Fungal Ecology 28 (2017) 86–96

Contents lists available at ScienceDirect

Fungal Ecology

journal homepage: www.elsevier.com/locate/funeco

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

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ARTICLE INFO

Article history:
Received 24 January 2016
Received in revised form
6 March 2017
Accepted 27 April 2017

Corresponding Editor: Luke Barrett

Keywords:
Competition
Disease diagnostics
Niche partitioning
Stramenopila
Oomycota
Phytophthora ramorum
Phytophthora nemorosa
qPCR
Sudden Oak Death
Umbellularia californica

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, *Phytophthora nemorosa* and *Phytophthora 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.

1. 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

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http://dx.doi.org/10.1016/j.funeco.2017.04.006
1754-5048/

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; Grünwald 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 *Phytophthora infestans* (Nowicki et al., 2012), Port Orford Cedar root disease caused by *Phytophthora lateralis* (Hansen et al., 2000), and Jarrah dieback of *Eucalyptus* caused by *Phytophthora cinnamomi* (Jung et al., 2013). *P. 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, *Phytophthora nemorosa* and *Phytophthora 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 and Rizzo, 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, 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. pseudosyringae* 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 (reviewed by Grünwald et al., 2012, Garbelotto and Hayden, 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 the 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;

H2c. Or, 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.

2. Materials and methods

2.1. 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 9300 ha watershed contains two major drainages, Crystal Springs and Pilarcitos, with elevations ranging between 95 and 1050 . 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 8.8–21.5° 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°, 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.

2.2. 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.

2.3. 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 fall (September/October) have triggered pathogen dormancy (Eyre et al., 2013).

2.4. 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 s 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 1× 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 s 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).

2.5. 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).

2.6. 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 and 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 3×10^4 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 5 M HCl and rinsed 3 times in deionized water.

2.7. 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 × 60 × 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, 2011; Linzer, 2009; 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.

2.8. 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.

3. Results

3.1. 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 Fig. 2A and 2B). The Pilarcitos drainage is higher in elevation, with plots at an altitude ranging between 197 and 311 m, while the altitude of plots in the Crystal Springs drainage ranges between 95 and 187 m. 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, $p = 0.0028$).

3.2. 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). *P. 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

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; ×2 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	<0.0001*
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*

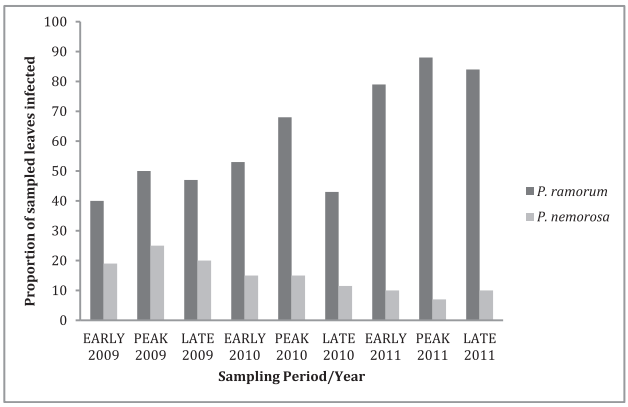

Fig. 1. Proportions of sampled Bay Laurel leaves with *P. ramorum* or *P. nemorosa* infection in each of the nine seasonal sampling events, measured using molecular diagnostic qPCR assays.

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 (Figs. 2B and 3). In the peak/spring and late/fall 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 the 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*.

3.3. 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.

4. 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 Lindow, 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, *P. ramorum*, was only discovered in 2000 (Werres et al., 2001; Rizzo et al., 2002). Since 2000 (Svihra, 1999; Rizzo et al., 2002), 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,

