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# Phenolic Chemistry of Coast Live Oak Response to *Phytophthora ramorum* Infection

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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. In this study, the chemical basis of host resistance was investigated. Three field experiments were carried out in California between December 2004 and September 2005. The levels of nine phenolic compounds (gallic acid, catechin, tyrosol, a tyrosol derivative, ellagic acid, and four ellagic acid derivatives) extracted from the phloem of trees that had been either artificially inoculated with *P. ramorum* or trees putatively infected with *P. ramorum* (based on canker symptoms) were quantified by high-performance liquid chromatography (HPLC). Significant differences in phenolic profiles were found between phloem sampled from the active margins of cankers, healthy phloem from asymptomatic trees, and phloem sampled 60 cm away from canker sites, 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. Gallic acid

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and tyrosol were tested in *in vitro* bioassays and showed strong dose-dependent inhibitory effects against *P. ramorum*, *P. cinnamomi*, *P. citricola*, and *P. citrophthora*. These results suggest that phloem chemistry varies in response to pathogen infection in California coast live oak populations and that changes in phloem chemistry may be related to apparently resistant phenotypes observed in the field.

**Keywords** Sudden oak death · Resistance · *Quercus agrifolia* · Canker · *In vitro* bioassay

## Introduction

*Phytophthora ramorum* Werres, de Cock, and Man in't Veld, the causal agent of sudden oak death (Werres et al. 2001; Rizzo et al. 2002), was first observed killing tanoaks (*Lithocarpus densiflorus*) in Marin County, California in the mid 1990s (Svihra 1999). Since then, the pathogen has been found in 14 coastal counties in California and 1 southwestern county in Oregon. To date, the pathogen has been responsible for widespread mortality of tanoaks, as well as several oak species, including coast live oak (*Quercus agrifolia*), California black oak (*Q. kelloggii*), and Shreve oak (*Q. parvula* var Shrevei) (Garbelotto et al. 2001). The pathogen is known to occur in North America and Europe, and has a wide host range of over 40 plant genera (Rizzo et al. 2005). In the US, the impact of the disease on tanoaks and coast live oaks has been particularly significant with *P. ramorum* in some sites killing 100% of the mature tanoaks and 45% of coast live oaks (McPherson et al. 2005). At such sites, the death of these trees is having a dramatic ecological impact as these species provide food and habitat for animals, serve as fire breaks, and host mycorrhizal networks (Pavlik et al. 1991; Davidson et al. 2005). The destruction of keystone oak species could ultimately lead to reduced biodiversity in these ecosystems and changes in forest composition (Rizzo and Garbelotto 2003).

In oaks and tanoaks, the primary observable symptom of infection is the development of stem cankers that produce a deep red exudate from intact bark (Garbelotto et al. 2001; Rizzo et al. 2002; McPherson et al. 2005). The canker is caused by the pathogen infecting phloem tissue, and frequently extends into the outer portions of the xylem (Rizzo et al. 2002; Brown and Brasier 2007). A black line is usually evident at the margins of the infected areas of the phloem and xylem (Davidson et al. 2003). Other symptoms include brown and white boring dust on the surface of the bark and/or moss caused by ambrosia and bark beetles tunneling into the bleeding cankers (McPherson et al. 2005).

The apparent resistance to *P. ramorum* infection of some individuals within coast live oak populations has been observed in artificial inoculation studies (Dodd et al. 2005; McPherson et al., unpublished data). For example, from artificial branch-cutting inoculation trials, Dodd et al. (2005) found 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 small proportion of infected coast live oaks have survived for extended periods in populations with high mortality levels (McPherson et al. 2005).

Elevated levels of secondary metabolites, specifically phenolic compounds in infected tissue, are often associated with resistance to fungal pathogens in angiosperms (Ostrofsky et al. 1984; Bennett and Wallsgrove 1994). It is possible that these apparently resistant coast live oaks may have increased amounts of phenolic compounds in the *P. ramorum* infected tissue, which are inhibiting the growth of the pathogen. However, no data are available showing 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 (e.g., Mammela et al. 2000; Salminen et al. 2004; De Simon et al. 2006).

