

## Suppressive Influence of *Laccaria laccata* on *Fusarium oxysporum* and on Douglas-fir Seedlings

D. M. Sylvia and W. A. Sinclair

Former graduate research assistant and professor, Department of Plant Pathology, Cornell University, Ithaca, NY 14853-0331. Present address of senior author: Department of Plant Pathology, University of Florida, Gainesville 32611.

We acknowledge the contribution of H. E. Wilcox to the design of the wick-culture system. We thank W. J. Bloomberg for isolate T841 of *Fusarium oxysporum*, L. F. Grand for isolate T1036 of *Laccaria laccata*, the Weyerhaeuser Company for Douglas-fir seed, and A. O. Larsen and H. H. Lyon for technical help.

Accepted for publication 23 August 1982.

### ABSTRACT

Sylvia, D. M., and Sinclair, W. A. 1983. Suppressive influence of *Laccaria laccata* on *Fusarium oxysporum* and on Douglas-fir seedlings. *Phytopathology* 73:384-389.

Some strains of the ectomycorrhizal fungus *Laccaria laccata* can invade primary roots of seedlings of Douglas-fir (*Pseudotsuga menziesii*) and protect them from lethal attack by *Fusarium oxysporum*. We appraised the potential of *L. laccata* to inhibit *F. oxysporum* and to influence the development of young seedlings. In dual cultures on agar media with pH relatively less favorable for growth of *F. oxysporum* than *L. laccata*, diffusible metabolites of a mycorrhizal strain of *L. laccata* inhibited growth and caused distortion of hyphae of the pathogen. Cell-free fluid from cultures of this strain of *L. laccata* delayed germination of microconidia and

chlamydospores of *F. oxysporum*. Only slight suppression occurred under conditions that favored rapid growth of *F. oxysporum*. A nonmycorrhizal strain of *L. laccata* inhibited growth of *F. oxysporum* over a wide range of conditions on agar media, but culture filtrates did not affect spore germination. Antibiosis may be a partial basis for root protection by the mycorrhizal strain of *L. laccata*. On agar plates and in gnotobiotic systems without soil, *L. laccata* or its cell-free metabolites suppressed root growth of Douglas-fir. In sterile or pasteurized soil, however, no suppression occurred.

Some strains of the ectomycorrhizal fungus *Laccaria laccata* (Scop.: Fr.) Berk. & Br. can protect primary roots of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) against invasion by the pathogen, *Fusarium oxysporum* Schlecht. emend Snyd. & Hans (21,24). Protection is due in part to resistance induced in the cortex of primary roots by *L. laccata* or its metabolites (25). During studies of the nature of root protection, we also examined the possibility that under exacting conditions, *L. laccata* may exert an antibiotic influence on *F. oxysporum*. Further, since induced resistance implies an energy cost to the seedling, we studied the influence of *L. laccata* and its cell-free metabolites on the growth of very young Douglas-fir seedlings. This paper reports a suppressive influence of the mycorrhizal fungus on the pathogen and on young seedlings in soil-free systems.

**Background.** Root rot of Douglas-fir seedlings by *F. oxysporum* is lethal if the seedlings become infected while very young. Bloomberg (2) found an inverse relationship between seedling age at inoculation and subsequent mortality. Few lethal infections occurred in seedlings that were more than 25 days old when transplanted into soil infested with *F. oxysporum*. During the period of highest susceptibility, only the primary root is available for inoculation. Lateral roots emerge from the primary root 3 wk or more after the onset of germination (4). Thus, the primary root is the site of most lethal infections and is the site of any protective influence. *L. laccata* can colonize and infect primary roots rapidly (4). In or on primary roots it can induce resistance and perhaps also inhibit fungal pathogens through antibiosis, competition, or an influence on other rhizosphere organisms. We considered the first of these types of inhibition.

Previous workers regarded antibiosis and competition as inadequate explanations for root protection by *L. laccata* because this fungus did not inhibit *F. oxysporum* in paired-culture tests on media rich in nutrients (14,24). However, Park (18) suggested that high levels of nutrients may mask an antibiotic effect. Smiley and Cook (23) and Elad et al (5) noted that low vigor of a pathogen may reduce its tolerance to antagonism. We, therefore, reappraised the

antibiotic potential of *L. laccata* under conditions that selectively favored its growth and/or retarded the growth of *F. oxysporum*.

When *L. laccata* forms mycorrhizae with Douglas-fir seedlings, they grow more rapidly than nonmycorrhizal seedlings (21). However, mycorrhizal fungi sometimes suppress plant growth (1,8,10,13,19,29,30). This influence may be associated with colonization of very young or stressed seedlings that are unable to limit the fungal symbiont or to produce sufficient carbohydrate for normal development of both organisms. The balanced reciprocal parasitism (7) that characterizes most mycorrhizal associations is delayed or absent. When primary roots of Douglas-fir are invaded by *L. laccata*, they never become mycorrhizal, but they do react with increased synthesis of phenolics (25). We measured initial seedling response to *L. laccata* in terms of elongation of the primary roots and accumulation of dry mass under conditions both stressful and favorable to seedlings.

### MATERIALS AND METHODS

**Conditions differentially favorable to mycorrhizal and pathogenic fungi.** The influences of pH, glucose concentration, and temperature on growth of two isolates of *L. laccata* and one of *F. oxysporum* were investigated in vitro. Isolate T1025 of *F. oxysporum* was a root-rotting strain from a conifer nursery in New York State (FO-NY). Isolate T813 of *L. laccata*, which grew slowly and produced lavender pigment in vitro, had previously been shown capable of colonizing radicles of Douglas-fir seedlings (4), providing root protection (21), and forming ectomycorrhizae (LL-M). Isolate T1036 of *L. laccata*, which grew rapidly and produced no pigment, had been nonmycorrhizal in tests with Douglas-fir and red pine (*Pinus resinosa* (Ait.)) (LL-N).