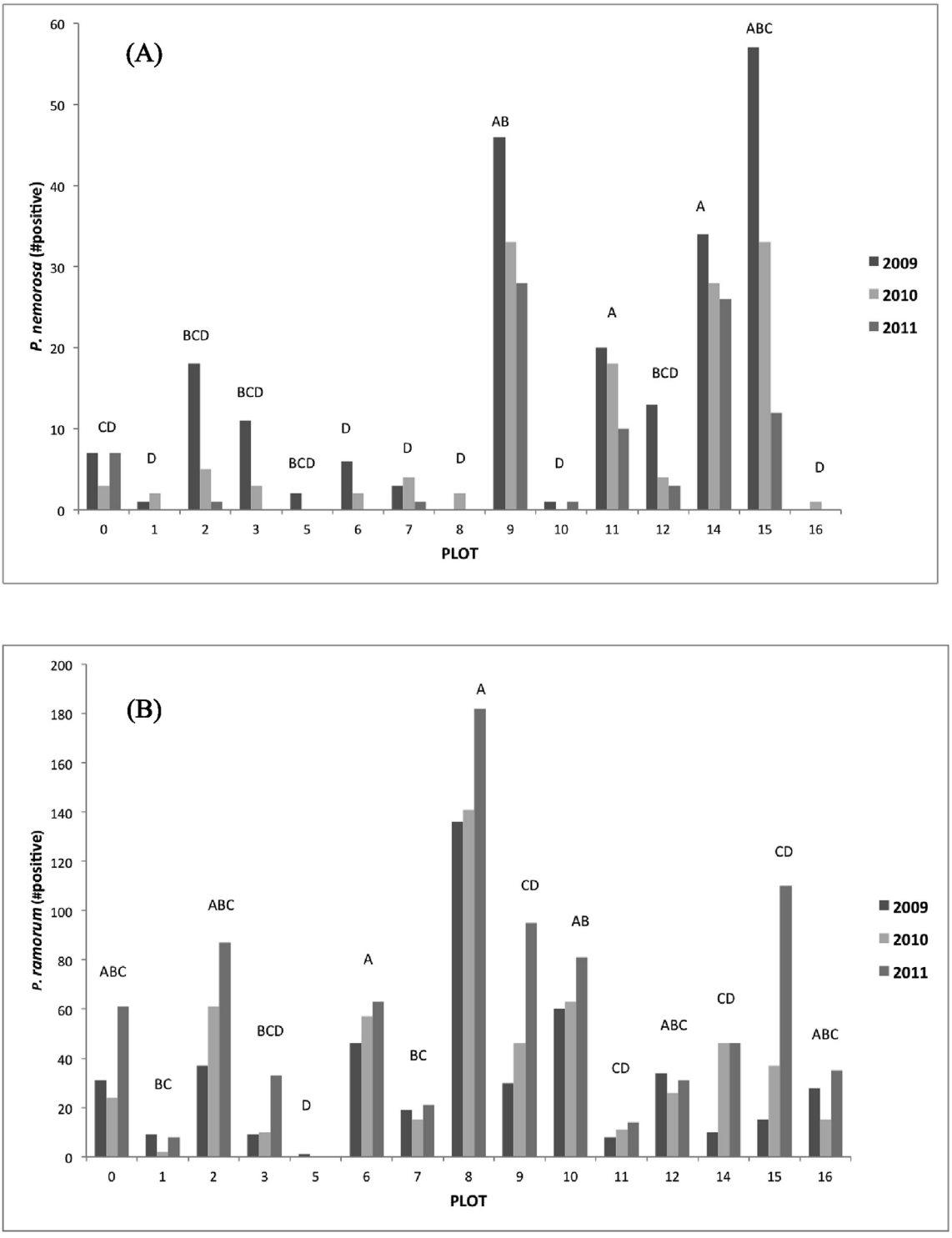

Fig. 2. Positive sample counts for *P. nemorosa* (A) and *P. ramorum* (B) by plot and year. Statistical comparisons of means are depicted by letters A-D; totals not designated by the same letter are significantly different at a 0.05 significance level. Plots 9, 11, 14 and 15 are located in the Pilarcitos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

