...rrhizae

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By Ernest Wright

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Forest Research Laboratory
School of Forestry
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SUMMARY

Tests in field demonstrated that high temperatures of soil caused by direct insolation do not seriously interfere with normal mycorrhizal formation or growth of newly established Douglas-fir seedlings except to increase initial depth of occurrence of mycorrhizae.

Tests in field also demonstrated that high temperatures of soil from insolation were never extreme enough to inhibit mycorrhizal formation on Douglas-fir seedlings, but initial depth of mycorrhizae correlated directly with increase in soil temperature.

Depth of mycorrhizae on seedlings varies directly with the intensity of previous slash burns. Slash fires decrease the subsequent number of mycorrhizae and sometimes delay mantle formation. Season of burn had no consistent effect on formation of mycorrhizal mantles.

In lightroom tests, *Cenococcum graniforme* predominated in both wet and dry soil under short-day insolation (9 hours) at 60-78 F. Most mycorrhizal fungi, however, increased significantly under long-day illumination (15 hours), which indicates that only under certain conditions can *C. graniforme* compete successfully with other mycorrhizal fungi. There were also significant increases in mycorrhizae related to soil temperature and photoperiod, but neither was independent of the other. There was no significant interdependence found between soil moisture and soil temperature. Douglas-fir seedlings with 15-hour illumination developed highly significant increases in mycorrhizal mantles in wet soil compared to dry soil. High soil moisture favored increase in ectendomycorrhizae, especially on ponderosa pine seedlings.

With soil moisture at field capacity and 15 hours of insolation, soil temperatures of 50-75 F and 45-90 F reduced ectomycorrhizal formation on ponderosa pine seedlings, compared to seedlings grown at 56-80 F.

High, naturally balanced fertility of soil stimulated mycorrhizal formation on Douglas-fir seedlings. Seedlings growing in soil from a poor site (Site V) regularly developed mycorrhizal relations with *C. graniforme* but also with a pseudomycorrhizal fungus. These seedlings were stunted and inferior to those growing on better sites; *Cenococcum*, therefore, appears of restricted benefit, but the presence of pseudomycorrhizal mycelia complicates this conclusion. Conversely, heavy application of nitrogen reduced mycorrhizae other than *C. graniforme*. Mycorrhizal frequency on Douglas-fir seedlings decreased when available nitrogen was increased. Seedlings grown in such soil were inferior to those grown in soil containing less nitrogen but with better balance between potassium and phosphorus.

Abundance of mycorrhizae and survival of Douglas-fir seedlings were not satisfactorily correlated in plantations. The benefits of mycorrhizae are difficult to evaluate because of many complicating factors. Good development of mycorrhizae, however, apparently enhances survival of seedlings in plantations.

Stunted, chlorotic Douglas-fir and ponderosa pines from some fumigated nursery seedbeds lacked mycorrhizae and were unsuitable for planting.

Fumigation of seedbeds in a nursery with sandy soil restricted mycorrhizal formation and caused chlorosis of 2-year-old ponderosa pine and Douglas-fir seedlings.

In both field and greenhouse, southernmost seed sources of Douglas-fir and ponderosa pine produced seedlings that formed mycorrhizae later than did seedlings from northern seed. Elevation or aspect, however, had no significant effect on development of mycorrhizae on Douglas-fir seedlings from southern Oregon seed sources in lightroom tests.

Mycorrhizae on Douglas-fir and Ponderosa Pine Seedlings

By Ernest Wright[1]

INTRODUCTION

In this study no attempt has been made to determine the complex functions of mycorrhizae. Rather, the emphasis is on the external factors affecting the occurrence of mycorrhizae on Douglas-fir and ponderosa pine seedlings. A mycorrhiza is defined here as an organ formed by a symbiotic association of the mycelium of certain fungi with roots of higher plants (Figures 1-4).

The literature on mycorrhizae is so voluminous that it would be unwise, even foolhardy, to attempt a comprehensive review. Only directly pertinent references will be cited. Those who are interested in a critical appraisal of the biology of mycorrhizae are referred to Harley's (5) recent text.

In classifying mycorrhizal types, I have followed the abbreviated terminology suggested by Peyronnel *et al.* (18). Thus, the term, ectotrophic mycorrhizae, is shortened to ectomycorrhizae and ectendotrophic mycorrhizae become ectendomycorrhizae. Endomycorrhizae were not studied.

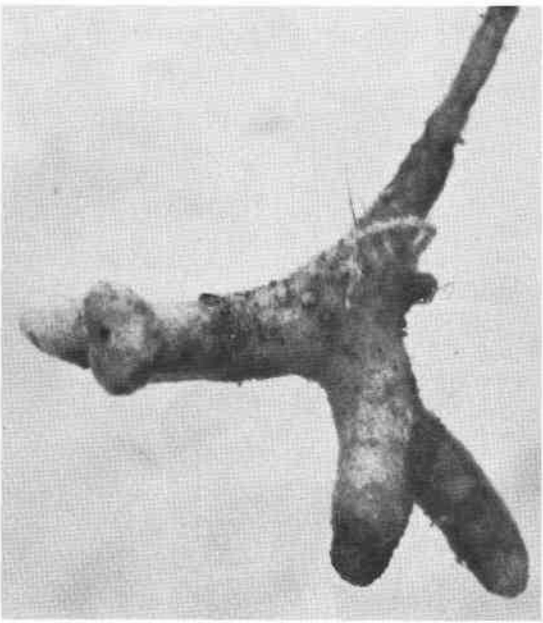


Figure 1. Typical dichotomous white ectomycorrhizae on 2-year-old ponderosa pine.

[1] Formerly Forest Pathologist, Forest Research Laboratory, School of Forestry, Oregon State University

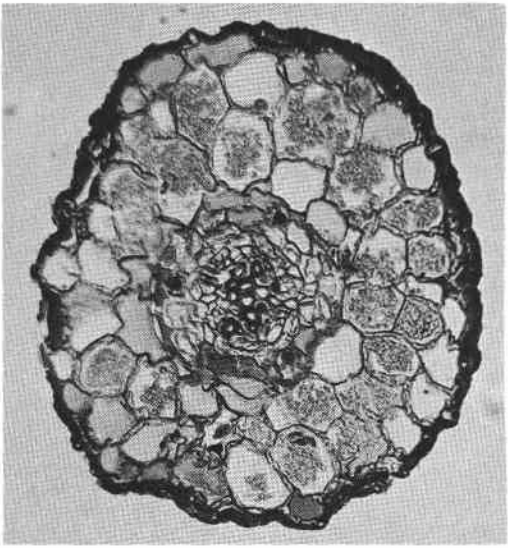


Figure 2. Magnified cross section of the mycorrhizal root tip of 2-0 ponderosa pine seedling, which shows the Hartig net and ectotrophic and endotrophic fungi.

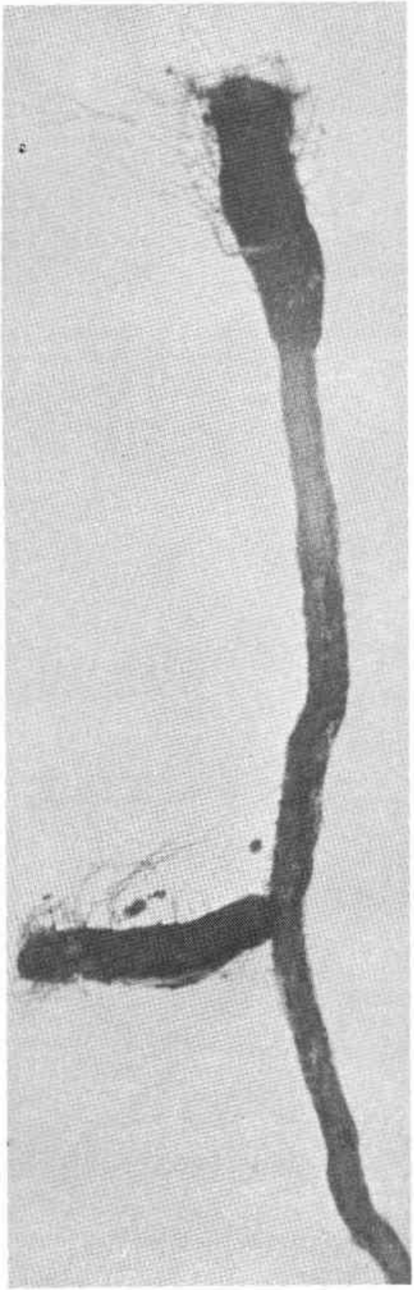
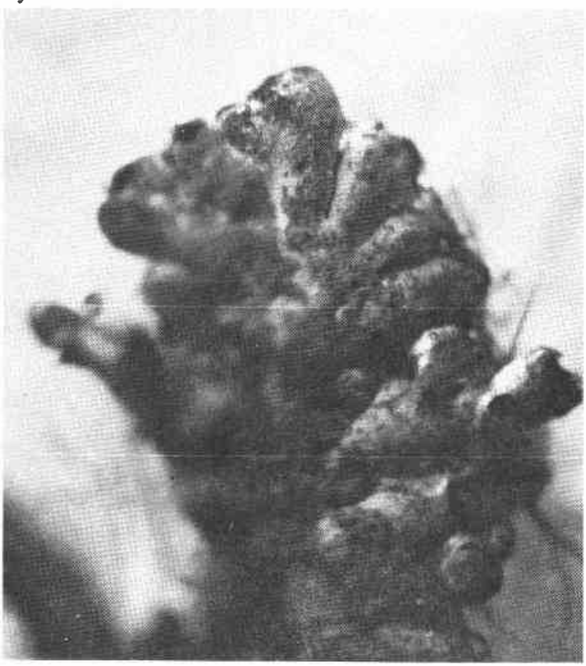


Figure 3. Digitate mycorrhizae composed of *C. graniforme* on 9-month-old Douglas-fir root.

Figure 4. Bluish-gray ectomycorrhiza on Douglas-fir root, 1½ years old.



EXPERIMENTAL

Mycorrhizal studies reported here are the result of several years of extensive research of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and ponderosa pine (*Pinus ponderosa* Laws.) seedlings. Pure cultural synthesis was not tried, and no attempt was made to maintain aseptic growing conditions, except for cultural isolation and dilution plate studies. This approach is justified on the basis that conclusions of the researchers cited have already established the symbiotic character of mycorrhizae (3, 19).

Mycorrhizae are classified as to color, form, quantity, and depth of occurrence. Absolute identification was not made except for *Cenococcum graniforme* (Sow.) Ferd. and Winge, which is distinctive and easily recognized (Figure 3).

Mycorrhizae with perceptible mantles were counted. This presented no problem except for occasional coralloid, racemose, or other compound mycorrhizae, for which mycorrhizal tips were tallied to obtain a comparable estimate of abundance (Figure 1).

Field plots and greenhouse tests were established by sowing seed of known origin, at constant depths. Artificial watering was not resorted to except in certain specific tests. In all field plots the seedlings were protected from deer and cattle by V-shaped tents of fine-mesh hardware cloth fastened at the base to metal rings (7). In some tests, illumination was regulated (Figure 5). All tests were replicated and randomized.

Seedlings were dug periodically and the roots carefully washed to free them of debris in preparation for detailed microscopic examinations. Rootlets were then examined with a 13-40 power binocular microscope as they floated in water in shallow dishes.

The effect of slash burning on mycorrhizal formation was studied only on Douglas-fir seedlings on sites where previously prepared slash piles had been burned in the spring or fall.

