Tanoak Resistance: Can it be Used to Sustain Populations?[1]

Katherine J. Hayden,[2] Alex Lundquist,[2] Douglas J. Schmidt,[2] Richard A. Sniezko,[3] Susan J. Frankel,[4] and Matteo Garbelotto[2]

Abstract

Tanoak (*Lithocarpus densiflorus*) trees are among *Phytophthora ramorum*'s most susceptible hosts. Extensive mortality in this species has led researchers to question whether selective breeding for resistance can be used to sustain populations; the answer depends on the extent and heritability of pathogen resistance within the host. Consequently, we have undertaken a multi-year common garden study of resistance to *P. ramorum* in tanoak seedlings grown from acorns collected by collaborators at sites in California and southern Oregon.

We have sown 12,650 acorns from nine unique sites in a common garden since 2006. The resulting seedlings have been assayed for resistance to *P. ramorum* by both detached leaf inoculations using plugs of mycelia as the infective agent, and seedling tip inoculations using a zoospore suspension. Both assays revealed variable resistance with significant heritability.

In addition to the laboratory assays, a subset of 800 seedlings from 50 different family groups were planted in a heavily infested, forested site in Monterey County, California. These seedlings are currently being monitored to determine whether there is a correlation between family-level variation in resistance in the laboratory setting to survivorship in the field. After 1 year, the survival rate was 82.5 percent, with no discernable effect of family; however, there were positive identifications of natural infection by *P. ramorum*.

Together, the data from these studies provide not only background knowledge crucial to predicting the evolutionary and ecological outcomes of the *P. ramorum* epidemic in tanoak populations, but also for ascertaining any potential for genetic resistance in tanoak to be used as a management tool.

Introduction

Tanoak (*Lithocarpus densiflorus*) trees are among *Phytophthora ramorum*'s most susceptible hosts. Incidence rates have been measured at 30 to 90 percent in infested areas (Maloney and others 2005, McPherson and others 2005, Meentemeyer and others 2008), and the median survival time for a symptomatic tree has been estimated to be 2.9 to 8.7 years (McPherson and others 2005). The high incidences coupled with high mortality have led researchers to question whether selective breeding for resistance can be used to sustain populations. Assessing the feasibility of a breeding program requires a great deal of background information, most of which is lacking

[1] A version of this paper was presented at the Fourth Sudden Oak Death Science Symposium, June 15-18, 2009, Santa Cruz, California.
[2] Dept of Environmental Science, Policy, and Management, 137 Mulford Hall, University of California, Berkeley, CA 94720.
[3] USDA Forest Service, Dorena Genetic Resource Center, Cottage Grove, OR 97424.
[4] USDA Forest Service, Pacific Southwest Research Station, Albany, CA 94710.
Corresponding author: khayden@nature.berkeley.edu.

for tanoaks. First and foremost, there must be heritable variation for pathogen resistance within the host in order for there to be any evolution of greater disease resistance in the tree population, whether by natural or artificial selection (Parker and Gilbert 2004, Simms 1996). Furthermore, for this variation to be used in management, it must be able to be appropriately assayed (Carson and Carson 1989, Sniezko 2006). In order to address these primary questions, we have undertaken a multi-year common garden study of resistance to *P. ramorum* in tanoak seedlings grown from acorns collected by collaborators at sites in California and southern Oregon.

Methods and Materials

Since 2006, we have sown 12,650 acorns from nine unique sites in pots in a common garden at the Oxford Tract Research Greenhouses in Berkeley, California. Collections have been made yearly, beginning with acorns provided by R. Dodd (University of California) and C. Roessler (Midpeninsula Regional Open Space District) from five sites in 2006, with sites ranging from Monterey County, California in the south to Curry County, Oregon, in the north, and El Dorado County, California (Blodgett Forest Research Station, in the western Sierra Nevada foothills) in the east. In 2007, the Blodgett collection was repeated (R. Dodd) because of poor growth the prior year. Further collections were made in 2008; these seedlings will be tested for resistance in future trials.

Resistance Assays, Laboratory

At 1 year of age, the 2006 and 2007 seedling cohorts were each assayed for resistance to *P. ramorum* by detached leaf inoculations using plugs of mycelia (isolate Pr52, CBS110537, ATCC MYA-2436) as the infective agent. Briefly, agar plugs were set on the freshly cut petiole of detached leaves (two to four replicate leaves per seedling), and incubated in moist chambers at 18 to 20 °C for 2 weeks. Replicates from each tree were incubated in different chambers. Lesions extended up the midrib, and lesion and leaf lengths were measured with Assess (APS Press, St. Paul, MN); the natural-log transformed ratio of lesion to leaf length was used for analyses. The 1033 seedlings from 71 families in the 2006 cohort were assayed in February 2008, and 448 seedlings from 22 families in the 2007 cohort were assayed in November 2008.

The 2007 cohort was assayed using a whole-seedling tip inoculation in December 2008. A suspension of Pr52 zoospores (1×10^4 spores/ml) was dropped onto a wax cup wrapped around the wounded seedling tip. Lesion length was measured monthly, as were the development of symptomatic leaves and mortality. The data presented here were taken 4 months after inoculation; data collection will continue for up to 1 year.