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

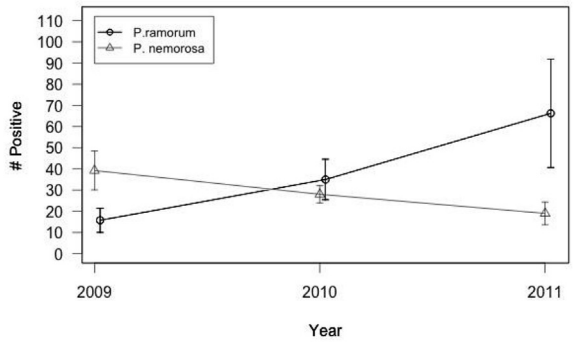

Fig. 3. Mean occurrence (number of positive leaf samples) of *P. ramorum* and *P. nemorosa* in plots located in the Pilarcitos drainage (plots 9, 11, 14, 15) where the highest levels of *P. nemorosa* were observed. Occurrence of *P. nemorosa* decreased in contrast to *P. ramorum* over the three-year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

(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 (Wickland 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 (Wickland 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 fall 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 and Rizzo, 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

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	<0.0001*	0.0030*	0.1934	0.0551	0.1617	0.4064	0.0142*	0.1407

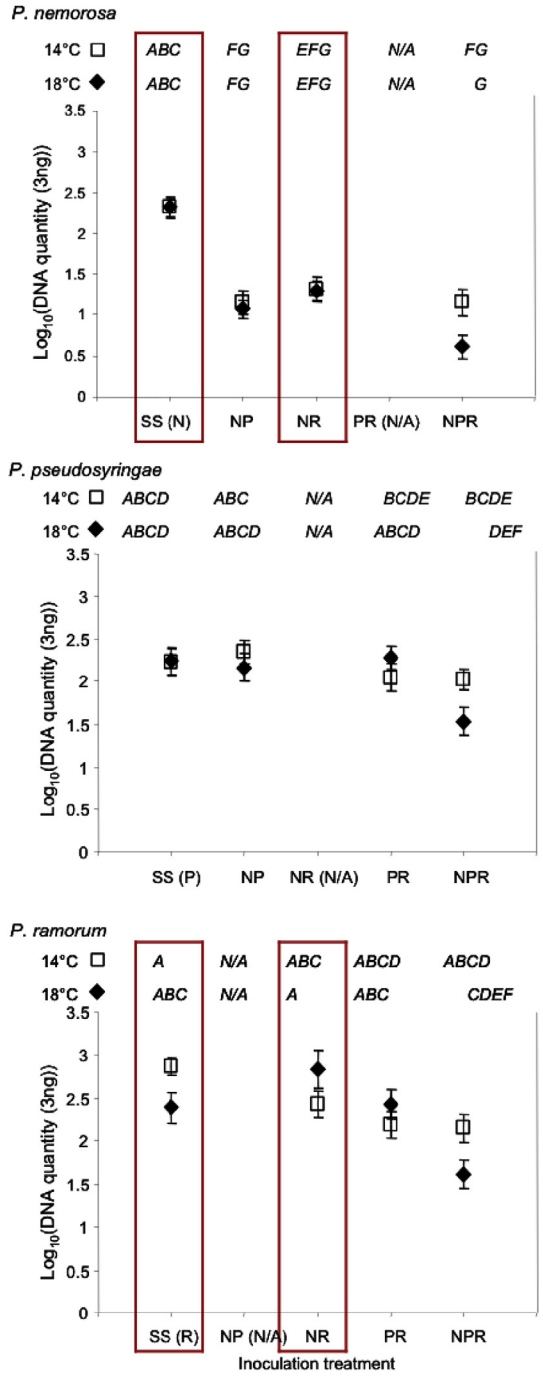

Fig. 4. Total DNA quantity in leaf lesions estimated by qPCR for *P. nemorosa* (N), *P. pseudosyringae* (P) and *P. ramorum* (R); SS = single-species inoculation. The 3 panels represent the results of an ANOVA and Tukey-HSD *post-hoc* test, divided by species. Symbols represent means ± SE; $Log_{10}(x)$-transformed 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.

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 through 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 drier 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 the 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.

