Resistance to *Phytophthora ramorum* in tanoak

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Introduction

Tanoak (*Lithocarpus densiflora*) is among the hosts most heavily impacted by sudden oak death; individual sites show up to 70% of tanoak infected, with correspondingly high mortalities [1] . However, patches of healthy tanoak are often observed immediately adjacent to patches with heavy mortality, suggesting that there may be variability for resistance in the system. We report on a preliminary study of resistance to infection by *Phytophthora ramorum* within a single tanoak population.

Fig. 1. Tanoaks infected with *P. ramorum*. Both leaves (a) and bole (b) contract lesions.

Materials and methods

- Host: 60 commercially-obtained *L. densiflora* saplings grown from seed collected from a single population in Six Rivers National Forest.
- Pathogen: *P. ramorum* isolate Pr75 (first isolated on *Quercus agrifolia* in California). Transferred through *L. denisflora*, then grown at 20°C for 2 wk on 10% V8A.

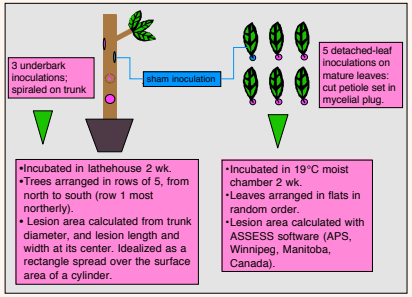

Fig. 2. Experimental scheme. Three underbark and 5 leaf inoculations were performed per tree, as well as a sham inoculation per method per tree. Leaves were inoculated using a 5 mm diameter agar plug cut from the colony edge, and placed either in a wound under the tree bark (stem), or immediately adjacent to the cut petiole (leaf).

Results

Lesion size, log-transformed before taking means to correct for heteroscedasticity, showed a continuous, log-normal distribution in both leaves and stems (Fig. 3). Leaf lesions occurred along the petiole, with slight spreading into the leaf, and ranged from 4 mm^2 to 105 mm^2. Stem lesions ranged from 2.7 cm^2 to 20.8 cm^2.

There was a significant effect of individual tree on lesion size in both leaves and stems (Table 1, Table 2). Because there was also a significant area effect, we nested tree within lathehouse row number for analysis of variance (ANOVA). Trees were arranged from north to south in 10 east-west rows of 5 trees, with row 1 the most northerly. Thus, "lathehouse row" is a proxy for microclimate.

Table 1. ANOVA demonstrates significant effects of position within the lathehouse (row) and individual (tree), nested within position, on stem lesion area. Statistical analyses were performed on log-transformed data in JMP v.5.01.

Source	DF	SS	MS	F Ratio	Prob > F
Row	7	6.2403	0.8915	12.1642	<.0001
Tree[Row]	32	5.5392	0.1731	2.362	0.0013
Error	72	5.2766	0.0733		

Table 2. Nested ANOVA showing significant effects of lathehouse position (row) and individual (tree) on leaf lesion area.

Source	DF	SS	MS	F Ratio	Prob > F
Row	7	6.2006	0.8858	3.2635	0.0029
Tree[Row]	32	26.5780	0.8306	3.06	<.0001
Error	159	43.1572	0.2714		

We used multiple regression to determine relationships between leaf lesion area and stem lesion area, while taking into account the effect of lathehouse position. There was a significant, positive correlation of stem and leaf lesion areas, and each were significantly correlated with lathehouse position (Table 3, Fig. 4). The strongest effect was lathehouse position on stem lesion area. The negative correlation coefficient of lathehouse position with both stem lesion area and leaf lesion area indicates that south-facing trees had smaller lesions than those facing north.

Table 3. Multiple regression indicates a significant correlation between leaf and stem lesion sizes within individual trees, as well as a strong area effect.

Variable	by Variable	Spearman Rho	Prob>|Rho|
log stem lesion area	log leaf lesion area	0.3932	0.0121
lathehouse row	log leaf lesion area	-0.3422	0.0307
lathehouse row	log stem lesion area	-0.5142	0.0007

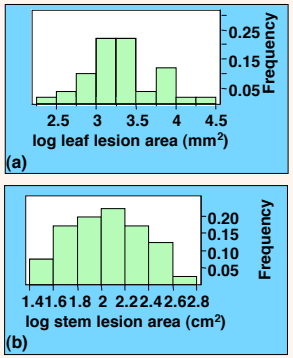

Fig. 3. Distribution of log-transformed lesion area for leaves (a) and stems (b). Values are the means of log-transformed values from all assays per tree.

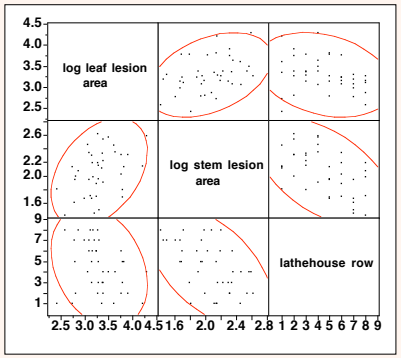

Fig. 4. Multivariate analysis of leaf lesion area (log transformed, from mm^2), stem lesion area (log transformed, from cm^2), and lathehouse position. Each square depicts the relationship of two of the variables with the effects of the third subtracted out. Red lines are 95% density ellipses.

Conclusions

Despite the high mortality frequently observed in tanoak populations, there appears to be significant variation in resistance to *P. ramorum* among individuals. Because both leaves and stems were challenged with wounding inoculations, the observed resistance is to spread of the pathogen once it has gained entry to the host. Resistance to infection by zoospores may show a different pattern, yet may be less robust to large quantities of inoculum.

Because mortality from sudden oak death in tanoak occurs after lesions girdle the host, under-bark inoculations are the obvious assay for resistance to the pathogen. However, they are not practical for widespread surveys of natural populations. Leaf inoculations are easily replicable, and leaf and stem lesion sizes are significantly correlated. While other researchers have found zoospore inoculations of tanoak leaves to yield inconsistent results [2], the agar-plug inoculation reliably yielded a midrib lesion that mimics those commonly observed in California woodlands. Moreover, while both leaf and stem lesions were strongly influenced by microclimate, the effect was strongest on stems, most likely because leaves were incubated in a controlled environment.

Microclimate strongly influenced lesion development, even in detached leaves. All studies of resistance, even in a glass- or lathehouse, should therefore account for microclimate, by design and in analysis.

Future work will extend the work discussed here to a range-wide survey of resistance within and among populations of tanoak in California and Oregon. Resistance structure will be compared to genetic structure, and the usefulness of laboratory-determined resistance for predicting natural infection in the field will be determined.

Literature cited

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2. E.M. Hansen, J.L. Parke, & W. Sutton. 2005. Susceptibility of Oregon forest trees and shrubs to *Phytophthora ramorum*: A comparison of artificial inoculation and natural infection. *Plant Disease* **89**: 63-70

Acknowledgments

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