Variances in lesion lengths were analyzed by mixed-model, nested ANOVA. For detached leaves, the model was:

$y_{ijklm} = \mu + C_i + S_j + P_{k(j)} + T_{l(k,j)} + E_{ijklm}$

where y_{ijklmn} is the predicted lesion value for m^{th} observation of the l^{th} seedling of the k^{th} parent from the j^{th} site, μ is the grand mean, C is the i^{th} incubation chamber, S_j is the source site, $P_{k(j)}$ is the parent, nested within site, $T_{l(k,j)}$ is seedling, nested within

parent and site, and E_{ijklm} is the residual variation. Site, parent, and seedling were modeled as random effects; chamber was fixed. The 2007 cohort included only a single source site, so site was omitted from that model.

Lesions resulting from seedling tip inoculations were modeled as:
$y_{kl} = \mu + P_k + E_{kl}$
where y_{kl} is the predicted lesion value for the l^{th} seedling from the k^{th} parent family, and E_{kl} is the residual; parent was a random effect. Seedling stem height, diameter, and block were originally included in the model as fixed effects, but were non-significant and were removed.

Because these open-pollinated families are expected to contain a mixture of full- and half-siblings, narrow-sense heritability of lesion size was calculated as $h^2 = 3V_P/V_T$, where V_P/V_T is the proportion of total variance due to shared parent.

Resistance Assays, Field

In addition to the laboratory assays, 800 seedlings from 50 families in the 2006 cohort were planted out in 10 different plots in two *P. ramorum*-infested canyons in the Santa Lucia Preserve, Carmel Valley, California in January 2008. Four seedlings were randomly placed in each of 4 different plots, for a total of 16 seedlings planted per family. These seedlings will be followed for at least 3 years to track natural infection rates and symptom development. Data were taken quarterly, including growth, number of symptomatic leaves, stem lesions, and dieback; results reported here are from the first year of monitoring.

Small mammal herbivory, consistent with rabbits, was observed at half of the plots, with 26 percent of all seedling severely herbivorized. As of May 2009, all seedlings were individually caged, and 30 percent of the herbivorized plants showed regrowth.

Results

Resistance Assays, Laboratory

Maternal family contributed significantly to variance in resistance, measured by lesion size, in all assays (table 1, table 2). There was a trend toward greater heritability of resistance to lesion expansion 4 months after seedling tip inoculation ($h^2 = 0.51$, 95 percent CI 0.26-1.52) than in either detached leaf assay (2006 $h^2 = 0.14$, 95 percent CI 0.04-0.26; 2007 $h^2 = 0.14$, 95 percent CI 0.05-0.46); notably, all confidence intervals overlap. There was no significant geographic trend in resistance by the detached-leaf assay.

In detached leaves, shared-parent family mean lesion lengths ranged from 22 to 44 percent of the leaf length. After tip inoculation, family mean lesion lengths ranged from 3.4 cm to 7.5 cm, and infection resistance (percentage of seedlings with no apparent symptoms after inoculation, as of May 2009) ranged from 0 to 22 percent. Infection in detached leaves was 100 percent; there was no infection resistance by detached-leaf assay.

As of April 2009, 4 months after inoculation, overall survivorship of tip-inoculated seedlings was 86.5 percent. Mortality was exponentially associated with median family lesion length (fig. 1, $P < 0.0001$, exponential fit $R^2 = 0.76$).

Table 1—Analysis of variance: random effects on lesion as proportion of leaf length in detached leaf inoculation assays. Parent refers to open-pollinated maternal seed parent

Random Effect	Variance Ratio	Variance Component (95% CI)	% Total
2006 cohort			
Site	0.167	0.011 (0.003-0.180)	10.78
Parent(Site)	0.071	0.005 (0.003-0.009)	4.59
Seedling (Site, Parent)	0.312	0.016 (0.016-0.025)	20.13
Residual		0.064	
Total		0.100	
2007 cohort			
Parent	0.075	0.003 (0.001-0.010)	4.73
Seedling (Parent)	0.521	0.021 (0.017-0.027)	32.63
Residual		0.041	62.64
Total		0.065	

Table 2—Analysis of variance: random effects on lesion length in seedling tip inoculation assay

Random Effect	Variance Ratio	Variance Component (95% CI)	% Total
Parent	0.205	2.28 (1.123-6.788)	17.01
Residual		11.125	83.00
Total		13.404	

