Disease Progression of *Phytophthora ramorum* and *Botryosphaeria dothidea* on Pacific Madrone

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ABSTRACT

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Infection by *Phytophthora ramorum* was associated with stem and leaf lesions of Pacific madrone (*Arbutus menziesii*) seedlings and saplings. In addition, a common and native pathogen, *Botryosphaeria dothidea*, caused similar leaf and stem lesions. When exposed to natural levels of inoculum in forests infested with *P. ramorum*, 50 to 66% of madrone saplings used as bait died. Recovery of *P. ramorum* from colonized plant tissue on culture media was generally low. From initial infection, *P. ramorum* was not culturable from leaf tissue after a mean of 3.5 weeks or from stem tissue after a mean of 8 weeks. Generally, *B. dothidea* was recovered more frequently from necrotic stems and leaves than was *P. ramorum*. Experimental inoculations of madrone seedlings showed that leaf and stem lesion lengths were, on average, greater on tree seedlings inoculated with *P. ramorum* than on those inoculated with *B. dothidea. P. ramorum* and *B. dothidea* appear to coexist in stem and leaf tissue, forming a novel pathogen complex, affecting growth and reproduction of Pacific madrone.

Phytophthora ramorum S. Werres. A.W.A.M. de Cock & W.A. Man in't Veld is a recently described pathogen that causes sudden oak death, as well as a leaf and twig blight of many other plant species (4,10,13,14,18). This pathogen appears to have been introduced into native forests of California and Oregon, where it infects nearly all woody plant species and several herbaceous species. Although several studies have been established to monitor disease progression on oak and tanoak (17), few have provided a detailed description of disease progression caused by *P. ramorum* on non-oak hosts from these forests (5). Experiments to date on non-oak hosts have focused mostly on completing Koch's postulates to demonstrate pathogenicity of *P. ramorum* (10,14), while symptomatology and disease progression have not been well characterized. Because of this, we are unable to predict the future effects of *P. ramorum* on individual populations of non-oak hosts and on the overall plant communities in which these hosts occur.

Based on previous studies, Pacific madrone (*Arbutus menziesii*) appears to be one of the most susceptible plant species in terms of dieback and potential mortality (10,14). Madrone is an important overstory tree in several forest types along the Pacific Coast from California to British Columbia (11). In California, it is a major component of the mixed-evergreen forest type, along with coast live oak (*Quercus agrifolia*), California bay laurel (*Umbellularia californica*) and Douglas-fir (*Pseudotsuga menziesii*) (11). It also is associated with coast redwood-tanoak (*Sequoia sempervirens/Lithocarpus densiflorus*) forests in California (11). Along the Central Coast of California, each of these forest types has been invaded by *P. ramorum*, and in many areas these forests have experienced extensive mortality of coast live oak and tanoak (13).

The presence of *Phytophthora ramorum* has been confirmed in leaf lesions and stem cankers on madrone using molecular and, to a lesser extent, culture techniques (5). Difficulty in culturing *P. ramorum* from lesions, in addition to lack of unique symptomatology of the disease, has complicated diagnosis of infection by *P. ramorum* on madrone. To further complicate field diagnosis, madrone also may be infected with *Nattrassia mangiferae* Nattrass (synanamorph = *Scytalidium dimidiatum*) and *Botryosphaeria dothidea* (Moug.:Fr.) Ces. & De Not., most often identified by its anamorphic state *Fusicoccum aesculi* (7). These two pathogens overlap in geographic range with *P. ramorum* in the coastal ranges of California (12,15). *N. mangiferae* causes distinct sunken cankers on the branches and trunk and is considered to be a relatively weak pathogen; hot, dry weather favors disease development of this pathogen (7). *B. dothidea* causes twig and leaf dieback that could be confused with infection caused by *P. ramorum*. Generally, neither *N. mangiferae* nor *B. dothidea* is thought to cause death of madrone unless coupled with severe drought conditions or other causes of water stress, such as pathogens or insects (7). *Botryosphaeria* spp. are known to be endophytic on a number of species in the family *Proteaceae* as well as on *Fagus* spp. (3,6), and some *Botryosphaeria* spp. have a latent phase and are not pathogenic until environmental or physiological conditions are favorable (16).

Given that *P. ramorum* and *B. dothidea* overlap in geographic range in Northern California and cause similar symptoms on madrone, laboratory and field studies were established to characterize symptoms of the diseases caused by *P. ramorum* and *B. dothidea*. Because the geographic range of madrone extends well beyond the current range of *P. ramorum*, we need to begin to understand the short- and long-term effects of this non-native pathogen, as well as how it interacts with native pathogens. The objectives of this research were to (i) determine the natural susceptibility of madrone in forests known to be infested with *P. ramorum,* (ii) assess disease development and recovery from host tissue infected by *P. ramorum* and *B. dothidea*, and (iii) determine whether there are positive or negative interactions between *P. ramorum* and *B. dothidea*, because the two potentially may coexist and compete for the same resources.