Stock cultures of *F. oxysporum* were maintained on potato-dextrose agar (PDA) and those of *L. laccata* on modified Melin-Norkrans agar medium (MMN) (14) amended with 50 ppm biotin. Plugs from plate cultures were stored in sterile distilled water (SDW) at 2 C (16). Cultures for inoculum were prepared by transferring plugs to plates of MMN at 22 C and incubating 1 wk for FO-NY and LL-N or 2 wk for LL-M. Plugs 5 mm in diameter were then transferred from culture margins to test media (MN) containing Melin-Norkrans salts, thiamine (100 ppm), biotin (50 ppm), and agar (1.5%). Vitamins were added and pH adjusted

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

aseptically by additions of 1 N HCl or 1 N NaOH after autoclaving. The medium was amended to provide the following experimental parameters in separate experiments: pH (3–7 at 1-unit intervals) in 10 mM glucose at 21 C, glucose (3, 10, and 50 mM) at pH 6.5 and 21 C, temperature (9–30 C at 3-degree intervals) in 10 mM glucose at pH 5.0. Colony diameters along two perpendicular axes were measured after 12 days, except that colonies of FO-NY and LL-N in the glucose test were measured after 7 days. Each treatment was replicated at least three times. Data were fitted by the least squares method to curves representing linear or quadratic equations.

**Antibiosis on solid media.** A paired-culture test was designed to reevaluate antibiosis *in vitro* under conditions that differentially favored growth of the test fungi. Two-week-old stock cultures of both isolates of *L. laccata* were comminuted for 5 sec in a small blender with sufficient SDW to yield a slurry. This was dispensed (1.5 ml per plate) as a streak at one side of the agar surface in petri plates containing MN medium at various glucose concentrations and pH. A slurry of MMN agar served as the control. Plates were incubated 2 wk at 21 C. Plugs from margins of 1-wk-old cultures of *F. oxysporum* (FO-NY and isolate T841, a root rotting strain from British Columbia [FO-BC]) were then placed 40 mm from the edges of *L. laccata* colonies and growth was observed periodically for 1 mo. Factorial experiments were conducted involving pH (4 and 6), glucose concentration (3, 10, and 50 mM), and temperature (15, 21, and 27 C). Antagonism in each treatment was evaluated in terms of an index obtained by dividing the distance that *F. oxysporum* grew toward *L. laccata* by the distance it grew toward the agar slurry on a control plate. In a similar experiment with isolates LL-M and FO-NY, activated charcoal was incorporated at 0.1% into MN medium containing 10 mM glucose at pH 4, and fungal growth was monitored at 15, 21, and 27 C. At the conclusion of each experiment, the pH of the medium was determined with a surface electrode placed in the center of the plate in a drop of 10 mM CaCl<sub>2</sub>. Culture margins were stained with 1.0% phloxine, and hyphal tips were observed microscopically. Each treatment had at least three replicates. Experiments were done twice, and data were subjected to analysis of variance.

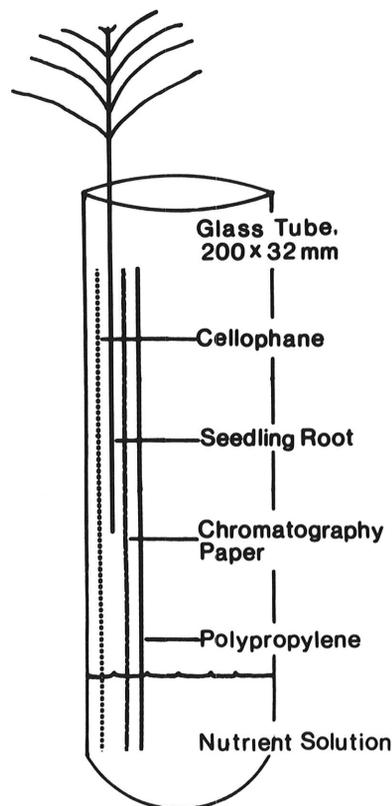
**Antibiotic influence of culture filtrates.** Extracellular metabolites of both isolates of *L. laccata* were assayed for activity against spore germination of FO-NY. Metabolites of isolate T1067 of *Trichoderma harzianum* Rifai (TH) from the rhizosphere of a Douglas-fir seedling were used for comparison. This isolate had previously produced metabolites inhibitory to the pathogen (*unpublished*). Fungi were grown in 125-ml Erlenmeyer flasks with 60 ml of MN liquid medium made with either SDW or 0.2 M sodium phosphate buffer (both adjusted to pH 6.0) at three glucose levels (3, 10, and 50 mM). Fungal inocula in 1-ml amounts of slurry from stock cultures were added to flasks and incubated 14 days for LL-M or 8 days for TH and LL-N at room temperature on a reciprocating shaker at 100 excursions per minute. Control flasks were incubated without fungi. The contents of each flask were then poured through Whatman No. 1 filter paper and the pH of the filtrate was determined. The filtrate was sterilized by aspiration through 0.2- $\mu$ m Nalgene membrane filters and stored at 2 C for up to 9 mo.

The influences of culture filtrates on microconidial and chlamyospore germination were determined by methods modified from Brian and Hemming (3). Sterile culture filtrate (0.9 ml), full strength for both spore types or diluted 1:8 with nutrient solution for microconidia only (pH adjusted to that of the filtrate), was combined with 0.1 ml of spore suspension (10<sup>7</sup> spores per milliliter). This resulted in a spore concentration of 10<sup>6</sup>/ml and final filtrate concentrations 90 and 10% of the original. Microconidia were harvested from 10-day-old cultures grown on PDA slants at room temperature with 12 hr of artificial light (~25  $\mu$ E·m<sup>-2</sup>·sec<sup>-1</sup>). Chlamyospores were obtained as described by Short and Lacy (20) except that SDW was used in place of soil extract. Abundant chlamyospores formed in SDW from germinated microconidia. Concentrated chlamyospore suspensions were stored in SDW at 2 C for up to 9 mo without loss of germinability. Filtrate dilutions with spores were added dropwise to sterile microscope slides and incubated in moist chambers at 15 C for microconidia and 21 C for

chlamyospores. Spore germination (germ tube length >25% the length of the spore) was monitored periodically for 24 hr by placing a coverslip on a drop and observing 30 spores. Each treatment had at least four replicates, experiments were done twice, and data from each experiment were subjected to analysis of variance.