*P. ramorum* is a newly discovered pathogen (see review by Rizzo et al. 2005), and there is a need for knowledge concerning the biology of the disease, including a better understanding of the defense responses of oaks infected with *P. ramorum*. We investigated the chemical defense response of California coast live oaks to *P. ramorum* infection by comparing concentrations of the soluble phenolics extracted from healthy and infected trees, and we tested the inhibitory activity of differentially expressed phenolics against pathogenic *Phytophthora* species (including *P. ramorum*) in *in vitro* bioassays.

## Methods and Materials

Phloem was sampled from artificially inoculated, naturally infected, and healthy coast live oaks at three different times, December 2004, May 2005, and September 2005 at field sites located in Deer Island, Open Space Preserve (38° 6′ N, 122° 32′ W) and China Camp State Park, Marin County (38° 0′ N, 122° 30′ W), California.

The main goals in the following experiments were to induce cankering (or detect natural cankers) and study the associated host response to the necrogenic activity of the pathogen. Therefore, reisolations of the pathogens were not attempted. Previous studies have shown that *P. ramorum* is the only *Phytophthora* species detected in the cankers produced by using the same inoculation technique and samples cultured from natural cankers found in Marin County ($N$=44) (McPherson, unpublished data). Furthermore, the appearance of cankers resulting from our inoculations was entirely consistent with the appearance of natural cankers.

The phloem lesions (i.e., the cankers) resulting from artificial inoculations in experiments 1 and 3 covered vertical distances ranging from a few centimeters to over 50 cm (unpublished data). However, exact canker sizes were not measured because, on large stems, the cankers are extremely irregular and the pathogen appears to weave in and out of the phloem (Brown and Brasier 2007). Consequently, cankers are often a collection of smaller lesions with no well-defined edge to the infection, which makes it difficult to accurately measure lesion size.

*Set-up for experiment 1: comparison between artificially inoculated and apparently healthy trees* Coast live oaks that did not display bleeding cankers or irregular crowns, i.e., that looked healthy, were randomly selected at Deer Island. The diameter at breast height (i.e., approximately 1.5 m above ground) of the selected trees ranged from approximately 28 to 69 cm. The GPS coordinates were recorded for one tree, and the rest of the trees were located in this general area. The trees were then randomly assigned to no treatment (control) and treatment with *P. ramorum*. Each treatment and control consisted of five independent experimental units (trees), for a total of ten trees.

On June 29, 2004, five randomly selected trees were inoculated with *P. ramorum* isolate FOP 10-9-1 with three inoculations per tree at breast height by using a modification of the procedure described by Rizzo et al. (2002). A hole (2 cm diameter) saw was used to remove plugs of bark and phloem tissue down to the sapwood. Five-millimeter agar plugs colonized with an actively growing culture of *P. ramorum* on V-8 agar were placed against the sapwood, and the bark plugs were replaced. Aluminum foil was placed over the wounds, and plugs were held in place with duct tape to minimize contamination and desiccation of cut tissue.

On December 13, 2004, the outer bark over and around the inoculation sites was removed with a hand axe. Samples of inner phloem from the margins of the cankers were harvested, placed in plastic bags, and stored at ambient temperature in the field. Samples of

inner phloem were harvested in the same way from corresponding positions on the trunk of healthy trees. All samples were stored at 4°C upon returning to the laboratory and until extracted.

*Set-up for experiment 2: comparison between naturally infected and apparently healthy trees* Seven naturally infected trees and five apparently healthy asymptomatic trees were randomly selected for sampling in May 2005 at the China Camp State Park. Trees putatively infected with *P. ramorum* were selected based on symptoms, i.e., the presence of exudate from a canker. Using the same harvesting method described above, phloem was sampled from the active margins of the cankers as well as asymptomatic phloem located at least 60 cm above the cankers on each infected tree (these samples will be henceforth referred to as AFC samples for "away from the canker"). Phloem was sampled from five trees showing no symptoms of *P. ramorum* infection. Samples were placed on dry ice in the field and stored at 4°C upon returning to the laboratory and until extracted.

*Set-up for experiment 3: comparison between cankered and noncankered phloem within trees* The five uninoculated control trees that were sampled in experiment 1 at Deer Island were used in experiment 3. They had been wounded for sampling on December 13, 2004. These five trees were inoculated with *P. ramorum* (isolate from Jack London State Park, Glen Ellen, California) on June 30, 2005 in areas of the trunk that had not been wounded previously. Phloem samples were collected on September 12, 2005 from canker margins and AFC positions as described for experiment 2. Samples were placed on dry ice in the field, then transported to the laboratory, and stored at 4°C upon returning to the laboratory and until extracted.

