Sources of Inoculum for *Phytophthora ramorum* in a Redwood Forest

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Accepted for publication 24 March 2008.

ABSTRACT

Davidson, J. M., Patterson, H. A., and Rizzo, D. M. 2008. Sources of inoculum for *Phytophthora ramorum* in a redwood forest. *Phytopathology* 98:860–866.

Sources of inoculum were investigated for dominant hosts of *Phytophthora ramorum* in a redwood forest. Infected trunks, twigs, and/or leaves of bay laurel (*Umbellularia californica*), tanoak (*Lithocarpus densiflorus*), and redwood (*Sequoia sempervirens*) were tested in the laboratory for sporangia production. Sporangia occurred on all plant tissues with the highest percentage on bay laurel leaves and tanoak twigs. To further compare these two species, field measurements of inoculum production and infection were conducted during the rainy seasons of 2003–04 and 2004–05. Inoculum levels in throughfall rainwater and from indi-

vidual infections were significantly higher for bay laurel as opposed to tanoak for both seasons. Both measurements of inoculum production from bay laurel were significantly greater during 2004–05 when rainfall extended longer into the spring, while inoculum quantities for tanoak were not significantly different between the 2 years. Tanoak twigs were more likely to be infected than bay laurel leaves in 2003–04, and equally likely to be infected in 2004–05. These results indicate that the majority of *P. ramorum* inoculum in redwood forest is produced from infections on bay laurel leaves. Years with extended rains pose an elevated risk for tanoak because inoculum levels are higher and infectious periods continue into late spring.

Additional keywords: plant–host interaction, generalist pathogen.

Redwood forests are one of the ecosystems most threatened by *Phytophthora ramorum*, the causal agent of sudden oak death (13,19–21,25). These forests are range-limited and occur only in a narrow ≈700 km long band on the Pacific Coast from southwestern Oregon to southern Monterey County, California (18,31). Tanoak, the most susceptible of the canopy trees infected by *P. ramorum*, co-occurs with redwood throughout this region (13, 18,20). With mortality levels potentially reaching millions of trees (15), the loss of tanoak severely threatens the integrity of these forests that provide habitat to over 200 vertebrate species, 350 vascular plants, 90 lichens, and 300 macrofungi (18). Unfortunately, edaphic factors that support the growth of redwoods such as summer fog, moderate temperatures, and predominantly moist soil, also favor survival and transmission of *P. ramorum*.

Within redwood forests, *P. ramorum* infects virtually all woody canopy and understory plant species, as well as some herbaceous plants (2,4,9). Dominant hosts in redwood tanoak forest include redwood (*Sequoia sempervirens*), tanoak (*Lithocarpus densiflorus*), bay laurel (*Umbellularia californica*), Douglas fir (*Pseudotsuga menziesii*), grand fir (*Abies grandis*), huckleberry (*Vaccinium ovatum*), rhododendron (*Rhododendron macrophyllum*), salal (*Gaultheria shallon*), and salmonberry (*Rubus spectabilis*). In spite of the threatened nature of this ecosystem, field studies have not been conducted to quantify which of these hosts are most important for spread of *P. ramorum* within the redwood forest environment.

Disease symptoms and plant tissues infected vary among hosts (2,4,9). Most infection, including that on redwoods, is nonlethal

and consists of foliar or twig blight. However, stem cankers on huckleberry, rhododendron, and especially tanoak, can lead to mortality. Inoculum production is also likely to vary among hosts. Owing to the high number of hosts and the diversity of symptoms, understanding which hosts are able to effectively produce inoculum and transmit disease is a complex question. Yet this knowledge is essential for predicting the transmission dynamics of *P. ramorum* and implementing control measures within redwood forests.

Weather conditions will affect inoculum production from each host, and may do so differently (5,30). The range of *P. ramorum* within the central portion of the redwood forest belt experiences a maritime Mediterranean climate, with cool wet winters and warm to hot summers (18). Moisture is an essential factor in survival and sporulation of *P. ramorum*, and the duration, frequency, and timing of rain events during the winter months appear to play a key role in inoculum production and the infection cycle. El Niño events and years with extended rains have correlated with surges in inoculum levels in mixed-evergreen forest (5), and increases in numbers of infected trees across different forest types (5,19,21). However, little is known about the mechanisms underlying this increase in inoculum levels on a per host basis. This information is especially crucial because hosts that drive infections often become exceptionally competent for spore production under favorable conditions (1).

