Chemistry of Coast Live Oak Response to *Phytophthora ramorum* Infection [1]

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Abstract

Since the mid 1990s, *Phytophthora ramorum* has been responsible for the widespread mortality of tanoaks, as well as several oak species throughout California and Oregon forests. However, not all trees die, even in areas with high disease pressure, suggesting that some trees may be resistant to the pathogen. The apparent resistance to *P. ramorum* infection of some individuals within coast live oak populations has been observed in artificial inoculation studies. For example, from artificial branch-cutting inoculation trials, Dodd and others (2005) found significant variation (up to eightfold difference in lesion sizes) in susceptibility to *P. ramorum*. In addition, apparent resistance has also been observed in naturally infected forests, where a number of coast live oaks have survived for more than seven years despite being infected (McPherson and others 2005 and unpublished data).

Elevated levels of secondary metabolites, specifically phenolic compounds in infected tissue, are often associated with resistance to fungal pathogens in angiosperms (Bennett and Wallsgrove 1994; Ostrofsky and others 1984). It is possible that these apparently resistant coast live oaks may have increased amounts of phenolic compounds in the *P. ramorum* infected tissue, which is inhibiting the growth of the pathogen. However, there are no reports that describe the changes in secondary metabolites of coast live oaks infected with *P. ramorum*. To date, the majority of studies investigating phenolic chemistry in oak have focused on constitutive wood and foliage chemistry.

Three field experiments were carried out in Deer Island and China Camp State Park, CA between December 2004 and September 2005 on large trees (DBH approx. 28 to 69 cm). Trees were either artificially inoculated (experiments 1 and 3) or naturally infected with *P. ramorum* (experiment 2). Phloem was sampled from the margin of active lesions and also from healthy phloem at least 60 cm away from lesion margin (AFC, away from cankers) of some of the same inoculated trees and from apparently healthy trees. Phenolics were extracted in methanol, identified by HPLC-mass spectrometry or matched to standards and quantified by HPLC-UV analysis. Nine phenolic compounds (gallic acid, catechin, tyrosol, a tyrosol derivative, ellagic acid and four ellagic acid derivatives) were analyzed in this way.

Significant differences in phenolic profiles were found between phloem sampled from the active margins of cankers, healthy phloem from asymptomatic trees, or AFC phloem,

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although the magnitude and direction of the responses was not consistent across all experiments. Concentrations of gallic acid, tyrosol, and ellagic acid showed the greatest differences in these different tissues, but varied considerably across treatments. Specifically, significantly greater amounts of ellagic acid and gallic acid were observed in infected phloem than non-infected phloem in experiments 1 and 2. In contrast, significantly higher amounts of tyrosol and ellagic acid were present in infected phloem than in corresponding controls in experiment 3 (tables 1-3). Interestingly, catechin levels were significantly reduced in infected tissue in two of the three experiments, i.e. experiments 1 and 3.

Table 1—Effect of artificial *P. ramorum* inoculation on the concentration of nine phenolic compounds extracted from the phloem of coast live oak in experiment 1

	Experimental factors*	
Compound	Infected (N = 5)	Healthy (N = 5)
Gallic Acid	1.97 (0.93) a	0.09 (0.03) b
Tyrosol	0.91 (0.35)	1.01 (0.18)
TY1[x]	2.88 (0.77)	4.08 (0.69)
Catechin	0.63 (0.16) a	2.87 (0.35) b
EA1[y]	0.11 (0.05)	0.39 (0.16)
EA2[y]	0.03 (0.01) a	0.37 (0.17) b
Ellagic acid	1.47 (0.69) a	0.02 (0.01) b
EA3[y]	0.11 (0.06	0.35 (0.09)
EA4[y]	0.09 (0.06)	0.10 (0.02)

*All concentrations expressed as mg/g fresh weight (SE).
[x] Compound quantified in terms of tyrosol equivalents.
[y] Compounds quantified in terms of ellagic acid equivalents.
Values in each row followed by different letters are significantly different ($P < 0.05$).

