Relationship Between Resistance to *Phytophthora ramorum* and Constitutive Phenolic Chemistry in Coast Live Oaks and Northern Red Oaks[1]

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Abstract

Phytophthora ramorum causes lethal canker diseases and extensive mortality in coast live oak (CLO) (*Quercus agrifolia*) and tanoak (*Lithocarpus densiflorus*). No practical controls are available for this disease in non-urban environments. Therefore, characterization of natural resistance is highly desirable. Variation in resistance to *P. ramorum* has been observed in CLO in both naturally infected trees and controlled inoculation trials. The persistence of asymptomatic CLOs in naturally infested disease progression plots established in 2000 has been reported (McPherson and others 2005; McPherson and others, unpublished). Around a third of CLOs in a population directly inoculated with the pathogen failed to develop symptoms or appeared to recover following initial symptom development (McPherson, unpublished). Previous studies suggested that phloem phenolic chemistry may play a role in induced defense responses to *P. ramorum* in CLO (Ockels and others 2007). However, in those studies, a relationship was not established between phenolic defense responses and actual resistance, and constitutive phenolic levels may also play a role in resistance, tolerance, or mitigation of initial infection.

The escape of *P. ramorum* into native forests outside of its current range is also highly feared. *Quercus* spp. are dominant throughout eastern North American forests and are extremely important from both ecological and economic standpoints. Laboratory inoculations have demonstrated susceptibility to *P. ramorum* in many eastern tree species, with northern red oak, *Quercus rubra* (NRO), being the third most susceptible species tested (Tooley and Kyde 2007). The pathogen has also been isolated from bleeding cankers on landscape NROs in The Netherlands (Brasier and others 2004), but there is no information concerning variation in susceptibility within the species.

Phenolics are an extremely diverse class of highly bioactive, and in many cases, highly toxic secondary metabolites. Accumulation (both constitutive and induced) of certain phenolics has been implicated in defense strategies, particularly in conifers, where they have been more intensively studied, but in a few angiospermous species as well (reviewed by Witzell and Martin 2008).

Here we describe investigations aiming to elucidate the role of constitutive phenolics in resistance by quantifying the relationship between concentrations of individual and total phenolics (quantified by HPLC analysis) to actual resistance in CLO and NRO. Our long-term

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[1] A version of this paper was presented at the Fourth Sudden Oak Death Science Symposium, June 15-18, 2009, Santa Cruz, California.

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goal is to identify easily screenable biomarkers of resistance. Three experiments were conducted towards this goal. In Experiment one, we used cohorts of CLOs that had previously been characterized as relatively resistant (R) or susceptible (S) (D. Huberli, personal communication). Constitutive (pre-inoculation) phenolics were extracted from branches harvested from R and S trees on three different dates (October 2007, April 2008, November 2008). Additional branches from the same trees were inoculated in the greenhouse at the time of phloem sampling to confirm relative resistance. In Experiment two, concentrations and variation in phenolics of CLOs exhibiting apparent field resistance, in other words, that remained asymptomatic (PR: putatively resistant) under high disease pressure in the field and/or after artificial inoculation during 5 to 8 years of continuous observation (McPherson and others 2005, 2008) were compared to symptomatic (S: susceptible) trees and trees that had shown symptoms at one time and then recovered (PS: previously symptomatic). We tested two cohorts of remnant CLOs, both in stands with elevated infection levels, one representing remnant trees in an infection center with high mortality (China Camp) and the other (Nike) subjected to artificial inoculations in 2002. In Experiment three, 10 half-sib families of 1-year-old NRO were inoculated and their relative resistance evaluated against concentrations of constitutive phenolics.

In Experiment one, there was a significant effect of date on average lesion length ($F_{2, 36}$ = 3.52, P = 0.040). Dodd and others (2005) observed a similar seasonal trend. In October 2007, trees in the R group had significantly shorter lesions than trees in the S group, confirming their *a priori* rankings, and though R and S trees did not differ in concentrations of any phenolics tested, there was a significant negative correlation between average within-tree concentrations of a tyrosol derivative and lesion lengths (Spearman's $\rho = -0.733$, P = 0.012). In April 2008 and November 2008 lesions in R and S trees were not significantly different, suggesting that branch bioassays under controlled conditions did not provide a reliable evaluation of relative field resistance in our study. However, Dodd and others (2008) showed consistency in relative susceptibility rankings during multiple springtime inoculations. Because our cohort of trees did not display consistent levels of relative resistance in the second and third trials, we cannot compare trends in phenolic chemistry, and substantiate the October 2007 results.

In Experiment two, there were no significant differences between PR, PS, and S for any individual compounds or for total phenolics in the China Camp cohort. In the Nike study, total phenolics did not differ between tree categories, but levels of a tyrosol derivative and ellagic acid varied significantly between PR, PS, and/or S. Biochemical activity of ellagic acid against *P. ramorum* is unknown, and the tyrosol derivative will need to be chemically characterized before activity can be postulated. Nevertheless, Ockels and others (2007) described a strong, dose-dependent inhibitory effect of tyrosol on *P. ramorum* and other *Phytophthora* spp. *in vitro*, and antifungal activity of tyrosol has been described elsewhere (Slininger and others 2004, Baidez and others 2006). The UV spectra of tyrosol and the unknown derivatives were identical, and they varied only by their elution times. Results from the Nike study showed that the constitutive concentration of certain, perhaps critical, phenolics were higher in PR and PS trees. This pattern becomes particularly interesting when trees in PR and PS are conceptualized as "more resistant" than currently symptomatic trees.

