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SYSTEMIC EFFECTS OF *Heterobasidion annosum* ON FERULIC ACID GLUCOSIDE AND LIGNIN OF PRESYMPTOMATIC PONDEROSA PINE PHLOEM, AND POTENTIAL EFFECTS ON BARK-BEETLE-ASSOCIATED FUNGI

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Abstract—Concentrations of soluble phenolics and lignin in the phloem of ponderosa pines inoculated with the pathogen *Heterobasidion annosum* were assessed over a period of 2 years in a 35-year-old plantation in northern California, USA. The major effect of the pathogen on phloem-soluble phenolics consisted of a significant accumulation of ferulic acid glucoside: 503 ± 27 μg/g fresh weight (FW), compared with 366 ± 26 μg/g FW for mock-treated and 386 ± 27 μg/g FW for control trees. Lignin content was negatively correlated with ferulic acid glucoside concentration, and there was an indication of lignin reduction in the

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cell walls of inoculated trees. Lignin had a negative effect on the *in vitro* growth of two common bark beetle fungal associates, *Ceratocystiopsis brevicomi* and *Ophiostoma minus*. For this reason it, is hypothesized that lower lignification may facilitate the growth of beetle-associated fungi, resulting in greater susceptibility of the presymptomatic host to bark beetle colonization.

Key Words—Pine root rot, induction, systemic accumulation, phenolics, lignin depletion.

INTRODUCTION

A considerable body of evidence points to predisposition of root-diseased conifers to colonization by tree-killing bark beetles (Cobb et al., 1974; Goheen and Cobb, 1980; Livingston et al., 1983; Goheen and Hansen, 1993). This appears to be particularly important in the maintenance of endemic beetle population densities (summarized in Paine and Baker, 1993), presumably because the availability of disease-weakened trees may shorten the time required by colonizing beetles to find a suitable substrate and, thus, reduce the risks of losses due to predation or exposure (Wood, 1982). However, root disease centers are also often foci of bark beetle outbreaks, because once diseased trees are colonized, pheromone production is induced and amplification of bark beetle attack occurs on adjacent healthy trees (Wood, 1982). Death of these healthy trees apparently results from the overwhelming of tree defenses brought about by the dynamic combination of insect attack and infection by bark-beetle-associated fungi (Paine et al., 1997). Thus, understanding how diseased trees become susceptible to bark beetle attack will provide insight into mechanisms by which bark beetle outbreaks occur. In particular, an understanding of systemic physiological changes in the phloem of root-diseased-trees and potential effects of those changes on the fungi associated with bark beetles, is lacking (Paine et al., 1997; Wood, 1982).

Prelanding olfactory cues may or may not aid in the location of a host tree (Moeck et al., 1981; Nebeker et al., 1995; Brattli et al., 1998; Macias-Samano et al., 1998; Bonello et al., 2001b). In the postlanding environment, close range olfactory and/or gustatory cues (Elkington and Wood, 1980; Wood et al., 1986; Raffa et al., 1993), as well as constitutive and induced plant defenses against insects and associated fungi (Harrington, 1993), such as resinosis (summarized in Lorio, 1993) and lignification, are important in the host-selection process. In several reports, secondary metabolites, notably phenylpropanoid derivatives, terpenoids, and host cell wall lignin have been demonstrated to have significant effects on bark beetles infesting conifers (Wainhouse et al., 1990; Klepzig et al., 1996; Storer and Speight, 1996; McNee, 2000). Because an attack can occur away from the direct influence of the root pathogen, interaction between host tree and bark beetles may be affected by systemic chemical changes induced by the pathogen in the phloem of the host tree; similar systemic chemical changes may explain the induced

resistance phenomenon recently observed in Monterey and Austrian pine (Blodgett and Bonello, 2001; Bonello et al., 2001a).

Systemic effects of disease on plant metabolism are well known, particularly in the case of secondary metabolism associated with systemic acquired resistance (SAR) (Heil, 1999). Secondary metabolites in the phenolic and isoprenoid pathways have been studied in great detail in many plant species, including herbaceous and arboreal angiosperms, and gymnosperms (Strack, 1997). Many of these pathways and compounds can be profoundly affected by disease. A phenolic derivative, salicylic acid, has been implicated in signaling in the SAR phenomenon (Metraux, 2001).