To determine which hosts are sources of inoculum for *P. ramorum* in redwood forest and to characterize inoculum production, inoculum levels and infection rates were monitored for the dominant hosts in a well-studied redwood forest in central, coastal California (13). We chose to focus on dominant host species because an understanding of *P. ramorum* transmission from the most common species would contribute significantly toward explaining the overall transmission dynamics of this generalist pathogen. The study was conducted in two consecutive rainy sea-

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doi:10.1094/PHYTO-98-8-0860

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sons to compare the quantity of sporulation and infection on these hosts. The following questions were addressed: (i) From which of the dominant hosts can *P. ramorum* produce spores? (ii) How does the quantity of inoculum production in throughfall rainwater or from individual infections compare between bay laurel and tanoak trees? (iii) What is the timing and quantity of infections on bay laurel leaves and tanoak twigs? (iv) How does year to year variation in rainfall affect inoculum production and infection levels on bay laurel and tanoak trees?

MATERIALS AND METHODS

Site. This research was conducted in a redwood forest spanning Jack London State Park and the contiguous Sonoma Developmental Center (122°W, 38°N) in the central portion of the redwood range in Sonoma County, California (18). The research site encompassed 65 ha at 235 to 280 m elevation (13). Yearly rainfall during 2003-04 was 1,200 mm and in 2004-05 was 1,525 mm (measured from July to June) (Jack London State Park, *unpublished data*). The key difference between the two study years was that rainfall extended into May and June in 2005 while minimal amounts of rainfall were detected after February in spring 2004 (Fig. 1). Total rainfall from March to June was 99 and 536 mm for spring of 2004 and 2005, respectively. The average rainfall at the study site from 1973 to 2000 during March to June was 346 mm (Jack London State Park, *unpublished data*). Temperature during the study period ranged from -0.2 to 35.2°C (P. Maloney, *unpublished data*).

Soils in this forest are volcanic in origin and the dominant series are Spreckles and Goulding (3). Soil pH ranged from 5.5 to 6.1 (8). Like most extant redwood forests, these stands were logged several times during the past 160 years and are likely to be in a 50 year-old stage of re-growth (6,28). Dominant tree species in this forest were tanoak, redwood, and bay laurel, with relative densities of 40.0, 23.7, and 26.0%, respectively (13). Based on a 2002 study of 30 500 m² plots, mean percent infection levels with 95% confidence intervals for tanoak, redwood, and bay laurel at the study site were 44.6 ± 11.2, 1.3 ± 2.5, and 47.8 ± 11.1%, respectively (13; R. Cobb, *unpublished data*). In addition, the mean percentage of *P. ramorum*-mediated mortality of tanoak saplings and adults with 95% confidence intervals was 26.3 ± 5.9% (13; R. Cobb, *unpublished data*). Other common species in this redwood forest included Douglas fir, big leaf maple (*Acer macrophyllum*), madrone (*Arbutus menziesii*), huckleberry, and hazelnut (*Corylus cornuta*); each of these species is also a host for *P. ramorum*.

Sporulation in moist chambers. To determine the likelihood of sporulation from infected tissue from the dominant woody plant species in this redwood forest, we incubated 20 symptomatic samples each of bay laurel leaves, tanoak leaves with petioles, tanoak twigs with leaves and petioles removed (terminal branches <1 cm in diameter), and redwood twigs with needles (terminal branches <0.5 cm in diameter) in laboratory moist chambers (crispers 17 cm long by 12 cm wide by 7 cm high) at 15°C. All 20 samples for each plant tissue came from separate individuals that were spatially distributed throughout the study site. Symptoms on infected tissues were consistent with published descriptions (4,14). Each sample was placed on a plastic grid in a separate moist chamber and diH₂O was added to the level of the grid. Samples were examined under the dissecting scope at 3 days, and then weekly thereafter for 6 weeks for the presence of sporangia and chlamydospores. Infected tissue was plated on pimaricin-ampicillin-rifampicin-PCNB selective agar (PARP) (7) at the end of the trial to verify viability of *P. ramorum*. Plates were incubated for 1 month. The two trials of this study began on 20 February and 31 October 2003. These dates are representative of the middle of the 2002-03 rainy season and the beginning of the 2003-04 rainy season, respectively.