Acknowledgments

Thank you to Dora Barbosa, Lydia Baker, Catherine Eyre, and Katy Hayden for invaluable technical assistance and/or advice, as well as Alex Lundquist, Jennifer Tobener, Natalie Lowell, Jerry Lin, Mochi Lui and Kiana Ward for aiding with data collection and processing. We would also like to thank Ellen Natesan at the SFPUC for providing access to the field site and data archives. Funding for this project was provided by the San Francisco Public Utility Commission (SFPUC), the National Science Foundation- Ecology and Evolution of Infectious Diseases (EEID) Initiative, the NSF Doctoral Dissertation Improvement Grant (DDIG), and the United States Forest Service (USFS) Pacific Southwest (Region 5).

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.funeco.2017.04.006.

References

Al-Naimi, F.A., Garrett, K.A., Bockus, W.W., 2005. Competition, facilitation, and niche differentiation in two foliar pathogens. Oecologia 143, 449–457.
Clément, J.A., Magalon, H., Glais, I., Jacquot, E., Andrivon, D., 2012. To be or not to be solitary: *Phytophthora infestans*' dilemma for optimizing its reproductive fitness in multiple infections. PLoS One 7, e37838.
Cobb, R.C., Rizzo, D.M., Hayden, K.J., Garbelotto, M., Filipe, J.A.N., Gilligan, C.A., Dillon, W.W., Meentemeyer, R.K., Valachovic, Y.S., Goheen, E., Swiecki, T.J., Hansen, E.M., Frankel, S.J., 2013. Biodiversity conservation in the face of dramatic forest disease: an integrated conservation strategy for Tanoak (*Notholithocarpus densiflorus*) threatened by Sudden Oak Death. Madroño 60, 151–164.
Connell, J.H., Slatyer, R.O., 1977. Mechanisms of succession in natural communities and their role in community stability and organization. Am. Nat. 111, 1119–1144.
Croucher, P.J.P., Mascheretti, S., Garbelotto, M., 2013. Combining field epidemiological information and genetic data to comprehensively reconstruct the invasion history and the microevolution of the sudden oak death agent *Phytophthora ramorum* (Stramenopila: Oomycetes) in California. Biol. Invasions 15, 2281–2297.
De Martonne, 1926. L'indice d'aridite. Bull. 725 l'association Geogr. Fr. 9, 3–5.
Davidson, J.M., Werres, S., Garbelotto, M., Hansen, E.M., Rizzo, D.M., 2003. Sudden oak death and associated diseases caused by *Phytophthora ramorum*. Online. Plant Health Prog. http://dx.doi.org/10.1094/PHP-2003-0707-01-DG.
Davidson, J.M., Wickland, A.C., Patterson, H.A., Falk, K.R., Rizzo, D.M., 2005. Transmission of *Phytophthora ramorum* in mixed-evergreen forest in California. Phytopathology 95, 587–596.
Ditmore, M., Moore, J.W., TeBeest, D.O., 2008. Interactions of two selected field isolates of *Colletotrichum gloeosporioides* f. sp *aeschynomene* on Aeschynomene virginica. Biol. Control 47, 298–308.
Dobson, A.P., 1985. The population dynamics of competition between parasites. Parasitology 91, 317–347.
Englander, L., Browning, M., Tooley, P.W., 2006. Growth and sporulation of *Phytophthora ramorum in vitro* in response to temperature and light. Mycologia 98 (3), 365–373.
Erwin, D.C., Ribeiro, O.K., 1996. *Phytophthora* Diseases Worldwide. American Phytopathological Society Press, St. Paul, MN.