MATERIALS AND METHODS

Field studies. To observe disease development over time, healthy madrone saplings were exposed to natural inoculum of *P. ramorum* in redwood forests at Jack London State Park, Sonoma County, CA. The presence of *P. ramorum* previously had been confirmed in native vegetation in each plot. The dominant tree species in the plots were redwood, tanoak, and bay laurel, with madrone as an associated species.

In California's Mediterranean climate of predominantly winter rainfall, *P. ramorum* has been shown to sporulate most actively during December to May (3; P. E. Maloney, *unpublished*). Madrone saplings, approximately 4 years old, 1.0 m tall, and 1.0 cm in diameter (purchased from Suncrest Nurseries, Watsonville, CA) in individual 18.9-liter pots were placed in each

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of 10 500-m^2 permanent plots (one sapling/plot) at the end of November 2001 and 2002. In each plot, a sapling was placed adjacent to a nearby madrone. Two more saplings were placed in two additional plots (one sapling/plot) in April 2003, for a total of 12 plots for the 2002–03 season. All saplings were removed from all plots for each year of the experiment.

Plants were visited monthly throughout the winter and spring of 2001–02, and every 3 weeks throughout the winter and spring of 2002–03. Madrone saplings from 2001–02 were sampled for the presence of *P. ramorum* and, in 2002–03, for the presence of both *P. ramorum* and *B. dothidea.* Isolation of *B. dothidea* was attempted for samples from the first four visits of the 2002–03 season. Samples were taken from canker and lesion margins, then cut up into smaller pieces and placed into petri dishes containing a *Phytophthora* selective medium, pimaricin-ampicillin-rifampicin-pentachloronitrobenzene agar (PARP; 9) or on acidified potato dextrose agar (APDA) to isolate for *B. dothidea.* Plant tissue sampled for *B. dothidea* was surface sterilized with 10% bleach (6% NaOCl) for 10 s and rinsed in sterilized water before plating onto APDA.

In 2001–02 and 2002–03, two to three symptomatic stem sections and two to three leaves or leaf sections were sampled on each visit. Lesion length was measured on each symptomatic stem section (new and old) on each visit until disease was well advanced throughout the plant (most if not all tissue appearing necrotic), after which a subsample of stem sections was collected for isolation and lesion measurement for the remaining visits. Stems were not destructively sampled, and only small stem samples were taken so that lesion lengths could be continuously measured. For stem lesions, branch health (dead or wilted) and coalescence with other stem lesions was noted. Leaf lesions (new and old) were measured at each visit, and lesion characteristics (active or dry), leaf status (dead, wilted, or missing), and lesion enlargement toward the stem were noted. When diseased leaf tissue was necrotic, plants were no longer measured for lesion expansion but were sampled for presence of *P. ramorum*. A portion of the leaf lesion was cut from the selected leaf for isolation. The same leaves were revisited and sampled in the same manner for the remaining visits.

Disease progression experiments. Two inoculation trials were conducted in a growth chamber on 1-year-old seedlings (approximately 0.5 cm in diameter and 0.3 m tall) to monitor independently disease progression of *P. ramorum* and *B. dothidea*. Seedlings from a California Sierra Nevada population of madrone were purchased from the California Department of Forestry (CDF) nursery in Davis, CA. Each of the madrone seedlings came in a 656-cm^3 container. Sand was layered over peat to fill 1,570-cm^3 pots approximately half full. The seedling, which remained in its original container, was placed in the pot and the remainder of the pot filled with sand. Seedlings were maintained in a lathhouse until used for the experiment and then placed in the growth chamber 1 week prior to the start of the experiment.

The isolate of *P. ramorum* (isolate no. Pr 431, American Type Culture Collection no. MYA-3297) used in the experiment was recovered from a madrone sapling used as bait from Jack London State Park. The *B. dothidea* isolate came from an ornamental redwood in Kings County, CA (isolate no. 288 and 291, Kearney Agricultural Center, Parlier). Cultures are maintained in the culture collection of D. M. Rizzo (University of California, Davis).

Inoculum was prepared by growing *P. ramorum* and *B. dothidea* on clarified V8 juice agar (CV8) and PDA, respectively, for 5 to 7 days, on which mycelium and spores (for *P. ramorum*) were well established. Agar plugs of each pathogen were cut from the advancing margin of the mycelium using a sterile 5-mm-diameter cork borer. For wound treatments, the middle of the leaf or stem was pierced with a sterile needle. Plugs then were placed mycelium side down onto the wounded tissue. For nonwound treatments, plugs were simply placed onto the plant tissue. Each plug was sealed around the stem or leaf by wrapping with parafilm. Three leaves per plant were selected at random, with plugs placed on the upper leaf surface, and only one plug was used for each stem. For each pathogen treatment, there were five replicate seedlings. The nontreated control was replicated on three seedlings. Each seedling was placed in an individual plastic bag (with open top) and randomly placed within the growth chamber (Conviron PGR-15, Winnipeg, Manitoba, Canada). Plants were sprayed with deionized water for 25 s every Monday, Wednesday, and Friday using a polyethylene tank sprayer. At 2 weeks, all plugs were removed from each plant.