In addition to the previous study, four pieces of tanoak bark canker from the main trunk of each of 14 mature trees were placed in laboratory moist chambers (crispers 17 cm long by 12 cm wide by 7 cm high) at 15°C to test for sporulation. To avoid impacting tanoak trees in Jack London State Park, tanoak trunk cankers were collected from Camp Maymack (9 trees) and the Santa Lucia Preserve (5 trees) in Santa Cruz and Monterey counties, respectively. Collection occurred in May, 2004, the end of the rainy season. Study trees had previously tested positive for *P. ramorum* and ranged in diameter breast height (dbh) from 5.5 to 46 cm. Canker pieces measured ≈5 by 9 cm at the bark surface, and extended ≈6 cm into the tree. Therefore, canker pieces contained outer bark, phloem, and xylem. In the moist chamber, water was added to within 0.5 to 1 cm of the top surface of the bark, bark was misted regularly with diH₂O to maintain a film of water on the top surface, and crispers were sealed with Vaseline to maintain high moisture levels. The outer surface of the bark was examined under the dissecting scope on a weekly basis for 6 weeks for the presence of sporulation. In addition, the cut submerged sides of the piece were examined for hyphae and sporulation. Hyphae extending from the exposed side surfaces of the phloem were plated on PARP to verify the presence of *P. ramorum*. Infected tissue from all cankers was replated at the end of the experiment to verify viability of *P. ramorum*. Only one trial was conducted because of the destructive nature of canker removal on adult tanoak trees.

Inoculum in rainwater. Results from laboratory sporulation in moist chambers indicated that bay laurel leaves and tanoak twigs had the highest percentage of sporulation. Consequently, subsequent studies in this paper focused on inoculum production and rates of infection in these two plant tissues. To compare the amount of inoculum in rainwater flowing through bay laurel trees versus tanoak trees, raintraps (2-gallon buckets) were placed beneath the canopies of these two species at seven approximately 20 m² sites. At each site, three buckets were placed under bay canopies and three buckets were placed under tanoak canopies for a paired design. Rainwater was collected from individual rain events (duration 1 to 8 days) approximately every 3 weeks, resulting in 9 and 15 collections during the winter rainy seasons of 2003-04 and 2004-05, respectively. Rainwater was collected within 1 day of the end of the rain event. From each site, equal portions of rainwater from the three buckets were pooled into one sample that ranged in volume from 0.5 to 3.8 liters, depending on the quantity of rainwater available from a given rain event. Therefore, seven samples of rainwater were collected for both species during each rain event. Rainwater was then taken back to the laboratory, filtered through miracloth (VWR 80058394) to remove coarse debris, and filtered through a Millipore glass fiber pre-filter (Fisher APFD09050) with a 2.7 μm nominal pore size to collect *Phytophthora* sporangia and zoospores. Filters were plated, filtrate side down, on PARP, and removed after 3 to 4 days. Colonies were counted at that point, and then at regular intervals until 21 days. Final counts were based on morphological verification of *P. ramorum* and results expressed in terms of colony forming units (CFU) per liter rainwater. For additional details on methods, see Davidson et al. (5).

Separate statistical analyses were used to test for differences in inoculum concentration in rainwater from bay laurel versus tanoak in the two seasons because heteroscedasticity could not be controlled in a full analysis of variance (ANOVA) model including main effects of species, year, species by year interaction, and raindate nested within species by year interaction. Differences in inoculum concentration in paired samples of rainwater from bay laurel versus tanoak were tested separately for both of the sample years using a Wilcoxon signed-rank test because data did not meet the assumptions for a paired *t* test. Comparisons in CFU/liter for the high spring rainfall versus low spring rainfall seasons were conducted separately for both species using ANOVA with main effects

year, and raindate nested within year. The ANOVA was weighted by the inverse of the standard deviation of the residuals squared because standard transformations did not resolve unequal variances among treatment means (17,29). A standard Bonferroni cor-

rection was applied to each of the four analyses in this section and significance was evaluated at $P \leq 0.0125$ to correct for Type I error due to multiple tests of a common hypothesis (23). All statistical analyses in this paper were conducted using JMP IN 5.1 (22).