In Experiment three, NROs were screened for familial variation in phenolic chemistry and susceptibility to *P. ramorum*, and the relationship between peak area of individual phenolics and lesion length was examined. We found up to five-fold variation in mean lesion lengths between families. Although there was not an overall effect of family on lesion length (Kruskal-Wallis, $\chi^2 = 13.39$, P = 0.146), average lesion length was significantly different between some families in pair-wise comparisons, suggesting there may be useful variation in susceptibility within the species. We screened individual compounds (HPLC peaks) for a possible defensive role by correlating peak area with lesion length. Those compounds correlating negatively with lesion length may be involved in fungal growth inhibition and thus resistance. Significant, negative correlations were found for six peaks. We also tested each

peak for a family effect. A significant family effect may indicate that regulation or expression of the compound in question is heritable. Phenolics showing both negative correlations with lesion length and significant family effects are particularly good candidates for use as biomarkers in breeding resistant NROs. Four peaks met both of these requirements, but will need to be chemically characterized and tested in bioassays to determine potential biological activities.

Some of the inconsistency in our CLO results may be due to our inability to identify, post epidemic, trees that are truly resistant and susceptible to *P. ramorum*. The fact that we were unable to obtain representative samples of the most susceptible CLOs prior to their infection and death makes comparing chemical defenses between R and S trees problematic. This issue may only be resolved by banking information on constitutive phenolics from large numbers of trees prior to infestation, with comparisons made following death of the most susceptible trees.

In spite of these limitations, it does appear that production of tyrosol derivatives is upregulated in the more resistant CLOs examined. Taken together, significantly higher levels of ellagic acid and a tyrosol derivative observed in the Nike trees (the most reliable of the two field studies), the negative correlation between a different tyrosol derivative and lesion length in October 2007 in Experiment one (the only trial where R and S trees could be statistically separated), and the strong *in vitro* anti-*Phytophthora* activity of tyrosol (Ockels and others 2007) suggest that these compounds may be especially good candidates for further examination as potential biomarkers for resistance of CLO to *P. ramorum*. Similarly, four unidentified phenolic compounds in NRO were identified as biomarker candidates based on lesion length and family effect, but chemical characterization of these compounds must occur before their potential defense role can be evaluated. Studies on both remnant CLO and NRO must be repeated to validate results, and data from winter and spring dates may be especially ecologically relevant, given that this is when natural infection appears to take place in coastal California forests (Rizzo and others 2005).

Acknowledgments and Funding

The authors would like to thank members of the Garbelotto lab: Lydia Baker, Alex Lundquist, Ellen Crocker, Doug Schmidt, and Alex Yiu, for generous assistance in the field and lab, and especially Katy Hayden for organization and management of projects at University of California, Berkeley. Special thanks to Duan Wang for her assistance in sample collection and processing and BSL3 work, and Nathan Kleczewski and Justin Whitehill for assistance with HPLC and statistical analyses.

Funding was provided by U.S. Department of Agriculture, Forest Service Research Joint Venture Agreement #07-JV-11242300-075, an Ohio Agricultural Research and Development Center grant to A. Nagle, and state funds appropriated to the Department of Plant Pathology, Ohio State University.

Literature Cited

Baidez, A.G.; Gomez, P.; Del Rio, J.A. and Ortuno, A. 2006. Antifungal capacity of major phenolic compounds of *Olea europaea* L. against *Phytophthora megasperma* Drechsler and *Cylindrocarpon destructans* (Zinssm.) Scholten. Physiological and Molecular Plant Pathology. 69(4-6): 224–229.

Brasier, C.; Denman, S.; Brown, A. and Webber, J. 2004. Sudden oak death (*Phytophthora ramorum*) discovered on trees in Europe. Mycological Research. 108(10): 1108–1110.

Dodd, R.S.; Huberli, D.; Douhovnikoff, V.; Harnik, T.Y.; Afzal-Rafii, Z. and Garbelotto, M. 2005. Is variation in susceptibility to *Phytophthora ramorum* correlated with population genetic structure in coast live oak (*Quercus agrifolia*)? New Phytologist. 165(1): 203–214.

McPherson, B.A.; Erbilgin, N.; Wood, D.L.; Svihra, P.; Storer, A.J. and Standiford, R.B. 2008. Attraction of ambrosia and bark beetles to coast live oaks infected by *Phytophthora ramorum*. Agricultural and Forest Entomology. 10(4): 315–321.

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(1-3): 71–89.

Ockels, F.S.; Eyles, A.; McPherson, B.A.; Wood, D.L. and Bonello, P. 2007. Phenolic chemistry of coast live oak response to *Phytophthora ramorum* infection. Journal of Chemical Ecology. 33(9): 1721–1732.

Rizzo, D.M.; Garbelotto, M. and Hansen, E.M. 2005. *Phytophthora ramorum*: integrative research and management of an emerging pathogen in California and Oregon forests. Annual Review of Phytopathology. 43: 309–335.

Slininger, P.J.; Burkhead, K.D. and Schisler, D.A. 2004. Antifungal and sprout regulatory bioactivities of phenylacetic acid, indole-3-acetic acid, and tyrosol isolated from the potato dry rot suppressive bacterium Enterobacter cloacae S11 : T : 07. Journal of Industrial Microbiology and Biotechnology. 31(11): 517–524.

Tooley, P.W. and Kyde, K.L. 2007. Susceptibility of some eastern forest species to *Phytophthora ramorum*. Plant Disease. 91(4): 435–438.

Witzell, J. and Martin, J.A. 2008. Phenolic metabolites in the resistance of northern forest trees to pathogens - past experiences and future prospects. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere. 38(11): 2711–2727.