The growth chamber was programmed for 15°C at night and 20°C during the day with 90% relative humidity and 12 h of light and 12 h of dark for both trials, which ran from July through August and October through December 2002, respectively. Lesion lengths were measured at 7, 10, 14, 18, 21, 28, 35, and 42 days. In the second trial, plants were sampled at 28, 35, and 42 days by taking tissue from the advancing lesion margin on leaves and stems to reisolate *P. ramorum* and *B. dothidea*.

All data were analyzed using a two-way analysis of variance (ANOVA) for stem and leaf data using the software program JMP (SAS Institute, Duxbury, Pacific Grove, CA). Based on initial ANOVA, there was no trial effect; therefore, data were pooled from the two trials for both stem and leaf data. For all analyses, wound and pathogen were treated as fixed effects. Data were log transformed to meet the assumptions of an analysis of variance, and where variances were unequal, a Welch ANOVA was used in which observations are weighted by the reciprocals of the estimated variances. Orthogonal contrasts were used to test for differences between pathogens for each wound treatment and between wound treatments for each pathogen species.

Pathogen interaction experiments. Two growth chamber trials were conducted with madrone seedlings to determine the degree of interaction between *P. ramorum* and *B. dothidea*. Plants were obtained from the same source and potted in the manner described above.

Preparation of inoculum and inoculation of leaves and stems were done as in the previous experiment. All plants were wound inoculated at the start of the experiment. Five replicates each of *P. ramorum* followed by *B. dothidea* and *B. dothidea* followed by *P. ramorum* were used. Three replicates each of *P. ramorum* and *B. dothidea* alone were used as checks. Plants that were inoculated with both pathogens received plugs with the first pathogen, the plugs were sealed with parafilm, and the plugs removed after 7 days. The second

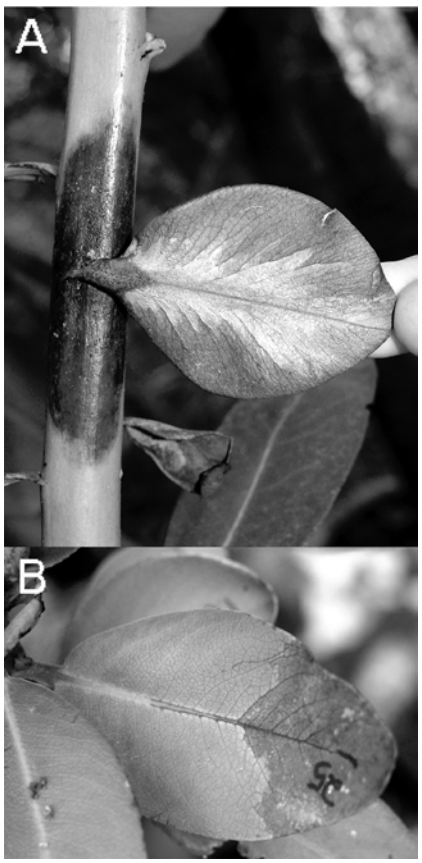

Fig. 1. Madrone saplings exposed in the field with **A,** stem and **B,** leaf lesions caused by *Phytophthora ramorum.*

pathogen then was placed on the original wound, the plug sealed with parafilm, and the plug removed after 7 days. Plugs that were placed onto madrone trees inoculated with only one pathogen (i.e., controls) were removed after 7 days.

Seedlings were placed in the growth chamber and incubated at the environmental settings described above. Lesion lengths were measured at 7, 14, 21, 28, 35, 42, and 49 days. Small pieces of leaf and stem tissue were sampled at the advancing lesion margin of leaves to reisolate *P. ramorum* and *B. dothidea* at 14 days after the initial inoculation and on subsequent measurement days. Stems were sampled at both the center of the lesion and at the advancing lesion margin starting with the 21-day measurement.

Data were analyzed using a one-way ANOVA for stem and leaf data, and pathogen recovery data were analyzed using a two-way ANOVA for each species. Based on initial ANOVA, there was no trial effect; therefore, data were pooled from the two trials. All analyses were performed using the software program JMP (SAS Institute, Duxbury). For the analyses, pathogen and plant location (stem or leaf) were treated as fixed effects. Pathogen interaction data were log transformed, and pathogen recovery data were arcsine transformed to meet the assumptions of ANOVA. To test for differences in lesion length between pathogen treatments, we used orthogonal contrasts. Orthogonal contrasts also were used to test for differences in recovery