**Influence of *L. laccata* on initial seedling growth.** Seedlings of Douglas-fir or red pine were grown gnotobiotically (*sensu* Kreutzer and Baker [11]) with each isolate of *L. laccata* in soil-culture tubes (4) or in wick-culture tubes.

The wick-culture system was constructed by lining a 200 × 32-mm culture tube with a trilaminar support structure consisting of polypropylene, Whatman No. 3 chromatography paper, and cellophane, the latter outermost (Fig. 1). The chromatography paper acted as a wick that carried nutrients to the root. The cellophane and polypropylene provided a sandwich from which the root could be removed without damage. To allow introduction of fungi and the radicles of intact seedlings, a stainless steel rod was positioned between paper and cellophane. Forty milliliters of nutrient solution was added, and the tube was closed with a foam plug, autoclaved, and allowed to equilibrate for at least 1 wk before test organisms were added. The nutrient solution, adjusted to pH 5.5 with 1 N NaOH, contained the following: 6.2 mM NH<sub>4</sub>NO<sub>3</sub>, 1.8 mM KH<sub>2</sub>PO<sub>4</sub>, 0.3 mM CaCl<sub>2</sub>, 0.4 mM NaCl, 0.6 mM MgSO<sub>4</sub>·7H<sub>2</sub>O, 1.2 ml of 1% aqueous Fe-citrate, micronutrients (9), 0–100 mM glucose, and deionized H<sub>2</sub>O. Seeds were surface sterilized in 30% H<sub>2</sub>O<sub>2</sub> for 2 hr (if Douglas-fir) or 30 min (if red pine) and allowed to germinate aseptically on moist filter paper. Plate cultures of *L. laccata* were leached twice in SDW and comminuted to produce a slurry, which was injected in 1-ml amounts into the tubes. Control tubes received a slurry of leached MMN or leached MMN plus steam-killed inoculum. After 2 or 3 days, the radicle (30–40 mm long) of a 10-day-old seedling was aseptically placed on the slurry in each tube. The shoots were enclosed in plastic bags for 2 wk to prevent desiccation while the radicles became established.



**Fig. 1.** Diagrammatic representation of a wick-culture system. Tube is lined with cellophane, chromatography paper, and polypropylene. To insert radicle, cellophane and paper are separated by a stainless steel rod. Assembly is closed with a foam plug that holds hypocotyl against lip of tube.

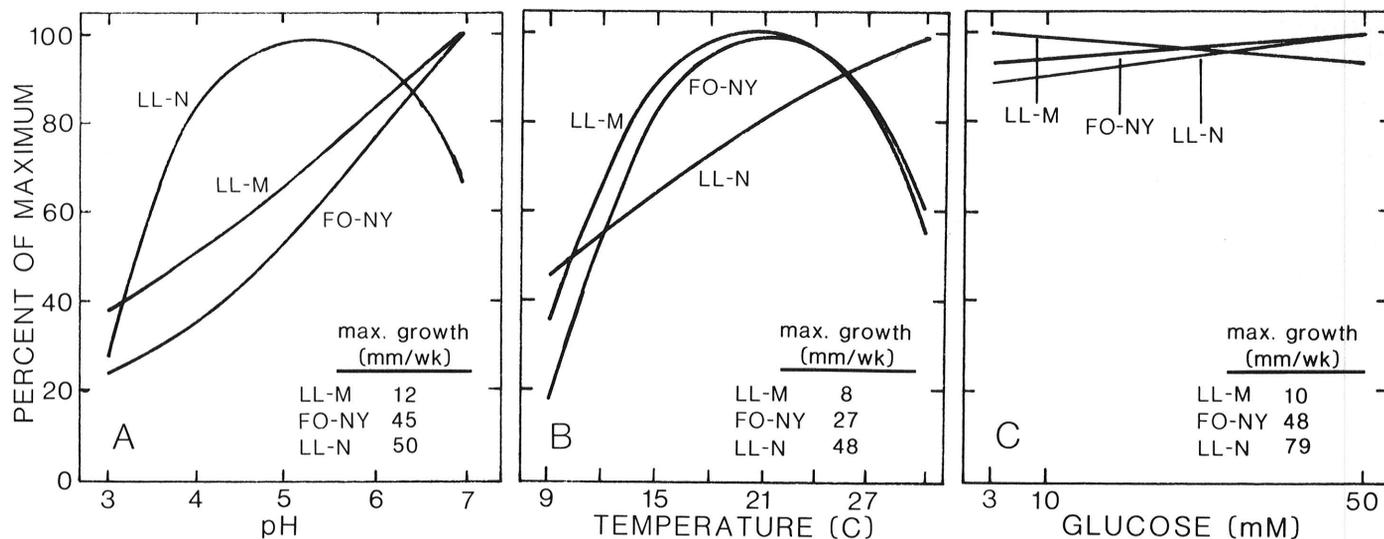


Fig. 2. Relative rates of mycelial growth of a mycorrhizal (LL-M) and nonmycorrhizal (LL-N) strain of *Laccaria laccata* and *Fusarium oxysporum* (FO-NY) on solid media over ranges of A, pH; B, temperature; and C, glucose concentration. Tests for conformity of data to quadratic equations (A and B) or straight lines (C) resulted in significant ( $P < 0.01$ ) sample correlation coefficients  $\geq 0.75$  for all relationships except LL-M and FO-NY in Fig. 2C, which are not significant.