Fig. 1. A and B, Mean colony forming units (CFU)/liter rainwater $\pm$ standard error (SE) collected under bay laurel (Δ) or tanoak trees ($\circ$), C and D, mean number of zoospores $\pm$ SE per individual infection on bay laurel leaves or tanoak twigs, and E and F, percentage of bay laurel leaves or tanoak twigs infected during rainy seasons with low (2003–04) and high (2004–05) spring rainfall. Daily precipitation for each season is indicated by the dashed lines.

Sporulation from individual infections under natural conditions. To compare inoculum potential from infected individual bay laurel leaves and tanoak twigs under natural forest conditions, the quantity of zoospores produced on these plant tissues was measured during rainstorms. One infected leaf tip or twig piece was collected from each of 15 bay laurel and tanoak trees located throughout the Jack London State Park study area at ≈ 24 h into a rainstorm approximately every 2 weeks during the rainy seasons of 2003-04 and 2004-05. Leaf and twig infections had approximately the same surface area. Tanoak twig pieces did not include petioles or leaves. Infected leaf tips and twig pieces were immediately placed in 1.5 ml of dH_2O in 2-ml Eppendorf tubes and 3.5-ml screw cap tubes, respectively. Care was taken to place bay laurel leaves with the adaxial side touching the tube wall, as sporangia form on the lower side of the leaf. After collection from all trees, tubes were placed on ice for 45 min to shock sporangia into releasing zoospores. In the laboratory, samples were incubated at 22°C for 1.5 h to complete zoospore release, and then plant tissue was removed from tubes, and the solution pipetted over the tissue piece to wash adhering zoospores into solution. Samples were placed at 15°C overnight to allow zoospores to encyst to achieve a more homogeneous mixture for determining concentration. Subsequently, two 100- μl aliquots were plated on PARP and colonies counted at 24 and 48 h. The average of the final counts from the two aliquots was used to calculate the total number of zoospores in the 1.5-ml sample, and hence produced on the plant part.

As described in the previous section, a complete statistical model evaluating the effect of host species and year on inoculum production could not be completed. Separate analyses were conducted to test for differences in inoculum production on bay laurel leaves and tanoak twigs in the two seasons, and a standard Bonferroni correction was applied to the four analyses. Differences in the quantity of zoospores produced on bay laurel leaves versus tanoak twigs were tested separately for both of the rainy seasons using a Wilcoxon one-way test blocked by date. This test required that the data were balanced with equal counts in each date by species cell (22). The number of zoospores produced in the high spring rainfall versus low spring rainfall seasons was compared separately for both of the species using ANOVA with main effects year and raindate nested within year. The ANOVA analyses were weighted as previously described because standard transformations did not correct for unequal variance among treatment means.

Infection rates. The timing and quantity of infection on bay laurel leaves and tanoak branches were compared during the rainy seasons of 2003-04 and 2004-05. At the beginning of each season before *P. ramorum* spores were detected in rainwater, 10 uninfected bay laurel leaves on each of 10 terminal branches on each of 10 trees were marked for observation, for a total of 100 branches and 1,000 leaves. Ten 30 cm terminal branches were also marked on each of 10 tanoak saplings, for a total of 100 branches. Tanoak branches were approximately the same length as bay branches, so that a roughly equal amount of tissue was

surveyed. Bay laurel leaves and tanoak branches were censused for infection every 2 weeks during the two rainy seasons. For symptomatic lesions, a small piece of leaf or branch tissue was excised for plating on PARP to confirm the presence of *P. ramorum*. Time until infection was compared between bay laurel leaves and tanoak branches using a proportional hazards analysis, and the overall rate of infection between the two species was analyzed with a chi-square test.