Eyre, C.A., Kozanitas, M., Garbelotto, M., 2013. Population dynamics of aerial and terrestrial populations of *Phytophthora ramorum* in a California forest under different climatic conditions. Phytopathology 103, 1141–1152.
Eyre, C.A., Hayden, K.J., Kozanitas, M., Grünwald, N.J., Garbelotto, M., 2014. Lineage, temperature, and host species have interacting effects on lesion development in Phytophthora ramorum. Plant Dis. 98, 1717–1727.
Garbelotto, M., Hayden, K.J., 2012. Sudden oak death: interactions of the exotic oomycete *Phytophthora ramorum* with naive North American hosts. Eukaryot. Cell 11, 1313, 1123.
Grünwald, N.J., Garbelotto, M., Goss, E.M., Heungens, K., Prospero, S., 2012. Emergence of the sudden oak death pathogen *Phytophthora ramorum*. Trends Microbiol. 20, 131–138.
Hamelin, F.M., Bisson, A., Desprez-Loustau, M.-L., Fabre, F., Mailleret, L., 2016. Temporal niche differentiation of parasites sharing the same plant host: oak powdery mildew as a case study. Ecosphere 7, e01517.
Hansen, E.M., Goheen, D.J., Jules, E.S., Ullian, B., 2000. Managing port-orford-cedar and the introduced pathogen *Phytophthora lateralis*. Plant Dis. 84, 4–14.
Hansen, E.M., Reeser, P., Davidson, J.M., Garbelotto, M., Ivors, K., Douhan, L., Rizzo, D.M., 2003. *Phytophthora nemorosa*, a new species causing cankers and leaf blight of forest trees in California and Oregon, U.S.A. Mycotaxon 88, 129–138.
Harman, G.E., 2006. Overview of mechanisms and uses of *Trichoderma* spp. Phytopathology 96, 190–194.
Hayden, K., Ivors, K., Wilkinson, C., Garbelotto, M., 2006. TaqMan chemistry for *Phytophthora ramorum* detection and quantification, with a comparison of diagnostic methods. Phytopathology 96, 846–854.
Hayden, K.J., Rizzo, D., Tse, J., Garbelotto, M., 2004. Detection and quantification of *Phytophthora ramorum* from California forests using a real-time polymerase chain reaction assay. Phytopathology 94, 1075–1083.
Hood, M.E., 2003. Dynamics of multiple infection and within-host competition by the anther-smut pathogen. Am. Nat. 162, 122–133.
Hüberli, D., Hayden, K.J., Calver, M., Garbelotto, M., 2011. Intraspecific variation in host susceptibility and climatic factors mediate epidemics of sudden oak death in western US forests: susceptibility variation of bay laurel to sudden oak death. Plant Pathol. 61, 579–592.
Hüberli, D., Van Sant, W., Tse, J.G., Garbelotto, M., 2003. First report of foliar infection of starflower by *Phytophthora ramorum*. Plant Dis. 87, 599.
Johnston, S.F., Cohen, M.F., Torok, T., Meentemeyer, R.K., Rank, N.E., 2016. Host phenology and leaf effects on susceptibility of California bay laurel to *Phytophthora ramorum*. Phytopathology 106, 47–55.
Jung, T., Colquhoun, I.J., Hardy, G.E.S.J., 2013. New insights into the survival strategy of the invasive soilborne pathogen *Phytophthora cinnamomi* in different natural ecosystems in Western Australia. For. Pathol. 43, 266–288.
Jung, T., Nechwatal, J., Cooke, D.E.L., Hartmann, G., Blaschke, M., Oßwald, W.F., Duncan, J.M., Delatour, C., 2003. *Phytophthora pseudosyringae* sp. nov., a new species causing root and collar rot of deciduous tree species in Europe. Mycol. Res. 107, 772–789.
Keane, R.M., Crawley, M.J., 2002. Exotic plant invasions and the enemy release

