finding of *greater antagonistic competitive ability* of *P. ramorum* vs. *P. nemorosa* and/or *P. pseudosyringae* as measured in the *in vitro* assays. This hypothesis is consistent with an explanation of competitive displacement by *P. ramorum*;

H2b: Plots dominated by *P. nemorosa* or *P. pseudosyringae* will *not* shift to *P. ramorum* dominance. This shift in dominance will be accompanied by a finding of *equal or lesser competitive ability* of *P. ramorum* vs. *P. nemorosa* and/or *P. pseudosyringae* as measured in the *in vitro* assays. This hypothesis is consistent with an explanation of lack of competitive displacement due to priority effects in addition to weak or absent direct competition between pathogens;

Or, H2c: Plots dominated by *P. nemorosa* or *P. pseudosyringae* will *not* shift to *P. ramorum* dominance. This shift in dominance will be accompanied with a finding of *greater competitive ability* of *P. ramorum* vs. *P. nemorosa* and/or *P. pseudosyringae* as measured in the *in vitro* assays. This hypothesis is consistent with an explanation of lack of competitive displacement by *P. ramorum* due to priority effects, *despite* superior competitive ability of *P. ramorum*.

H3: Seasonal shifts in dominance will not occur during the dry (off-peak) seasons, when high levels of pathogen dormancy occur.

Given the differences in host mortality caused by these three *Phytophthora* species, understanding the nature, determinants, and outcomes of competition among them has important implications both for understanding the epidemiology of Sudden Oak Death and for the possible implementation of appropriate disease control strategies. In broader terms, the study sheds light on how the same niche may be used by different organisms, and how climate, priority effects, and competition may mediate the result of such interactions.

Methods

Plot Selection and Design

A network of 15 research plots was established within the San Francisco Public Utility Commission (SFPUC) lands in San Mateo County (latitude: 37.520°, longitude: –122.369°), approximately 30 km south of San Francisco (Supplementary Fig. 1; Supplementary Table 1). This 9,300 hectare watershed contains two major drainages, Crystal Springs and Pilarcitos, with elevations ranging between 95 and 1050 m. Higher elevations occur primarily in the Pilarcitos drainage, located west of the Crystal Springs drainage (i.e., in closer proximity to the Pacific Ocean). The average annual temperature range is 8.8-21.5º C, with an average annual precipitation of 62 cm, occurring most frequently between November and April. The entire watershed, which has been closed to the public for over a century, includes one of the earliest known SOD infections in San Mateo County, with confirmed reports of infection as early as 2001 (Croucher et al. 2013). Plot locations were selected based on the presence of appropriate vegetation types (i.e., suitable habitat for *Phytophthora* species), as well as accessibility. All plots were separated by a minimum of 2 km to avoid mixing of local pathogen populations (Mascheretti et al. 2008). Each plot consisted of three 100 m transects, 10 m wide and separated by 120 degrees, radiating from the plot center. A bay laurel stem was selected for repeated sampling at 10 m intervals along each transect. A stem was defined as any major branch

separating from a tree's main stem below breast height (1.4 m). All bay laurel stems occurring in each plot were tallied to determine plot level density.

Climate Data Collection

Temperature data were collected from two Remote Automated Weather Stations (RAWS): Pulgas (Lat: 37.47500, Long: -122.29810) in the Crystal Springs drainage, and Spring Valley (Lat: 37.5625, Long: -122.436389) in the Pilarcitos drainage. Microclimate data were collected via three HOBO data loggers (Onset Computer Corporation, MA, USA) per major drainage. Rainfall data were retrieved from the CA Department of Water resources and SFPUC data archives of the Lower Crystal Springs rain gauge (Lat: 37.5325, Long: -122.363) and the Pilarcitos rain gauge (Lat: 37.547, Long: -122.421). Using temperature and rainfall values, a monthly Aridity Index (AI) was calculated as AI =12*P/(T+10), where P is the precipitation in mm and T is the mean air temperature in degrees C (De Martonne 1926). A high index value represents a low level of aridity, whereas a low index indicates high aridity.

Sampling of Infected Plant Material

Each sampling event began with surveys of the 388 bay laurel stems for the presence of foliar symptoms. Symptoms consist of darkened necrotic leaf tissue normally found at the tip or along the leaf edge, bordered by a dark zone line and a chlorotic (yellowish) halo or as dark pixelated spots scattered about the leaf, more common in active infection (Davidson et al. 2003). Six leaves, approximately 2 y in age and displaying symptoms of foliar *Phytophthora* spp., were sampled from the lower canopy of each tree. Infection by aerial *Phytophthoras* is not systemic, even within a single leaf; hence, the best experimental unit is the individual lesion. However, to obtain sufficient sampling resolution at the scale of the study, our sampling design included pooling lesions from multiple leaves on the same tree into a single sample, to ensure adequate sampling density without dramatically increasing sampling effort. This approach may theoretically overestimate co-infection by different species, but such an outcome was not observed in the present study (see Results).

Bay laurel assessments were conducted 9 times over 3 y in 2009, at the end of a 3 y drought, and in 2010 and 2011, both characterized by above-average rainfall levels. Surveys were conducted 3 times per year to capture seasonal variation of pathogen incidence: an "Early" season sampling in late winter/early spring (February/March) when temperatures warm enough for sporulation have not yet been reached, a "Peak" season sampling in late spring/early summer (May/June) when rainfall coupled with warmer temperatures provides ideal conditions for sporulation and transmission, and a "Late" season sampling after the hot and dry months of late summer/early autumn (September/October) have triggered pathogen dormancy (Eyre et al. 2013).

Molecular Sampling

It has been shown that the presence of aerial *Phytophthora* spp. in leaves can only be reliably detected from symptomatic portions of a leaf (Hayden et al. 2004). Accordingly, samples for molecular diagnostics were obtained from the margin of putative *Phytophthora* lesions on symptomatic bay laurel leaves using a surface-sterilized 6 mm hole punch. From each set of 6 leaves, 3 leaf punches were randomly selected from a collection comprising one punch per leaf and combined in a 2 ml screw-top tube containing a sterile glass bead; the samples were lyophilized and then pulverized using a FastPrep®-24 homogenizer (MP Biomedicals) for a

minimum of 30 sec at 4 m/sec. DNA was extracted using either the CTAB and phenol/chloroform extraction protocol of Hayden *et al.* (2004), or the ROSE extraction method of Steiner *et al.* (1995) as implemented by Osmundson *et al.* (2013). Presence of *P. ramorum* in culture-negative leaves was assessed using the DNA-based nested qPCR assay described by Hayden *et al.* (2006). Samples yielding a negative result with regard to *P. ramorum* infection in the qPCR assay were then tested for the presence of *P. nemorosa* and *P. pseudosyringae* using species-specific primers designed by Linzer (2009). Existing TaqMan primers and probes specific to *P. ramorum* (Schena et al. 2006) were used to capture any false negatives from the original qPCR diagnostic. This multiplex qPCR assay (*nemorosa-pseudosyringae-ramorum*, or N-P-R), optimized by Linzer (2009), was conducted as follows. The target locus selected for this assay is a nuclear, single copy region of the ras-related protein *ypt1*, that has been shown to be polymorphic between, but not within, *Phytophthora* species (Schena and Cooke 2006). Primer and probe sets for *P. nemorosa* and *P. pseudosyringae* were designed following guidelines described by Schena *et al.* (2006) and using *Phytophthora* sequences from GenBank (Supplementary Table 2). Fluorophore and quenching pairs for primer/probe sets were selected using the multiplexing compatibility criteria described by Marras (2007). Cross reactivity of species-specific primers and probes was tested using a dilution series. Amplification potential of *Phytophthora* DNA was tested with and without the addition of *U. californica* DNA, which can be inhibitory (Hayden et al. 2006), and the sensitivity of multiplexed and simplex reactions was compared. It was determined empirically that 1:10 dilutions of phenol/chloroform-extracted DNA and undiluted ROSE-extracted DNA yielded the most consistent results – and that the two extraction techniques yielded the same results when compared directly – when analyzing the single-copy *ypt1* locus in an *U. californica*-leaf background, so all N-P-R multiplex qPCR assays were performed using these template dilutions. All PCR reactions were performed in 20 µl volumes containing 1x TaqMan Universal PCR master mix (No AmpErase UNG) (Applied Biosystems, Foster City, CA), 900 nM of each primer, 200 nM of each probe (Operon Biotechnologies, Huntsville, AL), and 5 µl of template DNA (or ultrapure PCR H_2O for negative controls). PCR reactions were performed on a CFX96 Touch Real Time PCR Detection system (Bio-Rad Laboratories, Inc.; Hercules, CA). Amplification conditions consisted of one 10-min denaturation at 95°C followed by 45 cycles of 15 sec at 95°C and 1 min at 58°C. Real-time data were collected during each 58°C step and analyzed using qbasePLUS software (Bio-Rad). Additionally, all samples were amplified using a 1:50 dilution with a universal primer and probe set from a conserved region of the 18s rDNA as a means of confirming DNA extraction success for samples that were negative for the species-specific reactions. This primer set amplifies DNA from many taxa, including both plants and Oomycetes (Schena et al. 2006).

Co-infection Experiment

To determine how these three foliar *Phytophthora* species might interact during the infection process, we designed a controlled co-infection experiment on detached leaves of bay laurel. Seven possible combinations were tested using a single droplet containing equally concentrated solutions of suspended zoospores from one to three species. The treatment groups were as follows: (1) *P. nemorosa* alone (N); (2) *P. pseudosyringae* alone (P); (3) *P. ramorum* alone (R); (4) N + P; (5) N + R; (6) P + R; (7) N + P + R. To prepare inoculum solutions for the co-infection treatments (i.e., treatments 4-7), a partial replacement series design commonly used in competition experiments was applied (Silvertown and Doust 1993); i.e., the concentration of zoospore solution prepared from each infecting species was constant, as was total zoospore

density for each treatment, the latter with varying ratios of constituent components based on the treatment group. Each treatment was replicated at both 14 and 18° C, corresponding to optimal growth temperatures of the various species (see Inoculum Preparation, below).

Inoculum Preparation

Inoculum solutions of suspended *Phytophthora* zoospores were prepared as follows. Cultures of each species based on comparable pathogenicity levels and sporulation ability of isolates, as determined through a preliminary experiment (Linzer 2009), were selected and grown on V8A media (Erwin and Ribeiro 1996). Because intraspecific genetic variation is minimized by the exclusively clonal reproductive mode of *P. ramorum*, and by the selfing reproductive mode of the other two species (Hansen et al. 2003; Jung et al. 2003; Linzer et al. 2009), sporulation ability and healthy appearance of colonies *in vitro* rather than genetic variation *per se* were employed as the two main selection variables of genotypes to be employed. Between 20-30 plugs of agar roughly 0.5 cm^2 were excised from the margin of each culture, placed face-up in an empty Petri dish, and flooded with a 2% soil-water infusion (Linzer 2009) until the liquid level was even with the top of the agar plugs. Each species was incubated for 3-5 d at or near its optimum growth temperature (*P. ramorum* and *P. pseudosyringae* at 18°C, *P. nemorosa* at 14°C). Plug suspensions were then placed in a 4° C ice bath for 30 min, then incubated at room temperature for 2 h to induce zoospore release from sporangia. Concentrations of each zoospore solution were quantified using a hemocytometer and adjusted to 3 x 10^4 zoospores mL^{-1} to ensure each species had sufficient zoospores in the co-infection treatments. Negative control inoculum (sterile distilled H_2O and clean V8A media) was prepared simultaneously using the same methodology but using an uncolonized V8A plate. To prevent premature zoospore encystment, all labware contacting the zoospore suspension was soaked for 24 h in 5M HCl and rinsed 3 times in deionized water.

Inoculation

Host leaves of intermediate age (approximately 2 years), were detached from a single uninfected bay laurel tree on the University of California, Berkeley campus previously determined to be of intermediate susceptibility to *P. ramorum* (Meshriy et al. 2006). All 366 leaves were surface-sterilized with 70% EtOH, and each of the 7 treatments was randomly assigned and applied on 25 leaves, while an additional 8 negative control leaves were included at each temperature. Using acid washed pipette tips, 35 µl of zoospore suspension was placed on the abaxial surface of each leaf, approximately 1 cm from the tip. This inoculation method is comparable to the presumed natural infection pathway of bay laurel leaves. It is believed that windborne sporangia land on the surface of a leaf and release zoospores into accumulated water droplets that coalesce together at the edges of leaves (Davidson et al. 2005). Leaves were incubated in moist chambers constructed from two 40 x 60 x 10 cm nursery trays. The bottom tray of each box was lined with paper towels and moistened with sterile distilled H_2O. Inoculated leaves were placed upon a 1 cm-gridded plastic rack inserted into the bottom of each box. Boxes were sealed in a plastic bag and placed into one of two growth chambers set at either 14 or 18°C. Every other day, boxes were opened and misted with sterile distilled H_20 to ensure that leaves remained moist. Visible lesions had formed on nearly all inoculated leaves after 12 d: based on our experimental conditions and on other published work on inoculations of detached leaves (Hüberli et al. 2003; Linzer 2009; Hüberli et al. 2011; Eyre et al. 2014), this was regarded as an optimal length for the experiment. Leaves were removed from the trays, surface-sterilized, and

scanned. Lesion size was then quantified using ASSESS v 1.01 (Lamari 2002). A small section of each leaf was excised, taking care to encompass the entire lesion; the section was placed in a 2 ml tube and stored at -20° C until qPCR diagnostics were used to test for both presence and quantity of each *Phytophthora* species.

Statistical Analyses

The following statistical analyses were carried out in JMP 10 (SAS Institute). A generalized linear model (GLM) assuming a Poisson distribution was used to compare prevalence of *P. nemorosa* and *P. ramorum* infection, represented as the proportion of infected trees detected out of all surveyed trees, by plot, season, and season nested within year. No *P. pseudosyringae* was detected in the plot network, therefore it was not included in the analysis. A Student's 2-sample t-test was used to compare mean *P. nemorosa* and *P. ramorum* occurrence by plot and by year. A linear regression was used to examine proportions of both *P. nemorosa* and *P. ramorum* as a function of overall bay laurel density per plot. A logistic regression analysis on the frequency of *P. ramorum* and *P. nemorosa* detection was performed using the monthly Aridity Index at each sampling point as the independent variable. Finally, a Spearman's rank order correlation test was used to compare the frequencies of *P. nemorosa* and *P. ramorum* in all plots at each sampling event, producing a non-parametric Spearman's Rho coefficient for each sampling event ranging from -1 to 1. For the co-infection experiment, APS ASSESS (Lamari 2002) was used to quantify lesion size expressed as necrotic area. To determine the effect of the treatments on overall leaf damage and pathogen load, lesion area and estimated quantities of DNA by treatment were analyzed using two-way ANOVA, with temperature and species-combination treatment as fixed factors. Analyses of estimated DNA quantity by lesion were conducted on Log_{10} transformed data. To determine the result of competitive interactions between species, a three-way ANOVA with species, temperature and species-combination treatment as fixed factors was used to analyze DNA concentrations of individual species. *Post hoc* means comparisons were performed using Tukey-Kramer HSD tests.

Results

Climate and Environmental Data Analysis

The California Department of Water Resources reported annual rainfall in 2009 to be only 60% of the 50 y average, with little to no rainfall in the spring months. An increase was reported for 2010 and 2011, to 115% and 135% of the 50 y average, respectively, with above average rainfall occurring in the spring of both years Plots in the Pilarcitos drainage exhibited a slightly cooler and wetter microclimate than plots in the Crystal Springs drainage (Supplementary Figs 2A and 2B). The Pilarcitos drainage is higher in elevation, with plots at an altitude ranging between 197 and 311m, while the altitude of plots in the Crystal Springs drainage ranges between 95 and 187m. The Aridity Index (AI) was calculated for each of the six plots where microclimate data were available by using cumulative precipitation and mean daily temperature data from the 30 d prior to each sampling event (Supplementary Fig. 3). The aridity indices for all six plots were comparable during the dry periods, yet plots in Pilarcitos had higher AI values in the wet periods, indicating a slightly wetter environment during the rainy season (Supplementary Figs 2A and 3). A logistic regression of *P. nemorosa* and *P. ramorum* observations against varying values of the AI through time showed a significant and positive relationship between AI and prevalence of *P. ramorum* (Chi-square =16.2, $p < 0.0001$) and a

negatively significant relationship with prevalence of *P. nemorosa* (Chi-square = 8.96, p = 0.0028).

Molecular Diagnostics (N-P-R Assay)

The nine surveys of 388 bay laurels in the 2009-2011 period resulted in the sampling of 3026 symptomatic leaves. Of those 3026 samples, 1890 were determined to be positive for *P. ramorum* while 10 samples yielded inconclusive results. The remaining 1126 *P. ramorum*-negative samples were tested using the N-P-R diagnostic assay, and 446 samples yielded positive results for *P. nemorosa* (Supplementary Table 3). *Phytophthora pseudosyringae* was not detected in any of the samples. A subset of the 1890 *P. ramorum*-positive samples was tested for presence of the two additional *Phytophthora* species. The percentage of samples found to contain DNA of more than one species was 1.5%. Thus, while it is possible for a leaf to contain lesions from more than one species of *Phytophthora*, it was very uncommon at the scale of a single lesion, the experimental unit targeted for this assay. A Generalized Linear Model (GLM) shows significant differences in the abundance of both *P. nemorosa* and *P. ramorum* between years (*P. nemorosa*: Chi-square = 17.84, $p < 0.0001$; *P. ramorum*: Chi-square = 40.41, $p < 0.0001$) and plots (*P. nemorosa*: Chi-square = 110.12, $p < 0.0001$; *P. ramorum*: Chi-square = 117.85, $p < 0.0001$), but not by season nested within year (*P. nemorosa*: Chi-square = 0, p = 1.000; *P. ramorum*: Chi-square = 5.68, p = 0.4599) (Table 1). Two plots (8 and 10, both in Crystal Springs drainage) contained less-than-expected occurrence of *P. nemorosa*, while four plots (9, 11, 14, and 15, all in Pilarcitos drainage) contained greater-than-expected *P. nemorosa* occurrence ($p < 0.0001$). Four plots (2, 6, 8, and 10), none of which matched plots with highest levels of *P. nemorosa,* contained greater-than-expected prevalence of *P. ramorum* (Table 1). In 2009, the driest year during our study, prevalence of *P. nemorosa* was at its highest level (Fig. 1). Prevalence of *P. nemorosa* began to decrease in the wetter 2010, and more drastically so after the heavy rains of 2011. The opposite relationship was observed for *P. ramorum* (Fig. 1), whose prevalence increased as the weather conditions changed from dry to wet over the course of the three-year study.

Examination of occurrence by plot and year (Fig. 2A) indicated significantly higher rates of *P. nemorosa* occurrence in plots 9, 11, 14, and 15, all located within the Pilarcitos drainage. In these four plots, a decrease in the mean occurrence of *P. nemorosa* was observed as the mean occurrence of *P. ramorum* increased with changing weather conditions (Fig. 2B, 3). In the Peak/spring and Late/autumn seasons of 2009 and the Peak/spring season of 2011 ($\rho = -0.8774$, $p < 0.0001$; $\rho = -0.7096$, $p = 0.003$; $\rho = -0.6172$, $p = 0.0142$, respectively), a significant negative correlation occurred between the proportions of the two pathogens (Table 2).

Significant correlations were found between plot-level bay laurel density and proportions of trees testing positive for either *P. nemorosa* or *P. ramorum* (Supplementary Table 1, Supplementary Fig. 4). However, while *P. ramorum* prevalence was positively correlated with bay laurel density ($p < 0.0001$) (Linear fit equation : proportion *P. nemorosa* positive = 0.173 – 0.0005 * bay laurel density), *P. nemorosa* prevalence was negatively correlated with bay laurel density ($p = 0.0232$) (Linear fit equation : proportion *P. ramorum* positive = 0.433 + 0.0019 * bay laurel density).

To examine dynamics of *P. nemorosa* and *P. ramorum* on individual trees, longitudinal patterns of infection were compared in trees sampled within four plots with significant *P. nemorosa* prevalence (plots 9, 11, 14, and 15; Supplementary Fig. 5). These comparisons show an overall shift in dominance from *P. nemorosa* to *P. ramorum*. Of 131 *Phytophthora*-positive

trees, a total of 76 trees showed evidence of only *P. nemorosa* infection in 2009, declining to 39 in 2010 and 11 in 2011. In contrast, 18 trees showed evidence of only *P. ramorum* infection in 2009, increasing to 50 in 2010 and 77 in 2011. However, *P. nemorosa* maintained presence in the plots, with 56 trees showing presence of *P. nemorosa* in at least one sampling event following detection of *P. ramorum*.

Three-species co-infection experiment

With the exception of all negative control leaves, which remained asymptomatic, all leaves developed a single lesion where the inoculum droplet was applied. DNA of each inoculated species was detected in all experimental leaves using the universal primer/probe set, while no *Phytophthora* DNA was detected in negative-control leaves. Univariate regression between leaf lesion size and total amount of *Phytophthora* DNA showed a significant, positive relationship (parameter estimate = 12.17, $p < 0.0001$). No significant differences between lesion sizes among species combinations were observed within temperatures, but the 18° C N+P+R treatment exhibited lower lesion size and recovered DNA amounts compared to the *P. ramorum*-only treatment at 14° C ($F = 2.40$, $p = 0.0043$) (Supplementary Fig. 6A). Total *Phytophthora* DNA quantity, estimated by real-time PCR (quantity of *P. nemorosa* + *P. pseudosyringae* + *P. ramorum*), were correlated with those for lesion area (Supplementary Fig. 6B). Similarly, the only differences between treatments in terms of the total amount of DNA present were between the 18° C N+P+R treatment and the 14° C *P. ramorum*-only treatment ($F = 3.03$, $p = 0.0003$) (Supplementary Fig. 6B).

For individual species, significant differences in *Phytophthora* DNA quantity were detected by species-treatment and temperature ($F = 17.18$, $p < 0.0001$). There were no significant differences in the quantity of DNA detected in the individual-species treatments at either temperature; however, mean DNA quantity for *P. nemorosa* was reduced more than tenfold in all co-infection treatments with *P. pseudosyringae* and/or *P. ramorum* (Fig. 4). Neither the quantities of *P. pseudosyringae* nor *P. ramorum* DNA were significantly different from those in the single-species treatments in any two or three-species combination within one temperature (Fig. 4). Within species, there was a trend toward the lowest amount of DNA being detected in the three-species treatment at 18° C, with some comparisons being significant at $p = 0.05$ (Fig. 4). There were no significant differences in identical treatments between temperatures.

Discussion

Colonization by an introduced pathogen is likely to be shaped by a number of factors, including the distribution and susceptibility of suitable hosts; the presence of environmental conditions suitable for establishment, growth, reproduction, and dispersal; and the nature (synergistic, neutral, or antagonistic) of the pathogen's interactions with other non-host organisms, including other pathogens. While the first two of these factors fall under the regular purview of plant pathology research, the third is less frequently considered, but may be extremely important for the development of disease outbreaks. Coexistence between organisms colonizing the same plant may occur as a result of nutritional (Wilson and Lindau 1994) or temporal (Hamelin et al. 2016) niche partitioning; alternatively, competitive exclusion may eliminate one or more competitors (Lopez-Villavicencio et al. 2007, 2011; Siou et al. 2015). The outcome of these interactions may be affected by factors such as climate, priority effects, and

genotype (Hood 2003; Simpson et al. 2004; Al-Naimi et al. 2005; Laine 2011; Clément et al. 2012; Hamelin et al. 2016).

Sudden Oak Death has been present in California since at least the mid-1990s, but the causal agent, *Phytophthora ramorum*, was only discovered in 2000 (Werres et al. 2001; Rizzo et al. 2002). Since 2000 (Svirha 1999, Rizzo et al. 2001), field surveys in the western United States have led to the discovery of two additional aerially-dispersed *Phytophthora* species, *P. nemorosa* and *P. pseudosyringae*, with similar host ranges and symptoms to *P. ramorum*, but causing less oak and tanoak tree mortality (Wickland et al. 2008). Improved understanding of the current distribution of *P. ramorum*, its ecological limits and response to environmental conditions, and the nature of its interactions with *P. nemorosa* and *P. pseudosyringae* are likely to be important for tracking the Sudden Oak Death epidemic and developing predictive models of disease spread.

We used extensive field surveys over a drought/recovery cycle coupled with measurements of abiotic characteristics to establish fine-grained estimates of the distribution of *P. ramorum*, *P. nemorosa* and *P. pseudosyringae*, track their dynamics in response to changing environmental conditions, and predict the environmental conditions likely to favor the establishment of the different species. Given that significant habitat overlap exists for these species, the role of competitive interactions between them is also likely to have a critical influence on establishment. However, disentangling the effects of competition from those of environmental heterogeneity is difficult under field conditions. Therefore, we also examined the competitive outcomes arising from co-inoculation of these species under controlled conditions, using a detached leaf assay previously shown to suitably replicate natural pathogen infection (Johnston et al., 2016), combined with a qPCR-based assay for detecting and distinguishing the three species in leaf samples (Linzer 2009).

Results of field surveys showed that both *P. ramorum* and *P. nemorosa* are present in our study sites on the San Francisco peninsula. The limited genetic variation within each species (Linzer et al. 2009) and negative screens of herbarium specimens (Monahan et al. 2008) indicate that all three *Phytophthora* species may be introduced to California. Nonetheless, the broader geographic range of *P. nemorosa* and its presence in regions where *P. ramorum* has not yet been detected (Wickand et al. 2008) suggest that *P. nemorosa* has been established in the western United States longer than *P. ramorum*. Surprisingly, the significantly wider distribution of *P. ramorum* in the SFPUC field sites suggests that *P. ramorum* has a higher ecological amplitude than, and may be able to outcompete, *P. nemorosa*; this finding also suggests that the smaller overall geographic range currently occupied by *P. ramorum* in California is due to a more recent colonization history rather than to lower colonization potential. The apparent lack of *P. pseudosyringae* in these sites suggests either that environmental conditions are unsuitable for its establishment (unlikely given its overall geographic distribution; Wickland et al. 2008), that it has not been introduced to this specific area, or that it has been outcompeted by *P. ramorum* and/or *P. nemorosa*.

Both *P. nemorosa* and *P. ramorum* were found to occupy the same niche; i.e., portions of *U. californica* leaves prone to coalescence of water droplets. However, although coexistence of the two species is possible (Wickand et al. 2008), coexistence on the same leaf was rarely observed in our samples. *P. nemorosa* was abundant in only four of our sampling plots. These plots are characterized by higher elevation, lower overall bay laurel density, and less aridity (i.e., wetter and cooler conditions). There is no overlap between plots where *P. ramorum* is most abundant and plots where *P. nemorosa* is most abundant, suggesting that the two species are fine-tuned to different ecological conditions (i.e., hypothesis H1 is supported by these data). Our

data further suggest that the slightly warmer temperatures of the lower elevation plots in which *P. ramorum* is most abundant - coupled with spring rainfall - may disproportionately favor its transmission potential, as indicated by Hüberli *et al.* (2011).

The occurrence of a significant climatic shift in 2009-2010 from a period of sustained drought to 2 y of above-average rainfall during the wet seasons of 2010 and 2011, in combination with our survey design encompassing the major periods of annual seasonal weather fluctuation, provided an opportunity to observe shifts in the prevalence of *P. ramorum* and *P. nemorosa* in response to abiotic conditions. In dry conditions such as the majority of 2009 as well as the autumn sampling events of 2010 and 2011, the frequency of *P. nemorosa* in cooler and wetter sites amenable to its establishment was comparable to that of *P. ramorum*. This indicates that *P. nemorosa* does have the ability to persist in a *P. ramorum* dominated region, but only in some sites and to a greater extent when overall conditions are dry. When conditions became favorable to sporulation of *P. ramorum* – i.e., increased winter/spring rainfall (Davidson et al. 2005; Hüberli et al. 2011; Eyre et al. 2013) – contrasting prevalence of the two pathogens was observed, with an increase in *P. ramorum* and a decrease in *P. nemorosa* even in those plots where *P. nemorosa* was previously dominant (i.e., hypothesis H3 is supported by the observed peak 2009 and 2011 results, though contradicted by the observed shift in the Late 2009 sampling). Given that it is the cooler and wetter plots that support persistence of *P. nemorosa* during dry conditions, it is likely that the observed shifts result from a greater competitive ability of *P. ramorum* during wet periods known to foster its sporulation (i.e., hypothesis H2a is supported over H2b and H2c).

Examination of individual-level colonization patterns over the duration of the study for trees in the four plots with significant *P. nemorosa* infection highlights the contrasting prevalence of the two pathogens while also revealing a more complex dynamic. Although the dominant trend was a majority of trees (76/131, or 58%) with only *P. nemorosa* detected in 2009 and a majority (58.8%) with only *P. ramorum* detected in 2011, *P. nemorosa* was detected in a sampling period following a *P. ramorum* positive result in nearly half (42.7%) of these trees. This suggests that competitive exclusion of *P. nemorosa* by *P. ramorum* is not absolute at the whole-tree level. As our sampling design involved testing of multiple leaves per tree, molecular screening is likely to indicate the dominant pathogen on the tree at the given sampling period when only a single pathogen is detected; however, the presence of many evergreen leaves provides the opportunity for both pathogens to persist. Whether *P. ramorum* establishes and outcompetes preexisting *P. nemorosa* on leaves under natural conditions was not established in the present study, nor was whether *P. nemorosa* recurrence results from new leaf colonization or from persistence on older leaves. However, the contrasting trends in colonization prevalence strongly suggest that *P. ramorum* is gaining dominance on individual trees through indirect (more extensive colonization of new leaves) and/or direct displacement of *P. nemorosa*.

Two additional results support the conclusion of competitive interactions as a major factor influencing the observed prevalence of the two species. The first is the contrasting relationship between bay laurel density and prevalence of the two species; while *P. ramorum* prevalence was positively correlated with bay laurel density, *P. nemorosa* prevalence was negatively correlated with it. However, previous studies have shown occurrence of both *P. ramorum* and *P. nemorosa* to be positively correlated with bay laurel density (Murphy and Rizzo 2006; Wickland et al. 2006; Murphy et al. 2008). This seemingly paradoxical result could be explained by the higher competitive ability of *P. ramorum* due to increased sporulation levels, which result in more effective plant to plant transmission levels than those of *P.nemorosa*. Lower

bay laurel density may thus favor *P. nemorosa* persistence indirectly by reducing sporulation potential of *P. ramorum*. The second line of supporting evidence is provided by the results of the *in vitro* co-inoculation studies. These results show an antagonistic relationship between *P. ramorum* and *P. nemorosa*, with *P. ramorum* co-inoculation resulting in a significant decrease in colonization of *P. nemorosa*.

Because competition can be mediated by environmental conditions (Marín et al. 1998; Liancourt et al. 2005), and because *in vitro* growth of these three pathogens suggests differing optimal growth conditions, we replicated the co-infection experiment at two temperatures, 14° C and 18° C, reflecting growth optima previously determined for the three species *in vitro* (Hansen et al. 2003; Jung et al. 2003; Englander et al. 2006; Wickland et al. 2008). Results indicate that the effect of these selected temperatures was small, as there were no significant differences between the same treatments at different temperatures.

The observation of only one *Phytophthora* species' DNA in the vast majority of leaf samples suggests that a direct competitive displacement of existing *P. nemorosa* colonization by *P. ramorum* on individual leaves is unlikely. Rather, it is more likely that as leaves drop and new ones develop, they may be colonized either by one or both species. If colonization is simultaneous and inoculum amounts are similar, our *in vitro* experimental evidence suggests that *P. ramorum* would outcompete *P. nemorosa*. If *P. nemorosa* arrives first and becomes well-established, it appears possible that it could persist via priority effects, but mostly under environmental conditions unfavorable to *P. ramorum* sporulation. Several additional studies could address these hypotheses, including mark-recapture to study disease dynamics on individual leaves, sequential (rather than simultaneous) *in vitro* inoculation studies to study priority effects, and observation of inoculum production to assess the hypothesis that *P. ramorum* can displace *P. nemorosa* at a plot level though higher inoculum load. Repeated follow-up surveys of currently asymptomatic trees (Supplementary Table 4), as well as other sites in which the two pathogens have been reported to co-occur (e.g., Murphy and Rizzo 2006), would shed additional light on the results of their interactions.

Based on the results of the field and laboratory studies, we suggest the following scenario: *P. nemorosa* and *P. ramorum* can coexist at a landscape level during drought conditions; however, when conditions are favorable for sporulation, *P. ramorum* is a formidable competitor that can outcompete *P. nemorosa* in colonizing new leaf surfaces. Inoculum load appears to be the major factor in this success, as evidenced by greater persistence of *P. nemorosa* in plots with lower bay laurel density and the shift toward greater persistence of *P. ramorum* in years with adequate rainfall that promotes sporulation. In dryer conditions, lower bay laurel density appears to keep *P. ramorum* prevalence in check. However, when environmental conditions become favorable to high inoculum production of *P. ramorum*, this higher inoculum in addition to better competitive ability swamps out the effect of low host density. Along with previous results showing a positive correlation between bay laurel density and *P. ramorum* occurrence, and that leaves rather than soil are the major source of disease persistence and transmission (Eyre et al. 2013), our results highlight the importance of host density in expanding the Sudden Oak Death epidemic. These results also provide support for recommendations of local bay laurel removal to help prevent infection of high-value oaks on a small scale (California Oak Mortality Task Force; http://www.suddenoakdeath.org/diagnosis-and-management/treatments/).

Given that the geographic range of *P. pseudosyringae* includes all surrounding directions that contain terrestrial habitat (Wickland et al. 2008), lack of detection of this species in our field

sites was somewhat surprising. *In vitro* co-inoculation showed neutral coexistence of *P. pseudosyringae* with *P. ramorum* and competitive dominance of *P. pseudosyringae* over *P. nemorosa*; therefore, we conclude that competitive interactions alone cannot account for the observed field patterns. Similarly, inability to detect *P. pseudosyringae* DNA does not explain these results, as the molecular assay was successful in our laboratory-inoculated leaves, and a similar assay was validated on field-collected leaves by Tooley *et al.* (2006). As competitive interactions alone appear insufficient to explain the lack of observed occurrence of *P. pseudosyringae*, we hypothesize that this absence can be attributed either to historical factors or to the absence of microsite differences that might favor this pathogen. As such ecological differences have not been previously identified, further research in this area may be important for understanding the distribution of *P. pseudosyringae*.

Although all leaves collected in the field showed lesion symptoms characteristic of infection by one of the three target pathogens, none of these pathogens were detected in approximately 22.5% of samples. This is likely either due to the absence of all three pathogens or due to samples containing degraded DNA. A number of other pathogens, including *Colletotrichum gloeosporioides*, the fungus responsible for bay laurel anthracnose (Davidson et al. 2003), can cause similar symptoms. Expanding field and laboratory studies to include these pathogens may offer insights into the role of additional competitive interactions in the spread of *Phytophthora* diseases. If DNA degradation is the cause, it is nonetheless unlikely to result in biased detection of any of the three *Phytophthora* species, based on the demonstrated ability of our assays to detect all three species from leaf material as well as previous results by Tooley *et al.* (2006) demonstrating detection of *P. ramorum* and *P. pseudosyringae* from field material.

By combining multi-season, multi-year field surveys with measurements of environmental conditions and controlled laboratory experiments, this study expands our understanding of Sudden Oak Death dynamics by highlighting the environmental and biotic factors that influence spread of the SOD epidemic, including conditions that favor the persistence of ecologically similar *Phytophthora* species and those that favor competitive dominance of *P. ramorum*. An implication of this study is that prediction and management of the disease should consider environmental conditions, host range, and the presence of potentially interacting *Phytophthora* species, particularly *P. pseudosyringae*, which based on our *in vitro* results may have better competitive ability than *P. nemorosa* against *P. ramorum*. These conclusions are important not only from a theoretical perspective, but also because these three species have extremely different end-effects in the ecosystems they colonize, with *P. nemorosa* and *P. pseudosyingae* being pathogens causing limited host mortality and *P. ramorum* being one of the most destructive forest pathogens currently known. Our findings may help to better predict the outcome of this expanding pathogen in light of different ecological conditions, presence of competitors, and changing climatic conditions.

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Tables and Figures

Table 1. Results of a Generalized Linear Model assessing proportions of *P. nemorosa* and *P. ramorum* detected from total trees sampled as a function of year, plot, and season (nested within year) (Poisson distribution; X2 goodness-of-fit test). Single asterisks denote significant positive correlations, and double asterisks denote significant negative correlations, at p = 0.05.

Effect	*P. ramorum*			*P. nemorosa*		
	DF	Chi square	P> Chi Square	DF	Chi square	P> Chi Square
Year	2	40.41	0.0001*	2	17.84	0.0001*
Plot	14	117.85	0.0001*	14	110.12	<.0001*
Season (Year)	6	5.68	0.4599	6	0	1.000
PLOT	Estimate	Std Error	P value	Estimate	Std Error	P value
0	0.137	0.129	1.000	-0.123	0.243	1.000
1	-0.062	0.142	1.000	-0.046	0.234	1.000
2	0.337	0.118	0.0087*	-0.025	0.232	1.000
3	-0.068	0.142	1.000	0.053	0.224	1.000
5	-1.600	0.296	<0.0001*	0.048	0.225	1.000
6	0.543	0.108	<0.0001*	-0.470	0.286	1.000
7	-0.064	0.142	1.000	-0.303	0.264	1.000
8	0.535	0.108	<0.0001*	-0.978	0.364	0.0151**
9	-0.090	0.143	1.000	0.775	0.163	<0.0001*
10	0.506	0.109	<0.0001*	-0.910	0.352	0.0343**
11	-0.313	0.172	0.142	1.175	0.145	<0.0001*
12	0.021	0.136	1.000	-0.013	0.231	1.000
14	-0.136	0.146	1.000	0.990	0.149	<0.0001*
15	-0.113	0.145	1.000	0.746	0.165	<0.0001*

Table 2. Results of a Spearman's rank order correlation test used to compare the frequencies of *P. nemorosa* and *P. ramorum* in all plots at each sampling event. The non-parametric Spearman's Rho coefficient ranges from -1 to 1. Significant negative correlations between the proportions of the two pathogens in a given sampling period are denoted by asterisks ($p \leq 0.05$).