There are many reports of secondary metabolite changes induced locally in conifers by microbial infection. For example, accumulation of soluble and cell-wall-bound phenolics has been shown in mycorrhizal roots of Douglas fir (Sylvia and Sinclair, 1983; Strobel and Sinclair, 1988), while in European larch the phenomenon is more variable, with accumulation (Weiss et al., 1997) or decreased concentration (Muenzenberger et al., 1995) of phenolics in mycorrhizal roots, depending on the particular host–mycobiont combination. Localized pathogenic induction of changes in secondary metabolism are also well known. Stilbenes, a class of phenylpropanoid derivatives (Strack, 1997) with antimicrobial properties (Hart, 1981; Kemp and Burden, 1986), are present constitutively in spruce secondary phloem (Woodward and Pearce, 1988) and in pine heartwood (Hart, 1981; Kemp and Burden, 1986), but are synthesized *de novo* in pine sapwood and juvenile tissues following microbial challenge (Prior, 1976; Bonello et al., 1993; Lieutier et al., 1996). Some of these phenomena may be related to localized (Christiansen et al., 1999; Krokene et al., 1999; Franceschi et al., 2000) or SAR (Bonello et al., 2001a) phenotypes. Studies specifically designed to induce systemic effects of disease on the secondary metabolism of large coniferous trees in controlled experiments are unknown, although in one case systemic effects of natural infection were demonstrated (Klepzig et al., 1995a).

In plants, localized lignification of host cell walls is another documented constitutive and inducible defense mechanism against pathogens (Vance et al., 1980; Aist, 1983; Ride and Barber, 1987). At the tissue level, lignification can occur as an early response in the periderm-restoration process in woody plants (Woodward, 1992). Little is known about the systemic effects of disease on lignification, with most information deriving from studies of cell wall appositions in SAR induced in crop plant species (Hammerschmidt and Kuc, 1982). No information is available on the systemic effects of disease on lignification in coniferous trees and, more generally, woody plants.

To contribute to a better understanding of these interactions, we tested the hypothesis that root disease of ponderosa pine (*Pinus ponderosa* Dougl. ex Laws), caused by *Heterobasidion annosum* (Fr.) Bref (an important root rot of conifers, see Woodward et al., 1998), induces systemic changes in the phenolic constituents

of the phloem, which may directly or indirectly affect bark beetle behavior. The relevance of this model system follows from the important role played by *H. annosum* as a predisposing factor to bark beetle outbreaks (summarized in Paine and Baker, 1993). In particular, we report results highlighting systemic effects induced by *H. annosum* on soluble low-molecular-weight phenolics; as well as on the lignin content of the phloem. Because enhanced lignification is a known defense response against fungi, we also investigated the effect of lignin on the *in vitro* growth rates of two bark beetle-associated fungi: *Ceratocystiopsis brevicomi* Hsiau et Harrington and *Ophiostoma minus* (Hedgcock) Sydow et Sydow.

METHODS AND MATERIALS

Field Site and Inoculation. Two adjacent stands of 35-year-old ponderosa pines (east and west plots) at the University of California's Blodgett Forest Research Station (BFRS) (El Dorado County, California, 38°55′ N, 120°39′ W) were selected in the spring of 1997. Diameter at breast height was determined for a random sample of 30 trees in each plot.

In each of the two plots (i.e., among a total of 60 trees), 10 trees were assigned at random to each of three treatments: inoculation with *H. annosum* (strain AWR-282: intersterility group P, isolated from ponderosa pine in the Modoc National Forest, California), mock inoculation, and unwounded control. Inoculation with the root pathogen was carried out by insertion of colonized wood dowels at four orthogonal positions around the base of the trunk by using a modification of a previously reported method (Garbelotto et al., 1997). Dead outer bark was pared away using a hatchet to produce a flat area, and inoculation holes 0.9 cm in diameter, 6–7 cm deep, were made with a cordless drill at approximately 5 cm above litter line. One wood dowel, either colonized by the pathogen or autoclaved, but not colonized (mock), was inserted into each hole. Autoclaved cork stoppers were inserted into each hole, and the infection court was coated with pruning seal (Ace Hardware, USA). Inoculations and mock inoculations were carried out on June 14–19, 1997.