RESULTS

Sporulation in moist chambers. Sporangia production occurred on some samples of all plant tissues tested. However, the percentage of samples with sporangia varied greatly among the plant tissues tested, and there was a seasonal effect of early versus mid rainy season collection time (Table 1). The highest percentage of positive samples occurred with bay laurel leaves (80%) and tanoak twigs (65%) collected during the mid-rainy season in February 2003. Sporangia production occurred on 25% or fewer of the redwood twigs (plus needles) and tanoak leaves from the same collection period. Recovery of the pathogen from the February collection was also low from redwood twigs and tanoak leaves, indicating that *P. ramorum* may not survive well in these host tissues. Sporangia production was lower on all plant tissues collected following the summer dry season in October 2003. Sporangia were produced on 30% of the bay laurel leaves collected in October, while sporulation from tanoak twigs (60%) was similar to the February collection. Pathogen recovery rates from plating infected tissue of these two plant parts were similar to the first collection in February, indicating that *P. ramorum* survived the summer dry season in these tissues. Sporangia production for both tanoak leaves and redwood twigs was zero for the October collection. Recovery of *P. ramorum* from these plant parts was also lower than the February collection, suggesting that pathogen viability decreased through the summer in these host tissues. For both collection periods, the percentage of samples for each host tissue with chlamydospores was generally the same as that with sporangia.

For tanoak trunk cankers, sporangia and chlamydospores only were observed on the intact, top surface of bark for one of the 14 individuals (7.1%). This individual was the smallest tree of the group, with a dbh of 5.5 cm. Hyphae with chlamydospores formed on the exposed, submerged phloem on the sides of bark pieces for 10 of the 14 individuals. The 5.5 cm diameter tree also produced sporangia from the submerged, cut sides of canker pieces. Recovery of *P. ramorum* at the end of the monitoring period occurred from the same set of 10 trees (71.4%) that formed hyphae in the dH_2O .

Inoculum in rainwater. In both 2003-04 and 2004-05, rainwater samples collected under bay laurel trees had significantly higher spore concentrations than samples collected under tanoak trees (Wilcoxon signed-rank: 2003-04: $P < 0.0001$; 2004-05: $P < 0.0001$) (Fig. 1A and B). In 2003-04, the low spring rainfall year,

TABLE 1. Percentage of naturally infected samples that produced *Phytophthora ramorum* sporangia or chlamydospores after incubation in laboratory moist chambers

Plant tissue type ^a	Collection period ^b					
	Mid-rainy season			Early rainy season		
	% Sporangia ^c	% Chlamydospores ^c	% Recovery ^d	% Sporangia ^c	% Chlamydospores ^c	% Recovery ^d
Bay laurel leaves	80%	80%	90%	30%	30%	90%
Tanoak twigs	65%	50%	70%	60%	60%	75%
Tanoak leaves	10%	5%	15%	0%	0%	5%
Redwood twigs + needles	25%	25%	25%	0%	0%	0%

^a Twenty samples of each tissue type were incubated in moist chambers for 6 weeks.

^b Mid-rainy season plant tissues collected 17 February 2003; early rainy season plant tissues collected 30 October 2003.

^c Percentage of samples that produced sporangia or chlamydospores on exposed surfaces.

^d Percentage of samples from which *P. ramorum* was isolated at the end of the experiment.

