Mycorrhizal Deficiency in a Douglas-Fir Region Nursery

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Abstract. Severely stunted, phosphorus-deficient Douglas-fir seedlings grown in a newly developed western Oregon nursery failed to respond to fertilization during their second growing season. The seedlings proved to be nonmycorrhizal; ectomycorrhizal fungi were apparently sparse in the soil due to the previous long period of agricultural use of the tract combined with the land leveling and soil fumigation during development of the nursery. Inoculation of affected beds with mycorrhizal fungi in soil from a nearby bed of ectomycorrhizal transplant seedlings significantly increased phosphorus uptake and growth of seedlings during the third growing season. Seedling growth was further enhanced by combining inoculation with fertilization. By the end of the third growing season, most surviving seedlings on uninoculated plots had begun to form mycorrhizae from natural sources of infection.

Additional key words. *Pseudotsuga menziesii*, soil fumigation, phosphorus nutrition.

MANY conifers require ectomycorrhizae for normal growth and development beyond the first year after seed germination. Where symbiotic fungal associates are naturally lacking, foresters have recognized the need to import them for inoculation into nursery soils (Bowen 1965). Nurseries growing native tree species in forested regions, however, have rarely had mycorrhizal problems because native fungi usually provide reliable and continuing inoculation. But even in these nurseries circumstances can combine to produce a deficit of mycorrhizal fungi, resulting in serious loss of seedlings. In this paper we report such a situation and the results of an experiment conducted to provide a basis for prescribing corrective measures.

Background

In August 1961, the Industrial Forestry Association (IFA) purchased a tract in the Willamette Valley near Canby, Oregon, as a forest nursery site. The highly productive soil, a Sifton fine sandy loam, had been used for agriculture for years, the most recent use being for turkey and grain crops. The first beds for the 1962 seedling crop were constructed in a fairly level portion of the tract, which included a recently uprooted filbert (*Corylus* sp.) orchard. Much of the remaining area, first sown in 1963, was gently rolling and required leveling to construct nursery beds. Top soil had been removed prior to leveling and restored as practicable.

From previous experiences in the IFA Greeley Forest Nursery at Nisqually, Washington, the nursery staff concluded that soil fumigation prior to sowing was desirable to reduce weeding costs and losses from root pathogens. So, at Canby, similar soil fumigation with a chloropicrin-ethylene bromide mixture was applied to both the 1962 and 1963 sowings.

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings sown in 1962 grew slowly and became chlorotic on

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FIGURE 1. *Beds of 2-0 Douglas-fir seedlings at Canby nursery. Tall seedlings are mycorrhizal; stunted seedlings lack mycorrhizae.*

FIGURE 2. *Stunted, nonmycorrhizal, 2-0 Douglas-fir seedlings averaging 2 to 3 cm tall. Relatively large seedling in the center had overcome its growth check and had mycorrhizae on its deeper roots.*

localized areas during the second growing season. They apparently recovered after application of ammonium sulfate at 100 lb per acre (112 kg per ha).

The 1963 sowing in the leveled part of the nursery likewise produced areas of discolored and stunted seedlings (Figs. 1 and 2). These areas of stunting were more extensive than in the 1962 sowing, and the seedlings did not respond to similar fertilization. Also, affected areas seemed to be associated with exposure of subsoil from the leveling operation. A subsequent fertilizer trial of essential elements failed to improve seedling color or restore normal growth rate. Islands of seedlings did recover in affected beds, but with no relationship to fertilizing (Fig. 1). Soil and foliage from both these islands and the stunted seedlings were sampled for comparison by chemical analysis. Soils were generally comparable in all respects (Table 1), but foliar contents of elements were distinctly lower for stunted seedlings—especially phosphorus (less than one-third that of recovered seedlings) (Table 2). Foliage of stunted seedlings showed the purplish cast typical of severe phosphorus deficiency. Subsequent root examination revealed good ectomycorrhizal development on normal seedlings and none on stunted seedlings.

