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Actinomycetes Inducing Phytotoxic or Fungistatic Activity in a Douglas-fir Forest and in an Adjacent Area of Repeated Regeneration Failure in Southwestern Oregon

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Abstract. Actinomycetes were isolated from the upper 1 – 3 cm of the soil layer in a well-developed forest and in an adjacent clearcut area where Douglas-fir [*Pseudotsuga menziesii* (MIRB.) FRANCO] regeneration had been impaired for two decades. The population density in the clearcut area was two times as high as that in the forested area. The percentage of actinomycetes that inhibited seed germination of the test plants was significantly higher in isolates obtained from the clearcut area than in those obtained from the forested area, and isolates from the clearcut showed five times the phytotoxic effect of those from the forest. Some actinomycete isolates, 4 % from the clearcut and 2.6 % from the forest, significantly reduced *in vitro* growth of two common ectomycorrhizal fungi of Douglas-fir, *Laccaria laccata* and *Hebeloma crustuliniforme*. Two actinomycete isolates from the clearcut reduced fungal growth by 40 % and 73 %. Reduction of the nutrient in the growth medium did not affect the antifungal activity of the actinomycetes. The data support the idea that, along with other factors, phytotoxic and antifungal actinomycetes may suppress natural regeneration or establishment of planted seedlings – either directly or indirectly – through inhibition of seed germination or of mycorrhizal fungi.

Regeneration of Douglas-fir [*Pseudotsuga menziesii* (MIRB.) FRANCO] after clearcutting is successful in most regions of the Pacific Northwest. However, in some areas with well-developed indigenous forests, many successive replantings have failed. This has been a particular problem in droughty sites in southwestern Oregon and northern California (AMARANTHUS and PERRY 1987). In southwestern Oregon alone, poor regeneration has resulted in withdrawal of more than 68 000 ha of public forest land from the timber-production base (US Department of Interior 1978,

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1979). In some areas, the soil remains nearly bare (Fig. 1). Field examinations have shown that seasonal drought may account for only part of this failure (AMARANTHUS and PERRY 1989) in areas with skeletal soils (at least 35 % rock fragments by volume; HOBBS and WEARSTLER 1983). Studies in other areas of the United States indicate that allelopathic agents may contribute to regeneration failures (FISHER 1980, HORSLEY 1987), and preliminary work in southwestern Oregon suggests that allelochemicals produced by soil actinomycetes contribute to regeneration failures (PERRY and ROSE 1983). The production of phytotoxins by actinomycetes has been reported by DEFRANK and PUTNAM (1985) and HEISEY *et al.* (1985). In Israel, phytotoxin-producing soil actinomycetes have been implicated in the failure of annual plants to occupy certain areas within desert plant communities (KATZ *et al.* 1987).

Like any other allelopath, microorganisms may affect plants directly or indirectly through inhibition of mycorrhizal associates or other beneficial organisms. Most important commercial tree species in the temperate zone, including Douglas-fir, are dependent on mycorrhizae (PERRY *et al.* 1987). Recent work suggests that ectomycorrhizal associations may be susceptible to inhibition by allelopathic agents such as living plants or the litter of dead plants (PERRY and ROSE 1983, ROSE *et al.* 1983, PERRY and CHOQUETTE 1987). BEVEGE (1968) suggested that microorganisms may be involved in the production of bioactive compounds in forest soils in Queensland, and microorganisms have also been implicated in allelopathic phenomena related to forest regeneration in northeastern Tasmania. It is possible that allelopathy occurs in the presence of a succession of microorganisms that retard the sequence of breakdown and permit accumulation of detrimental concentrations of phytotoxic compounds.

This study was designed to determine whether soil-borne actinomycetes having phytotoxic or antifungal effects were present in a clearcut area in which tree regeneration had failed, and to evaluate the role of actinomycetes in the failure.