*Chemical analyses* Phloem tissue was frozen in liquid nitrogen and then ground to a powder with a mortar and pestle. Soluble phenolics were extracted with 1 ml of high-performance liquid chromatography (HPLC) grade methanol (Fisher) from 100 mg FW ground phloem tissue for 24 h at 4°C. Particulate matter was removed by centrifugation (5 min at 12,000×*g*). Soluble phenolics were analyzed by HPLC on an Alliance 2690 separation module equipped with an autosampler and a 996 model photo diode array detector (Waters, Milford, MA, USA). A Waters Xterra RP18, 5 μm, 4.6×150 mm column was used. With the exception of a different acidified water–methanol solvents system, the same operating conditions as detailed by Bonello and Blodgett (2003) were performed. Here, 2% formic acid was replaced with 2% acetic acid, and no bases were added to either water or methanol. The photo diode array detector was set to scan between 237 and 400 nm (Bonello and Blodgett 2003), and simultaneous monitoring was performed at 280 nm (flavan-3-ols) and 370 nm (bicyclic coumarin derivatives).

*Peak identification and quantification* To identify peaks that showed a treatment-dependent behavior, samples within treatments were pooled by combining 50 μl from each sample. The pooled samples were analyzed by HPLC to provide a representative chromatogram for each treatment (Bonello and Blodgett 2003). Nine of these peaks (Table 1) were then analyzed for all individual samples.

UV spectra and retention times of the individual peaks were compared to the following standards: benzoic acid, caffeic acid, catechin, ellagic acid, 4-ethylguaiacol, 4-ethylphenol, gallic acid, ferulic acid, 4-methoxy-4-propylphenol, vanillin, vanillic acid, catechin, taxifolin, tyrosol (Sigma Aldrich, St. Louis, MO, USA), *trans*-4-cinnamic acid, *trans*-4-coumaric acid,

**Table 1** Summary of nine phenolic compounds identified in the crude extracts of coastal live oak phloem

| Identity (Abbreviated Name, Where Indicated) | RT (min) | Detector Channel for Quantification (nm) |
|---|---|---|
| Gallic acid | 4.7 | 280 |
| Tyrosol | 7.0 | 280 |
| Tyrosol-like (TY1) | 8.7 | 280 |
| Catechin | 9.5 | 280 |
| Ellagic acid derivative (EA1) | 18.4 | 370 |
| Ellagic acid derivative (EA2) | 19.2 | 370 |
| Ellagic acid | 19.4 | 370 |
| Ellagic acid derivative (EA3) | 21.2 | 370 |
| Ellagic acid derivative (EA4) | 22.6 | 370 |

pinosylvin, pinosylvin monomethyl ether (Apin Chemicals, Abingdon, UK), peltatoside, and hyperoside (Extrasynthese, Genay, France).

Gallic acid, tyrosol, catechin, and ellagic acid (see Fig. 1 for structures) were unequivocally confirmed by spiking samples with the respective standards and comparing retention times and UV spectra. These four compounds were quantified by using a four-point regression curve (in a range of 0.005 to 5 mg/ml for catechin, gallic acid, tyrosol, and 0.001 to 1 mg/ml for ellagic acid) constructed with the available standards. Calibration curves with $r^2$ of 0.99 or better were used. One compound with RT of 8.7 min was identified as a tyrosol derivative based on its UV spectrum, which was similar to that of tyrosol. Similarly, four compounds with RT of 18.5, 19.2, 21.2, and 22.6 min were identified as ellagic acid derivatives based on their UV spectra, which were similar to that of ellagic acid. These compounds, henceforth referred to as TY1, EA1, EA2, EA3, and EA4, respectively, were quantified in terms of tyrosol and ellagic acid equivalents with UV detection at 280 nm for tyrosol and 370 nm for ellagic acid (see Table 1 for a summary of the nine compounds identified and quantified by HPLC).