rainwater was sampled during nine rain events. For the purpose of analysis, the first and last rain events were excluded given that no CFUs were detected in any of the samples for either species, indicating that conditions may not have been appropriate for sporulation. Dates for which only one of the species failed to produce spores were included in analyses. For the remaining seven dates, the mean CFU/liter for samples collected under bay laurel and tanoak trees were 30.97 ± 7.08 (median: 3.50; range: 0 to 210.86) and 3.46 ± 1.87 (median: 0; range: 0 to 80.80), respectively. In 2004-05, the high spring rainfall year, there were 15 sample periods. The first three of these were excluded from analyses because all samples were zero. For the 12 remaining rain events, the mean CFU/liter for rainwater samples collected under bay laurel trees and tanoak trees were 94.69 ± 22.45 (median: 7.24; range: 0 to 945.33) and 4.85 ± 1.84 (median: 0; range: 0 to 106.40), respectively. The same sets of dates were used for between year comparisons for each species. On dates in which all samples were zero, 0.001 was added to one of the seven samples in order to execute weighted ANOVA (weight = [standard deviation of residuals]⁻²). CFU/liter was significantly higher for rainwater collected under bay laurel trees in 2004-05 versus 2003-04 but not significantly different for rainwater from tanoak trees during these 2 years (bay laurel: LSM 2003-04: 30.97 ± 6.40 versus 2004-05: 94.69 ± 20.02 ; $F = 9.19$, $df = 1$, $P = 0.0030$, $n = 133$; tanoak: LSM 2003-04: 3.46 ± 1.89 versus 2004-05: 4.85 ± 1.75 ; $F = 0.2892$, $df = 1$, $P = 0.5918$, $n = 133$) (Fig. 1A and B). The mean concentration of CFUs for rainwater collected under bay laurel was 3.06 times higher in 2004-05 than in 2003-04, as opposed to only a 1.40 times increase in the mean concentration of propagules for rainwater collected under tanoak. Inoculum production varied significantly among raindates within years for bay laurel ($P < 0.0001$), but not for tanoak ($P = 0.0471$; Bonferroni corrected).

Sporulation from individual infections under natural conditions. Consistent with the rainwater results, the number of zoospores produced on bay laurel leaves was significantly higher than that produced on tanoak twigs in both 2003-04 and 2004-05 (2003-04: $\chi^2 = 21.06$, $df = 1$, $P < 0.0001$, $n = 180$; 2004-05: $\chi^2 = 40.29$, $df = 1$, $P < 0.0001$, $n = 240$) (Fig. 1C and D). In the low spring rainfall year, 2003-04, the first two rain events sampled were excluded from analysis because no samples produced any spores. In addition, three events during the season were excluded due to drying of plant parts before they could be collected. For the remaining six raindates, the mean number of zoospores per bay laurel leaf was 267.47 ± 61.80 (median: 0; range: 0 to 3,645) versus 5.83 ± 3.20 (median: 0; range: 0 to 240) for tanoak twigs. In 2004-05, the high spring rainfall year, one date was excluded from analysis because all samples from both species were zero. For the eight sample dates remaining, the mean number of zoospores per bay laurel leaf and per tanoak twig were 1019.12 ± 238.88 (median: 15; range: 0 to 17,475) and 38.62 ± 25.67 (median: 0; range: 0 to 2,955), respectively. For within species between year comparisons, the same set of dates were used and 0.001 was added to 1 of the 15 samples for dates in which all samples were zero in order to execute weighted ANOVA. The number of zoospores produced on bay laurel leaves was significantly higher in 2004-05 than in 2003-04, but the numbers of zoospores produced on tanoak twigs were not significantly different in the 2 years (bay laurel: LSM 2003-04: 267.47 ± 58.46 versus 2004-05: 1019.12 ± 218.98 ; $F = 11.00$, $df = 1$, $P = 0.0011$, $n = 210$; tanoak: LSM 2003-04: 5.83 ± 3.22 versus 2004-05: 38.62 ± 25.76 ; $F = 1.59$, $df = 1$, $P = 0.2082$, $n = 210$) (Fig. 1C and D). Zoospore production varied significantly on different raindates for bay laurel ($P < 0.0001$), but not for tanoak ($P = 0.2143$).

Infection rates. The difference in infection rates on bay laurel leaves and tanoak terminal branches varied between the two seasons (Fig. 1E and F). In 2003-04, a year with low spring rainfall and low inoculum levels, the final infection rate for tanoak

branches (41.0%) was greater than that for bay laurel leaves (8.6%) ($\chi^2 = 65.35$, $df = 1$, $P < 0.0001$). The time until new infections was not significantly different between the two species as indicated by the proportional hazards analysis ($\chi^2 = 2.90$, $df = 1$, $P < 0.0887$). However, in 2004-05, a high spring rainfall year, overall infection rates were not significantly different for the two species (bay laurel: 63.6% versus tanoak: 62.0%; $\chi^2 = 0.100$, $df = 1$, $P < 0.7520$). In this year, bay laurel leaves were more likely to be infected at an earlier point in time than tanoak branches (proportional hazards: $\chi^2 = 33.68$, $df = 1$, $P < 0.00001$). The number of infected bay laurel leaves increased over sevenfold from 2003-04 to 2004-05, while the number of infected tanoak branches only increased 1.5 times. Isolation of other *Phytophthora* species during the two seasons was extremely low; *P. nemorosa* was isolated from only 1 of 881 bay laurel leaves cultured.