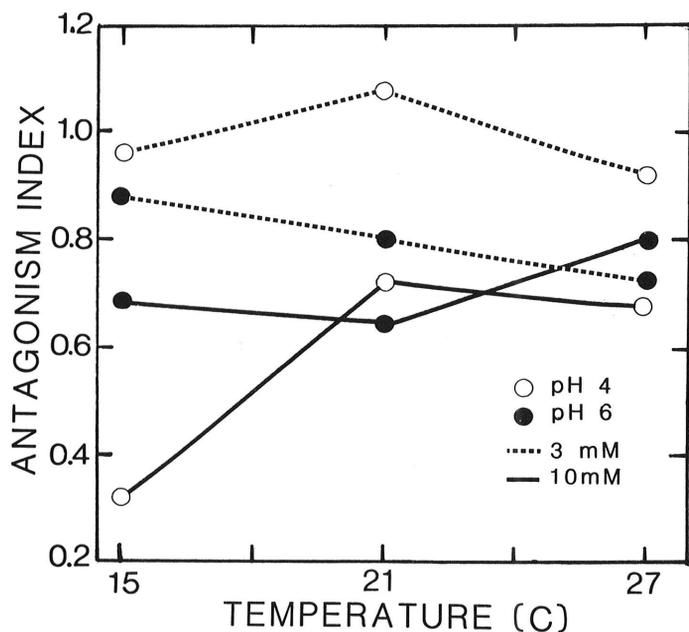


Fig. 3. Suppression of hyphal growth of *Fusarium oxysporum* (FO-NY) on solid media by a mycorrhizal strain of *Laccaria laccata* (LL-M). Antagonism index was obtained by dividing the distance that *F. oxysporum* grew toward *L. laccata* by the distance it grew toward an agar slurry on the control plate. Each data point represents the mean of at least three replicates. LSD at  $P = 0.05$  is 0.21.

Shoots were subsequently exposed to the ambient conditions of an air-conditioned chamber within a greenhouse. The chamber contained a water bath that maintained roots at 22 C while shoots were subject to diurnal temperature fluctuations (18–27 C). Natural light was supplemented by mercury metal halide lamps to provide 300–450  $\mu\text{E}/\text{m}^2/\text{sec}$  for at least 14 hr/day. In some experiments, wick tubes were modified by placing a dialysis barrier (12,000–14,000 molecular weight cut off) between the root and the fungus. This allowed observation of the effects of diffusible metabolites. It was necessary to wash tubes thoroughly to prevent growth suppression associated with the dialysis sheet. Assembled tubes were soaked 3 hr in dilute EDTA at 98 C, washed with deionized water, soaked in dilute  $\text{NaHCO}_3$ , and washed five times

with deionized water. Length of the primary root was measured weekly. Dry weights of shoots and the final pH of the nutrient solution were determined at the conclusion of each experiment (4–6 wk). Experiments were designed to test effects of LL-M, LL-N, and glucose concentration on seedling development. Experiments initially had 10–20 replicates per treatment, but seedlings often perished after transfer to wick-tube culture so final replication was variable. Experiments were done at least twice, and measurements at the conclusion of an experiment were subjected to analysis of variance.

Development of primary roots as influenced by LL-M was also monitored on plates of MN medium with 10 mM glucose, pH 4, with or without 0.1% activated charcoal. As in the paired-culture tests, a slurry (1.5-ml) of mycelium and agar or agar alone was dispensed to one side of each plate and incubated for 2 wk at 21 C. Four Douglas-fir germinants with radicles 5–10 mm long were placed on the agar, with radicles toward LL-M, 4 cm from the fungal colony. Plates were sealed with Parafilm® and incubated 5 days at 15 C in the dark. During incubation the plates were inclined at 80 degrees with the radicles oriented downward. There were five replicates per treatment, and the experiment was done twice. Data from each experiment were subjected to analysis of variance.

## RESULTS

Representative data from each phase of the study are presented.

**Differentially favorable conditions for fungi.** Maximum growth rates over ranges of pH, temperature, and glucose concentration were greatest for LL-N, followed by FO-NY and LL-M (Fig. 2A–C). Tests for conformity of data to quadratic or linear equations resulted in significant ( $P < 0.01$ ) sample correlation coefficients  $\geq 0.75$  for all relationships except for effects of glucose concentration on LL-M and FO-NY. At pH 5 and below, growth of FO-NY was suppressed more than that of either isolate of *L. laccata* (Fig. 2A). These differences were significant except at pH 3. At pH 4, LL-N grew at ~77% of maximum rate, LL-M at 48%, and FO-NY at only 30%. Maximum growth for LL-M and FO-NY occurred at 21 C. At 30 C, the growth rate curve for LL-N was still rising (Fig. 2B). Glucose concentration between 3 and 50 mM had little effect on radial growth rate of any of the fungi (Fig. 2C).

**Antibiosis on solid media.** Factorial experiments at three temperatures (15, 21, and 27 C), two pH levels (pH 4 and 6), and two glucose levels (3 and 10 mM) demonstrated that antibiosis in vitro could be substantial (Fig. 3). Inhibition of FO-NY by LL-M was consistently greatest at 15 C on media initially containing 10

mM glucose at pH 4. Antagonism was generally greater at 10 mM than at 3 mM glucose, but significant interactions occurred among all three factors. Similar results were obtained when FO-BC was tested. Final pH values of control media and those on which *L. laccata* grew did not differ significantly from each other. In further tests, antagonism did not occur on MN media containing 50 mM glucose. Hyphal tips of *F. oxysporum* on control plates (Fig. 4A) elongated rapidly and were unbranched for several millimeters, whereas hyphal tips of cultures inhibited by LL-M (Fig. 4B) were branched, highly vacuolate, had swollen apices, and remained separate from LL-M. Incorporation of activated charcoal into the test media lessened the extreme inhibition of FO-NY by LL-M at 15 C, 10 mM glucose, pH 4, but not the more moderate suppression at 21 and 27 C (Table 1). Inhibition of FO-NY by LL-N occurred over the full range of conditions tested, resulting in an average 40% suppression of growth.