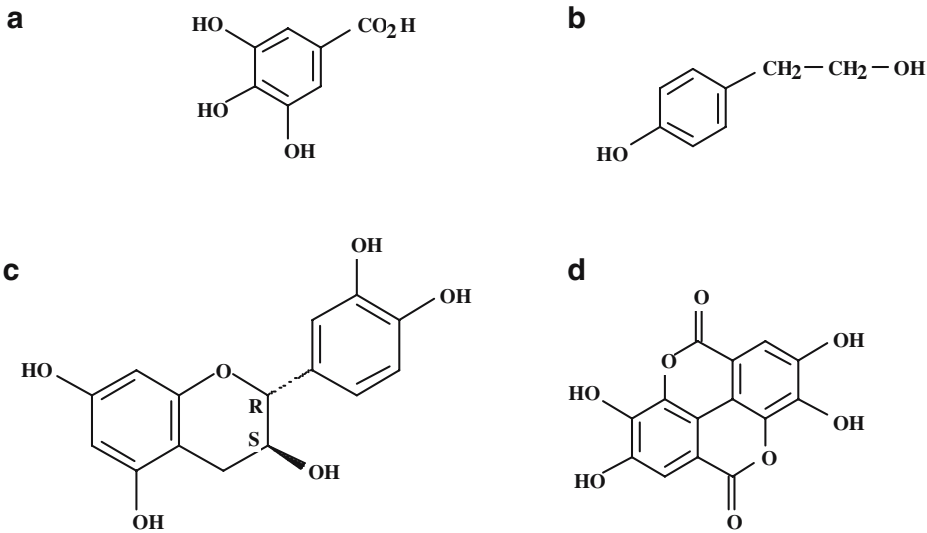


**Fig. 1** Structures of the compounds identified in phloem extracts. **a** Gallic acid, **b** tyrosol, **c** catechin, **d** ellagic acid

*In vitro bioassay* 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*. Solubility problems prevented us from testing the activity of ellagic acid under the conditions of our assays. For each compound, the concentrations incorporated into dilute frozen lima bean agar (LBA) (Schmitthenner and Bhat 1994) (using the equivalency of 1 ml medium=1 g fresh weight of phloem) were based on the range of concentrations found in the crude extract across all experiments and treatments, i.e., gallic acid, 0.0–5.63 mg/g FW; tyrosol, 0.0–3.25 mg/g FW. In practice, we selected a range of concentrations including approximately twice the highest concentration found in the tissues. This was based on the rationale that individual tissue measurements resulted from the homogenization of tissue that included both necrotic and nonnecrotic areas at the canker margins. Thus, compound concentrations in phloem cells located at the pathogen's invasion front may be underestimated by the "mean" concentration found in the tissue sample analyzed.

Gallic acid changed the medium pH to 3.9, so a corresponding control of LBA, pH 3.9, was used (medium acidified with HCl). Tyrosol did not change the medium pH, so the control for this treatment was unamended LBA (pH=6.7). The same unamended LBA was used as a baseline control in the gallic acid treatment (Fig. 2). In each case, a 3-mm plug of actively growing mycelium was placed onto the center of LBA plate, and mycelial growth (colony diameter) was measured after 4 days incubation at room temperature in the dark. Three replicate Petri dishes/species/chemical/concentration were used. The bioassays with *P. ramorum* were conducted in the laboratory of Dr. David Rizzo at UC Davis.

*Statistical analyses* For experiment 1, the data on the effect of infection status on concentrations of the identified compounds were analyzed by using multivariate ANOVA. For experiment 2, the data on the effects of infection status and sampling position within trees (canker or AFC, for the infected trees only) on compound concentrations were analyzed with ANOVA by using an unbalanced split plot model with infection status as the whole-plot and sampling position as the subplot. For experiment 3, our data on the effect of sampling position (canker or AFC) on compound concentrations were analyzed with a paired *t*-test. Data from the bioassay were analyzed with univariate ANOVA with compound/concentration and species used as factors. Fisher's least significant difference (LSD) was used to compare the means in all tests. In all analyses, Levene's test was used to examine variance equality, and log or square root transformations were used to eliminate or minimize variance heterogeneity. All tests were conducted using SPSS 11.0 for Windows or MacOS X.

## Results

### Quantification of Phenolic Compounds

*Experiment 1: comparison between artificially inoculated and apparently healthy trees* Infected phloem contained greater amounts of gallic acid (20-fold) and ellagic acid (64-fold) than healthy phloem ($F_{1,9}$=32.491, $P$<0.001; $F_{1,9}$=8.312, $P$<0.03, respectively). In contrast, infected phloem contained 22% of the catechin and 7% of the EA2, present in healthy phloem ($F_{1,9}$=24.893, $P$<0.002; $F_{1,9}$=17.048, $P$<0.002, respectively). Infection status did not affect the concentrations of tyrosol, TY1, EA1, EA3 or EA4 (Table 2).