Table 2—Effect of natural *P. ramorum* infection on the concentration of nine phenolic compounds extracted from the phloem of coast live oak in experiment 2

	Experimental factors*		
Compound	Infected (N = 7)	AFC (N = 7)	Healthy (N = 5)
Gallic Acid	0.94 (0.29) a	0.09 (0.02) b	0.07 (0.01) b
Tyrosol	0.82 (0.25)	1.13 (0.20)	1.26 (0.17)
TY1[x]	2.89 (0.56)	2.71 (0.34)	2.58 (0.23)
Catechin	3.52 (0.59)	3.32 (0.54)	2.07 (0.19)
EA1[y]	0.36 (0.13)	0.43 (0.15)	0.32 (0.06)
EA2[y]	0.15 (0.05)	0.31 (0.16)	0.14 (0.05)
Ellagic acid	0.16 (0.05) a	0.04 (0.02) b	0.04 (0.02) ab
EA3[y]	0.21 (0.10)	0.20 (0.12)	0.10 (0.10)
EA4[y]	0.13 (0.06)	0.15 (0.07)	0.09 (0.08)

*All concentrations expressed as mg/g fresh weight (SE).
[x]Compound quantified in terms of tyrosol equivalents.
[y]Compounds quantified in terms of ellagic acid equivalents.
Values in each row followed by different letters are significantly different ($P < 0.05$).

Table 3—Effect of artificial *P. ramorum* inoculation on the concentration of nine phenolic compounds extracted from the phloem of coast live oak in experiment 3

	Experimental factors*	
Compound	**Infected**	**AFC**
Gallic Acid	0.42 (0.30)	0.47 (0.21)
Tyrosol	2.23 (0.33) a	0.75 (0.57) b
TY1[x]	2.19 (0.87)	4.25 (1.32)
Catechin	1.18 (0.34) a	3.84 (0.88) b
EA1[y]	0.21 (0.13)	0.40 (0.16)
EA2[y]	0.18 (0.12)	0.37 (0.18)
Ellagic acid	0.53 (0.16) a	0.04 (0.01) b
EA3[y]	0.07 (0.03)	0.23 (0.10)
EA4[y]	0.14 (0.06)	0.12 (0.05)

*All concentrations expressed as mg/g fresh weight (SE).
[x] Compound quantified in terms of tyrosol equivalents.
[y] Compounds quantified in terms of ellagic acid equivalents.
Values in each row followed by different letters are significantly different ($P < 0.05$).

The soluble phenolic compounds identified in the phloem extracts of infected coast live oak have been implicated as playing key roles in defense against fungi and herbivores in many woody species, including other *Quercus* spp. (Feucht and Treutter 1999; Malterud and others 1985, Pearce 1996). For example, the durability of some hardwood species against microbes has been directly attributed to the elevated presence of hydrolysable tannins (Barry and others 2001, Hillis 1999, Klumpers and others 1994, Vivas and others 2004).

The antifungal activities of gallic acid (2, 3, 6 and 10 mg/mL) and tyrosol (1, 2, 3 and 6 mg/mL) were tested against *P. ramorum, P. cinnamomi, P. citricola,* and *P. citrophthora in vitro*. Both compounds showed strong dose-dependent inhibitory effects against all four species (fig. 1).

In conclusion, this study demonstrated clear host secondary metabolite responses that may be implicated in resistance of coast live oak to attack by *P. ramorum*. Further studies involving correlation of compound concentrations with disease resistance *in planta* will be necessary to establish a potential defensive role for any of these compounds. If such a role is established, then some of these compounds could be used as biomarkers in the selection of resistant coast live oak genotypes. These studies, however, are contingent on developing reproducible techniques that can be used routinely to obtain quantitative measures of host resistance (e.g. Blodgett and others. 2007), which are lacking at present.

Key words: *Phytophthora ramorum,* sudden oak death, defense, resistance, phenolics.