Slash piles for burning were constructed 6 feet high as follows:

Light burn—Douglas-fir slash up to 2 inches in diameter
Medium burn—slash and debris up to 6 inches in diameter
Hard burn—slash up to 1 foot or more in diameter

Soil temperatures at different depths were measured during burning by means of buried thermocouples, registering maximum thermometers, or by asbestos stakes coated with thermo-label paint.

In spring burns, seed was sown after the soil was cool.

Seed was sown in fall burns the succeeding spring at the same time spring burns were sown. (Soil had over-wintered.)

Replicated soil dilution plates were made before and after burning in several tests.

The study has been divided into the following phases:

1. Effect of soil temperatures on mycorrhizae and seedling growth.
    a. In forest soil, with and without shade
    b. After fall and spring burns
2. Relation of light and soil moisture to mycorrhizal development
3. Relation of soil fertility to mycorrhizal formation
4. Effect of seed source on chronological development and abundance of mycorrhizae.
5. Influence of soil fumigation and other nursery practices on mycorrhizae.

Figure 5. Field plot, showing hardware cloth tents. Insolation was varied by several thicknesses of muslin over the hardware cloth.

Effects of High Temperatures of Soil on Mycorrhizae

The effect of high temperature on the development of mycorrhizae was studied both in field and greenhouse tests.

Field Tests

Roots of 1-year-old Douglas-fir and ponderosa pine seedlings growing in plots exposed to direct sunlight were examined for mycorrhizae. The plots were located mostly on cleared south slopes in the Coast Range of western Oregon. Plot surfaces were bare of litter. Maximum soil temperatures were recorded during July and August at a standardized depth of 2 inches.

Seedlings were growing in these field plots at the time the soil temperatures were recorded, so there was dual effect of sunlight and soil temperature. The average depth of mycorrhizae, however, increased directly with increase of soil temperature (Table 1).

There was no indication that the most extreme temperature from insolation recorded for soil in these tests had any inhibitory effect on mycorrhizal development on Douglas-fir except depth of occurrence.

A quite different effect, however, may result when the soil is heated by slash burning before establishment of the seedlings.

Table 1. Average Numbers of Mycorrhizae and Short Roots on Unshaded 1-0 Seedlings of Douglas-fir and Ponderosa Pine in Mineral Soil.

Location	Slope	Seed-lings	Mycorrhizae: *C. graniforme*	Mycorrhizae: Other	Mycorrhizae: Total	Mycorrhizae: Avg depth	Short roots: Total	Short roots: Mycorrhizae
						Mm		%[1]
			DOUGLAS-FIR					
Burnt Woods[2]	North	8	3.7	23.0	26.7	53	14	77
	South	9	6.0	13.0	19.0	79	32	59
Black Rock[3]	Level	5	2.2	2.8	5.0	59	14	56
Mary's Peak[4]	South	5	1.6	8.2	9.8	76	--	--
			PONDEROSA PINE					
Black Rock[3]	Level	5	0.6	9.2	9.8	61	23	43

[1]Based on avg number of short roots on seedlings in quadruplicated plots.
[2]Lincoln County; elev., 1,000 ft; max. soil temperature, 78 F at 2-in. depth.
[3]Polk County; elev., 1,500 ft; max. soil temp., 82 F at 2-in. depth.
[4]Benton County; elev., 1,700 ft; max. soil temp., 114 F at 2-in. depth.

To determine the effect of slash burning on the development of mycorrhizae on Douglas-fir seedlings, a series of triplicate plots burned over in fall was established also in the Coast Range of Oregon.

On these fall-burned plots, the harder the burn the deeper mycorrhizae formed initially on the roots of 1-year-old Douglas-fir seedlings developing from seed sown the following spring (Table 2). Reduction in the number of mycorrhizae was evident, particularly on the roots of seedlings growing in hard burns. This agrees with Mikola *et al.* (15) for pine seedlings growing in hard burns in Finland.

During burning tests, soil temperature varied in intensity and duration at 2-3 inch depths. Light burns produced quick, hot, temperatures that reached 100-120 F at 2-inch depths. Medium burns, at the same depth, showed temperatures of 150-165 F. For hard burns, the temperatures increased more slowly to 182 F (17). Soil temperatures cooled to normal most quickly in light burns, required 3 hours longer in medium burns, and took 15 hours longer from hard burns to cool to normal than did medium burns. There evidently could be effects both from intensity of burn and duration of burn on soil microflora. The effect of burning on mycorrhizae is most recognizable 1 year after burning and becomes less clear on 2-year-old seedlings. Soil pH was also changed by slash burning from 5.0 in natural soil to less acid in medium burns (6.0-6.5) and in hard burns—such as occur when slash is burned in large piles (Figure 6)—to a pH of 7.5.

Comparison of Fall and Spring Burns

Burning slash in the spring was also compared with fall burning to determine whether there is a different effect on mycorrhizae and other soil microflora.

Triplicate fall and spring-piled slash of similar classes was burned on the same sites as previously described (Table 3).

Table 2. Average Number and Depth of Mycorrhizae on Douglas-fir Seedlings Planted Where Slash Had Been Burned in the Fall.

Seedlings	Burn class	Mycorrhizae: C. graniforme Number	C. graniforme Depth	Other Number	Other Depth	Total
			Mm		Mm	
6-7 months old[1]						
50	None	3.2	73	15.2[2]	59	18.4
32	Light	1.7	67	25.9[2]	52	27.6
31	Medium	0.8	95	11.3[2]	92	22.5
20	Hard	3.8	109	5.3[2]	118	9.1
14-18 months old[3]						
26	None	3.5	76	28.3[4]	68	31.8
18	Light	3.5	87	23.5[4]	62	27.0
19	Medium	4.2	92	13.3[4]	53	17.5
13	Hard	0.0	--	8.2[4]	65	8.2

[1]About 13-14 months after burning at five sites in the Coast Range.
[2]White, gray, and occasional red mycorrhizae.
[3]About 21 to 25 months after burning.
[4]White and gray mycorrhizae.

Table 3. Effect of Fall and Spring Burning on Average Height, Root Length, and Mycorrhizal Development of Douglas-Fir Seedlings.

Burn	Seedlings Number	Seedlings Height	Root length	Mycorrhizae: *C. graniforme*	White	Brown	Total
		Mm	Mm				
			SEVEN MONTHS OLD				
Fall							
None	4	41.2	107.5	1.2	47.7	0.0	48.9
Light	4	66.2	112.5	0.0	4.5	5.0	9.5
Hard	4	62.5	155.0	0.0	19.0	0.5	19.5
Spring							
None	4	40.0	91.2	0.5	29.2	0.0	29.7
Light	4	51.2	87.5	0.0	6.2	0.0	6.2
Hard	4	55.0	122.5	0.0	37.2	0.0	37.2
			ONE YEAR OLD				
Fall							
None	10	42.2	95.0	0.4	39.5	0.0	39.9
Light	7	60.4	99.3	0.0	0.0	16.4	16.4
Hard	7	61.4	135.7	0.0	20.6	0.0	20.6
Spring							
None	6	40.4	100.8	0.0	32.2	3.0	35.2
Light	5	53.0	143.7	1.0	5.4	0.0	6.4[1]
Hard	5	55.2	107.0	0.2	41.7	0.0	41.9

[1]Some roots, with no visual ectomycorrhizal mantles, when sectioned showed the beginning of Hartig nets.

Figure 6. An example of the burning of piled logging debris, which included large branches, chunks, and tops. Such hard burns change the texture of the soil and influence mycorrhizal formation.

Soil temperatures resulting from slash burning in the spring were higher than for the same class of fires burned in the fall. Temperatures of 120-150 F. were recorded at 2-inch depths in light burns and as high as 350 F in hard burns in the spring. These high temperatures were maintained after light burns for only 20-40 minutes and after hard burns, 40-60 minutes. This was considerably less prolonged than for fall burns.

Cenococcum graniforme did not appear on 7-month-old seedlings in all burns and was not observed until the seedlings were 1 year old and then only in light burns in spring. Effect on soil microflora in spring and fall burns may be caused by differences in the moisture content of slash and soil.

Triplicate burns of similar intensities were made on the same site in fall and spring.

Light burning in spring decreased the total number of mycorrhizae somewhat on seedlings, compared to fall burns, but Douglas-fir seedlings growing where there had been hard burns in fall showed significantly fewer mycorrhizae than for similar-age seedlings growing where there had been a hard burn in spring (Table 3). This definitely appears to be a matter of heat penetration and duration.

Areas lightly burned in the fall frequently contained denser stands of seedlings than in areas of medium or hard burns, sometimes denser than check plots as noted by Bever (1) and others.

Soil-Dilution-Plate Counts

Dilution counts were made to determine whether soil microflora generally showed a trend in burned areas similar to that of mycorrhizae.

Soil from areas lightly burned in spring showed a pronounced reduction in bacteria and streptomycetes at the beginning of the first growing season (Table 4). Generally, areas lightly burned in fall had marked increases in bacteria and streptomycetes. By the end of the first growing season, however, soil from both fall and spring burns had developed marked reductions in soil bacteria and streptomycetes. The increase in soil bacteria and streptomycetes in lightly fall-burned soil at the beginning of the growing season may have suppressed pathogenic fungi, which permitted better emergence and denser stands of seedlings, as noted.

In surface soil (0-3 inches) lightly burned in spring, the principal fungus was identified as *Cephalosporium humicola* Oud.; in soil lightly burned in fall, *Mucor rammannianus* Moller predominated. At greater depths (3-6 inches), *Penicillium decumbens* Thom accounted for

Table 4. Counts of Microorganisms on Soil Dilution Plates at Beginning and End of Growing Season.

Burn	Sample depth	Spring burns pH	Spring burns Bact.+ strep.	Spring burns Fungi	Fall burns pH	Fall burns Bact.+ strep.	Fall burns Fungi
	Inches		*Millions*	*Thousands*		*Millions*	*Thousands*
Beginning of first growing season							
None	0-3	--	100.6	78.2	--	24.3	75.7
None	3-6	--	20.5	86.0	--	6.6	48.0
Light	0-3	--	3.2	15.7	--	38.5	42.2
Light	3-6	--	2.9	31.5	--	23.9	61.2
Medium	0-3	--	5.2	6.5	--	20.6	39.0
Medium	3-6	--	0.6	26.5	--	34.6	62.5
End of first growing season							
None	0-3	6.0	15.6	74.0	6.5	13.0	67.0
None	3-6	6.5	18.2	82.5	6.5	12.0	52.0
Light	0-3	6.8	4.0	58.0	7.4	2.8	38.0
Light	3-6	6.6	2.2	16.5	6.8	3.1	36.5
Medium	0-3	6.8	3.5	59.0	7.4	3.7	8.0
Medium	3-6	6.6	4.2	60.0	6.9	2.0	31.0

about 20 percent of the soil molds after light burns in both spring and fall, with *Cephalosporium* spp. predominating.

In medium burns in spring, the surface soil contained principally *Cephalosporium* spp. At 3- to 6-inch depths, *Pythium vexans* de Bary comprised 90 percent of the mold count. After medium burns in fall, there was a great complexity of fungus flora. *Penicillium decumbens* was abundant both in the surface soil and at 3- to 6-inch depths.

In unburned check plots, *Penicillium decumbens* was also abundant in both surface and in 3- to 6-inch soil samples. Many other fungus mold species were present, particularly *Trichoderma lignorum* (Tode) Harz.