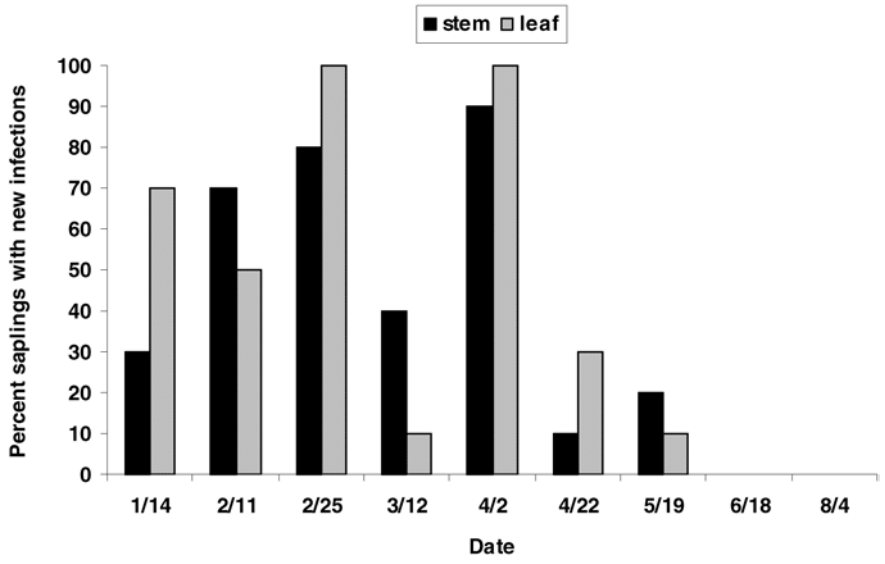

Fig. 2. Percentage of madrone saplings infected with *Phytophthora ramorum* on stems (n = 10) and leaves (n = 10) at different dates at Jack London State Park in 2003. Percentage based on the number of individual saplings with new leaf or stem infections from each of the 10 plots.

