Environmental limits to infection by *Phytophthora ramorum* in tanoak and California bay laurel

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Introduction

Despite widespread concern about risk factors associated with *Phytophthora ramourm*, very little is know about the conditions that might limit infection. This study examines two factors hypothesized to limit both infection rates and the extent of disease caused by *Phytophthora ramorum* in intermediately resistant tanoak (*Lithocarpus densiflora*) and California bay laurel *(Umbellularia californica*). The objective is to find an upper and lower limit for the temperature and incoulum levels at which *P. ramorum* infects tanoak and bay host species. Additionally, we will determine how lesion size varies within these environmental conditions. We hypothesized that as inoculum concentration increased leaves would develop larger lesion sizes and have increased chance of infection. Additionally, at ideal growth conditions the infection rate for leaves would be the greatest and that the pathogen would favor cooler temperatures over warmer temperatures. Lastly, at higher and lower temperatures there would be a significant difference in infection rates and lesion extent.

Materials and methods

To determine the maximum and minimum inoculum zoospore concentrations for leaf infection, leaves of each species were inoculated at five concentrations ranging from $3.3x10^4$ spores/mL to 33 spores/mL, and incubated at 19°C for ten days. To find the maximum and minimum temperatures that resulted in zoospore infection of leaves we inoculated leaves of each species with a constant zoospore solution of $3x10^4$ spores/mL for one day at five temperatures ranging from 5°C to 33°C, and then incubated at ideal growth conditions (19°C) for 10 days. Concentrations were chosen to be meaningful in terms of the number of zoospores per leaf. For example, 33 spores/ml translates to 10 spores/leaf inoculation. All leaves were inoculated by pipetting zoospore solution into 50 mL conical Falcon tubes with the sample leaves. Bay laurel leaves were inoculated at the tips and tanoak leaves were inoculated by wounding the petiole and placing it directly in zoospore solution (Fig. 1) To determine how lesion size varied within these conditions, lesion area was measured on each infected leaf. Data were analyzed both in terms of infection rates (plus or minus infection) and infection extent (lesion size).

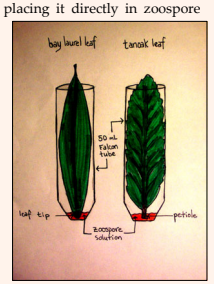

Fig. 1. Diagram showing inoculation set up scheme. Bay leaves were inoculated with zoospore solution at tips while tanoak were inoculated at the petiole

Results

Concentration Experiment

Bay leaves were infected at higher rates with increasing zoospore concentration across hosts ($p<0.0001$) (Fig. 2). Infection did occur at the lowest zoospore concentration (1.7% infection at 33 spores/mL). Additionally, increasing zoospore concentration for bay leaves correlated with an increase in lesion size (Fig. 3).

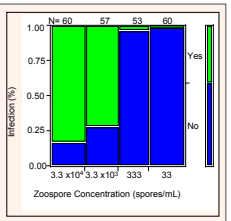

Fig. 2. Infection rates for bay leaves at four different zoospore concentrations. "Yes" represents leaf infection; "No" signifies no infection

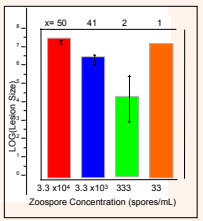

Fig. 3. Mean lesion size in bay leaves at four different zoospore concentrations. "x" represents the number of leaves infected. Error bars represent one standard error of the mean

Generally, for tanoak leaves infection rates increased with increasing zoospore concentration (Fig. 4). However, at 33 spores/mL infection was greater than at 333 spores/mL (13.7% and 9.3% respectively).

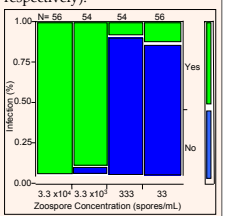

Fig. 4. Infection rates for tanoak leaves at four different zoospore concentrations. "Yes" represents leaf infection' "No" signifies no infection

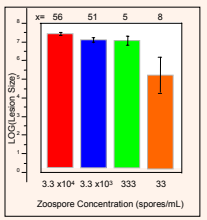

Fig. 5. Mean lesion size in tanoak leaves at four different zoospore concentrations. "x" represents the number of leaves infected. Error bars represent one standard error of the mean

Fig. 5. shows an increasing lesion size in tanoak leaves with increasing zoospore concentration. However, only the lesion size at 33 spores/mL was significantly different from the other concentrations in tanoak leaves (Tukey-Kramer test). Moreover, for one individual tanoak host zoospore concentration did not play a significant role (results not shown.)