*Experiment 2: comparison between naturally infected and apparently healthy trees* As in experiment 1, the concentration of gallic acid was higher in the cankers of infected trees than in

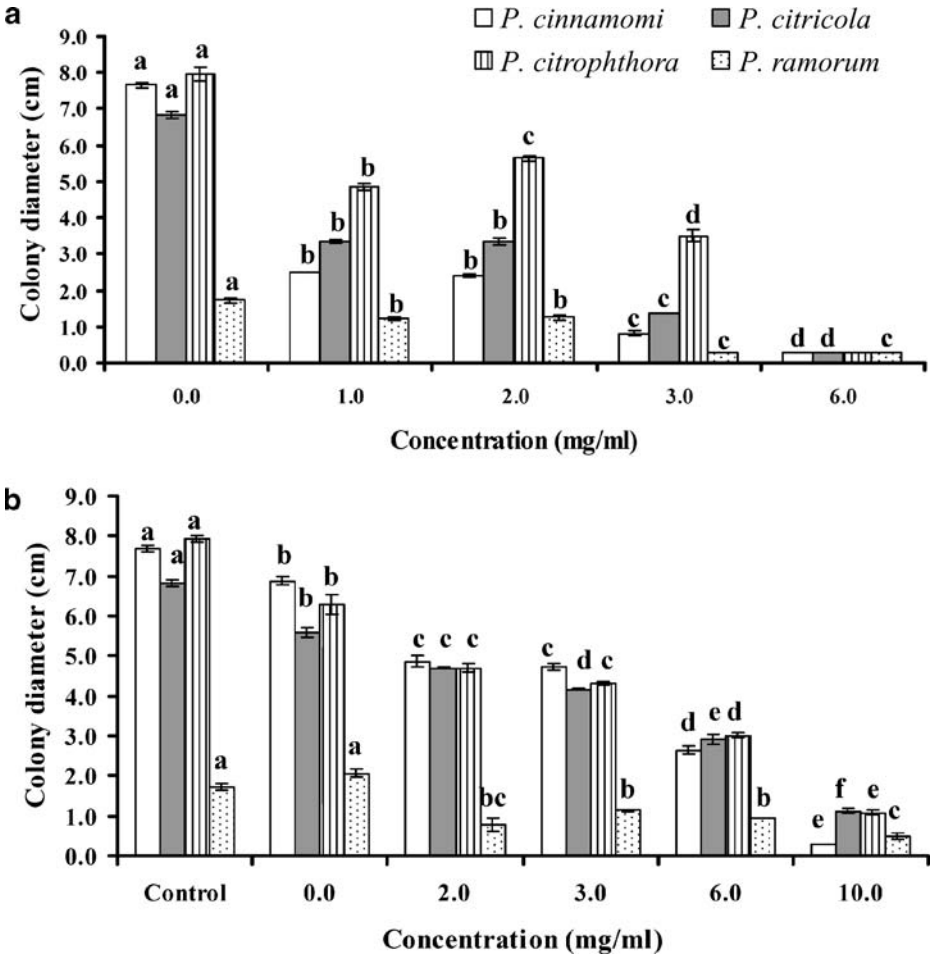


**Fig. 2** Effects of tyrosol (**a**) and gallic acid (**b**) on colony growth rates for *P. cinnamomi*, *P. citricola*, *P. citrophthora*, and *P. ramorum*. Each bar represents the mean of three replicates ±1 SEM and includes the diameter of the original inoculum plug (3 mm). Different letters indicate significant differences ($P<0.05$, univariate ANOVA, 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 **b**

either the phloem of asymptomatic trees (13-fold) ($F_{1,15.9}=28.34$, $P<0.001$) or AFC phloem (10-fold) ($F_{1,8.3}=36.74$, $P<0.001$). AFC and asymptomatic phloem did not differ significantly in gallic acid levels ($F_{1,15.9}=0.01$, $P>0.93$) (Table 3). Ellagic acid was present in greater amounts (4-fold) in cankers of infected trees than in AFC phloem ($F_{1,9.1}=6.14$, $P<0.05$).A trend of greater levels of ellagic acid in infected phloem compared with healthy phloem was also detected ($F_{1,13.7}=3.57$, $P=0.08$). The difference between AFC and healthy phloem was not significant ($F_{1,13.7}=0.84$, $P>0.37$). The concentrations of tyrosol, TY1, catechin, EA1, EA2, EA3, and EA4 were not different among infected, AFC, and healthy phloem (Table 3).