Numerically, soil fungi were reduced by light burns in spring more than by similar fall burns at the start of the growing season. By the end of the growing season, however, this trend was not so evident. Dilution plate populations showed the same decrease resulting from slash burning as did mycorrhizal formations.

On the basis of these tests, the conclusion was that mycorrhizal fungi and soil microflora are reduced by the thermal effect of slash burning. Seasonal variations appear of minor importance (27).

Mycorrhizae Observed

Black, white, and gray ectomycorrhizae were frequently observed on Douglas-fir seedlings growing in burned areas as well as in check plots (Figures 3, 4). Brown, yellow, and reddish mycorrhizae occurred occasionally. Single and double clamp connections were observed on ectomycorrhizae (Figures 7, 8). Clamp connections were never seen on *Cenococcum graniforme* hyphae.

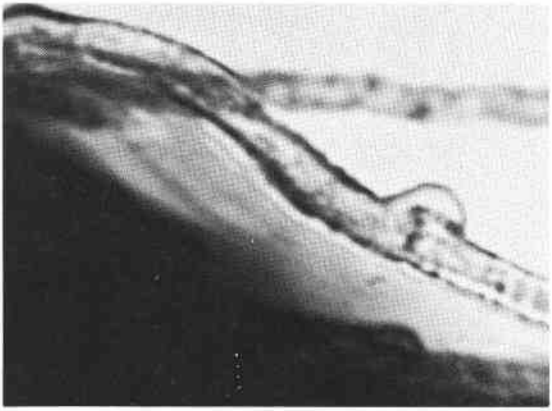
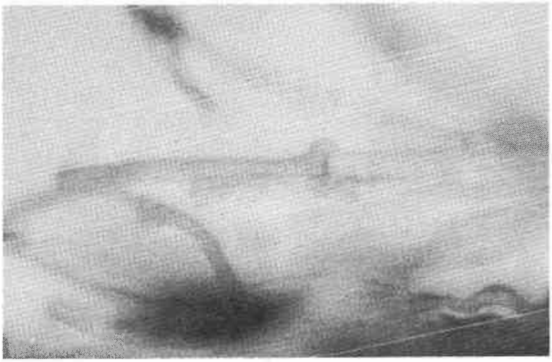


Figure 7. Single clamp connection on ectomycorrhizal hyphae growing on 2-year-old Douglas-fir.

Figure 8. Double clamp connection on ectomycorrhizal hyphae growing on 3-year-old Douglas-fir.



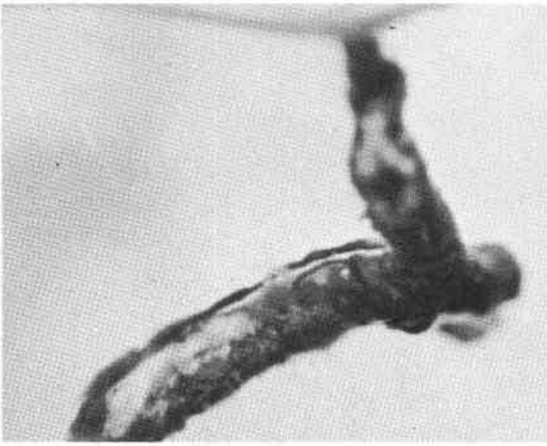
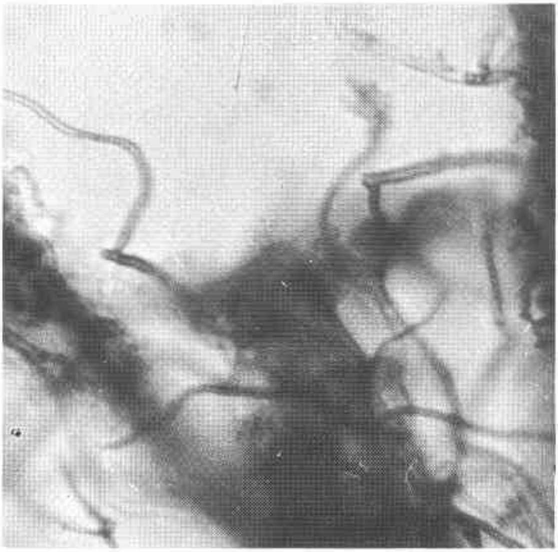


Figure 9. Fuzzy, black coenocytic hyphae of *Mycelium radicis atrovirens* (pseudomycorrhiza) over the white mycorrhizal mantle, on 1½-year-old Douglas-fir.

A phenomenon sometimes noted on root tips of overwintered Douglas-fir seedlings was a black-brownish, fuzzy, mycelial growth that covered the true mantle (Figure 9). This superficial growth was composed of fine coenocytic, olive-colored hyphae branching at right

Figure 10. Right angle branching characteristic of *Mycelium radicis atrovirens* on 1½-year-old Douglas-fir.



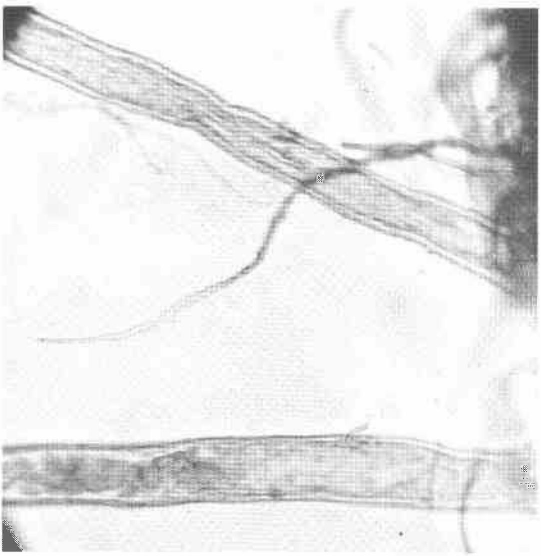


Figure 11. Small size of *Mycelium radicis atrovirens* shown with normal root hairs on 1½-year-old Douglas-fir.

angles (Figure 10). The function of this growth is unknown. The mycelial growth apparently is the weakly parasitic fungus classified by Melin as *Mycelium radicis atrovirens* (11). In the present study it was definitely not a part of the mycorrhizal mantle and appeared to be epiphytic. No haustoria of this fungus penetrated the root tissues. This sheath could, however, influence absorption of soil solutes. *Mycelium radicis atrovirens* has been aptly labeled as a pseudomycorrhizal fungus. The fineness of the hyphae contrasts with the size of the root hairs (Figure 11).

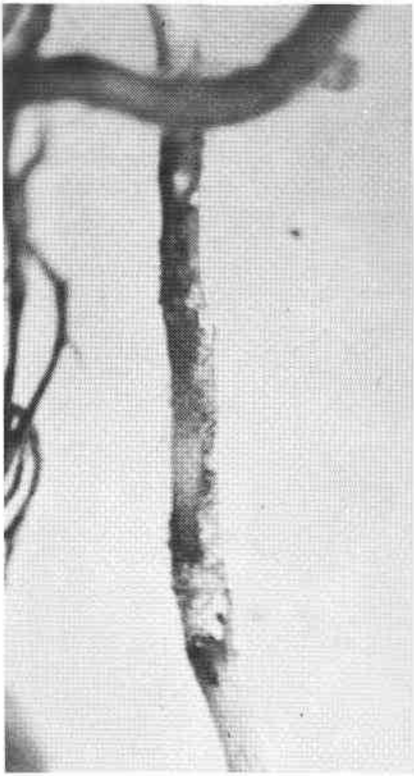


Figure 12. External appearance of mycorrhizal mantle on long root of 9-month-old Douglas-fir seedling.

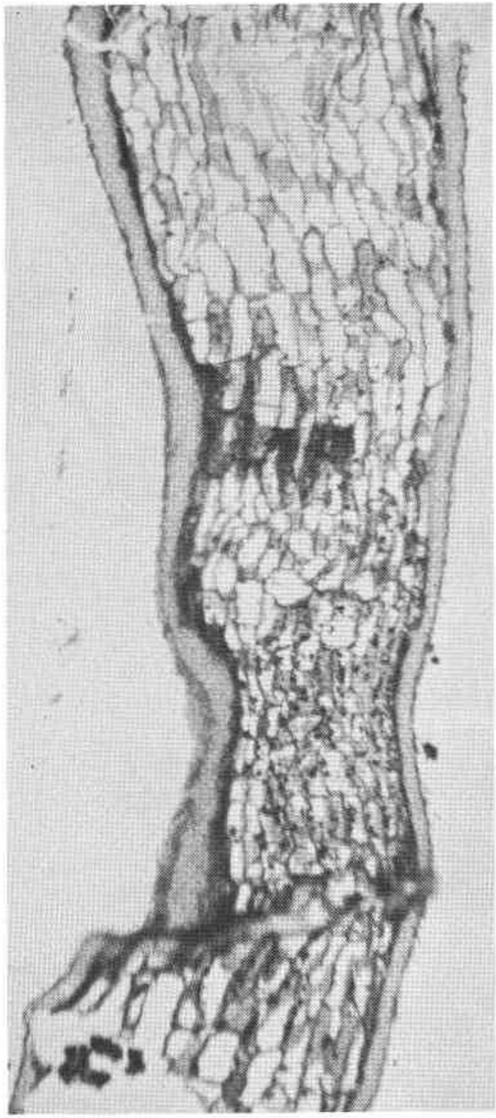


Figure 13. Longitudinal section of a long root from a 9-month-old Douglas-fir seedling showing extensive development of mycorrhizal mantle and Hartig net.

In agreement with Goss's findings (4), pseudomycorrhizae were not observed on overwintered seedlings of ponderosa pine.

Mycorrhizae on Douglas-fir seedlings were digitate, racemose, or fan-like (Figure 3), and some mycorrhizal mantles covered long roots (Figures 12, 13). Ponderosa pine mycorrhizae were white and dichotomously branched (Figure 1). Smooth, swollen root tips of ponderosa pine seedlings sometimes showed ectendomycorrhizae (Figure 2). For Douglas-fir, ectendomycorrhizae were less common, but did occur occasionally in very wet soil.

Greenhouse Tests

Seasonal and environmental factors are known to influence seedling growth and may thereby affect mycorrhizal formation (23). To minimize these influences, soil from several burns was removed to a greenhouse for further testing. The soil used was taken only from fall burns, to a depth of 3 inches. Crocks were filled with soil kept at field capacity and sown with seed of the same source planted in field trials. Artificial illumination of 1,000 foot-candles for 15 hours was provided. All pots were triplicated and randomly distributed. Table 5 summarizes the data from this series of tests.

Table 5. Effects of Fall Slash-Burning on Average Numbers of Mycorrhizae and Short Roots on Douglas-fir Seedlings Growing in Oregon Coast Range Soil,[1] from Greenhouse Tests.

Seedlings	Burn	Mycorrhizae		Short roots	
		C. graniforme	Other	Total	With mycorrhizae[2]
					Percent
		SIX MONTHS OLD			
Wolf Creek, Lincoln County					
5	None	0	278.0	571.0	48.6
5	Medium	0	0	388.4	0
		SEVEN MONTHS OLD			
Gold Creek, Polk County					
9	None	13.7	17.0	42.9	67.4
9	Light	7.5	24.6	73.7	40.8
10	Medium	1.7	20.5	85.0	25.8
10	Hard	0	12.3	73.6	12.3
Black Rock, Polk County					
5	None	0.4	105.2	105.6	57.7
5	Light	0	15.4	13.1	15.4
5	Medium	0.2	0	1.2	0.2

[1]Collected after overwintering except for Wolf Creek soil which was collected in the fall 48 hours after slash burning.
[2]Based on mean number of short roots.

