Influence of Temperature and Pathogen Aggressiveness on Biological Control of Fusarium Root Rot by *Laccaria bicolor* in Douglas-fir

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ABSTRACT

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The biological control of Fusarium root rot of Douglas-fir seedlings by *Laccaria bicolor* was studied in vitro. Primary roots of Douglas-fir seedlings were exposed to *L. bicolor* for 1 wk and then inoculated with *Fusarium oxysporum*. Resistance sufficient to prevent lethal infection was expressed to a nonaggressive strain of *F. oxysporum* at 17 but not at 22 C. Resistance was not expressed against two aggressive strains of *F. oxysporum* at either temperature. The pathogen entered all inoculated roots but was restricted to cortical intercellular spaces in which effective resistance had been induced. Cortical cell walls with effective induced resistance contained tan-to-brown pigmentation, which darkened during challenge with *F. oxysporum*. The modest degree and conditional nature of the resistance induced by *L. bicolor* may preclude the practical application of this fungus as a biological control for Fusarium root rot in forest nurseries.

Additional keywords: ectomycorrhizal fungus, root protection.

In forest nurseries, *Fusarium oxysporum* Schlecht.:Fr. emend. Snyd. and Hans. causes pre- and postemergence damping-off, root rot, stunting, and death of first-year seedlings of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (1,16). Infections that occur on primary roots in the first month after sowing seed often are fatal (2). Bloomberg (3) inferred that the root tip is the principal site of infection of primary roots. Different isolates of *F. oxysporum* show specialization in the types of symptoms they incite, and they vary in aggressiveness (1).

Temperature governs the incidence and severity of root rot of conifer seedlings by *F. oxysporum* (2,22). Death of Douglas-fir seedlings due to *F. oxysporum* in greenhouse experiments was fourfold greater in temperature regimes exceeding 23 C for more than 6 hr/day than in regimes with cooler temperatures during the first month after sowing seed (2).

Some strains of the ectomycorrhizal fungus *Laccaria bicolor* (Maire) Orton (syn. *Laccaria laccata* (Scopoli *ex* Fries) Berkeley *et* Broome [25]) have given temporary or partial protection of primary roots of Douglas-fir from infection by *F. oxysporum* in the field (16) and in controlled environments (18,21), apparently by enhancing resistance to the pathogen (21). This resistance was expressed before or in the absence of mycorrhizal development. *L. laccata* (*L. bicolor*) also protected roots of *Pinus sylvestris* from damage by *Cylindrocarpon destructans*, *Fusarium moniliforme*, and *Rhizoctonia solani* (5–7), and roots of *Picea abies* from *F. oxysporum* (14). Additionally, *L. laccata* promoted growth and slightly suppressed root rot caused primarily by *F. oxysporum* in seedlings of Douglas-fir and Norway spruce grown in fumigated nursery beds (15). Protection of primary roots of *Pinus resinosa* from *F. oxysporum* by *Paxillus involutus* before mycorrhizal formation also has been reported (8).

Protection of Douglas-fir roots from *F. oxysporum* by *L. bicolor* has been variable and of limited duration, usually being expressed as a suppression or delay of seedling death (16–18). Sinclair et al (16) obtained root protection with low, but not high, inoculum densities of *F. oxysporum* in soil. Protection was consistent and effective in controlled environments in which roots were exposed to *L. bicolor* for 2–4 wk before challenge with *F. oxysporum* (21). This consistent root protection apparently resulted from a fortuitous combination of environmental and biotic factors, because root protection was erratic when conditions were varied in our preliminary experiments (*unpublished data*).

The present studies were undertaken to identify possible sources of variability in root protection by *L. bicolor*. Susceptibility of primary roots to infection by *F. oxysporum* and, presumably, root protection by *L. bicolor* are influenced by seedling age (2) and nitrogen supply (16). We hypothesized that susceptibility and root protection also may be conditioned by the stage and rate of primary root development, the specific tissues inoculated with *F. oxysporum*, temperature during challenge with *F. oxysporum*, and aggressiveness of the pathogen strain. Thus, we investigated

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the effects of temperature and aggressiveness of isolates of *F. oxysporum* on disease development and the induction of resistance by *L. bicolor* in vitro.