Sampling Event	Early 2009	Peak 2009	Late 2009	Early 2010	Peak 2010	Late 2010	Early 2011	Peak 2011	Late 2011
Spearmans ρ	-0.4458	-0.8774	-0.7096	-0.3556	-0.5045	0.3806	-0.2315	-0.6172	-0.4144
P-value	0.0958	<.0001*	0.0030*	0.1934	0.0551	0.1617	0.4064	0.0142*	0.1407

Figure 1. Proportions of sampled bay laurel leaves with *P. ramorum* or *P. nemorosa* infection in each of the nine seasonal sampling events, measured using molecular diagnostic qPCR assays.

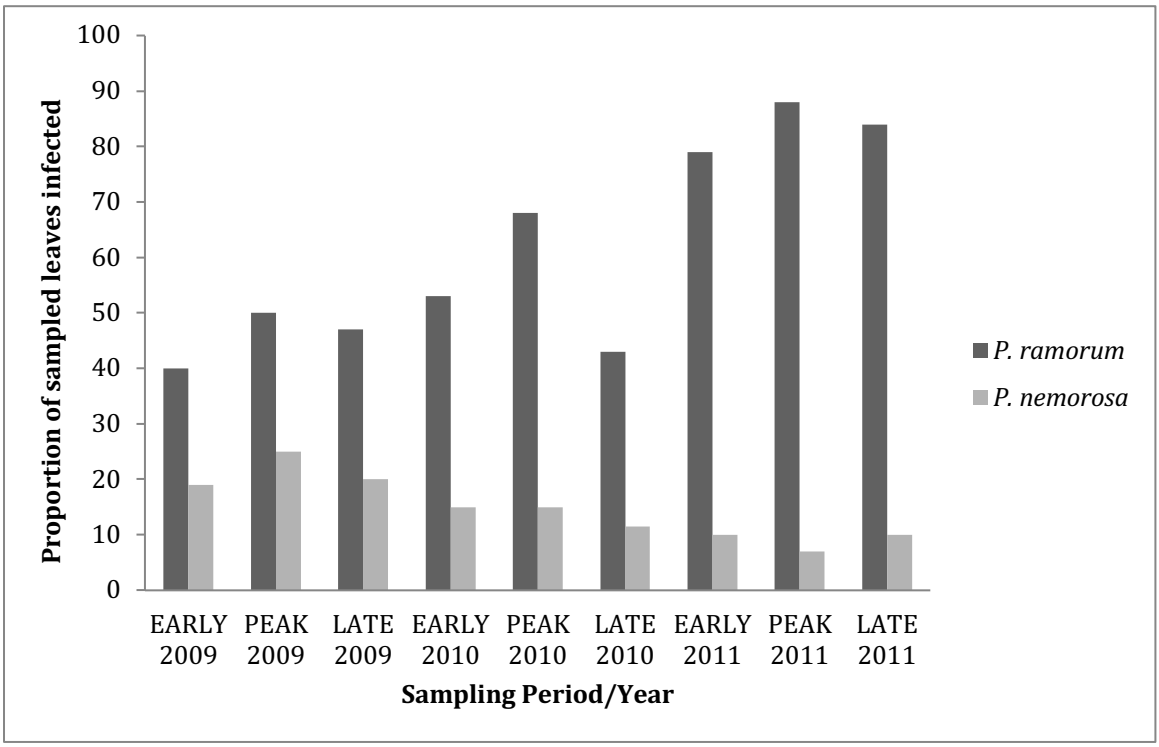
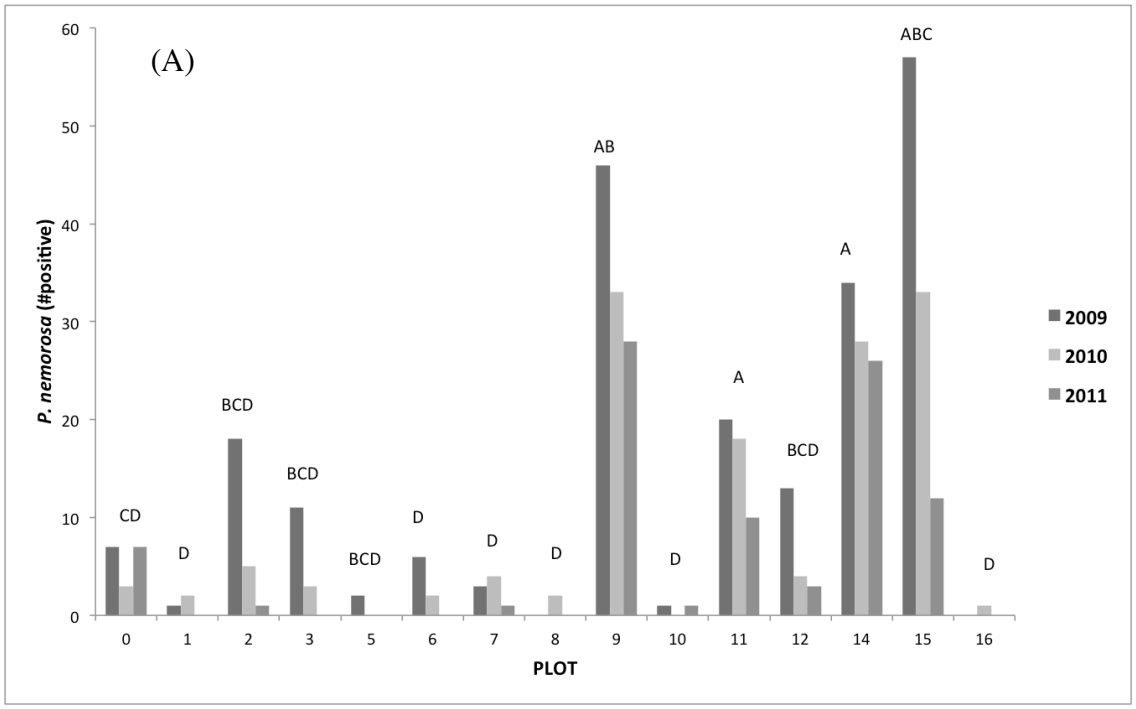


Figure 2. Positive sample counts for *P. nemorosa* (A) and *P. ramorum* (B) by plot and year. Statistical comparisons of means are depicted by letters A-D; totals not designated by the same letter are significantly different at a 0.05 significance level. Plots 9, 11, 14 and 15 are located in the Pilarcitos drainage; the remaining plots (excluding plot 5) are located in the Crystal Springs drainage.



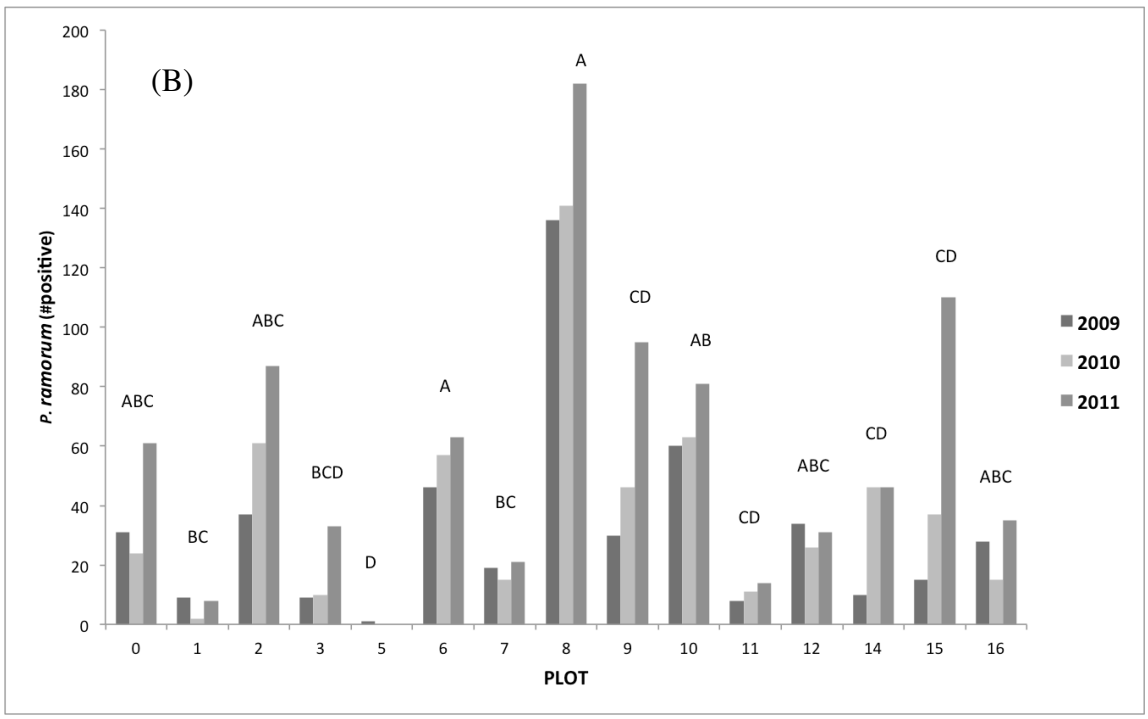


Figure 3. Mean occurrence (number of positive leaf samples) of *P. ramorum* and *P. nemorosa* in plots located in the Pilarcitos drainage (plots 9, 11, 14, 15) where the highest levels of *P. nemorosa* were observed. Occurrence of *P. nemorosa* decreased in contrast to *P. ramorum* over the three-year study period.

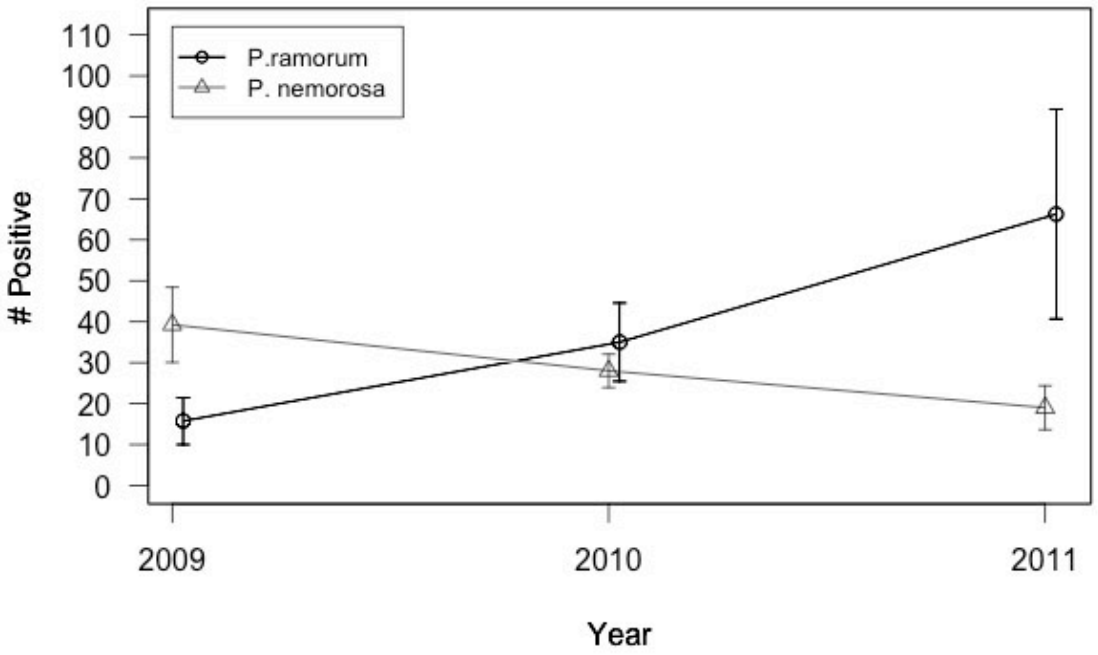
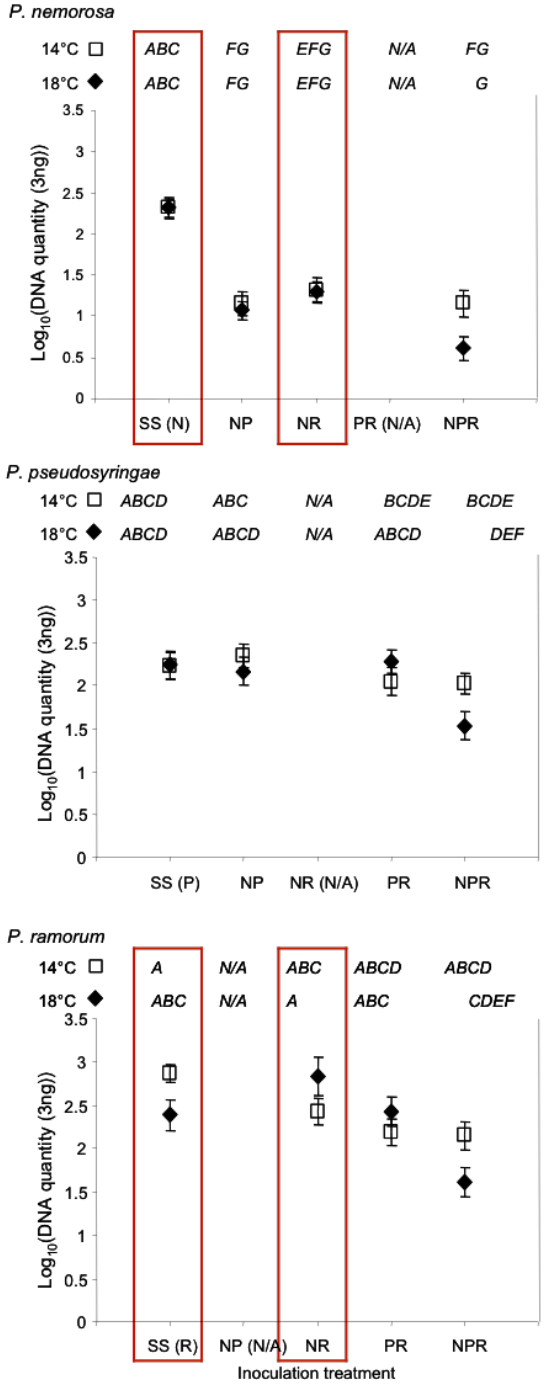


Figure 4. Total DNA quantity in leaf lesions estimated by qPCR for *P. nemorosa* (N), *P. pseudosyringae* (P) and *P. ramorum* (R); SS = single-species inoculation. The 3 panels represent the results of an ANOVA and Tukey-HSD post-hoc test, divided by species. Symbols represent means ± SE; $Log_{10}(x)$-transformed data were plotted. Open squares are results at 14°C; filled diamonds at 18°C. Results not sharing the same letter in Tukey HSD Test results tables were significantly different at $p < 0.05$). Results of interactions between *P. nemorosa* and *P. ramorum* are highlighted in boxes.



Supplementary Table 1. Locations of sampling plots located within the SFPUC Peninsula watershed and associated plot data including geographical coordinates, elevation, and density of California bay laurel (*Umbellularia californica*) trees.

Drainage/Area	Plot	Latitude	Longitude	Elevation (m)	Bay Density (# /0.33ha)
CS	0	37.53420725	-122.3765705	115	59
CS	1	37.47667587	-122.3240183	131	8
CS	2	37.57758904	-122.4122678	155	56
CS	3	37.48812832	-122.3420616	107	39
NA	5	37.45370812	-122.3306575	457	2
CS	6	37.54543221	-122.4033964	104	113
CS	7	37.46138871	-122.3038186	140	25
CS	8	37.55632374	-122.3955187	113	299
PL	9	37.53828797	-122.4080535	252	70
CS	10	37.56582756	-122.4038403	187	42
PL	11	37.53001152	-122.3908418	295	27
CS	12	37.51191507	-122.3585746	95	51
PL	14	37.54701261	-122.4284439	311	39
PL	15	37.52467467	-122.3933726	197	70
CS	16	37.55031173	-122.3838935	129	33

CS=Crystal Springs drainage; PL=Pilarcitos drainage; NA=Not associated with specific drainage.
Elevation measured in meters.

Supplementary Table 2. Primers and TaqMan probes used for the 3-species (N-P-R) qPCR assay.

Target taxa	Target locus	Type*	5' conjugate	Name and sequence (5' - 3')	3' conjugate	Source	Amplicon (bp)
Phytophthora	*ypt*1	FW primer	none	Yph1F	none	Schena	
				CGACCATKGGTGTGGACTTT		*et al.*(2006)	~450
Phytophthora	*ypt*1	RV primer	none	Yph2R	none	Schena	
				ACGTTCTCMCAGGCGTATCT		*et al.*(2006)	
P. nemorosa	*ypt*1	FW primer		Ynem1F		this study	
			none	CGATAGGTGCGTTGGACTTTAGC	none		
P. nemorosa	*ypt*1	RV primer		Ynem1R		this study	81
			none	GTATCTGCAAGTCACAGCGG	none		
P. nemorosa	*ypt*1	Taqman probe		Ynem1P		this study	
			HEX	ACCTTGGAGGACCACGGAAAGGATCTAACTG	BHQ1		
P. pseudosyringae	*ypt*1	FW primer		Ypseud1F		this study	
			none	GACTTTGTAAGTGCCTCCG	none		
P. pseudosyringae	*ypt*1	RV primer		Ypseud1R		this study	89
			none	CGTCAGCAATCCTGATAGGAAG	none		
P. pseudosyringae	*ypt*1	Taqman probe		Ypseud1P		this study	
			Texas Red	CACATCGGCTCTTCTCTAGTCGGCG	BHQ2		
P. ramorum	*ypt*1	FW primer		Yram4F		Schena	
			none	TTTGTCAGTGACCTCTCTCTCTCTC	none	*et al.*(2006)	
P. ramorum	*ypt*1	RV primer		Yram3R		Schena	87
			none	GCATAAGTATAAGTCAGCAAGCCTGT	none	*et al.*(2006)	
P. ramorum	*ypt*1	Taqman probe		YramP		Schena	
			FAM	AGAACACGATCCCCTCGTCAGCAGTC	BHQ1	*et al.*(2006)	
universal	18S rRNA	FW primer		LS-F		Schena	
			none	GGATCCATTGGAGGGCAAGT	none	*et al.*(2006)	
universal	18S rRNA	RV primer		LS-R		Schena	69
			none	CTTAAATATACGCTATTGGAGCTGGAA	none	*et al.*(2006)	
universal	18S rRNA	Taqman probe		LS-P		Schena	
			FAM	TACCGCGGCTGCTGGCACCA	BHQ1	*et al.*(2006)	

FW = forward, RV = reverse.

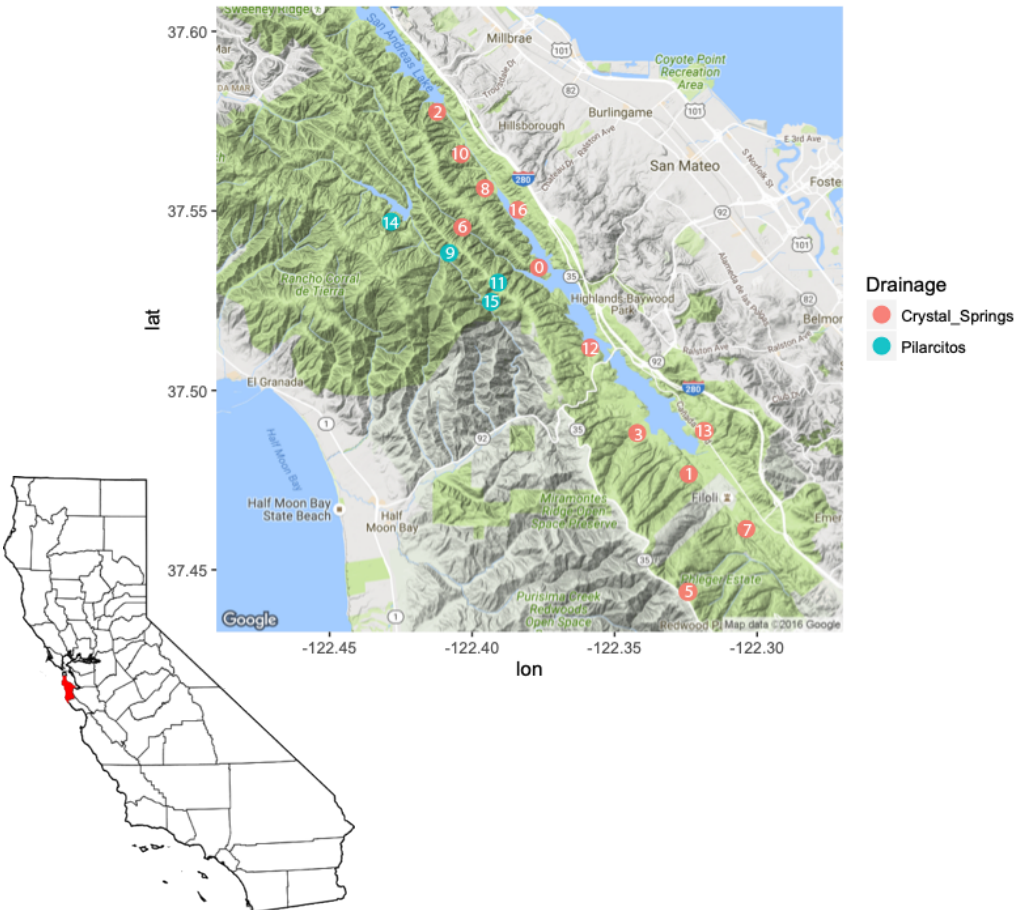
Supplementary Table 3. Results of molecular detection assays for the three target taxa, compared by sampling event. The total number of symptomatic leaves tested for *Phytophthora* DNA and the number of *P. nemorosa* and *P. ramorum* positive samples detected from each sampling event are provided. *Phytophthora pseudosyringae* was not found in any sample.

Sampling Event	Early 2009	Peak 2009	Late 2009	Early 2010	Peak 2010	Late 2010	Early 2011	Peak 2011	Late 2011
Total # leaves sampled	259	374	377	328	346	317	341	356	321
Total Assayed (N-P-R)	165	187	193	142	111	179	62	39	48
P. nemorosa # positive	50	93	76	49	52	37	33	24	32
P. ramorum # positive	105	188	180	181	235	138	278	318	271
No detectable *Phytophthora*	104	93	112	98	59	142	30	14	18

Supplementary Table 4. Asymptomatic bay laurel occurrence by plot and sampling event. Number of bay laurel trees surveyed is presented, followed by the proportion of that number that were surveyed and found to be negative for infection by *Phytophthora ramorum*, *P. pseudosyringae*, and *P. nemorosa*.

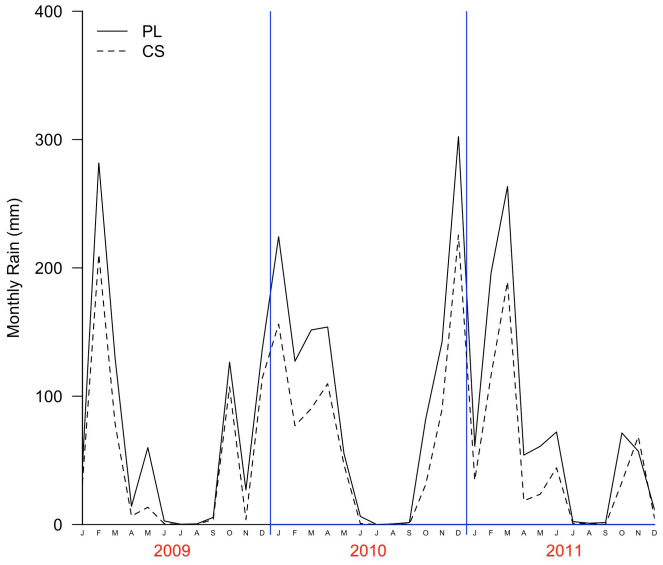
Sampling Event		Early 2009	Peak 2009	Late 2009	Early 2010	Peak 2010	Late 2010	Early 2011	Peak 2011	Late 2011
Plot	Bays Surveyed									
0	28	50.0	3.6	10.7	35.7	32.1	17.9	17.9	10.7	17.9
1	8	62.5	37.5	0.0	62.5	75.0	37.5	37.5	25.0	62.5
2	30	46.7	0.0	0.0	3.3	6.7	3.3	3.3	0.0	0.0
3	16	56.3	18.8	0.0	37.5	37.5	31.3	31.3	12.5	18.8
5	2	0.0	0.0	0.0	50.0	100.0	100.0	100.0	100.0	100.0
6	21	14.3	0.0	4.8	0.0	0.0	0.0	0.0	0.0	0.0
7	19	36.8	0.0	0.0	36.8	42.1	36.8	36.8	31.6	47.4
8	66	36.4	3.0	7.6	18.2	21.2	9.1	9.1	7.6	6.1
9	45	26.7	0.0	0.0	13.3	4.4	2.2	2.2	2.2	8.9
10	28	3.6	7.1	0.0	14.3	7.1	3.6	3.6	0.0	3.6
11	13	7.7	7.7	7.7	0.0	0.0	0.0	0.0	0.0	100.0
12	24	29.2	0.0	0.0	12.5	16.7	16.7	16.7	8.3	50.0
14	28	28.6	0.0	0.0	0.0	0.0	10.7	10.7	10.7	7.1
15	45	35.6	0.0	4.4	2.2	11.1	8.9	8.9	8.9	8.9
16	15	53.3	13.3	6.7	13.3	40.0	33.3	33.3	13.3	20.0

Supplementary Figure 1. Top: Map showing locations of sampling plots, denoted by drainage, within the San Francisco Public Utility Commission Peninsula watershed. Bottom: Map of California showing San Mateo County highlighted in red

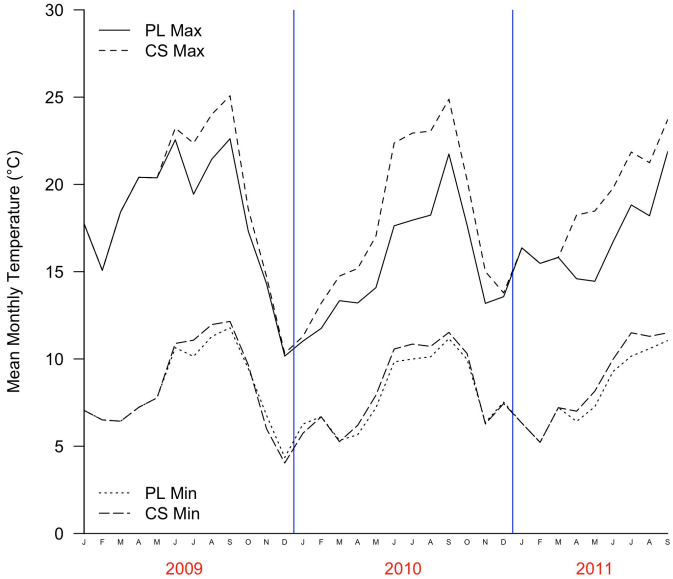


Supplementary Figure 2. (A) Average monthly rainfall (mm) from 2009-2011 in both the Crystal Springs (CS) and Pilarcitos (PL) drainages within the SFPUC Peninsula watershed. (B) Average monthly maximum and minimum temperatures (degrees C) from the Crystal Springs and Pilarcitos drainages.

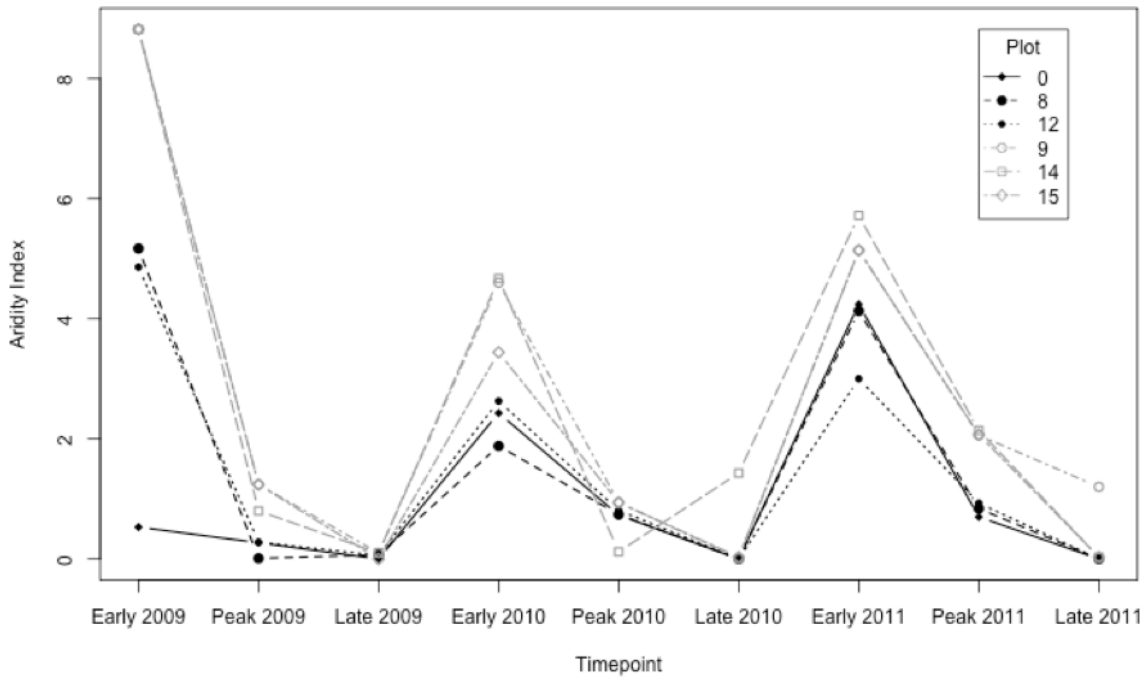
(A)



(B)

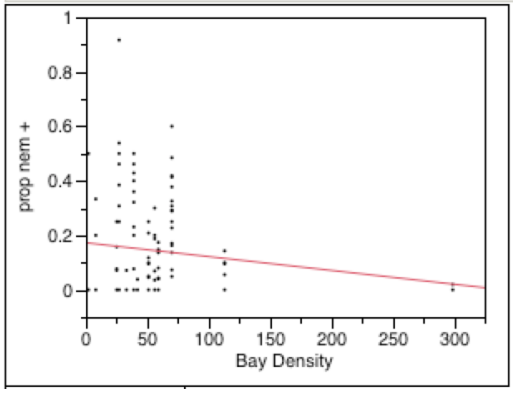


Supplementary Figure 3. De Martonne Aridity Index values from three plots per drainage, calculated using daily mean temperature and cumulative rainfall measurements from 30 days prior to each of the nine seasonal sampling events spanning 2009-2011. Open circles represent plots in the Pilarcitos drainage and closed circles represent plots in the Crystal Springs drainage.

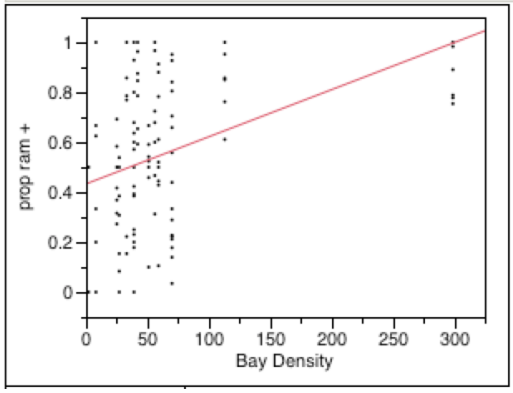


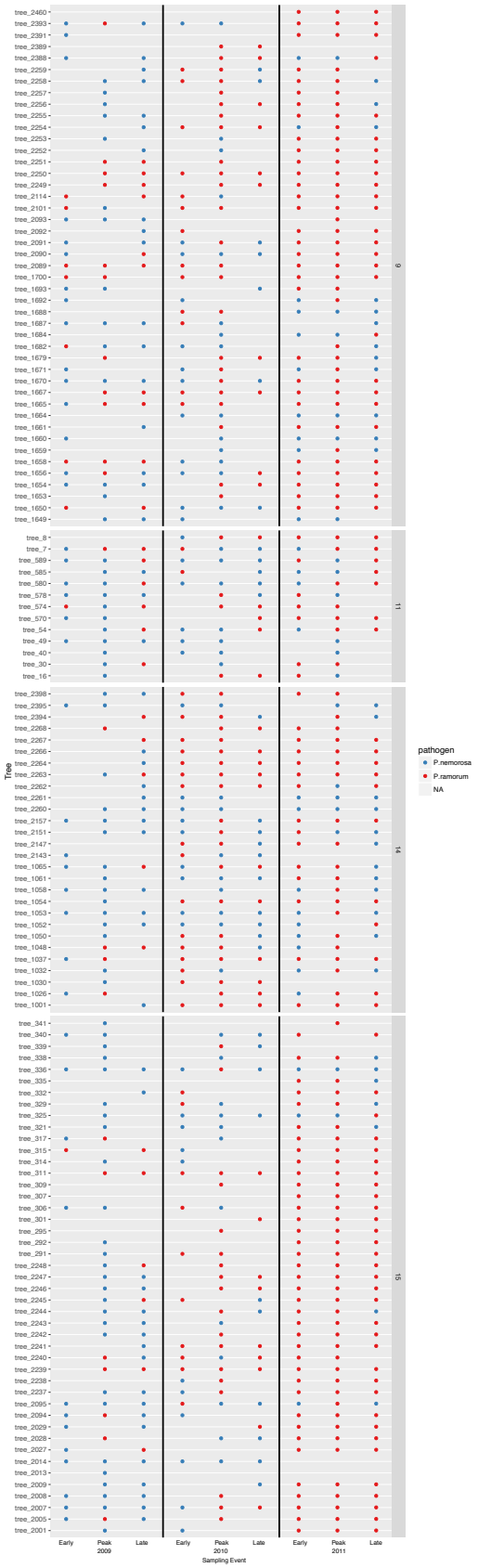
Supplementary Figure 4. Relationship between bay laurel density and proportion of (A) *P. nemorosa* and of (B) *P. ramorum* positive trees. *P. ramorum* prevalence is positively correlated with bay laurel density ($p < 0.0001$) Linear fit equation : proportion *P. nemorosa* positive = 0.173 – 0.0005 * bay laurel density) and *P. nemorosa* is negatively correlated with bay laurel density ($p = 0.0232$) (Linear fit equation : proportion *P. ramorum* positive = 0.433 + 0.0019 * bay laurel density).

(A)



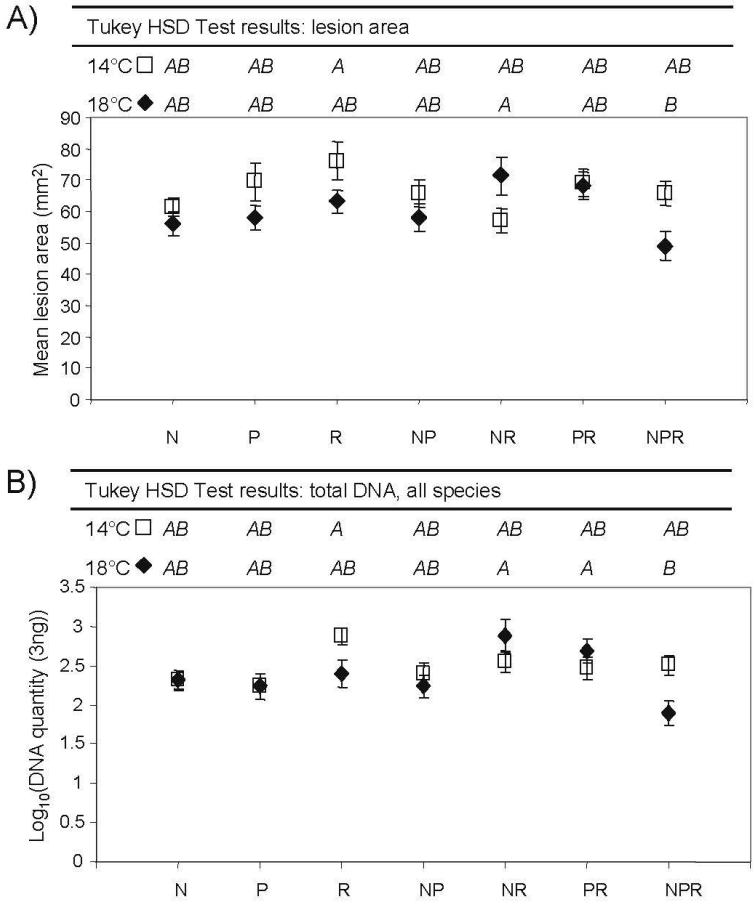
(B)





Supplementary Figure 5.
Dotplot showing individual tree-level infection across the four plots with significant amounts of *P. nemorosa* infection (plots 9, 11, 14, 15) in order to track the course of infection in individual trees over time. An overall shift in dominance from *P. nemorosa* to *P. ramorum* is indicated.

Supplementary Figure 6. Results from the *in vitro* co-inoculation experiments of *P. nemorosa* (N), *P. pseudosyringae* (P) and *P. ramorum* (R), showing effects of treatment on lesion area and pathogen DNA quantity. Symbols are means ± SE. Open squares are results at 14°C; filled diamonds at 18°C. Points not connected by the same letter in Tukey HSD Test results tables were significantly different at $p < 0.05$. A) Lesion area on *Umbellularia californica* leaves. B) Total quantity of *Phytophthora* DNA in leaf-lesions, all species, as estimated by real-time PCR. $Log_{10}(x)$-transformed data were plotted.



Chapter 2

Disease ecology and epidemiology of the Sudden Oak Death pathogen *Phytophthora ramorum* in a mixed bay laurel-oak woodland.

Abstract

The application of epidemiological models plays an important role in understanding disease progression and in determining effective strategies for disease control; specifically when features such as broad host range, high inoculum load, cryptic spread, large geographic scales, and dormant infection make control efforts difficult. Heterogeneity in transmission among individuals, regions and years complicates efforts to predict outbreaks. Targeting important spatial or temporal hotspots containing multiple superspreading or reservoir hosts, could lead to substantial gains in efficiency and reductions in costs of control efforts. *Phytophthora ramorum* relies on California bay laurel as the major transmissive host in coastal California mixed forests; however, patterns and drivers of heterogeneity in bay laurel infection, oak infection, and oak mortality have not been examined. Intensive, repeated surveys of individual bay laurel and oak hosts were conducted over a multi-year period spanning a dry to wet climatic transition. These data were used to develop predictive models of bay laurel infection, including transitions between dormant and active infection, as well as infection and mortality in oaks. The results of these models affirm the importance of seasonal and annual rainfall shifts in driving the prevalence of active infection, and reveal the importance of plot-level heterogeneity, particularly in bay laurel density, size and aspect. For oaks specifically, infection rates are greater among larger trees, yet levels of mortality are greater among smaller trees. Proximity to infected bay laurel foliage is an important factor in predicting oak infection, (as oaks with shorter distance to nearest infected bay laurel foliage exhibit greater rates of infection). These results suggest avenues for control efforts and aid in obtaining a better understanding of this pathogen's disease ecology.

