Role of Flavanolic Wall Infusions in the Resistance Induced by *Laccaria bicolor* to *Fusarium oxysporum* in Primary Roots of Douglas-fir

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ABSTRACT

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When primary roots of newly germinated seedlings of Douglas-fir (*Pseudotsuga menziesii*) were inoculated with *Laccaria bicolor*, yellow-to-brown infusions were detected in many cortical cell walls beginning 13 days after initial contact with the fungus. Control roots developed only faint pigmentation in a few cortical walls. When control roots were challenged with a nonaggressive isolate of *Fusarium oxysporum*, the pathogen colonized intercellular spaces of the cortex and then usually penetrated cell walls to initiate intracellular colonization and lesion formation. Control roots that resisted *F. oxysporum* developed intense brown infusions in cortical walls adjacent to intercellular hyphae. Histochemical tests indicated that these wall infusions were phenolic and were probably derived from flavanols. Peroxidase activity, independent of exogenous H_2O_2, was also detected in walls undergoing infusion. The pathogen also colonized roots exposed to *L. bicolor*, but intracellular colonization and lesion formation usually were prevented. The pathogen was restricted to intercellular spaces in protected roots. This restriction was associated with the stimulation of flavanolic wall infusion by *L. bicolor*. When in one test *L. bicolor* did not stimulate wall infusion, it also did not induce resistance to *F. oxysporum*. Flavanolic wall infusions also were associated with resistance of intact root tips to degradation by cellulase. Thus, in this system, the prevention of lesion formation in living roots appeared to be a consequence of flavanolic wall infusions, induced by *L. bicolor*, which restricted *F. oxysporum* to intercellular spaces by inhibiting degradation of host cell walls. Variable stimulation of flavanolic wall infusion may contribute to inconsistent root protection by *L. bicolor*.

Additional keyword: ectomycorrhizal fungus.

The ectomycorrhizal fungus, *Laccaria bicolor* (Maire) Orton (syn. *Laccaria laccata* (Scopoli *ex* Fries) Berkeley *et* Broome [42]), can enter into an early close association with primary roots of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (8) and induce resistance to the root-rotting fungus, *Fusarium oxysporum* Schlecht. emend. Snyd. and Hans., before the formation of mycorrhizae. *L. bicolor* has shown promise as a biological control for root diseases incited by *F. oxysporum* in both field (28) and controlled environments (29,30,39), but root protection has been variable in both incidence and extent. Thus, research was undertaken to identify possible sources of variation in and the mechanism of root protection. Root protection was found to be influenced by temperature during challenge and aggressiveness of pathogen isolate (36). A high degree of root protection (prevention of lesion formation) was obtained only against a relatively nonaggressive isolate of *F. oxysporum* at a temperature below the optimum for disease development (36).

Ectomycorrhizal fungi elicit in plant roots the accumulation of phenolic materials that may enhance resistance to root-pathogenic fungi (9–11), but the precise role of these phenolics in root protection has not been extensively investigated. Sylvia and Sinclair (39) found that exposure of primary roots of Douglas-fir to *L. bicolor* did not inhibit subsequent external colonization by *F. oxysporum*, but did induce resistance to internal colonization by the pathogen. These authors observed that *L. bicolor* stimulated the accumulation of condensed tannins within cortical cells; the authors proposed that these phenolics were the basis of root protection (39).

Our studies (33,34) indicated that the amounts of extractable *o*-dihydroxyphenols and proanthocyanidins present in roots before challenge with *F. oxysporum* are not directly related to

resistance induced by *L. bicolor*. Rather, the association of cortical wall pigmentation with the restriction of *F. oxysporum* to intercellular spaces in roots that had resisted the pathogen, and the greater intensity of wall pigmentation in roots exposed to *L. bicolor* before *F. oxysporum* than in roots not exposed (36), indicated that wall pigments might play a role in resistance. In this paper, we report on the inducibility by *L. bicolor* and *F. oxysporum*, and the chemical nature of wall pigmentation in primary roots of Douglas-fir. We relate enhancement by *L. bicolor* of cortical wall pigmentation to the induced resistance of the roots to *F. oxysporum*. The influence of pigmentation on the enzymatic digestibility of cortical walls also is examined. An abstract of this work has been published (35).