Five inoculated trees from each plot ($N = 10$) were sampled on May 5, 1998, approximately 11 months after inoculation, to test for *H. annosum* activity. Cores from 3 cm above and 3 cm below each inoculation site were taken to the lab, where they were surface-sterilized by immersion in 1% sodium hypochlorite for 2 min. The cores were then incubated in moist chambers and determined to support active growth of *H. annosum* when the characteristic conidiophores were formed (Woodward et al., 1998).

A separate cohort of 40 trees in the same plantation, located at least 30 m away from any of our test trees, was similarly inoculated in October 1997, and felled at the inoculation level in October 1999 (McNee et al., 2003). Extent of fungal

invasion was estimated visually based on resin soaking and/or discoloration/drying of the host sapwood and phloem, and considered representative of the trees in the main experiment.

Sampling. Trees were sampled for analysis of phenolics five times over 2 years, on October 28, 1997, July 21 and 30 October, 1998, and July 12 and September 24, 1999. One core per tree, comprising outer bark, phloem, and 3–5 cm of sapwood, was taken with an increment borer at breast height on the first three dates. Phloem plugs were taken with a 2 cm diameter cork borer on the last two dates from the same relative positions. On each occasion, the distance around the tree circumference at breast height between new and previous samples was maximized to avoid the effects of localized host responses to the wounds. Additionally, in September 1999, phloem samples were taken from the base of the trees, next to one of the inoculation sites selected at random. The cores and plugs were immediately frozen in liquid nitrogen and taken to the laboratory for analysis of phenolics. In the laboratory, phloem was separated from the sapwood of each core and homogenized at liquid nitrogen temperature by using a Mikrodismembrator S (BBraun Biotech, Allentown, PA). Celite (Sigma, St. Louis, MO) was used as a carrier for phloem homogenization (Rosemann et al., 1991) when this came from cores, because of the low amounts of fresh material. Phloem from plugs was homogenized without celite.

Analysis of Methanol-Soluble Phenolics. The homogenized biomass ($\sim$50 mg (FW)) was extracted twice in 0.5 ml of HPLC grade methanol overnight at 4°C; the supernatants were combined, centrifuged, and the extracts analyzed directly by HPLC or stored at -20°C (or -80°C) until analysis. Compounds were separated on a Beckman Ultrasphere 5 μm, ODS C-18 reverse phase, 4.6 $\times$ 250 mm column, using the water/methanol-based gradient system described by Rosemann et al. (1991) at a flow rate of 1 ml/min.

Analysis of Cell Wall-Bound Phenolics. Pellets left over from the analysis of the October 1998, and July and September 1999 soluble phenolics were processed and extracted for determination of cell wall-bound phenolics by using either a modification of the method of Strack et al. (1988), as described in Bonello et al. (1993), or the following. Briefly, the pellets were washed three times with methanol and once with water, 100 μl of 1 N NaOH containing 100 mM ascorbic acid and 0.2% sodium borohydride ($NaBH_4$) (to limit oxidation of the phenolic metabolites) were added, and the samples were hydrolyzed overnight on a shaker. Formic acid (1.5 m; 100 μl) was added to the mixture to stop the reaction, followed by 200 μl of methanol. The mixture was centrifuged for 10 min at 11,000 rpm, and the supernatant was analyzed by HPLC, or stored at -80°C until analysis.

Analysis of Lignin-Like Material. Phloem collected from the stem in October 1998 and July and September 1999, and from the tree base next to inoculation sites in September 1999 was processed and extracted for quantitative determination of lignin-like material complexed to the cell wall by using a modification

of the method of Bruce and West (1989), as described by Bonello et al. (1993). This method involves derivatization of lignin polymers with thioglycolic acid (Sigma–Aldrich, St. Louis, MO) and solubilization with alkali. Lignin concentration was determined spectrophotometrically against a standard of pure spruce lignin (Sigma–Aldrich) using a Shimadzu UV-260 spectrophotometer.