MATERIAL AND METHODS

The Study Area

Soil samples for the study were collected from Cedar Camp (42,05′; 123,20′) in the Siskiyou Mountains of southwestern Oregon at 1706 m above sea level. Average annual precipitation in the area is about 1650 mm, more than 50 % in snow, and a drought period commonly occurs from July through September. Forests are dominated by Douglas-fir, accompanied by white fir [*Abies concolor* (GORD. &GLEND.) LINDL.] and Shasta red fir (*Abies magnifica* var. *shastensis* LEMM. – AMARANTHUS and PERRY 1987). The site has a south-facing aspect, with an inclination of about 30°. Soil is a sandy, skeletal, excessively drained, mixed Entic Cryumbrept formed from quartz diorite parent material (AMARANTHUS and PERRY 1987).

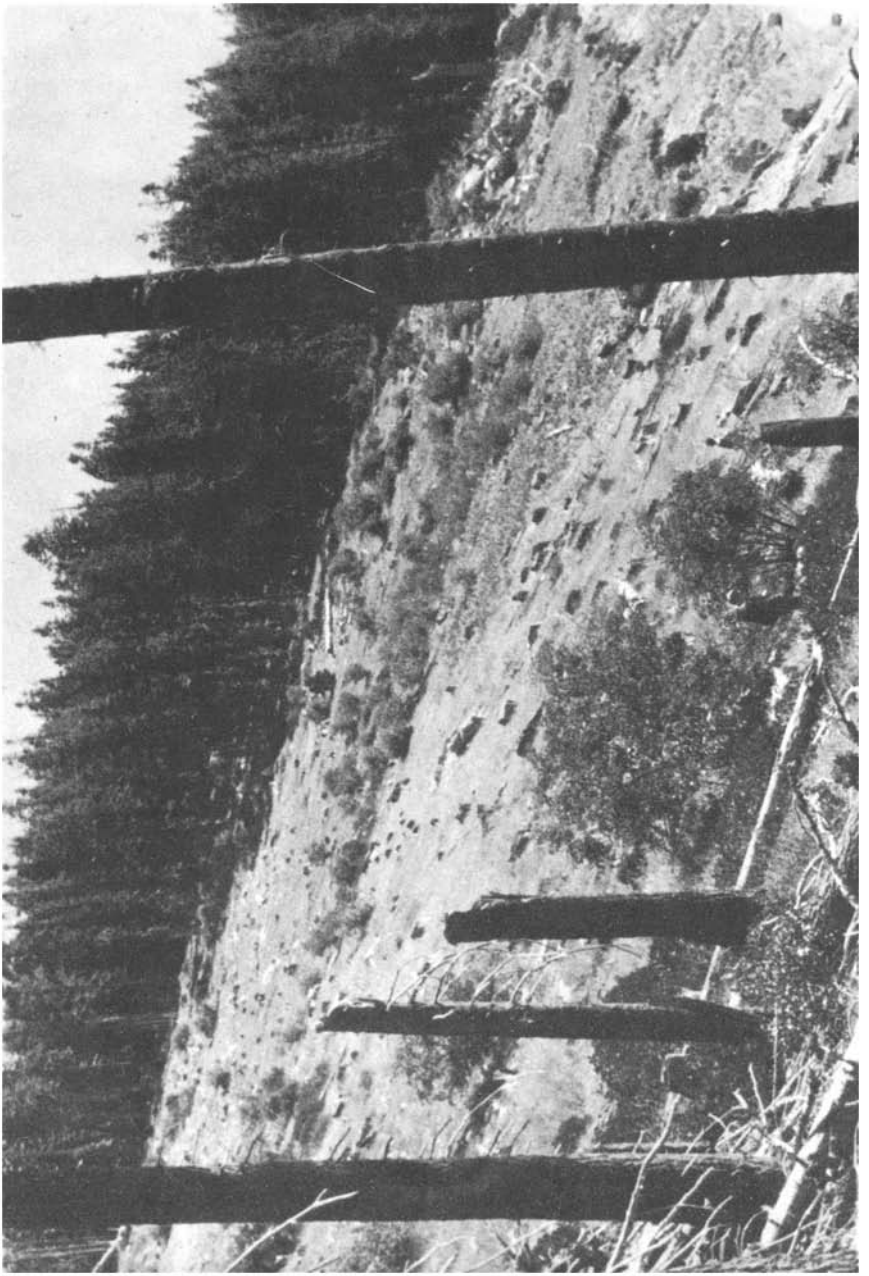

Fig. 1. A south-facing clearcut area in Cedar Camp in the Siskiyou Mountains of Oregon 18 years after trees harvest and after several replanting attempts. In the background is the untouched, indigenous forest.