MATERIALS AND METHODS

Fungal cultures. Isolate 1064 of *F. oxysporum* from a diseased Douglas-fir seedling in a forest nursery was provided by W. J. Bloomberg of the Canadian Forestry Service, Victoria, BC. Isolates 3064 and 3074 of *F. oxysporum* from a diseased Douglas-fir seedling and from nursery soil, respectively, were provided by P. B. Hamm of Oregon State University, Corvallis. Stock cultures were grown on potato-dextrose agar (PDA) and stored as plugs from plate cultures in sterile distilled water at 2 C (11). The isolates were used to inoculate roots of Douglas-fir seedlings and reisolated from diseased roots every 6 mo to maintain pathogenicity. Preliminary tests in vitro indicated that these isolates varied in aggressiveness *sensu* Vanderplank (24), based on the incidence and size of lesions induced during a 13-day incubation period at 17 C. Isolate 3074 was most aggressive and 1064 the least.

Isolate 813 of *L. bicolor* was used. This isolate (derived from a collection of basidiocarps filed as *L. laccata* no. 53,000 in the Mycological Herbarium, Cornell University) is capable of colonizing radicles of Douglas-fir seedlings (4), inducing phenolic accumulations in Douglas-fir roots, protecting roots from *F. oxysporum*, and forming ectomycorrhizae (17,21). The fungus was last reisolated from a Douglas-fir ectomycorrhiza in 1984, 2–3 yr before the experiments described here.

Seed sources and germination. Seed of Douglas-fir from sources in the Pacific Northwest was provided by Weyerhauser Co. (Tacoma, WA) or purchased from F. W. Schumacher Co. (Sandwich, MA), and stored in sealed plastic bottles at <0 C. To produce axenic seedlings, seeds were immersed in 30% H_2O_2 for 2 hr, then transferred aseptically to petri dishes containing moist filter paper. After 5 days in a dark incubator at 22 C, early germlings (from 10–20% of the seeds) were transferred to fresh germination dishes to avoid fungal contaminants, which typically began emerging from internally infested seeds within 4 days, despite H_2O_2 treatment. Dishes were returned to the dark incubator for 2 more days, after which germlings with radicles 1–2 cm long were selected for experiments.

Plant growth and challenge with *F. oxysporum*. A petri-dish system in which roots of intact seedlings grew through a slurry composed of agar, nutrient salts, and distilled water was used to maintain gnotobiotic conditions (Fig. 1A). The slurry was prepared by blending to homogeneity equal volumes of 2% water agar and a nutrient salts solution used by Sylvia and Sinclair (20). Slurry (1.8–2.0 ml) was dispensed into 9-cm-diameter plastic petri dishes with a syringe, forming a truncated triangular lane, approximately 5 cm long and 0.2 cm thick, for root growth. The root tip of a sterile germling was inserted into the slurry near the center of the dish, and slurry and root were covered with a single 3.5- × 4.5-cm layer of sterile cheesecloth to hold them in place. Dish lids then were secured with paraffin film. Root-tip positions were marked on the dish bottom, and the dishes were incubated in a horizontal position in darkness for 7 days. Darkness was maintained, with the exception noted below, because roots that received prolonged exposure to light developed green plastid-like structures within cells of the pericycle, and, thus, it was thought that light might influence physiological processes relevant to root responses to fungi. Incubation was interrupted briefly after 3 and 6 days to mark root-tip positions and record root elongation. The period of culture in petri dishes hereafter is called the growth phase of an experiment.