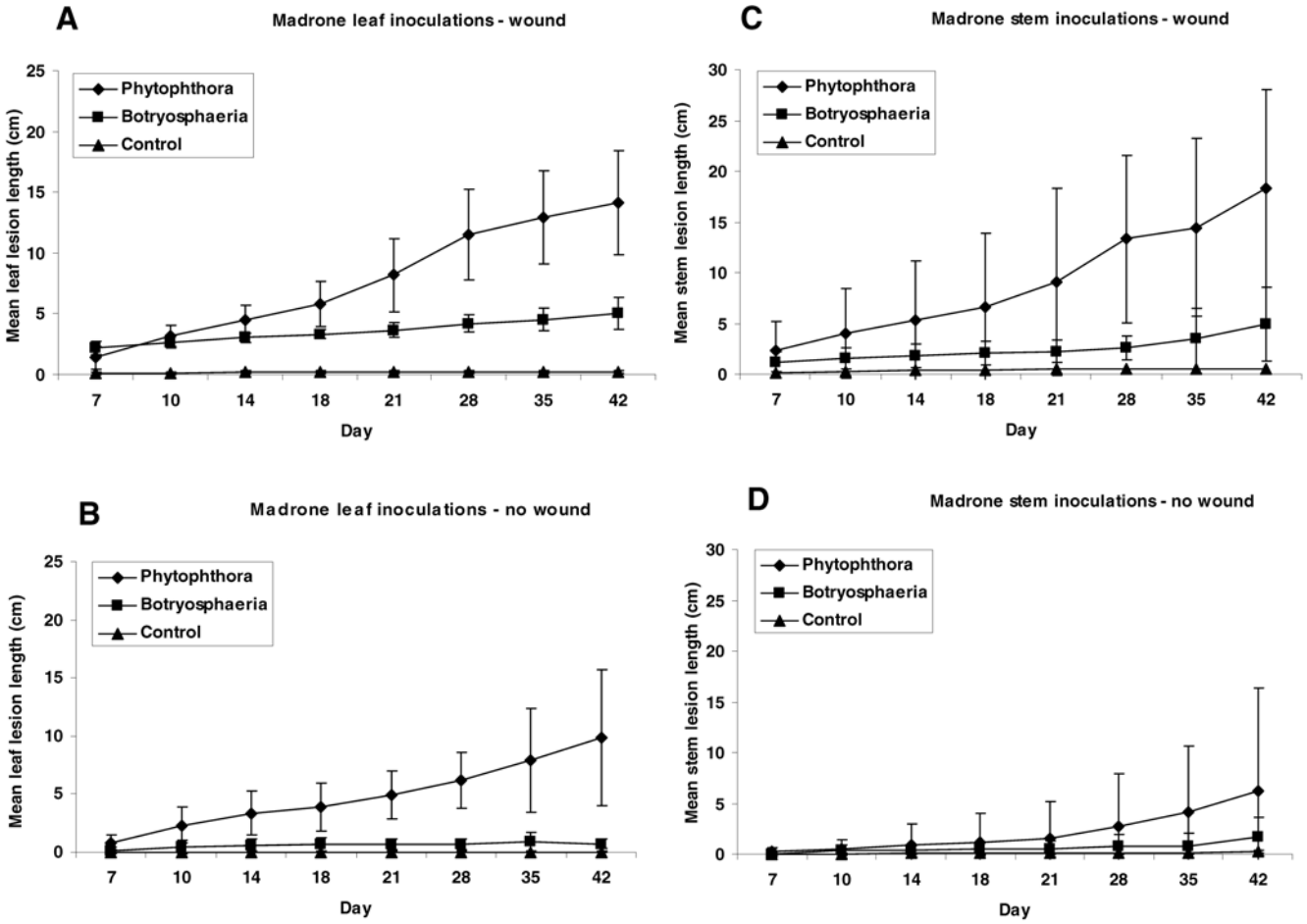

Fig. 3. Lesion lengths for madrone seedlings inoculated with *Phytophthora ramorum* or *Botryosphaeria dothidea* on **A,** wounded and **B,** unwounded leaves, and **C,** wounded and **D,** unwounded stems. Mean lesion lengths for each pathogen treatment and wound treatment for leaves and stems based on n = 10 and n = 6, respectively, with standard deviations.

frequency between pathogen treatments for each species.

RESULTS

Field studies. When exposed to natural inoculum, 70 and 100% of madrone saplings became infected with *P. ramorum* within 2 to 5 months during the winters of 2001–02 and 2002–03, respectively. *B. dothidea* also was isolated from all exposed madrone saplings sampled in 2003. Both stems and foliage were infected with *P. ramorum* (Fig. 1). *P. ramorum* was isolated from stems up to 12 weeks after initial infection (mean, 8 weeks) and from leaves up to 6 weeks after initial infection (mean, 3.5 weeks). Infections occurred on both stems and leaves throughout the winter and spring of 2002–03 (Fig. 2). Many stem lesions coalesced after initial appearance within 3 to 8 weeks, forming large continuous lesions along the main stem. In addition, leaf lesions coalesced with stem lesions as leaf lesions enlarged down through the petiole and into the main stem. A total of 50 to 66% of the madrones died due to infection by *P. ramorum*.

Disease progression experiments. On inoculated stems and leaves, lesions caused by *P. ramorum* and *B. dothidea* appeared water-soaked or dark after 7 days. *P. ramorum* caused greater leaf lesion lengths by day 42 than *B. dothidea* in both the wound and no-wound treatments (Fig. 3). Mean leaf lesion lengths were significantly different between the pathogens; there also was a significant wound–pathogen interaction effect (Table 1; Fig. 3). Wounded leaves inoculated with *P. ramorum* had significantly greater lesion lengths at day 42 than the unwounded leaves with *P. ramorum* (orthogonal contrast, $F = 34.48$, $P < 0.0001$; (Fig. 3A and B). Similarly, wounded leaves inoculated with *B. dothidea* had significantly greater lesion lengths at day 42 than the unwounded leaves with *B. dothidea* (orthogonal contrast, $F = 28.21$, $P < 0.0001$; Fig. 3A and B). For both wound and no-wound leaf treatments, leaf lesions length caused by *P. ramorum* at day 42 were significantly greater than the corresponding wound and no-wound leaf lesion lengths caused by *B. dothidea* (orthogonal contrasts, $F = 21.31$, $P < 0.0001$ and $F = 26.71$, $P < 0.0001$, respectively; Fig. 3A and B).

On stems, the mean stem lesion length at day 42 varied between the wound treatments and pathogen treatments, and there was a significant wound–pathogen interaction effect (Table 1; Fig. 3). Wounded stems with *P. ramorum* had significantly greater lesion lengths at day 42 than the unwounded stems with *P. ramorum* (orthogonal contrast, $F = 19.98$, $P = 0.001$; Fig. 3C and D). Wounded stems with *P.*