DISCUSSION

Although sporangia of *P. ramorum* formed on all dominant hosts in this redwood forest, they formed most frequently on bay laurel leaves and tanoak twigs. Of these two hosts, infections on bay laurel are clearly driving inoculum production in this forest ecosystem. Zoospore production from individual infections was significantly higher on bay laurel leaves than tanoak twigs in both seasons studied, with overall mean numbers of zoospores that were 46 and 26 times higher on bay laurel leaves for 2003-04 and 2004-05, respectively. Consistent with this result, rainwater collected under bay laurel trees contained up to 9 and 20 times the overall mean concentration of propagules as that found in rainwater collected under tanoak trees for the two consecutive rainy seasons. Ultimately, death of infected tanoak trees will also limit the importance of transmission of *P. ramorum* from this host, whereas infected bay laurel trees survive infection and remain an ongoing source of spores (4,13).

Although more inoculum was found under bay laurel trees in the two rainy seasons studied, tanoak terminal branches were equally or more susceptible than bay laurel leaves in terms of infection rates. In the low spring rainfall season, unfavorable for *P. ramorum*, a greater percentage of tanoak branches (41%) were infected than bay laurel leaves (8.6%), suggesting that the lower inoculum levels recorded during this season did not effectively infect bay laurel leaves. In 2004-05, a year with extended rains and higher inoculum levels, an almost equal percentage of tanoak branches (62%) and bay laurel leaves (63.6%) were infected. Taken together with the results on inoculum levels in rainwater, infection data indicate that although tanoak is more susceptible, bay laurel trees produce more inoculum, in part due to higher sporangia production from each infection. Although not measured in this study, a greater number of possible infection sites on many drip tips on bay laurel trees as opposed to fewer infection sites on branches on tanoak trees may also contribute to the higher inoculum levels found in rainwater flowing through bay laurel trees. In addition, in extended rainfall years such as 2004-05, the earlier onset of infection in bay laurel leaves would provide a longer period from which to produce spores, leading to an earlier onset of an exponential increase in infection levels within the tree (Fig. 1F).

Inoculum concentration in rainwater increased significantly in the higher spring rainfall year for bay laurel but not for tanoak. This increase was due in part to a sevenfold increase in the percentage of infected bay laurel leaves. However, it was also due to an almost fourfold increase in mean numbers of zoospores produced from each infection, ranging up to 17,475 zoospores/leaf in 2004-05. In essence, although bay laurel is a less susceptible host than tanoak in years with low rainfall, it becomes a significantly more competent host in years with high rainfall due to both higher levels of infection and inoculum produced per infection. Higher

numbers of spores produced on bay laurel leaves under favorable conditions may be key to reaching an inoculum threshold that results in additional infections and an exponential increase in the number of infected bay laurel leaves. While the mechanisms for this increase in sporulation are unknown, they may indicate that infections are initiated by a greater number of propagules, a greater quantity of hyphae is able to grow within the leaf, and/or more sporangia are produced when rain events are more frequent, longer in duration, or extend into warmer months at the end of the spring. Regardless of mechanism, the overall increase in inoculum from bay laurel trees in high rainfall years may have large negative effects on tanoak populations. The tanoak branches monitored in this forest showed a 50% increase in infection rate in the high spring rainfall year.