Introduction

The role of transmission heterogeneity at individual, species-level, and spatial scales is increasingly recognized as an important driver of plant disease epidemics (Madden and Hughes 1995; Mundt et al. 2011; Cobb et al. 2017; Garbelotto and Gonthier 2017). Particular individuals and/or sites may have an outsized effect on transmission, earning the designations "superspreading" hosts and "hotspots," respectively (Paull et al. 2012). A host's susceptibility, ability to harbor latent infection, environment, and degree of connectivity to other susceptible hosts can significantly influence its effect on the spread of a disease. This is well documented in zoonotic disease systems, as exemplified by the infamous "Typhoid Mary," who was believed to have infected over 100 people with typhoid fever due to her ability to maintain a latent asymptomatic infection and her occupation as a cook with an itinerant job history (Brooks 1996). Problematic disease spread during an asymptomatic phase is also know to occur in plant disease systems as with Huanglongbing disease, a widespread bacterial infection of citrus trees (Lee et al. 2015). Identifying superspreading hosts, hotspots, and the conditions that create them is therefore important for accurate disease modeling and developing control strategies (Lloyd-Smith et al. 2005; Paull et al. 2012).

Unlike with zoonotic disease systems, plants are sessile hosts, which provides an interesting contrast to traditional epidemiological models. Models of plant disease systems must therefore rely much more heavily on spatial epidemiology (Ostfeld et al. 2005). The disease triangle, a tenet of plant pathology, states that environmental factors, along with host susceptibility and pathogen virulence, play a strong role in potential outbreaks as host plants are rooted in their environment and are thus subject to both biotic and abiotic factors which may or may not change through time (Francl, 2001; Garbelotto and Gonthier 2017). In a given forest stand, there can be variation in susceptibility among individual hosts perhaps due to differences at the genetic level but also because of the specific environment they are rooted in. Variation at larger scales due to the environment, microclimate and host density are all factors that may contribute to a host achieving status as either an infection "superspreader" (Stein 2011) or as inoculum reservoir, or "refugial" host (Haydon et al. 2002*).* Because trees are sedentary, sites where superspreaders and/or refugial hosts are abundant can be identified as hotspots for disease spread. These hotspots can be sites of importance for disease epidemiology either because infection is elevated due to the presence of superspreading hosts or because the pathogen can survive in these sites during climatically unfavorable periods due to the presence of refugial hosts.

Many forest diseases, such that those caused by invasive fungi and oomycetes, have the ability to cause large-scale landscape transformation (Gibbs 1978; Paillet 2002; Rizzo et al. 2005). Control of these diseases on a broad scale is expensive and often impractical, and treatment of symptomatic hosts alone is therefore unlikely to control disease epidemics (Gilligan et al. 2007). Management interventions for forest diseases could be efficiently focused in limited geographic areas if it were possible to identify stand or weather variables correlated with higher infection levels, hotspots where superspreading individuals or host species might increase pathogen transmission, and where refugial hosts may allow for pathogen survival during unfavorable weather conditions. Recognition of new infection foci is complicated by the large geographic scales involved, the difficulty of identifying infected hosts across such scales, the potentially large dispersal capacity of pathogens, and the cryptic nature of early infections

(Filipe et al. 2012). Identifying both current and potential superspreading hosts and hotspots can play an important role in identifying priority sites and individuals for targeted control.

Sudden Oak Death (SOD), caused by the introduced and exotic oomycete *Phytophthora ramorum*, is a disease that has caused widespread mortality of coast live oak (*Quercus agrifolia*) and tanoak (*Notholithocarpus densiflorus*) in Oregon and California (Rizzo et al. 2005). In this multi-host pathosystem, *P. ramorum* can infect over 100 species (USDA APHIS 2008), but there is great heterogeneity among species in both their rates of inoculum load and transmission to susceptible hosts and in the lethality of infection for particular hosts. The primary amplifying host responsible for pathogen transmission is California bay laurel (*Umbellularia californica*) (Garbelotto et al. 2003; Davidson et al. 2005). *Phytophthora ramorum* produces sublethal lesions on bay laurel leaves, from which it can produce both infective spores (zoospores) and survival structures (chlamydospores). The pathogen exhibits strong seasonal behavior on bay laurel, producing zoospores during the wet spring season and surviving in either a viable or dormant state during the dry summer months in California's Mediterranean climate (Davidson et al. 2005; Rizzo et al. 2005; Fichtner et al. 2007; Garbelotto et al. 2017). Coast live oaks are dead-end hosts for the disease, as they contract lethal infections but cannot transmit the pathogen. Establishment of the pathogen on oaks generally causes large "bleeding" cankers on the trunk as the tree produces a sap that weeps in response to infected cambial tissue. Necrosis of the cambial tissue eventually leads to girdling and death of the tree (Rizzo et al. 2002). High levels of disease incidence on a landscape level is most often correlated to stand dominance of bay laurel, which acts as a superspreading species in the disease system (Davidson et al. 2005, 2008; Maloney et al. 2005).

Because of the strong link between bay laurel dominance and SOD incidence, it is important not only to identify factors that promote colonization of bay laurel trees which can harbor high levels of infectious propagules and factors that correlate with disease impacts, in this case higher levels of mortality in oaks, but also to understand sources of heterogeneity in transmission potential from bay laurel to bay laurel trees and from bay laurel trees to oak and tanoak trees. Previous studies of heterogeneity in this system indicated that in vitro susceptibility of bay laurel leaves to *P. ramorum* infection is genetically based, but overall prevalence of infection in the field (number of affected leaves per plot) correlates more strongly with environmental characters (Anacker et al. 2008). Similarly, the ability of the pathogen to survive throughout the summer season is correlated with site-level characteristics (DiLeo et al. 2014). Finally, reversal of infectious status of bay laurels from infected to uninfected has been shown to be strongly correlated with topography, weather, and stand structure (Lione et al. 2017).

Heterogeneity among individuals is likely to exist even within forest stands, with important implications for management. Furthermore, individual-level differences may be amplified by changes in weather conditions; for example, bay laurel trees that regularly carry viable infection during the specific season that favors zoospore release are likely to be more important as infection foci or superspreading individuals, and trees harboring dormant pathogen (as chlamydospores) across years may be more important as reservoirs or refugial hosts for the disease. The degree of linkage between this heterogeneity and the ultimate effect of the epidemic, measured in rates of oak and tanoak mortality, and the environmental or other factors that influence this linkage are not well understood.

This study examined bay laurel infection at the individual and plot levels and its association with oak and tanoak infection and mortality across seasons and years in order to understand the role of individual, spatial, environmental, and temporal heterogeneity in

propagating the SOD epidemic. Specifically, the following questions and associated hypotheses were addressed:

1) Which characteristics of the biotic and abiotic neighborhood are most important in predicting infection of the transmissive host bay laurel?
    a. Disease prevalence on bay laurel is correlated with bay laurel basal area, and bay laurel density.
    b. Site-level characteristics, including slope, aspect, and canopy cover, are correlated with levels of disease prevalence on bay laurel, and the presence of superspreading and refugial host trees.
    c. Intrinsic characters of oak individuals such as size and proximity to infected bay laurels are correlated with levels of oak infection and mortality.
2) Are oak infection and oak mortality correlated with high levels of bay laurel infection, and close proximity to infected bay laurels?
    a. Oak infection and mortality are correlated with high levels of bay laurel infection, high bay laurel density and close proximity to infected bay laurels.
    b. Oak infection and oak mortality are correlated with intrinsic characters of oak individuals, such as oak size, and oak mortality is also correlated with the presence of visible SOD symptoms i.e. bleeding bole cankers.
3) Do hosts exhibit intraspecific variation in their likelihood of harboring the pathogen?
    a. Heterogeneity in infection occurs between individual bay laurel trees; both superspreader hosts and refugial hosts exists, creating hotspots for infection.
    b. Heterogeneity in disease incidence on bay laurels and oaks is correlated with seasonal and yearly weather changes; these changes may interact with individual-level and/or site-level characteristics to result in different plots and/or individual trees exhibiting different responses to shifts in weather .
    c. Survival of active *P. ramorum* infection between seasons depends upon the specific seasons examined, as well as upon yearly differences in weather.

To test these hypotheses, a network of sampling transects were constructed within a single watershed that included stands characterized by varying host composition, density, and site characteristics (e.g., slope, aspect), but a comparable history of exposure to the disease. The data obtained were then analyzed by running a series of generalized linear mixed effects models designed to predict likelihood of infection on bay laurel and oak, to predict the likelihood of survival on infected oaks and to predict the likelihood of survival of the pathogen on bay laurel hosts in varying seasonal weather shifts. Results from the models were then used to describe seasonal cycles of the disease and to infer the overall ecology of SOD, its impact on hosts, and the nature of temporal disease dynamics in mixed evergreen forests. More specifically the data were used to study yearly and seasonal fluctuations in disease incidence at the landscape level and to study the relationships between infection and composition of surveyed forest stands. Additionally, further investigations were carried out to study seasonal change in infection status of the transmissive host California bay laurel, and to study the relationship between tree size, infection and mortality in oaks.

As a disease system, Sudden Oak Death is characterized by a number of features that make control efforts difficult, including a very broad host range, the ability to produce an extremely high inoculum load, the ability to spread cryptically, distribution of potential hosts over a large geographic range, and the ability to produce dormant propagules. Given these complexities, the application of epidemiological models can play an important role in

understanding disease progression and in determining the most effective strategies for disease control (Filipe et al. 2012). Heterogeneity in transmission among individuals, spatial regions, and years complicates efforts to predict disease outbreaks (Paull et al. 2012). However, predicting and targeting important spatial or temporal hotspots, and individual superspreading or refugial hosts, could lead to substantial gains in efficiency and reductions in cost of ongoing control efforts. Using the results from this study, we aim to shed light on the role of transmission heterogeneity in the survival and spread of the epidemic, and suggest potential avenues for response.

Methods

Site Selection and Experimental Design

This study was conducted within the San Francisco Public Utility Commission (SFPUC) peninsula watershed (37°31'10.3"N 122°22'08.2"W) in central San Mateo County, California (Supplementary Table 1; Supplementary Fig. 1). This site encompasses 9,300 hectares (23,000 acres), ranges in elevation from 95-1050 m, and hosts a variety of habitats, some of which include rare and endangered species (Graves and Schrader 2008). The watershed has maintained limited public access for nearly a century, thus reducing the amount of anthropogenic disturbance. Mean annual temperatures range from 8.8º - 21.5 ºC, with the coldest temperatures occurring in January and the warmest in September. The mean annual precipitation is 62 cm, with the majority of rainfall occurring from November through April (Kozanitas et al. 2017). This site was likely to have been infested by *P. ramorum* 10 - 20 years prior to plot establishment, with confirmed reports of *P. ramorum* dating as early as 2001 (Croucher et al. 2013).

In the summer of 2008, 16 research plots within the watershed were established in locations with suitable plant hosts present. The plots were spaced at least 2 km apart to minimize potential spatial autocorrelation interference between sites. This distance was based on results of previous population genetics studies and spatial autocorrelation analyses on the mobility of infectious propagules that suggested the vast majority of airborne inocula move only tens or hundreds of meters in rain events and up to 1km in high winds (Mascheretti et al. 2008). Three transects were laid out in each plot, while in six plots (three per major drainage) the number of transects was doubled, with the aim of obtaining more isolates of the pathogen for a concurrent population genetics study. In total, there were 66 transects nested within the 16 research plots. Each transect was 100m in length and 10m in width, radiating from a plot center. All bay laurel and coast live oak stems with a minimum diameter at breast height (DBH) of 1 cm were tagged, and the DBH recorded in cm. Any major branches of a tree separated from one another below breast height (1.4m) were considered independent stems. At 10m increments along each transect, a single bay laurel stem (when present) was selected for repeated surveying throughout the year, while all oak stems occurring within each 100 x 10 m transect corridor were inspected once per year for five years.

Field Surveys

Following plot network establishment and preliminary data collection in the summer of 2008, bay laurel assessments were repeated 10 times over a four-year period spanning 2009-2012. Surveys were conducted three times per year in 2009-2011 during predetermined seasonal periods in order to capture snapshots of pathogen viability throughout the year. These seasonal

periods were defined as: an “Early” season sampling in late winter/early spring (February/March) to capture pathogen dynamics when temperatures were not yet warm enough for sporulation; a “Peak” season sampling in late spring/early summer (May/June), when rainfall coupled with warmer temperatures provide ideal conditions for sporulation and infection; and a “Late” season sampling in late summer/ early autumn (September/October) after the hottest and driest months have typically urged the pathogen into dormancy (Eyre et al. 2013; Kozanitas et al. 2017). A single end point survey took place in the late spring, or “Peak” season of 2012.

Bay laurel sampling consisted of surveying a total of 388 stems that occurred at 10m intervals along each transect. If a tree was determined to be symptomatic following an initial visual assessment, six symptomatic leaves were then sampled from the lower canopy and tested for presence of the pathogen via culturing and qPCR. Symptomatic leaves were recognized by areas of tissue necrosis often congregated at the tip, or along the lower edge of a leaf where droplets of water congregate and remain. These lesions are classically characterized by a yellow halo just beyond an active zone of infection that appears as a dark irregularly shaped line, or as dark pixilated spots scattered about the leaf, more common of active infection (Garbelotto et al. 2002; Davidson et al. 2003). Only leaves from trees with visible symptomatic tissue were collected and processed, as it has been documented that in the absence of visible symptoms a positive ID via molecular diagnostics is unlikely (Hayden et al. 2006). A total of 3880 data points (from 388 trees) were obtained for determining bay laurel infection across the 10 sampling events.

An initial total of 950 oaks across all transects were surveyed once per year, during the autumn or “Late” season, over a five-year period spanning 2008-2012. Oak tissue was sampled only if the oaks showed visible signs of infection such as bleeding cankers (Garbelotto et al. 2002; Davidson et al. 2003). Cankers were sampled by removing the outer layer of bark, excising the margin of infected cambial tissue with a sterile scalpel and embedding chips from the margins of each canker directly into a selective medium. A positive pathogen identification required growth of a colony that could be morphologically identified as *P. ramorum* (Gallegly and Hong 2008). Infected stems were also surveyed for the presence of bark beetles and the secondary sapwood fungus *Annulohypoxylon thouarsianum*, the approximate level of canopy dieback for each tree was noted. At each surveyed oak, the distance to the nearest infected bay laurel foliage was measured in meters in a straight line from the trunk of the oak to the leaves of the bay laurel branch . Canopy cover was assessed at each surveyed tree using a spherical densitometer comprised of 24 squares, held at waist height 12-18 inches from the body. Four readings were taken, one for each cardinal direction using the following method; four dots spaced evenly across each of the 24 squares were assumed and the number of dots not covered by the reflecting canopy were tallied, averaged, multiplied by 1.04 and subtracted from 100, resulting in the percent of canopy coverage (Lemmon 1957).

The slope and aspect at each oak and bay laurel tree were measured using a Sunto MC-2 compass with clinometer. Geographic location and elevation were recorded using a Garmin GPSmap 60Cx. Rainfall data were retrieved from the CA Department of Water Resources (CA-DWR ; RAWS database, Western Regional Climate Center, wrcc@dri.edu) and SFPUC data archives of the Crystal Springs Cottage rain gauge (37°28'08.4"N 122°19'44.4"W)

Pathogen isolation and *in vitro* culturing.

Within 72 hours of sampling, each collected leaf had a small section of symptomatic tissue excised along the advancing margin of a single lesion and embedded in the *Phytophthora-*

selective medium PARP (Rizzo et al. 2002). Plates were then incubated in the dark at 20°C for 7-10 days. All plates exhibiting mycelial growth were examined using microscopy and morphologically determined to be *P. ramorum* positive or negative (Gallegly and Hong 2008). Positive isolates were then subcultured to eliminate contaminants. Oak canker samples were plated directly onto PARP in the field, incubated for 3-5 days, and sub-cultured to reduce contaminants before colonies were identified as *P. ramorum* based on morphology. Positive isolates were then subcultured onto clean plates.

Molecular Diagnostics

Visibly symptomatic leaf samples that yielded a culture-negative result could either have been infected with another species of *Phytophthora* (Kozanitas et al. 2017) or the *P. ramorum* infection had entered dormancy and was therefore not culturable. In order to differentiate between the two alternatives, tissue samples for molecular diagnostics were obtained from all symptomatic bay laurel leaves that did not yield a positive culture, using a surface-sterilized 6 mm hole punch to excise leaf sections from the margin of putative *P. ramorum* lesions. From each set of six leaves, three leaf punches were selected and combined in a 2 ml screw-top tube containing a sterile glass bead; wood samples were processed by excising three 5 mm sections from the margin of infected cambial tissue and placed in a 2ml tube with two sterile glass beads. The samples were lyophilized and then pulverized using a FastPrep®-24 homogenizer (MP Biomedicals) for a minimum of 30 seconds at 4 rmps. DNA was extracted using either the CTAB and phenol/chloroform extraction protocol of Hayden *et al.* (2004), or the ROSE extraction method of Steiner *et al.* (1995) as modified by Osmundson *et al.* (2013). Presence of *P. ramorum* in culture-negative leaves was assessed using the DNA-based nested qPCR assay described by Hayden *et al.* (2006). PCR reactions were performed on a Bio-Rad (Hercules, CA) CFX96 Touch Real Time PCR Detection machine.

Statistical Analyses

Analyses were divided into the following three categories, and run in five separate models. The first category focused on factors able to predict infection of coast live oak and/or bay laurel by *P. ramorum*, the second on factors predicting the survival potential of infected oaks, and the third examined factors predicting the survival potential of *P. ramorum* on bay laurel in varying seasonal shifts. All statistical analyses were conducted in the R statistical environment, version 3.1.0 (R Core Team 2014). Generalized linear mixed effects models (GLMM) were constructed using the *lme4* package (Bates and Maechler 2010). Akaike's Information Criterion (AIC) was used to select the models that best fit the data.

The likelihoods of host infection were modeled within a GLMM using logit transformations in a logistic regression with a binomial error structure. The fixed effect variables included topographic characteristics (slope, aspect), the biotic neighborhood (canopy cover, bay laurel density, bay laurel basal area of the transect), or individual descriptors (DBH, proximity to nearest bay laurel). Basal area of each individual bay laurel tree within a transect was calculated using the following equation [$BA = \pi \times (DBH \text{ in cm} \times 0.01 \times 0.5)^2$], summed to determine the basal area per transect, then scaled to a per hectare measurement. Circular aspect variables were transformed to the linear variables of northness = cos (aspect) and eastness = sin (aspect) (Roberts, 1986) where values range from -1 to 1 and all additional variables used in the models were centered and scaled (subtracting from the mean and dividing by the standard deviation) prior to analysis. Stems missing data for any variable were excluded from the analyses.

The likelihoods of host infection were predicted using two separate models, one for bay laurel and one for coast live oak. The likelihood of active infection occurring on bay laurel was predicted using a repeated measures GLMM analysis to investigate how neighborhood effects and temporal variation contributed to *P. ramorum* prevalence on bay laurel. Active bay laurel infection was the dependent variable, with each tree scored for having viable *P. ramorum* obtained through culture methods. The model included the independent predictor variables of bay laurel DBH, bay laurel density, bay laurel basal area, aspect, and canopy cover with sampling period (Early, Peak, or Late), and year (2009-2012) in which a tree was sampled as fixed effects. The random effect variable of stem ID accounted for the repeated measure of stems through time. To account for spatial non-independence of stems located within the same plot or along the same transect, the random variables of transect nested within plot were included. The intercept of the model was allowed to shift/vary among groups denoted by the random variables.

Likelihood of oak infection (confirmed via cultured isolates from symptomatic canker tissue) was predicted with a similar model to the bay laurel infection, model outlined above but excluded the sampling period term, as oaks were only sampled once per year. Added to the model were the fixed predictor variables of oak DBH and distance (m) to the nearest infected bay laurel foliage.

A third model was run in order to predict the survival of infected oaks over the course of the study. Oak mortality at each sampling point was used as the dependent predictor variable in a repeated measures logistic regression GLMM, identical to the models described above, but in this case the presence of a visible canker was added as a fixed predictor variable to the model.

The final two models were used in order to make predictions of seasonal *P. ramorum* survival. Yearly patterns of change in the infection status of bay laurels were examined using two different repeated measure GLMMs to predict the likelihood of the pathogen itself surviving from one season to the next on a bay laurel host. The first model took each bay laurel stem with a culture-positive score (active infection) at any time point and used pathogen viability at the next time point as the dependent variable. The second model took each bay laurel stem with a PCR positive but culture negative score (dormant infection) and identified those individuals that switched to a culture positive score at the following sampling point. This model was assumed to analyze the shift from dormant to active status. The Peak 2012 data was included in the model so isolates in the Late 2011 sampling period could be traced to an endpoint.

To better understand the effect of plot on the likelihood of bay laurel infection and variation in infection levels across the landscape, comparisons of cumulative disease incidence (CDI) – defined in this study as the number of times out of ten possible sampling events that an individual bay laurel tree provided a positive isolate in culture, were conducted between plots containing superspreaders. A CDI of 70% or higher was set as the threshold for trees to be considered superspreader hosts (7/10 culture positives yielded). Although there is no official threshold in place (Lloyd-Smith et.al, 2005), an individual or a group of individuals responsible for 80% of the transmission has been used in other epidemiological studies *(*Galvani et. al, 2005). This threshold was chosen because 7/10 culture positives would mean a tree was infectious in all of the Early and Peak season sampling events when and/or at least one or more of the Late season sampling events.

Results

Pathogen viability on bay laurel was significantly correlated with rainfall across both seasons and years. Levels of active infection were highest in the Peak sampling period of each

year (Fig. 1). With the exception of one Late sampling period (autumn 2010), Early season sampling periods yielded higher isolation success than Late season sampling periods. Total isolation success increased each year, and then dropped in the Peak sampling period of 2012, which was characterized by less spring rainfall than 2011 with rainfall at 135% of the 50 year average (Kozanitas et al. 2017). Total infection (active and dormant combined) levels exhibited an overall increase over the course of the study, however, with each subsequent sampling event the proportion of dormant infection decreased relative to active infection until the Late 2011 sampling period when dormant infection and active infection levels were nearly equal (Fig. 2).

Active infection on bay laurel infection was found to be heterogeneous at both spatial and individual scales. A comparison of cumulative disease incidence (CDI) across plots showed significant heterogeneity among plots, as well as among individual trees within plots (Fig. 3). In general, disease "hotspots" with the highest median CDI, also contained potential "superspreader" trees, defined as individual trees in which active infection was detected in at least seven of the ten sampling periods. One exception was found in which superspreader trees were detected in a non-hotspot and hotspot presence was not specifically linked to any particular drainage or elevation (Supplementary Table 1). Hotspots were also more likely to contain "refugial" trees defined as individual trees in which active infection persists in the drier late season. Six of the seven plots that contained superspreader trees also contained individuals from which active infection was detected in at least two of the three Late seasons, and the three plots with the highest median CDI each contained refugial trees that were positive in all three of the Late seasons (Fig. 3). Overall, superspreader host trees were present in more plots that refugial host trees, 7/15 and 4/15 respectively. (Fig. 3). Three plots (6, 8, and 10) had a significant number of superspreaders: 12/21, 18 /66, and 13/28 trees sampled, respectively. However, the same three plots only contained 2, 2 and 3 refugial trees, respectively. All refugial host trees present in the three aforementioned plots were also superspreaders.

Bay laurel infection was also found to vary with bay laurel density, topography, and year. Results of the first GLMM (Table 1) indicated that the probability of a bay laurel being infected by *P. ramorum* significantly increased with bay laurel density (Fig. 4A), and decreased as bay laurel basal area increases (Fig. 4B); i.e., being surrounded by a larger number of smaller trees led to higher probability of infection for any given bay laurel tree. Infection was also more likely to occur on eastern facing slopes than on slopes with a different aspect (Table 1). Likelihood of active infection was significantly higher during the Peak season than either the Early or Late season, and infection was more likely to occur in the Early season than in the Late season (Peak > Early > Late) (Table 1). The likelihood of bay laurels being infected was found to be significantly different each year (Table 1). Infection probability was highest in 2011, and lowest in 2009, mirroring the proportion of positive isolates (Fig. 1) (2011 > 2012 > 2010 > 2009). The likelihood of a single, average-sized bay laurel tree becoming infected, with varying bay laurel density and basal area, under opposing climatic conditions was determined by comparing results from the wettest (spring 2011) and driest (autumn 2009) seasons observed in the study, and in both instance infection probability increased with bay laurel density (Fig. 4C.). Infection probability was also shown to increase with increased moisture as the association with bay laurel density occurred independent of season.

The next two models indicated that the likelihood of oak infection and oak survival varied significantly with oak size and differing bay laurel neighborhood effects. Oak infection probability was positively correlated with oak size (DBH), bay laurel density, and proximity to the closest infected bay laurel foliage (Table 1). The proximity effect was strongest when oaks

were between 0-5 meters from infected bay laurel foliage, with the likelihood of infection decreasing when that distance was greater than 10m (Fig. 4D). Oak mortality was significantly positively correlated with the presence of a canker and negatively correlated with oak DBH (Fig. 4E). The association with oak DBH was opposite for oak infection. While a smaller DBH led to a higher likelihood of mortality in oaks, a larger DBH was positively correlated with a higher probability of oak infection (Fig. 4E). Oak mortality exhibited an overall increase over the course of the study, while oak infection assessed through the presence of disease symptoms decreased between 2008 and 2011, and then increased in 2012 (Fig. 5).

Finally, a strong positive correlation was found to exist between rainfall and bay laurel infection levels, both active and dormant as the proportion of active bay laurel infection mirrored the yearly increase in spring rainfall (Fig. 4F). An association with levels of symptomatic oaks and oak mortality was also found to exist however in this case the number of cankered oaks progressively decreased during drier spells (2008-2010) due to mortality of infected oaks and lack of new infections. With the with the onset of significant rain events, as seen in 2011, the number of cankered oaks, and therefore new oak infections began to increase from 2011-2012 for the first time over the course of the study (Fig. 4F).

Results from the statistical models presented thus far have focused on predicting *viable* infection of bay laurel, coast live oak and oak survival. A second set of GLMMs was constructed to predict survivability of *P. ramorum* (likelihood of remaining viable) in different seasons and years and found that pathogen survival was associated with both annual and seasonal weather shifts. The first of these models started with a culture positive tree, took into account all repeated measures, and found those samples that were still viable (i.e. culture positive) at the next time point. In order to understand the results of this model, consider any shift from one season to the next in terms of the following three categories: Early->Peak (E->P); Peak ->Late (P->L); Late -> Early (L->E).

A culture-viable, or active infection on a bay laurel was least likely to survive a shift from the wet spring (Peak season) into the dry autumn (Late season) after months without moisture (Table 1). An individual was most likely to survive from winter into spring. The probability of a viable infection detected in the autumn (Late season) surviving on the same individual into the winter (Early season) was intermediate to the first two scenarios. Additionally, active *P. ramorum* infection was more likely to remain viable during any seasonal shift in 2010 or 2011 than any shift in 2009 (2009<2010≈2011).

Dormant *P. ramorum* infections were more likely to shift to active infection during the transition from the Late season to the Early season (i.e., from least favorable to moderately favorable conditions), and least likely to switch from dormant to active when going from the Peak to the Late season (i.e., from most favorable to least favorable conditions). Transitions from dormant status were most obvious when moving from early spring to late spring but the magnitude of the event was lower than that of individuals that become active at the beginning of the rainy season after a long dry spell (C > A > B). (L->E) > (E->P) > (P->L). The year effect in this model showed that changing from dormant to active status during any seasonal shift in the dry 2009 (driest year of study) was less likely than during any seasonal shift in 2010 or 2011. Conversely, a shift in status from dormant to active was more likely to happen in 2011 than in 2010 (the two wetter years of the study), regardless of season (2011 > 2010 > 2009).

Discussion

Sudden Oak Death is a highly destructive exotic forest disease with a relatively young history in California (Garbelotto and Rizzo 2005), a different mechanism of spread depending on the forest ecosystem affected (Rizzo and Garbelotto 2003) and with a still relatively limited understanding of its epidemiology (Garbelotto and Hayden 2012). This limited knowledge in turn has hampered the development and implementation of effective disease management approaches (Rizzo et al. 2005). Although the body of literature on the topic is growing and providing increasing information on aspects of the disease such as progression of tree mortality and its correlation with forest stand metrics (McPherson et al. 2010; Cobb et al. 2012), no study has yet explicitly tackled temporal and landscape-level patterns of infection on California bay laurel and coast live oak, nor identified drivers and spatial-temporal patterns of oak mortality. In coastal California's mixed hardwood forests, California bay laurel has previously been identified as the major transmissive host for SOD and the impacts of bay laurel infection on coast live oak have been well documented (Garbelotto et al. 2003; Rizzo and Garbelotto 2003; Davidson et al. 2005). Hence, understanding patterns and drivers of heterogeneity in bay laurel or oak infection and in mortality of dead-end oak hosts, as well as uncovering patterns of bay laurel-to-bay and bay laurel-to-oak disease transmission is critical.

In this study, we used intensive and repeated surveys of individual bay laurel and oak hosts totaling over 8,600 observations over a multi-year period spanning a drought to wet to drought weather transition to examine the dynamics of both active and dormant infection. These data were used to calculate the likelihood of bay laurel infection, transitions between dormant and active bay laurel infection, and infection and mortality in oak hosts based on individual tree, overall stand, seasonal and temporal variables. The results of these models confirm several previous observations, offer new insights, and suggest potential avenues for control.

The field data confirmed the importance of seasonal rainfall shifts in driving the prevalence of active infection in bay laurel hosts (Hüberli et al. 2011). Viable infection levels were highest in the spring and lowest in the autumn, corresponding to wet and dry periods, respectively, in California's Mediterranean climate. This patterns holds regardless of year; however, disease prevalence is higher in each seasonal survey period in years with higher overall rainfall (Fig. 1). The probability of bay laurel infection was affected by plot-level heterogeneity: infection increased with high bay laurel density and low bay laurel basal area regardless of year (Fig. 4A, 4B) – i.e., in dense stands of smaller trees – and on eastern facing slopes that are protected from cooler marine winds from the west and where fog dissipating off of ridge tops increases humidity locally. Such plots include disease hotspots containing active infection more frequently (Fig. 3). In addition, these plots have a higher occurrence of superspreader host trees maintaining active infection over most of the Peak sampling periods, as well as refugial trees acting as reservoirs of infection in the dry autumn season or during droughts. Three plots had a sufficient number of superspreaders and refugial trees to draw a comparison between the two categories: refugial trees were in numbers almost one order of magnitude lower than superspreaders, and all refugial trees were also superspreaders. This finding has important implications for control strategies and for future surveying strategies. Any future survey interested in identifying refugial trees will require an intensive survey design, given their low frequency. Within years, survival of active infection is most likely from late winter/early spring to late spring, least likely from late spring over summer into autumn, and intermediate from autumn to the next spring. If an individual had active infection in late winter, when moisture was

available but temperatures were not yet optimal, then it was very likely to remain active as conditions improved in the spring. In years characterized by higher overall rainfall, survivorship increases across all seasonal shifts (Table 1). Because leaf populations (as opposed to soil populations) of *P. ramorum* appear to be most important in terms of disease progression (Eyre et al. 2013), understanding heterogeneity in maintaining active infection is likely to be important in controlling disease outbreaks. From a management perspective, this study clearly indicates that dry years, or the driest periods of years with regular rainfall, may be the best times to devise disease containment practices for bay laurels, given the lower and spatially concentrated infection frequency. Conversely, years with above average rainfall may lead to disease incidence on bay laurel that is too high to allow for cost effective disease control.

Because oaks, and not bay laurels, suffer the highest mortality in SOD outbreaks, understanding the factors that most strongly contribute to oak infection and mortality – including the relationship between these factors and the characteristics of nearby bay laurel trees – is critical. Oak infection was predicted in one model using oak basal area and bay laurel density, as well as proximity to infected bay laurel foliage. Larger oaks had an increased probability of infection, made higher or lower by density of bays laurels on the transect where they occurred, and the closer a tree was to an infected bay laurel, the more likely it was to become infected. This effect was strongest when oaks were between 0-5 meters from an infected bay laurel; after about 10m, the likelihood of infection began to lessen (Fig. 4D.) Oak mortality increased with the presence of cankers and decreased with increasing oak size; smaller cankered oaks had a higher risk of mortality than large oaks, despite large oaks becoming infected more easily (Fig. 4E). Trees with a DBH of less than 10cm had a higher probability of dying regardless of infection status, but trees with a canker were roughly 60% more likely to die than uninfected trees, and all trees were less likely to die as they increased in size, up to 60cm. This could be because smaller trees that do become infected will die more quickly than larger ones within a five-year window; those smaller trees could take less time to girdle, or it could be that cankers expand at different rates in different sized trees, in this case more rapidly in smaller trees.

These results suggest several avenues for control efforts. Dense stands of younger bay laurel trees, especially those situated on east-facing slopes in habitats similar to that of the current study, should be prioritized for survey and possibly prophylaxis including stand thinning. Additionally, a 10m no-bay laurel buffer zone around oak hosts may aid in preventing infection as suggested by Garbelotto *et al.* (2017) based on measurements of airborne inoculum; however, at the landscape level, buffers may not be effective if they are narrower than the dispersal capacity of the pathogen, or if cryptic infection has already proceeded beyond the buffer before it was erected (Filipe et al. 2012). Nonetheless the relationship between likelihood of oak infection, distance from bay laurel, and oak diameter identified in this study can be used to determine a safe size for an effective no-bay laurel buffer around target oaks or oak stands. Another element that could decrease the efficacy of a buffer approach is the potential for *P. ramorum* to be spread by non-bay laurel hosts; however Garbelotto *et al.* (2017) have experimentally determined that oak infection requires very high inoculum pressure, hence any host that may be relevant to oak infection must support very high levels of sporulation .

Refugial hosts are bound to exists in almost any natural pathosystem and this study convincingly shows that refugial trees are present in SOD infested forest stands. However these host trees may often be overlooked because of the extensive, repeated sampling over multiple seasons, including seasons where disease transmission may not be occurring, that is required to identify them. During periods of drought the number of refugial trees may be lowest making

their removal most cost effective. Removal of refugial trees may affect future disease cycles by lowering the number of trees capable of starting an outbreak at the onset of the wet season, while removing superspreaders may reduce the number of trees becoming infected through contagion at the peak of the disease cycle. Focusing on these two classes of epidemiologically relevant host types, may be more cost effective than randomly removing bay laurels to reduce stand density, because of the large effects the removal of these types of trees may have on the disease cycle. This may be particularly true for the removal of refugial trees, given that they are significantly less numerous than superspreaders, and while their removal will not prevent a future outbreak rates of transmission can potentially be lowered. This study also identifies the characteristics of sites with high incidence of superspreading and refugial trees: these areas should be prioritized for disease management, because they are likely to be key areas for the recolonization of, in this particular case, the entire watershed, and more generally, larger stands encompassing sites that contain superspreaders and refugial trees, by *P. ramorum* during favorable climatic conditions. Since the number of refugial trees may differ among sites, even during the most unfavorable conditions for the pathogen, targeting sites characterized by the smallest number of refugial trees could optimize this management approach.

The increase in likely inoculum load over the course of the years 2009-2011, three consecutive years with increasing levels of rainfall, the close association between bay laurel traits and oak infection, and the lack of significant increase in new oak infection over this time period suggest that a significant lag period may exist between bay laurel infection and oak infection, and between oak infection and the ability to see infection symptoms on oaks. Specifically, these results show that oak infection is not a yearly event, but only occurs when rainfall levels are above average as indicated by the fact that new symptoms of infection were detected in oaks only in 2011 and 2012, or a year after the wet spring of 2010 and the extremely wet spring of 2011 respectively (Fig. 4F). Research conducted by Garbelotto *et al.* (2017) has suggested a similar pattern by quantifying airborne inoculum and matching it to inoculum levels theoretically necessary to infect oak: this study confirms with field data this critical aspect of the SOD cycle. Models predicting SOD-induced oak mortality must take into account the clear presence of this threshold effect, and not simply correlate rainfall levels with oak infection levels. Such correlation may instead be valid for other hosts, and is supported for instance, in bay laurel by the data presented in this study. Based on this study, the onset of oak mortality and of visible symptoms other than tree death gradually increases over time since infection: symptoms start appearing 1 year after infection (based on the 2010-2012 results) while oak mortality appears to continue for at least 5 years after infection, assuming the symptomatic oaks early in the study were infected during the last period with above average rainfall (2006) (CA-DWR) (Fig. 4F). Thus, oak infection and oak mortality, although inextricably interconnected, do not have matching time frames: oak infection occurs in a relatively short window of time (the spring of a very wet year), but the mortality that results will be staggered for at least 5 years after infection (Fig. 4F). However it is important to consider the hosts phenology as well. When considering oaks, the spring season is not only the time with the most inoculum potential and highest rate of pathogen transmission (Garbelotto et al. 2017) but the time when oaks are most susceptible due to increases in growth rates and thus availability of nutrients for the pathogen (Dodd et al. 2008) as well as the formation of bark furrows which in particularly wet years may allow for pathogen pooling and a mode of entry into the cambial tissue. It is the combination of warm temperatures coupled with high levels of rainfall and high levels of inoculum levels that

occur in the late spring that allow infection courts to take hold and lead to outbreaks of oak infection in coastal California forests.