MATERIALS AND METHODS

Fungal cultures, plant growth, and challenge with *F. oxysporum*. The materials and methods used in this study are as previously described (36). Plants were grown axenically for 7 days in petri dishes in which roots were elongated through a nutrient-agar slurry amended or not with living hyphal fragments of a mycorrhizal isolate of *L. bicolor* (813). Roots were transferred to moist chambers for challenge with a nonaggressive isolate of *F. oxysporum* (1064) for 13 days. Growth and challenge of roots with the pathogen were conducted at 17 C. Roots were inoculated with *F. oxysporum* by placing a plug of potato-dextrose agar (PDA), colonized by the pathogen, 3–5 mm to one side and 5 mm proximal to the root tip. Controls received a fungus-free plug of PDA. Lesion formation in primary roots of Douglas-fir by isolate 1064 of *F. oxysporum* was previously shown to be inhibited by isolate 813 of *L. bicolor* in this system (36).

Histochemistry and observation of internal colonization by *F. oxysporum*. For observation of phenolics, roots were fixed at 4 C in 50 mM sodium cacodylate buffer, pH 6.8, containing 1% (v/v) glutaraldehyde and 0.5% (w/v) caffeine to prevent the leaching of phenolics (22). Sections of tissues frozen in an embedding matrix (M-1; Lipshaw Manufacturing Corp., Detroit, MI) were cut to 30 μm thickness with a freezing-stage microtome and transferred to 0.1–0.2 ml of distilled water. Within 2–5 min, sections were treated with histochemical reagents for the localization of phenolics (Table 1). After 3–5 min (10 min for sections subjected to the butanol-HCl reagent), sections were mounted directly in the staining reagent and examined by light microscopy. Autofluorescence of unstained sections in distilled water was evaluated with an epifluorescence microscope fitted with an exciter filter that allowed peak transmission at 360 nm (long-wave cutoff at 400 nm) and a barrier filter with a short-wave cutoff at 420 nm.

Peroxidase (EC 1.11.1.1.7) activity in fresh sections was localized with 3,3′,5,5′-tetramethylbenzidine (TMB; [16]). Sections cut to 30 μm thickness with a freezing-stage microtome were incubated for 10 min in 0.1 mM TMB in 50 mM sodium acetate buffer, pH 4.5. They then were mounted directly in this medium and examined by light microscopy. No exogenous hydrogen peroxide was required for this reaction.

TABLE 1. Histochemical staining characteristics of wall infusions induced by *Laccaria bicolor* and *Fusarium oxysporum* in the cortex of primary roots of Douglas-fir

Reagent	Reference[a]	Detects	Results[b]
Sudan black B	2	Suberin	−
Phloroglucinol-HCl	23	Lignin	−
Nitroso	17	*o*-Dihydroxy phenolics	+
Vanillin-HCl	13	Flavanols	+
Dimethoxybenzaldehyde-HCl	20	Flavanols	+
Butanol-HCl	37	Proanthocyanidins	−
HCl	27	Dihydrochalcones, anthocyanins	−

[a] See Literature Cited section.
[b] − Denotes no reaction; + denotes positive reaction (reddish coloration).

Influence of *L. bicolor* on wall infusions and colonization by *F. oxysporum*. To find out whether prior exposure to *L. bicolor* affected flavanolic cortical wall infusions in a manner consistent with root protection, plants were exposed to *L. bicolor* and subsequently challenged with *F. oxysporum*. Some roots were harvested 6 days after the beginning of the challenge period for assessment of cortical wall infusions, whereas other roots were allowed to interact with *F. oxysporum* for 13 days to assess the influence of prior exposure to *L. bicolor* on colonization by *F. oxysporum*. Wall infusions in sections cut from the site of inoculation with *F. oxysporum* and stained with dimethoxybenzaldehyde (DMB)-HCl (to enhance visualization of infusions) were evaluated on a scale of 0–3, in which a score of 0 indicated no visible infusion and a score of 3 indicated intense wall color. Infusions were scored in both the outer cell layers (persistent root-cap cells, rhizodermis, and outermost layer of cortical cells) and inner layers (the remaining two to three cell layers) of the cortex. A minimum of three samples per replicate and four to 10 replicates per treatment were observed in each of three trials.