hypothesis. Trends Ecol. Evol. 17, 164–170.
Laine, A.L., 2011. Context-dependent effects of induced resistance under co-infection in a plant-pathogen interaction. Evol. Appl. 4, 696–707.
Lamari, L., 2002. Assess. Image Analysis for Plant Disease Quantification. The American Phytopathological Society. APS Press, St. Paul, MN.
Liancourt, P., Callaway, R.M., Michalet, R., 2005. Stress tolerance and competitive-response ability determine the outcome of biotic interactions. Ecology 86, 1611–1618.
Linzer, R.E., 2009. Population Biology, Invasiveness and Coinfection of Exotic Forest Pathogens in Two Temperate-Forest Pathosystems [Ph.D.]. United States – California. University of California, Berkeley.
Linzer, R.E., Rizzo, D.M., Cacciola, S.O., Garbelotto, M., 2009. AFLPs detect low genetic diversity for *Phytophthora nemorosa* and *P. pseudosyringae* in the US and Europe. Mycol. Res. 113, 298–307.
Lopez-Villavicencio, M., Jonot, O., Coantic, A., Hood, M.E., Enjalbert, J., Giraud, T., 2007. Multiple infections by the anther smut pathogen are frequent and involve related strains. PLoS Pathog. 3, 1710–1715.
Lopez-Villavicencio, M., Courjol, F., Gibson, A.K., Hood, M.E., Jonot, O., Shykoff, J.A., Giraud, T., 2011. Competition, cooperation among kin, and virulence in multiple infections. Evolution 65, 1357–1366.
Marín, S., Companys, E., Sanchis, V., Ramos, A.J., Magan, N., 1998. Effect of water activity and temperature on competing abilities of common maize fungi. Mycol. Res. 102, 959–964.
Marras, S.A.E., 2007. Interactive fluorophore and quencher pairs for labeling fluorescent nucleic acid hybridization probes. Mol. Biotechnol. 38, 247–255.
Martin, F.N., Tooley, P.W., 2003. Phylogenetic relationships of *Phytophthora ramorum*, *P. nemorosa*, and *P. pseudosyringae*, three species recovered from areas in California with sudden oak death. Mycol. Res. 107, 1379–1391.
Mascheretti, S., Croucher, P.J.P., Kozanitas, M., Baker, L., Garbelotto, M., 2009. Genetic epidemiology of the sudden oak death pathogen *Phytophthora ramorum* in California. Mol. Ecol. 18, 4577–4590.
Mascheretti, S., Croucher, P.J.P., Vettraino, A., Prospero, S., Garbelotto, M., 2008. Reconstruction of the Sudden Oak Death epidemic in California through microsatellite analysis of the pathogen Phytophthora ramorum. Mol. Ecol. 17, 2755–2768.
Meshriy, M., Hüberli, D., Harnik, T., Miles, L., Reuther, K., Garbelotto, M., 2006. Variation in susceptibility of *Umbellularia californica* (bay laurel) to *Phytophthora ramorum*. In: Frankel, S.J. (Ed.), Proceedings of the Sudden Oak Death Second Science Symposium: the State of Our Knowledge. Pacific Southwest Research Station, Forest Service, U.S.Department of Agriculture, Albany, CA, pp. 87–89. General Technical Report PSW-GTR-196.
Monahan, W.B., Tse, J., Koenig, W.D., Garbelotto, M., 2008. Preserved specimens suggest non-native origins of three species of *Phytophthora* in California. Mycol. Res. 112, 757–758.
Murphy, S.K & Rizzo, D.M. 2006. Incidence of *Phytophthora ramorum*, *P. nemorosa* and *P. pseudosyringae* in three coastal California forest communities. Proceedings from the Second Sudden Oak Death Science Symposium, January 18-21, 2005. Monterey, California. Gen. Tech Rep. PSW-197. Albany, CA: Pacific Southwest Research Station, Forest Service, U.S. Dept. of Agriculture, pp. 69–71.
Murphy, S.K., Wickland, A.C., Lynch, S.C., Jensen, C.E., Maloney, P.E. & Rizzo, D.M. 2008. Distribution of *Phytophthora ramorum*, *P. nemorosa*, and *P. pseudosyringae* in native coastal California forest communities. *Sudden Oak Death Third Science Symposium. USDA Forest Service General Technical Report PSW-GTR-214, Santa Rosa, California* pp. 51–54.