Seedlings grown in unburned soil collected in the fall at Wolf Creek developed an extraordinary abundance of short roots, with heavy mycorrhizal formation. In these burning tests, hard burns in fall significantly reduced the percentage of mycorrhizae and substantiated the results for similar burns in field plots.

Seedlings grown in potted soil collected immediately after moderate burning, such as that from Wolf Creek, showed no external mycorrhizal mantles at 9 months of age. Mycorrhizae on seedlings in soil from light burns were largely white or brown, but those on seedlings in unburned soils were white and gray. Selective killing of mycorrhizae-forming fungi appears related to intensity of burn, but this was not clearly evident in soil from burns collected after overwintering.

Field test plots have already shown that high temperatures of soils in summer on severely exposed sites are not drastic enough to inhibit mycorrhizal formation on Douglas-fir seedlings. Similar results were reported for ponderosa pine (24). Bjorkman (2), however, has demonstrated that decreasing light also has an important influence on mycorrhizal formation by influencing photosynthesis and subsequent accumulation of carbohydrates in the roots. Testing the effect of different photoperiods on mycorrhizal formation appeared desirable, therefore.

Effects of Light and Moisture on Formation of Mycorrhizae

Field Tests

A series of quadruplicated, randomly arranged test plots was established on cleared, level plots at Black Rock (site II). Shade was varied by covering hardware-cloth cages with different thicknesses of muslin (Figure 5). The plots were unwatered except by natural precipitation. Daily soil temperatures were recorded at 2-inch depth on thermographs. All plots were thinned to the same seedling density. These plots were kept under observation for 18 months. Table 6

Table 6. Influence of Shade on Average Numbers and Depth of Mycorrhizal Formation on Douglas-fir and Ponderosa Pine Seedlings Planted in Site II Soil, from Field Tests.

Shade[1]	Seedlings	Mycorrhizae					
		C. graniforme		Other		Total	
		Number	Depth	Number	Depth	Number	Depth
Percent			*Mm*		*Mm*		*Mm*
				DOUGLAS-FIR			
Six months old							
0	5	2.2	53	2.8	59	5.0	56
45	4	1.8	47	4.6	53	6.4	50
56	5	1.8	51	2.8	50	4.1	50
78	5	3.0	61	5.0	58	8.0	59
Twelve months old							
0	5	2.6	81	4.8	71	7.4	76
45	4	2.7	72	4.7	75	7.4	73
56	4	0.7	52	7.0	57	7.7	54
78	4	0.7	60	0.2	50	0.9	55
Twenty months old							
0	3	5.3	115	7.0	89	12.3	102
45	5	0.6	133	11.6	69	12.2	101
56	3	9.6	116	18.8	97	28.4	106
78	3	1.7	89	7.3	100	9.0	94
				PONDEROSA PINE			
Six months old							
0	5	0.7	120	9.0	98	9.7	109
45	4	0.5	80	3.5	106	4.0	93
56	4	0.0	--	3.5	122	3.5	122
78	4	0.0	--	2.0	77	2.0	77
Twelve months old							
0	4	0.2	70	0.0	--	0.2	70
45	4	3.5	79	0.0	--	3.5	79
56	4	2.5	99	27.7	85	30.2	92
78	4	3.5	63	0.0	--	3.5	63

[1]No shade, 6,200 ft-c; 45 percent, 3,400 ft-c; 56 percent, 2,720 ft-c; 78 percent, 1,360 ft-c.

shows the influence of shade on mycorrhizal formation on Douglas-fir and ponderosa pine seedlings.

Reducing illumination to 22 percent did not consistently reduce the number of *Cenococcum graniforme* or other mycorrhizae. Because sunlight was no less than 1,300 foot-candles, however, this was not sufficient to verify either Bjorkman's (2) or Mikola's findings (14). These tests showed that both Douglas-fir and ponderosa pine form abundant mycorrhizae with only 25 percent of normal illumination. Depth of mycorrhizae, however, decreased with decreasing light except for the youngest Douglas-fir seedlings. A few pseudomycorrhizae (Figure 9) were observed on unshaded seedlings at 115 mm.

The highest summer temperature recorded was 90 F at 2-inch depth, which persisted for 2 hours. The minimum soil temperature recorded in winter was 34 F, which persisted for a week. The variation between shaded and unshaded plots was 2 degrees F, up or down, for lowest soil temperatures. For highest soil temperature, the range was 11 degrees F, up or down.

Because of variations in soil moisture and recorded differences in soil temperatures, studies were made on the effect of reduced illumination in a controlled environment.

Lightroom Tests

Triplicated, randomly distributed flats of seedlings of site II soil were exposed to fluorescent lights (1,000 foot-candles, more or less) in rooms with controlled temperature. Length of day ranged from 9 to 15 hours. Seedlings were subjected to several temperatures ranging from 40 to 78 F. In some tests, the soil was watered to field capacity weekly; in others, only once a month. Mycorrhizae were counted when terminal buds were formed, usually on 4-month-old Douglas-fir and on 5-month-old ponderosa pine. Data were subjected to analysis of variance based on the transformed values of the square root of mycorrhizal mantle counts.

Variation in soil temperature and moisture in these tests made no significant difference in number of mycorrhizae (Table 7). There was, however, a significant difference in number of mycorrhizae when variation in soil moisture was correlated with duration of photoperiod (Table 8). These data show that seedlings growing in wet soil with a 15-hour photoperiod had significantly more mycorrhizal mantles at the 1 percent level than seedlings in either wet or dry soil with 9- and 11-hour photoperiods. Seedlings with a 15-hour photoperiod in dry soil had significantly more mycorrhizae at the 5 percent level than those in wet or dry soil with a

Table 7. Relation of Temperature and Soil Moisture to Averages of Mycorrhizal Mantle Counts on Douglas-fir Seedlings, 4 Months Old.

Temperature	Wet soil	Dry soil	Differential
Deg F			
Nine-hour photoperiod			
50-78	10.6	15.8	-5.2
60-78	9.8	5.8	+4.0
70-78	6.4	10.6	-4.2
Fifteen-hour photoperiod			
50-78	60.4	45.4	+15.0
60-78	65.0	34.4	+30.6
70-78	21.2	14.0	+7.2

Table 8. Relation of Soil Moisture and Photoperiod to Mycorrhizal Counts for Douglas-fir Seedlings, 4 Months Old.[1]

Soil	Photoperiod, hours				
	9	11	13	15	Means
Wet	3.66	5.40	6.71	8.81	6.14
Dry	4.18	5.32	6.59	6.64	5.68
Means	3.92	5.36	6.65	7.72	---

[1]Significance at the 1 percent level 3.06, and at the 5 percent level 2.18; coefficient of variation 17.1 percent.

Table 9. Relation of Soil Temperature and Photoperiod to Mycorrhizal Counts for Douglas-fir Seedlings, 4 Months Old.[1]

Temperature	Photoperiod, hours				
	9	11	13	15	Means
Deg F					
50-78	4.62	6.76	8.61	9.32	7.33
60-78	3.52	4.80	6.46	8.93	5.86
70-78	3.62	4.78	4.86	4.91	4.54
Means	3.62	5.45	6.64	7.71	---

[1]Significance at the 1 percent level, 0.89 and at the 5 percent level, 0.64; coefficient of variation 17.1 percent.

9-hour photoperiod. Thus, soil moisture and photoperiod proved interrelated; neither was independent of the other.

Analysis of variance indicated that there was also an interrelation between soil temperature and length of photoperiod. Seedlings grown at 50-78 F with a 15-hour photoperiod had significantly more mycorrhizae at the 1 percent level than seedlings subjected to 9- and 11-hour insolation periods (Table 9). At 60-78 F and 15 hours of light there were also more mycorrhizal mantles at the 1 percent level than at any of the other photoperiods. At the highest temperature range (70-78 F), only those seedlings grown under the shortest photoperiod developed significantly fewer mycorrhizae than those with longer exposures to light; otherwise there were no significant increases or decreases. Here again, temperature and length of light-day were interrelated and neither was independent of the other.

A similar light-room test for 5-month-old Douglas-fir seedlings with a somewhat wider temperature range (40-78 F) and the same photoperiods showed similar interrelations between moisture, temperature, and duration of photoperiod.

Because photoperiod was related to both temperature and soil moisture for Douglas-fir seedlings, data were analyzed for kinds of mycorrhizae. The percentages of *C. graniforme* and other mycorrhizae were determined for short- and long-day insolation and for soils of different moisture content (Table 10 and Figures 14 and 15).

On the basis of percentage of mycorrhizae (Table 10), *C. graniforme* predominated only in wet soil with a short-day (9-hour) illumination. Perhaps of even more significance was the effect of a long photoperiod on *C. graniforme*, which formed less than a third of the total mycorrhizal population in both wet and dry soil. These findings agree with Mikola's (14) conclusions on the effect of reduced light on *Cenococcum* on pine in Finnish forests.

Table 10. Effect of Photoperiod and Soil Moisture on Kind of Mycorrhizae on Douglas-fir Seedlings, 4 Months Old.

Soil	9-Hour photoperiod		15-Hour photoperiod	
	Cenococcum	Other	*Cenococcum*	Other
	Percent	*Percent*	*Percent*	*Percent*
Wet	58	42	31	69
Dry	41	59	28	72

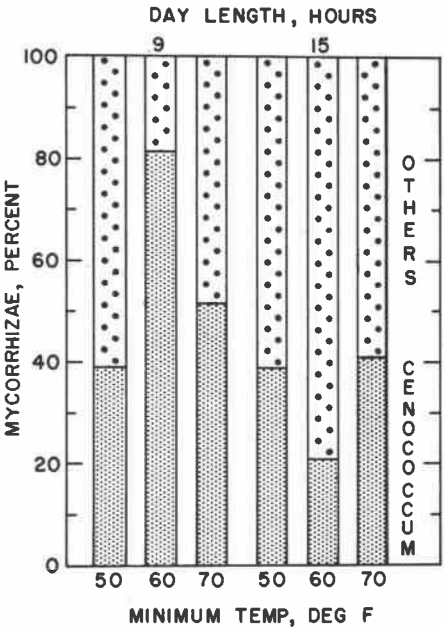


Figure 14. Relation of day length and minimum temperature of the soil to the percentage of *Cenococcum* among mycorrhizae on 4-month-old Douglas-fir in moderately dry soil (one-half field capacity). Maximum temperature was 70 F.

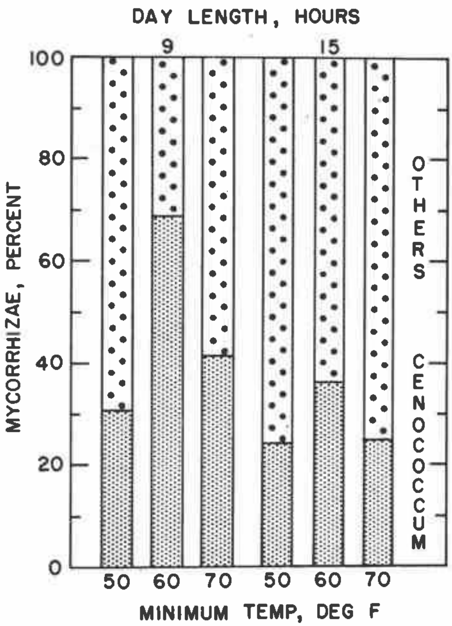


Figure 15. Relation of day length and minimum temperature of the soil to the percentage of *Cenococcum* among mycorrhizae on 4-month-old Douglas-fir in soil maintained at field capacity. Maximum temperature was 70 F.