The extent of internal colonization by *F. oxysporum* at 13 days after inoculation was determined in all remaining inoculated roots (eight to 15 replicates per treatment in each of three trials). Microscopic observations were done on transverse, 30 μm sections cut from successive 4- to 5-mm segments beginning at the root cap until sections free of internal colonization were found. At least five sections per segment were examined for the presence of hyphae in inter- and intracellular locations. The patterns of root colonization by *L. bicolor* and *F. oxysporum* in this system are described in a companion paper (36). Because of the infrequent and sparse internal colonization of roots by *L. bicolor*, internal hyphae were ascribed to *F. oxysporum*.

Results of trials A and B were similar. Data of these trials were pooled to improve cell sizes for chi-square analysis of lesion incidence data, and because variances of wall infusion rating means were homogeneous for the two trials. Results of trial C were dissimilar, and these data are presented separately.

Enzymatic digestibility of roots. Whole roots were incubated in 4 ml of a 1:19 aqueous dilution of a liquid cellulase (EC 3.2.1.4) preparation, derived from *Trichoderma reesei* Simmons (GC105L, Genencor, South San Francisco, CA), in 13- × 100-mm glass tubes for 24 hr at 22 C. Each root was then examined visually, within the tube, for physical evidence of tissue degradation. Degradation was readily detected as a collapse of tissue, manifest as a "pinched" appearance, which sometimes led to complete separation of root tips from shanks in a region 2–5 mm proximal to the root cap.

Effect of simulated wall infusions on resistance to *F. oxysporum*. Simulated wall infusions were produced by immersing roots of intact plants in filter-sterilized, 1 mM aqueous catechin solution for 1 hr. Control roots were immersed in sterile distilled water. After the first immersion, roots were soaked for a second hour in sterile distilled water to remove catechin that had not become bound to the root. Wall infusions also were produced by allowing seedling roots to grow for 1 wk in a standard nutrient-agar slurry amended with filter-sterilized catechin to give a final concentration of 1 mM and a pH of 5.5. Roots were subsequently challenged with *F. oxysporum*.

RESULTS

Influence of *L. bicolor* on physical properties and colonization of roots by *F. oxysporum*. Nearly all roots inoculated with *F. oxysporum* developed superficial light tan-to-brown discoloration during the 13-day challenge period, but this became more pronounced if roots had previously been exposed to *L. bicolor*. Roots examined microscopically after challenge with *F. oxysporum* for 13 days fell into two groups: those with limited intercellular colonization of living cortex by the pathogen (no intracellular colonization or lesions) and those with extensive inter- and intracellular colonization throughout the cortex and stele (lesions). In lesions, cortical cell protoplasts were disrupted (cytoplasmic materials absent or aggregated and browned) and the walls of

stelar parenchyma cells were extensively digested. The incidence of intracellular colonization and lesion formation was lower (Table 2; $P \leq 0.05$ by chi-square analysis) where roots had been exposed to *L. bicolor* before challenge with *F. oxysporum* than in roots not exposed to *L. bicolor*. In roots challenged with *F. oxysporum* but lacking lesions, cortical cell walls adjacent to intercellular spaces containing hyphae of *F. oxysporum*, and occasionally walls one to two cell diameters away, were infused with a tan-to-brown pigment that had the histochemical properties summarized in Table 1.

Chemical nature and timing of the wall pigmentation in relation to colonization by *F. oxysporum*. Intercellular colonization of the outer cortex by *F. oxysporum* was detected in many root tips 6 days after inoculation, whereas intracellular colonization was rarely observed at this time. Cell walls adjacent to intercellular hyphae of *F. oxysporum* were infused with a yellow-to-tan or brown pigment (Fig. 1A). Observations made on other roots 3–10 days after inoculation with *F. oxysporum* alone suggested that the observed range in pigmentation indicated stages of wall infusion: yellow represented the early stage of infusion and brown represented the latest stage of infusion. Intensity of staining with DMB-HCl also increased with time after inoculation. Walls adjacent to *F. oxysporum* also reacted strongly with TMB (Fig. 1B), indicating peroxidase activity (16). Other cortical walls usually were free of pigmentation and usually did not react with TMB, although pigmentation and reaction with TMB were occasionally detected in walls one or two cells distant from hyphae of *F. oxysporum*. Cortical cell walls in lesions (13 days after inoculation with *F. oxysporum*) did not react with TMB. Positive reaction with TMB, independent of inoculation with *F. oxysporum*, was detected in walls of immature xylem vessels, in the persistent root-cap and underlying rhizodermal cells (5) and in cortical walls of older tissues (> 15 mm proximal to the root cap).