Nowicki, M., Foolad, M.R., Nowakowska, M., Kozik, E.U., 2012. Potato and tomato late blight caused by *Phytophthora infestans*: an overview of pathology and resistance breeding. Plant Dis. 96, 4–17.
Osmundson, T.W., Eyre, C.A., Hayden, K.M., Dhillon, J., Garbelotto, M.M., 2013. Back to basics: an evaluation of NaOH and alternative rapid DNA extraction protocols for DNA barcoding, genotyping, and disease diagnostics from fungal and oomycete samples. Mol. Ecol. Resour. 13, 66–74.
Rizzo, D.M., Garbelotto, M., 2003. Sudden oak death: endangering California and Oregon forest ecosystems. Front. Ecol. Environ. 1, 197–204.
Rizzo, D.M., Garbelotto, M., Davidson, J.M., Slaughter, G.W., Koike, S.T., 2002. *Phytophthora ramorum* as the cause of extensive mortality of *Quercus* spp. and *Lithocarpus densiflorus* in California. Plant Dis. 86, 205–214.
Scanu, B., Linaldeddu, B.T., Franceschini, A., 2010. First report of *Phytophthora pseudosyringae* associated with ink disease of *Castanea sativa* in Italy. Plant Dis. 94, 1068.
Scanu, B., Jones, B., Webber, J.F., 2012. A new disease of *Nothofagus* in Britain caused by Phytophthora pseudosyringae. New Dis. Rep. 25, 2044, 0588.
Schena, L., Cooke, D.E.L., 2006. Assessing the potential of regions of the nuclear and mitochondrial genome to develop a “molecular tool box” for the detection and characterization of *Phytophthora* species. J. Microbiol. Methods 67, 70–85.
Schena, L., Hughes, K.J.D., Cooke, D.E.L., 2006. Detection and quantification of *Phytophthora ramorum, P.kernoviae, P.citricola* and *P.quercina* in symptomatic leaves by multiplex real-time PCR. Mol. Plant Pathol. 7, 365–379.
Silvertown, J.W., Doust, J.L., 1993. Introduction to Plant Population Biology. Blackwell Scientific Publications, Oxford, UK, pp. 116–140.
Simpson, D.R., Thomsett, M.A., Nicholson, P., 2004. Competitive interactions between *Microdochium nivale* var. *majus, M-nivale* var. *nivale Fusarium culmorum* planta vitro. Environ. Microbiol. 6, 79–87.
Siou, D., Gelisse, S., Laval, V., Elbelt, S., Repincay, C., Bourdat-Deschamps, M., Suffert, F., Lannou, C., 2015. Interactions between head blight pathogens: consequences for disease development and toxin production in wheat spikes. Appl. Environ. Microbiol. 81, 957–965.
Steiner, J.J., Poklemba, C.J., Fjellstrom, R.G., Elliott, L.F., 1995. A rapid one-tube genomic DNA extraction process for PCR and RAPD analyses. Nucleic Acids Res. 23, 2569.
Svihra, P., 1999. Sudden Death of Tanoak, Lithocarpus Densflora. Pest Alert #1. UC Cooperative Extension, Marin Co, San Rafael, CA.
Tooley, P.W., Martin, F.N., Carras, M.M., Frederick, R.D., 2006. Real-time fluorescent polymerase chain reaction detection of *Phytophthora ramorum* and *Phytophthora pseudosyringae* using mitochondrial gene regions. Phytopathology 96, 336–345.
Werres, S., Marwitz, R., Man In't veld, W.A., De Cock, A.W.A.M., Bonants, P.J.M., De Weerdt, M., Themann, K., Ilieva, E., Baayen, R.P., 2001. *Phytophthora ramorum* sp. nov., a new pathogen on *Rhododendron* and Viburnum. Mycol. Res. 105, 1155–1165.
Wickland, A.C., Jensen, C.E., Rizzo, D.M., 2008. Geographic distribution, disease symptoms and pathogenicity of *Phytophthora nemorosa* and *Phytophthora pseudosyringae* in California, USA. For. Pathol. 38, 288–298.
Wickland, A.C. & Rizzo, D.M. 2006. Ecology of *Phytophthora nemorosa* and *Phytophthora pseudosyringae* in Mixed-Evergreen Forests1. Sudden Oak Death Second Science Symposium: the state of our knowledge: January 18-21, 2005, Monterey, California p. 73. US Dept. of Agriculture, Forest Service, Pacific Southwest Research Station.
Wilson, M., Lindow, S.E., 1994. Coexistence among epiphytic bacterial populations mediated through nutritional resource partitioning. Appl. Environ. Microbiol. 60, 4468–4477.