*Experiment 3: comparison between cankered and noncankered phloem within trees* Tyrosol, catechin, and ellagic acid were found in different amounts in infected and AFC phloem

**Table 2** 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[a] | |
|---|---|---|
| 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[b] | 2.88 (0.77) | 4.08 (0.69) |
| Catechin | 0.63 (0.16) a | 2.87 (0.35) b |
| EA1[c] | 0.11 (0.05) | 0.39 (0.16) |
| EA2[c] | 0.03 (0.01) a | 0.37 (0.17) b |
| Ellagic acid | 1.47 (0.69) a | 0.02 (0.01) b |
| EA3[c] | 0.11 (0.06) | 0.35 (0.09) |
| EA4[c] | 0.09 (0.06) | 0.10 (0.02) |

Values in each row followed by different letters are significantly different ($P$<0.05); multivariate ANOVA, LSD test.

[a] All concentrations expressed as mg/g fresh weight (SE).

[b] Compound quantified in terms of tyrosol equivalents.

[c] Compounds quantified in terms of ellagic acid equivalents.

collected in May 2005. Infected phloem had higher concentrations of tyrosol (3-fold) and ellagic acid (14-fold) than AFC tissue ($t_4$=2.86, $P$<0.05; $t_4$=6.84, $P$<0.01, respectively), whereas catechin was present in infected tissue at 31% of the amount found in AFC phloem ($t_4$=3.09, $P$<0.05) (Table 4). No differences were found between infected and AFC samples for the following compounds: gallic acid, TY1, EA1, EA2, EA3, and EA4 (Table 4).

*In vitro bioassay* Results of the bioassay are summarized in Fig. 2. All treatments had significant effects on colony growth rates for all four *Phytophthora* species. Tyrosol

**Table 3** 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[a] | | |
|---|---|---|---|
| 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[b] | 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[c] | 0.36 (0.13) | 0.43 (0.15) | 0.32 (0.06) |
| EA2[c] | 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[c] | 0.21 (0.10) | 0.20 (0.12) | 0.10 (0.10) |
| EA4[c] | 0.13 (0.06) | 0.15 (0.07) | 0.09 (0.08) |

Values in each row followed by different letters are significantly different ($P$<0.05), ANOVA, LSD test.

*AFC*: asymptomatic phloem located at least 60 cm above the canker.

[a] All concentrations expressed as mg/g fresh weight (SE).

[b] Compound quantified in terms of tyrosol equivalents.

[c] Compounds quantified in terms of ellagic acid equivalents.

**Table 4** 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[a] | |
|---|---|---|
| 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[b] | 2.19 (0.87) | 4.25 (1.32) |
| Catechin | 1.18 (0.34) a | 3.84 (0.88) b |
| EA1[c] | 0.21 (0.13) | 0.40 (0.16) |
| EA2[c] | 0.18 (0.12) | 0.37 (0.18) |
| Ellagic acid | 0.53 (0.16) a | 0.04 (0.01) b |
| EA3[c] | 0.07 (0.03) | 0.23 (0.10) |
| EA4[c] | 0.14 (0.06) | 0.12 (0.05) |

Values in each row followed by different letters are significantly different ($P<0.05$), paired *t*-test.

*AFC*: asymptomatic phloem located at least 60 cm above the canker.

[a] All concentrations expressed as mg/g fresh weight (SE).

[b] Compound quantified in terms of tyrosol equivalents.

[c] Compounds quantified in terms of ellagic acid equivalents.

completely inhibited growth of all four species at the highest concentration, but significant effects could be observed at lower concentrations. *P. citrophthora* appeared to be the least sensitive of the four species to tyrosol. An apparently linear dose response for all four species was observed with gallic acid, although medium acidity also appeared to be a factor (albeit minor) in slowing growth rates for *P. cinnamomi*, *P. citricola*, and *P. citrophthora*, but not *P. ramorum*.