For experiments in which the slurry was amended with *L. bicolor*, this fungus was cultured in 500-ml flasks containing 100 ml of modified Melin-Norkrans (MMN) broth (10) amended with 200 μg/ml each of biotin and thiamine after autoclaving. Cultures were incubated at 21–25 C on a shaker for 14–18 days. In some experiments, the MMN broth and vitamins were sterilized by filtration because the fungus produced more biomass in media not treated with steam. Mycelium of *L. bicolor* was collected aseptically and washed with distilled water by suction filtration. Moist mycelium was weighed and blended at low speed with 50 ml of nutrient salts solution (18) for 8 sec and then with 50 ml of solid 2% water agar for 20 sec to produce the slurry. Hyphal fragments of mycelia treated in this way retained viability, as indicated by subculture on solid or liquid MMN. Control roots

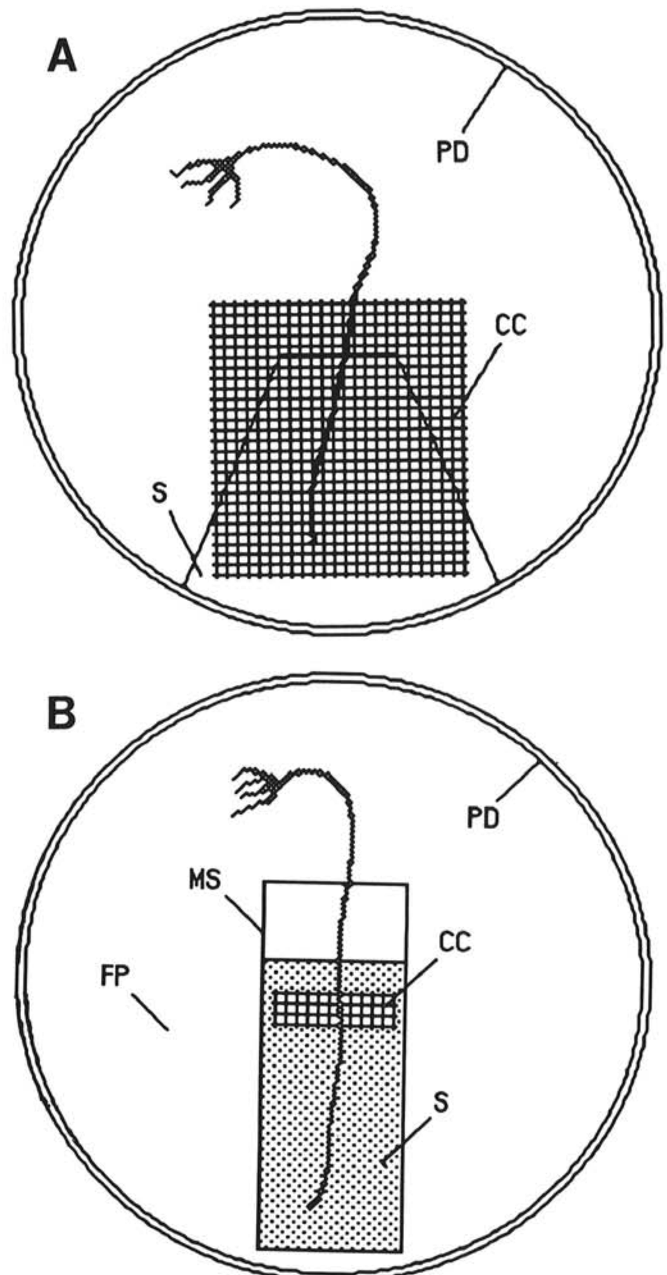

Fig. 1. Schematic representations of petri-dish systems used for initial growth of Douglas-fir seedlings (**A**) and incubation with *Fusarium oxysporum* (**B**). **A,** Growth unit composed of a 9-cm-diameter plastic petri dish (PD) containing 1.8–2 ml of a nutrient-agar slurry (S) in a lane from the center to one edge of the dish. The tip of a germling root was inserted into the slurry near the center of the dish, and root and slurry were covered with a single layer of cheesecloth (CC) to hold them in place and to constrain the root so that it would grow through the slurry. For some experiments, the slurry was amended with moist living hyphal fragments of *Laccaria bicolor*. **B,** Moist chamber for challenge of a seedling root with *F. oxysporum*. Chamber consists of a glass petri-dish (PD) containing moist filter paper (FP) and a glass microscope slide (MS) with its upper surface covered with nutrient-agar slurry (S). The primary root of an intact seedling from the growth phase was placed onto the slurry, held in place by a small piece of cheesecloth (CC), and inoculated with *F. oxysporum*.

were grown in slurry not amended with *L. bicolor*. After the growth phase, seedlings were transferred aseptically to moist chambers for inoculation with *F. oxysporum*. These chambers consisted of petri dishes, each with moist sterile filter paper and a glass microscope slide (Fig. 1B). Over two-thirds of the slide's upper surface was covered with 1 ml of agar-nutrient slurry on which the root of an intact germling was placed. To inoculate a root, a plug cut with a 14-gauge cannula from PDA (controls) or from the margin of a 3- to 5-day-old petri dish culture of *F. oxysporum* on PDA was placed 3–5 mm to one side of the root. Dish lids were secured with paraffin film, and dishes were incubated in darkness for 13 days.