The significance of bay laurel as a major source of inoculum in redwood forests is consistent with a number of other studies that suggest bay laurel is the driver of *P. ramorum* infection in oak woodlands (5). Infection on bay laurel is known to precede infection on oak and tanoak trees as *P. ramorum* invades new forest land (16; P. Maloney, unpublished data; A. Wickland, unpublished data); the presence of bay laurel is correlated with mortality levels in oak trees (12,13,24,26); and recovery of *P. ramorum* from forest soil is highest under bay laurel trees (8). The 9- to 20-fold difference in rainwater inoculum from bay laurel over tanoak trees may explain some of the slower spread of *P. ramorum* in southwestern Oregon where there are fewer bay laurel trees and infection is spreading primarily among tanoak trees (10) (although eradication efforts have also played a major role in slowing the epidemic in Oregon). These results may also guide predictions on the spread of *P. ramorum* into new forestlands in California based on the density of bay laurel within tanoak populations.

Given the central importance of bay laurel for the spread of *P. ramorum*, methods are being tested to limit the impact of this host. These include screening for genetic resistance (11) and thinning or eradication of bay laurel from forests (27; Y. Valachovic, personal communication). Attempts to lower inoculum production from bay laurel must be guided by knowledge of the effects of weather conditions on spore production, the dispersal potential of spores from bay laurel within closed forest, and the inoculum thresholds necessary for infection on key susceptible species, such as tanoak, in order to implement programs that will decrease the number of propagules arriving at keystone hosts to a level below infection thresholds. Unfortunately, the inoculum threshold for tanoak may be quite low. In this study, 41% of tanoak branches became infected even in a year with rainfall patterns unfavorable to *P. ramorum*.

In spite of the justifiable emphasis on transmission of *P. ramorum* from bay laurel, this host species is less prevalent in some regions of the redwood belt, and epidemiological studies are needed to understand transmission in forests with low densities of bay laurel. Besides tanoak, which other overstory or understory hosts can spread *P. ramorum*? Contrasts between low and high-density bay laurel forests in terms of inoculum production, infection levels on keystone hosts, and differences in forest community structure for plants, animals, and microorganisms, also may help clarify the ecological gains and losses associated with bay eradication efforts.

Climate varies throughout the range of redwood forest, and transmission studies are necessary to determine the potential spread from dominant hosts in these different microclimates. Our study site represents one of the hotter, drier regions within the redwood belt. While the Sonoma field site experiences approximately 4 months of winter rain followed by summer fog and temperatures ranging up to 35°C, other more northern regions of California and southwestern Oregon receive 8 months of winter rain with more frequent summer rains and cooler temperatures year around. In contrast to our site, inoculum in rainwater from

these cooler wetter sites can be present during summer rains and with little delay after the onset of winter rains (E. Hansen, unpublished data). Patterns of inoculum production for all hosts within the northern redwood forests may differ from those in the redwood forests of central California. Sporulation from tanoak and redwood may increase under more favorable northern conditions, making these hosts potentially more competent for spread of inoculum than would be predicted by the results of our study alone.

Our study provides one of the few examples of epidemiological tracking of inoculum and infection levels of a major, generalist pathogen in a natural ecosystem. Of the dominant hosts in this redwood forest, bay laurel produced the most inoculum in both the low and high spring rainfall years, although tanoak exhibited greater susceptibility in the low spring rainfall year and provided low levels of inoculum in both years. Levels of inoculum production tended to be higher for both hosts in the high spring rainfall year, but exhibited a significant increase only for bay laurel. Increased numbers of infected bay laurel leaves and spores produced per leaf in the high spring rainfall year accounted for the majority of the inoculum increase in the forest during this extended rainy season, and substantiated the role of bay laurel as the main driver of *P. ramorum* transmission in redwood forests in California.

ACKNOWLEDGMENTS

We thank Jack London State Park, the Sonoma Developmental Center, Camp Maymack, and the Santa Lucia Conservancy for permission to conduct research in their forests. Rainfall data was contributed by Jack London State Park and P. Maloney. K. Falk, A. Wickland, A. Adie, R. Albright, J. Bienapfl, K. Covington, L. Douhan, K. Huryn, C. Jensen, S. Kane, S. Langley, S. Lynch, M. Voigt, G. Walden, and J. Zanzot provided excellent assistance. The manuscript was improved by constructive comments from R. Cobb, N. Grunwald, and two anonymous reviewers. We are grateful for funding from the USDA Forest Service Pacific Southwest Research Station and the Gordon and Betty Moore Foundation.

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