Trees on about 40 acres were harvested, and the area was subsequently slashburned. A sparse cover of annuals, herbs, *Pteridium aquilinum* (L.) KUHN., *Sambucus racemosa* L., and some scattered *Arctostaphylos viscida* (manzanita) survives in the area (AMARANTHUS and PERRY 1987). In the adjacent undisturbed forest, a 3- to 5-cm organic layer covers the inorganic soil.

Soil Samples

Samples of mineral soils were collected in September 1986 on three areas at each of the two sites, from the first 1–3 cm of the upper soil layer in the clearcut area and from beneath the organic layer in the adjacent undisturbed forest. Samples were preserved in a cold room at 8 °C in 100-mg portions that were later air dried, sieved through a 0.5-mm screen, and shaken in 100 ml of sterile, distilled water for 20 min. Aliquots (25 µl) of soil suspension were spread on the surfaces of two agar media: tap water agar (TWA) adjusted to pH 10.5 (BEVEGE 1968), and sodium albumenate agar (SAA) adjusted to pH 6.8.

The total number of colony-forming units of actinomycetes per g dry soil was determined on TWA after 12 days of incubation or on SAA after 6 days. For each sampling site, 50 agar plates were used; thus there were 150 plates from both the clearcut area and the undisturbed forest. Pure isolates were transferred to malt-yeast agar (MYA) or glucose-molasses-peptone agar.

Phytotoxic Activity

The actinomycete isolates were streaked on MYA plates. After a week, four rows of six seeds each were placed opposite the isolate in the pattern illustrated in Fig. 2a.

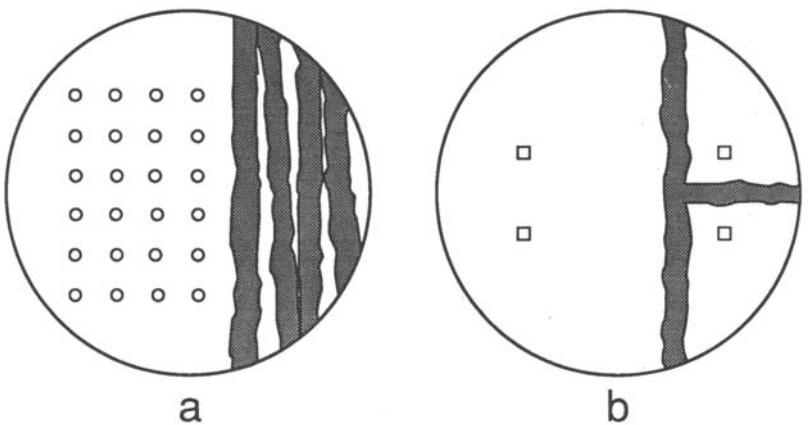

Fig. 2. Pattern of actinomycete isolates and seed planting for evaluating inhibition of seed germination (a) and of mycorrhizal fungi (b).

Because seeds of Douglas-fir germinate slowly and are difficult to surface sterilize, the fast-germinating seeds of *Anastatica hierochuntica* L. or *Lactuca sativa* L. were used. These enabled germination to be recorded 12 or 24 h after seed wetting; thus, interference by associated seed-coat microflora could be minimized. Three replicates were used to screen the isolates for phytotoxic activity. Isolates exhibiting phytotoxicity were further evaluated in a second series of tests that was replicated ten times. Values for intensity of inhibition (percentage of reduction in germination relative to that of controls) were calculated from the latter test series.

Antifungal Activity

For evaluating the effect of actinomycetes on mycorrhizal fungi, two common Douglas-fir ectomycorrhizal fungi, *Laccaria laccata* and *Hebeloma crustuliniforme*, were placed both on MYA plates at a short distance from 10-day-old cultures of actinomycetes (Fig. 2b) and on MYA plates without actinomycetes. Diameter growth of fungal colonies was measured after 7 days of incubation, and growth of colonies plated with actinomycetes was compared to growth of colonies without actinomycetes. As in the seed germination study, three replicates of each isolate were first screened for antifungal activity; then intensity of inhibition of isolates exhibiting antifungal activity was determined in a second test with 10 replications.