Quantification and analysis of disease development. Roots were evaluated for disease incidence (+/−) and, in some cases, severity (length of lesions). In roots not previously treated with *L. bicolor*, lesions were apparent where tissues colonized by *F. oxysporum* collapsed and darkened to tan or light brown. In experiments in which roots were treated with *L. bicolor* before challenge with *F. oxysporum*, disease was evaluated microscopically as the incidence and linear extent of intracellular colonization by *F. oxysporum*. Microscopic evaluation was necessary because roots treated with *L. bicolor* and inoculated with *F. oxysporum* sometimes became brown, although not colonized intracellularly by the pathogen, or developed little tissue collapse despite intracellular colonization. Roots for this examination were fixed in 50 mM sodium cacodylate buffer, pH 6.8, containing 1% glutaraldehyde and 0.5% caffeine (w/v) to prevent leaching of phenolics (13). Successive segments (4–5 mm long) were placed in an embedding matrix (M-1; Lipshaw Manufacturing Corp., Detroit, MI), frozen, and sectioned with a freezing-stage microtome. A minimum of three sections per segment were observed to detect the presence or absence of intracellular hyphae of *F. oxysporum*. (Hyphae of *L. bicolor* were not found in intracellular locations.) Incidence data were subjected to Chi-square analysis, and severity data to analysis of variance with a procedure of SAS (Statistical Analysis Systems, Cary, NC) for linear models. Treatment effects with $P \leq 0.05$ were declared significant.

Susceptibility to *F. oxysporum* as related to stage of tissue development and rate of root elongation. The susceptibility of immature tip and maturing shank tissues of primary roots (5 or 15 mm proximal to the root cap, respectively) to isolates 1064 and 3074 of *F. oxysporum* at 17 C was investigated in an experiment with a completely randomized design and a minimum of nine and eight replicate plants per treatment in the first and second trials, respectively. Disease incidence data were pooled to improve cell sizes for Chi-square analysis. Roots of Douglas-fir germlings grew at rates of 0–5 mm/day during the 6-day growth period. Less than 20% of roots grew <2 mm/day, but preliminary observations indicated that these slowly growing roots might be unusually susceptible to *F. oxysporum*. Therefore, the relationship of root-elongation rate during the growth phase to susceptibility to *F. oxysporum* was investigated. Approximately equal numbers of slowly growing (≤10 mm in 6 days) and more rapidly growing (>10 mm in 6 days) roots were challenged with isolate 1064 of *F. oxysporum*, which was inoculated 5 mm proximal to the root tip at 17 C. A completely randomized design with 23 or 24 plants per treatment was used. Two trials were performed.

Influence of temperature and isolate of *F. oxysporum* on disease development. The combined influences of temperature and pathogen isolate on disease development were tested in a 2 × 2 × 4 factorial experiment arranged in a randomized complete block design. Temperatures were 17 or 22 C during the growth phase, followed by incubation at 17 or 22 C with isolates 1064, 3064, or 3074 of *F. oxysporum* inoculated 5 mm proximal to the root tip, or without the pathogen. These temperatures were selected because preliminary experiments indicated that *L. bicolor* was capable of protecting roots at 17 C, and that at 22 C it was highly conducive to disease development. Nine or more replicate plants per treatment were used in each of two trials that yielded similar results. Because no disease occurred in roots not inoculated with *F. oxysporum*, statistical analyses of disease incidence and severity were done only on data from roots inoculated with the pathogen. Data of the trials were pooled to improve cell sizes for Chi-square analysis, and because variances of lesion length means were homogeneous for the two trials. The influence of temperature on root elongation and increase in colony diameter of *F. oxysporum* on PDA were assessed separately.