No reaction with phloroglucinol-HCl or with Sudan black B was observed in cortical walls at any stage of infusion (Table 1). Cortical cell walls did not display autofluorescence typical of lignin or suberin, although a pale green autofluorescence, uniformly distributed in these walls, sometimes was observed in immature tissues regardless of treatment with *F. oxysporum*. These tests did indicate the expected presence of lignin in xylem vessels and of suberin in the endodermis. Cell walls in the later stages of pigmentation (tan-to-brown) gave a red product with a nitroso reagent, indicating the presence of *o*-dihydroxy phenolics (32), and with vanillin-HCl and DMB-HCl (Fig. 2). The color of wall pigments was unaltered by exposure to HCl alone, indicating the absence of anthocyanins and dihydrochalcones, which can give a false positive with the acid component of vanillin-HCl and DMB-HCl (27). Thus, positive reaction with these reagents indicated the presence of flavanols (27). Walls in the early stages of infusion (yellow) gave no reaction with any of the histochemical reagents. Proanthocyanidins (polymeric flavanols or condensed tannins with molecular configurations that yield anthocyanins upon treatment with hot butanol-HCl [31]) were present in granular bodies within the cytoplasm but were not detected in cortical cell walls.

Influence of *L. bicolor* on wall infusions and colonization by *F. oxysporum*. Treatment with *L. bicolor* induced wall infusions

TABLE 2. Influence of *Laccaria bicolor* on cortical wall infusion and on the incidence of intracellular colonization by *Fusarium oxysporum*[a]

L. bicolor[b]	*F. oxysporum*[b]	Wall infusion rating[c] Outer cortex	Wall infusion rating[c] Inner cortex	Intracellular colonization[d]
Trials A and B				
−	−	1.5 ± 0.2	1.0 ± 0.2	···
+	−	2.5 ± 0.2	2.0 ± 0.2	···
−	+	1.8 ± 0.1	0.7 ± 0.2	15/23
+	+	2.7 ± 0.1	2.5 ± 0.2	3/22
		L[e]	L × F[e]	L[e]
Trial C				
−	−	1.5 ± 0.3	0.7 ± 0.3	···
+	−	1.2 ± 0.2	0.7 ± 0.3	···
−	+	1.4 ± 0.2	0.7 ± 0.1	5/8
+	+	2.0 ± 0.3	1.2 ± 0.3	3/8
		N[e]	N	N

[a] Seedlings were grown in petri-dish culture for 1 wk with roots unexposed to *L. bicolor* or to 1 g of moist, living mycelium of *L. bicolor* per 100 ml of nutrient-agar slurry before challenge in moist chambers with *F. oxysporum* inoculated to the root tip.

[b] − Indicates no exposure to *L. bicolor* or *F. oxysporum*; + indicates exposure to *L. bicolor* or *F. oxysporum*.

[c] Six days after inoculation with *F. oxysporum* at 17 C, tissues adjacent to the inoculum were fixed, sectioned, stained with dimethoxybenzaldehyde-HCl to detect flavanols, and the intensity of the staining reaction within cortical cell walls was rated on a scale of 0 (no infusion)–3 (intense infusion). Numbers of replicates ranged from four to 10 per treatment. Results in trials A and B were similar and homogeneity of variances permitted pooling of data for these trials. Results of trial C were dissimilar and are presented separately.

[d] Thirteen days after inoculation, roots were fixed, sectioned beginning at the root tip, and the sections were examined for the presence of intracellular hyphae of *F. oxysporum*. Fractions are the numbers of roots colonized over the numbers inoculated. Numbers of replicates ranged from eight to 15 per treatment. Results in trials A and B were similar, and incidence data were pooled to obtain sufficient cell sizes for chi-square analysis.