## Discussion

Increased concentrations of secondary compounds, especially phenolic compounds, have been reported to occur in the lesion margins of many woody tree species and are generally considered to be implicated in protection against pests and diseases (Hart and Hillis 1974; Woodward and Pearce 1988; Evensen et al. 2000; Viiri et al. 2001, Eyles et al. 2003; Bonello and Blodgett 2003; Blodgett et al. 2007). In the present study, significant differences in phenolic profiles were demonstrated between phloem sampled from the active margins of cankers, healthy phloem from asymptomatic trees, and phloem sampled 60 cm above canker sites in coast live oaks. In particular, five compounds (gallic acid, tyrosol, catechin, ellagic acid, and EA2) were present in differing concentrations in the various treatments. Given that these compounds were present in the healthy phloem as well as in the infected phloem, they are considered constitutive compounds, in contrast to *de novo* produced compounds that are synthesized only in response to a pathogen attack and that would only be detected in infected tissue. This result confirms the lack of evidence, to our knowledge, for phenolics produced *de novo* in response to pathogen attack in oaks.

It should be noted that the production of phenolic compounds was not consistent across all experiments. Specifically, greater amounts of ellagic acid and gallic acid were observed in infected phloem than noninfected phloem in experiments 1 and 2. In contrast, higher amounts of tyrosol and ellagic acid were present in infected phloem than corresponding controls in

experiment 3. It is interesting to note that catechin levels were reduced in infected tissue in two of the three experiments, i.e., experiments 1 and 3. In general, the artificially inoculated trees accumulated more phenolics in response to the infection than the naturally infected trees. We suggest that the artificially inoculated trees, which had been more recently infected with an active pathogen, stimulated a more active host response. As we were unable to determine when the naturally infected trees were inoculated, we cannot speculate on the effect that timing of inoculation has on phenolic production and accumulation. Furthermore, it is possible that other factors such as seasonality (i.e., phenological status) or even the method used to store tissue (room temperature vs dry ice) might have contributed to the observed variability. These factors should be investigated further. The use of controlled branch inoculations to correlate elevated phenolic levels with certain infection characteristics including canker size and host resistance (e.g., Blodgett et al. 2007) may also be helpful.

The soluble phenolic compounds identified in the phloem extracts of infected coast live oak have been implicated as playing key roles in the defense against fungi and herbivores in many woody species including other *Quercus* spp. (Malterud et al. 1985; Pearce 1996; Feucht and Treutter 1999). For example, the durability of some hardwood species against microbes has been directly attributed to the elevated presence of hydrolyzable tannins (Klumpers et al. 1994; Hillis 1999; Barry et al. 2001; Vivas et al. 2004). Hydrolyzable tannins refer to both ellagitannins and gallotannins. The former are esters of hexahydroxydiphenoyl groups with a sugar core and often contain galloyl groups. In contrast, gallotannins consist of a sugar substituted only with galloyl groups (Okuda et al. 1995; Barry et al. 2001).

Hart and Hillis (1972) conducted bioassays with white oak (*Quercus alba* L.) heartwood extracts containing ellagitannins and observed inhibition of the growth of *Poria monticola* Murrill, a wood decay fungus. The capacity of tannins to inhibit the growth of microbes has been credited to the ability of tannins to bind to proteins (Field and Lettinga 1992; Kawamoto et al. 1997) and their antioxidant activity (Okamura et al. 1993; Hagerman et al. 1998). In another study, tyrosol, catechin, and oleuropein were among the main phenolic compounds identified in the stem cortex of olive trees (Del Rio et al. 2003). The stem cortex of olive tree is commonly attacked by a *Phytophthora* sp. causing rot and necrosis, and Del Rio et al. (2003) showed that of the three compounds, tyrosol was the most active growth inhibitor of *Phytophthora* in an *in vitro* bioassay. Of particular note, the greatest antifungal effect was achieved with all three compounds combined. The authors suggested that the increased accumulation of phenolic compounds in the cortex was a defense mechanism of olive plants against the pathogen. Our bioassay results confirmed the toxicity of tyrosol against *Phytophthora* spp., including *P. ramorum*, and indicate that gallic acid also has antimicrobial activity. It is possible that both compounds (and others we have not tested) may have synergistic activities.

In summary, this study demonstrated clear host secondary metabolite responses that may be implicated in the 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 techniques to obtain quantitative measures of host resistance (e.g., based on lesion size as in Blodgett et al. 2007), which are lacking at present in a form that is conducive to concurrent characterization of defense mechanisms.

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