Methods

To test the effects of inoculation with mycorrhizal fungi and possible interactions between fertilization and inoculation, we established three replicate blocks of plots in the most severely affected part of the nursery on September 10, 1964 (Fig. 1). Eight plots, 45 cm long and bed-wide (each bed had eight rows of Douglas-fir seedlings), were laid out in each block in spots selected for preponderance of stunted seedlings. Seed-

TABLE 1. Chemical analysis of soil[1] from under stunted and spontaneously recovered seedlings; Canby Nursery, second growing season, July 1964.

Seedlings	pH	Organic matter	Total N	P	Cation exchange capacity	Exchangeable K	Exchangeable Ca	Exchangeable Mg
		Percent	*Percent*	*Kg/Ha*	*Meq/100 g ovendry soil*			
Recovered	5.0	7.63	0.19	101	15.7	0.73	2.2	0.9
Stunted	5.0	8.20	.18	72	16.6	.80	2.2	.9

[1] Determinations from the Oregon State University Soil Test Laboratory (Alban and Kellogg 1959)—P determined by sodium bicarbonate extraction.

TABLE 2. Chemical analysis of foliage[1] from stunted and spontaneously recovered seedlings; Canby Nursery, second growing season, July 1964.

(In percent)

Seedlings	Sample no.	N	P	K	Ca	Mg
Recovered	1	1.82	0.17	1.17	0.26	0.12
	2	1.76	.18	1.14	.25	.11
Stunted	1	1.49	.05	.86	.13	.10
	2	1.48	.05	.85	.13	.10

[1] Analyses by R. L. Carmichael, Oregon State University Forest Research Laboratory.

lings in rows 3 and 6 of each plot were dug out with minimal disturbance to adjacent rows. A narrow ditch, similar to those left in rows 3 and 6, was dug outside the outer row of both sides of the bed. By this procedure, each remaining seedling row was adjacent to a ditch, which served as a receptacle for inoculum.

Each of four inoculation treatments was established in two randomly selected plots within each block: (1) control—ditches refilled with soil originally removed; (2) mycorrhizal soil—ditches filled with soil taken from a bed of vigorous, heavily ectomycorrhizal, 2–1 transplant seedlings; (3) interplanted transplants—vigorous, 2–1 ectomycorrhizal seedlings from beds mentioned in treatment 2, planted at approximately 5-cm spacing in ditches which were refilled with the soil originally removed; and (4) interplanted ectomycorrhizal seedlings from scattered vigorous patches in beds of otherwise stunted seedlings (Fig. 1), planted at approximately 5-cm spacing in ditches which were refilled with the soil originally removed (Fig. 3, left).