Effects of Lignin on in Vitro Growth Rates of C. brevicomi and O. minus. *C. brevicomi* and *O. minus* used in this study were isolated from western pine beetles, *Dendroctonus brevicomis* LeConte, collected from ponderosa pines at BFRS by Tom Harrington (Iowa State University), who donated them to us. Cultures were maintained on 2% malt extract agar (MEA) at room temperature.

Pure spruce lignin (Sigma–Aldrich) was incorporated into 2% MEA at the following rates: 0, 2, 4, 8, 16, and 20 mg/ml. This range includes the *in vivo* lignin concentrations described in the results, if one assumes correspondence between 1 ml of culture medium and 1 g FW phloem. The two fungal species were tested on the lignin-amended medium by placing 5-mm mycelial plugs (mycelium side down), taken from the margins of actively growing cultures, at the edge of 60-mm diameter Petri dishes. The cultures were maintained at room temperature in the dark for the duration of the experiment. Cumulative radial growth was measured at 6, 12, and 21 days for *C. brevicomi* (a slow growing species), and at 6 and 9 days for *O. minus* (a fast growing species).

Identification and Synthesis of Ferulic Acid Glucoside. Ferulic acid glucoside was identified by diode array spectroscopy before and after co-chromatography with prepared reference material. The compound eluted at 20.93 min and showed two maxima at 290 and 313 nm.

Ferulic acid glucoside was prepared by using a slight modification of the methods of Pearl and Beyer (1951) and Schuster et al. (1986). A solution of 157 mg vanillin glucoside (0.5 mmol; Apin, UK) and 115 mg malonic acid (1.1 mmol) in 1 ml dry pyridine and 20 μl piperidine (Fluka, Germany) was kept at 45°C for 8 days, when only one product, and no vanillin glucoside, was detected by HPLC (Turunen et al., 1999). The solvents were removed in a rotary evaporator at 40°C and the pale yellow residue was partly dissolved by vigorously vortexing in 10 ml dry ethanol–acetic acid (95:5, v/v) for 1 min. It was not possible to dissolve 100% of the residue. The dissolved product was then precipitated from the supernatant by dropwise addition of 10 ml dry diethyl ether–acetic acid, (95:5, v/v). The supernatant was carefully removed and the residue dried in a stream of nitrogen. The washing procedure was repeated twice with abs. ethanol and diethyl ether. The dry powder (150 mg, 0.42 mmol, yield 84%) was identified as ferulic acid glucoside by ^{1}H NMR spectroscopy (DMSO-d_6, 500 MHz, DMX-500, Bruker, Germany).

Data Analysis. Data were analyzed using SPSS 10.0 for Windows. Differences in mean diameter of trees between the two plots (east and west) were tested using analysis of variance (ANOVA) (Sokal and Rohlf, 1981). Differences in the

mean amounts of metabolites in the phloem samples were tested using ANOVA, following appropriate data transformation (Sokal and Rohlf, 1981). Where correlation coefficients between data for each compound extracted from phloem collected on different dates were significant, repeated measurements ANOVA was used (SPSS 10.0 for Windows). For each compound extracted from phloem samples, orthogonal contrasts (Sokal and Rohlf, 1981) compared the inoculated treatment to the mock-inoculated and control treatments combined, followed by a comparison between the mock-inoculated and the control trees. Relationships between the compounds found in the phloem were tested by calculating correlation coefficients (Sokal and Rohlf, 1981).

The relationships between the radial growth of *C. brevicomi* and *O. minus* and the concentration of lignin in MEA were tested by using linear and exponential regressions.

RESULTS

Tree Size and Pathogen Activity. When the experiment was set up in 1997, the difference in the mean diameters of trees in the east plot (29.2 ± 1.1 cm) and in the west plot (34.3 ± 1.3 cm) was significant ($F = 9.08$; df: 1, 59; $P = 0.004$). Active mycelium of the pathogen was recovered from at least one inoculation site in three trees from the west plot and four from the east plot (from five sampled in each plot). Thus, $70 \pm 14.5\%$ (SE) of tested inoculated trees were actively infected 11 months after inoculation. Visual assessment of the infection sites on the stumps of the separate trees felled in October of 1999, showed that limited development of the infection occurred over the 2 years of the study, with sapwood discoloration evident only just around the inserted inoculation dowels (Figure 1).