The tests so far presented have been on environmental factors affecting mycorrhizal development on Douglas-fir seedlings. Similar tests were also made for ponderosa pine, though less extensively than for Douglas-fir. Ponderosa pine seedlings were grown under lights in sandy loam soil from a natural pine stand at Indian Ford, Deschutes County, Oregon. The soil was held at field capacity. Temperatures ranged from 40 to 90 F.

Ponderosa pine seedlings grown with a 15-hour photoperiod at 56-80 degrees F and soil moisture at field capacity showed significantly more mycorrhizae than those grown at 50-75 F

Table 11. Relation of Soil Temperature and 15-Hour Photoperiod to Formation of Mycorrhizal Mantles on Ponderosa Pine Seedlings, 5 Months Old.[1]

Seedlings	Temperature	Mean no. mantles
	Deg F	
12	50-75	8.0
12	56-80	23.4
12	40-90	18.2

[1]Least significant difference was 5.84 at the 1 percent level and 4.34 at the 5 percent level; coefficient of variation, 31.9 percent.

with the same day length and soil moisture (Table 11). There was also a significant increase at the 5 percent level of probability in number of mycorrhizal mantles on seedlings grown at 56-80 F compared with those grown at 40-90 F.

Analysis of variance for ponderosa pine seedlings indicated that soil temperatures and duration of photoperiod are interrelated in mycorrhizal formation.

The development of *C. graniforme* mycorrhizae on ponderosa pine was so sparse in lightroom tests as to preclude analyses on effects of temperature and soil moisture.

Lightroom and greenhouse tests have thus helped to clarify the irregularities of mycorrhizal observations on seedlings in field plots.

Effects of Soil Fertility on Formation of Mycorrhizae

Nutritional requirements of mycorrhizal fungi have been widely investigated (3, 5, 16). In the present study, soil-fertility tests were undertaken to gain supplemental information applicable to mycorrhizal formation only on Douglas-fir seedlings.

Field Tests

A poor forest soil (site V quality) at Black Rock was selected for treatment with various amounts of ammonium nitrate before sowing Douglas-fir seed. The effect of these treatments on mycorrhizal counts is shown in Table 12.

In the tests with ammonium nitrate, it was not always possible to positively separate *C. graniforme* mycorrhizae from dark colored hyphae of *Mycelium radicis atrovirens*. As amounts of ammonium nitrate increased from 150 to 450 pounds per acre, there was an inconsistent increase of all black mycorrhizae and a decline in abundance of other mycorrhizae. Pseudomycorrhizae were frequently mixed with the mantle sheath of true mycorrhizae (Figure 16, bottom). These observations correspond to reports by Levisohn (9)

Table 12. Effect of Ammonium Nitrate on Average Number of Mycorrhizal Formations on 2-0 Douglas-fir Seedlings Growing in Poor Soil (Site V) from Field Test.

Ammonium nitrate	Mycorrhizae		
	Blackish	Other	Total
Lb/acre			
None	26.2	25.4	51.6
150	24.0	24.0	48.0
300	47.8	2.8	50.6
450	21.8	9.0	30.8

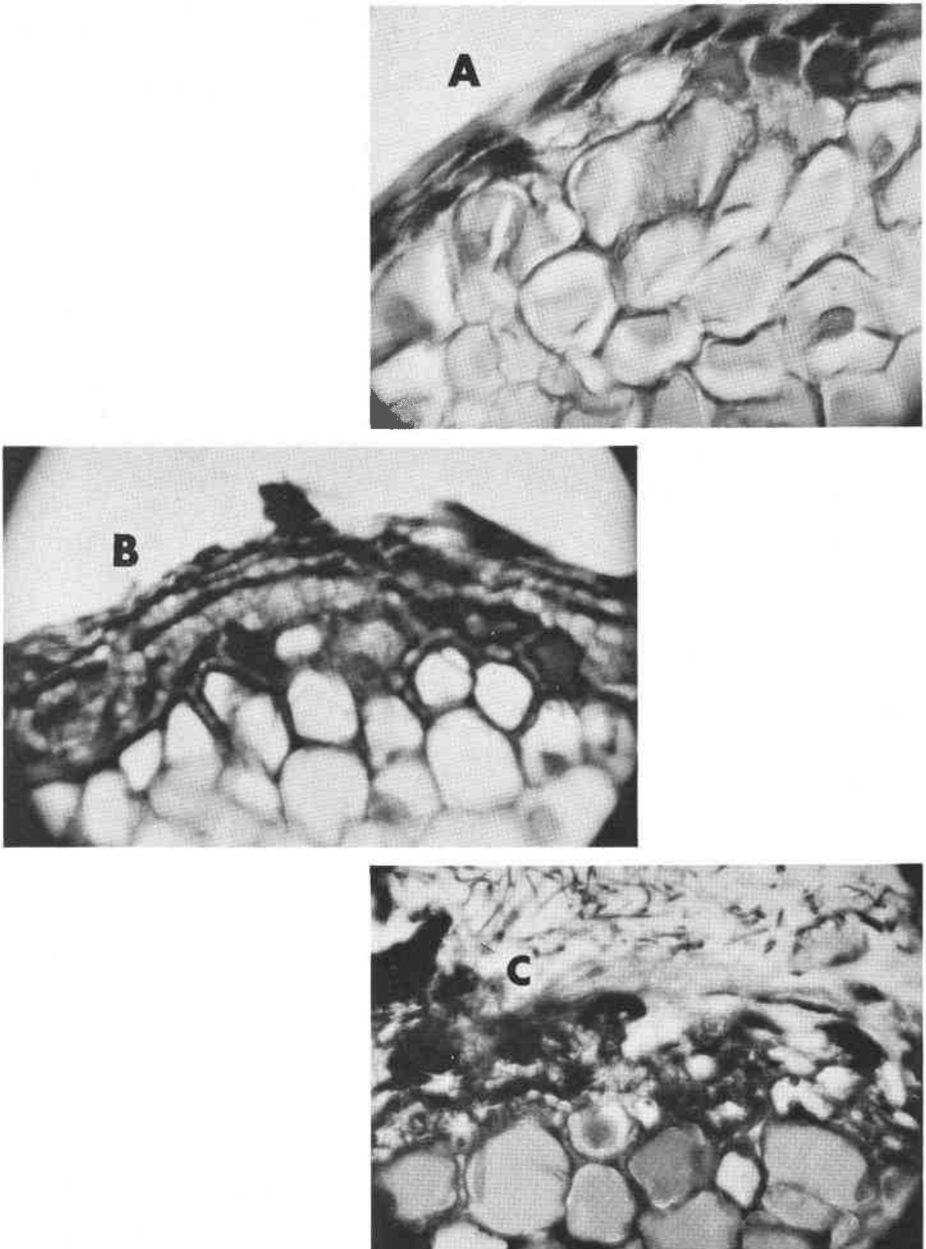


Figure 16. Mycorrhizal mantle and Hartig net on 2-0 Douglas-fir seedlings grown in three soils: A, site I soil from Burnt Woods; B, site II soil from Black Rock; C, site V soil from Black Rock.

and others who found weakly parasitic fungi especially common on pine and spruce seedlings in soils with high levels of available nitrogen. At 150 pounds of nitrogen to the acre, there were also a few brown mycorrhizae (Figure 17).

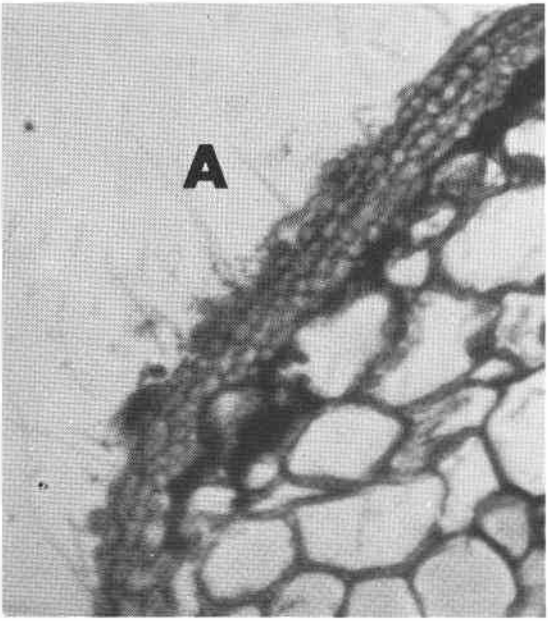
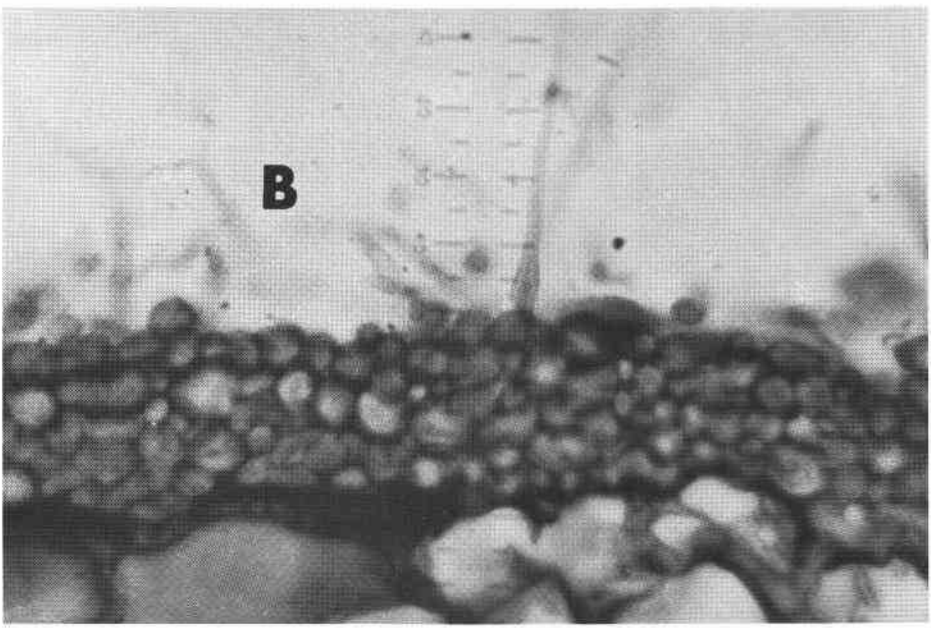


Figure 17. Brown hyphal mantle on Douglas-fir seedlings grown in site V soil at Black Rock, to which ammonium nitrate had been added at 150 pounds to the acre. A, profuse development of external hyphae aroung the mantle; B, detail of hyphal strands, showing attachment of external hyphae to mantle cells.



Microtome sections of mycorrhizal roots of seedlings from all three soils failed to show consistent differences in mantle thickness or Hartig-net formation.

The interesting mycorrhizal associations of the 2-year-old seedlings in plots where ammonium nitrate was applied led to extensive tests under uniform environment in light rooms.

Lightroom Tests

Soil from the surface foot of three different forest sites was collected, sieved, and placed in foot-tall ceramic pots. Wilting point was determined for each soil, and moisture levels were held at field capacity. The same number of Douglas-fir seeds from hand-pollinated mid-Willamette trees were placed in each pot and subjected to daily illumination of 15 hours at 1,000 foot-candles. After the seedlings had passed through 4 cycles in 2 years and formed the fourth set of terminal buds, they were harvested and the roots examined for mycorrhizae (Table 13). In some tests, the soil and seedlings were analyzed for nutritional properties. Percentage of mycorrhizae was determined from counts of short roots. Size of seedlings was calculated from measurements of roots and shoots and from ovendry weight.