In summary, this study has identified not only those site and weather variables associated with infection levels by *Phytophthora ramorum* both on California bay laurel and coast live oak, but also those variables that explain bay laurel to oak disease transmission. Additionally, due to the repeated sampling over time, superspreader trees that remain infectious for longer periods of time were identified, as well as refugial trees that act as a reservoir of inoculum during dry periods. Also identified were those sites dubbed "hotspots" in which superspreader and refugial trees are present in large numbers. Finally, the models present the likelihood of pathogen survival or death between seasons, within each year, information that is extremely important to help further the understanding of the life cycle of the pathogen and the epidemiology of the disease and is relevant to the formulation of novel cost-effective and scientifically sound disease control options. In order to manage forest stands infected with the Sudden Oak Death pathogen, we recommend selective bay laurel removal around oaks in a buffer area whose size can be determined using the data presented in this study, by considering the diameter and density of those bay laurels to determine the size of the removal area. Additionally, when possible we recommend the identification (via culturing diagnostics) and removal of refugial trees during periods of drought, or the wholesale removal of bay laurel in areas characterized by dense stands of smaller bay laurels especially those occurring on Eastern facing slopes. Removal of the pathogen from the landscape is not possible but targeted management and knowledge of transmission patters and disease cycles can help to curb landscape level spread.

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Figures and Tables

Table 1. Models predicting infection of bay laurel *(U. californica*) and oak (*Q. agrifolia*) hosts, survival of *P. ramorum* infection on bay laurel both active (A) and dormant (D), and survival of infected oak hosts. D->A considers *P.ramorum* isolates that shifted from dormant to active, A->A considers isolates that remained active. Bold text indicates a significant result. Significant results with a negative sign denote lower infection or survival likelihood with increases in that variable. Results with a positive sign denote higher infection or survival likelihood with increases in that variable.

	INFECTION PREDICTION						**SURVIVAL PREDICTION**								
Group	***U. californica***			***Q. agrifolia***			***P.ramorum***			***P.ramorum***			***Q. agrifolia***		
RANDOM EFFECTS	**Variance**	**(Std. Dev.)**					**D->A**			**A->A**					
Tree ID	0.4499	0.6707					5.91e-12	2.43e-06		2.26e-11	4.76e-06				
Transect (Plot)	0.3325	0.5766		0.8221	0.9067		2.12e-01	4.61e-01		1.25e-01	3.54e-01		0.000	0.0000	
Plot	0.8729	0.9343		0.2211	0.4702		1.02e+00	1.01e+00		8.83e-02	2.97e-01		1.1172	0.3424	
FIXED EFFECTS	**Estimate**	**(SE)**	**p-value**												
(Intercept)	**1.05**	**(0.40)**	**0.009**				**-2.68**	**(0.43)**	**3.91e-10**	0.61	(0.45)	0.174			
Bay DBH	-0.08	(0.07)	0.235				-0.06	(0.06)	0.33	-0.05	(0.10)	0.649			
Bay Basal Area	**-0.31**	**(0.14)**	**0.031**	-0.03	(0.33)	0.923	**-0.29**	**(0.13)**	**0.03**	-0.22	(0.13)	0.087	0.05	(0.22)	0.83
Bay Density	**0.44**	**(0.17)**	**0.010**	**0.83**	**(0.35)**	**0.017**	**0.31**	**(0.16)**	**0.04**	0.19	(0.12)	0.108	0.09	(0.23)	0.71
Northness	0.09	(0.08)	0.303	-0.19	(0.17)	0.250	0.13	(0.09)	0.18	-0.03	(0.10)	0.771	-0.22	(0.17)	0.19
Eastness	**0.20**	**(0.09)**	**0.022**	-0.17	(0.16)	0.311	**0.25**	**(0.09)**	**0.005**	0.01	(0.11)	0.953	0.05	(0.17)	0.78
Canopy Cover	-0.003	(0.003)	0.347				**-0.002**	**(0.003)**	**0.50**	0.003	(0.004)	0.428			
Oak DBH				**0.94**	**(0.15)**	**4.41e-10**							**-0.29**	**(0.14)**	**0.04**
Prox. to Inf. Bay				**-1.20**	**(0.15)**	**1.81e-15**							-0.10	(0.16)	0.511
Cankered													**3.027**	**(0.30)**	**<2e-16**
Prior PCR +							**0.43**	**(0.13)**	**0.001**						
Season Peak							**-1.91**	**(0.20)**	**< 2e-16**	**-2.01**	**(0.20)**	**< 2e-16**			
Season Early	**-1.21**	**(0.12)**	**< 2e-16**												
Season Late	**-1.38**	**(0.12)**	**< 2e-16**				**0.37**	**(0.12)**	**0.003**	-1.26	(0.23)	2.54e-08			
Year 2009	**-3.18**	**(0.14)**	**< 2e-16**												
Year 2010	**-1.48**	**(0.11)**	**< 2e-16**				**1.87**	**(0.16)**	**< 2e-16**	1.04	(0.25)	4.35e-05			
Year 2011	**-0.57**	**(0.15)**	**0.0002**				**2.81**	**(0.18)**	**< 2e-16**	0.96	(0.24)	8.75e-05			
Model AIC	3434			617.5			1897			1204			518.4		

Figure 1. Isolation success of *P. ramorum* from bay laurel leaf samples collected over 10 sampling events , three times per year for three years from 2009-2011, and in the spring (Peak) season of 2012. Early, Peak and Late sampling events represent late winter, late spring and autumn respectively.

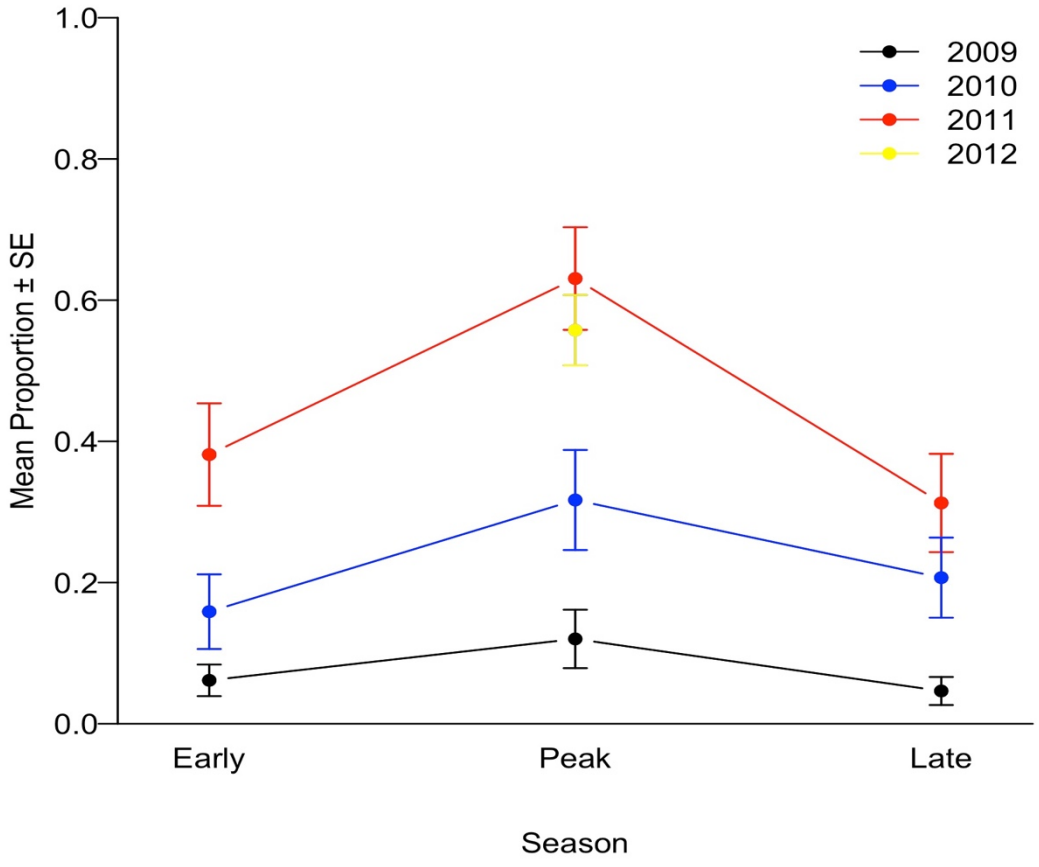


Figure 2. Annual and seasonal heterogeneity in proportions of bay laurel infection with viable (culture +) and dormant (culture -/PCR +) *P. ramorum* over three years. Blue bars represent the proportion of bay laurel trees that yielded a positive *P. ramorum* isolate. Red bars represent the proportion of samples that were negative in culture, but positive when tested with a PCR diagnostic assay.

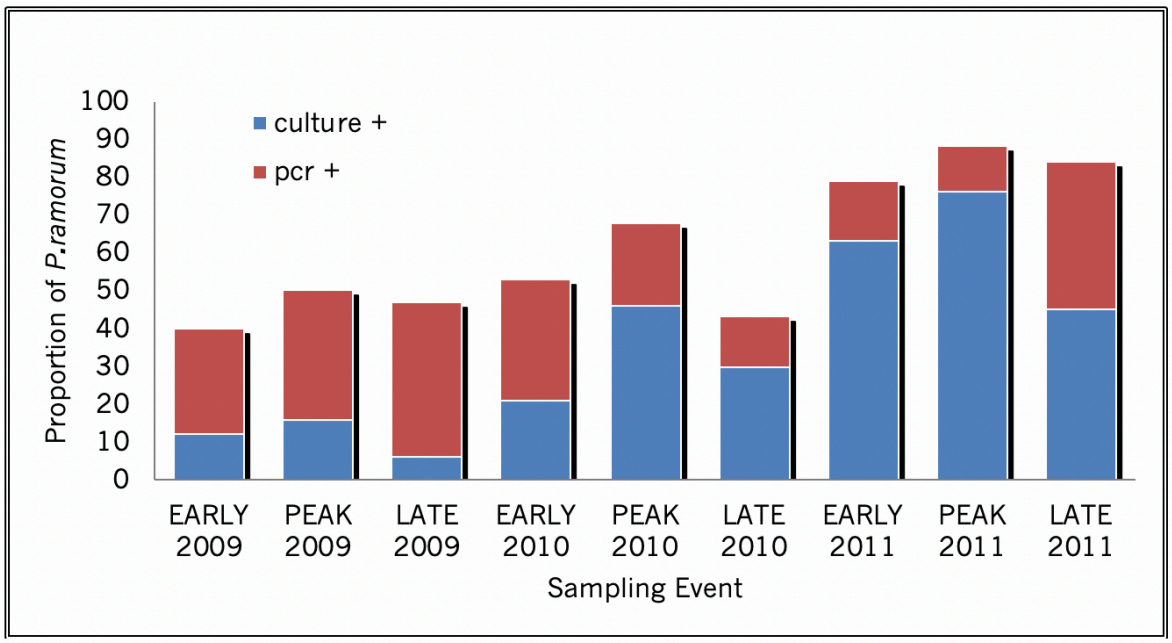


Figure 3. Comparisson of *P. ramorum* disease incidence on bay laurel between plots across ten sampling events over three years. Plots are ranked in order from lowest to highest incidence. The bold line in each box represents the median number of times a tree in that plot yielded a positive culture. Red colored bars indicate the presence of "superspreader" trees , bay laurels that yielded a positive isolate of *P. ramorum* >7/10 times. Plots denoted with an asterisk contain one or more "refugial" trees that harbor viable infection in all three of the autumn (Late season) sampling events, have the highest level of infection and the most "superspreader" trees.

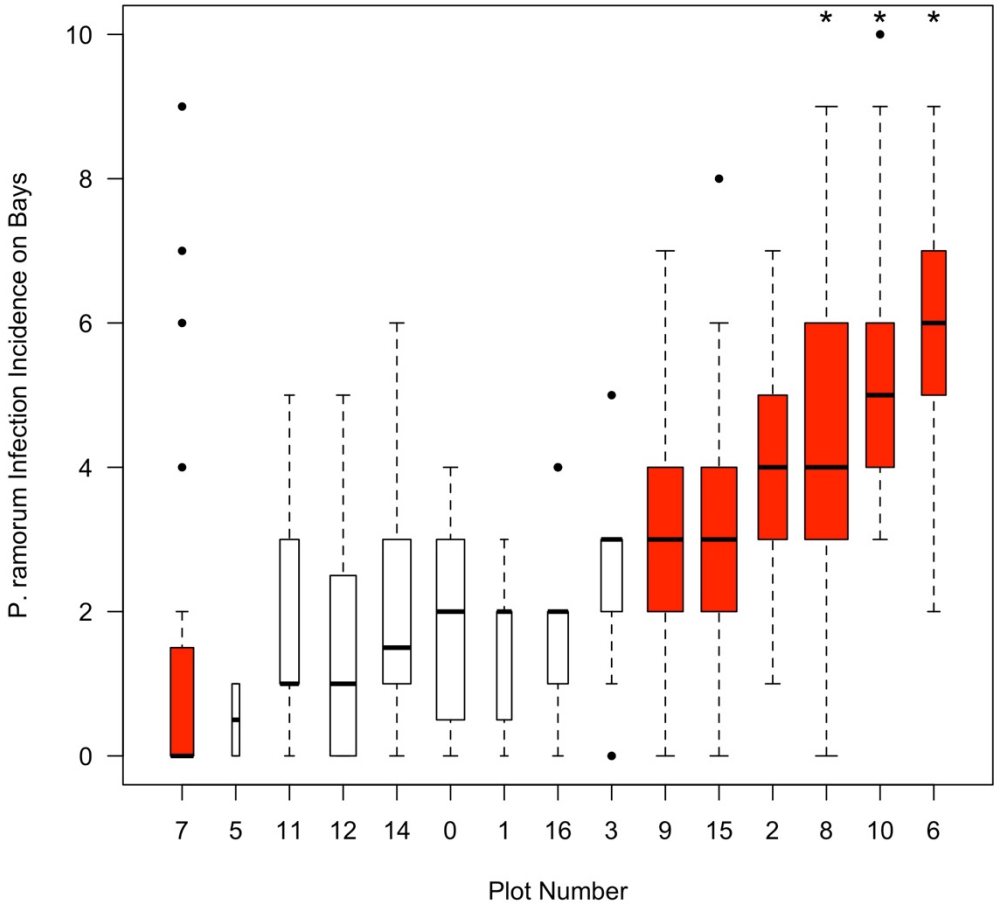


Figure 4: A.) Probability of active *P.ramorum* infection on bay laurel with respect to bay laurel density, with bay laurel basal area held constant), in each year of the study. B.) Probability of active *P.ramorum* infection on bay laurel in relation to bay laurel basal area, with bay laurel density held constant, in each year of the study. C.) Probability of active *P.ramorum* infection on an average-sized bay laurel in the wettest and driest conditions encountered during the study (driest: autumn 2009; wettest: spring 2011) with respect to bay laurel density. Bold center line represents median bay laurel basal area, the "cloud" around the center lines represent the 90th and 10th percentiles of the data. D.) Probability of oak infection as a function of size (DBH), bay laurel density, and distance to nearest infected bay laurel tree for three size classes of oaks. The cloud around each line represents bay laurel density. E.) Predicted oak mortality based on oak DBH and presence of symptomatic infection (canker). The solid line represents oaks symptomatic for *P. ramorum* infection, and the dashed line represents asymptomatic trees. F.) Association of rainfall with active *P.ramorum* infection on bay laurel, oak infection and oak mortality in each year of the study. The blue line represents the amount of rainfall during the spring of each year. The solid green line shows the proportion of active infection on bay laurel from 2009-2012, and the dashed green line includes the proportion of dormant bay laurel infection detected from PCR diagnostics. The red solid line represents the proportion of infected oak trees each year, the black solid line represents the proportion of dead oaks. The red dashed line combines the proportion of cankered oaks and dead oaks each year.

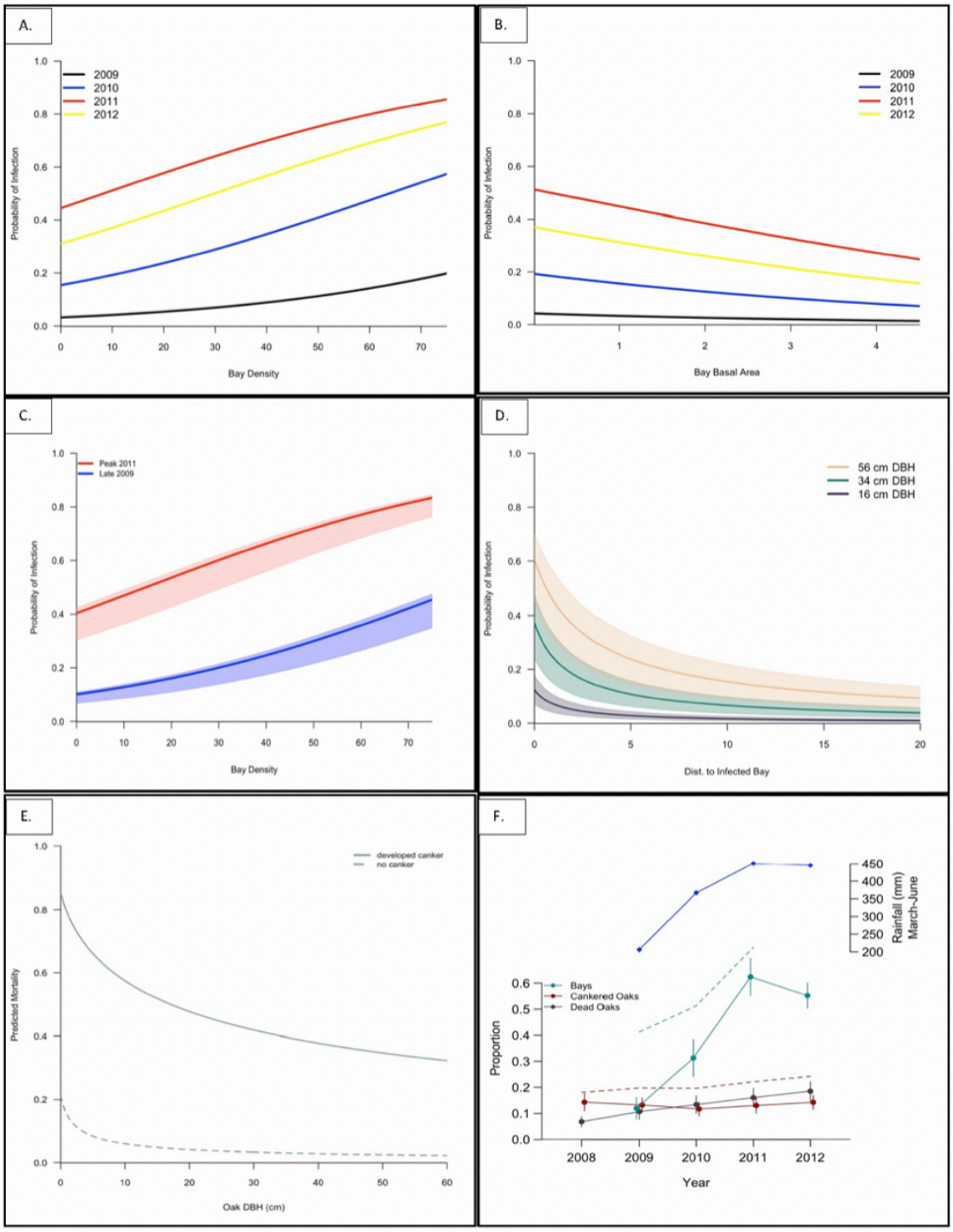
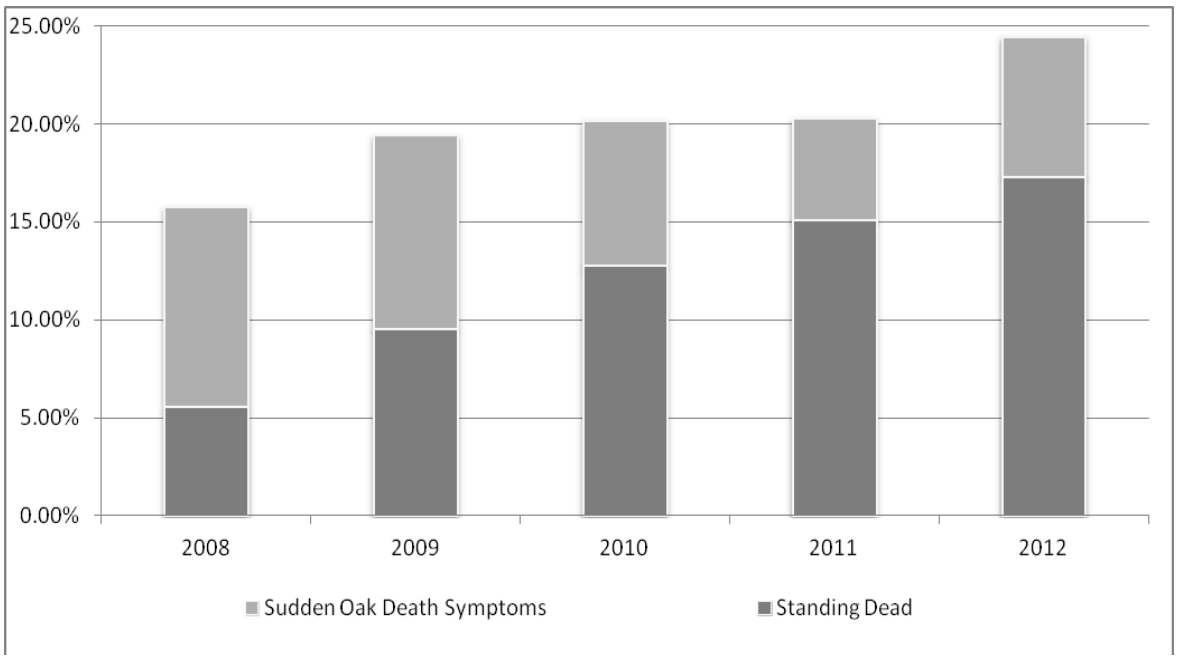

A.
2009
2010
2011
2012
Probability of Infection
Bay Density
B.
2009
2010
2011
2012
Probability of Infection
Bay Basal Area
C.
Peak 2011
Late 2009
Probability of Infection
Bay Density
D.
56 cm DBH
34 cm DBH
16 cm DBH
Probability of Infection
Dist. to Infected Bay
E.
developed canker
no canker
Predicted Mortality
Oak DBH (cm)
F.
Bays
Cankered Oaks
Dead Oaks
Proportion
Rainfall (mm)
March-June
Year

Figure 5. Yearly percentage of oak infection (light grey) and mortality (dark grey) over the course of the study.

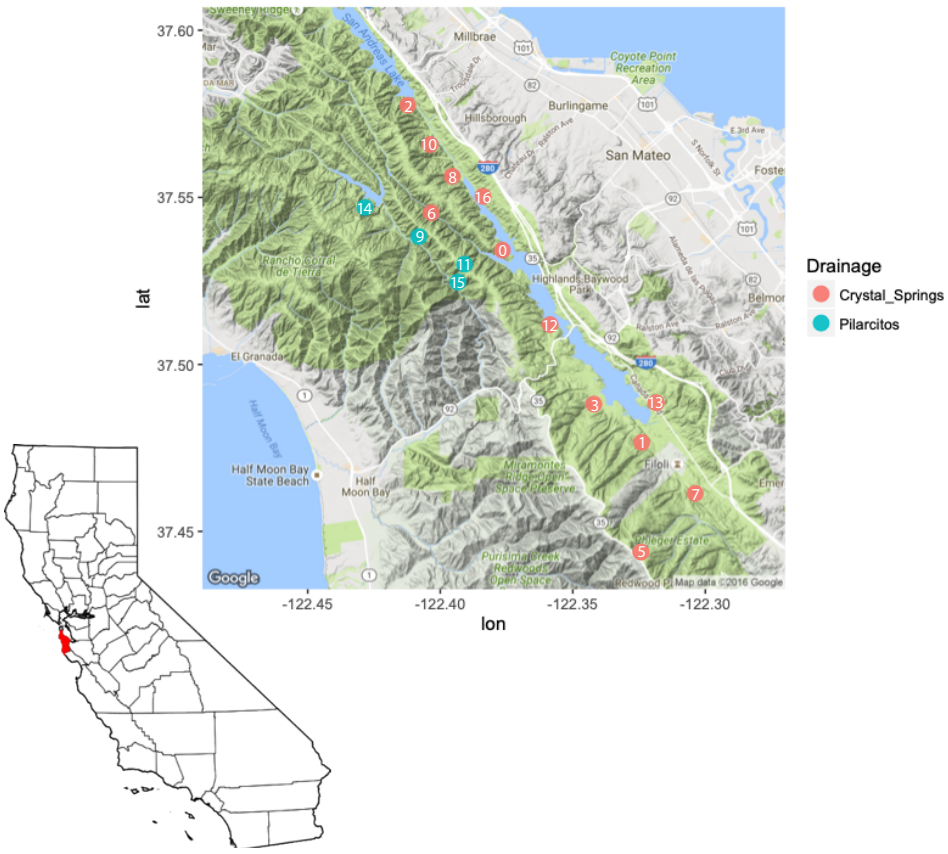


Supplementary Table S1. Locations of individual plots located within the SFPUC Peninsula watershed.

Drainage/Area	Plot	Latitude	Longitude	Elevation (m)
CS	0	37.53420725	-122.3765705	115
OC	1	37.47667587	-122.3240183	131
CS	2	37.57758904	-122.4122678	155
OC	3	37.48812832	-122.3420616	107
NA	5	37.45370812	-122.3306575	457
CS	6	37.54543221	-122.4033964	104
OC	7	37.46138871	-122.3038186	140
CS	8	37.55632374	-122.3955187	113
PL	9	37.53828797	-122.4080535	252
CS	10	37.56582756	-122.4038403	187
PL	11	37.53001152	-122.3908418	295
OC	12	37.51191507	-122.3585746	95
NA	13	37.48925057	-122.3155543	149
PL	14	37.54701261	-122.4284439	311
PL	15	37.52467467	-122.3933726	197
CS	16	37.55031173	-122.3838935	129

CS=Crystal Springs drainage; PL=Pilarcitos drainage; OC=Old Canada area;
NA=Not associated with specific drainage/area. Elevation measured in meters

Supplementary Figure S1. Location of sampling plots. Top: Map showing locations of sampling plots, denoted by drainage, within the San Francisco Public Utility Commission Peninsula watershed. Bottom: Map of California showing San Mateo County highlighted in red.



Chapter 3

Population dynamics of aerial and terrestrial populations of *Phytophthora ramorum* in a California forest under different climatic conditions.

Abstract

Limited information is available on how soil and leaf populations of the Sudden Oak Death pathogen, *Phytophthora ramorum*, may differ in their response to changing weather conditions, and their corresponding role in initiating the next disease cycle after unfavorable weather conditions. We sampled and cultured from 425 trees in six sites, three times at the end of a 3-year long drought, and twice during a wet year that followed. Soil was also sampled twice with similar frequency and design used for sampling leaves. Ten microsatellites were used for genetic analyses on cultures from successful isolations. Results demonstrated that incidence of leaf infection tripled at the onset of the first wet period in 3 years in the spring of 2010, while that of soil populations remained unchanged. Migration of genotypes among sites was low and spatially limited under dry periods, but intensity and range of migration of genotypes significantly increased for leaf populations during wet periods. Only leaf genotypes persisted significantly between years, and genotypes present in different substrates distributed differently in soil and leaves. We conclude that epidemics start rapidly at the onset of favorable climatic conditions through highly transmissible leaf genotypes, and that soil populations are transient and may be less epidemiologically relevant than previously thought.

Introduction

The success of an invasive exotic pathogen may be determined by the ecological amplitude of the organism, i.e. a combination of the range of hosts available, aggressiveness on each host (Heger and Trepl 2003; Parker and Gilbert 2004), release from natural enemies and competitors (Keane and Crawley 2002), and reproductive rate and dispersal potential of infectious propagules in a new environment (May and Anderson 1983; Williamson and Fitter 1996; Sakai et al. 2001). Further complexity is encountered when dealing with pathogens that may completely change their lifestyle in different hosts or habitats, e.g. when plant pathogens switch from a saprotrophic to a pathogenic state depending on the substrate (Olson et al. 2012), a host species jump occurs (Woolhouse et al. 2005), or zoonotic diseases transition from transmissive to dead-end hosts (Morens et al. 2004;).

Although only recently discovered in temperate forests (Rizzo et al. 2002; Hensen et al. 2003; Hansen 2008; Brown et al. 2005), aerial *Phytophthora* species (order: Peronosporales; kingdom: Stramenopila) represent a unique opportunity to study the epidemiology of infectious forest pathogens deeply affected by environmental conditions such as temperature and rainfall (Erwin and Ribeiro 1996). These organisms are capable of colonizing completely different substrates including leaves, branches, trunks, soil, and water (Garbelotto and Hayden 2012). The epidemiology of these aerial *Phytophthora* species is largely unresolved and the role played by each substrate in their population dynamics is unknown. To date, the majority of research on aerial *Phytophthora* species has focused on *P. ramorum*, the causal agent of Sudden Oak Death in North America, of Sudden Larch Death in Great Britain, and of Ramorum Blight in nurseries worldwide (Grünwald et al. 2012; Garbelotto and Hayden 2012), and *Phytophthora infestans*, the pathogen causing potato blight (Granke et al. 2009; Skelsey et al. 2009).

Studies of *P. ramorum* have focused on understanding the ecological and environmental parameters that facilitate infection (Davidson et al. 2005; Anacker et al. 2008; Tooley et al. 2009; Hüberli et al. 2011), on describing the effects of the disease on individual plant species and/or plant communities (Linderman et al. 2006; Cobb et al. 2010; McPherson et al. 2010) and on reconstructing the evolutionary and recent history of both wild and nursery populations of the organism (Ivors et al. 2006; Mascheretti et al. 2008, 2009; USDA APHIS 2008). However, limited information has been generated on how climatic patterns, both within and between years, affect the active size ("active" here is meant loosely as the portion of the population that sporulates and thus is infectious), the diversity, and the migration potential of *P. ramorum* genotypes. Although some studies have shown how virulence and sporulation are traits greatly affected by the plant hosts colonized by *P. ramorum* (Hüberli and Garbelotto 2011; Kasuga et al. 2012), little information has ever been provided, for this or any other mixed-substrate (e.g soil-air-water) plant pathogen, on how populations from different substrates may interact and differently contribute to the epidemiology and to the microevolution of a species. The genetic structure in years characterized by limiting unfavorable environmental conditions, the change experienced by such populations as conditions become favorable, and the epidemiology of populations from different substrates are elements essential to the understanding of infectious diseases, including those caused by plant pathogens.

Although there are over 100 species of plants that are either natural hosts of *P. ramorum* (Garbelotto et al. 2003; Hansen et al. 2005; USDA APHIS 2008; Tjosvold et al. 2005; Davidson et al. 2003), or have been shown to be susceptible in lab trials, the leaves of California bay laurel (*Umbellularia californica*) provide the most prolific substrate for sporulation of the pathogen in

California forests, with limited to no effect on the overall health of the host (Davidson et al. 2005; Di Leo et al. 2009; Cobb et al. 2010) *Phytophthora ramorum* populations infecting bay laurel leaves are strongly affected by seasonal temperatures and rainfall in California (Davidson et al. 2005; Anacker et al. 2008; Di Leo et al. 2009, Hüberli and Garbelotto 2011). The Californian climate is typically Mediterranean, with generally moderate temperatures year round. The winter and spring months are characterized by mild temperatures and rain, which are followed by long periods of dry weather during the summer months (Fig. 1). In the years preceding this study (2007-2009), the San Francisco Bay Area experienced an atypical drought period in which the usual spring rains were much reduced. This drought was broken in 2010, with a return to wetter spring conditions. This drought-non drought climatic transition, as well as the relative fluctuations of climate within each year, are both the focus of this study. *P. ramorum* moves through the landscape primarily via airborne sporangia produced on leaves and twigs of infectious hosts including but not limited to bay laurel (Garbelotto et al. 2003; Davidson et al. 2005; Maloney et al. 2005), while thick walled chlamydospores, also produced on leaves or twigs, are suspected to be the main source for soil inoculum (Fichtner et al. 2009). It is unknown whether soil represents a reservoir of inoculum for this pathogen, relevant to its survival during the unfavorable dry summer months or during extended drought periods. It has been postulated that leaves may be the substrate where *P. ramorum* oversummers (Fichtner et al. 2008) but direct evidence on this aspect of the disease is still lacking.

Most plant pathogens require precise environmental conditions for transmission to occur, and consequently, the density of pathogen populations and disease severity fluctuate significantly (Marchetti et al. 1989; Papastamati et al. 2007; Madgwick et al. 2011). While this dependence of dispersal on environmental conditions implicitly suggests that unfavorable conditions will lead to a bottleneck in pathogen populations, in the case of *P. ramorum*, it is pivotal to understand whether both soil and leaves are effective reservoirs of inoculum necessary to start the next disease cycle, whether survival occurs everywhere or only in refugial sites, and whether all genotypes respond equally to the onset of warmer and wetter conditions, that are more favorable for the pathogen.

Additionally, when a pathogen colonizes different substrates, it is important to determine whether some genotypes may show adaptation to a specific substrate, and whether the effect of the substrate on pathogen fitness differs across substrates. While this issue of distinct populations of the same pathogen in different hosts and substrates has been widely studied for parasitic or zoonotic diseases (Hide et al. 2004; Annan et al. 2007; Brinkerhoff et al. 2010; Salathe and Schmid-Hempel 2011), it has only rarely been investigated in plant pathogens (Zahn et al. 2002; Richardson et al. 2007; Mahdizadeh et al. 2011).

Specific goals of this study included: 1) to determine how disease incidence, size, and genotypic composition of populations of the pathogen in bay laurel leaves are affected by the onset of favorable rainy conditions after a persistent drought; 2) to compare the genotypic diversity, migration, presence, and evenness of individual foliar genotypes during the transition between a drought and a wet year; 3) to determine whether soil and leaf populations sampled at the same time in the dry spring of 2009 and the wet spring of 2010 represent genetically isolated groups displaying differential adaptation to the two substrates and comparable spatial aggregation of alleles in the presence of different climatic conditions, and, finally; 4) to determine whether populations from both substrates may be a significant source of inoculum for next year's disease cycle.

Methods

Habitat/plot selection

Six study plots were established in San Mateo County within the 9300 ha watershed (Lat: 37.519539º, Long: -122.368952º) controlled by the San Francisco Public Utility Commission (SFPUC). Plots were equally divided between the Crystal Springs and Pilarcitos drainages, ranging 95-320 m in elevation; Pilarcitos is on average higher in elevation than Crystal Springs and is the only drainage to include tanoaks (*Notholithocarpus densiflorus*). The entire watershed is closed to the public, relatively undisturbed, and *P. ramorum* has been present at least since 2001 (Rizzo, personal communication). Sufficient time has passed for the pervasive infestation of all appropriate habitats in the area selected for this study, thus eliminating possible effects of infestation age on the analysis. Plots were separated by at least 2 km, a distance gap only occasionally bridged by the pathogen (Garbelotto and Hayden 2012), and equally distributed in two drainages. Each plot consisted of six 100 m transects, 10m wide, emanating at 60º from the plot center. Each bay laurel stem was tagged to enable repeated sampling of the same trees. Stems were defined as major branches separating from the main stem below breast height, measuring upwards of 1 cm diameter. Local climate data were acquired from the Remote Automated Weather Station (RAWS) at Pulgas (Lat: 37.47500º, Long:-122.29810º), SFPUC data archives and local temperature collected in each plot using HOBO data loggers (Onset Computer Corporation, MA, USA).

Leaf sampling

Bay laurel trees were surveyed every 10m along the six transects in each plot; six symptomatic leaves were sampled from the lower canopy of each tree. Leaves were classified as 'symptomatic' if they displayed lesions characteristic of *P. ramorum* infection i.e. a black or brown irregularly shaped lesion at the tip and or edges of leaves, often with a pixelated appearance and with edges characterized by a dark border line or a yellow halo. Younger leaves were sampled if possible, irrespective of size, with the aim of sampling current viable pathogen infections. Sampling of symptomatic leaves occurred up to three times per year: 'Early' sampling in late winter/early spring (February/March), when conditions were not yet ideal for pathogen sporulation and infection (Davidson et al. 2011; Hüberli et al. 2011); 'Peak' sampling in early June when sporulation and infection levels are generally highest; 'Late' sampling in the hot and dry autumn (September) when the pathogen is the least active and least infectious. Leaves were sampled three times in 2009 (Early, Peak, Late) and twice in 2010 (Early and Peak). Rainfall levels in 2009 and 2010 were approximately 50% and 110% of the 30-year average, respectively (CA Dept. Water Resources 2010), with almost no rainfall in the spring of 2009 and with higher than average spring rainfall in 2010. The monthly Aridity Index (De Martonne 1926) was calculated as: $AI_M = P/(T+10)$, where P is the precipitation (mm) and T is the mean air temperature (ºC). This index provides a unified term of reference to define whether overall weather conditions were unfavorable or favorable to pathogen dispersal; index values are low when aridity is high.

The edges of lesions from the six leaves collected from each surveyed tree, were plated in PARP selective medium [400µl/L Pimaricin; 250mg/L Ampicillin; 10mg/L Rifampicin; 25mg/L PCNB] (Vettraino et al. 2009). Plates were incubated in the dark, at 18ºC for 5-7 days. *P. ramorum* growth was identified from mycelial morphology and then subcultured onto clean PARP medium to eliminate contaminants.

Soil sampling and baiting

Soil was sampled concurrently with leaf collections for two consecutive years during the two 'Peak' sampling efforts of 2009 and 2010. Approximately 500g of soil were taken from three points around the base of each tree/branch from which leaves were sampled, and pooled together. Surface duff was removed and collection was from the surface to approximately 20 cm depth. Samples were stored at 10ºC for a maximum of 48 hours, before being mixed thoroughly and flooded with dH_2O until the soil was completely covered. Ten leaf discs of uninfected *Rhododendron* var. Cunningham's White were placed in mesh bags and floated on the surface of the water. Baited soil was incubated in the dark at 18ºC for 5-7 days. Following incubation, leaf discs were plated into PARP+H selective medium (PARP+25 mg/L Hymexozol) (0), with discs completely submerged into agar. Plates were incubated in the dark at 18ºC for 1-4 days: growth usually occurred from leaf discs within 1-3 days. *P. ramorum* colonies were selected based on microscopic morphology and subcultured onto clean PARP medium.

DNA extraction

Mycelial isolates maintained on PARP were inoculated into 12% pea broth (120g peas/L dH_2O, autoclaved for 20 min at 121ºC, 1.05 kg/cm^2, strained and re-autoclaved under the same conditions) liquid culture and grown for 1 week at room temperature, followed by lyophilization. Lyophilized mycelium was ground using 5mm glass beads and a bead amalgamator. DNA was extracted by addition of 200µl 0.5M NaOH to approximately 10ng of crushed mycelium, mixing thoroughly, followed by dilution of 5µl of this in 495µl 10mM Tris-HCl pH 8.0 (Wang et al. 1993; Osmundson et al. 2012).