FIG. 1. Extent of colonization of ponderosa pine stems by *Heterobasidion annosum* 2 years after artificial inoculation. Note the limited sapwood area occupied by discolored/resin-soaked tissue (arrows).

Oct. '97	r = 0.432 P = 0.001 N = 60	r = 0.661 P < 0.001 N = 55	r = 0.742 P < 0.001 N = 59	r = 0.508 P < 0.001 N = 59
	July '98	r = 0.312 P = 0.020 N = 55	r = 0.508 P < 0.001 N = 59	r = 0.423 P = 0.002 N = 53
		Oct. '98	r = 0.548 P < 0.001 N = 54	r = 0.471 P = 0.001 N = 49
			July '99	r = 0.588 P < 0.001 N = 53
				Sept. '99

FIG. 2. Bivariate correlations between ferulic acid glucoside concentrations in the phloem of ponderosa pines at breast height at different combinations of dates. All correlations between dates were significant and positive ($P \leq 0.028$).

Phloem Samples from the Stem at Breast Height. No qualitative changes were detected throughout the study. However, the concentration of the constitutive compound ferulic acid glucoside was affected by treatment. Bivariate Pearson correlations between ferulic acid glucoside concentrations in all trees at different dates were all positive and significant (Figure 2, $P \leq 0.028$), indicating that trees producing higher amounts of this compound did so consistently over time. Thus, repeated measurements ANOVA was performed. The difference in ferulic acid glucoside concentration between trees in the east and west plots was not significant, and there were no significant interaction between plot and treatment. However, differences in the mean concentrations of ferulic acid glucoside among treatments were not significant. Ferulic acid level in all inoculated trees pooled over plots was 503 ± 27 μg/g FW, compared with 366 ± 26 μg/g FW for mock-treated and 386 ± 27 μg/g FW for control trees ($F = 3.68$; df : 2, 43; $P = 0.033$. *Contrasts*: inoculated vs. (mocks + controls): $P = 0.010$; mocks vs. control: $P = 0.784$). Other constitutive compounds, e.g., taxifolin-3′-glucoside and an unidentified

cinnamic acid derivative, also showed some changes, but not consistently and not in the same magnitude, and were not considered further in the analysis. No significant qualitative or quantitative differences in cell wall-bound phenolics were observed among treatments throughout the study. Both of the methods used worked well and gave comparable results.

Correlations between lignin contents at different dates were not significant, so the data were analyzed at individual dates. Differences among treatments were not statistically significant, either in one-way or two-way ANOVAs, except for the east plot in October 1998, when the phloem of inoculated trees contained less lignin than the other treatments: inoculated, 3.87 ± 1.59 mg/g FW; mock-inoculated, 18.93 ± 5.58 mg/g FW; controls, 7.0 ± 3.09 mg/g FW ($F = 4.25$; df: 2, 25; $P = 0.027$; inoculated vs. (mocks + controls): $P = 0.041$; mocks vs. control: $P = 0.065$). The mean concentrations of lignin from all trees pooled over plots and all three dates were inoculated, 7.92 ± 1.29 mg/g FW; mock-inoculated, 9.66 ± 1.39 mg/g FW; controls, 10.49 ± 2.37 mg/g FW (differences not significant).

There was an apparent negative association between lignin content and ferulic acid glucoside concentration up the stem, so a partial correlation controlling for plot and treatment was carried out between these two variables averaged for each tree over the last three dates. The correlation between lignin and ferulic acid glucoside was negative and significant ($r = -0.30$, $P = 0.021$, $N = 56$).

Phloem Samples from Base of Trees (September 1999). Differences between treatments in ferulic acid glucoside at the base were not significant. The mean concentrations of ferulic acid glucoside from all trees pooled over plots were inoculated, 372 ± 55 μg/g FW; mock-inoculated, 253 ± 49 μg/g FW; controls, 303 ± 58 μg/g FW. Similarly, effects of treatment on lignin content at the base were not significant.