In order to determine the effects of nutrient concentration on antifungal activity, the two isolates exhibiting the greatest inhibition of fungal growth were further tested in a dilution series: 12.5 %, 25 %, and 50 % of the complete MYA. Each dilution was tested with three replications. Except for dilution, all other procedures were as described above.

RESULTS AND DISCUSSION

Recovery of Actinomycetes from Soil Samples

More than twice as many actinomycete colony-forming units formed from clearcut soil as from forest soil (Table 1). Twenty per cent more colonies formed on SAA than

TABLE 1
Numbers of actionomycete colony-forming units developing from soils of the clearcut and the adjacent forested areas on two different agar media

Soil habitat	Colony-forming units/g soil[a]	
	Tap-water agar	Sodium albumenate agar
Clearcut	3.5×10^{5} [a]	4.2×10^{5} [a]
Forest	1.6×10^{5} [b]	1.9×10^{5} [b]

[a] Numbers in each column or row followed by the same letter are not significantly different at the $P = 0.05$ level (t-test).

on TWA; however, the ratio of forest-soil colonies to clearcut-soil colonies did not differ in the two media.

Effect of Actinomycete Isolates on Seed Germination

Of the 150 actinomycete isolates from each area, 22.8 % of those from the clearcut and only 9.2 % of those from the forest inhibited seed germination of *A. hierochuntica* or *L. sativa* (Table 2). Inhibitory isolates from both areas had similar effects on seeds of both plant species, reducing germination 12 % to 19 % below that of controls (seeds without actinomycetes). When both total colony-forming units and the percentage inhibiting seed germination are taken into account, the population density of phytotoxic actinomycetes in clearcut soil (3.5×10^5 colonies/g $\times$ 21.3 % = 7.5×10^5) was nearly 5 times that in forest soil (1.6×10^4 colonies/g $\times$ 9.2 % = 1.5×10^4).

TABLE 2
Mean percentage (±SE) of inhibitory actinomycetes and of reduction in germination of *Anastatica hierochuntica* and *Lactuca sativa* on malt yeast agar[a]

	Inhibitory actinomycetes [%]		Reduction in seed germination[b]	
Soil habitat	*A. hierochuntica*	*A. sativa*	*A. hierochuntica*	*L. sativa*
Clearcut	22.8 ± 4.5[a]	21.3 ± 5.7[a]	18.7 ± 4.9[a]	12.1 ± 6.6[a]
Forest	9.2 ± 4.1[b]	9.2 ± 4.1[b]	12.8 ± 3.2[a]	15.1 ± 7.5[a]

[a] Numbers in each column or row followed by the same letter are not significantly different ($P > 0.05$; t-test).
[b] Percentage of reduction relative to that without actinomycetes (controls).

Effect of Actinomycete Isolates on Mycorrhizal Fungi

Of the 150 actinomycete isolates from each area, six (4 %) from the clearcut and four (2.6 %) from the forest inhibited *in vitro* growth of *L. laccata* and *H. crustuliniforme* (Table 3). The two fungi did not differ markedly in their response to the actinomycetes. Two isolates from the clearcut (CC15d and SH) had the strongest effect on fungal growth, inhibiting diameter growth of *L. laccata* colonies by 40 % and 73 %, respectively, and *H. crustuliniforme* colonies by 35 % and 62 %. These were the only isolates that inhibited both fungal growth and seed germination.

In contrast, reducing concentration of the medium below 50 % increased inhibitory activity of isolate CC15d from about 45 % to 55 % (Fig. 3). It is possible that the isolate SH induces fungal inhibition by releasing antifungal substances, while the isolate CC15d inhibits by competing with the fungi for nutrients, as decribed by HSU

TABLE 3
Mean percentage (±SE) of inhibitory actinomycetes isolated from clearcut and forest soils and of inhibition of colony diameter growth of Douglas-fir ectomycorrhizal fungi[a]

Soil habitat	Inhibitory actinomycetes		Reduction in fungal growth[b]	
	H. crustuliniforme	*L. laccata*	*H. crustuliniforme*	*L. laccata*
Clearcut	4.0 ± 2.9	4.0 ± 3.1	23.0 ± 19.7	23.0 ± 0.4
Forest	2.6 ± 1.8	2.6 ± 2.2	15.5 ± 0.5	23.0 ± 6.4

[a] Data are average of six replicates each.
[b] Percentage of reduction relative to that without actinomycetes (controls).

and LOCKWOOD (1969). Each fungus was similarly susceptible to the isolate SH; the isolate CC15d was relatively more inhibitory toward *H. crustuliniforme* (Fig. 3).