Microsatellite data generation

P. ramorum DNA was amplified using 6 sets of primers amplifying a total of 10 microsatellite loci; Ms18 and 64 (Ivors et al. 2006), Ms39, 43, 45 (Prospero et al. 2007) and MsILVO145 (Vercauteren et al. 2010) (Table S1). 10µl PCR reactions were set up with final concentrations for each reaction as follows: 1X PCR Buffer, 1.5mM $MgCl_2$, 200µM dNTPs, 0.2µM F primer (labeled with HEX or FAM), 0.2µM R primer, 1u/µl GoFlexiTaq (Promega). Thermal cycling programs varied for different primers and were taken from Ivors *et al.* (2006), Prospero *et al.* (2007) and Vercauteren *et al.* (2010). A 3730 ABI Sequencer was used for fragment analysis using LIZ 500 size standard (Applied Biosystems) and analysed using Peakscanner v1 (ABI Biosystems). Fragment sizes were converted to numbers of microsatellite motif repeats for downstream analysis. Multilocus genotypes (MLGs) were assigned to each isolate using Gimlet v1.1.3 (Valière 2002).

Genetic diversity indices and minimum spanning networks

The following indices of diversity were calculated: (i) Clonal genotype diversity, R=(G-1)/(N-1) where *G* is the number of MLGs present in a sample and *N* is the sample size (Mascheretti et al. 2009); (ii) Stoddart and Taylor's index, $G=1/\Sigma p_i^2$ where p_i is the frequency of the *i*th MLG (Stoddart and Taylor 1988); (iii) Evenness index, $E_5 = (1/\lambda)-1/e^{H'}-1$ where λ = Simpson's index of diversity and H' = Shannon-Wiener diversity index (Shannon and Weaver 1949; Ludwig and Reynolds 1988; Grünwald et al. 2003). E_5 tends towards 0 as a single genotype becomes more dominant, when $E_5=1$ all genotypes are equally represented; (iv) Allelic richness, A_r, was calculated using rarefaction to account for unequal sample sizes of populations

using ADZE v1.0 software (Szpiech et al. 2008). ADZE v1.0 was also used to produce genotype accumulation curves, for each sampling time for both leaf and soil.

Genetic distances were calculated according to Bruvo *et al.*, (2004) (Bruvo et al. 2004) in GenoDive 2.0b10 (Meirmans and Van Tienderen 2004) using the stepwise mutation model. When calculating Bruvo distances, mutation is exponentially related to step length, and thus this metric has been deemed ideal for the comparison of closely related individuals (Mascheretti et al. 2008). Bruvo distances were also used to produce a minimum spanning network, to depict the relationship and interconnectedness of genotypes, using MINSPNET (0) and visualized using Graphviz v2.28 (www.graphviz.org). To examine the origin of the high genotypic diversity observed in the soil, we generated a MSN for the more diverse 2010, in which MLGs were identified as present in (i) leaf only, (ii) both soil and leaf, and (iii) soil only (Fig. 6). The most likely progenitor of each soil-only genotype was then selected as the closest MLG towards the center of the MSN as described in Mascheretti *et al.* (2008). The number of progenitors of soil-only MLGs in each of the three possible classes was computed to understand the possible origin of the genotypic diversity detected in soil in 2010. For MLGs present both in soil and leaves, the contribution of each substrate was assigned based on the frequencies in Fig. 6.

Analyses of genetic structure and spatial autocorrelation

Populations were tested for genetic structure using Arlequin v3.5.1.2 (Excoffier and Lischer 2010). Standard AMOVAs were performed on an external distance matrix of Bruvo distances among MLGs, and 10000 permutations were performed. AMOVAs were employed to study; (i) temporal genetic differentiation of populations collected at different times, (ii) spatial genetic structure of populations collected at the same time from different sites, and, (iii) to infer the presence or absence of significant migration between foliar and soil populations collected from the same site.

Spagedi v1.3 (Hardy and Vekemans 2002) was used to determine presence of significant clustering of alleles using spatial autocorrelation independently for soil and leaf samples. Data were analyzed at the individual level and spatial coordinates for each tree along transects were supplied in UTM format (WGS84). Moran's index of genetic similarity, *I* (Sokal and Oden 1978), was used to correlate allelic repeats with spatial distance classes. A total of 20000 permutations were used for significance testing of Moran's *I*.

Statistical analyses

Pearson's chi-squared, Fisher's Exact, and Spearman's Rank Correlation Coefficient tests were performed in R (R Core Team 2011), to test the significance of differences between soil and leaf isolation success among sampling times, and to compare the abundance of MLGs between leaf and soil substrates and sampling times. Spearman's Rank Correlation Coefficient test and Fisher's Exact test were used to analyze persistence of MLGs between years by comparing the rankings of MLGs within substrates between years.

The leaf data were also grouped by whether the aridity index (AI) in the plot for 15 and 30 d prior each sampling time was above or below the average aridity index calculated for the whole duration of the study. Using JMP software (JMP, Version 10.0.0. SAS Institute Inc., Cary, NC, 1989-2013), a Kruskall Wallace non-parametric ANOVA was used to compare the isolation success (%), diversity (G) and Evenness (E_5) between samples above and below average aridity.

Results

Climatic conditions

Weather data (Fig. 1) for the study area showed a cyclic fluctuation in air temperature throughout the year. The lowest average daily temperatures recorded each year were in February 2009 (10.3ºC) and January 2010 (9.7ºC), and highest daily average in September both years (2009: 20.2ºC; 2010: 19.4ºC). The average daily minimum temperature was in December each year (2009: 5.4ºC; 2010: 6.6ºC), and average daily maximum was in September (2009: 30.9ºC; 2010: 28.4ºC). The highest individual temperature recorded on any day was in August 2010 at 42.8ºC, and the lowest in December 2009 (-1.6ºC). Monthly total rainfall peaked each year in the winter months; February 2009 (246 mm) and January 2010 (195 mm). Monthly rainfall in 2009 peaked in February (246 mm), and decreased in the following months to virtually nothing between April and September (ranged between 0 mm -12.7 mm). In 2010, the period of rain was more prolonged, peaking in January 2010 (195 mm), with rainfall remaining above 20 mm until May (ranging from 21mm – 120 mm), before reducing to nothing. There was an absence of rain in both years during the summer months, with rain returning in the autumn/winter (Fig. 1).

Sampling success and genetic diversity

A total of 425 trees were surveyed five times. From these, 1665 symptomatic leaf samples were collected cumulatively in five sampling times, and 610 soil samples were obtained in the two 'Peak' sampling times. Table 1 summarizes results by sampling time for soil and leaf sampling. Isolation success from leaves ranged from 10 % in the very dry autumn/Late season of 2009 to 49 % in the rainy and warm late spring/Peak season of 2010 (Fig. 1); incidence was found to be significantly different among sampling times (χ^2=146.320, df=4, p<0.0001). Soil isolation success was slightly higher in 2009 (24%) than in 2010 (19%), but this difference was not significant.

For foliar populations, diversity expressed by *G* was relatively stable during 2009, ranging between 8.715-9.377. *G* increased in Early 2010 (10.249), and reached its highest value in Peak 2010 (14.687). *R* decreased overall in 2009, dipping lowest in Peak 2009 (0.333) and increasing slightly in Late 2009 (0.378). In 2010, *R* had an inverse pattern to *G*, decreasing from Early 2010 (0.393) to its lowest overall value in Peak 2010 (0.282) (Table 1, Fig. 3). Evenness, as measured by E_5, was greatest in Late 2009, and decreased in Early 2010 and reached its lowest value in Peak 2010. Genetic structuring, measured by Fst, increased through 2009 (0.152-0.244), and peaked in Early 2010 (0.321), before decreasing in Peak 2010 (0.242) (Table 1, Fig.3).

Changes in soil diversity indices were less marked than those recorded for foliar populations. Both *R* and *G*, and allelic richness diversity indices were higher in soil in 2010 than in 2009 (2009: R=0.268, G=9.063, Ar=2.77; 2010: R=0.386, G=12.27, A*r*=3.316) (Table 1, Fig. 3), however Fst remained relatively unchanged between years (2009: 0.216, 2010: 0.202), while the evenness of genotypes (E_5) showed a slight increase (2009: 0.653, 2010: 0.726) indicating lack of dominance of any particular soil genotype across sites.

Isolation success from leaves was significantly greater when the Aridity index in the 15d prior to the sampling period was below average (i.e. wetter, e.g. Peak 2010) (Kruskall Wallace non-parametric ANOVA: p=0.0297). Diversity, G, and evenness, E_5, compared between the two groups were approaching significance at the 0.05 level (G: p=0.0825; E_5: p=0.0825). When calculations were performed using weather data from a 30d period prior to sampling, there was

no significant difference in isolation success (Sampling Month AI: 0.2089; Month prior AI: p=0.3778), G (Sampling Month AI: p=0.527; Month prior AI: p=0.5277) or Evenness (Sampling Month AI: 0.4807; Month prior AI: p=0.5277).

Distribution of genotypes by substrate and time

Genotype accumulation curves for the mean number of distinct alleles indicate that despite unequal numbers of isolates obtained from each sampling time and different substrates, the intense field sampling effort was sufficient to capture a good representation of the diversity present in both substrates in the overall dataset (Fig. 4) and in the two drainages considered independently (data not shown). The sample size where each population can be assessed evenly (g=76) falls in the plateau of each accumulation curve (Fig. 4). The rarefied allelic richness index, *Ar*, for Soil Peak and Leaf Peak are very similar when compared within each year; 2009 Peak Leaf and Soil are both approximately 2.7, and increases equally in 2010 for both Leaf Peak and Soil Peak to 3.07 and 3.13 respectively (Table 1, Fig. 2).

A total of 33 and 20 MLGs were identified from leaves and soil respectively in 2009; that number increased to 55 and 23 respectively, in 2010 (Table 1, Table S2). Many of the MLGs identified were singletons, i.e. distinct MLGs only represented by a single isolate. In leaves, singleton MLGs represented 43% of all leaf MLGs in 2009 and 57% in 2010. However, in terms of individuals, singletons represent only a small proportion of the population (2009: 9%, 2010: 14%). In soil, the same pattern is seen, 35% of soil MLGs were singletons in 2009, 48% in 2010, representing 10% and 19% of individuals respectively.

A number of MLGs were found in both soil and leaves each year; 13 in 2009 and 15 in 2010. When the abundances (as a percentage of the whole population in each substrate) of the MLGs present in both soil and leaves were compared, no significant difference was observed in 2009 (χ^2=10.5222, df=12, p=0.5702), but a significant difference was detected between the two substrates in 2010 (χ^2=27.0098, df=14, p=0.0192). (Fig.5)

Temporal turn-over of genotypes in leaves and soil

A total of 63 MLGs were found in leaf populations during the study. Of these, 27 were isolated in both 2009 and 2010 (42.8% of MLGs). When the relative abundance of each leaf MLG in each year was ranked, there was significant correlation between years (Spearman's rank: ρ=0.76, n=27, p=0.0000) indicating a persistent foliar population between years. In soil, 35 MLGs were found during the study (total for 2009 and 2010 together). Only 8 of these MLGs (22.8% of all MLGs) were found in both years. In contrast to the leaf populations, the overall rank of these persistent soil MLGs varied between years (Spearman's rank: ρ=-0.18542, n=8, p=0.704). The relative abundances of certain soil MLGs changed dramatically between years. For example, MLG 66 was present in very low numbers in 2009 (n=2, 2% of all isolates), but was the most abundant MLG found in soil in 2010 (n=10, 12%). Similarly, although in the reverse direction, MLG43 was most common in soil in 2009 (n=19, 26% of all isolates) but its abundance was reduced to only 3 individuals in 2010, reduced 20% from 2009 to just 5% of all isolates (Table 2). This same pattern was seen occasionally in individual leaf MLGs (e.g. MLG66 in leaf populations had 3 individuals in 2009, increasing to 27 in 2010), but generally the most abundant leaf MLGs in 2009 remained the most abundant in 2010.

Out of 64 trees that yielded viable leaf isolations in 2009, 51 (80%) also yielded viable cultures in 2010, and 16 (25%) yielded the same MLGs both years. Out of 72 viable soil isolations from 2009, only 13 (18%) were successful again in 2010, and the same MLG was

recovered from the same location in only 2 (3%) soil samples in consecutive years. A Fisher's exact test determined persistence of identical MLGs was significantly higher in leaves than in soil (2-tailed p-value<0.0001).

An AMOVA analysis was performed in which all leaf isolates obtained at the same time within a year (Early, Peak, Late) represented a population, independent of site of origin but hierarchically grouped in the year according to the time when sampling was performed. In leaf populations, there was no significant genetic structuring between 2009 and 2010 samplings (Fct=0.00434, p=0.11603), or among sample times within each year (Fsc=0.00176, P=0.27663). Conversely, AMOVA analysis of soil samples revealed significant structure between 2009 and 2010 (Fst=0.04654, p≤0.001).

Genetic comparison between soil and leaf samples

The presence of genetic structure between soil and leaf samples was analyzed using AMOVA. Peak season soil populations were compared with leaf populations obtained at concurrent or earlier samplings. Results showed significant genetic structure (significant Fsc values) between soil and leaf samples in 2010, but not in 2009 (Table 3).

When comparing genetic diversity indices between the two substrates sampled at the same time in Peak time, comparable *G* values were obtained in soil and leaves in the dry 2009. In the wet 2010, *R* from leaves (Peak: 0.282) is lower than *R* from 2009 leaves (Peak: 0.333), and is also lower than *R* from soil (0.386) calculated for the same year (Table 1, Fig. 3).

The minimum spanning network analysis showed that 4 of the soil-only MLGs most likely derived from soil progenitors, and the 3 other soil-only MLGs came from leaves. Soil and leaf genotypes did not segregate in different portions of the network. Singleton MLGs were generally located around the periphery of the network.

Spatial analysis within leaf and soil samples

Examining each substrate separately, genetic structure was identified among different plots both in leaves (variation=4.7%, Fsc=0.05436, p=0.01287) and soil (variation=11%, Fsc=0.122, p=0.000), while drainage was not significantly different for either substrate. When isolates from both substrates were grouped together by plot, there was still overall significant structure both between plots (variation=16.71%, FCT=0.16715, p=0.0002), and also between the substrates within those plots (variation=2.46%, FSC=0.02951, p=0.0005). Fst values among leaf populations sampled at the same time from different plots ranged between 0.152 and 0.321 (p-values≤0.001) (Table 1, Fig. 3). Fst was lowest (0.15) in the dry and cold winter/early spring sampling of 2009, and gradually increased throughout all successive samplings until the winter/early spring of 2010, when Fst values peaked at 0.32. A much lower Fst value (0.24) was recorded in late spring 2010 sampling, only three months after the previous 2010 sampling, and in conjunction with the first period of abundant rainfall and warm temperatures in three years. When the same analysis was performed on soil samples from both years, the Fst values among plots were found to be highly significant in the analysis both in 2009 and 2010 (Table 1).

For leaf populations, number and frequencies of MLGs present in at least two plots were compared between the Early and Peak 2010 samplings. The number of MLGs in at least two plots increased from 5 MLGs (representing 21 out of 57 isolations) in the 2010 winter, to 9 MLGs (representing 105 of 171 isolations) in the late 2010 spring (Fisher's exact test late spring > winter = P<0.001). For 8 of the 9 MLGs (89% of isolations) present in two or more sites in

Peak 2010, a specific MLG had never been detected in at least one of the sites during any of the four earlier samplings.

Spatial autocorrelation analyses

Analysis of how genetic distance correlated with spatial distance found the general patterns of genetic similarity vs. distance were similar for soil and leaves in 2009 (Fig. 7a,c), with significant aggregation of identical alleles until approximately 4m for both soil and leaves. In 2010, aggregation of identical alleles increased for both substrates, but at different rates. Significant aggregation was in fact detected up to 12m for soil and up to just over 200m for leaves (Fig. 7b,d).

Discussion

This is one of the first studies to examine the natural population dynamics for *Phytophthora ramorum*, and for a plant pathogen in general, in different substrates (soil and leaves) through time, with sampling encompassing a transition from a drought to a wet year. This study was not designed to determine an exact correlation between climatic parameters and population dynamics, but rather was focused on identifying shifts in pathogen dynamics at the transition between unfavorable and favorable conditions i.e. the abundant rainfall between the Early and Peak samplings of 2010 following the 2009 drought. This transition is clearly highlighted by the values of the Aridity index, a composite of rainfall and temperature data, which show a substantial change in conditions in 2010, from 2009, mirrored by significant changes in isolation success and in values of the several indices calculated for leaf populations. During this climatic transition, all indices of genetic diversity varied in the same direction in the entire dataset, and in the two drainages considered independently, indicating that a reliable generalized response had been identified. However, seasonal climatic variations during the drought often triggered differential responses in the two drainages, which can be addressed in future studies.

The ability to culture the pathogen from bay laurel leaves was taken as an indicator of its viability. Although viability may underestimate pathogen presence, due to symptomatic but non-culturable bay laurel leaves potentially harboring dormant *P. ramorum* infections (Chimento et al. 2011), it has been commonly used to study the portion of a pathogen population that responds rapidly to changing weather conditions and that is responsible for outbreaks and new disease cycles (Davidson et al. 2011; Cobb et al. 2012). It is clear that *P. ramorum* can recover extremely rapidly even after a long dry period. Viable infections were low and comparable throughout 2009 and in Early 2010, but incidence of viable infections almost tripled in only three months, as the wet period of prolonged rainfall occurred in the spring of 2010. This is one of the first quantifications of population recovery of this pathogen after an unfavorable period lasting three years.

Viable pathogen populations were larger during 2010, when the more prolonged and abundant rainfall in the spring generated conditions conducive to pathogen proliferation. The fast response of the pathogen to shifts in climatic conditions is supported by the significance of analyses using a 15d period prior to sampling, but not a 30d period. Indeed, isolation success was significantly greater in plots and sampling times that were wetter than average in the 15d period prior to sampling, i.e. Peak 2010. Concomitantly, overall allelic and genotypic richness were also slightly increased, while evenness of genotypes was reduced (i.e. a few highly abundant

genotypes dominate), and their migration levels increase more markedly. This correlation between genetic diversity and population size is to be expected in a clonally reproducing species such as *P. ramorum* or *P. cinnamomi*, where new genotypes arise through mutations and somatic recombination events which will happen in direct relationship to population size (Dobrowolski et al. 2001; Mascheretti et al. 2008).

These results agree with much work on infectious diseases and pests both of plants and animals (Koelle et al. 2006; Chapuis et al. 2008; Gordo et al. 2009). In an epidemic phase, favorable environmental conditions not only lead to increased reproductive rate, but also to the dominance of a few genotypes, potentially better adapted to transmission during outbreaks (Frank 1992). In our study, several MLGs clearly followed this pattern, e.g. MLG66 which went from being relatively rare in 2009 to being dominant in both soil and leaves in 2010. Although post-epidemic chronic conditions are characterized by the presence of relatively few genotypes, lack of transmission and migration of dominant genotypes in unfavorable conditions (Frank 1992) leads to higher relative local diversity, as reported for Dutch Elm Disease (Santini et al. 2005; Solla et al. 2008).

Soil populations did not display the increased levels of migration and the reduction in genotype evenness between 2009 and 2010 that were observed in leaf populations. It has been suggested that soil populations of *P. ramorum* are derived from foliar infestations (Fichtner et al. 2009): consequently our expectations were that trends in both substrates would have mirrored each other, and that source populations (i.e. infectious leaves where most sporulation occurs) would have been genotypically significantly more diverse than sink populations (e.g. soil). The minimum spanning network showed that MLGs found in soil and leaves do not segregate in different parts of that network, confirming that populations in the two substrates intermix. Furthermore, based on our minimum spanning network analysis, genotypes only found in the soil did not originate exclusively from either soil or leaf progenitor genotypes, but were generated in equal numbers from both. Alone, these analyses suggest that leaf and soil populations are overlapping in terms of genotype composition, as it would be expected if soil populations were derived from foliar ones. However, AMOVA identified a small but significant structure between populations in the two substrates, and statistical analyses determined that while frequencies and ranks of genotypes were comparable in the dry 2009, that was not the case in the wet 2010. We interpret this as the consequence of the dynamics of inoculation of the two different substrates, with infection of leaves being ecologically more stringent and strongly mediated by density of inoculum and ability of the pathogen to rapidly and competitively infect leaves. In contrast, soil inoculation may simply be a function of inoculum density and escape from predation.

A major factor in determining the extent and spread of disease is the volume and spatial distribution of sporangia spreading from infected plants, as well as host susceptibility and weather conditions. *P. infestans* and *P. capsici* are two other species known to produce aerial sporangia. *P. infestans* sporangiophores release wind-borne sporangia under dry conditions, thus facilitating medium to long-range dispersal (Skelsey). In contrast, sporangial release by *P. capsici* occurs in wet conditions, allowing sporangia to remain turgid (Granke et al. 2009, 2012) and resulting in their limited spatial spread. Our data and published literature (Davidson et al. 2005; Granke et al. 2009, 2012) suggest *P. ramorum* is similar to *P. capsici* in terms of dispersal, with sporangial release and distance covered by sporangia being strongly mediated by intensity and length of rain events. Spatial autocorrelation results show convincingly that in dry conditions, spatial aggregation of identical alleles stops at 4m from a source, independent of substrate. In wet conditions, that range increases to 12m for soil populations, and to over 200m

for leaf populations. This short to medium-range movement may be essential to cross the gaps in forest vegetation that are extremely common in California coastal woodlands. The ability of the pathogen to spread longer distances in environmentally favorable conditions is an additional component that may reduce Fst values, by increasing the pathogen's range of migration.

Previous estimates of cumulative multi-year movements for *P. ramorum* range between 0–350m, based on genetic (Mascheretti et al. 2009) and landscape level observations (Kelly and Meentemeyer 2002). This contrasts to *P. infestans*, which is extremely widely dispersed, facilitated both by human transport (across continents) and aerially dispersed over long distances (kilometers) via wind (Fry et al. 1992; Brown, and Hovmøller 2002). *P. ramorum* has also been introduced on multiple occasions into California by human transport, but from these introduction points it has spread relatively slowly via environmental dispersal (Mascheretti et al. 2009). Thus, in favorable environmental conditions, *P. ramorum* genotypes will arrive from more distant sources (medium range), but with lower inoculum densities.

Our results also show that in a natural setting, soil genotypes are capable of spreading either over equal (in a dry year) or much lower (in a wet year) distances than foliar genotypes, and thus soil populations may be less epidemiologically relevant than previously thought, with regards to spread of the pathogen within a forest or even between distinct forest stands. Of course, soil has been shown to be infectious (Fichtner et al. 2009) and infested soil has been reported past the margins of infested forests, especially along trails used by humans and ungulates (Davidson et al. 2005; Cushman and Meentemeyer 2008). Thus, there may be a significant epidemiological role for soil infestations in artificial settings or at the edges of forest stands.

Population size and genetic structure were both greatly affected by yearly climatic variation, and to a smaller degree by seasonal variations. This raises the issue of whether the bottlenecks experienced during the dry season, or during a drought, may lead to a significant change in the microevolutionary trajectory of the pathogen in the SFPUC watershed. In other words, was there a significant shift in genotypic composition, in particular following the 2007-2009 drought? Additionally, were both soil and leaf populations reservoirs providing pathogen genotypes that started the 2010 outbreak? AMOVAs indicated a very minimal change in the genetic structure of foliar populations, supported by a strong correlation between the rankings of MLGs found in consecutive years i.e. the few most dominant MLGs found in leaves in 2009, were also dominant in 2010. Conversely, soil populations displayed a significant difference between the 2009 and the 2010 samplings. Furthermore, instances where isolations from the same trees/area of soil in consecutive years yielded the same genotype were much more frequent in leaves than in soil, suggesting that infection persists more readily in leaves between years. Carry-over of identical genotypes between years may ensure an overall stable genetic composition of populations on leaves. In the case of soil, the low frequency of samples yielding the same genotype indicates that the soil population may not be as genotypically stable through time as that of leaves, and that pathogen survival is less than a year meaning that that soil was re-inoculated during the 2010 rainy season. Other studies of survival of *P. ramorum* in forest soil have indicated that viability of the pathogen was less than 1 year, and that survival was negatively correlated with temperature and organic richness of the top layer (Fichtner et al. 2009). While this apparent turnover in soil could be thought to be an artifact of insufficient sampling from a much more diverse pool of genotypes, our rarefaction analysis indicates that our soil sampling efforts were sufficient for the average allele frequencies to be comparable and to capture the extent of diversity. It should be noted that survival in soil may be longer than

recorded in this study if two consecutive wet years were to be considered. However two wet years are a relatively infrequent event in California, thus we believe that the results presented here identify a general pattern of persistence that differs significantly between leaves and soil. We conclude that oversummering of the pathogen occurs in leaves and not in soil; thus next year's disease cycle is started by genotypes that survive in bay laurel leaves, while soil genotypes may be epidemiologically less relevant in the natural disease cycle.

The six study plots were located 2-7.3 km apart, much closer than distances between plots in other previous studies in California (Mascheretti et al. 2008, 2009). To ensure good spatial and ecological coverage, study plots were equally distributed within two different drainages. While AMOVA detected no significant effect of drainage, there were significant genetic differences among the populations found at each plot. This is consistent with limited migration of the pathogen among even closely spaced sites, leading to strong founder effects (Mascheretti et al. 2008, 2009). It has been estimated that three founding genotypes are responsible for most California infestations (Mascheretti et al. 2008, 2009), hence it is likely that few founding genotypes started the infestations within the SFPUC watershed, giving rise to the observed differences between plots, simply because of stochastic effects, potentially amplified by the different rate of successful isolation among plots. However, when plots within a drainage are grouped and drainages are compared, the increased sample size and spatial coverage result in an obvious increase in the chances of capturing all or most of the founding genotypes and their progeny, thus eliminating the source of the genetic differences observed at the individual plot level. This interpretation points to a local diversity that is generated not by a successful process of local diversification, but rather by a lack of equilibrium among sites, due to limited levels of migration between spatially discrete infestations.

The reasons why the lowest Fst value was obtained in the winter/Early season of 2009 (the first sampling of this study) are not clear, however this result may be the legacy of the extremely favorable weather conditions of 2005 and 2006, when a major outbreak occurred. Fst values increased at each sampling time following that first sampling time until Early 2010. This is an interesting result showing the cumulative effects of persisting environmentally unfavorable conditions. In unfavorable conditions, genotypes that emerge locally are less likely to be challenged by successful and fast-spreading genotypes coming from other sites within the watershed. The first onset of favorable environmental conditions in the late spring/Peak season of 2010 resulted in a significant drop in Fst (Fig. 3). We surmise this lower Fst value may be the result of a) increased reproductive rate, and b) enhanced movement of successful genotypes among sites. Increased number of genotypes and tripled infection incidence in the wetter 2010 both support the validity of hypothesis a. The validity of hypothesis b is supported by the significant increase in the presence of the same genotype in two or more sites. The fact that these genotypes were missing from at least one of the sites in 89% of the cases, suggests they are not originating locally, but are migrating among sites (although obviously not just the six sites included in this study).

Results from this study can be unified into a schematic summary/model for the interaction between soil and leaf populations under contrasting environmental conditions. During an unfavorable year, population sizes are small both in soil and on leaves, due to repression of sporulation and transmission caused by the lack of favorable environmental conditions (Davidson et al. 2005; Hüberli et al. 2011). Infected leaves are a source of inoculum for both uninfected leaves and soil, hence a depression in sporulation will results in small population size (P_L) in both substrates. Similarly, these unfavorable conditions reduce both the

amount and distance of migration (M_L), leading to only moderate levels of competition between local and migrant genotypes (C_M) and resulting in high local genetic diversity (R_H), greater evenness (E_{5H}), and high genetic structure between sites (Fst_H), both for soil and leaves. In a rainy and warm year, favorable conditions facilitate an increase both in population size (P_H), and in migration/transmission between foliar hosts (M_H). In favorable conditions, high population size (P_H) and high migration (M_H, Fst_L) lead to a high level of competition for space and resources (C_H) resulting in the dominance of the more fit genotypes and in low genotypic diversity in leaves (R_L), and less evenness (E_{5M}), where transmission ability will be directly correlated with success of infection and establishment. A larger population in leaves during favorable conditions will also result in a more diverse soil population (P_H, R_H), but lack of long distance soil to soil movement (M_L) will result in high soil diversity and high evenness both within (R_H, E_{5H}) and among (Fst_H) sites. Although possible (Fichtner et al. 2009), it is unknown whether soil genotypes do actually infect leaves in the canopies of bay laurels.

We conclude that the epidemiology of Sudden Oak Death in California may be significantly altered by identifying what ecological factors characterize foliar refugial sites during droughts, and by attempting to reduce disease incidence on bay laurel leaves in these refugial sites during a dry year, when natural survival of the pathogen is lowes

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Figures and Tables

Table 1. Sampling and Isolation Summary, Monthly Aridity Index, AI_M, and Gene diversity indices for Soil and Leaf samples: Stoddart and Taylor's Index, *G*; Clonal genotype diversity, *R*; Evenness index, E_5; allelic richness with rarefaction, *Ar*; Fst values among sites calculated by AMOVA (p=0.0001).

Sampling Time	Aridity (AI_M)	Trees surveyed	Symptom Incidence	Sampled	Isolations	Isolation Success (%)	MLGs Detected		*G*	*R*	E_5	*Ar*	Fst
Soil and Leaf Totals				**2275**	**523**	**23**	**84**						
Leaf Early 2009	2.64	425	0.69	295	56	19	**35**	25	8.805	0.429	0.638	2.875	0.152
Leaf Peak 2009	**0.22**	**425**	**0.93**	**394**	**63**	**16**		**22**	**8.715**	**0.333**	**0.632**	**2.709**	**0.208**
Leaf Late 2009	2.22	425	0.91	388	39	10		15	9.377	0.378	0.798	2.6	0.244
Leaf Early 2010	44.17	425	0.79	334	57	17	**55**	23	10.249	0.393	0.654	2.831	0.321
Leaf Peak 2010	**0**	**425**	**0.83**	**354**	**173**	**49**		**49**	**14.687**	**0.282**	**0.593**	**3.075**	**0.242**
Soil 2009				**302**	**72**	**24**	**20**		**9.063**	**0.268**	**0.653**	**2.77**	**0.216**
Soil 2010				**308**	**59**	**19**	**23**		**12.277**	**0.386**	**0.726**	**3.136**	**0.202**

Table 2. Incidence of MLGs found in both years in leaf and soil populations.

Leaf				Soil			
MLG	**2009**	**2010**	**+/- % of 2009**	**MLG**	**2009**	**2010**	**+/- % of 2009**
43	35	27	-10.2	43	19	3	-21.2
52	24	37	1.1	57	6	8	5.5
57	15	11	-4.6	36	6	4	-1.4
102	13	15	-1.6	52	6	3	-3.2
59	11	5	-4.7	102	4	2	-2.1
48	8	5	-2.8	59	3	6	6.2
118	8	8	-1.5	48	3	1	-2.4
132	6	3	-2.5	66	2	10	14.5
106	4	6	0.1				
36	3	13	3.8				
66	3	27	10.0				
62	2	5	0.9				
149	2	1	-0.8				
19	2	7	1.8				
70	2	2	-0.4				
26	2	1	-0.8				
97	1	2	0.2				
9	1	1	-0.2				
137	1	2	0.2				
56	1	7	2.4				
11	1	2	0.2				
68	1	2	0.2				
94	1	2	0.2				
37	1	1	-0.2				
40	1	1	-0.2				
61	1	1	-0.2				
153	1	1	-0.2				

Table 3. Composite table of AMOVA results from 4 analyses where soil and leaf populations of *P. ramorum* are compared.

AMOVA Analysis	Samples compared	Source of Variation	df	Sum of squares	Variance component	% variation	Fixation index	F value	P value
i	**Early leaf 2009 vs Soil 2009**	Among plots: a plot is early leaf 2009 pooled with peak soil 2009	3	0.605	0.0096	19.95	Fct	0.1995	0.0191
		Between early leaf 2009 and peak soil 2009 by plot	3	0.093	-0.0005	-0.99	Fsc	-0.0124	0.4445
		Among individuals within plot from both early leaf 2009 and peak soil 2009	108	4.195	0.0389	81.04	Fst	0.1896	0.0000
		Total	114	4.893					
ii	**Peak leaf 2009 vs Soil 2009**	Among plots	3	0.693	0.0092	18.55	Fct	0.1855	0.0180
		Between leaf and soil	3	0.179	0.0011	2.23	Fsc	0.0274	0.0679
		Among individuals within plot	116	4.575	0.0394	79.22	Fst	0.2078	0.0000
		Total	122	5.448	0.0498				
iii	**Early leaf 2010 vs Soil 2010**	Among plots	3	1.048	0.0137	24.14	Fct	0.2414	0.0115
		Between leaf and soil	4	0.272	0.0021	3.74	Fsc	0.0493	0.0400
		Among individuals within plot	101	4.124	0.0408	72.12	Fst	0.2788	0.0000
		Total	108	5.444	0.0566				
iv	**Peak leaf 2010 vs Soil 2010**	Among plots	3	1.800	0.0114	19.73	Fct	0.1973	0.0083
		Between leaf and soil	4	0.367	0.0024	4.21	Fsc	0.0525	0.0009
		Among individuals within plot	213	9.330	0.0438	76.05	Fst	0.2395	0.0000
		Total	220	11.496	0.0576				

In the first hierarchical level (Fct) soil and leaf populations for each plot are pooled and plots are compared. In the second hierarchical level (Fsc) soil and leaf populations within each plot are compared to one another. In the third level (Fst) all individuals are compared by plot independent of substrate. Each of the four analyses independently compares an individual population of leaves sampled at a particular time with the soil sampled in the same year

Figure 1. Monthly climate data for the SFPUC watershed study area, taken from the RAWS weather station at Pulgas: (i) total rainfall (mm), (ii) air temperature – daily average and average monthly minimum and maximum.

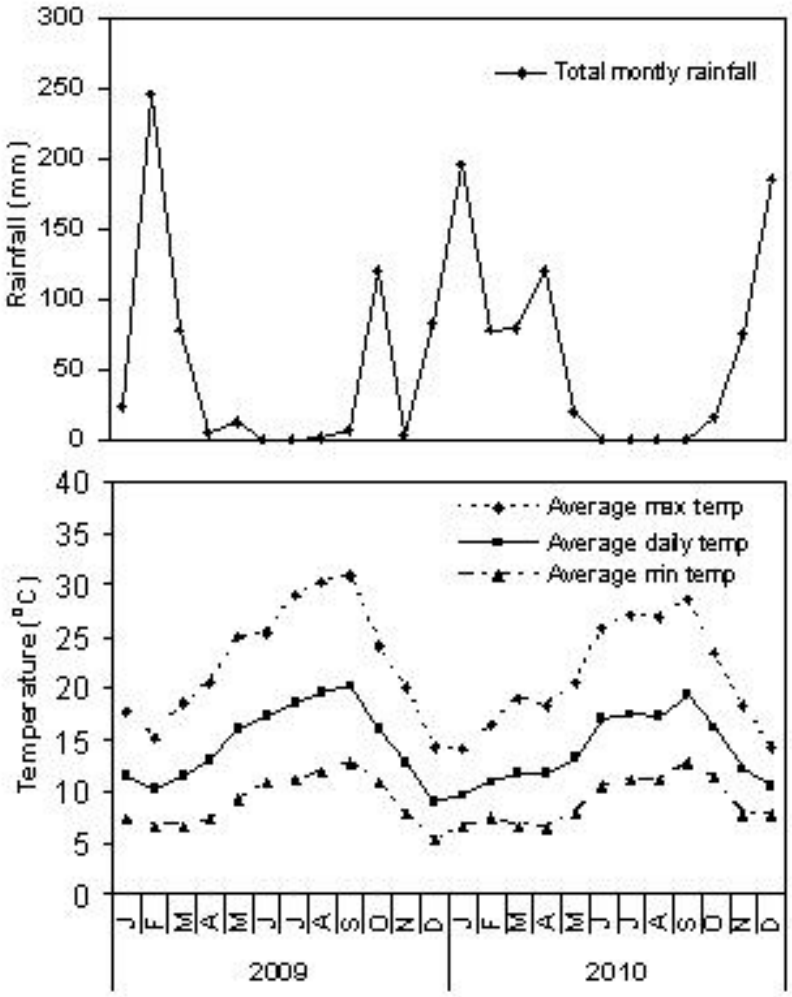


Figure 2. Success of isolation (%) of *P. ramorum* from leaf samples, number of multilocus genotypes (MLG) detected and the Monthly Aridity for each sampling time (2009: Early, Peak, Late; 2010: Early, Peak).

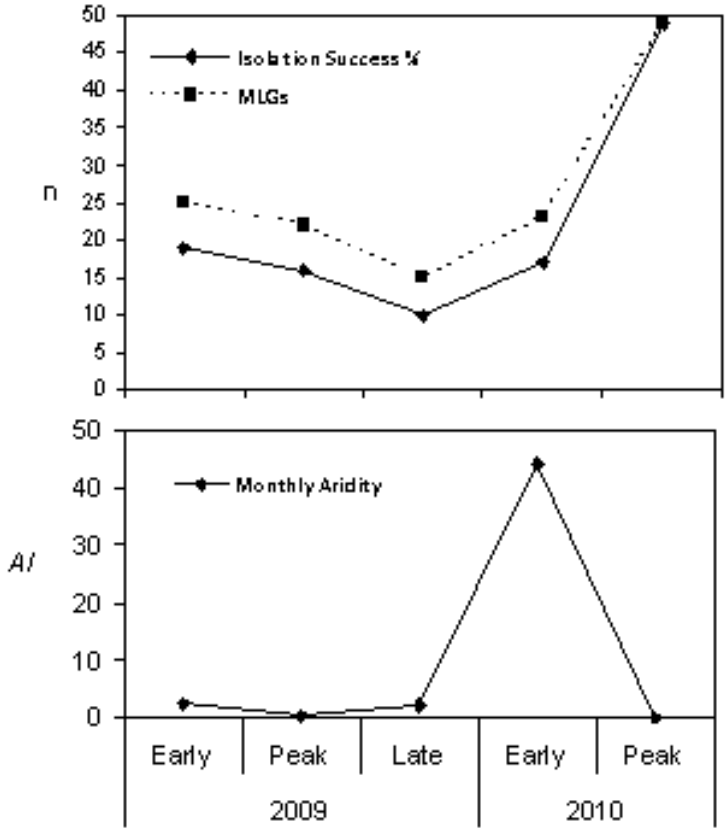


Figure 3. Genetic diversity indices compared between soil and leaf populations for each sampling time (2009: Early, Peak, Late; 2010: Early, Peak): (i) Allelic richness, *Ar*, (ii) Stoddart and Taylor's index, *G*; (iii) Clonal genotypic diversity, *R*; (iv) Evenness index, E_5; (v) Genetic structure, Fst, of individual populations partitioned by plot calculated by AMOVA.