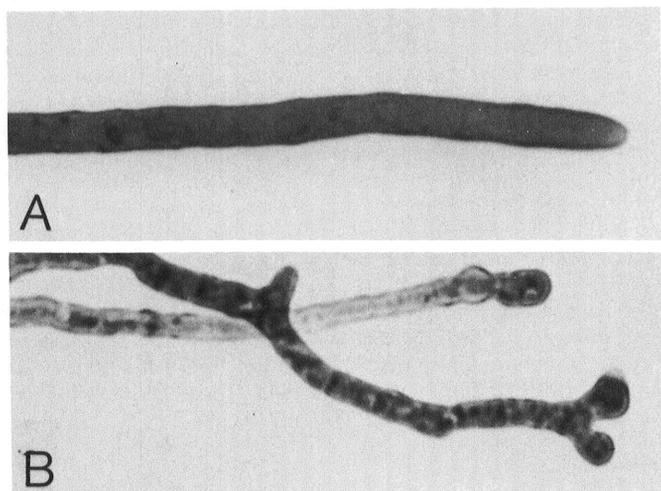


Fig. 4. Hyphal tips of *Fusarium oxysporum* grown on solid media. A, Control culture. B, Culture inhibited by a mycorrhizal strain of *Laccaria laccata*, showing vacuolate, highly septate, swollen appearance ( $\times 1,240$ ).

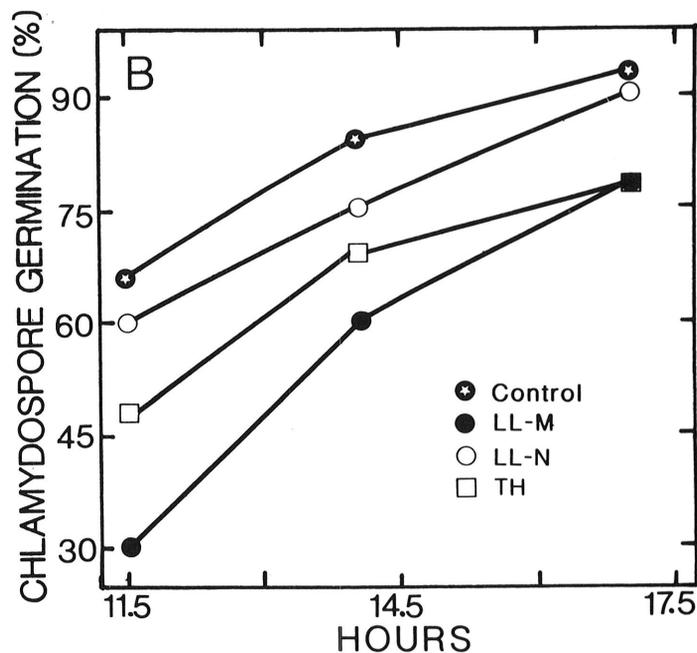
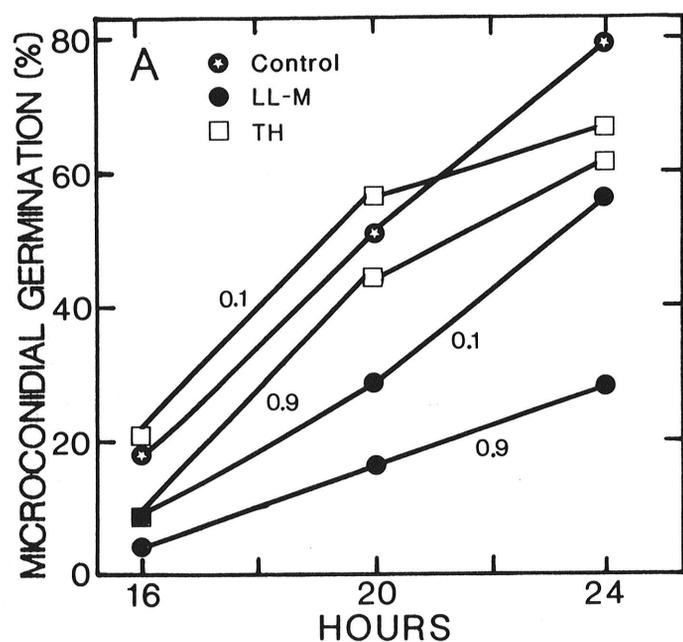


Fig. 5. Effects of culture filtrates of a mycorrhizal (LL-M) and a nonmycorrhizal (LL-N) strain of *Laccaria laccata* and *Trichoderma harzianum* (TH) on spore germination of *Fusarium oxysporum*. Fungi were grown in Melin-Norkrans medium with 10 mM glucose. Each data point represents the mean of at least four replicates. A, Microconidial germination at 15 C in 90 and 10% initial filtrate concentration. LSD at  $P=0.05$  is 3%. B, Chlamydospore germination at 21 C. LSD at  $P=0.05$  is 10%.

**Antibiosis of culture filtrate.** After growth of LL-M, LL-N, and TH, pH of culture fluids ranged from 2.5 to 5.5 depending on the presence of sodium phosphate buffer. Preliminary tests indicated that neither buffer nor pH in this range affected the rate of spore germination. Therefore, unbuffered filtrates were used and the pH of each was adjusted to the mean pH of filtrates in the experiment. At 15 C, filtrate of LL-M at either 90 or 10% of the original concentration delayed microconidial germination of FO-NY significantly (Fig. 5A). Filtrate from TH delayed germination at 90%, but not at 10%, of the original concentration, and this influence was significantly less than that of LL-M. Results were similar at 24 C. Germination of chlamydospores of FO-NY was significantly delayed by filtrate from LL-M and TH, whereas filtrate from LL-N had no effect (Fig. 5B). Inhibitory properties of sterile filtrate were unaffected by storage for up to 9 mo.