McComb (10, 11) and Mitchell *et al.* (16) found that additions of phosphorus increased absorption and stimulated metabolic sequences for seedlings with good mycorrhizal development, which increased seedling growth.

The present tests also indicated that phosphorus stimulated mycorrhizal formation on Douglas-fir seedlings. Hence, the increase in number of beneficial mycorrhizae was at least partially responsible for improved seedling growth as indicated by increased ovendry weights.

Heavy applications of nitrogen promoted development of black mycorrhizae, which included the parasitic type (13). The presence of *Mycelium radicis atrovirens*, therefore, may help explain the reduced weight of seedlings growing in heavily nitrogen-enriched soil.

The explanation for these differences in mycorrhizal formation is related, at least partially, to photosynthesis and carbohydrate formation (2). The brown mycorrhizae, in particular, extended hyphae only to a limited extent into the soil. On the other hand, there was evidence of ectendomycorrhizae, especially on seedlings in soil with moderate additions of nitrogen (Figure 18).

Table 13. Relation of Site and Growth of Douglas-Fir Seedlings to Number of Mycorrhizae after Four Growth Cycles.

Soil	Weight		Mycorrhizae			Short roots	
	Top	Roots	*Cenococcum*	Other	Total	Total	With mantles
	Grams	*Grams*					*Percent*
Site II very good	2.08	1.64	0	921	921	1,039	88.6
Site II good	1.29	0.96	0	1,208	1,208	1,283	94.2
Site V poor	0.32	0.32	153[1]	115	268	577	46.4

[1]Thirty-five pseudomycorrhizae (*Mycelium radicis atrovirens*) not included.

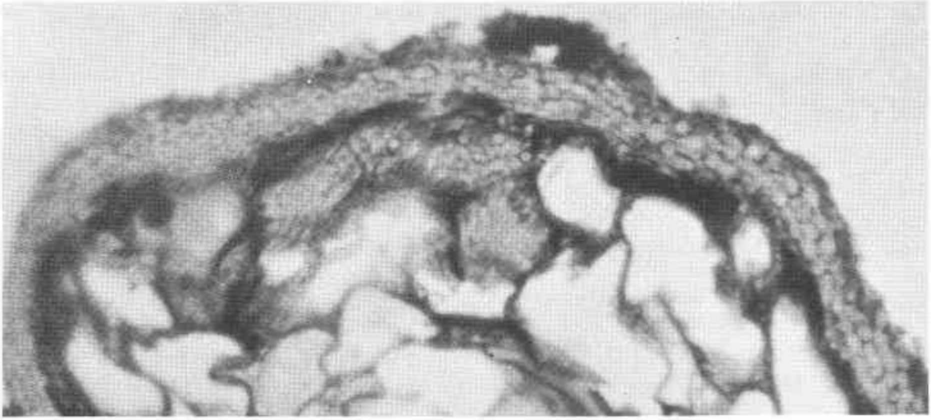


Figure 18. Section across a root tip of 2-0 Douglas-fir grown in site V soil from Black Rock, to which ammonium nitrate had been added at 150 pounds to the acre.

The chemical composition of the soils in which the seedlings were grown is given in Table 14. The mineral uptake of the Douglas-fir seedlings grown on the three sites is shown in Table 15.

Table 14. Chemical Composition of Soils.

Soil[1]	Total Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Total bases
	Percent	*Lb/acre*	*Lb/acre*	*Mg/100g*	*Mg/100g*	*Mg/100g*
Site II very good	0.436	41	1,856	7.7	5.1	15.18
Site II good	0.252	37	686	8.3	2.1	11.28
Site V poor	0.154	8	515	2.8	1.5	4.96

[1]High site II, pH 6.2; good site II, 5.8; poor site V, 5.5.

Table 15. Mean Uptake of Five Minerals by Douglas-Fir Seedlings after Four Growth Cycles.

Soil	Nitrogen[1]	Phosphorus[2]	Potassium[3]	Calcium[3]	Magnesium[4]
	Mg	*Mg*	*Mg*	*Mg*	*Mg*
Site II very good	31.56	7.55	28.43	11.18	4.26
Site II good	17.10	5.36	16.42	5.49	2.34
Site V poor	3.96	0.78	3.05	0.77	0.58

[1]Total nitrogen by Kjeldahl process.
[2]Fiske-Subbanow method.
[3]Potassium and calcium by flame photometer.
[4]Titan yellow method (Drosdoff-Nearpass).

Average weight of root and top growth on an ovendry basis is given in Table 16 and difference in size is shown in Figure 19.

The data in Tables 13 to 16 showed that soil with high natural fertility had superior seedlings (tops and roots) with the highest mineral uptake and exceptionally good development of mycorrhizae (29). The difference in pH of the soils was slight and was of no apparent significance in this test.

Table 16. Ratio of Weight of Tops and Roots to Number of Mycorrhizae Listed in Table 13.

	Tops	Roots
Site II very good	0.0023	0.0018
Site II good	.0012	.0008
Site V poor	.0012	.0012

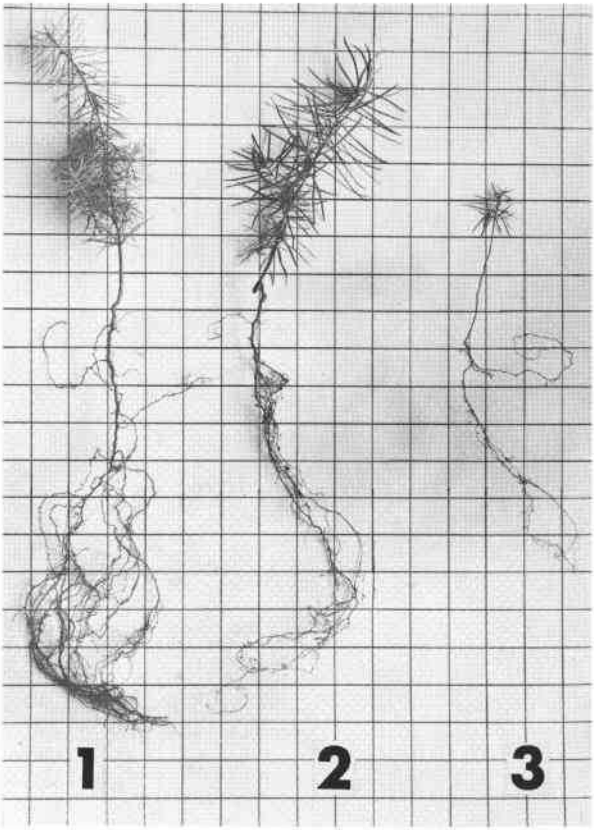


Figure 19. Comparative size of 24-month-old seedlings of Douglas-fir, grown on an accelerated schedule in a controlled-environment room. 1, Seedlings grown in site I soil from Burnt Woods; 2, in site II soil from Black Rock; and, 3, in site V soil from Black Rock.

Douglas-fir seedlings were also grown on soil from the poor site (V) to which various amounts of nitrogen, potassium, and phosphorus were added (Table 17).

Application of phosphorus and potassium without addition of nitrogen increased other than black mycorrhizae, but increase in nitrogen beyond 150 parts per million eliminated all other ectomycorrhizae and left only *C. graniforme.*

Heavy applications of nitrogen (300 pounds per acre) did not increase seedling weight unless phosphorus was also added. The greatest number of mycorrhizal mantles occurred on seedlings growing in the poor soil with moderate additions of phosphorus plus potassium but no supplemental nitrogen.

Microtomed sections of mycorrhizal roots of seedlings from all three soils failed to show consistent differences in mantle thickness and formation of Hartig nets.

The mean mineral uptake of Douglas-fir seedlings may, however, show different relations between soil fertility and number of mycorrhizae (Tables 13, 15).

An interesting feature of these tests is that seedlings in soil of highest natural fertility had significantly more mycorrhizae than did seedlings in soil of low fertility and the seedlings were definitely more vigorous. Also, Douglas-fir seedlings growing in the richest soil had more kinds of mycorrhizae than did those in the poorest soil. On the basis of color alone, poor soil had primarily two kinds of mycorrhizae—black and white—but six kinds were observed on seedlings in the most fertile soil—white, black, gray, brown, orange, and yellow. Seedlings from site II soil, which was not so fertile as the best soil, had almost exclusively white mycorrhizae. Phosphorus uptake by Douglas-fir seedlings grown in poor soil was particularly low (Table 15). The significance of these observations can be, of course, only speculative.

Differences in mycorrhizal formation and better seedling growth as related to soil fertility indicate the need for careful determination of soils and fertilizers in nurseries to obtain maximum benefits and good mycorrhizal development.

On the basis of experimental results, Hatch (6) suggested that internal nutrient status, especially with respect to nitrogen, potassium, and phosphorus, was the prime factor in determining the intensity of infection. When the internal concentrations of these elements are high and balanced, the seedlings became resistant to infection by mycorrhizal fungi.

Table 17. Effect of Nitrogen, Phosphorus, and Potassium on Formation of Mycorrhizal Mantles and on Mean Weight of 7-Month-Old Douglas-Fir Seedlings Planted in Poor Soil (Site V) from Light-Room Tests.

Nitrogen	Phosphorus	Potassium	Mycorrhizae			Seedling weight
			Black[1]	Other	Total	
Ppm	*Ppm*	*Ppm*				*Grams*
0	0	0	62.3	185.4	247.7	0.58
0	100	50	60.0	252.0	312.0	0.97
150	100	0	13.3	139.6	152.9	1.29
150	0	50	9.0	36.3	45.3	1.66
300	0	0	15.6	0.0	15.6	0.90
300	200	0	10.3	0.0	10.3	1.33
300	200	100	38.0	0.0	38.0	0.58

[1] *Cenococcum graniforme + Mycelium radicis atrovirens.*

Effects of Seed Source on Formation of Mycorrhizae

The one factor responsible for early mycorrhizal formation that appears independent of all others is seed source (28). To expand the basis for this conclusion, additional tests in field and light room were made on the development and abundance of mycorrhizal mantles on Douglas-fir and ponderosa pine. Several phenotypic forms of Douglas-fir and ponderosa pine are known.

Field Tests

Five replicated plot series were located in widely separated areas (Table 18). Douglas-fir and ponderosa pine seed were obtained from six locations for each species (Table 19). Douglas-fir seeds from several altitudes on north and south slopes in a southern Oregon locality also were tested.

Douglas-fir. The most complete previous test of seed source noted for Douglas-fir was for seedlings grown at an Oregon State forest nursery near Corvallis (28). Seedlings from far north and seed sources at high elevation had significantly greater numbers of mycorrhizal mantles on 11-month-old seedlings than on the same age seedlings from a southern Oregon seed source. Seedlings 18 and 24 months old, however, did not show significant statistical differences in mantle counts.

To supplement earlier tests made in nursery seedbeds, plots were established in cleared forest areas 15 miles north of Corvallis, near Monmouth, and about 70 miles south, at Dorena Dam near Cottage Grove, Oregon. These plots were at low elevations (about 300 feet) (Table 20).

The tests again showed that young Douglas-fir seedlings from seed source 14 had significantly fewer mycorrhizae than seedlings from the other seed sources tested, except in the Monmouth plots where delayed germination influenced seedling age.