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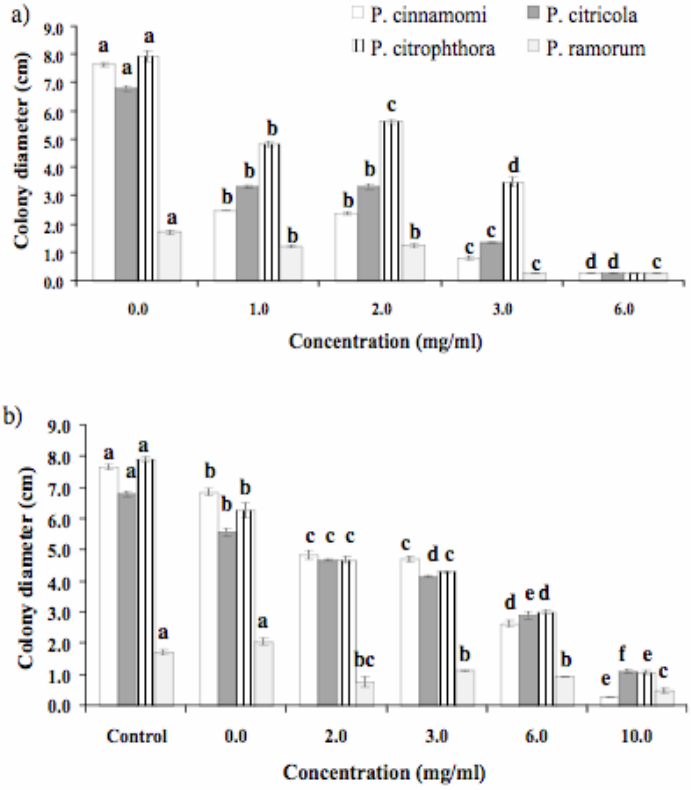

Figure 1. Effects of tyrosol (a) and gallic acid (b) on colony growth rates for *P. cinnamomi, P. citricola, P. citrophthora,* and *P. ramorum*. Each bar represents the mean of three replicates ± 1 SEM and includes the diameter of the original inoculum plug (3 mm). Different letters indicate significant differences ($P < 0.05$, LSD test) within species only. Species was a significant factor in all ANOVAs, indicating that the four *Phytophthora* species are affected differentially by both treatments. The control for tyrosol (0.0 mg/ml) was used as a baseline and is reproduced as the control bars in chart B.

Literature Cited

Barry, K.M.; Pearce, R.B.; Evans, S.D.; Hall, L.D.; Mohammed, C.M. 2001. Initial defence responses in sapwood of *Eucalyptus nitens* (Maiden) following wounding and fungal inoculation. Physiological and Molecular Plant Pathology. 58: 63–72.

Bennett, R.; Wallsgrove, R.M. 1994. Tansley Review No. 72: Secondary metabolites in plant defense mechanisms. New Phytologist. 127: 617–633.

Blodgett, J.T.; Eyles, A.; Bonello, P. 2007. Organ-dependent induction of systemic resistance and systemic susceptibility in *Pinus nigra* inoculated with *Sphaeropsis sapinea* and *Diplodia scrobiculata*. Tree Physiology. 27: 511–517.

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

Feucht, W.; Treutter, D. 1999. The role of flavan-3-ols in plant defense. In: Dakshini K.M.M., Foy C.L. (eds) Principles and practices in plant ecology: Allelochemical Interactions. CRC Press, Boca Raton: 307–338.

Hillis, W.E. 1999. Heartwood and tree exudates. Springer-Verlag, Berlin; New York.

Klumpers, J.; Scalbert, A.; Janin, G. 1994. Ellagitannins in European oak wood - Polymerization during wood aging. Phytochemistry. 36: 1249–125.
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Malterud, K.E.; Bremnes, T.E.; Faegri, A.; Moe, T.; Dugstad, E.K.S. 1985. Flavonoids from the wood of *Salix caprea* as inhibitors of wood-destroying fungi. Journal of Natural Products. 48: 559–563.

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

Ostrofsky, W.D.; Shortle, W.C.; Blanchard, R.O. 1984. Bark phenolics of American beech (*Fagus* grandifolia) in relation to the beech bark disease. European Journal of Forest Pathology. 14: 52–59.

Pearce, R.B. 1996. Antimicrobial defences in the wood of living trees. New Phytologist. 132: 203–233.

Vivas, N.; Nonier, M.F.; de Gaulejac, N.V.; de Boissel, I.P. 2004. Occurrence and partial characterization of polymeric ellagitannins in *Quercus petraea* Liebl. and *Q. robur* L. wood. Comptes Rendus Chimie. 7: 945–954.