[e] Denotes significant ($P \leq 0.05$) effect of factor indicated (analysis of variance or chi-square test). N = No significant effect; L = *L. bicolor*; and F = *F. oxysporum*.

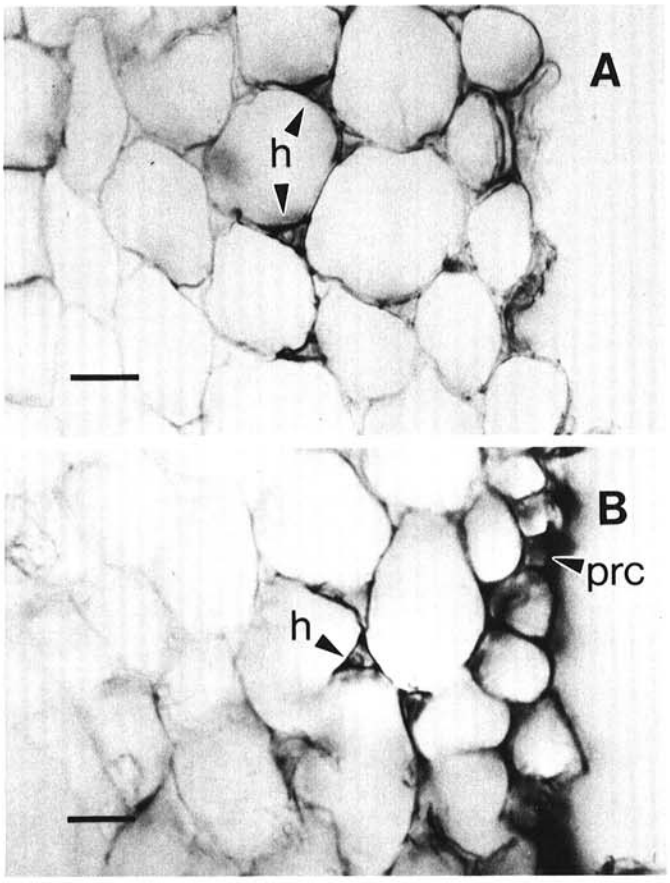

Fig. 1. Wall infusions and peroxidase activity induced by *Fusarium oxysporum*. The figures represent transverse sections from fresh tissues of apparently healthy roots that had been challenged with *F. oxysporum* for 9–10 days. Bars = 40 μm. **A,** Unstained section. Cortical cell walls adjacent to intercellular spaces containing hyphae (h) of the pathogen are pigmented (brown, seen as dark gray to black in this figure). This is representative of the most intense pigmentation in response to *F. oxysporum* seen at this time. **B,** Section incubated with TMB for the detection of peroxidase activity. Positive reaction (green) has occurred in cortical cell walls adjacent to intercellular spaces containing hyphae (h) of *F. oxysporum*, and in walls and cytoplasm of persistent root-cap (prc) and rhizodermal (rh) cells.

and protected against lesion development by *F. oxysporum* in two of the three trials (Table 2). In these trials, *L. bicolor* alone induced intense flavanolic wall infusions within 13 days. Only faint, scattered infusions were detected in control roots. Intercellular colonization by *L. bicolor* at this time was rare and limited to the outer cortex. *L. bicolor* did not colonize roots in the intracellular region. Infusions in the inner cortex were most intense in roots treated with *L. bicolor* (Table 2; Fig. 2). *F. oxysporum* alone usually had little effect on overall wall infusion scores, although intense infusion was occasional in the outer cortex. Intense wall infusions in roots treated with *L. bicolor* and challenged with *F. oxysporum* were associated with a significantly ($P \leq 0.05$; chi-square analysis) lower incidence of lesions (three of 22 roots diseased in trials A and B combined) than developed in roots exposed to *F. oxysporum* alone (15 of 23 roots). Lesion length was unaffected by *L. bicolor*.