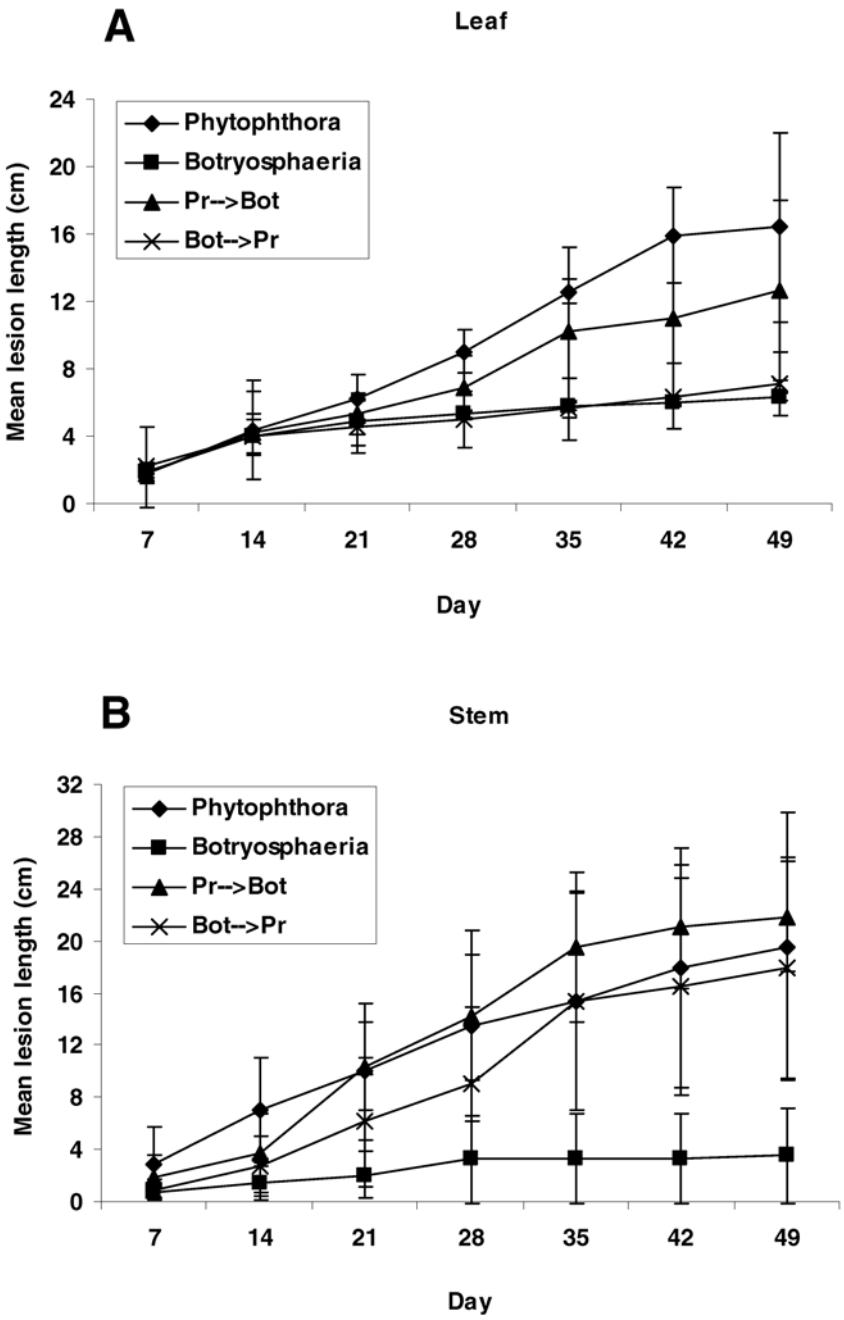

Fig. 4. Mean lesion lengths on madrone seedlings inoculated with *Phytophthora ramorum*, *Botryosphaeria dothidea*, *P. ramorum* followed by *B. dothidea* (Pr → Bot), or *B. dothidea* followed by *P. ramorum* (Bot → Pr) on **A,** leaves and **B,** stems with standard deviations.

Table 1. Analysis of variance of mean leaf and stem lesion lengths at 42 days after inoculation with *Phytophthora ramorum* or *Botryosphaeria dothidea*, and control inoculations on wounded and nonwounded tissue[a]

	Leaf				Stem			
Source	**df**	**SS**	***F***	***P***	**df**	**SS**	***F***	***P***
Wound	1	<0.01	<0.01	0.99	1	0.19	10.84	0.002
Pathogen	2	1.95	17.36	<0.0001	2	0.62	17.43	<0.0001
Wound–pathogen	2	3.55	31.50	<0.0001	2	0.13	3.89	0.027
Residual	44	2.48	…	…	46	0.82	…	…

[a] Data were log transformed prior to analysis.

ramorum also had significantly greater lesion lengths at day 42 than the corresponding wounded stems with *B. dothidea* (orthogonal contrast, $F = 18.8$, $P = 0.001$; Fig. 3C). Orthogonal contrasts showed no significant difference between wound treatments on stems inoculated with *B. dothidea*, or between unwounded stems inoculated with *P. ramorum* and *B. dothidea* (Fig. 3C and D).

For inoculations with *P. ramorum,* almost the entire length of seedling stem tissue was colonized, and 15 of 20 seedlings died. For inoculations with *B. dothidea,* a smaller percentage of stem tissue was colonized, and there was less seedling mortality (5 out of 20 died). Neither *P. ramorum* nor *B. dothidea* were recovered from control seedlings.