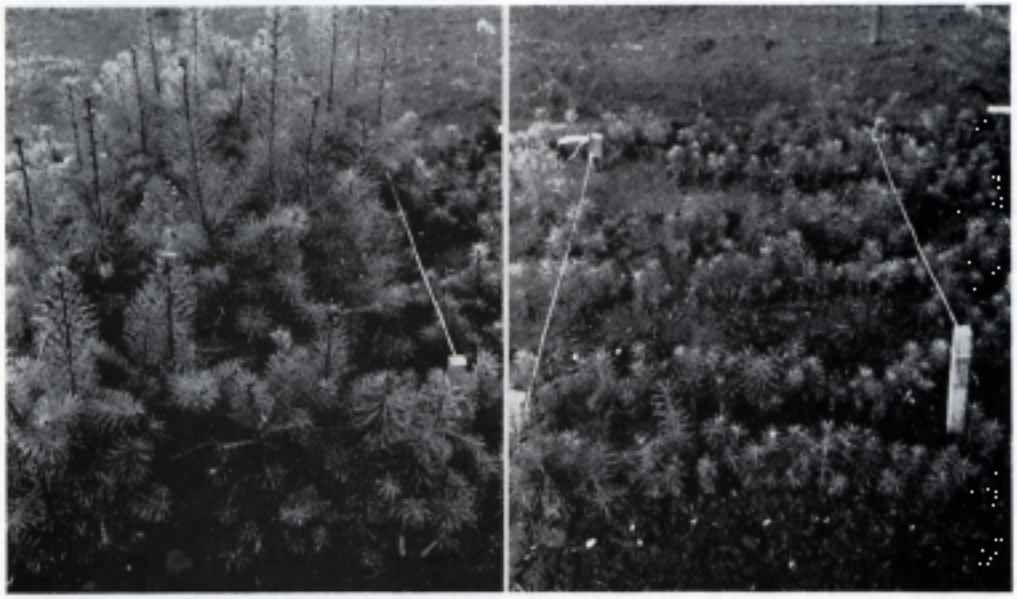

FIGURE 3. Left: *Plot inoculated with mycorrhizae by interplanting with vigorous mycorrhizal seedlings from patches occurring in beds of stunted seedlings; Block 1, unfertilized, 1 year after treatment. The lush growth of the large, interplanted seedlings hides the treated seedlings originally growing in the bed.* Right: *Original 3-0 Douglas-fir seedlings in the same inoculated plot as shown at left after interplanted seedlings were clipped off at ground line.*

One of the two plots for each inoculation treatment within each block was broadcast-fertilized with a mixture of urea, treble-superphosphate, and muriate of potash at a per-acre rate of 50 lb of nitrogen, 100 lb of phosphorus, and 25 lb of potassium (respectively 56, 112 and 28 kg per ha). The other plot of each pair was left unfertilized.

In October 1965, about 13 months after treatment, seedlings were lifted from the central 15-cm portion of all seedling rows of each plot. Twenty of these seedlings were randomly selected for determining ovendry weights of roots, tops, and foliage. The dried foliage was subsequently analyzed for content of nitrogen, calcium, potassium, phosphorus, and magnesium. An additional 10 seedlings were randomly selected for measurement of length of terminal shoot and needles grown before (1964) and after (1965) treatment and for frequency of mycorrhiza formation. The five longest needles from each year's complement were measured. Presence of mycorrhizae was spot checked microscopically on hand-cut rootlet sections. Estimating frequency of mycorrhizae on individual seedlings proved unnecessary, since mycorrhizae were either uniformly abundant or only sparsely present on deeper roots.

All numerical data were submitted to variance analysis.

Results

Means of plots, blocks, or treatments for lengths of shoots and needles grown in 1964, the season preceding treatment, revealed no significant differences. All plots could accordingly be assumed to have, on the average, seedlings at equivalent stages of development at the outset of the experiment.

When lifted, at least some surviving seedlings in all plots had begun to recover from the stunting. Seedlings from inoculated plots, however, were uniformly dark green and thrifty (Fig. 3, right). Most seedlings from control plots were

FIGURE 4. *3-0 Douglas-fir seedlings in a plot that was fertilized but not inoculated with mycorrhizal fungi; Block 1, 1 year after treatment.*

at best only in an early stage of recovery (Fig. 4), and usually substantial mortality was evident.

Inoculated seedlings had abundant, well-formed ectomycorrhizae uniformly distributed throughout their root systems, whereas a few mycorrhizae had begun to form only on the deeper roots of control seedlings. Apparently, inoculation treatments had rapidly provided a good population of mycorrhizal fungi, but in control plots the fungi from residual sources or natural inoculation had spread slowly and erratically.

Although morphological classification of the ectomycorrhizae was not attempted, we noted four distinctly different types, apparently formed by different fungi. Microscopic check of root tips having abundant root hairs and lacking any sign of fungal mantle showed absence of fungal infection in all cases. No ectendomycorrhizae were found.

Inoculation. Mean phosphorus content of foliage and 1965 shoot length were both significantly higher with inoculation (average of treatments 2, 3, and 4) than without.

TABLE 3. Mean values of Douglas-fir seedling characteristics measured to evaluate effects of mycorrhizal inoculation and fertilization in nursery beds.

Characteristic	Units	Uninoculated	Inoculated w/transplant bed soil	Unfertilized	Fertilized
Top dry weight	g	0.24	0.49*	0.27	0.36*
Root dry weight	g	.28	.48*	.29	.36
Foliage dry weight	g	.14	.27*	.15	.21*
Height growth, 1965	cm	2.8	4.9*	4.1	5.1*
Length of 1965 needles	cm	1.4	1.8*	1.7	1.9
Foliage content:					
P	% wt	.17	.24*	.20	.23
N	% wt	1.68	1.88	1.72	2.00*
K	% wt	.72	.86	.82	.87
Ca	% wt	.56	.56	.46	.59*
Mg	ppm	770	530	590	554

* Significant at the 5-percent confidence level.