WRIGHT and BOLLEN (1961) noted that the high population densities of actinomycetes in soil under stands of 25-year-old Douglas-fir (near Burnt Woods, 20 miles west of Corvallis, Oregon) occured during the rainy period (March–June) and decreased during drought. In areas with extreme fluctuations of precipitation and drought, extreme changes in the density and composition of actinomycete populations can be expected. Therefore, quantitative isolations and the rate of

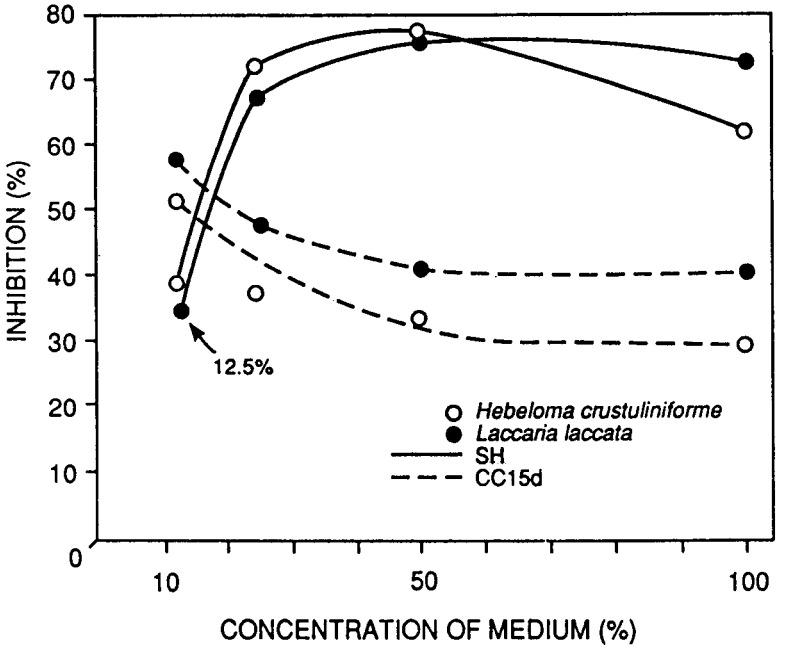

Fig. 3. Inhibition of the mycorrhizal fungi *Laccaria laccata* and *Hebeloma crustuliniforme* by actinomycete isolates SH and CC15d, as affected by medium concentration of malt-yeast agar (MYA).

biological activities should be recorded during the different seasons. Development of actinomycetes is generally enhanced in soils with relatively low water content (ALEXANDER 1970), which may explain their relatively high density in the clearcut area.

It is plausible that after clearcutting in southwestern Oregon, particularly on the southern aspects, the vegetation-free upper soil layer dries out much more than soil in forested areas, and eventually the concentration of actinomycetes increases. However, the inhibition of seed germination of *A. hierochuntica* or *L. sativa* does not necessarily indicate inhibition of Douglas-fir. Further studies are needed to clarify the relative importance of actinomycetes in the suppression of Douglas-fir regeneration under different soil conditions and to determine their indirect inhibitory effect on mycorrhizal associations. Nevertheless, the use of test plants in this study gave information on phytotoxicity produced in the area, and it is likely that such toxicity is partially responsible for the sparse vegetation cover. This view supports, to some degree, the finding that planting immediately after broadcast burning increases survival and growth of Douglas-fir seedlings; delay of planting for nearly a year negates improvements in seedling performance (TESCH *et al.* 1987). During delay, as the upper soil layer of an exposed clearcut area dries out in short alternating periods of drought and humidity, the population density of actinomycetes may increase, while the establishment of vegetation is impaired.

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