Pathogen interaction experiments. On both leaves and stems, there were significant pathogen treatment effects ($F = 5.12$, $P = 0.006$ and $F = 9.14$, $P = 0.0002$, respectively; Fig. 4). Specifically, leaves inoculated with *P. ramorum* followed by *B. dothidea* had significantly greater mean lesion lengths at day 49 than leaves inoculated with *B. dothidea* followed by *P. ramorum* (orthogonal contrast, $F = 4.07$, $P = 0.05$; Fig. 4A). As expected, leaf lesion lengths caused by *P. ramorum* were significantly greater than leaf lesion lengths caused by *B. dothidea* at day 49 (orthogonal contrast, $F = 10.75$, $P = 0.002$; Fig. 4A). Orthogonal contrasts showed no significant difference in leaf lesion lengths between inoculations with *B. dothidea* and *B. dothidea* followed by *P. ramorum* or between *P. ramorum* and *P. ramorum* inoculation followed by *B. dothidea* (Fig. 4A). Plants inoculated with *B. dothidea* followed by *P. ramorum* had greater stem lesion lengths at day 49 than did plants inoculated only with *B. dothidea* (orthogonal contrast, $F = 15.58$, $P = 0.001$; Fig. 4B). As we found for leaves, stem lesion lengths caused by *P. ramorum* were significantly greater than stem lesion lengths caused by *B. dothidea* at day 49 (orthogonal contrast, $F = 25.20$, $P = 0.001$; Fig. 4B). Contrasts showed that stem lesion lengths caused by *P. ramorum* did not differ from those caused by *P. ramorum* followed by *B. dothidea* or *B. dothidea* followed by *P. ramorum* (Fig. 4B).

Recovery frequency for *P. ramorum* at day 49 showed a significant plant location (stems versus leaves) effect ($F = 103.47$, $P < 0.0001$) but no pathogen or pathogen–location interaction effect (Fig. 5A). However, *P. ramorum* was recovered significantly more frequently from stems and leaves inoculated with *P. ramorum* than from stems and leaves inoculated with *B. dothidea* followed by *P. ramorum* (orthogonal contrasts, $F = 4.21$, $P = 0.046$; Fig. 5A). There was no significant difference in recovery frequencies for *B. dothidea* at day 49 between the pathogen treatments or plant location, and there was no pathogen–plant location interaction effect. *B. dothidea* was recovered from both stems and leaves of all pathogen treatments (Fig. 5B). However, *B. dothidea* was recovered significantly more frequently from the treatment with *B. dothidea* alone than from the pathogen interaction treatment of *P. ramorum* followed by *B. dothidea* (orthogonal contrasts, $F = 4.21$, $P = 0.045$; Fig. 5B).