Two additional seed-source tests were made in plots located farther north and south where differences in day length could have a more pronounced effect. To obtain uniform edaphic conditions, these plots were established in a British Columbia forest nursery at Duncan on Vancouver Island and in an Oregon State forest nursery at Elkton, Oregon. Four

Table 18. Location of Field Plots Used for Tests of Seed Sources.

Locality	North Latitude	Elevation
	Deg:Min	*Feet*
Douglas-fir		
Elkton, Douglas Co., Ore.	43°30'	150
Dorena Dam, Lane Co., Ore.	43°45'	300
Monmouth, Polk Co., Ore.	44°45'	200
Duncan, Vancouver Island, B.C.[1]	48°45'	1,500
Ponderosa pine		
Shasta, Shasta Co., Calif.	41°30'	3,500
Elkton, Douglas Co., Ore.	43°30'	150
Bend, Deschutes Co., Ore.	44°	4,000
Monmouth, Polk Co., Ore.	44°45'	200

[1]In cooperation with the British Columbia Forest Service and Duncan Nursery Supervisor, Jack Long.

Table 19. Data on Seed Sources.

No.	Locality	North Latitude	Elevation
		Deg:Min	*Feet*
Douglas-fir			
14	Siskiyou Co.,	42°30'	3,000
8	Marion Co., Ore.	45°	200
7	Tillamook Co., Ore.	45°30'	2,000
6	Thurston Co., Wash.	47°	500
15	Vancouver, B.C.	48°	800
17	Bella Coola, B.C.	51°30'	1,100
Ponderosa pine			
1[1]	Custer National Forest, S.D.	44°30'	5,000
2	Coconino National Forest, Ariz.	36°	5,000
3	Shasta National Forest, Calif.	41°30'	3,900
4	Deschutes National Forest, Ore.	44°	5,000
5	Colville National Forest, Wash.	48°30'	4,000
6	Chelan National Forest, Wash.	48°30'	3,500

[1]Used in only a few tests.

Table 20. Douglas-Fir Seed Source and Mantles Formed in Forest Soil.[1]

Seed source	Seedling age, *months*	
	8 (at Monmouth)	13 (at Dorena Dam)
6	1.96[2]	1.95[3]
8	3.24[3]	0.0
14	0.01	0.43
15	6.80[3]	2.85[3]
17	0.60	2.13[3]

[1]Transformed values of the square roots of mycorrhizal counts.
[2]Significantly greater than seed source 14 at the 5 percent level of probability.
[3]Significantly greater than seed source 14 at the 1 percent level of probability.

replications were made of randomly distributed plots. The results of these tests are presented in Table 21.

The plots at Duncan, British Columbia, were fairly close to the collection sites for seed sources 15 and 17. The nursery plots at Elkton were under climatic conditions similar to those of the collection site for seed source 14 (Southern Oregon).

Seedlings from seed source 14 again had fewer mycorrhizae in both nurseries, but the difference was not so statistically significant as in previous tests (28). Seedlings from seed source 8, collected in the Willamette Valley at a low elevation, showed surprisingly few mycorrhizae in the Elkton test. The greatest number of mycorrhizal mantles was observed on seedlings from seeds collected at low elevation in Thurston County, Washington.

Ponderosa pine. Differences in size and color of ponderosa pine seed are common. Ponderosa pine seeds used in these tests were obtained from a wide geographic area by courtesy of the U.S. Forest Service (Table 19). Limited use of seed from the Black Hills

(Custer National Forest) precluded its evaluation. The seeds most thoroughly tested came from Arizona (Coconino), California (Shasta), Oregon (Deschutes), and Washington (Colville) national forests. Table 22 shows locations of test plots.

The field test plots indicated no significant difference in mycorrhizal mantle counts on Arizona and California ponderosa pine seedlings grown at a nursery near Shasta, California. Seedlings grown from Oregon and Washington seeds, however, did show significantly more mycorrhizae than those from either of the southern seed sources. At the Bend nursery, east of the Cascade Mountains and about 175 miles due north of Shasta, 1-year-old seedlings from the most northern seed source (Colville) showed significantly more mantles (at the 1 percent level) than the Arizona seedlings. Both Bend and Shasta nurseries are at 5,000-foot elevation. At Monmouth, Oregon, about 75 miles farther north than Bend but west of the Cascade Mountains, there was also a highly significant increase in number of mycorrhizae for seedlings from all three northern seed sources as compared to the Arizona seedlings. Again, when the seedlings were 2 years old, these differences were less pronounced.

Table 21. Douglas-Fir Seed Source and Mantles Formed in Nursery.[1]

Seed source	Seedling age, *months*	
	6	12
At Duncan, B.C., (latitude 45° 45' N.)		
6	36.3[1]	--
8	20.5	--
14	10.0	--
15	19.8	--
17	26.5[1]	--
At Elkton, Oregon, (latitude 43° 45' N.)		
6	5.4	156.2[1]
8	0.0	4.8
14	0.0	16.0
15	0.0	29.0
17	3.8	25.6

[1]Transformed values of the square roots of mycorrhizal counts.
[2]Significantly greater than seed source 14 at the 5 percent level.

Table 22. Mycorrhizal Mantles on Ponderosa Pine[1] Seedlings.

Plot location	Seedling age	Seed source			
		Arizona	California	Oregon	Washington
	Years				
Shasta, California	1	5.46	4.73	31.94	15.11
Bend, Oregon	1	11.60	27.78	27.98	33.67
	2	42.24	49.86	40.84	45.70
Monmouth, Oregon	1	0.00	24.61	8.73	27.89
	2	3.86	35.96	14.06	40.32
Elkton, Oregon	2	0.00	35.23	23.98	33.54

[1]Least significant difference was 3.966 at the 1 percent level and 2.918 at the 5 percent level; coefficient of variation was 59.8 percent.

Table 23. Relation of Seed Source, Temperature, and Photoperiod to Formation of Mycorrhizal Mantles in Douglas-fir Seedlings, 4 Months Old. Soil Moisture Was at Field Capacity.[1]

Temp	Time	Source		
		7	14	17
Deg F	*Hours*			
40-78	9	4.48	3.00	4.69
	15	31.45	9.47	27.01
50-78	9	10.93	9.34	5.02
	15	21.11	18.93	9.01
70-78	15	44.44	36.61	40.53

[1]Least significance was 1.679 at the 1 percent level and 1.269 at the 5 percent level; coefficient of variation was 17.7 percent.

The Arizona ponderosa pine seedlings had significantly fewer mycorrhizal mantles than the seedlings from other seed sources. Differences between seedlings from seed sources did vary, but seedlings from the northern seed (Colville) most consistently produced seedlings with significantly more abundant mycorrhizae. Seedlings from the southern seed sources for ponderosa pine, therefore, showed the same late trend toward fewer mycorrhizal mantles as found for Douglas-fir seedlings.

Further evaluation of seed source and mycorrhizal formation obviously required additional light-room tests.

Lightroom Tests of Douglas-Fir Seeds

Previous tests did not show clearly that variation in formation of mycorrhizal mantles on seedlings from northern and southern seed sources was caused by light requirements. To test further the effect of light, tests were made in light chambers where not only insolation, but also soil moisture and temperature were controlled. Three Douglas-fir seed sources were tested: one from British Columbia, 17; another from northern Oregon, 7; and a third from southern Oregon, 14 (Table 23).

Southern seed source 14 showed significantly fewest mycorrhizal mantles at 40-78 F and 70-78 F in both short-day and long-day illumination. At 50-78 F, however, northern source 17 seedlings had significantly fewer mycorrhizal mantles than seedlings from source 14 or 7, as was also noted for seedlings from seed source 17 planted in the Monmouth, Oregon, field plots.

Under long-day photoperiods, seedlings of the northernmost seed source, 17, were expected to form mycorrhizal mantles earlier than seedlings from seed source 14, from southern Oregon. Because mycorrhizal mantles were fewer on seedlings from source 17 under short as well as long photoperiods at 50-78 F, seed collected from different elevations and aspects in the same southern geographic area as source 14 was tested for mycorrhizal development (Table 24).

There were no statistically significant differences shown in total mycorrhizal mantle counts on seedlings grown from southern Oregon seed from different elevations or from north and south slopes. Neither was there any significant correlation found between number of short roots, growing root tips, and laterals or length of main root with the number of mycorrhizae on these Douglas-fir seedlings.

On the basis of field and light-room tests, the conclusion was that for Douglas-fir seedlings, differences in rate of mycorrhizal mantle formation are caused by genetic characters

Table 24. Statistical Mean for Mycorrhizal Mantles as Related to Photoperiod, Elevation, and Aspect for Douglas-fir Seedlings, 4 Months Old, from the Same Southern Area as Seed Source 14.

Temp	Time	Elevation		
		1,500	3,000	5,000
Deg F	*Hours*	*Feet*	*Feet*	*Feet*
North slope				
40-78	9	0	0.68	0.47
	15	6.10	5.71	3.92
50-78	9	4.64	2.40	5.05
	15	6.24	2.61	2.74
70-78	15	8.52	8.30	8.37
South slope				
40-78	9	0.85	0.35	1.05
	15	3.61	2.88	3.16
50-78	9	3.87	2.12	3.80
	15	2.15	4.46	4.40
70-78	15	6.22	10.23	5.75

Table 25. Relation of Elevation and Aspect to Classification of Mycorrhizae on 4-Month-Old Douglas-Fir Seedlings from Seed Source 14, Based on 9 Seedling Averages.

Temperature	Lighted period	Elevation, *feet*					
		1,500		3,000		5,000	
		C.g.[1]	Other	*C.g.*[1]	Other	*C.g.*[1]	Other
Deg F	*Hours*						
North slope seed							
40-78	9	0.0	0.0	0.7	0.0	0.7	0.0
	15	7.3	35.3	7.5	37.5	5.0	14.5
50-78	9	2.8	19.7	1.8	5.5	3.8	23.3
	15	9.5	36.2	5.7	4.0	6.0	3.5
70-78	9	1.2	0.2	2.8	0.0	1.5	1.3[2]
	15	9.0	68.0	6.0	70.6	11.2	63.0
South slope seed							
40-78	9	1.0	0.0	0.5	0.0	1.7	0.0
	15	7.0	13.0	5.0	3.8	2.0	11.2
50-78	9	4.5	12.8	2.7	2.2	3.0	15.2
	15	1.5	5.2	6.0	14.0	5.8	14.3
70-78	9	2.3	0.0	0.2	0.0	0.1	0.0[2]
	15	5.3	34.3	5.7	101.3	3.2	35.0

[1]*Cenococcum graniforme*.
[2]Roots not dormant, data unreliable.

related to seed source. Genetic influence, however, may be masked by the effect of the environment.

The relation of elevation and aspect of seed source to classification of mycorrhizae is given in Table 25. In most counts, *C. graniforme* comprised a significantly small part of the mycorrhizal population except on seedlings from seed collected at 3,000-foot elevation, and even then it could hardly be classified as dominant. Less than 50 percent of *C. graniforme* mycorrhizae were branched, however, which may be of some consequence. Conversely, all other mycorrhizae were more than 50 percent forked, as shown in the tabulations of 15 seedlings and three seed sources (Table 26).

Table 26. Classification of Compound Mycorrhizae.

Seedling age	Branching in *Cenococcum*	Branching in other mycorrhizae
Months	*Percent*	*Percent*
6	0	54
12	14	81
18	45	92
24	39	79
27[1]	0	100

[1]A white oak site, all others Douglas-fir land.