The intensity of wall infusions induced by *L. bicolor* and *F. oxysporum* varied within treatments and between experiments. In trials A and B, infusion ratings of outer cortical cell walls ranged from 1 to 3 in roots inoculated with *L. bicolor* alone, and from 2 to 3 in double-inoculated roots. In trial C, the corresponding ranges were 1–2 and 1–3. In trial C, *L. bicolor* did not significantly stimulate wall infusion, and did not protect roots from *F. oxysporum*, influencing neither lesion incidence (Table 2) nor length (data not presented). The pathogen also did not significantly influence wall infusion ratings in this experiment.

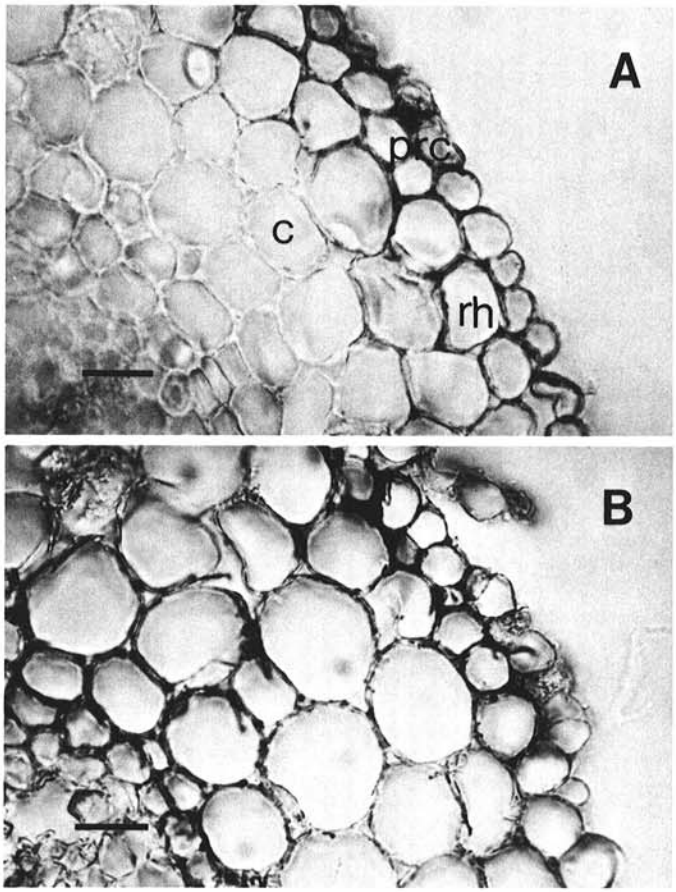

Fig. 2. Wall infusions induced differentially by *Fusarium oxysporum* and *Laccaria bicolor*. The figures represent transverse sections from fresh tissues of apparently healthy roots that had been challenged with *F. oxysporum* for 6 days, then sectioned and stained with DMB-HCl for detection of flavanols. Bars = 20 μm. **A,** Section of root not exposed to *L. bicolor* before challenge with *F. oxysporum*. A positive staining reaction (reddish brown, seen as dark gray in this figure) indicates the presence of flavanols in walls of persistent root-cap (prc), rhizodermal (rh), and outer cortical (c) cells. The section is representative of the most intense wall infusion seen in response to *F. oxysporum* alone at 6 days postinoculation. **B,** Root grown with *L. bicolor* for 7 days, then challenged with *F. oxysporum* for 6 days. Staining of cell walls extends throughout the cortex.

Influence of wall infusions on enzymatic digestibility of root tissues. Commercial cellulase caused degradation of tissues in a region 2–5 mm behind the cap of approximately 50% of the roots tested. This degradation caused collapse of the affected region or removal of the root tip. Older cortical tissues remained intact and developed flavanolic wall infusions during enzyme treatment. Similar infusions also developed in cortical walls of the tip region of the roots that were not degraded during enzyme treatment. When living roots were incubated in cellulase plus 10 mM ascorbate (pH 5.5), an antioxidant that inhibited the development of wall infusions, the proportion of root tips degraded by the enzyme was significantly increased (data not shown). Ascorbate did not enhance the digestibility of older cortical tissues, which resisted degradation by this cellulase in the presence or absence of flavanolic wall infusions. Walls of stelar parenchyma cells, which never developed flavanolic wall infusions, were readily degraded by the enzyme, even in segments cut from more mature tissue, 15–20 mm from the root tip.