Influence of temperature and isolate of *F. oxysporum* on root protection by *L. bicolor*. The influences of challenge temperature and pathogen isolate on disease development and root protection by *L. bicolor* were investigated in a 2 × 2 × 4 factorial experiment arranged in a randomized complete block design. Factors investigated were *L. bicolor* (absent or present at 1 g of moist hyphal fragments per 100 ml of root-growth medium), challenge temperature (17 or 22 C), and isolate of *F. oxysporum* (none, 1064, 3064, or 3074). Temperature during the growth phase was 17 C, and *F. oxysporum* was inoculated 5 mm proximal to the root tip. Because no disease occurred in roots not inoculated with *F. oxysporum*, statistical analyses of disease incidence and severity were done only on data from roots inoculated with the pathogen. The experiment was done twice, with a minimum of six and eight replicate plants per treatment in the first and second trials, respectively. Similar results were obtained in both trials. Data of the two trials were pooled to improve cell sizes for Chi-square analysis of disease incidence, and because variances of lesion length means were homogeneous for the two trials.

RESULTS

Susceptibility to *F. oxysporum* as related to stage of tissue development and rate of root elongation. Root tips were infected with comparable frequency (no significant difference by Chi-square analysis) by the two isolates (10 of 19 colonized by isolate 1064 and seven of 20 by isolate 3074). Shanks were resistant to 1064 (one of 20 colonized) but not to 3074 (six of 20 colonized). Root tips, because of their greater susceptibility, were selected as the site of inoculation for further studies.

Slowly growing roots developed lesions significantly more often than did more rapidly growing roots (Chi-square analysis, $P < 0.05$ in each of two trials). Lesions formed within the 13-day challenge period on 87% of roots that had elongated ≤10 mm, but on only 36% of roots that had elongated >10 mm during the 6-day growth period before challenge with *F. oxysporum*. Lesion lengths were not measured. Because of their atypically high degree of susceptibility to *F. oxysporum*, slowly growing roots were excluded from experiments dealing with root protection.

Influence of temperature and isolate of *F. oxysporum* on disease development. Temperature strongly influenced disease development. The incidence of root lesions caused by each isolate of *F. oxysporum* was significantly greater ($P < 0.01$) when incubation was at 22 rather than 17 C (Table 1). Growth temperature did not significantly influence disease incidence. Incubation temper-

TABLE 1. Influence of temperature and isolate of *Fusarium oxysporum* on the incidence of root lesions

Isolate of *F. oxysporum*	Lesion incidence[a] at temperature regimes (C)[b]			
	17–17	22–17	17–22	22–22
1064	8/20	12/20	19/19	20/20
3064	11/19	11/20	20/20	18/20
3074	17/20	14/20	19/19	20/20

[a] Lesion incidence was assessed visually. Fractions denote numbers of diseased roots over numbers inoculated. The experiment was performed twice with similar results; the data were pooled to improve cell sizes for Chi-square analysis. The incidence of colonization by each isolate was significantly greater ($P \leq 0.01$, by Chi-square analysis) at an incubation temperature (main effect) of 22 rather than of 17 C. Colonization by 3074 was significantly greater ($P \leq 0.03$, by Chi-square analysis) than by 1064 at 17 C.

[b] Growth temperature–incubation temperature. Seedlings were grown for 7 days at 17 or 22 C, then incubated for 13 days at either 17 or 22 C with *F. oxysporum* inoculated to the root tips. Isolate 1064 was previously selected as nonaggressive, 3064 as moderately aggressive, and 3074 as aggressive.

ature interacted significantly ($P = 0.01$) with growth temperature in its effect on disease severity (lesion length; Fig. 2). The lengths of lesions induced by all three isolates of *F. oxysporum* were greater at an incubation temperature of 22 C, and the increase in lesion length due to elevation of the incubation temperature was greater (2.7-fold) where roots had developed at 17 rather than 22 C (1.8-fold) during the growth phase. The effect of an elevated incubation temperature on disease development was most marked with the nonaggressive isolate (1064), with which disease incidence more than doubled and lesions were 2.5–4.7 times longer at 22 than at 17 C. Roots elongated 1.4 times more rapidly, and isolates of *F. oxysporum* grew 1.5 times more rapidly, on average, at 22 than at 17 C.