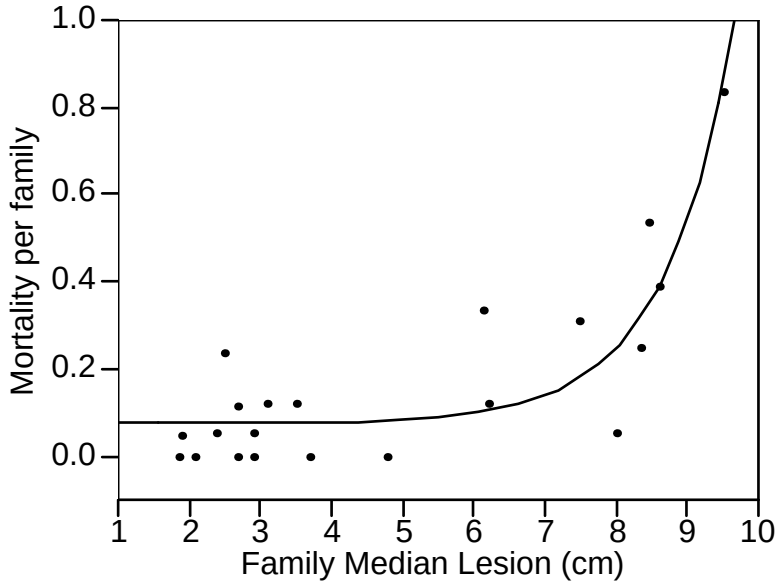

Figure 1—Association between mortality and family median lesion length in tanoak seedlings tip-inoculated with *P. ramorum*. Mortality = 0.075 + 0.000058 Exp(Median Lesion), $P < 0.0001$, $R^2 = 0.76$.

Resistance Assays, Field

At 6 months after planting, *P. ramorum* infection was confirmed in 26 seedlings using TaqMan detection (Hayden and others 2006). After 1 year, the pathogen was identified in an 11 additional seedlings using morphological identification of isolates on selective media, with molecular diagnostics ongoing. The 1-year survival rate was 82.5 percent, with no discernable effect of family.

Conclusions

The data from these studies help to provide the background knowledge crucial both to predicting the evolutionary and ecological outcomes of the *P. ramorum* epidemic in tanoak populations, and for ascertaining any potential for genetic resistance in tanoak to be used as a management tool. The inoculation assays revealed heritable genetic variation in resistance to *P. ramorum* in tanoak seedlings in a laboratory setting, and provide two methods with which to identify candidate families for further study. These assays each have advantages: while the tip inoculation most closely mimics natural infections, it is destructive and therefore not replicable. The detached leaf inoculation is replicable, but may have a smaller genetic component. The assays likely measure different resistance mechanisms; both may be useful for predicting disease outcomes. Tip-inoculated seedlings will continue to be monitored; differences between seedlings and families may be more apparent after a longer period of pathogen growth.

Further acorn collections are planned. While the first collections were designed to sample randomly in order to include as much natural variation as possible, collections from 2008 onwards are targeted towards individuals that may be expected to have greater than average resistance, such as surviving trees in areas of high mortality. The seedlings from these collections will be assayed by both detached-leaf and seedling tip assays, with an emphasis on tip inoculations. Seedlings from the 2006 collection were tip-inoculated in May 2009; the results of this assay will be compared with those of both detached-leaf inoculations and field studies.

There was infection of seedlings under natural conditions in the first year of the field resistance study; we expect symptom development to intensify in the second autumn. This field component will provide a crucial avenue to validate applicability of lab studies to real-world survivorship. The presence of natural infection demonstrates the utility of the study design, and our experience will allow refinement of the technique for future use. Moreover, the establishment of a common garden population establishes a long-term resource that may continue to be used for genetic study.

Acknowledgments

This research was funded by grants from the U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station, the Midpeninsula Regional Open Space Preserve, and Point Reyes National Seashore. We thank Richard Dodd, Cindy Roessler, Stephen Underwood, Leonel Arguello, Monica Bueno, Chris Lee, Radowslaw Glebocki, Thom Sutfin, Ed Orre, and Alison Forrestel for acorn collections; Cheryl McCormick and Chris Hauser of the Santa Lucia Conservancy for land access and assistance; and Jessica Wright for assistance with study design.

Literature Cited

Carson, S.D. and Carson, M.J. 1989. Breeding for resistance in forest trees - a quantitative genetic approach. Annual Review of Phytopathology. 27: 373–395.

Hayden, K.J.; Ivors, K.; Wilkinson, C. and Garbelotto, M. 2006. TaqMan chemistry for *Phytophthora ramorum* detection and quantification, with a comparison of diagnostic methods. Phytopathology. 96: 846–854.

Maloney, P.E.; Lyncy, S.C.; Kane, S.F.; Jensen, C.E. and Rizzo, D.M. 2005. Establishment of an emerging generalist pathogen in redwood forest communities. Journal of Ecology. 93: 899–905.

McPherson, B.A.; Mori, S.R.; Wood, D.L.; Storer, A.J.; Svihra, P.; Kelly, N.M. and Standiford, R.B. 2005. Sudden oak death in California: disease progression in oaks and tanoaks. Forest Ecology and Management. 213: 71–89.

Parker, I.M. and Gilbert, G.S. 2004. The Evolutionary Ecology of Novel Plant-Pathogen Interactions. Annual Review of Ecology, Evolution, and Systematics. 35: 675–700.

Simms, E.L. 1996. The evolutionary genetics of plant-pathogen systems. BioScience. 46: 136–45.

Sniezko, R.A. 2006. Resistance breeding against nonnative pathogens in forest trees - current successes in North America. Canadian Journal of Plant Pathology. 28: S270–S279.