Exposure to *L. bicolor* did not affect digestibility of root tips by cellulase in the 7-day growth phase, during which flavanolic wall infusions did not develop. Similarly, infusion of cell walls at the root surface with flavanolic polymer (by immersion for 1 hr in 1 mM catechin) did not influence digestibility.

Effect of simulated wall infusions on resistance to *F. oxysporum*. Treatment of roots with catechin by either of two methods resulted in wall infusions in persistent root-cap and rhizodermal cells, but not in the inner cortex. These infusions had staining properties similar to infusions induced by *L. bicolor*, but they did not enhance cortical resistance to *F. oxysporum*.

DISCUSSION

Pigmented infusions were observed in the walls of cortical cells adjacent to hyphae of *F. oxysporum* in intercellular spaces, and were more generally distributed in the cortex of roots exposed to *L. bicolor* or to cellulase from *T. reesei*. We also have observed pigmented infusions in primary roots of Douglas-fir colonized in the intercellular region by a contaminating *Rhizopus* sp., and in roots inoculated with *Pseudomonas cepacia* (N. E. Strobel and W. A. Sinclair, *unpublished results*). *Trichoderma harzianum* and *P. cepacia* previously were reported to induce phenolic accumulations and resistance to *F. oxysporum* in Douglas-fir roots (39). Thus, cortical wall infusion with pigmented materials appears to represent a nonspecific response of Douglas-fir primary roots to diverse microorganisms and their metabolites.

Results of histochemical tests indicated that the cortical wall pigments formed in response to *F. oxysporum* and other organisms may have been derived by the action of peroxidase on flavanols in a process analogous to lignification. Stafford (31) has suggested that the occurrence of flavanols or condensed tannins in cell walls results passively from the loss of integrity associated with cell aging or death. Flavanolic wall infusion may also be an active, coordinated response (21) of living cells to stress in the cortex of Douglas-fir primary roots. Flavanolic infusion was, in many cases, highly localized in portions of cell walls adjacent to fungal hyphae. Positive reaction with TMB, obtained without exogenous hydrogen peroxide, was indicative of the endogenous generation of hydrogen peroxide at infusion sites. Although the relationship of nuclear condition to wall infusion status was not specifically studied, intact nuclei were observed in cells with flavanolic wall infusions. Constitutive peroxidase activity was detected in cell walls of the rhizodermis, a tissue analogous to the epidermis of roots of other plants (5). Constitutive peroxidase activity was reported in root epidermal cell walls of *Phaseolus vulgaris* (1).

Phenolic infusions were formed at a suitable time and place to have played a role in induced resistance to *F. oxysporum*. Exposure of roots to *L. bicolor* before challenge with *F. oxysporum* stimulated wall infusion during the first 6 days of the challenge period and enhanced resistance. When *L. bicolor* did

not stimulate wall infusion (trial C), it also did not reduce the incidence of lesion formation by the pathogen. Failure of *L. bicolor* to stimulate wall infusion may have been attributable to reduced responsiveness of roots to elicitation, because *F. oxysporum* also did not stimulate wall infusion in this case. Stimulation of wall infusion may thus be an essential component of the resistance induced by *L. bicolor*. Variability in the incidence and/or extent of wall infusion induced by *L. bicolor* may account for the occurrence of intracellular colonization and unrestricted lesion expansion in a small proportion of roots exposed to *L. bicolor* before isolate 1064 of *F. oxysporum* at 17 C (in these tests and in ref. 36), and for the inconsistent root protection we have generally observed with *L. bicolor*.