Effects of Lignin on in Vitro Growth Rates of C. brevicomi and O. minus. Soluble lignin had a significant negative effect on the growth rates of the two bark beetle-associated fungi (Figure 3, shows growth rates at 6 days). Assuming that 1 ml of lignin-amended MEA is equivalent to 1 g phloem FW, and based on the regression functions, it was found that a reduction in lignin concentration from 10.07 to 7.92 mg/g FW [corresponding to the observed decrease in the phloem of inoculated trees compared with that in control trees (mean of mock-inoculated and control trees)], results in 12% and 25.9% increases (at 6 days), in *in vitro* growth rates of *C. brevicomi* and *O. minus*, respectively. Similar growth rate changes were also observed for the other test dates (data not shown).

DISCUSSION

The activity of a pathogen can result in increased susceptibility to other pathogenic and nonpathogenic microbes, presumably because of weakened host

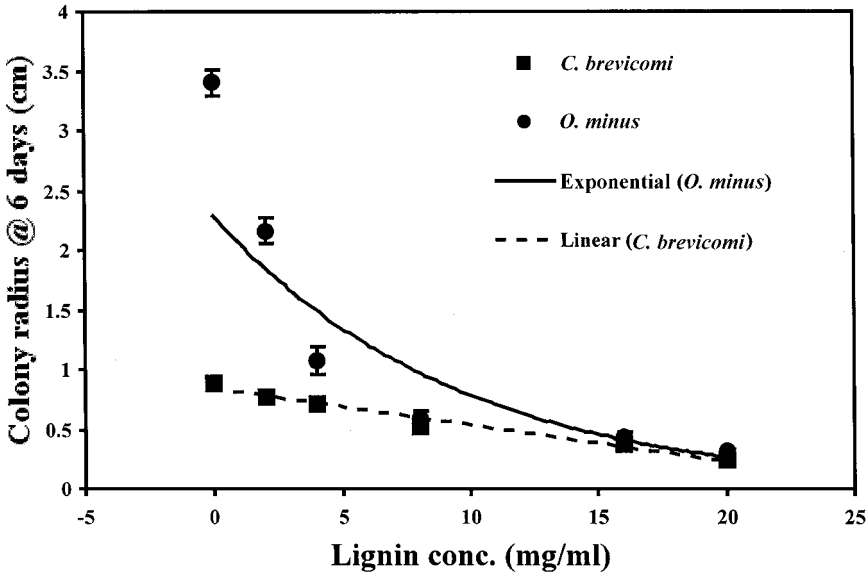

FIG. 3. Negative effect of lignin on *in vitro* growth rates of *Ceratocystiopsis brevicomi* and *Ophiostoma minus* at 6 days. Bars are SE and are not visible when smaller than the symbol. Linear and exponential regression equations fitting growth rates at 6 days for *C. brevicomi* and *O. minus* were $y = -0.03x + 0.84 (R^2 = 0.97, P < 0.001)$ and $y = 2.29\ e^{-0.107x} (R^2 = 0.87, P < 0.01)$, respectively (where "$x$" is lignin concentration and "y" is colony radius).

defenses. For example, establishment of the root pathogen *Armillaria* spp. on various conifers often follows attacks by other root, foliar, or stem pathogens (Wargo and Harrington, 1991). Successful attacks by most bark beetle species appear mediated, in part, by certain fungi that these insects vector (Whitney, 1982), such as species in the ophiostomatoid ascomycete genera *Ceratocystis*, *Ceratocystiopsis*, and *Ophiostoma*, and at least one species from the genus *Leptographium* (i.e., *L. terebrantis* Barras & Perris) (Owen et al., 1987; Klepzig et al., 1995b). Some of these fungi are capable of killing their hosts independently of their vectors (Owen et al., 1987; Parmeter et al., 1989; Harrington, 1993; Krokene and Solheim, 1998), while others are mycangial fungi that appear to confer a direct fitness advantage to the beetles without being injurious to the host tree (Barras, 1973; Bridges, 1983; Six and Paine, 1998). Consequently, one mechanism by which root disease affects bark beetle populations may be through increased susceptibility of the host to invasion by bark beetle-vectored fungal symbionts and plant pathogens.