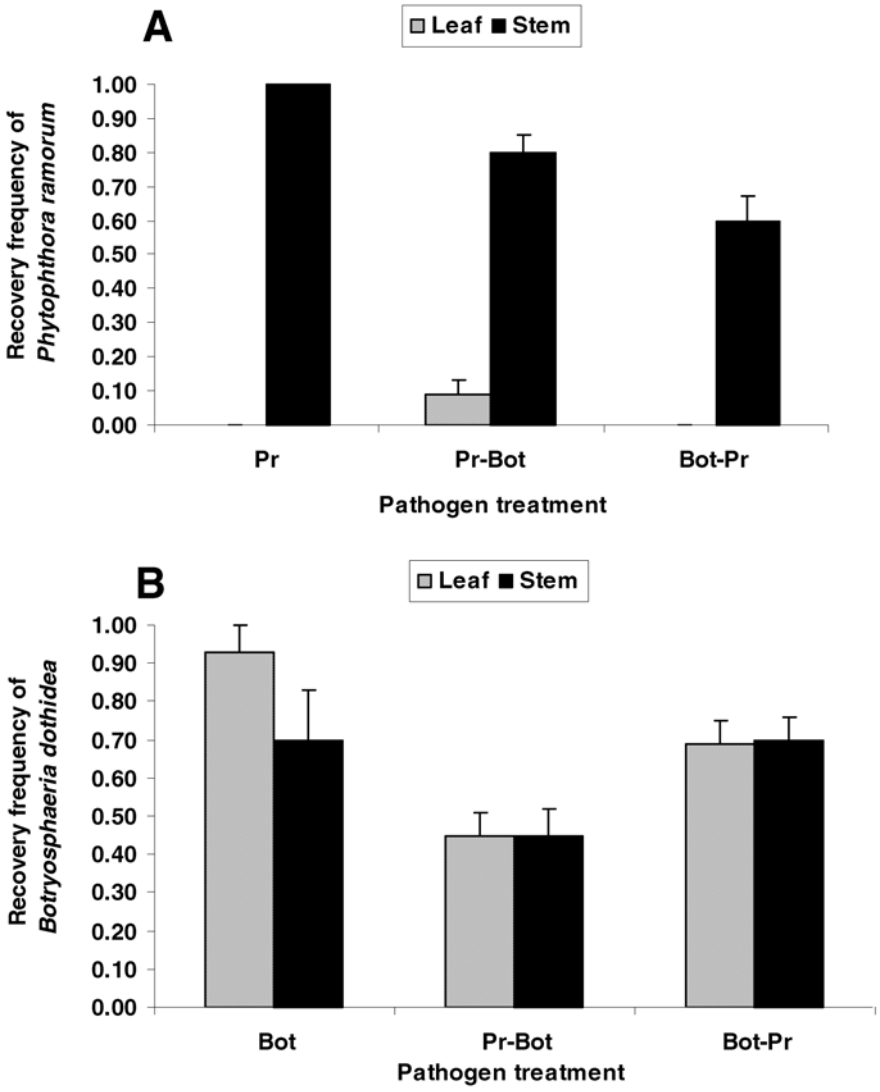

Fig. 5. Mean recovery frequency of *Phytophthora ramorum* **(A)** and *Botryosphaeria dothidea* **(B)** from leaves and stems at 49 days after inoculation from madrone seedlings inoculated with *P. ramorum*, *B. dothidea*, *P. ramorum* followed by *B. dothidea* (Pr → Bot), or *B. dothidea* followed by *P. ramorum* (Bot → Pr) with standard error bars.

DISCUSSION

Madrone seedlings and saplings exposed to artificial and natural inoculum of *P. ramorum* produced similar symptoms and, over time, most of the infected individuals died, both in the growth chamber and in the field. In growth chamber experiments, stem and leaf wounds enhanced symptom development and growth of *P. ramorum*. Recovery of *P. ramorum* was higher when isolating from stem tissue than from leaf tissue. The stem may be a more favorable environment for this pathogen to grow and survive in seedlings and saplings because the tissue dies more gradually. *P. ramorum* is much more difficult to culture from leaves because colonized tissue dies quickly. This was evident in both the field and growth chamber experiments, where *P. ramorum* was recovered on average up to 3.5 weeks after initial infection on saplings in the field and with little to no recovery at 49 days after initial infection in growth chamber experiments. Thus, isolation and identification of *P. ramorum* from madrone trees in the field may be possible if the infection is recent and active. Polymerase chain reaction techniques, however, can identify the presence or absence of *P. ramorum* in dead tissue (5). For larger madrone trees, detection is more difficult due to the inaccessibility of symptomatic branches and the similarity of the dieback symptoms to those associated with *B. dothidea*.

Overall symptom development and growth of *B. dothidea* in inoculated plants

was much less extensive than with *P. ramorum*. Similar to what we found with *P. ramorum,* stem and leaf wounds enhanced symptom development and growth of *B. dothidea*. Previous studies have shown that *B. dothidea* enters hosts through wounds and lenticels (12). Mortality of madrone seedlings caused by *B. dothidea* was low in our growth chamber experiments, but it had high recovery frequency from both stems and leaves at 49 days after initial infection. *B. dothidea* has exhibited limited growth when hosts are receiving adequate water, but it can grow saprophytically in necrotic tissue (1), which may explain why we were able to recover *B. dothidea* consistently from dead leaf tissue. In addition, if *B. dothidea* has an endophytic or latent phase in its life cycle (16), this also could potentially explain its ubiquity and high recovery frequencies from stem and leaf tissue of madrone.

Pathogen interaction experiments showed that *P. ramorum* and *B. dothidea* can coexist in leaf and stem tissue. On leaf tissue, *P. ramorum* grew best when alone. In the presence of *B. dothidea*, *P. ramorum* grew better if it infected leaf tissue prior to *B. dothidea* than if *B. dothidea* was previously inoculated. Presence of a strong interaction between these two pathogens still is not clear and it is only on leaf tissue where a possible interaction may be occurring. However, in stem tissue, growth of *P. ramorum* alone and in the presence of *B. dothidea* did not differ. *P. ramorum* grew better on stems than on leaves, possibly because the stem environment is more conducive for growth of this pathogen.

B. dothidea is considered an opportunistic pathogen that rarely attacks vigorous trees (7). However, an interaction with *P. ramorum* may result in a novel pathogen complex affecting growth and reproduction of madrone. Both *P. ramorum* and *B. dothidea* inoculum is dispersed during the winter rainy season in California (2,4; J. Davidson and P. E. Maloney, *unpublished data*). *P. ramorum* appears to survive in infected host tissue during the dry summer months, but is least active at this time of year. In contrast, the summer drought months of California are optimal for growth and activity of *B. dothidea* (2,8). If the two pathogens are present on madrone, *P. ramorum* may initiate branch dieback during the rainy season and *B. dothidea* could accelerate and extend the amount of branch mortality during the summer.

P. ramorum already has caused extensive mortality of oak and tanoak in California. The rapid death of saplings observed in growth chamber and field experiments suggests that impacts on madrone also could be extensive. Large madrones trees in our permanent plots have shown extensive dieback although, for the reasons discussed above, they have not been confirmed to be infected with *P. ramorum*. This pathogen may be especially important in areas where madrone is a major component of coastal forest communities and where weather conditions are conducive to *P. ramorum* biology. Further research is needed to study and monitor madrone populations in the field. Only then can we assess the full potential effects of this disease on growth and reproduction on madrone trees in forests infested with *P. ramorum*.

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