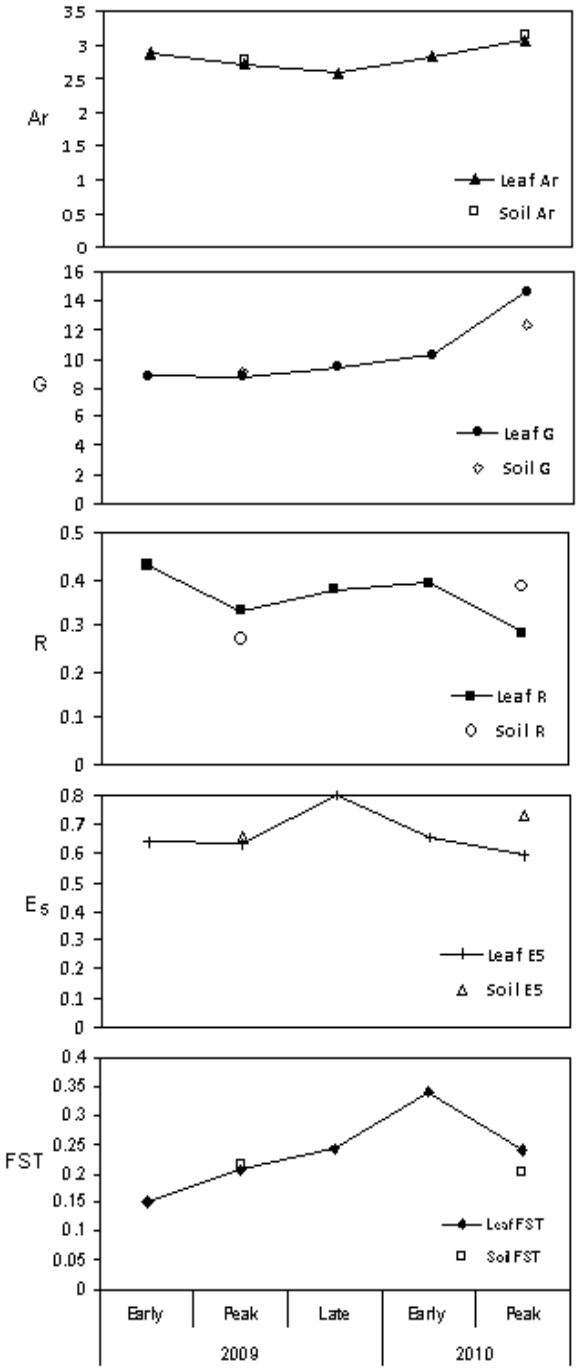


Figure 4. Genotype accumulation curves calculated using rarefaction for the mean number of distinct alleles per locus, for soil and leaf population at each sampling time.

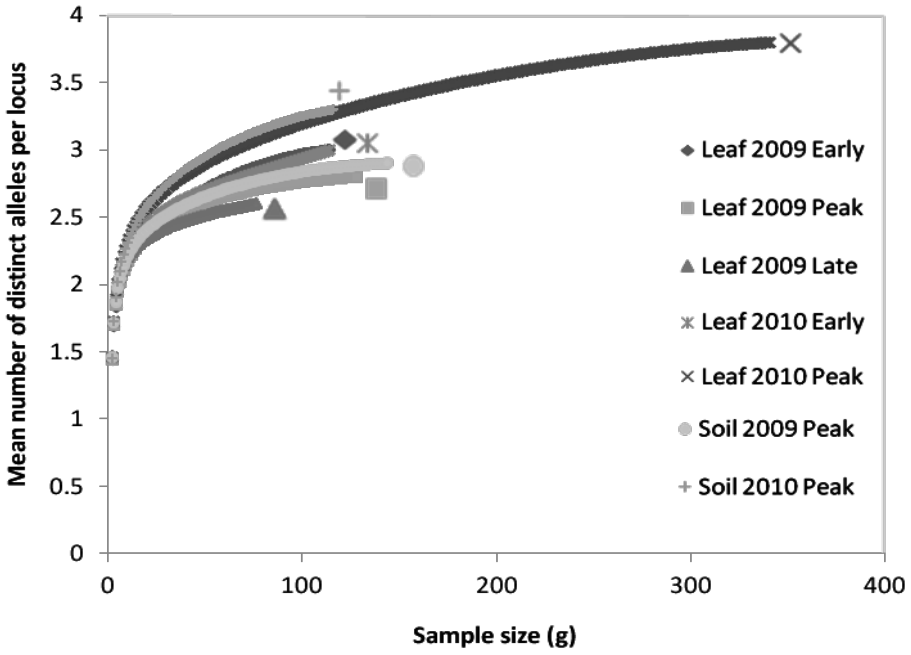


Figure 5. Comparison of the relative abundance of the overlapping multilocus genotypes (MLG) that were isolated from both soil and leaf samples in 2010. Values are the percentage of the whole population that each MLG represents in either soil or leaves.

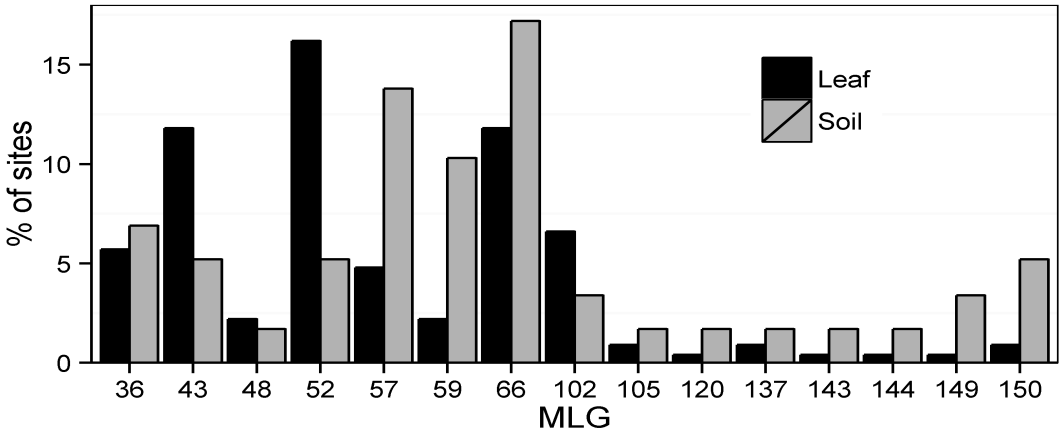


Figure 6. Minimum Spanning Network for all multilocus genotypes (MLG) detected in 2010. Each node represents a unique MLG. Node shape indicates the substrate from which an MLG was isolated: circle = leaf, square = soil, triangle = both soil and leaf. Nodes are shaded black for singleton MLGs (those represented by only 1 individual), and scaled proportionally to the number of individuals represented with that MLG: 1(black)=singletons, 2=2-5 individuals, 3=5-10, 4=11-20, 5=21-30, 6=31-40. Arrows mark the most likely progenitors of soil only MLGs.

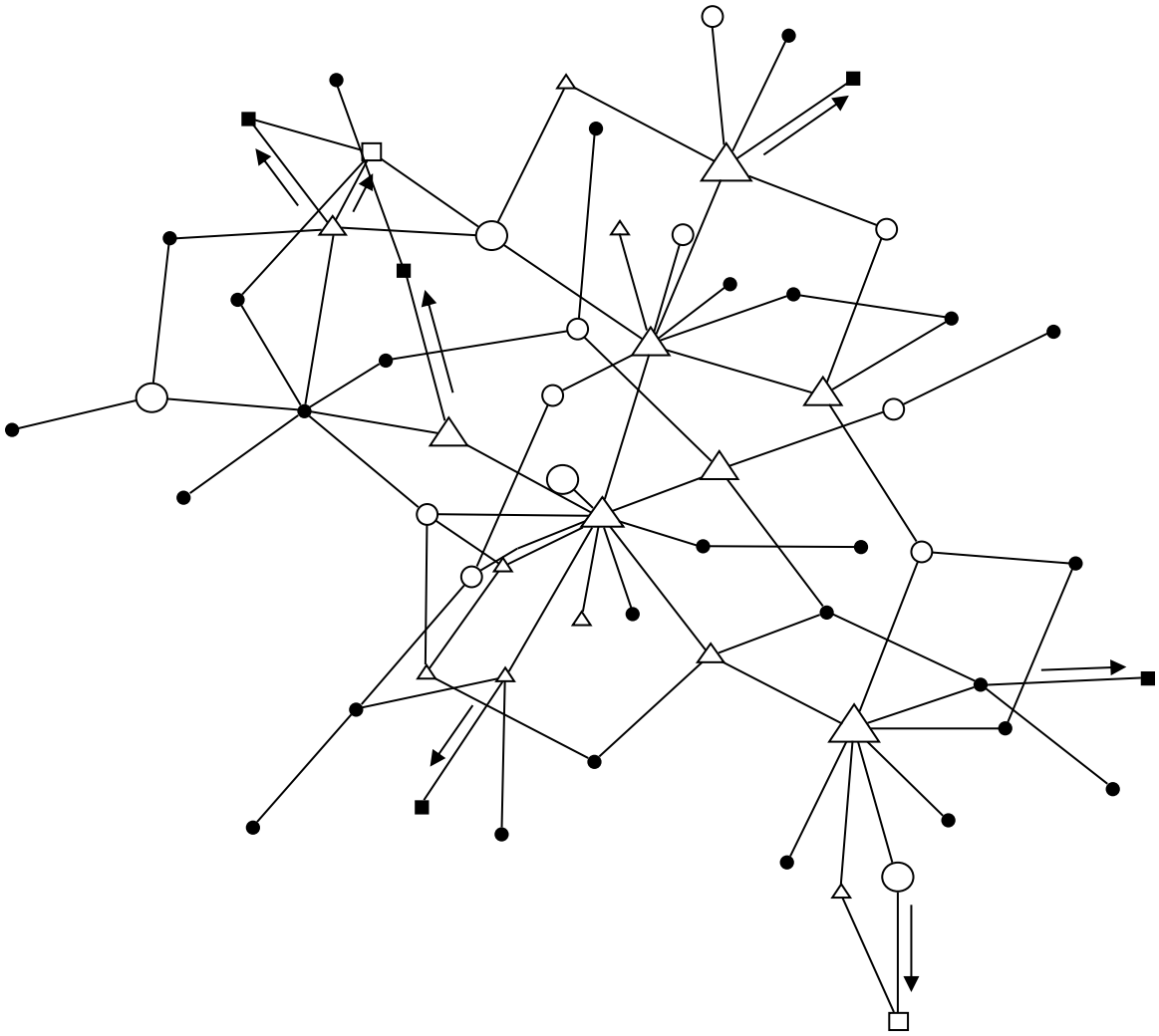


Figure 7. Genetic similarity, Moran's *I*, plotted against distance (m), with upper and lower significance thresholds plotted: (a) Soil 2009; (b) Soil 2010; (c) Leaf 2009; (d) Leaf 2010.

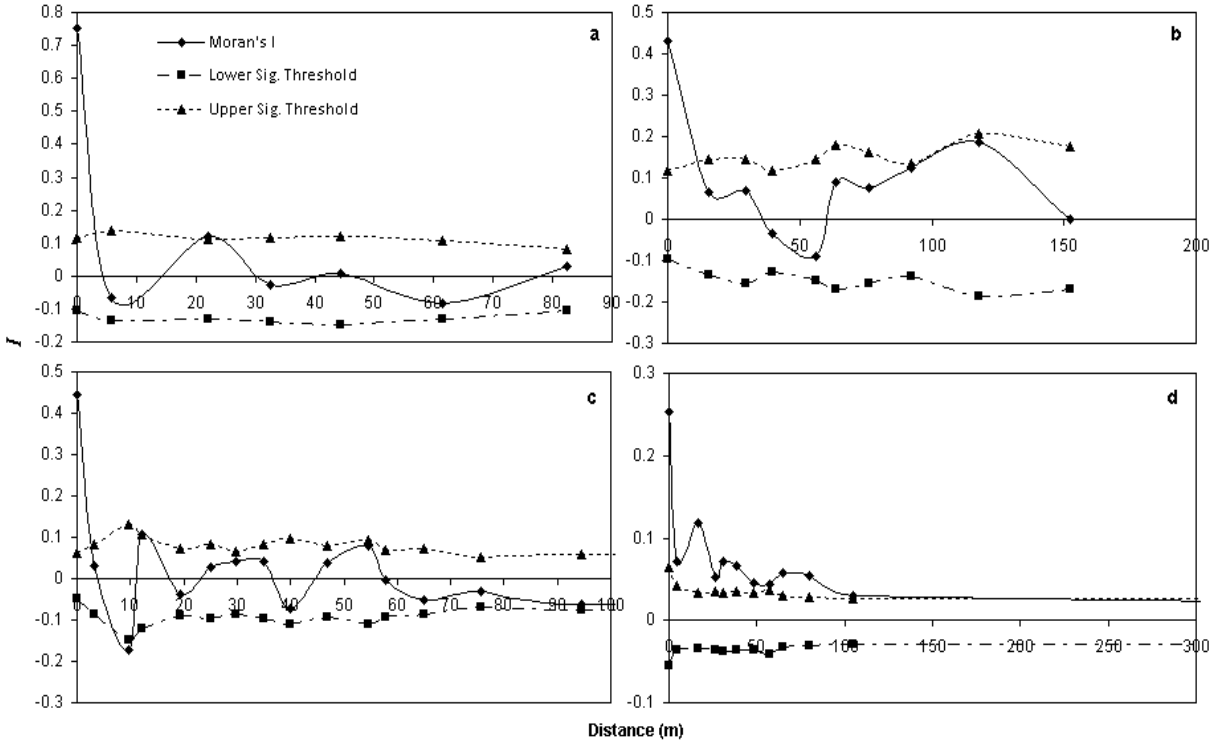
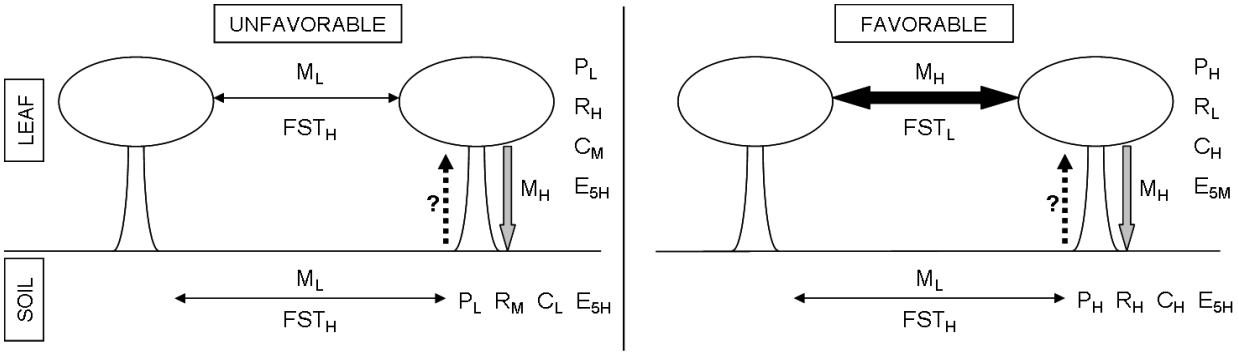


Figure 8. Schematic summary of the interaction between leaf and soil populations in contrasting environmental conditions, with respect to the effect on population size, diversity, genetic structure, migration and competition. P - Population size; R – Local genotypic diversity; C – Competition; M – Migration; E_5 - Evenness. Subscripts represent relative levels of each process taking place: L – Low; M – Medium; H – High.



Supplementary Table 1. Microsatellite primers used for amplification of *P. ramorum* DNA

ID	Locus	Repeat motif	Primer Sequence (5'-3')
18*	18	$(AC)_{39}$	F: [6~FAM]TGCCATCACAACACAAATCC
			R: TGTGCTATCTTTCCTGAACGG
39†	39a, 39b	$(GA)_{11}$	F: [6~HEX]GCACGGCCAGAGATTGATAG
			R: ATCTGCCGACGTGAAGAAGT
43†	43a	$(CAGA)_{71}$	F: [6~FAM]AAATATGCAAAAAGGCAGGA
		$(CAGA)_{75}(\ldots)(GAGA)_{17}$	R: CCGCGTAACCTAGTCTGCTC
45†	45	$(TCCG)_{11}$	F: [6~FAM]CGTGCTGCATCTGGTGTAGT
			R: GAAAGTCCGGATTTGCGTTA
64*	64	$(CT)_{16}$	F: [6~FAM]GCGCTAAGAAAGACACTCCG
			R: CAACATGTAGCCATTGCAGG
ILVO PrMS145‡	ILVO PrMS145a,b,c	$(AGCGAC)_{15}$	F: [6~FAM]CAGGAAACAGCTATGACCTG GCAGTGTTCTTCAACAGC
			R: ATTCCCGTGAACAGCGTATC

* from Ivors *et al.* (2006)

† from Prospero *et al.* (2007)

‡ from Vercauteren *et al.* (2010)

Supplementary Table 2. Allele sizes for the multilocus genotypes (MLG) identified in 2009 and 2010. Fragment analysis was conducted on an ABI 3730 sequencer (Applied Biosystems)

MLG	n	18	39a	39b	43a	43b	45	64	145b	145a	145c
2	1	222/-	131/131	248/248	366/366	478/478	167/187	343/381	168/179	168/200	168/243
9	3	222/-	131/131	248/248	370/370	478/478	167/188	343/381	168/179	168/200	168/243
11	3	222/-	131/131	248/248	370/370	482/482	167/187	343/381	168/179	168/200	168/243
17	2	222/-	131/131	248/248	374/374	478/478	167/188	343/381	168/179	168/206	168/243
18	1	222/-	131/131	252/252	370/370	474/474	167/187	343/381	168/179	168/200	168/243
19	9	222/-	131/131	252/252	370/370	478/478	167/187	343/381	168/179	168/200	168/243
22	1	222/-	131/131	248/248	374/374	478/478	167/188	343/381	168/179	168/200	168/238
23	2	222/-	131/131	252/252	370/370	478/478	167/188	343/381	168/179	168/200	168/243
25	2	220/271	131/131	252/252	366/366	490/490	167/187	343/381	168/179	168/200	168/243
26	3	220/271	131/131	248/248	366/366	490/490	167/187	343/381	168/179	168/200	168/243
33	1	220/275	131/131	248/248	354/354	478/478	167/187	343/381	168/179	168/200	168/243
36	26	220/275	131/131	248/248	366/366	482/482	167/188	343/381	168/179	168/200	168/243
37	2	220/275	131/131	248/248	366/366	486/486	167/187	343/381	168/179	168/200	168/243
40	2	220/275	131/131	248/248	366/366	490/490	167/187	343/381	168/179	168/200	168/243
41	1	220/275	131/131	248/248	370/370	466/466	167/187	343/381	168/179	168/200	168/243
42	2	220/275	131/131	248/248	370/370	474/474	167/188	343/381	168/179	168/200	168/243
43	84	220/275	131/131	248/248	370/370	482/482	167/188	343/381	168/179	168/200	168/243
44	1	220/275	131/131	248/248	370/370	482/482	167/187	343/379	168/179	168/200	168/243
45	1	220/275	131/131	248/248	370/370	482/482	167/187	343/381	168/179	168/222	168/243
48	17	220/275	131/131	248/248	370/370	486/486	167/188	343/381	168/179	168/200	168/243
51	1	220/275	131/131	248/248	370/370	482/482	167/187	343/381	168/179	168/206	168/243
52	70	220/275	131/131	248/248	370/370	490/490	167/188	343/381	168/179	168/200	168/243
54	1	220/275	131/131	248/248	370/370	490/490	167/187	343/381	168/179	168/200	168/249
55	1	220/275	131/131	248/248	370/370	490/490	167/187	343/385	168/179	168/200	168/243
56	8	220/275	131/131	248/248	370/370	494/494	167/187	343/381	168/179	168/200	168/243
57	40	220/275	131/131	248/248	374/374	482/482	167/188	343/381	168/179	168/200	168/243
58	3	220/275	131/131	248/248	374/374	482/482	167/187	343/381	168/179	168/200	168/238
59	25	220/275	131/131	248/248	374/374	486/486	167/188	343/381	168/179	168/200	168/243
60	1	220/275	131/131	248/248	374/374	482/482	167/187	343/381	168/179	168/200	168/249
61	2	220/275	131/131	248/248	374/374	482/482	167/187	343/383	168/179	168/200	168/243
62	8	220/275	131/131	248/248	374/374	490/490	167/188	343/381	168/179	168/200	168/243
63	1	220/275	131/131	244/244	374/374	486/486	167/187	343/381	168/179	168/200	168/249
65	1	220/275	131/131	248/248	378/378	482/482	167/187	343/381	168/173	168/200	168/243
66	42	220/275	131/131	248/248	378/378	482/482	167/188	343/381	168/179	168/200	168/243
67	2	220/275	131/131	248/248	378/378	482/482	167/187	343/381	168/179	168/200	168/238
68	3	220/275	131/131	248/248	378/378	486/486	167/187	343/381	168/179	168/200	168/243
70	4	220/275	131/131	252/252	370/370	482/482	167/187	343/381	168/179	168/200	168/243
71	1	220/275	131/131	252/252	370/370	482/482	167/187	343/381	168/179	168/200	168/238
72	4	220/275	131/131	252/252	370/370	486/486	167/188	343/381	168/179	168/200	168/243
73	1	220/275	131/131	252/252	374/374	486/486	167/188	343/381	168/179	168/200	168/243
74	1	220/275	131/131	252/252	374/374	490/490	167/187	343/381	168/179	168/200	168/243
75	1	220/275	131/131	252/252	370/370	490/490	167/187	343/381	168/179	168/200	168/243
77	1	220/275	131/131	244/244	370/370	482/482	167/188	343/381	168/179	168/200	168/243
87	1	220/275	131/131	248/248	362/362	478/478	167/188	343/381	168/179	168/200	168/243
88	1	220/275	131/131	248/248	362/362	482/482	167/187	343/381	168/179	168/200	168/243
89	1	220/275	131/131	248/248	362/362	482/482	167/187	343/383	168/179	168/200	168/243
94	3	220/275	131/131	248/248	366/366	478/478	167/187	343/381	168/179	168/200	168/243
97	5	220/275	131/131	248/248	366/366	482/482	167/188	343/383	168/179	168/200	168/243
102	34	220/275	131/131	248/248	370/370	478/478	167/188	343/381	168/179	168/200	168/243
105	3	220/275	131/131	248/248	370/370	482/482	167/188	343/381	168/179	168/200	168/238
106	10	220/275	131/131	248/248	370/370	482/482	167/187	343/381	168/179	168/200	168/249
108	1	220/275	131/131	248/248	370/370	482/482	167/187	343/381	168/179	168/227	168/238
118	19	220/275	131/131	248/248	374/374	478/478	167/188	343/381	168/179	168/200	168/243
119	2	220/275	131/131	248/248	374/374	482/482	167/188	343/381	168/179	168/227	168/243
120	2	220/275	131/131	248/248	378/378	478/478	167/188	343/381	168/179	168/200	168/243
129	1	220/275	131/131	252/252	370/370	478/478	167/187	343/381	168/179	168/200	168/243
132	14	220/275	131/131	252/252	374/374	482/482	167/188	343/381	168/179	168/200	168/243
135	1	220/275	131/131	248/248	351/351	482/482	167/188	343/383	168/179	168/200	168/243
136	1	220/275	131/131	248/248	370/370	482/482	167/188	343/381	168/179	168/200	168/232
137	4	220/275	131/131	248/248	370/370	482/482	167/188	343/383	168/179	168/200	168/243
138	1	220/275	131/131	248/248	374/374	478/478	161/188	343/381	168/179	168/200	168/243
139	1	220/275	131/131	248/248	378/378	482/482	147/188	343/381	168/179	168/200	168/243
141	2	220/277	131/131	248/248	370/370	494/494	167/188	343/381	168/179	168/200	168/243
142	1	220/277	131/131	248/248	366/366	490/490	167/188	343/381	168/179	168/200	168/243
143	2	220/277	131/131	248/248	370/370	482/482	167/188	343/381	168/179	168/200	168/243
144	2	220/277	131/131	248/248	370/370	489/489	167/188	343/381	168/179	168/200	168/243
146	1	220/279	131/131	248/248	370/370	486/486	167/187	343/381	168/179	168/200	168/243
149	5	220/279	131/131	248/248	370/370	482/482	167/188	343/381	168/179	168/200	168/243
150	2	222/-	131/131	248/248	374/374	478/478	167/187	343/381	168/179	168/200	168/243
151	2	222/-	131/131	248/248	374/374	482/482	167/187	343/381	168/179	168/200	168/249
152	1	222/-	131/131	252/252	370/370	482/482	167/187	343/381	168/179	168/200	168/238
153	2	222/-	131/131	252/252	374/374	478/478	167/187	343/381	168/179	168/200	168/243
159	1	230/275	131/131	248/248	370/370	456/456	167/187	343/381	168/179	168/200	168/243
160	1	222/-	131/131	248/248	370/370	478/478	167/187	343/381	168/179	168/200	168/238
161	1	222/-	131/131	248/248	370/370	478/478	167/187	343/381	168/179	168/206	168/243

Chapter 4

Population genetics clarify the epidemiological relationships among the three main California hosts of the pathogen *Phytophthora ramorum*, causal agent of Sudden Oak Death

Abstract

It has been demonstrated that California bay laurel (*Umbellularia californica*) is the major transmissive host of *Phytophthora ramorum,* the causal agent of Sudden Oak Death (SOD), in coastal California oak woodlands, causing mortality in both tanoak (*Notholithocarpus densiflorus*) and coast live oak (*Quercus agrifolia)*. However little is known about the population structure of the pathogen in each of these hosts, and no direct evidence has been provided on contagion pathways among these three main hosts. Here we employ a population genetics approach to identify the relationship among *P. ramorum* populations in bay laurels, oaks and tanoaks to clarify the contribution that each host may have on the epidemiology of SOD and on the microevolution of its causal agent. Additionally, we explore differences in population structure in wet vs. dry years and at sites with various levels of disease incidence and prevalence, within a single watershed where the disease is known to be in an endemic phase. We conclude that structure exists between hosts, that bay laurel is the source population for both tanoak and oak, and that tanoak contributes minimally to oak infection. We also conclude that in spite of their common source of inoculum, oaks and tanoaks are sinks that select for different pathogen genotypes due to the variance in selection pressure in each host type. Finally we conclude that different sites supported a dominance of different genotypes, more genotypes overall and more persistent genotypes, when compared to other sites, and that these 'hotspots' are likely to play a more significant epidemiological and evolutionary role for the pathogen.

Introduction

Sudden Oak Death (SOD) caused by *Phytophthora ramorum* is an introduced forest disease, recently become endemic in coastal Northern California and Southwestern Oregon (Garbelotto et al. 2020). The pathogen was introduced to California in the 1990's via infected nursery stock from Europe. To date, four clonal lineages have been detected worldwide outside the area where it is native (Mascheretti et al. 2009, Van Poucke et al. 2012), but only one lineage (NA1) has been detected in forest populations in California (Ivors et al. 2004), while two lineages (NA1 and EU1) are currently present in Oregon (Søndreli et al. 2019).

The Oomycete pathogen (Kingdom: Straminopila, Order: Peronosporales) (Werres et al. 2001) causes high levels of mortality in California forest settings, mostly of coast live oak (*Quercus agrifolia*) and tanoak (*Notholithocarpus densiflorus*). Bole infections of oaks and tanoaks cause lesions that eventually kill the host by girdling them, however these infections themselves are not transmissive (Garbelotto and Hayden 2012). Conversely, the primary infectious hosts and most commonly infected trees in California forests are California bay laurels (*Umbellularia californica*) and tanoaks (Garbelotto et al. 2003, Davidson et al 2005). California bay laurels and tanoaks in addition to over 100 other (minor) species thus serve as foliar hosts for the pathogen (USDA APHIS 2008), meaning sporulation and production of infectious sporangia occurs on the leaves of these hosts. Foliar and petiole infections cause localized tissue necrosis and branch die back in some hosts, but the plants themselves are not killed by the pathogen, if not after repeated infections in the course of multiple years (Garbelotto and Hayden 2012). On bay laurels, the tips of leaves, or any leaf margin where water pools will develop black pixilated lesions surrounded by a yellow discoloration when infected (Garbelotto et al. 2002), while on tanoaks, infection will damage the petiole and the mid vein of infected leaves: foliar symptoms become visible about one week after infection (Garbelotto et al. 2002, Davidson et al. 2005, Cobb et al. 2012). Bole cankers, on the other hand, may be visible mostly due to an excess of sap weeping out of the bark at the site of the canker, but only a few months after infection has occurred and not in all infected individuals. Eventually, as the cambial tissue is destroyed and the vascular system is compromised, the canopy will brown "suddenly", retaining the dead leaves, until the trees structural integrity is compromised by secondary agents and trees inevitably fail (Garbelotto et al. 2002, Davidson et al. 2005).

Several California oaks are considered to be epidemiological dead-end hosts, given that sporulation does not occur in their cambial tissue and the disease is not transmitted to other individuals. Interestingly, tanoaks act both as foliar/infectious and bole/dead-end hosts. Sporulation on foliar hosts is triggered by environmental cues and is markedly increased in late spring, when conditions are both warm and wet (Davidson et al. 2005, Hüberli et al. 2011). The majority of transmission occurs during this period and the pathogen survives in a dormant state until ideal conditions for sporulation return (Garbelotto et al. 2017, Kozanitas et al. 2020).

While it has been well documented that bay laurel populations drive the epidemiology of *P. ramorum* in oak woodlands by being the host supporting the greatest level of pathogen sporulation (Garbelotto et al. 2003, Davidson et al. 2005), it is also known that important levels of sporulation occur on twigs, petioles and leaves of tanoaks in California coastal forests. There is also a known and significant impact on tanoak in California, but rates of disease incidence and mortality are not nearly as severe in Oregon (Cobb et al. 2012), where targeted bay laurel removal in tanoak stands has been recommended. In California forests however, where bay laurel is a major player in disease dynamics, this is not a realistic option. Disease patterns have

been looked at from an ecological standpoint in California woodlands (Cobb et al. 2012, Garbelotto et al. 2017, Kozanitas et al. 2020) where research has improved our understanding of the transmission of inoculum from bay laurel onto oaks and tanoaks. Conclusions from that research (Garbelotto et al. 2017, Kozanitas et al. 2020) has indicated that there is a strong relationship between bay laurel disease prevalence and oak infection, but has also indicated that tanoak, which does not reach the levels of sporulation as observed on bay laurel, does not significantly contribute to oak infection. In fact, in forest stands dominated by tanoak, with low levels of bay laurel abundance, there is noticeably less infection of oak (Cobb et al. 2012, Garbelotto et al. 2017). Density of bay laurels and proximity between oaks or tanoaks and bay laurels are both strongly associated with disease transmission from bay laurel to oaks and tanoaks (Cobb et al. 2012, Kozanitas et al. 2020). The 'bay laurel as a major source of inoculum' epidemiological hypothesis has been in part confirmed by an experimental study testing *P. ramorum* inoculum potential in sites where bay laurels where removed. Results confirmed that inoculum levels high enough to infect oaks could be found only within 10m from bay laurels and only in plots where this transmissive host was not removed (Garbelotto et al. 2017). In the same study, a negative relationship between abundance of tanoak and incidence of oak infection was also identified (Garbelotto et al. 2017). Thus when bay laurel density is low and tanoak density is high, the levels of inoculum being produced on the leaves of tanoak are likely to be below the threshold necessary to successfully infect nearby oaks. However, infected tanoak leaves are able to infect themselves and their neighbors, causing bole cankers and petiole lesions (Davidson et al. 2005, Cobb et al. 2012, Garbelotto et al. 2017).

There is no understanding of how significant tanoak to bay laurel infection may be, nor has bay laurel to oak infection been proven using direct epidemiological evidence (e.g. the identification of contagion by the same Multilocus genotype (MLG) on both hosts). The close proximity of infected bay laurel branches to infected oaks and the decrease in inoculum to levels too low to infect oaks as bay laurels are removed both show that a correlation exists between bay laurel and disease incidence in oak (Kozanitas et al. 2020), but do not necessarily prove that bay laurel to oak infection is occurring.

Here, for the first time, we employ a population genetics approach to identify the relationship among *P. ramorum* populations in bay laurels, oaks and tanoaks to clarify the contribution that each host may have on the epidemiology of SOD and on the microevolution of its causal agent. Two similar studies on the population genetics of *P. ramorum* in different substrates, namely aerial populations found on the leaves of bay laurels, populations found in soil (Eyre et al. 2013) and populations found in water (Eyre et al. 2015) concluded that the epidemiology of SOD is driven by populations on bay laurel leaves, where, during favorable climatic conditions, a few pathogen genotypes are responsible for most of the infection of plants (Eyre et al. 2013), and for the inoculation of soil and water (Eyre et al. 2015). However, those studies also discovered that the pathogen is subjected to different selection pressures in soil and water than in leaves (Eyre et al. 2013, 2015). In soil, which is essentially a dead end for this pathogen with an unusual aerial biology (Garbelotto and Hayden 2012), selection pressure is laxed, while in leaves, individuals must be fit and are in competition to sporulate and infect new substrate. This differential selection pressure results in the survival of different genotypes in the three different substrates. Bay laurel populations are clearly more diverse and act as a source of inoculum, while soil and water act uniquely as a sink for the pathogen, where both dominant foliar pathogen MLGs as well as MLGs that are not detectable in leaves can be observed (Eyre et al. 2013, Eyre et al. 2015). Hüberli *et al.* (2011), Kozanitas *et al.* (2017), and Eyre *et al.* (2013,

2015) have all concluded that sporulation in bay laurel as well as bay laurel-dependent infection are strongly and positively mediated by increasing rainfall. However, Kozanitas *et al.* (2020) and Garbelotto *et al.* (2017) have shown that new oak infections only occur in years with extremely high rainfall level. Oak infection in fact requires very high levels of inoculum (Garbelotto et al. 2017) and is thus mediated by the presence of a high threshold in rainfall, when high precipitation levels and the presence of favorable climatic conditions result in high sporulation by the pathogen.

The previous works by Eyre *et al.* (2013 & 2015) and the field studies on the biology and sporulation potential of the pathogen in different forest ecosystems (Davidson et al. 2005, Cobb et al. 2012, Hüberli et al. 2011, Garbelotto et al. 2017, Kozanitas et al. 2020) provide the necessary framework to formulate the hypotheses below, aimed at providing more direct evidence on how the three main California forest hosts may be interrelated in terms of representing sources and/or sinks of infection:

1. Our experimental design, based on 63 transects in 15 sites at least 2 km apart from one another and within the same watershed, provide an excellent experimental design in which inoculum in one plot should not affect inoculum in another, given the documented spread potential of the pathogen being less than 2 km (Mascheretti et al. 2008, Eyre et al. 2013). At the same time, given that all plots share the same invasion history, establishment of the pathogen should have occurred at the same time across sites and genetic differentiation among study plots should be minimal. These are perfect requirements for a truly replicated study, with only minor effects of site and genetics of pathogen populations on the results. Our assumption was that site ecology and climate would also affect populations of the pathogen, with cooler sites characterized by higher density of infectious hosts (a.k.a. “hotspots”) supporting larger and more diverse pathogen populations. Population genetics indices should reflect these hypotheses as follows: a) Fst values should be very low among plots proving the similar genetic background of plots, but dominant MLGs may be different among sites proving that distance among sites is sufficient to regard them as not being interdependent; b) MLG and private allele diversity should be higher in the wet season and in wet years when the pathogen sporulates more heavily and populations increase in size; in dry years MLG evenness (i.e. equal MLG representation in a population) should be high given that conditions are not favorable to infectious outbreaks, while as rainfall levels increase a few dominant MLGs should cause most of the infections, drastically reducing evenness; c) Hotspots should have more diverse populations with dominance of one or few genotypes reflected by lower evenness index values.
2. The most abundant/ dominant MLGs in the bay laurel population will also be present in the oak population because the oak population is entirely derived from the bay laurel population and several MLGs will be found both in oaks and bay laurels. The Gst value (genetic distance) between bay laurel pathogen populations and oak populations should be extremely low, given that bay laurels are the main source of oak infections. Migration rates from bay laurel to oaks should be very high, if indeed bay laurels are the main source of infection for oaks, and oaks act simply as sink. Pathogen MLG evenness fluctuates in bay laurel populations due to the fact that infection and sporulation on bay laurel may occur every year in the presence of any precipitation, and populations grown and shrink accordingly on this host. Conversely, oaks are only infected by highly infectious and overrepresented MLGs during exceptionally wet years: hence MLG

diversity should be significantly lower on oaks while pathogen MLG evenness should be strikingly high, given that minor MLGs which are not competitive in wet years or proliferate in dry years will be never be able to attain the sporulation levels that are necessary to infect oaks.