Phenolic wall infusions were associated with the restriction of *F. oxysporum* to intercellular spaces in roots protected by *L. bicolor*. This restriction also occurred in the small proportion of roots not exposed to *L. bicolor* that nevertheless resisted *F. oxysporum*, probably as a consequence of greater expression of active defenses, including wall infusion, in these roots. Intercellular hyphae of *F. oxysporum*, never abundant in protected roots and observed only in transverse sections, may have gone undetected by Sylvia and Sinclair (39). Restriction of *F. oxysporum* to intercellular spaces in resistant roots may also account for the high frequency of isolation of *F. oxysporum* from apparently healthy Douglas-fir roots grown in a soil mixture nonconducive to root rot (4) and during temporary protection by *L. bicolor* in nursery tests (28). Phenolic modification of cortical cell walls was similarly associated with the restriction of *F. oxysporum* f. sp. *radicis-lycopersici* to intercellular spaces of tomato (6,7). Deposition of polyphenolic substances on the outer part of stelar parenchyma cell walls adjacent to hyphae of *Mycosphaerella fijiensis* Morelet growing within intercellular spaces of banana leaves was found to be more rapid and intense in a resistant than in a susceptible cultivar (26). Resistance to pathogens that colonize host tissues intercellularly prior to lesion development may thus depend in part on restriction of the pathogens to intercellular spaces as a consequence of phenolic wall modifications.

Flavanolic infusions induced by *L. bicolor* probably impair penetration of cortical cell walls by *F. oxysporum* by rendering the walls resistant to enzymatic degradation by the pathogen. Oxidized flavanols and condensed tannins have previously been reported to inhibit both the synthesis (40) and activity (9,15,24,44) of fungal hydrolytic enzymes. Degradation of the tips of intact roots by cellulase was enhanced by ascorbate, an inhibitor of wall infusion. Ascorbate in the challenge medium also greatly enhanced lesion formation by *F. oxysporum* in Douglas-fir (33). Because ascorbate was only mildly stimulatory to growth of *F. oxysporum* (33), inhibition of host resistance mechanisms likely accounted for the increased disease development. Flavanolic infusions induced in cortical cell walls by *P. cepacia*, in a wick-culture system based on that of Sylvia and Sinclair (38), also prevented degradation of root tips by pectinase (N. E. Strobel and W. A. Sinclair, *unpublished data*). Artificial phenolic infusions protected artificial and natural cell wall membranes from degradation by enzymatic and fungal attack (3). The failure of artificial wall infusions to protect roots from digestion by cellulase and colonization by *F. oxysporum* in the present study may have been due to the restriction of these artificial infusions to superficial cell layers. The contribution of infusions induced by *L. bicolor* to the resistance of cortical cell walls to enzymatic degradation could not be directly assessed by the methods employed in this study, because walls of the growing roots normally begin to undergo (unknown) maturational changes that render them incompletely digestible by cellulase and by *F. oxysporum* within 3–5 days after their formation at the root-tip meristem. A similar relationship of digestibility to cell age and state of differentiation was observed in roots of *Pinus resinosa* (12).

Chemical intermediates of the flavanolic wall infusion process also may have been inhibitory to development and pathogenesis by *F. oxysporum*. Phenoxy radicals and quinones generated by the action of peroxidase on phenolics (in the presence of hydrogen peroxide) have antimicrobial activity (41) and can bind covalently to proteins (18,43), often causing loss of enzyme activity (19). Peroxidase-generated reactive species and polymerized phenolics may act sequentially in the expression of resistance. By inhibiting pathogen growth and enzyme activity, peroxidase-generated reactive species may provide additional time for the accumulation of stable end-products, such as polymerized flavanols, that inhibit enzymatic digestion of host cell walls.

The timing and intensity of wall infusion with phenolics may be crucial determinants of resistance of Douglas-fir to *F. oxysporum*. Enhanced development of wall infusions was a prerequisite for root protection by *L. bicolor* in the present study. Ride (25) observed that although walls of wheat leaf cells developed some resistance to degradation by fungal enzymes after brief periods of induced lignification, this resistance increased with longer periods (24 hr). The rate and intensity of lignification are also thought to be important in the induced resistance of cucumber to *Colletotrichum lagenarium* (14). Roots that developed heavy wall infusions during a 4-wk preincubation with *L. bicolor* were completely resistant to *F. oxysporum* (39). The lesser degree of protection obtained by previous workers (28,30) may have been, in part, a consequence of limited exposure of roots to *L. bicolor* before encounter with *F. oxysporum*. Unfortunately, the highly specific nature of the requirements for this induced resistance (36) and variable expression of induced resistance mechanisms appear to preclude its practical exploitation.

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