Inoculation with soil from under mycorrhizal seedlings (treatment 2) additionally resulted in significantly higher mean dry weights of tops, roots, and foliage as well as longer 1965 needles than occurred in uninoculated plots (Table 3, Fig. 5). No such responses resulted from inoculation by interplanting mycorrhizal seedlings (treatments 3 and 4). We ascribed this lack of effect to suppression of treated seedlings by the densely overtopping interplants (Fig. 3, left). Even so, the treated seedlings did have abundant mycorrhizae, and, despite their relatively spindly form, much healthier appearing needles than did uninoculated seedlings (Figs. 3 right, and 4).

Inoculated seedlings did not differ significantly from uninoculated in foliage content of nitrogen, potassium, calcium, or magnesium.

Fertilization. Averaging out inoculation effects, a significant positive effect from fertilization occurred only for mean seedling top and foliage weight, 1965 height growth, and foliar content of nitrogen and calcium (Table 3). However, the mean data for top and foliage weights (Fig. 5) indicated that uninoculated plots did not respond to fertilization, while plots inoculated with soil containing mycorrhizal fungi (treatment 2) responded markedly. Since inoculation with mycorrhizal transplant seedlings (treatments 3 and 4) introduced the extraneous suppression of treated seedlings, an additional analysis of variance was computed to compare controls with treatment 2 alone.

Inoculation × Fertilization Interaction. Statistical comparison of controls with inoculation treatment 2 showed that foliage and top weights averaged significantly more for seedlings both inoculated and fertilized than for seedlings receiving only one of these treatments. Clearly, fertilization produced a growth response only on mycorrhizal seedlings (Fig. 5). No significant interactions occurred for the other treated variables.

A test for differences in top/root ratios between all treatments was nonsignificant. The higher the mean seedling top weight, the proportionately higher was the root weight, regardless of inoculation or fertilization (Fig. 5).

Observations on Mycorrhizal Fungi

We visited the nursery periodically throughout this study. On several occasions, four species of mycorrhizal fungi were fruiting abundantly in and near the