3. Bay laurels should also represent a significant source of inoculum for tanoaks, but differently from the oak-bay laurel relationship, dominance of MLGs and number of MLGs shared by both hosts may be less apparent, given the fact that tanoaks themselves also serve as a source of inoculum for other tanoaks. Consequently, Gst values should be intermediate and lower than for the bay laurel-oak comparison. Nonetheless, given the high level of pathogen sporulation on bays laurels, this host will still be a significant source of inoculum, and migration rates from bay laurel to tanoaks should be high, possibly even higher than between bay laurel to oaks because of the potential for reproduction on tanoaks of MLGs originated from bay laurel. Inverse tanoak to bay laurel migration should instead be much lower.
4. Tanoak-oak relationship may be in part affected by tanoaks being a marginal source of infection of oaks. This source-sink relationship may be identified by intermediate levels of tanoak to oak migration, much lower than levels of bay laurel-oak migration. However, intermediate migration levels may in part be the result of the fact that some of the same MLGs originated on bay laurel infect both oaks and tanoaks. If indeed tanoaks provided only a marginal source of inoculum for oaks, we would expect oak-tanoak Gst levels to be higher than for other pairwise host combinations. If bay laurels played a major role as a source for both oaks and tanoaks, the bay laurel population should be most diverse in terms of MLGs and private alleles, Gst between bay laurel and either host should be lower, and a principal components analysis should show bay laurel populations significantly overlapping with both oak and tanoak populations. However, given that selection pressures may be different between bay laurels and the other two hosts, and also between oaks and tanoaks, we expect the principal components analysis to identify a significant component of oak and tanoak populations that are different from bay laurel populations, but that are also not overlapping with one another.

Confirming the above hypotheses would greatly improve our understanding of the epidemiology of SOD in mixed California woodlands characterized by the presence of oaks, tanoaks and bay laurels and provide pivotal data for disease predictions and for the design of sound disease control strategies.

Methods

Field Site Selection/Habitat Description

The field portion of this study was conducted within an existing plot network, established in 2008 (Kozanitas et al. 2017, 2020), in the San Francisco Public Utility Commission (SFPUC) watershed in central San Mateo County, California (37°31'10.3"N 122°22'08.2"W). A total of 15 research plots were monitored repeatedly from 2008 to 2012, during pre-selected times of year (see below). The watershed ranges in elevation between 95 and 1050 m, and plots were located in one of two major drainages within the 9,300 hectare watershed (23,000 acres), either the Pilarcitos or Crystal Springs drainage, with the Pilarcitos drainage being on average higher in elevation than the Crystal Springs drainage. Each plot contained three transects 100m long and 10m wide, radiating from a center point, a bay laurel stem and tanoak stem (if present) was

selected and tagged for repeated surveying at each 10m increment along each transect. A stem was defined as any major branch of a tree separated from the main stem below breast height (1.4m) with a diameter at breast height (DBH) greater than 1cm. All oak stems along each transect were tagged and monitored once per year for five years. At three plots per drainage (total of six) three additional transects were added, as well as a circle 30m in diameter surrounding the six plot centers, where all host species were tagged for a more robust surveying schematic. All plots were located a minimum of 2km apart to avoid spatial autocorrelation between sites. This distance was based on a priori knowledge of infectious propagule mobility stating airborne inocula typically travel either locally (between 10-100m) in rain events or long distance (up to 1km) in high wind events (Mascheretti et al. 2008). Rainfall data were retrieved from the CA Department of Water Resources (CA-DWR; RAWS database, Western Regional Climate Center, wrcc@dri.edu) and SFPUC data archives of the Crystal Springs Cottage rain gauge (37°28'08.4"N 122°19'44.4"W)

Sampling Schematic

Three times per year, for three years, all bay laurels and tanoaks along transects and within the 30m circle surrounding plot center were surveyed for visible symptoms of *P. ramorum* infection and sampled if deemed symptomatic. Surveys were conducted three times per year in order to determine isolation success and therefore pathogen viability at different points throughout the year when sporulation levels were expected to vary due to temperature and availability of moisture based on a priori knowledge of the pathogens life cycle. Surveys took place in the late spring when sporulation and transmission from foliar hosts (bay laurel) to dead end hosts (coast live oak) is known to occur; in the autumn when pathogen viability was at its lowest after going dormant over the hot dry summer; and in the early winter when activity levels are intermediate (Eyre et al. 2013, Kozanitas et al. 2017, 2020). A bay laurel leaf was considered to be 'symptomatic' if it displayed the dark pixilated spots along the outer margin or lesions at the tip of the leaf, characteristic of infection by *P.ramorum,* while a tanoak was deemed symptomatic if necrotic tissue extended up the midrib of the leaf onto the petiole (Garbelotto et al. 2002; Davidson et al. 2003). Coast live oaks were assessed for symptoms once per year over a four-year period, and if bleeding bole cankers were present, the outer layer of bark was removed and the margin of the infected cambial tissue excised and embedded directly into selective media. In the final year of the study (2012) only isolates from oaks were genotyped and included in this study, this was done in order to capture any new infections on oak that potentially occurred during the peak transmissive season of the previous year.

Sample Processing

All sampled leaves from bay laurel and tanoak were processed within 72 hrs. of collection. Processing involved excising and embedding the advancing margin of a leaf or petiole lesion into the *Phytophthora*- selective media PARP (Rizzo et al. 2002). Plates were then incubated in the dark at 20°C for up to 7 days or until mycelial growth was observed and hyphae were subcultured onto clean plates. The oak samples, which were plated in the field, were subcultured onto clean PARP as soon as any mycelial growth was visible. All samples exhibiting mycelial growth were then scored under the microscope as *P.ramorum* positive or negative using distinguishable morphological features. Once mycelial isolates were large enough, they were inoculated into 12% pea broth liquid (Eyre et al. 2013) and grown for 7 days in 6 well culture plates at room temperature. Isolates were then transferred into 2ml screw cap tubes, lyophilized

and amalgamated using 5mm glass beads. DNA was extracted using an NAOH extraction method (Eyre et al. 2013).

Genotyping

Six sets of primers were used on the extracted *P. ramorum* DNA to amplify the following ten microsatellite loci known to be variable in the NA1 lineage; Ms18 and 64 (Ivors et al. 2006), Ms39a, Ms39b, Ms43b, Ms43b, Ms45 (Prospero et al. 2007) and MsILVO145a, MsILVO145b, MsILVO145c (Vercauteren et al. 2010). PCR reactions were carried out as described by Eyre et al. (2013) and the thermal cycling program for each primer was set by following the varying protocols outlined in Ivors *et al*. 2006; Prospero *et al*. 2007; Mascheretti *et al*. 2008, and Vercauteren *et al.* 2010. Fragment analysis was performed using a 3730 ABI Sequencer using a LIZ 500 size standard (Applied Biosystems, Foster City, California). Fragment sizes were scored using Peakscanner V1.0 (ABI Biosystems) and were then converted to the appropriate number of microsatellite motif repeats for analysis. Each isolate was assigned a Multilocus genotype (MLG) isolate using the Poppr package in R version 3.4.0 (R core team 2017) for genetic analysis of populations with clonal reproduction (Kamvar et al. 2014).

Statistical Analysis

All statistical analyses, with the exception of the coalescent analysis were conducted in the R statistical environment, version 3.4.0 (R Core Team 2017). Proportions of MLG abundance per plot were calculated and plotted using viridisLite package in R. Allelic richness A_r was calculated using rarefaction to account for unequal sample sizes and to visualize expected number of MLGs, or genotype accumulations curves by host species was plotted using the package vegan in R. Two metroplots were constructed in R, one to visualize the distribution of each unique MLG across sampling year and another to visualize the proportion of MLG abundance per plot as well as number of samples per MLG in each plot.

In order to view potentially existing clusters of MLGs, a Discriminant Analysis of Principal Components (DPAC), a cartesian method that uses linear combinations of alleles to infer the structure of putative populations was implemented using the R package adegenet. The DAPC is derived from a principal component analysis (PCA), and it differs from PCA in that PCA creates combinations of alleles that describe variation within the analysis, while DAPC creates combinations of alleles that seek to maximize differences among *a priori* assigned populations. The DAPC was executed by retaining 37 principal components that yielded 2 discriminant axes.

The following diversity indices were calculated using the Poppr package for R (R core team 2017). First, Nei's GST values were calculated from pairwise comparisons of the three host species, weighted by sample size to accommodate the unbalanced number of samples from each host. Nei's GST was then calculated by year, regardless of host. Also calculated was the Fst among plots, regardless of host or year. Additionally, the Shannon-Weiner Diversity Index (H), the Stoddard and Taylor's Index (G), the Simpson's index (lambda), evenness (E.5) which tends towards 0 as a single genotype becomes more dominant, and towards 1 when all genotypes are equally represented, and Nei's gene diversity (Hexp) were calculated for the three populations of *P.ramorum* isolated from each host and then again using the populations of *P.ramorum* isolated in each year of the study regardless of host. Finally we identified any private alleles, or alleles that are found only in a single host population by year.

To test the directional rate of *P. ramorum* migration among *Notholithocarpus densiflorus* (*Node*), *Quercus agrifolia* (*Quag*) and *Umbellularia californica* (*Umca*), coalescent analyses were performed using the software MIGRATE-N v3.7.2 (Beerli 2009). First, the length of both flanking regions of each microsatellite was subtracted from the total size of amplicons, and the resulting lengths were transformed into number of repeats, then a Bayesian inference method (Beerli 2006) was used to estimate direction and migration rates between the three host types. It has been shown that most infection and particularly cross-host infection in mixed oak woodlands occurs during the spring (Eyre et al. 2013, Hüberli et al. 2011, Garbelotto et al. 2017), therefore only genotypes isolated from bay laurel in the spring sampling events were used for this analysis. This also equalized the sample size and the timing of isolate collection from different hosts, because symptoms of infection occurring in the spring on oaks are only visible, at the earliest, in the autumn following infection, so isolates collected from oaks in the autumn likely originated from infections occurring in a previous spring season. Two runs were executed: one considering all possible routes and directions of migration and a second excluding a priori the routes (Beerli et al., 2019) from *Quag* to *Lide* and from *Quag* to *Umca* because of their demonstrated biological inconsistency. Migration from oaks to other hosts was regarded as nil a priori, considering it has been shown that oaks do not sporulate and thus can be regarded as epidemiological dead end hosts (Garbelotto and Hayden, 2012). For both runs the parameters set were the following: the microsatellite evolution model used in the analysis was the Brownian motion model; an assumption of a constant mutation rate for all loci, a burn in of 10,000, and a static heating scheme with four chains were used in the analysis. Migration (M) was estimated as the immigration rate *m* divided by the mutation rate *l*. For Theta and M, prior distribution parameters were assumed to be uniform: ranging between 0 and 0.1 (Mean 0.05, Delta 0.01, Bins 200) for Theta, and between 0 and 1000 (Mean 500, Delta 100, Bins 200) for M.

Results

A total of 491 bay laurel trees were surveyed nine times, 3972 were found to be symptomatic and sampled, resulting in 1320 isolates. 952 oaks were surveyed 4 times, with 251 sampled resulting in 138 isolates. 45 tanoaks were surveyed nine times, with 214 sampled resulting in 71 isolates. Cumulatively, a total of 1529 isolates from all three host types yielded 237 unique MLGs. The breakdown of MLGs per host is as follows: 71 isolates from tanoak were represented by 18 MLGs, 138 isolates from coast live oak were represented by 59 MLGs and 1320 isolates from bay laurel were represented by 206 MLGs. Of these, 169 MLGs were detected on bay laurel only, 28 MLGs on oak only and 4 MLGs on tanoak only. The number of MLGs detected on combinations of hosts is as follows: 22 MLGs on both bay laurel and oak, 4 on bay laurel and tanoak, 0 MLGs on both oak and tanoak and 10MLGs were found on all three hosts.

Allelic richness per host species was assessed using rarefaction and determined that sufficient observations of bay laurel populations were made via sampling efforts in order to get a reasonable estimate of unique MLG abundance per host species (Fig. 1). The rarefaction curves for oak and tanoak populations did not indicate sample saturation levels had been met, however these populations were sampled as extensively as the experimental design allowed for, as isolates from all individuals of these two host species that were exhibiting symptoms were included in the analysis. Isolation success from bay laurel varied greatly depending on season and year, due to the cyclical nature of the pathogens autecology in California's oak woodlands. Pathogen in

infected plant tissue alternates between viability and dormancy in association with shifts in temperature and rainfall, with the highest levels of viability occurring during the warm, wet spring sampling events (Eyre et al. 2013, Kozanitas et al. 2017, 2020). Due to this pattern, sizes of samples that yielded live cultures were uneven both throughout and across years. Tanoaks and oaks also had unequal sampling sizes as many infected individuals died, and uninfected individuals became symptomatic over the course of the study. Over the three years in which all three host species were sampled (excluding 2012) isolation success was highest in the wettest year (2011; 726 isolates), intermediate in the year with moderate rainfall (2010; 528 isolates) and lowest in the driest year of the study (2009; 240 isolates) (Table 3b.) The distribution of the ten most commonly occurring MLGs in each hosts is shown in Table 1. The most commonly occurring MLG overall (MLG 213) was also the most common MLG both in the bay laurel and in the oak population. However, that MLG was not the most common in the tanoak population, having been isolated only three times from that host. The most common MLG in tanoak (MLG 167) was the third most common MLG overall, and only the 7th most common in bay laurel. The top two MLGs found in the tanoak population MLG 167 and MLG 236 showed very low representation in the oak population (5 and 1 instances respectively), while the top two MLGs in oak (MLG 213 and MLG 153) were either barely observed or not detected at all in tanoak (3 and zero instances respectively). While the numbers of MLGs shared between bay laurels and oaks and between bay laurels and tanoaks were significant (22 and 4, respectively, representing 45% and 50% of all MLGs found on the non bay laurel host), no MLG was shared between oak and tanoak.

The two metroplots constructed to help visualize the abundance of MLGs both per year and per plot show the distribution of MLGs spatially and temporally. The first illustrated the pattern through time, and indicated that the most dominant MLGs persist and were detected in each year of the study (Supplementary Fig.1). Singletons, or those MLGs which show up only once in the population tended to only show up in one year, and usually in 2011, the year with the highest isolation success (Table 3b). Three main groups of MLGs emerge when considering abundance through time, those that are both abundant and persistent, those that are not abundant yet persist, and those that are neither abundant nor persistent study (Supplementary Fig.1). When considering the abundance of MLGs spatially, at the plot level in this case, it became apparent that different plots had different dominant MLGs and that different plots were not equal in terms number of MLGs detected. Additionally, some sites supported the presence of certain MLGs for longer periods of time than others (Figure 3). Site pairwise Fst values were variable depending on the pair of sites that was compared, however 98% of pairwise comparisons had a $Fst < 0.06$, suggesting very limited genetic structure. About 2% of comparisons had a $Fst > 0.06$ suggestive of moderate isolation and of presence of genetic structure. (Figure 4).

Results from the Discriminant Analysis of Principal Components (DAPC) showed overlap between bay laurel and oak populations, as well as between bay laurel and tanoak populations, while little to no overlap was detected between oak and tanoak populations. The overlap indicates that these populations are connected to one another, but that the oak and tanoak populations are not related. The genetic distance (Gst) between the three populations isolated from each host species was calculated and the interspecific pairwise measures of differentiation ranged from 0.0012, among coast live oak and bay laurel to, 0.02 among coast live oak and tanoak (Table 2a.). While these low inferred Gst values indicated little to no degree of differentiation among genotypes of *P. ramorum* in various host taxa, the pairwise Gst between oak and tanoak at 0.02 was an order of magnitude higher than any other pairwise comparison

between hosts. Pairwise comparisons among years were also conducted but showed little to no difference between populations (Table 2b.). The evenness of genotypes in the studied populations ranged from 0.4 to 0.7 along a scale of 0-1 with one being the most even. The oak population was the most even with a score of 0.7 (Table 3a.). While there was not differentiation in the yearly population with regard to genetic distance, there are differences in evenness among year with values ranging from 0.42 to 0.86 (Table 3b.) The highest value of 0.86 in 2012 is only reflective of isolates from the oak population as bay laurel and tanoak isolates were not collected in 2012, indicating another very even population of oak. The values for yeas 2009-2011 however include all three host populations. The most even population was detected in 2009 with an E_5 value of 0.57, which was also the driest year of the study, the least even population was detected in 2011, the wettest year of the study with an E_5 value of 0.42 and the population from 2010 had an intermediate E_5 value of 0.49, with reported rainfall levels slightly lower than that of 2011 (Eyre et al. 2013, Kozanitas et al. 2017, 2020). The Stoddard and Taylor index or G value in this situation is reflective of the number of MLGs analyzed taking sample size into account. The main result to focus on from these diversity indices is the pattern that emerges between the a priori rainfall levels, the measure of evenness and the abundance of MLGs detected in a year. With an increase in rainfall, there is an increase in the number of MLGs detected regardless of host, and a decrease in the evenness of the population, as some MLGs become dominant.

A search for private alleles unique to a particular host species found that bay laurel exhibited the highest number, although it should be noted that it was also the host taxon with the greatest sample size and is represented by the most MLGs (Table 4a.) The oak and tanoak population had private alleles in a much lower quantity than bay laurel. When looking at the number of private alleles in the yearly populations regardless of host species the following pattern emerged; the most private alleles were detected in 2011the wettest year of the study and the year with the highest number of MLGs (Table 4b.).

Coalescent analyses were performed to test the rate and direction of migration of *P. ramorum* among hosts. Given that oak infection only occurs in the spring and that oaks are not infectious, only isolates from bay laurel that had been collected during the spring sampling events were included, and any migration from oak was excluded. Migration was highest from bay laurel to both oak and tanoak, with virtually no migration occurring from tanoak to bay laurel and low to intermediate migration from tanoak to oak (Table 5).

Discussion

The SFPUC in San Mateo County was selected to examine the population genetics of *P. ramorum* among three key hosts of Sudden Oak Death due to the *a priori* knowledge of the pathogens invasion history in California (Croucher et al. 2013). In previous publications, it has been demonstrated that soil, water and bay laurel populations in the SFPUC were all interconnected, and that the genetic structure of all three was largely driven by the bay laurel population, with further differentiation likely to be accentuated by the differential selection processes in each of the three substrates (Eyre et al. 2013, 2015). Kasuga *et al*. 2012 is the only study to date showing no genetic structure among bay laurels, oaks and tanoaks, using a comparable set of SSR markers. That finding was important for two reasons: first, it determined that the SSR markers used were not affected by the significant genomic alteration occurring in oaks; second, it suggested that oaks being not infectious, may have been infected by the same pathogen population present on bay laurel and tanoak, the two transmissive hosts in California

mixed woodlands. However, sample size was rather limited in that study, and although sample size was balanced among hosts and counties, samples were not systematically collected from infected oaks and spatially adjacent bay laurels and tanoaks, as in the current study, making the inferences based on lack of structure unconvincing, in terms of proving direct bay laurel to oak infection. This is the first study designed to systematically and comprehensively sample oaks, bay laurels and tanoaks to obtain representative populations of the pathogen from each host in order to further our understanding of the epidemiology of Sudden Oak Death where the three main hosts for the disease coexist.

Other large scale studies have often ignored that exotic *P. ramorum* populations from different sites may have been introduced at different times, and may have been subject to significant founder effects, thus making results hard to compare. Here, all samples were obtained from three hosts within the same watershed, a watershed with a relatively long history of infection (Croucher et al. 2013), so that age of infestation should not represent a significant problem. The assumption of a homogeneous genetic background was confirmed by our population genetics analyses. Fst values among study plots were insignificant for 98% of pairwise comparisons among plots, while only 2% of comparisons had a Fst >6%, still a low value. To ensure that the pathogen populations studied were part of an ongoing active outbreak, number of MLGs and private alleles, as well as the evenness of representation of MLGs were studied through time, by sampling the same trees in different seasons and in different years. As expected, pathogen populations increased as rainfall increased, and the largest number of MLGs was identified using Stoddard's index in the late spring of 2011, when rainfall levels peaked during the course of the study (Table 3b). At the same time, the increase in MLGs fostered by conditions favorable to infection was mirrored by a decrease in evenness of representation of MLGs (Table 3b.). This decrease has been observed in other population genetic studies on *P. ramorum* in which multiple temporal samplings were performed (Eyre et al. 2013, 2015), and is normally explained by the dominance of a few infectious genotypes, as expected for an infectious disease (Frank et al. 1992). These infectious fit genotypes dominate during favorable weather conditions (e.g. temperatures around 20 ^{0}C and abundant rainfall typical of late spring in coastal California), but will also survive through time (Supplementary Fig. 1). Due to the survival of these epidemiologically relevant MLGs, we found little genetic structure when comparing SFPUC *P. ramorum* populations from different years (Table 2b). This last finding was in perfect agreement with the finding of Mascheretti *et al*. (2008, 2009) and Croucher *et al*. (2013). The study plots were selected to be at a minimum of 2km apart, to ensure that, although genetically similar, sites would be independent in terms of MLG migration between them. Our results indicated that dominant MLGs varied strikingly across the network of study plots (Figure 3), confirming pathogen populations were responding to different ecological and site conditions in spite of having the same history. Recently, Yuzon *et al*. (2020) have identified that site ecology drives evolutionary process, and that the same genomic variations occur in different sites characterized by similar ecology. Our results indicate that different ecology may allow different genotypes to become dominant, even if sites were invaded by the pathogen by the same founding individuals and at the same time.

Based on the knowledge that sporulation on bay laurel far exceeds levels observed on other hosts (Garbelotto et al. 2003, Davidson et al. 2005), and based on the strong association repeatedly identified between bay laurel density and infection rates on oaks and tanoaks (Cobb et al. 2012, Kozanitas et al. 2020) and between bay laurel density and inoculum loads (Garbelotto et al. 2017), our main assumption was that the California bay laurel population of *P. ramorum*

would represent a major source of inoculum for both oaks and tanoaks. Oaks would represent a sink, while tanoaks, would be a secondary source and also a sink. Our results convincingly show the primary role that bay laurels play in oak infection for the following reasons: the most abundant MLG in bay laurel was also the prominent MLG in oaks, proving for the first time direct contagion between bay laurel and oaks; MLG and private allele richness was much higher in bay laurels than in oaks further corroborating the role of bay laurels as a source population; 50% of MLGs found in oak were also found in bay laurel, Gst between bay laurel and oak populations was the lowest recorded in the study, and, finally, the number of migrants estimated through coalescent analysis was extremely high from bay laurel to oak. Based on other studies (Garbelotto et al. 2017, Kozanitas et al. 2020), it has been assumed that oak infection occurs only in exceptionally wet years, and supposedly only for MLGs that reach a high sporulation level. This selection process would equalize representation of isolates in oaks limiting it to those that meet the epidemiological requirements necessary for infection in this host. Accordingly, the evenness index was overall very high in oak populations and reached 0.85 in the 2012 sampling which was limited exclusively to oaks, suggesting the above assumption is correct.

Although migration was intermediate in scale between tanoak and oak populations, this may be in part due to the fact that oaks and tanoaks are infected by the same bay laurel source. In support of this interpretation, our analysis determined that no MLGs were shared between oaks and tanoaks, while about 50% of MLGs in tanoaks were shared with bay laurels, not unlike what was found for oaks. Furthermore, the 10 MLGs detected in oaks and tanoaks were all also found in bay laurels, suggesting once again a bay source for these MLGs in the two other hosts. Migration of the pathogen was also estimated to be very high from bay laurel to tanoak populations, giving credibility to other studies reporting a strong association between bay laurel density and tanoak infection (Cobb et al. 2012, Kozanitas et al. 2020), and further suggesting that the connection between oak and tanoak may be mediated by the bay laurel population that was the source of infection for both. The highest Gst value in the study was recorded between oak and tanoak populations, and the DAPC analysis shows clearly the oak and tanoak populations both overlap in part with the bay laurel population, but are clearly divergent from one another. This genetic difference between two populations that supposedly originated from the same source may be in part explained by the different selection pressure experienced by MLGs of the pathogen in the two hosts, in a way similar to what has been suggested to explain the genetic differences between bay laurel and soil or water populations (Eyre et al. 2013, 2015). The fact that the dominant MLG in tanoak is abundant in bay laurel but absent in oak, corroborates this hypothesis. Finally, if oaks were truly infected by MLGs coming directly from tanoaks, we would also expect tanoaks MLGs to infect bay laurels intermixed with oaks. However, the number of migrants from tanoaks to bay laurel was rather low, indicating that tanoak does not play a major epidemiological role in mixed oak woodlands with a significant component of bay laurels present. These results in general support the idea that the bay laurel population is the source of infection for the other two hosts, and that disease is primarily transmitted from bay laurel to both tanoak and oak, while transmission from tanoak to oak does not occur or is marginal.

In conclusion, here we provide direct evidence that in the mixed oak woodland of the SFPUC, bay laurels represent the most significant source of inoculum for both oaks and tanoaks, while tanoaks do not represent a significant source of inoculum for bay laurels. In spite of their common source of inoculum, oaks and tanoaks are sinks that select for different pathogen genotypes. This is a process likely to generate divergent evolutionary trajectories. A further

driver of heterogeneity was identified in the sites investigated. Some sites supported a dominance of different MLGs, more MLGs overall and more persistent MLGs, when compared to other sites. These sites are likely to play a more significant epidemiological and evolutionary role for the pathogen. Heterogeneity at the landscape level has been shown to drive disease progression, in relation to disease incidence and prevalence and now there is evidence to suggest it can also drive genetic structure within host populations. The sites dubbed hotspots by their ability to support higher levels of infection throughout the year (Kozanitas et al. 2020) were expected to be more diverse because of the larger populations they support, and our data supported this assumption. However, pathogen populations in these sites were less even due to the dominance of certain MLGs.

Previous studies on the population genetics in California lack an understanding of what happens to individual MLGs through time: our multi-year approach lends insight into the prevalence and persistence of specific pathogen genotypes. Genotypes fall into three main categories: those that are abundant and persistent (32/237; 13.5% of MLGs), those that are not abundant but persist through time (40/237; 16.9% of MLGs) and those which are neither abundant nor persistent (165/237; 69.6%MLGs). The data presented here show that MLGs from the first group are the most important epidemiologically being dominant in infections from the three hosts. Identifying MLGs in this group may be important to predict and manage disease outbreaks that may be more lethal than others. However it is possible that MLGs in the other groups may contribute to the adaptation and evolutionary process of the exotic pathogen in its new environment. With this study, we are the first to prove that MLGs from bay laurels are being transmitted to oak and tanoak, while tanoak is not a significant source of genotypes for either oaks or bay laurels. Finally, by showing the prevalence of different genotypes in both different hosts and sites, we also show, for the first time, that both landscape level variations and host species are not only able to drive disease dynamics at the ecological level, but at the genetic level as well. This information adds to data from previously published studies (Kasuga et al. 2012, 2016, Elliot et al. 2018) showing that oaks, but not bay laurel, trigger rapid evolutionary processes by causing genomic alterations and chromosomal copy number variations.

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Figures and Tables

Figure 1. A rarefaction curve to visualize allelic richness; to determine whether sufficient observations were made via sampling in order to get a reasonable estimate of unique MLG abundance per host species.

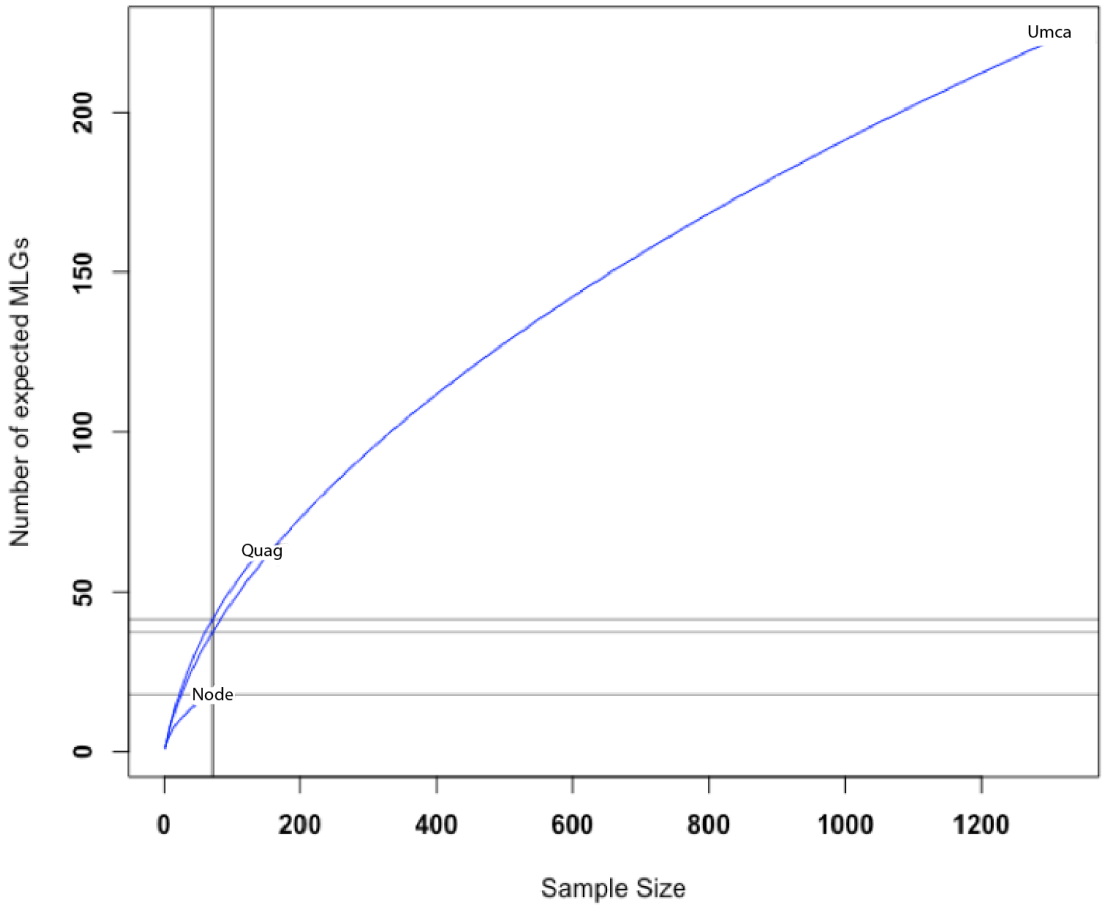


Figure 2. Results from a Discriminant Analysis of Principal Components (DAPC) as implemented in adegenet, showing any overlap that exists between the populations of *P. ramorum* isolated from the three different host species. The DAPC was executed by retaining 37 principal components that yielded 2 discriminant axes.

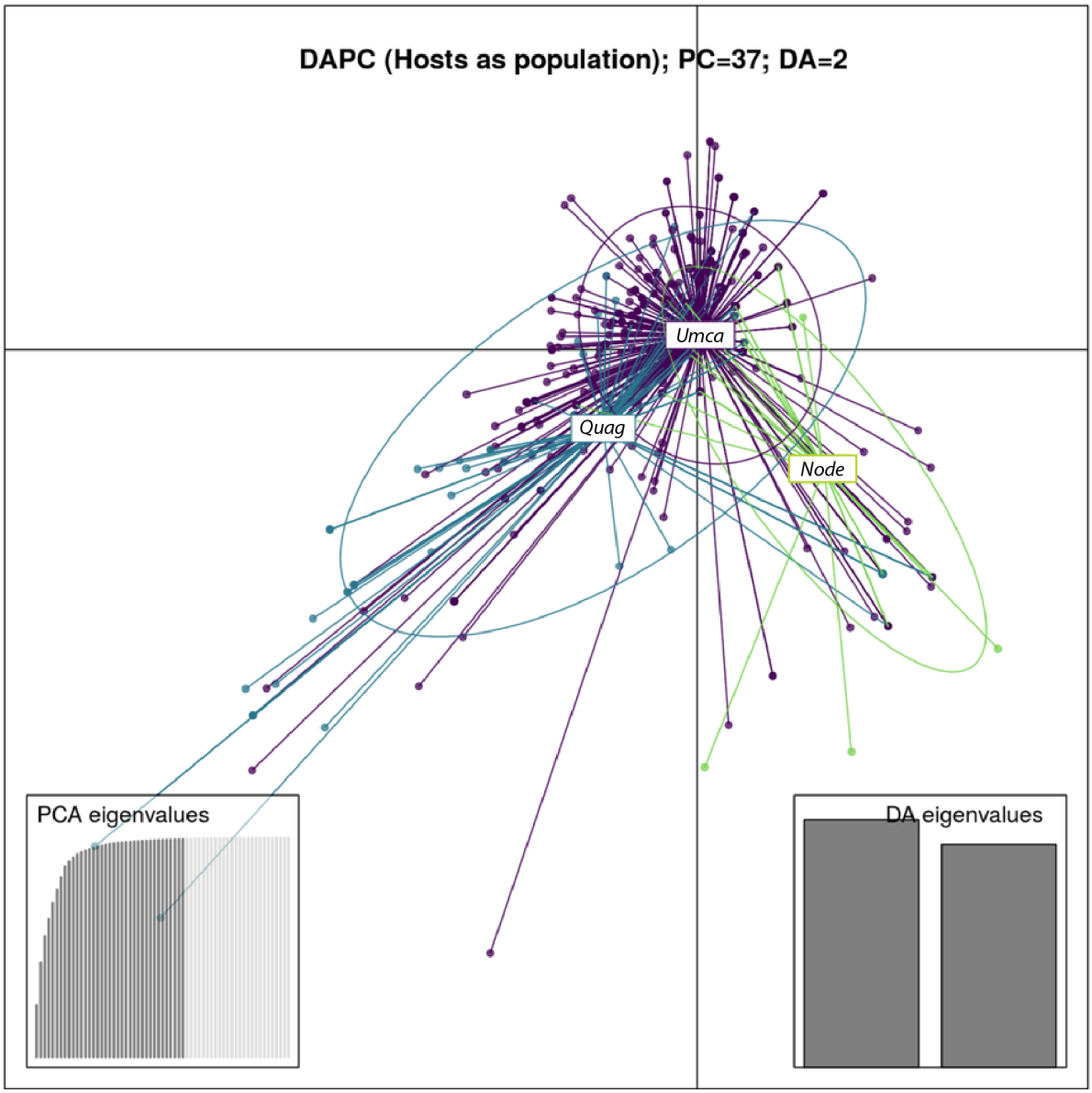


Figure 3. Proportion of MLG abundance per plot and number of samples per MLG. The figure only includes MLG's found in more than one plot.

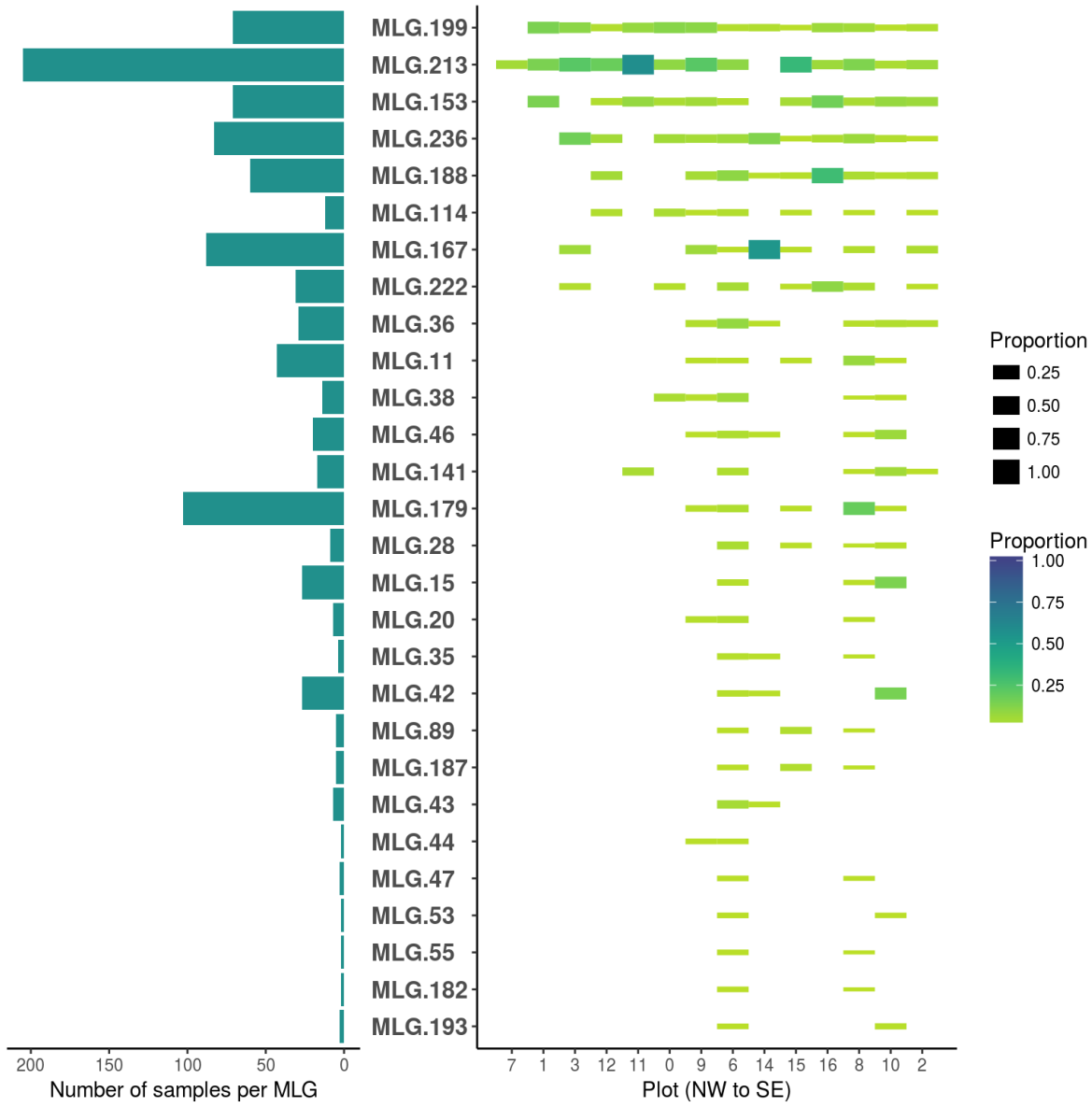


Figure 4. Histogram of pairwise fst by plot

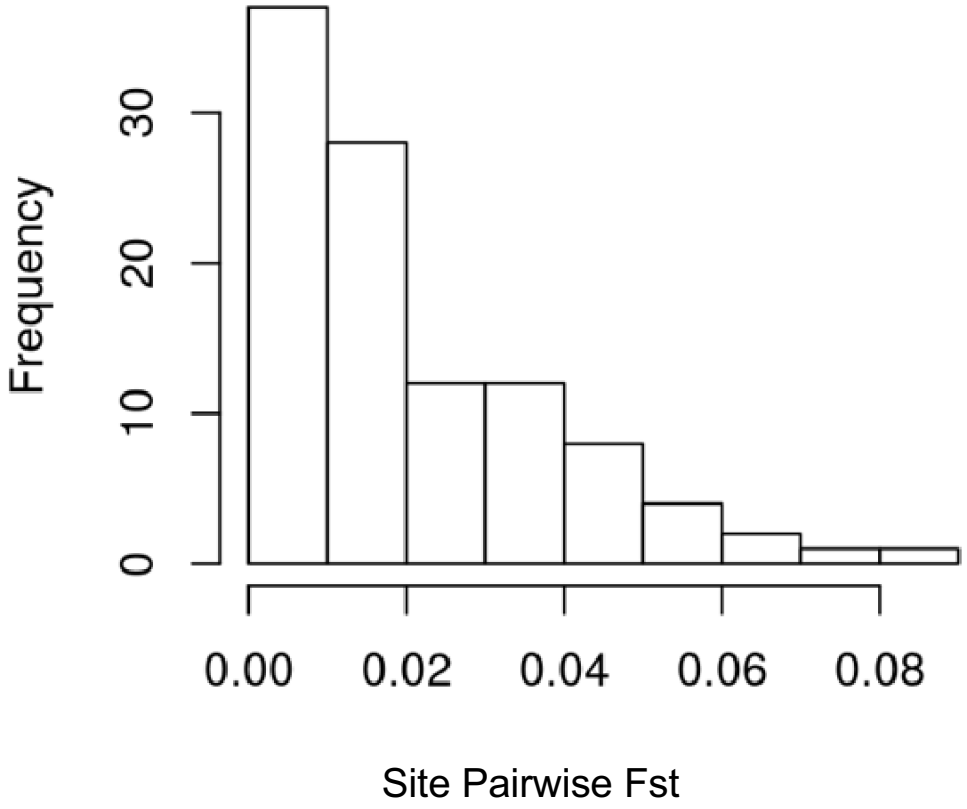


Table 1. Distribution of the ten most commonly occurring MLG in each host species

	MLG.213	**MLG.179**	**MLG.167**	**MLG.236**	**MLG.153**	**MLG.199**	**MLG.188**	**MLG.11**	**MLG.226**	**MLG.222**
Node	3	0	29	10	0	4	3	0	4	0
Quag	15	1	5	1	7	5	2	0	5	0
Umca	187	102	54	72	64	62	55	43	29	31

Table 2. Nei's Gst values from pairwise comparisons between a.) the three host species and b.) Gst by year

a.)