**Effects on initial seedling development.** Growth of Douglas-fir seedlings in wick culture was suppressed when these were inoculated with either strain of *L. laccata* (Fig. 6). Roots grew significantly slower and shoots tended to be smaller than those of controls. Red pine roots grown with LL-M for 5 wk were not significantly different from control roots while those grown with LL-N were only 18% as long as those of the controls.

Separation of the fungus from the seedling by a dialysis barrier did not alter suppression. The mean root length of control seedlings after 5 wk was 56% greater than that of seedlings incubated with

TABLE 1. Effect of activated charcoal on the inhibition of *Fusarium oxysporum* by a mycorrhizal strain of *Laccaria laccata*

Temp (C)	Antagonism index <sup>2</sup>	
	No charcoal	Charcoal, 0.1%
15	0.51 c	0.66 b
21	0.78 ab	0.66 b
27	0.76 ab	0.85 a

<sup>2</sup>Grown on Melin-Norkrans medium containing 10 mM glucose at pH 4. Antagonism index equals the distance *F. oxysporum* grew toward *L. laccata* divided by the distance it grew toward an agar slurry on the control plate in 14 days. Data are means of at least three replicates. Values followed by the same letter are not significantly different ( $P=0.05$ ) according to Duncan's multiple range test.

LL-M with or without dialysis separation (Table 2). The membrane barrier was not penetrated by LL-M within 6 wk. Deterioration of the membrane occurred within 2–3 wk, however, when LL-N was tested in this system. The pH of the nutrient solution fell ~1 pH unit during each wick culture experiment, but there were no significant pH differences between treatments.

The nutrient solution was amended with glucose in an attempt to lessen the suppressive effect on root growth. Up to 30 mM, glucose had no influence on suppression of Douglas-fir seedlings by LL-M (Table 3). At 50 mM, roots with LL-M were smaller than controls, but the difference was no longer significant. In another experiment, seedlings grown at two glucose concentrations with LL-M were classified as either suppressed (root length less than 60 mm at 6 wk) or not. An initial glucose level of 50 mM resulted in the suppression of 31% of seedlings with LL-M, while no seedlings were suppressed with 100 mM glucose.

Douglas-fir radicles also became suppressed when allowed to grow toward LL-M on agar (Table 4). Radicles either grew appressed to the agar or diverted off the surface. Roots that remained appressed in the presence of LL-M were on average only 55% as long as controls. Diverted roots were not significantly suppressed. More roots (58%) were diverted in the LL-M treatment than in the controls (21%). Activated charcoal at 0.1% in the medium prevented the suppressive influence.

Seedlings were not suppressed when grown with LL-M in soil-culture tubes. Emerging radicles were colonized rapidly by LL-M, and at 5 wk there were no significant differences between controls

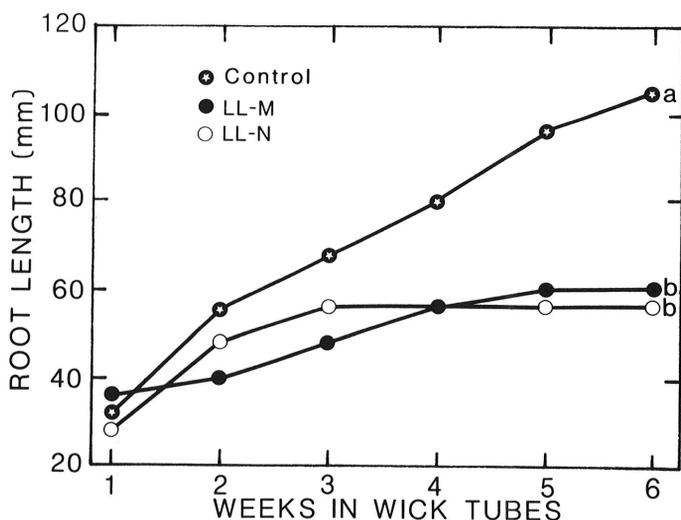


Fig. 6. Effects of a mycorrhizal (LL-M) and a nonmycorrhizal (LL-N) strain of *Laccaria laccata* on primary root growth of Douglas-fir. Seedlings were placed in wick-culture tubes when 10 days old and were allowed to grow for 6 wk. Root length equals total length of root minus length of root when introduced to tube culture. Each data point is the mean of at least five replicates. Root lengths at 6 wk followed by the same letter are not significantly different ( $P = 0.01$ ).

TABLE 2. Effect of a mycorrhizal strain of *Laccaria laccata* alone or separated from plants by dialysis membrane on root length and shoot weight of Douglas-fir<sup>2</sup>

Treatment	Dialysis	Root length (mm)	Shoot dry wt (mg)
Control	Yes	117 a	27 a
	No	124 a	22 a
<i>L. laccata</i>	Yes	79 b	23 a
	No	75 b	15 a

<sup>2</sup>Seedlings were grown in wick-culture tubes for 5 wk. Root length equals total root length minus length of root when introduced to culture tube. Values are means of at least four replicates. Within each column, values followed by the same letter are not significantly different ( $P = 0.05$ ).

and inoculated seedlings in root length (82–92 mm) or dry weight of shoots (11 mg). Stimulation of seedling growth by LL-M, previously reported (21), began 7 wk after germination in soil.

## DISCUSSION

A mycorrhizal strain of *L. laccata*, previously shown to protect premycorrhizal Douglas-fir seedlings from Fusarium root rot, had substantial antibiotic activity against the pathogen under some conditions in vitro. The mycorrhizal fungus also suppressed seedling development in soil-free, gnotobiotic systems and on agar plates, but not in soil.