The three isolates of *F. oxysporum* differed significantly in the incidence and severity of disease they incited. Isolate 3074 caused lesions more frequently than did isolate 1064 at an incubation temperature of 17 C ($P < 0.01$ by Chi-square analysis) and caused longer lesions than did 1064 at either incubation temperature. These differences reflected variation in aggressiveness of the isolates. Isolate 3064 expressed intermediate aggressiveness and did not differ significantly from 1064 or 3074 in the incidence or severity of disease it induced.

Influence of temperature and isolate of *F. oxysporum* on root protection by *L. bicolor*. Disease development was influenced by pathogen isolate and challenge temperature. Significantly fewer lesions were induced by isolates 1064 and 3064 of *F. oxysporum* at 17 C, regardless of treatment with *L. bicolor* (Table 2). The incidence of lesions induced by isolate 3074 did not differ between the two challenge temperatures. Lesion lengths were greater with all isolates at 22 rather than at 17 C, regardless of treatment with *L. bicolor* (Fig. 3). This influence of challenge temperature was most marked for isolate 1064 (significant temperature × isolate interaction, $P = 0.001$).

Root protection by *L. bicolor* was affected by challenge temperature and aggressiveness of pathogen isolate. When challenged with isolate 1064 of *F. oxysporum* at 17 C, roots previously exposed to *L. bicolor* were less often colonized in the intracellular spaces (and, thus, developed lesions less frequently) than were roots not so exposed (Chi-square analysis; $P < 0.05$; Table 2). Treatment with *L. bicolor* did not affect the incidence of lesions caused by isolate 3064 or 3074 at either challenge temperature. Treatment with *L. bicolor* also resulted in a modest suppression, relative to untreated roots, of the expansion of lesions formed

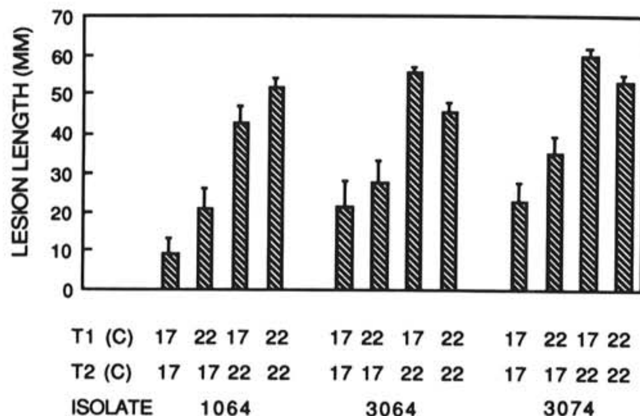

Fig. 2. Influence of temperature and of pathogen isolate on the length of lesions induced by *Fusarium oxysporum* on primary roots. Seedlings were grown in petri-dish culture at 17 or 22 C for 7 days (*T1*), then aseptically transferred to moist chambers for inoculation. Roots of intact seedlings were placed on glass slides coated with a nutrient-agar slurry and were inoculated with isolates 1064 (nonaggressive), 3064 (moderately aggressive), or 3074 (aggressive) of *F. oxysporum* at the root tip. Incubation was carried out for 13 days at either 17 or 22 C (*T2*). Means were calculated only with data for those roots that developed lesions. The experiment was done twice with similar results. Variances of lesion length means were homogeneous for the two trials, and pooled data are presented. Bars denote one-half the standard error of each mean. Analysis of variance indicated a significant interaction of growth and challenge temperatures ($P = 0.01$) and significant differences among isolates of *F. oxysporum* ($P = 0.001$). Temperature × isolate interactions were not statistically significant.