Despite this evidence, the physiological and biochemical mechanisms underlying these associations in coniferous hosts are imperfectly understood, particularly

with respect to systemic effects. We now have evidence, for the first time in a conifer, of sustained systemic induction over a period of 2 years of ferulic acid glucoside accumulation in the phloem of presymptomatic trees inoculated with the root pathogen *H. annosum*. The concentrations of ferulic acid glucoside proved to be elevated despite the fact that inoculated trees appeared to have effectively contained the pathogen (Figure 1).

Systemic ferulic acid glucoside accumulation in the phloem was mirrored by an overall average decrease in lignin content in the same trees. Lignin content of inoculated trees pooled over all dates was 21.4% lower than in the mock-inoculated and control trees combined. Correlative analysis showed an inverse relationship between ferulic acid glucoside and lignin concentrations. The mean reduction in stem lignin content was not significant, in part due to high variability within and between dates and treatments. However, this result, when coupled with the negative correlations between the concentrations of ferulic acid glucoside and lignin, may be biologically meaningful because ferulic acid glucoside is an indirect precursor in lignin biosynthesis (Strack, 1997). Glucosides are thought to be storable and transportable forms of otherwise reactive molecules, and ferulic acid glucoside is such an example. Following a hydrolytic reaction, the aglycone, ferulic acid, is available for further processing in the lignin biosynthetic pathway, which proceeds through enzymatic transformation of ferulic acid to coniferyl alcohol and eventual incorporation into the lignin polymer via condensation reactions with other precursors (Strack, 1997). Ferulic acid also functions as a major bridge in anchoring the lignin polymers to the carbohydrate constituents of the cell walls (Strack, 1997). We hypothesize that accumulation of ferulic acid glucoside in the phloem of inoculated trees indicates that this lignin precursor is diverted from lignin synthesis in noninfected areas, i.e., it is stored and transported to the infection courts to contribute to the localized, pathogen-induced lignification response. This hypothesis aims to explain the apparent contradiction between accumulation of this compound and concurrent reduction of lignin content in the same tissues, but would require tracer studies to be confirmed. Interestingly, systemic stresses like air pollution (e.g., ozone exposure) have also been shown to bring about a reduction in lignification rates and a concurrent increase in susceptibility to disease and bark beetle attacks in conifers (Stark et al., 1968; Bonello et al., 1993; Pearce, 1996), perhaps pointing to a generalized mechanism of stress-induced weakening of tree defenses.

Pathogen-induced systemic accumulation of ferulic acid glucoside and concurrent reduction in lignification may directly affect the success of bark beetle attacks on diseased trees. Preliminary evidence suggests that *Ips paraconfusus*, an important tree-killing bark beetle of pines, is not affected in its laboratory feeding behavior when fed a wide range of ferulic acid glucoside concentrations in an artificial diet (McNee, 2000). However, similar information on the effects of reduced lignification on *I. paraconfusus* feeding behavior is not presently available.

In addition to a possible effect of decreased lignification on adult beetle feeding, other indirect mechanisms may also render infected trees more susceptible to successful bark beetle establishment. Lignification of cell walls is one of the main disease resistance mechanisms in plants (Vance et al., 1980; Pearce, 1996b). However, the effect of systemic reduction in lignin content of inoculated ponderosa pine phloem on bark beetle—associated fungi remains untested. Our *in vitro* growth studies suggest that such reduction may contribute to faster growth of these fungi in root- and butt-infected trees (particularly *O. minus*, whose growth rate was 26% higher at the lower end of mean lignin concentration observed in the inoculated trees). However, this result has to be interpreted cautiously, because (1) the difference in lignification rates was not significant and (2) it does not provide information about the localized induced lignification response that would result from inoculation of pine stems with the ophiostomatoid fungi in the presence of root and butt infections with *H. annosum*. Indeed, it is possible that infection of pine phloem in the presence of elevated precursor concentrations observed in root- and butt-infected trees could result in higher lignification rates in response to infection by fungi such as *C. brevicomi* and *O. minus*. Furthermore, our tests with solubilized lignin may not accurately reflect the role of cell wall-bound lignin. Thus, the effect of root and butt rot fungi on the success of bark beetle colonization via a reduction in the host lignin deposition response remains to be clarified.

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