Temperature Experiment

Infection rates for bay leaves were higher at temperatures 5°C above and below the ideal growth temperature for *P. ramorum* (19°C) (Fig. 6) ($p<0.0001$). Also, we found that at the temperature extremes there still was significant infection; at 5°C there was a 56.7% infection rate and at 33°C there was a 16.9% infection rate. Surprisingly, at ideal growth conditions there was only a 40% infection rate. There were no significant differences between lesion sizes in bay leaves incubated at ideal growth conditions and those incubated at 5°C above and below 19°C.

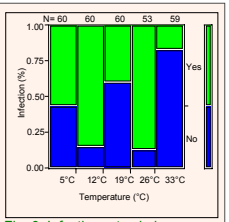

Fig. 6. Infection rates in bay leaves at five different temperatures. Notably, infection occurred at temperature extremes. "Yes" represents leaf infection; "No" signifies no infection

Lesion sizes in bay leaves incubated at the extreme temperatures were not significantly different. Significant differences in lesion sizes were found, however, in the leaves incubated at the extremes (5°C and 13°C) and those incubated in the mid-temperatures (12, 19, 26°C) (Fig. 7)

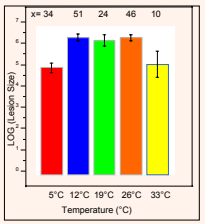

Fig. 7. Mean lesion sizes in bay leaves at five different temperature conditions. "x" represents the number of leaves infected. Error bars represent one standard error of the mean

In the temperature experiment, infection rates in tanoak leaves were greater than infection rates in bay leaves. Infection was greatest at 26°C, where we found a 91.5% infection rate (Fig. 8). There was a significant difference between lesion size of leaves incubated at 26°C and 33°C (Fig. 9)

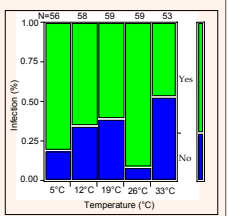

Fig 8. Infection rates in tanoak leaves at five different temperature conditions. "Yes" represents leaf infection; "No" signifies no infection

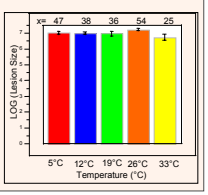

Fig. 9. Mean lesion sizes in tanoak leaves at five different temperature conditions. "x" represents the number of leaves infected. Error bars represent one standard error of the mean

Conclusions

Both experiments yielded leaf infection at all conditions. Leaf infection was especially remarkable at low concentration levels. At 33 spores/mL, or 10 spores/leaf inoculation, one bay leaf (1.7% of total) and 8 tanoak leaves (14.3%) were infected. Although infection did occur at these conditions, there was a significant decrease in infection rates for both species at 333 and 33 spores/mL (Fig. 2 and 4). Clearly, decreasing zoospore concentration decreases infection rates, but may not completely eliminate infection, even at very low spore loads. However, with concentration, 50 bay leaves were infected at the highest zoospore concentration and only one bay leaf was infected at the lowest concentration. This suggests that the lowest zoospore concentration (33 spores/mL) is close to the infection limit for bay leaves.

Infection rates in bay leaves were greatest at 5°C higher and lower than ideal growth conditions. Infection extent in bay leaves was similar; the three intermediate temperature treatments were indistinguishable, while significant differences leaves were found in intermediate temperature groups and those at the extremes. For tanoak, results were different. Unexpectedly, infection rates at 26°C were much higher than at 19°C (91.5% and 61% respectively). The next highest infection rate was found at 5°C (81%) (Fig. 8). There were significant differences in lesion sizes of tanoak leaves between 26°C and 33°C, suggesting an inhibitory effect of heat, but not of cold.

Future work will replicate this experiment to determine if the anomalous results were unique to this trial, perhaps due to an incubator idiosyncrasy. Further, zoospores and leaves were heated or chilled after inoculation was initiated. A future study will involve altering zoospore temperatures before inoculation, and will include temperatures beyond the range we tested. Together, our experiments demonstrate a remarkable ability of *P. ramorum* zoospores to infect leaves, even at suboptimal conditions.

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For further information

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