TABLE 2. Influence of challenge temperature, isolate of *Fusarium oxysporum*, and previous incubation with *Laccaria bicolor* on the incidence of root lesions[a]

Temperature	Isolate of *F. oxysporum*	*L. bicolor*[b]	Lesion incidence[c]
17 C	1064	−	11/18
		+	4/19[d]
	3064	−	11/19
		+	8/19
	3074	−	20/20
		+	16/17
22C	1064	−	20/20
		+	20/20
	3064	−	19/20
		+	15/16
	3074	−	20/20
		+	19/19

[a] Seedlings were grown at 17 C for 7 days in the absence or presence of *L. bicolor* incorporated as 1 g moist, living hyphal fragments per 100 ml of a nutrient-agar slurry through which roots elongated. Seedlings then were transferred to moist chambers for challenge with isolates 1064 (nonaggressive), 3064 (moderately aggressive), or 3074 (aggressive) of *F. oxysporum* at the root tip. Challenge was carried out for 13 days at either 17 or 22 C.

[b] − Indicates absence of *L. bicolor*; + indicates presence of *L. bicolor*.

[c] Incidence was assessed visually and microscopically on the basis of tissue collapse and intracellular colonization, respectively.The experiment was performed twice with similar results, and the data were pooled to improve cell sizes for Chi-square analysis. Fractions denote the number of diseased roots over the total inoculated.

[d] Significantly different ($P \leq 0.05$, by Chi-square analysis) from lesion incidence with 1064 at 17 C in the absence of *L. bicolor*.

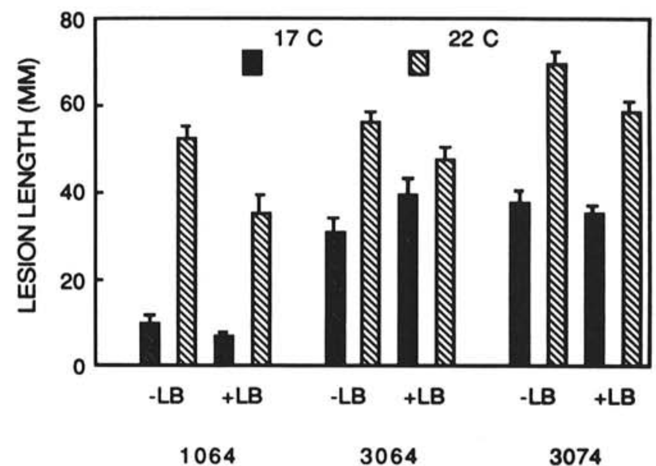

Fig. 3. Influence of previous treatment with *Laccaria bicolor* (LB), challenge temperature, and isolate of *Fusarium oxysporum* on the length of lesions induced on primary roots. Seedlings were grown in petri-dish culture at 17 C for 7 days in the absence or presence of *L. bicolor* incorporated as 1 g of moist, living hyphae per 100 ml of a nutrient-agar slurry through which roots grew. Seedlings then were aseptically transferred to moist chambers and their roots placed on glass slides coated with nutrient-agar slurry (without *L. bicolor*), and inoculated with isolates 1064 (nonaggressive), 3064 (moderately aggressive), or 3074 (aggressive) of *F. oxysporum* at the root tip. Challenge was carried out for 13 days at either 17 or 22 C. The experiment was done twice with similar results. Variances of lesion length means were homogeneous for the two trials, and pooled data are presented. Means were calculated only for those roots that developed lesions. Bars denote one-half the standard error of each mean. Analysis of variance indicated significant interactions of challenge temperature with isolate of *F. oxysporum* ($P = 0.001$) and with *L. bicolor* treatment ($P = 0.001$).

by all isolates of *F. oxysporum* at 22 C, but not at 17 C (significant *L. bicolor* × temperature interaction; $P = 0.001$; Fig. 3).