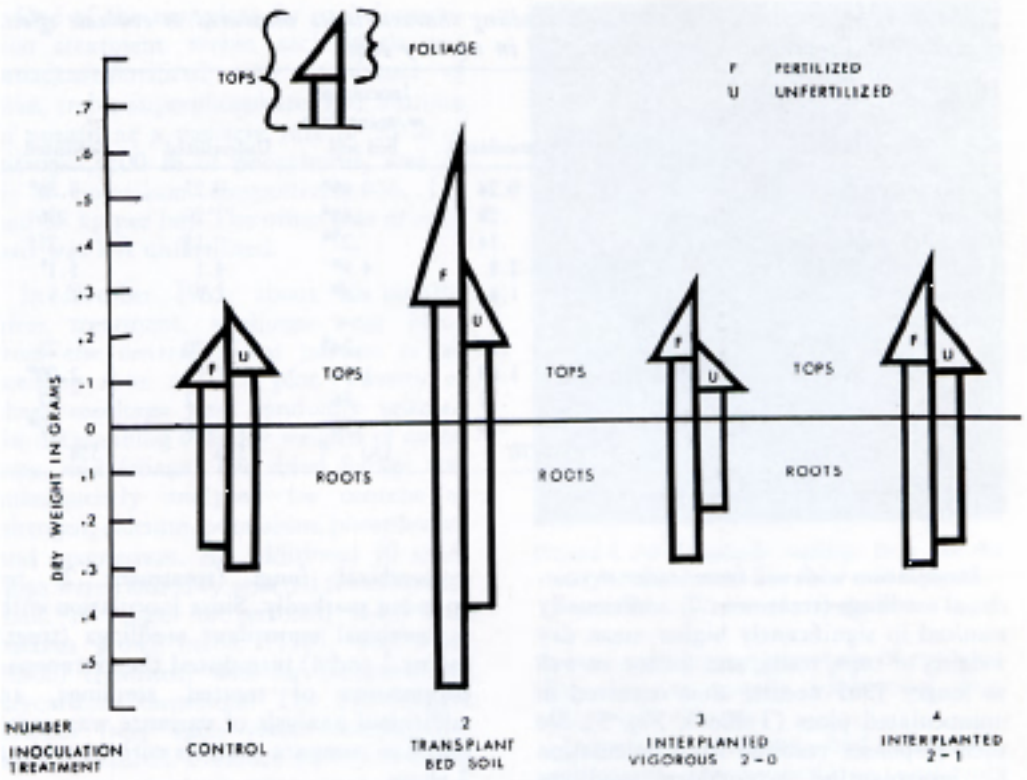

FIGURE 5. *Mean dry weights of 3-0 Douglas-fir seedling tops and roots with different types of mycorrhizal inoculation, with or without fertilizer.*

patches of vigorous seedlings dotting the beds of stunted seedlings. Collections of each, deposited in the herbarium of Oregon State University, are indicated by the italicized numbers following species names:

Hebeloma crustuliniforme (Bull. ex St. Am.) Quél.—Trappe *357*

Inocybe lacera (Fr.) Quél.—Trappe *365*

Laccaria laccata (Scop. ex Fr.) Berk. & Br.—Trappe *457*

Thelephora terrestris Ehrh. ex Fr.—Trappe *358*

These fungi are proven ectomycorrhiza formers (Bryan and Zak 1961, Marx 1966, Trappe 1967) with the exception of the *Inocybe*, which can be considered a probable ectomycorrhizal fungus pending experimental proof. All are nonselective in respect to mycorrhizal host associates (Trappe 1962, Hacskaylo 1965).

Judged by occurrence of mushrooms, *Inocybe lacera* was easily the most abundant and uniformly distributed of the four fungi. It frequently occurred in "fairy rings" outlining the edges of patches of vigorous seedlings (Fig. 6). In past years, Trappe found it fruiting abundantly in beds of Douglas-fir seedlings at the Forest Service Wind River Nursery at Carson, Washington (Trappe *60*), as well as in pioneering plant communities such as on the Kautz Creek flood alluvium in Mount Rainier National Park (Trappe *459*). Its apparent ability to thrive in nonforested soils should make it an aggressive competitor in nursery soils.

Thelephora terrestris is a common

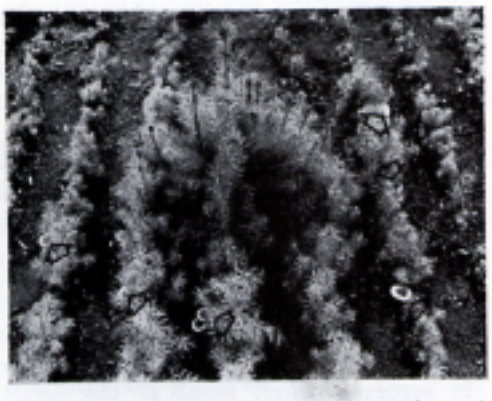

FIGURE 6. *Mushrooms of Inocybe lacera (arrows) fruiting in a ring around a patch of vigorous, mycorrhizal Douglas-fir seedlings.*

mycorrhizal fungus at Canby as well as in other nurseries (cf. Hacskaylo 1965), and it also occurs with mycorrhizal hosts in pioneer communities such as on the recent terminal moraines of Emmons Glacier in Mount Rainier National Park (Trappe *988* and *1011*) or on waste banks from anthracite mining in Pennsylvania (Schramm 1966). Like *I. lacera*, this species appears to be particularly well adapted to nursery conditions.

Hebeloma crustuliniforme and *Laccaria laccata* both occur in widely diverse habitats and thus can evidently adapt well to an environment so long as mycorrhizal hosts are present. *H. crustuliniforme* fruited abundantly in the Canby nursery, but *L. laccata* was found only in a few spots.

Expansion of Infection Centers

As described earlier, patches of a few to several hundred vigorous, mycorrhizal seedlings occurred seemingly at random among the severely stunted, nonmycorrhizal seedlings (Fig. 1). When the experiments were installed, we hoped to get quantitative data on rate of patch expansion as an index of rate of spread of mycorrhizal infection. Between the time of original and scheduled final measurement of selected patches, however, surrounding seedlings began to show general recovery from stunting. Examination of roots at time of second measurement revealed that mycorrhizal infections were generally well distributed throughout these particular beds. There were no discrete patch edges to be measured, so radial spread of mycorrhizal infection during the preceding year could not be determined.