	Umca	***Quag***	***Node***
Umca	0.0000000	0.0011751	0.0044148
Quag	0.0011751	0.0000000	0.0204225
Node	0.0044148	0.0204225	0.0000000

b.)

	2009	**2010**	**2011**	**2012**
2009	0.0000000	0.0010052	0.0019234	0.0040379
2010	0.0010052	0.0000000	0.0011183	0.0017303
2011	0.0019234	0.0011183	0.0000000	0.0013991
2012	0.0040379	0.0017303	0.0013991	0.0000000

Table 3. Diversity tables summarizing the diversity indices for populations of *P.ramorum* isolated from each of the three host species in a.) and in each year of the study in b.). Where **N** is the samples size, **H** is the Shanon-Weiner Diversity, **G** is the Stoddard and Taylor's Index, **lambda** is the Simpson's index, **E.5** is evenness, and **Hexp** is Nei's gene diversity.

a.)

Pop	N	MLG	eMLG	SE	H	G	lambda	E.5	Hexp
Node	71	18	18.00000	0.000000	2.169909	4.898931	0.7958738	0.5026026	0.4250025
Quag	138	59	39.75335	2.564092	3.731567	29.571429	0.9661836	0.7012349	0.4957181
Umca	1320	206	36.70857	3.409448	4.016354	23.486911	0.9574231	0.4126159	0.4766213
Total	1529	237	37.59686	3.454216	4.103311	25.144835	0.9602304	0.4055204	0.4780799

b.)

Pop	N	MLG	eMLG	SE	H	G	lambda	E.5	Hexp
2009	240	55	19.52723	2.232262	3.312310	16.02671	0.9376042	0.5681507	0.4643528
2010	528	115	21.69159	2.446747	3.767532	21.80385	0.9541365	0.4921291	0.4801571
2011	726	169	23.91651	2.456612	4.119303	26.38282	0.9620965	0.4194376	0.4795522
2012	35	18	18.00000	0.000000	2.718330	13.17204	0.9240816	0.8599117	0.4788406
Total	1529	237	23.03097	2.485942	4.103311	25.14483	0.9602304	0.4055204	0.4780799

Table 4. Private alleles occurring in populations of *P.ramorum* separated into groups by host species in a.) and by year in b.)

a.)

Host	ms18	ms39b	ms43a	ms43b	ms64	ms145a	ms145b	ms145c
Umca	11	12	18	12	6	5	4	3
Quag	0	0	0	8	0	0	0	0
Node	0	0	2	0	0	0	0	0

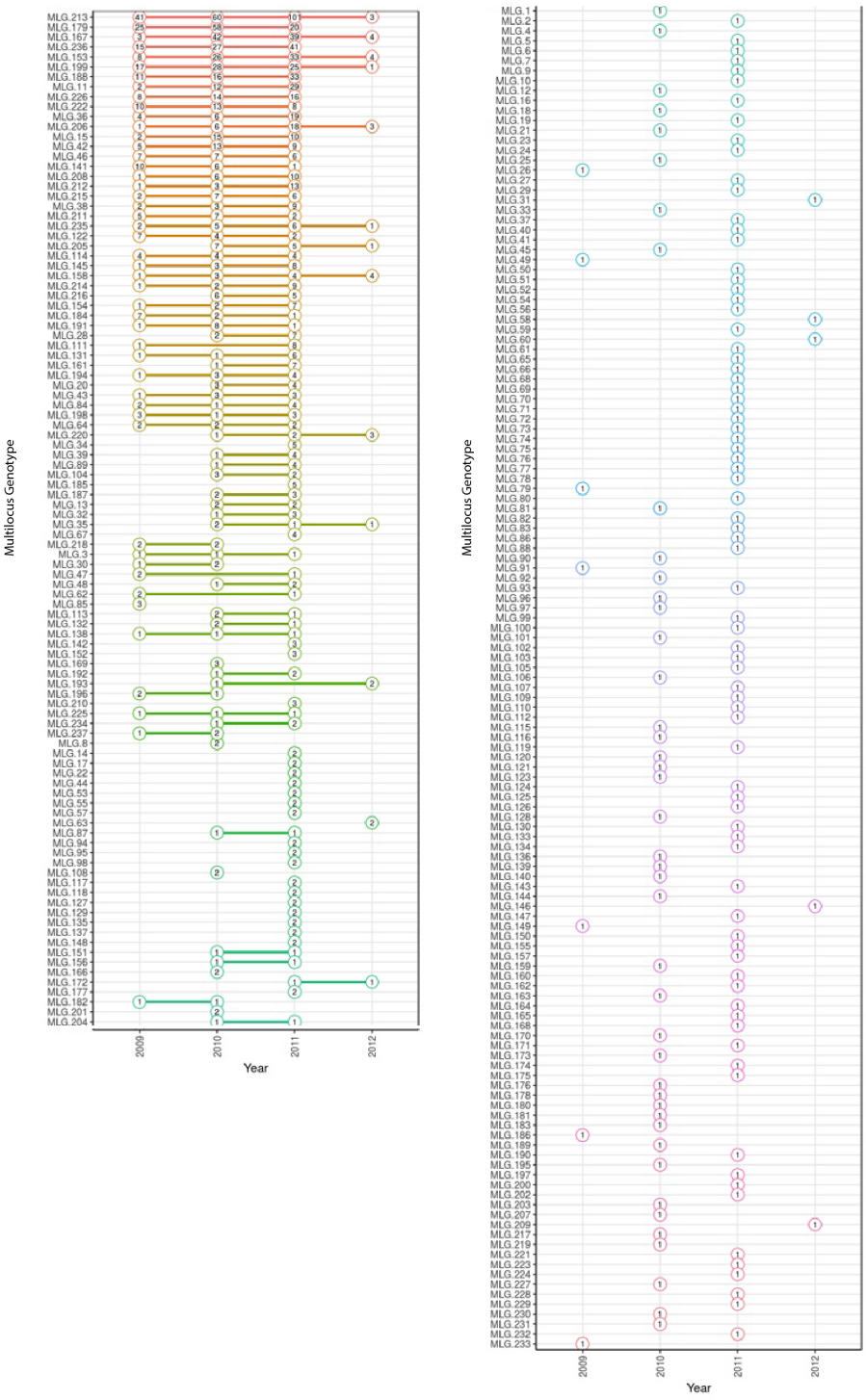
b.)

Year	ms18	ms39b	ms43a	ms43b	ms64	ms145a	ms145b	ms145c
2009	1	0	0	0	0	1	0	0
2010	0	4	2	8	2	3	3	8
2011	10	0	18	12	2	0	1	0
2012	0	0	0	0	0	0	0	0

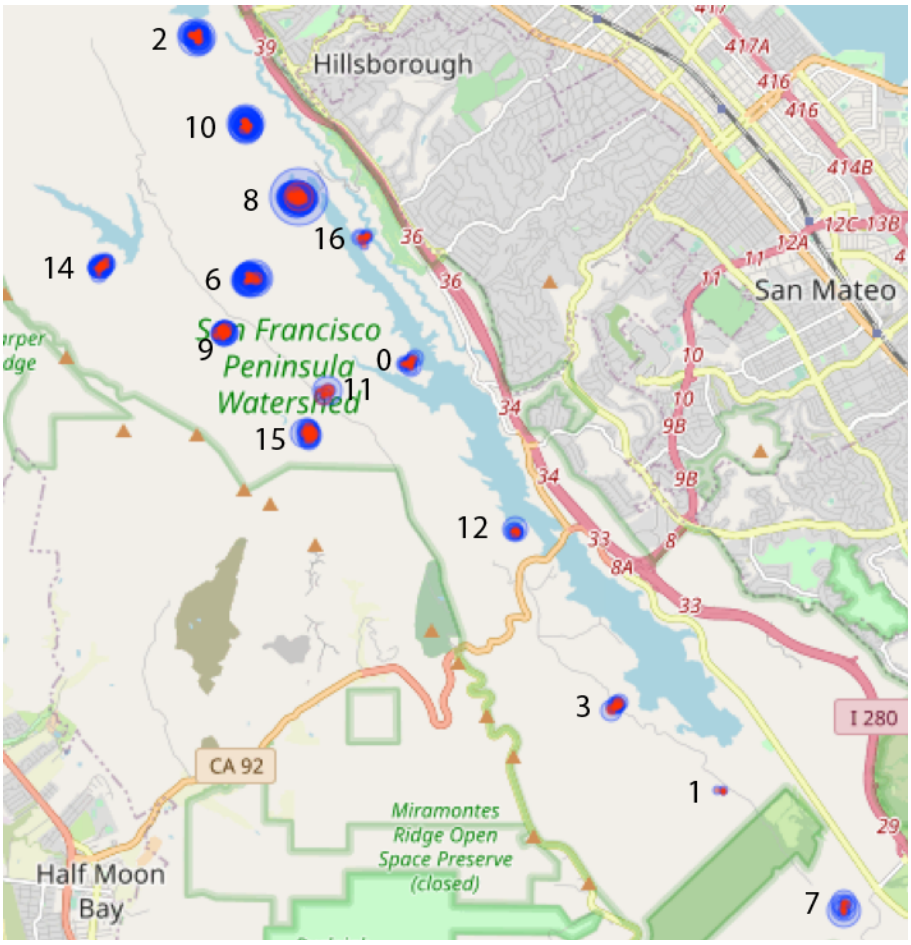
Table 5. Results of coalescent analysis from Migrate-N, using the mean data of all loci from bay laurel isolates collected during spring sampling events. The populations are as follows 1:tanoak (*Node*), 2: Coast Live oak (*Quag*), 3: bay laurel (*Umca*).

Theta							**Migration rate**					
Locus	Pop	2.5%	Mode	97.5%	Median	Mean	Direction	2.5%	Mode	97.5%	Median	Mean
All	1 (*Node*)	0.01400	0.01775	0.02600	0.01975	0.02066	3->1	860.000	927.000	1000.000	932.500	926.051
All	2 (*Quag*)	0.04400	0.04725	0.05900	0.04825	0.04019	1->2	40.0000	72.5000	100.000	427.500	306.705
All	3 (*Umca*)	0.09600	0.09875	0.10000	0.09875	0.09821	3->2	695.000	817.500	955.000	832.500	827.234
							1->3	45.0000	67.5000	90.0000	72.5000	69.6530

Supplementary Figure 1. A Metroplot showing the abundance and persistence of each multilocus genotype (MLG) detected per year.



Supplementary Figure 2. Map of plots within the SFPUC where each plot has been represented by a blue circle where the diameter of the circle is proportional to the abundance of Multilocus genotypes (MLGs). The red circle plotted on top of the blue circle indicates the abundance of the most abundant MLG.



Supplementary Figure 3. Allele sizes for the multilocus genotypes (MLG) identified in 2009 - 2012. Fragment analysis was conducted on an ABI 3730 sequencer (Applied Biosystems)

MLG	n	18	39a	39b	43a	43b	45	64	145a	145b	145c
1	1	222/222	131/131	256/256	374/374	478/478	167/187	343/381	168/179	168/200	168/242
2	1	222/222	131/131	268/268	370/370	474/474	167/187	343/381	168/179	168/200	168/242
3	3	222/222	131/131	268/268	370/370	478/478	167/187	343/381	168/179	168/200	168/242
4	1	222/222	131/131	252/252	362/362	482/482	167/187	343/381	168/179	168/200	168/242
5	1	222/222	131/131	252/252	366/366	478/478	167/187	343/379	168/179	168/200	168/242
6	1	222/222	131/131	252/252	366/366	478/478	167/187	343/381	168/179	168/200	168/242
7	1	222/222	131/131	252/252	378/378	478/478	167/187	343/381	168/179	168/200	168/242
8	2	222/222	131/131	252/252	370/370	474/474	167/187	343/381	168/179	168/200	168/242
9	1	222/222	131/131	252/252	370/370	478/478	167/187	343/381	168/179	168/206	168/242
10	1	222/222	131/131	252/252	370/370	478/478	167/187	343/381	168/179	168/200	168/248
11	43	222/222	131/131	252/252	370/370	478/478	167/187	343/381	168/179	168/200	168/242
12	1	222/222	131/131	252/252	370/370	482/482	167/187	343/381	168/179	168/200	168/236
13	4	222/222	131/131	252/252	370/370	482/482	167/187	343/381	168/179	168/200	168/242
14	2	222/222	131/131	252/252	374/374	474/474	167/187	343/381	168/179	168/200	168/242
15	27	222/222	131/131	252/252	374/374	478/478	167/187	343/381	168/179	168/200	168/242
16	1	222/222	131/131	252/252	374/374	482/482	167/187	343/381	168/179	168/200	168/242
17	2	222/222	131/131	248/248	362/362	478/478	167/187	343/381	168/179	168/200	168/242
18	1	222/222	131/131	248/248	366/366	486/486	167/187	343/381	168/179	168/200	168/242
19	1	222/222	131/131	248/248	366/366	478/478	167/187	343/381	168/179	168/200	168/248
20	7	222/222	131/131	248/248	366/366	478/478	167/187	343/381	168/179	168/200	168/242
21	1	222/222	131/131	248/248	366/366	482/482	167/187	343/381	168/179	168/194	168/242
22	2	222/222	131/131	248/248	366/366	482/482	167/187	343/381	168/179	168/200	168/242
23	1	222/222	131/131	248/248	378/378	474/474	167/187	343/381	168/179	168/200	168/242
24	1	222/222	131/131	248/248	378/378	478/478	167/187	343/381	168/179	168/206	168/242
25	1	222/222	131/131	248/248	378/378	478/478	167/187	343/381	168/179	168/200	168/242

26	1	222/222	131/131	248/248	370/370	490/490	167/187	343/381	168/179	168/200	168/242
27	1	222/222	131/131	248/248	370/370	474/474	167/187	343/381	168/179	168/206	168/242
28	9	222/222	131/131	248/248	370/370	474/474	167/187	343/381	168/179	168/200	168/242
29	1	222/222	131/131	248/248	370/370	478/478	167/187	343/377	168/179	168/200	168/242
30	3	222/222	131/131	248/248	370/370	478/478	167/187	343/381	168/179	168/194	168/248
31	1	222/222	131/131	248/248	370/370	478/478	167/187	343/381	168/179	168/206	168/248
32	4	222/222	131/131	248/248	370/370	478/478	167/187	343/381	168/179	168/206	168/242
33	1	222/222	131/131	248/248	370/370	478/478	167/187	343/381	168/179	168/200	168/246
34	5	222/222	131/131	248/248	370/370	478/478	167/187	343/381	168/179	168/200	168/248
35	4	222/222	131/131	248/248	370/370	478/478	167/187	343/381	168/179	168/200	168/236
36	29	222/222	131/131	248/248	370/370	478/478	167/187	343/381	168/179	168/200	168/242
37	1	222/222	131/131	248/248	370/370	482/482	167/187	343/381	168/179	168/200	168/248
38	14	222/222	131/131	248/248	370/370	482/482	167/187	343/381	168/179	168/200	168/242
39	5	222/222	131/131	248/248	374/374	470/470	167/187	343/381	168/179	168/200	168/242
40	1	222/222	131/131	248/248	374/374	474/474	167/187	343/381	168/179	168/194	168/242
41	1	222/222	131/131	248/248	374/374	474/474	167/187	343/381	168/179	168/200	168/248
42	27	222/222	131/131	248/248	374/374	474/474	167/187	343/381	168/179	168/200	168/242
43	7	222/222	131/131	248/248	374/374	478/478	167/187	343/381	168/179	168/206	168/242
44	2	222/222	131/131	248/248	374/374	478/478	167/187	343/381	168/179	168/200	168/248
45	1	222/222	131/131	248/248	374/374	478/478	167/187	343/381	168/179	168/200	168/236
46	20	222/222	131/131	248/248	374/374	478/478	167/187	343/381	168/179	168/200	168/242
47	3	222/222	131/131	248/248	374/374	482/482	167/187	343/381	168/179	168/200	168/248
48	3	222/222	131/131	248/248	374/374	482/482	167/187	343/381	168/179	168/200	168/242
49	1	275/230	131/131	248/248	370/370	456/456	167/187	343/381	168/179	168/200	168/242
50	1	275/222	131/131	252/252	366/366	486/486	167/187	343/381	168/179	168/200	168/242
51	1	275/222	131/131	252/252	370/370	478/478	167/187	343/381	168/179	168/200	168/242
52	1	275/222	131/131	252/252	374/374	482/482	167/187	343/381	168/179	168/200	168/242
53	2	275/222	131/131	248/248	366/366	486/486	167/187	343/381	168/179	168/200	168/242
54	1	275/222	131/131	248/248	370/370	486/486	167/187	343/381	168/179	168/200	168/248

55	2	275/222	131/131	248/248	370/370	486/486	167/187	343/381	168/179	168/200	168/242
56	1	275/222	131/131	248/248	370/370	478/478	167/187	343/381	168/179	168/210	168/242
57	2	275/222	131/131	248/248	370/370	482/482	167/187	343/381	168/179	168/200	168/242
58	1	275/222	131/131	248/248	374/374	486/486	167/187	343/381	168/179	168/200	168/264
59	1	275/222	131/131	248/248	374/374	478/478	167/187	343/381	168/179	168/200	168/242
60	1	275/222	131/131	248/248	374/374	482/482	167/187	343/381	168/179	168/200	168/236
61	1	220/259	131/131	248/248	374/374	478/478	167/187	343/381	168/179	168/200	168/242
62	3	220/271	131/131	252/252	366/366	490/490	167/187	343/381	168/179	168/200	168/242
63	2	220/271	131/131	248/248	362/362	490/490	167/187	343/381	168/179	168/200	168/242
64	6	220/271	131/131	248/248	366/366	490/490	167/187	343/381	168/179	168/200	168/242
65	1	220/271	131/131	248/248	370/370	486/486	167/187	343/381	168/179	168/200	168/242
66	1	220/271	131/131	248/248	370/370	482/482	167/187	343/381	168/179	168/200	168/230
67	4	220/271	131/131	248/248	370/370	482/482	167/187	343/381	168/179	168/200	168/242
68	1	220/271	131/131	248/248	374/374	478/478	167/187	343/381	168/179	168/200	168/242
69	1	220/271	131/131	248/248	374/374	482/482	167/187	343/381	168/179	168/200	168/242
70	1	220/270	131/131	248/248	366/366	482/482	167/187	343/381	168/179	168/200	168/242
71	1	220/273	131/131	244/244	374/374	529/529	167/187	343/381	168/179	168/194	168/260
72	1	220/273	131/131	252/252	370/370	478/478	167/187	343/381	168/179	168/200	168/242
73	1	220/273	131/131	248/248	366/366	478/478	167/187	343/381	168/179	168/200	168/248
74	1	220/273	131/131	248/248	378/378	482/482	167/187	343/381	168/179	168/200	168/242
75	1	220/273	131/131	248/248	370/370	486/486	167/187	343/377	168/179	168/200	168/242
76	1	220/273	131/131	248/248	370/370	486/486	167/187	343/381	168/179	168/200	168/260
77	1	220/273	131/131	248/248	370/370	482/482	167/187	343/381	168/179	168/200	168/236
78	1	220/273	131/131	248/248	370/370	482/482	167/187	343/381	168/179	168/200	168/242
79	1	220/279	131/131	248/248	366/366	486/486	167/187	343/381	168/179	168/200	168/242
80	1	220/279	131/131	248/248	366/366	482/482	167/187	343/381	168/179	168/200	168/242
81	1	220/279	131/131	248/248	370/370	486/486	167/187	343/381	168/179	168/200	168/242
82	1	220/279	131/131	248/248	370/370	482/482	167/187	343/381	168/179	168/206	168/242
83	1	220/279	131/131	248/248	370/370	482/482	167/187	343/381	168/179	168/200	168/236

84	7	220/279	131/131	248/248	370/370	482/482	167/187	343/381	168/179	168/200	168/242
85	3	220/279	131/131	248/248	374/374	482/482	167/187	343/381	168/179	168/200	168/236
86	1	220/279	131/131	248/248	374/374	482/482	167/187	343/381	168/179	168/200	168/242
87	2	220/277	131/131	248/248	370/370	490/490	167/187	343/381	168/179	168/200	168/242
88	1	220/277	131/131	248/248	370/370	478/478	167/187	343/381	168/173	168/200	168/242
89	5	220/277	131/131	248/248	370/370	482/482	167/187	343/381	168/179	168/200	168/242
90	1	220/275	131/131	236/236	366/366	482/482	167/187	343/381	168/179	168/220	168/230
91	1	220/275	131/131	236/236	370/370	482/482	167/187	343/381	168/171	168/222	168/242
92	1	220/275	131/131	250/250	374/374	482/482	167/187	343/381	168/179	168/200	168/242
93	1	220/275	131/131	244/244	366/366	482/482	167/187	343/383	168/179	168/200	168/242
94	2	220/275	131/131	244/244	374/374	529/529	167/187	343/381	168/179	168/194	168/260
95	2	220/275	131/131	244/244	374/374	490/490	167/187	343/381	168/179	168/200	168/242
96	1	220/275	131/131	244/244	374/374	530/530	167/187	343/381	168/179	168/194	168/260
97	1	220/275	131/131	244/244	374/374	486/486	167/187	343/381	168/179	168/200	168/248
98	2	220/275	131/131	244/244	374/374	478/478	167/187	343/381	168/179	168/200	168/242
99	1	220/275	131/131	244/244	374/374	482/482	167/187	343/381	168/179	168/200	168/260
100	1	220/275	131/131	252/252	362/362	474/474	167/187	343/381	168/179	168/228	168/242
101	1	220/275	131/131	252/252	366/366	474/474	167/187	343/381	168/179	168/228	168/242
102	1	220/275	131/131	252/252	366/366	478/478	167/187	343/381	168/179	168/200	168/242
103	1	220/275	131/131	252/252	366/366	478/478	167/187	343/383	168/179	168/200	168/242
104	5	220/275	131/131	252/252	366/366	482/482	167/187	343/381	168/179	168/200	168/242
105	1	220/275	131/131	252/252	378/378	474/474	167/187	343/381	168/179	168/200	168/242
106	1	220/275	131/131	252/252	378/378	482/482	167/187	343/381	168/179	168/200	168/242
107	2	220/275	131/131	252/252	370/370	440/440	167/187	343/381	168/179	168/200	168/242
108	1	220/275	131/131	252/252	370/370	490/490	167/187	343/381	168/179	168/200	168/242
109	1	220/275	131/131	252/252	370/370	486/486	167/187	343/381	168/179	168/206	168/242
110	1	220/275	131/131	252/252	370/370	478/478	167/187	343/381	168/179	168/228	168/236
111	9	220/275	131/131	252/252	370/370	478/478	167/187	343/381	168/179	168/200	168/242
112	1	220/275	131/131	252/252	370/370	482/482	167/187	343/381	168/179	168/206	168/242

113	3	220/275	131/131	252/252	370/370	482/482	167/187	343/381	168/179	168/200	168/236
114	12	220/275	131/131	252/252	370/370	482/482	167/187	343/381	168/179	168/200	168/242
115	1	220/275	131/131	252/252	370/370	482/482	167/187	343/383	168/179	168/200	168/242
116	1	220/275	131/131	252/252	374/374	490/490	167/187	343/381	168/179	168/200	168/242
117	2	220/275	131/131	252/252	374/374	486/486	167/187	343/381	168/179	168/200	168/242
118	2	220/275	131/131	252/252	374/374	478/478	167/187	343/381	168/179	168/200	168/242
119	1	220/275	131/131	252/252	374/374	482/482	167/187	343/381	168/179	168/228	168/242
120	1	220/275	131/131	252/252	374/374	482/482	167/187	343/381	168/179	168/206	168/242
121	1	220/275	131/131	252/252	374/374	482/482	167/187	343/381	168/179	168/200	168/270
122	13	220/275	131/131	252/252	374/374	482/482	167/187	343/381	168/179	168/200	168/242
123	1	220/275	131/131	248/252	370/370	478/478	167/187	343/381	168/179	168/206	168/236
124	1	220/275	131/131	248/248	280/280	482/482	167/187	343/381	168/179	168/200	168/242
125	1	220/275	131/131	248/248	382/382	482/482	167/187	343/381	168/179	168/200	168/236
126	1	220/275	131/131	248/248	382/382	482/482	167/187	343/381	168/179	168/200	168/242
127	2	220/275	131/131	248/248	340/340	482/482	167/187	343/381	168/179	168/200	168/242
128	1	220/275	131/131	248/248	354/354	478/478	167/187	343/381	168/179	168/200	168/242
129	2	220/275	131/131	248/248	362/362	486/486	167/187	343/381	168/179	168/200	168/242
130	1	220/275	131/131	248/248	362/362	478/478	167/187	343/381	168/179	168/228	168/242
131	8	220/275	131/131	248/248	362/362	482/482	167/187	343/381	168/179	168/200	168/242
132	3	220/275	131/131	248/248	362/362	482/482	167/187	343/383	168/179	168/200	168/242
133	1	220/275	131/131	248/248	358/358	482/482	167/187	343/379	168/179	168/200	168/242
134	1	220/275	131/131	248/248	358/358	482/482	167/187	343/385	168/179	168/200	168/242
135	2	220/275	131/131	248/248	358/358	482/482	167/187	343/381	168/179	168/200	168/242
136	1	220/275	131/131	248/248	366/366	470/470	167/187	343/381	168/179	168/194	168/242
137	2	220/275	131/131	248/248	366/366	470/470	167/187	343/381	168/179	168/228	168/242
138	3	220/275	131/131	248/248	366/366	490/490	167/187	343/381	168/179	168/200	168/242
139	1	220/275	131/131	248/248	366/366	486/486	167/187	343/359	168/179	168/200	168/242
140	1	220/275	131/131	248/248	366/366	486/486	167/187	343/381	168/179	168/206	168/242
141	17	220/275	131/131	248/248	366/366	486/486	167/187	343/381	168/179	168/200	168/242

142	3	220/275	131/131	248/248	366/366	494/494	167/187	343/381	168/179	168/200	168/242
143	1	220/275	131/131	248/248	366/366	478/478	167/187	343/381	168/179	168/228	168/242
144	1	220/275	131/131	248/248	366/366	478/478	167/187	343/381	168/179	168/200	168/248
145	12	220/275	131/131	248/248	366/366	478/478	167/187	343/381	168/179	168/200	168/242
146	1	220/275	131/131	248/248	366/366	478/478	167/187	343/383	168/179	168/200	168/242
147	1	220/275	131/131	248/248	366/366	482/482	167/187	343/366	168/179	168/200	168/242
148	2	220/275	131/131	248/248	366/366	482/482	167/187	343/381	168/173	168/200	168/242
149	1	220/275	131/131	248/248	366/366	482/482	167/187	343/381	168/179	168/228	168/242
150	1	220/275	131/131	248/248	366/366	482/482	167/187	343/381	168/179	168/200	168/254
151	2	220/275	131/131	248/248	366/366	482/482	167/187	343/381	168/179	168/200	168/248
152	3	220/275	131/131	248/248	366/366	482/482	167/187	343/381	168/179	168/200	168/236
153	71	220/275	131/131	248/248	366/366	482/482	167/187	343/381	168/179	168/200	168/242
154	10	220/275	131/131	248/248	366/366	482/482	167/187	343/383	168/179	168/200	168/242
155	1	220/275	131/131	248/248	378/378	470/470	167/187	343/381	168/179	168/200	168/242
156	2	220/275	131/131	248/248	378/378	490/490	167/187	343/381	168/179	168/200	168/242
157	1	220/275	131/131	248/248	378/378	474/474	167/187	343/381	168/179	168/206	168/242
158	12	220/275	131/131	248/248	378/378	486/486	167/187	343/381	168/179	168/200	168/242
159	1	220/275	131/131	248/248	378/378	478/478	167/187	343/381	168/179	168/200	168/246
160	1	220/275	131/131	248/248	378/378	478/478	167/187	343/381	168/179	168/200	168/236
161	8	220/275	131/131	248/248	378/378	478/478	167/187	343/381	168/179	168/200	168/242
162	1	220/275	131/131	248/248	378/378	482/482	167/187	343/377	168/179	168/200	168/242
163	1	220/275	131/131	248/248	378/378	482/482	167/187	343/381	168/173	168/200	168/242
164	1	220/275	131/131	248/248	378/378	482/482	167/187	343/381	168/179	168/220	168/242
165	1	220/275	131/131	248/248	378/378	482/482	167/187	343/381	168/179	168/200	168/264
166	2	220/275	131/131	248/248	378/378	482/482	167/187	343/381	168/179	168/200	168/236
167	88	220/275	131/131	248/248	378/378	482/482	167/187	343/381	168/179	168/200	168/242
168	1	220/275	131/131	248/248	370/370	396/396	167/187	343/381	168/179	168/200	168/242
169	3	220/275	131/131	248/248	370/370	466/466	167/187	343/381	168/179	168/200	168/242
170	1	220/275	131/131	248/248	370/370	456/456	167/187	343/381	168/179	168/200	168/242

171	1	220/275	131/131	248/248	370/370	408/408	167/187	343/381	168/179	168/228	168/242
172	2	220/275	131/131	248/248	370/370	470/470	167/187	343/381	168/179	168/228	168/242
173	1	220/275	131/131	248/248	370/370	490/490	167/187	377/339	168/179	168/200	168/242
174	1	220/275	131/131	248/248	370/370	490/490	167/187	343/379	168/179	168/194	168/242
175	1	220/275	131/131	248/248	370/370	490/490	167/187	343/379	168/179	168/200	168/242
176	1	220/275	131/131	248/248	370/370	490/490	167/187	343/385	168/179	168/200	168/242
177	2	220/275	131/131	248/248	370/370	490/490	167/187	343/381	168/179	168/194	168/242
178	1	220/275	131/131	248/248	370/370	490/490	167/187	343/381	168/179	168/200	168/248
179	103	220/275	131/131	248/248	370/370	490/490	167/187	343/381	168/179	168/200	168/242
180	1	220/275	131/131	248/248	370/370	490/490	167/187	343/383	168/179	168/200	168/242
181	1	220/275	131/131	248/248	370/370	474/474	167/187	343/381	168/179	168/200	168/260
182	2	220/275	131/131	248/248	370/370	474/474	167/187	343/381	168/179	168/200	168/242
183	1	220/275	131/131	248/248	370/370	486/486	167/187	343/381	179/164	200/164	242/164
184	10	220/275	131/131	248/248	370/370	486/486	167/187	343/381	168/179	168/206	168/242
185	5	220/275	131/131	248/248	370/370	486/486	167/187	343/381	168/179	168/200	168/260
186	1	220/275	131/131	248/248	370/370	486/486	167/187	343/381	168/179	168/200	168/248
187	5	220/275	131/131	248/248	370/370	486/486	167/187	343/381	168/179	168/200	168/236
188	60	220/275	131/131	248/248	370/370	486/486	167/187	343/381	168/179	168/200	168/242
189	1	220/275	131/131	248/248	370/370	486/486	167/187	343/383	168/179	168/200	168/242
190	1	220/275	131/131	248/248	370/370	494/494	167/187	343/379	168/179	168/200	168/242
191	10	220/275	131/131	248/248	370/370	494/494	167/187	343/381	168/179	168/200	168/242
192	3	220/275	131/131	248/248	370/370	478/478	167/187	343/381	168/179	168/194	168/242
193	3	220/275	131/131	248/248	370/370	478/478	167/187	343/381	168/179	168/228	168/236
194	8	220/275	131/131	248/248	370/370	478/478	167/187	343/381	168/179	168/228	168/242
195	1	220/275	131/131	248/248	370/370	478/478	167/187	343/381	168/179	168/206	168/236
196	3	220/275	131/131	248/248	370/370	478/478	167/187	343/381	168/179	168/206	168/242
197	1	220/275	131/131	248/248	370/370	478/478	167/187	343/381	168/179	168/200	168/248
198	7	220/275	131/131	248/248	370/370	478/478	167/187	343/381	168/179	168/200	168/236
199	71	220/275	131/131	248/248	370/370	478/478	167/187	343/381	168/179	168/200	168/242

200	1	220/275	131/131	248/248	370/370	482/482	167/187	343/357	168/179	168/200	168/242
201	2	220/275	131/131	248/248	370/370	482/482	167/187	343/379	168/179	168/200	168/242
202	1	220/275	131/131	248/248	370/370	482/482	167/187	343/377	168/179	168/200	168/242
203	1	220/275	131/131	248/248	370/370	482/482	167/187	343/381	179/164	200/164	164/248
204	2	220/275	131/131	248/248	370/370	482/482	167/187	343/381	168/179	168/222	168/242
205	13	220/275	131/131	248/248	370/370	482/482	167/187	343/381	168/179	168/228	168/236
206	28	220/275	131/131	248/248	370/370	482/482	167/187	343/381	168/179	168/228	168/242
207	1	220/275	131/131	248/248	370/370	482/482	167/187	343/381	168/179	168/206	168/236
208	17	220/275	131/131	248/248	370/370	482/482	167/187	343/381	168/179	168/206	168/242
209	1	220/275	131/131	248/248	370/370	482/482	167/187	343/381	168/179	168/200	168/254
210	3	220/275	131/131	248/248	370/370	482/482	167/187	343/381	168/179	168/200	168/260
211	14	220/275	131/131	248/248	370/370	482/482	167/187	343/381	168/179	168/200	168/248
212	17	220/275	131/131	248/248	370/370	482/482	167/187	343/381	168/179	168/200	168/236
213	205	220/275	131/131	248/248	370/370	482/482	167/187	343/381	168/179	168/200	168/242
214	12	220/275	131/131	248/248	370/370	482/482	167/187	343/383	168/179	168/200	168/242
215	15	220/275	131/131	248/248	374/374	490/490	167/187	343/381	168/179	168/200	168/242
216	11	220/275	131/131	248/248	374/374	474/474	167/187	343/381	168/179	168/206	168/242
217	1	220/275	131/131	248/248	374/374	474/474	167/187	343/381	168/179	168/200	168/270
218	4	220/275	131/131	248/248	374/374	474/474	167/187	343/381	168/179	168/200	168/242
219	1	220/275	131/131	248/248	374/374	486/486	167/187	343/381	168/179	168/194	168/222
220	6	220/275	131/131	248/248	374/374	486/486	167/187	343/381	168/179	168/200	168/264
221	1	220/275	131/131	248/248	374/374	486/486	167/187	343/381	168/179	168/200	168/248
222	31	220/275	131/131	248/248	374/374	486/486	167/187	343/381	168/179	168/200	168/242
223	1	220/275	131/131	248/248	374/374	478/478	167/187	343/377	168/179	168/200	168/242
224	1	220/275	131/131	248/248	374/374	478/478	167/187	343/381	168/179	168/200	168/248
225	3	220/275	131/131	248/248	374/374	478/478	167/187	343/381	168/179	168/200	168/236
226	38	220/275	131/131	248/248	374/374	478/478	167/187	343/381	168/179	168/200	168/242
227	1	220/275	131/131	248/248	374/374	478/478	167/187	343/383	168/179	168/200	168/242
228	1	220/275	131/131	248/248	374/374	482/482	167/187	343/379	168/179	168/200	168/242

229	1	220/275	131/131	248/248	374/374	482/482	167/187	343/377	168/179	168/200	168/242
230	1	220/275	131/131	248/248	374/374	482/482	167/187	343/381	179/164	200/164	242/164
231	1	220/275	131/131	248/248	374/374	482/482	167/187	343/381	168/179	168/194	168/248
232	1	220/275	131/131	248/248	374/374	482/482	167/187	343/381	168/179	168/194	168/242
233	1	220/275	131/131	248/248	374/374	482/482	167/187	343/381	168/179	168/206	168/242
234	3	220/275	131/131	248/248	374/374	482/482	167/187	343/381	168/179	168/200	168/248
235	14	220/275	131/131	248/248	374/374	482/482	167/187	343/381	168/179	168/200	168/236
236	83	220/275	131/131	248/248	374/374	482/482	167/187	343/381	168/179	168/200	168/242
237	3	220/275	131/131	248/248	374/374	482/482	167/187	343/383	168/179	168/200	168/242