Both *L. bicolor* and *F. oxysporum* colonized roots internally. Internal colonization of roots singly inoculated with *L. bicolor* was infrequent at both 17 and 22 C, and was then sparse and restricted to the intercellular spaces of the outer cortex. Internal colonization of roots singly inoculated with *F. oxysporum* was both inter- and intracellular, the latter occurring only in roots bearing lesions. All roots that were inoculated with *F. oxysporum* but did not develop lesions during the challenge period, regardless of exposure to *L. bicolor*, contained fungal hyphae in cortical intercellular spaces. The incidence of these internally colonized but apparently healthy roots was greatest where roots had been exposed to *L. bicolor* before challenge with isolate 1064 of *F. oxysporum* at 17 C. Intercellular hyphae of *F. oxysporum* and *L. bicolor* were not readily distinguishable in double-inoculated roots, because clamp connections of the latter fungus were rarely visible in the transverse sections used. However, because intercellular colonization of healthy roots was far more frequent and extensive in roots inoculated with *F. oxysporum* alone than in those inoculated with *L. bicolor* alone, intercellular hyphae in double-inoculated roots were assumed to be those of *F. oxysporum.* Cortical cell walls adjacent to intercellular spaces containing hyphae of *F. oxysporum* had a tan-to-brown pigment, which was darker in roots exposed to *L. bicolor* before inoculation with *F. oxysporum* than in those not exposed to the mycorrhizal fungus.

DISCUSSION

Temperature during challenge had profound effects on disease development. At 22 C, all three isolates of *F. oxysporum* caused lesions in nearly every inoculated root. At 17 C, many seedlings were able to resist the pathogen, particularly isolates 1064 and 3064; whereas, the aggressive isolate 3074 caused numerous large lesions at both challenge temperatures. Greater disease development at 22 C may have resulted from differential effects of temperature on host and pathogen. The higher temperature was only slightly more stimulatory to the linear growth of *F. oxysporum* on PDA than to the growth of roots, but the rapid development of the pathogen may have allowed intracellular colonization of roots before they could express active resistance. At 22 C, as compared with 17 C, the more rapidly growing and metabolizing roots, still dependent solely on seed stores, may have had fewer carbon reserves that could be diverted to defense functions. Also, elevated temperature may have increased the exudation of nutrients (9), which may enhance pathogenesis by *F. oxysporum* (23).

Apparently, seedlings of low vigor also were impaired in their ability to actively resist *F. oxysporum.* Seedlings with slowly growing roots were highly susceptible to infection by the non-aggressive isolate 1064 at 17 C, a temperature at which normal seedlings often are not infected by this isolate.

Temperature during challenge affected root protection by *L. bicolor*. The lesser protection by *L. bicolor* from isolate 1064 of *F. oxysporum* at 22 C was more likely a consequence of temperature effects on pathogen aggressiveness and/or host susceptibility (as demonstrated in the absence of *L. bicolor*) than of temperature effects on *L. bicolor*. Maximal growth of the isolate of *L. bicolor* (813) used in this study occurs at 22–23 C (20). The reason for the slight reduction by *L. bicolor* of lesion expansion at 22 C is unknown.

Our results indicate that *L. bicolor* induces only a modest increment in the resistance of primary roots of Douglas-fir to *F. oxysporum*, which can be overcome by aggressive pathogen isolates and at temperatures highly conducive to pathogenesis. Induced resistance was also variable (in that some roots always developed lesions) under conditions favorable for its expression (challenge with isolate 1064 of *F. oxysporum* at 17 C). Inhibition of lesion expansion by *L. bicolor* occurred only at 22 C, and then was insufficient to prevent eventual seedling mortality. In companion studies (19), substantial variability in the expression of induced resistance and of its probable mechanism (stimulation of infusion of cortical cell walls with flavanols) was observed both within treatments and between experiments. The conditional and variable nature of root protection by *L. bicolor* may account for the slight and inconsistent protection observed in nursery beds (16), as compared with that in a controlled gnotobiotic environment (21), and may preclude its practical application in forest nurseries primarily for biological control of Fusarium root diseases of Douglas-fir. However, *L. bicolor* does enhance the development of coniferous seedlings in forest nurseries (12) and, if used for this purpose, may confer as a secondary benefit some protection from root disease caused by *F. oxysporum.*

The association of cortical wall pigmentation with restriction of *F. oxysporum* to intercellular spaces of apparently healthy roots indicates a possible role of wall modifications in resistance to *F. oxysporum.* Wall pigments were darker in roots exposed to *L. bicolor* before challenge than in roots not exposed. Other studies (19) indicated that these wall pigments were derived from flavanols and that they reduced enzymatic digestibility of cortical cell walls.

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