Extracellular metabolites of the mycorrhizal strain of *L. laccata* inhibited and caused distortion of hyphae, and delayed germination of microconidia and chlamydozoospores of *F. oxysporum*. Such antagonism depended upon a medium with pH relatively less favorable for growth of *F. oxysporum* than *L. laccata*, but differentially favorable pH alone did not assure antagonism. At pH 4 and 3 mM glucose, for example, no inhibition occurred. Antagonism on solid media and inhibitory activity of culture filtrates were affected by the concentration of glucose on which the mycorrhizal strain of *L. laccata* grew. The suppressive influence was greatest when media contained 10 mM glucose. One possible explanation is that this glucose concentration provided a minimum energy requirement for antibiotic synthesis, but that higher levels either suppressed its production or masked the influence by causing rapid growth of the assay organism. Such data show that when requirements for antibiotic activity are exacting, test conditions that favor growth of an assay organism may prevent the detection of activity.

The nonmycorrhizal strain of *L. laccata* suppressed growth of *F. oxysporum* over the full range of conditions in paired-culture tests, and it was more inhibitory at 27 than at 15 C. However, culture filtrate from this fungus did not delay spore germination, and

TABLE 3. Length of the primary root of Douglas-fir seedlings after 5 wk in wick culture as influenced by a mycorrhizal strain of *Laccaria laccata* and glucose concentration<sup>2</sup>

Treatment	Root length (mm)			
	Initial glucose concn (mM)			
	0	5	30	50
Control	107 a	141 a	117 a	98 a
<i>L. laccata</i>	43 b	50 b	17 b	68 a

<sup>2</sup>Data are from two experiments, one at 0 and 5 mM and the second at 30 and 50 mM glucose. Root length equals total root length minus length of root when introduced to the culture tube. Numbers are means of at least five replicates. Values in the same column followed by the same letter are not significantly different ( $P = 0.05$ ).

TABLE 4. Length of primary roots of Douglas-fir after 5 days as influenced by a mycorrhizal strain of *Laccaria laccata*

Root growth <sup>2</sup>	Treatment				
	Controls			<i>L. laccata</i>	
	Agar slurry	No agar slurry	Charcoal in medium	Without charcoal	With charcoal
Radicles diverted from agar (%)	14	29	20	58	24
Length of appressed roots (mm)	23 a	19 a	19 a	11 b	23 a
Length of diverted roots (mm)	21 a	24 a	20 a	17 ab	18 ab

<sup>2</sup>Grown on Melin-Norkrans medium with 10 mM glucose, pH 4, at 15 C. Appressed and diverted refer to continuity of contact between root and agar surface. Means of at least five replicates. Length equals total root length minus length of root when placed on plate. Length values followed by the same letter are not significantly different ( $P = 0.05$ ) according to Duncan's multiple range test.

hyphal tips of *F. oxysporum* were not distorted by it on solid media. Because this strain grew very rapidly, depletion of nutrients may account for its suppressive influence.

Other workers have found that mycorrhizal fungi, including *L. laccata*, produce antibiotics. Krywolap (12) reported that *L. laccata* had antibiotic activity against Gram-negative bacteria, and Marx (14) found weak-to-moderate inhibition of some fungal pathogens (but not *F. oxysporum*) by *L. laccata*.

An extracellular metabolite that diffused in the liquid phase from *L. laccata* suppressed Douglas-fir seedling development in the wick-culture system. Others have found exudates from mycorrhizal and nonmycorrhizal fungi to retard root growth (27,28). This may be attributed to the production of hormones or toxins by the fungus. Hormones of fungal origin, especially auxin (22) and ethylene (6), are known to influence root development. Toxins produced by mycorrhizal fungi are not well documented, but Marx (15) found that diatretyne nitrile, the antibiotic produced by *Leucopaxillus cerealis* var. *piceina*, was toxic to pine seedlings at concentrations above 10 µg/g.

Seedling suppression by the mycorrhizal strain of *L. laccata* was eliminated by adding glucose to soil-free systems. Increased glucose levels may have suppressed production of the biostatic compounds and/or increased the capacity of seedlings to tolerate them.

Techniques for incorporating mycorrhizal inoculum into nursery soil (17) and encapsulating seed with basidiospores (26) will favor rapid colonization of elongating radicles. Little is known about the effect of these practices on the early stages of seedling development. Growth suppression in wick culture tubes suggested that establishment of a symbiotic relationship with *L. laccata* might represent an energy cost to the seedling. Suppression did not occur in soil, however, despite aggressive colonization of emerging radicles by the mycorrhizal fungus. Absence of seedling inhibition in soil may have been due to adsorption of active compound(s) to soil colloids or less luxuriant growth of the mycorrhizal fungus in soil than in wick culture. In open or nonsterile systems, leaching and microbial degradation may further reduce the influence of any biostatic factor. Apparently Douglas-fir seedlings in soil can be inoculated with *L. laccata* at their earliest stages of development without adversely affecting their growth.

Wick culture tubes provided a useful method for observing interactions between mycorrhizal fungi and primary roots of seedlings. Most roots remained unbranched and nonmycorrhizal throughout each experiment. The technique allowed nondestructive observation of the root zone and minimized tissue disruption at harvest. A disadvantage of the system was the apparent accumulation of phytotoxic substances produced by *L. laccata* and/or high sensitivity of seedlings to them.

No attempt was made to characterize the suppressive factor(s) produced by the mycorrhizal fungus. Such work should follow studies that show ecological significance of the suppression.