Auxin production by mycorrhizal fungi enhances dichotomous branching of pine roots (20) and also probably stimulates monopodial formations on Douglas-fir rootlets. This is, however, not always true, because even so-called long roots are sometimes covered with mycorrhizal sheaths (Figure 12). Mycorrhizae may also be of benefit by the extensive development of mycelial strands through the soil, which probably results in improved absorption of moisture and nutrients. *C. graniforme* is particularly proficient in this respect.

Mycorrhizae in Forest Nurseries

One of the objects of this study was to relate the importance of ectomycorrhizae to growth of Douglas-fir and ponderosa pine seedlings in nursery seedbeds.

Results in a previous paper (24) indicate that 2-year-old ponderosa pines with an abundance of visible ectotrophic mycorrhizal mantles grew better in nursery seedbeds and showed survival in field plantations superior to that of same-age stock lacking mycorrhizae. Better survival, however, could not be attributed entirely to mycorrhizae, because seedlings with mycorrhizae were larger than those without mycorrhizae. When seedlings of the same size and age were planted on a favorable site, the seedlings with mycorrhizae still showed a trend toward better survival than that of nonmycorrhizal stock. Seedlings of similar size transplanted on an unfavorable site showed no difference in survival (24). These tests indicate the offsetting effect of environmental factors.

Restricted development of ectomycorrhizae on 1-year-old ponderosa pine and Douglas-fir had no detrimental effect on survival when transplanted as 2-year-old seedlings (27). In these tests the 2-0 seedlings from fumigated seedbeds were larger than the mycorrhizal stock, but the smaller mycorrhizal stock showed no greater mortality. The transplant site, however, was favored with abundant rainfall and therefore, the seedlings were not subjected to drought stresses.

In another study (8), there was a positive correlation between survival and lifting time of Douglas-fir. When 2-year-old Douglas-fir were transplanted early in the fall before mycorrhizal mantles were discernible, survival of transplants was poor (26). The poor survival could not be attributed entirely to lack of mycorrhizae, however, but possibly also to the earliness of transplanting.

The effect of ectomycorrhizae on seedling growth and survival of transplants is extremely difficult to evaluate because of complicating environmental factors, but indications are that abundant mycorrhizae are beneficial.

Good mycorrhizal development on Douglas-fir has been found in parts of nursery seed beds, while stunting and yellowing of nonmycorrhizal stock are readily apparent in other parts of the same seedbeds (Figure 20).

A new forest nursery on former agricultural sandy loam soil, which had been sterilized with a commercial preparation of chloropicrin, produced 1-year-old Douglas-fir with three types of growth; vigorous and green, stunted and green, stunted and chlorotic (Figure 21) (22). All three types had terminal buds when dug for examination in mid-fall. Sectioning of root tips showed that normal green seedlings had thin mycorrhizal mantles, but well-developed Hartig nets in the root cortex. The stunted green seedlings had mycorrhizal mantles but no Hartig net. Stunted chlorotic stock had neither mycorrhizal mantles nor Hartig nets.

Chemical analyses were made of Douglas-fir foliage collected from vigorous green stock with mycorrhizae and chlorotic stunted seedlings without mycorrhizae. Collections were made in the summer, fall, and ensuing spring. Analyses of three seedlings are shown in percentages in Table 27.

Percentage of nitrogen and phosphorus increased with age of stock, especially for those seedlings with abundant mycorrhizae; potassium, however, showed a decline as phosphorus increased. These results are in agreement with several other investigators, who have shown that

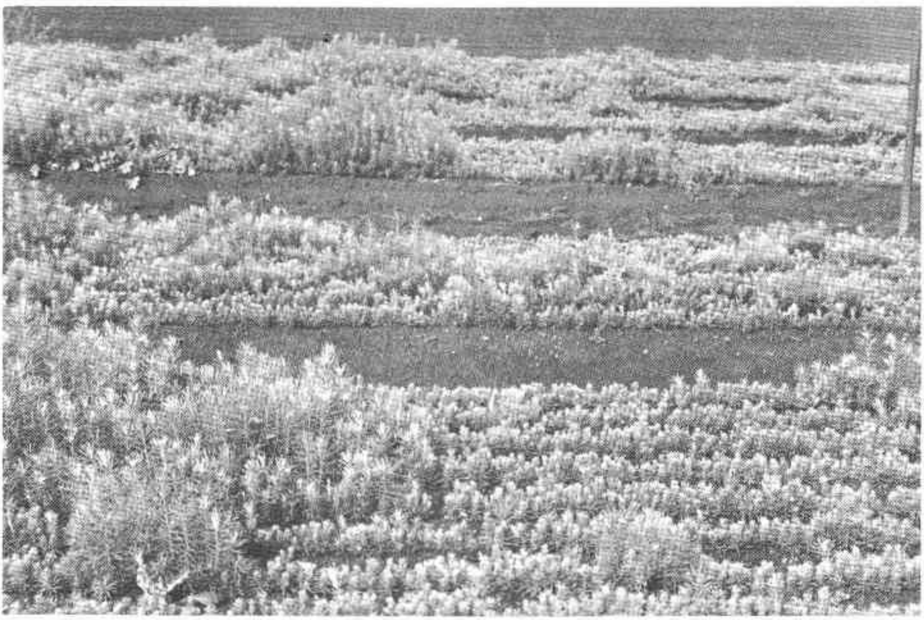


Figure 20. Relation of mycorrhizae to seedling growth of Douglas-fir in nursery beds. Small seedlings had very few mycorrhizae; large seedlings had abundant mycorrhizae.

Table 27. Foliage Analyses, Based on Ovendry Weight at 70 C, of Mycorrhizal and Nonmycorrhizal Douglas-fir Seedlings.

Mycorrhizae	Seedlings	Nitrogen	Potassium	Phosphorus	Calcium	Magnesium
		%	%	%	%	%
July 1964						
Abundant	Vigorous	1.79	1.15	0.176	0.255	0.113
None	Stunted	1.48	0.85	0.051	0.132	0.098
November 1964						
Abundant	Vigorous	2.70	1.09	0.210	0.470	0.117
None	Stunted	0.98	0.67	0.083	0.360	0.108
March 1965						
Abundant	Vigorous	2.27	0.84	0.278	0.410	0.077
Trace	Stunted	2.19	0.71	0.199	0.420	0.086

phosphorus is especially abundant in mycorrhizal plants. Harley summarized similar findings and verified them by use of radioactive phosphorus (5).

Another forest nursery with silt loam where seedbeds had been fumigated with methyl bromide showed 2-year-old chlorotic Douglas-fir and ponderosa pine seedlings (25). Only the normal-size seedlings had ectomycorrhizae (Figure 21). Foliage analyses of each kind of

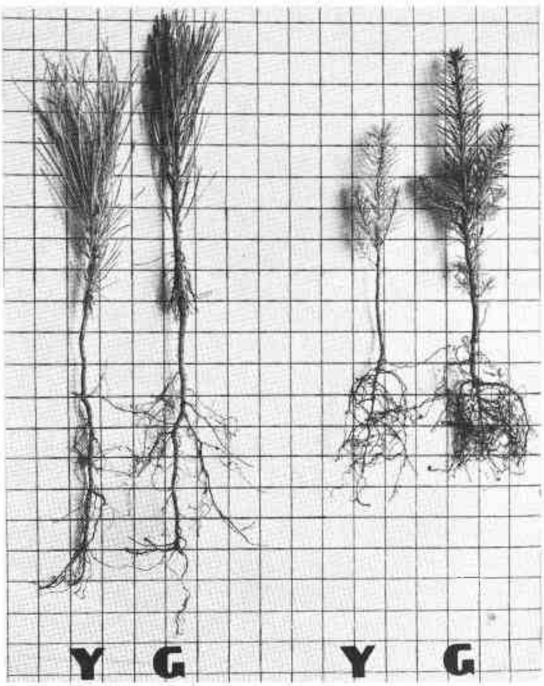


Figure 21. Yellow and green 2-0 seedlings of ponderosa pine (left) and Douglas-fir (right), grown in silt loam soil that had been fumigated with methyl bromide. Yellow seedlings had only a trace of mycorrhizae; green seedlings had well-developed mycorrhizal mantles.

Table 28. Chemical Analyses, Based on Ovendry Weight at 70 C, of Foliage Collected in the Fall from Silt Loam Soil.

Mycorrhizae	Seedlings	Nitrogen	Potassium	Phosphorus	Calcium	Magnesium
		%	%	%	%	%
Ponderosa Pine						
Abundant	Vigorous	1.84	1.02	0.214	0.200	0.135
Trace	Chlorotic	1.03	0.94	0.152	0.160	0.120
Douglas-fir						
Abundant	Vigorous	2.11	0.95	0.431	0.430	0.157
Few	Chlorotic	0.64	0.64	0.182	0.200	0.106

seedling are shown in Table 28. Microscopic examination of sectioned root-tips showed that the 2-year-old normal green seedlings had well-developed mycorrhizal mantles with Hartig nets, but the chlorotic plants had limited mantle formation and only an occasional Hartig net.

These analyses indicate that phosphorus was markedly more plentiful in green vigorous seedlings of ponderosa pine and Douglas-fir with abundant mycorrhizae than in chlorotic plants in the same seedbeds. Other elements were also more plentiful in green seedlings with abundant mycorrhizae. Equally vigorous seedlings with limited mycorrhizal development were not available for analysis. Hence, to state that the mycorrhizae alone caused the increase in phosphorus uptake is not justified, although this is probable.

The importance of internal development of mycorrhizal hyphae is impossible to analyze statistically. Microscopic examinations showed internal mycorrhizal development in healthy plant cells to be more abundant than in weak seedlings. Although the poorly developed seedlings cited were not dying, they were not acceptable as field transplants. The explanation for the failure of inter-mixed seedlings to develop good mycorrhizal associations is probably related to factors such as variable soil sterilization, differences in soil texture, leveling of seedbeds, and lack of ectomycorrhizae. Nutrient deficiences of nonmycorrhizal seedlings, therefore, appear at least partially related to decreased mycorrhizal development.

Soil of seedbeds had been uniformly fertilized before the seed was sown, so the differences in mycorrhizal development probably did not result from variation in soil fertility.

CONCLUSIONS

The extensive character of the research reported here does not justify unqualified conclusions. Tentative conclusions, however, are possible.

Slash burning before sowing does reduce soil microflora including mycorrhizal fungi. Reduction varies with the degree of burn and season of burn. In all instances the reduction is temporary. After slash burning, allowing at least one season to lapse before reseeding burned-over areas appears desirable so the soil can regain a more normal microflora.

To postulate that selection of a proper seed source would always lead to better growth and survival of seedlings would be unjustified. On the basis of these tests, however, certain seed sources almost invariably produced seedlings that formed mycorrhizal associations earlier and more abundantly than did other seed sources. Because most ectomycorrhizae are generally considered beneficial to seedling growth, seedlings with the earliest and greatest mycorrhizal growth apparently would stand a better chance of survival than seedlings with retarded mycorrhizal development—other things being equal.

In forest nurseries, especially those that grow 1-year stock for transplanting, selection of a proven seed source appears important because of possible earlier and better mycorrhizal formation.

Selection of the best seed source is, of course, not the only factor involved. The nursery soil must also have a suitable microflora so that early mycorrhizal associations will result. These tests as well as those by other researchers indicate that soil fertilization alone is not sufficient for good seedling growth (16). Likewise, there are additional factors such as moisture content of the soil and light intensity that also influence mycorrhizal development. These obviously should be kept at optimum for good growth of the species of seedling, which should also prove satisfactory for development of ectomycorrhizae.

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