#### LITERATURE CITED

- Beckjord, P. R., Adams, R. E., and Smith, D. W. 1980. Effects of nitrogen fertilization on growth and ectomycorrhizal formation of red oak. *For. Sci.* 26:529-536.
- Bloomberg, W. J. 1973. Fusarium root rot of Douglas-fir seedlings. *Phytopathology* 63:337-341.
- Brian, P. W., and Hemming, H. G. 1945. Gliotoxin, a fungistatic metabolic product of *Trichoderma viride*. *Ann. Appl. Biol.* 32:214-220.
- Brown, A. C., and Sinclair, W. A. 1981. Colonization and infection of primary roots of Douglas-fir seedlings by the ectomycorrhizal fungus *Laccaria laccata*. *For. Sci.* 27:111-124.
- Elad, Y., Chet, I., and Katan, J. 1980. *Trichoderma harzianum*: a biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. *Phytopathology* 70:119-121.
- Graham, J. H., and Linderman, R. G. 1981. Effect of ethylene on root growth, ectomycorrhiza formation, and *Fusarium* infection of Douglas-fir. *Can. J. Bot.* 59:149-155.
- Hacsakaylo, E. 1972. Mycorrhiza: The ultimate in reciprocal parasitism. *BioScience* 22:577-583.
- Hayman, D. S. 1974. Plant growth responses to vesicular-arbuscular mycorrhiza. VI. Effect of light and temperature. *New Phytol.* 73:71-80.
- Hoagland, D. R., and Arnon, D. I. 1950. The water-culture method for growing plants without soil. *Calif. Agric. Exp. Stn., Berkeley, Circ.* 347. 32 pp.
- Hyppel, A. 1968. Effect of *Fomes annosus* on seedlings of *Picea abies* in the presence of *Boletus bovinus*. *Stud. For. Suec.* 66:3-16.
- Kreutzer, W. A., and Baker, R. 1975. Gnotobiotic assessment of plant health. Pages 11-21 in: *Biology and Control of Soil-borne Plant Pathogens*. G. W. Bruehl, ed. *Am. Phytopathol. Soc., St. Paul, MN.* 216 pp.
- Krywolap, G. N. 1971. Production of antibiotics by certain mycorrhizal fungi. Pages 219-221 in: *Mycorrhizae*. E. Hacsakaylo, ed. *U.S. Dep. Agric. For. Serv. Misc. Publ.* 1189. 255 pp.
- Lundeberg, G. 1960. The relationship between pine seedlings (*Pinus sylvestris* L.) and soil fungi. *Svensk Bot. Tidskr.* 54:346-359.
- Marx, D. H. 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. *Phytopathology* 59:153-163.
- Marx, D. H. 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. II. Production, identification, and biological activity of antibiotics produced by *Leucopaxillus cerealis* var. *piceina*. *Phytopathology* 59:411-417.
- Marx, D. H., and Daniel, W. J. 1976. Maintaining cultures of ectomycorrhizal and plant pathogenic fungi in sterile water cold storage. *Can. J. Microbiol.* 22:338-341.
- Marx, D. H., Morris, W. G., and Mexal, J. G. 1978. Growth and ectomycorrhizal development of loblolly pine seedlings in fumigated and nonfumigated nursery soil infested with different fungal symbionts. *For. Sci.* 24:193-203.
- Park, D. 1960. Antagonism—The background to soil fungi. Pages 148-159 in: D. Parkinson and J. S. Waid, eds. *The Ecology of Soil Fungi*. Liverpool Univ. Press, Liverpool. 324 pp.
- Sands, R., and Theodorou, C. 1978. Water uptake by mycorrhizal roots of radiata pine seedlings. *Aust. J. Plant Physiol.* 5:301-309.
- Short, G. E., and Lacy, M. L. 1974. Germination of *Fusarium solani* f. sp. *pisi* chlamydospores in the spermosphere of pea. *Phytopathology* 64:558-562.
- Sinclair, W. A., Sylvia, D. M., and Larsen, A. O. 1982. Disease suppression and growth promotion in Douglas-fir seedlings by the ectomycorrhizal fungus *Laccaria laccata*. *For. Sci.* 28:191-201.
- Slankis, V. 1973. Hormonal relationships in mycorrhizal development. Pages 231-298 in: *Ectomycorrhizae, Their Ecology and Physiology*. G. C. Marks and T. T. Kozlowski, eds. Academic Press, New York. 444 pp.
- Smiley, R. W., and Cook, R. J. 1973. Relationship between take-all of wheat and rhizosphere pH in soils fertilized with ammonium-vs. nitrate-nitrogen. *Phytopathology* 63:882-890.
- Stack, R. W., and Sinclair, W. A. 1975. Protection of Douglas-fir seedlings against *Fusarium* root rot by a mycorrhizal fungus in the absence of mycorrhiza formation. *Phytopathology* 65:468-472.
- Sylvia, D. M., and Sinclair, W. A. 1983. Phenolic compounds and resistance to fungal pathogens induced in primary roots of Douglas-fir seedlings by the ectomycorrhizal fungus *Laccaria laccata*. *Phytopathology* 73:390-397.
- Theodorou, C., and Bowen, G. D. 1973. Inoculation of seeds with basidiospores of mycorrhizal fungi. *Soil Biol. Biochem.* 5:765-771.
- Turner, P. D. 1962. Morphological influence of exudates of mycorrhizal and non-mycorrhizal fungi on excised root cultures of *Pinus sylvestris* L. *Nature* 194:551-552.
- Wargo, P. M. 1966. Influence of fungal exudates on growth of excised roots of white oak. Ph.D. dissertation. Iowa State Univ., Ames. 107 pp. (Diss. Abstr. 27B:1018 [1966]).
- Wingfield, E. B. 1968. Mycotrophy in loblolly pine: I. The role of *Pisolithus tinctorius* and *Rhizoctonia solani* in survival of seedlings. II. Mycorrhiza formation after fungicide treatment. Ph.D. dissertation. Va. Polytech. Inst., Blacksburg. 78 pp. (Diss. Abstr. 29B:2707 [1969]).
- Zak, B. 1976. Pure culture synthesis of Pacific madrone ectendomycorrhizae. *Mycologia* 68:362-369.