Observations of these and other patches do permit speculation on a sequence of events which could begin any time during the growing season. One or a few adjacent stunted seedlings in a row suddenly burst their terminal buds and began to grow, a few mycorrhizae having been formed on deep roots (i.e., 15 to 20 cm below the surface). The seedlings' shoot growth accelerated and longer, greener needles formed as mycorrhiza formation spread over their root systems. Adjacent, stunted seedlings subsequently began to form mycorrhizae on roots intertwined with those of the now-growing neighbors. These adjacent seedlings, in turn, burst buds and commenced a growth pattern similar to the earlier released seedlings. Expansion of these centers of released growth progressed slowly at first but seemed to accelerate with increasing size of the patch. Once the process had begun within a row, nearby seedlings in adjacent rows began the sequence. In all cases, mycorrhiza formation seemed to precede bud burst. The overall effect was to produce dome-topped patches, with most advanced seedlings in the center and as yet unreleased seedlings at the edge (Figs. 1 and 6).

The source of these natural mycorrhizal infections was not determined. Because initial infection apparently started at a well-defined point in most cases, we suspect that the mycorrhizal fungi originated from spores rather than from buried residual vegetative inoculum left from initial development of the nursery site. Downward movement of spores from soil surfaces by water or soil

organisms is a well-documented phenomenon, especially in sandy soils such as found at Canby (Burges 1958).

Discussion

The radial spread with time of patches of mycorrhiza formation with the attendant vigorous growth of seedlings in beds of otherwise stunted seedlings, plus the failure of uninoculated seedlings to respond to fertilization, indicates that mycorrhizae played the causal role in renewal of growth. The increase of seedling foliage content of phosphorus following mycorrhiza formation reaffirms the well-established importance of ectomycorrhizae for phosphorus nutrition of host trees, as reviewed by Harley (1959). Indeed, the purple-bronze foliage of stunted seedlings and their very low P content indicated deficiency at the outset. Failure to increase P level in the foliage by fertilization alone clearly showed that P was available only to mycorrhizal Douglas-fir seedlings, regardless of its concentration in the soil. Our results closely parallel those reported for Douglas-fir by McComb and Griffith (1946), and for *Picea rubens* Sarg. by Mitchell *et al.* (1937).

Douglas-fir seedlings at Canby clearly suffered from delay of mycorrhiza formation early in the first growing season. The nonmycorrhizal seedlings that survived through the first season typically set a bud immediately above the cotyledons; many of the survivors had not even burst this bud by the end of the second growing season. Those that did burst bud grew a shoot only a few millimeters long with dwarfed, offcolor needles. Certain nonmycorrhizal pines, in contrast, have been reported to grow as well as or better than mycorrhizal ones during the first growing season in fumigated nursery soils (e.g., Hacskaylo and Palmer 1957, Laiho and Mikola 1964).

The results of our experiment at Canby are neither novel nor surprising. However, they illustrate that circumstances can combine to seriously deplete populations of mycorrhizal fungi in soils of a newly developed nursery, even when the nursery is located in a forested region abundantly endowed with endemic mycorrhizal fungi. At Canby, these circumstances were: (1) establishment of beds on soils with a prior history of agricultural use, (2) land leveling which removed topsoil from high ground, and (3) fumigation of a soil whose texture was ideal for deep and uniform penetration of the fumigant. In other words, a soil that was undoubtedly low in ectomycorrhizal fungi at the outset was divested of its upper layer, which likely contained the most viable spores and hyphae. Any remaining mycorrhizal fungi were effectively decimated by fumigation. The spontaneous recovery of the stunted 1962 seedlings can be attributed to the relatively little leveling done in that part of the nursery coupled with the previous crop history of a *Corylus* orchard. *Corylus* species have ectomycorrhizae; many of the associated fungi form mycorrhizae with conifers as well (Trappe 1962). Quite likely, some of these fungi carried over deep in the soil and formed mycorrhizae on the Douglas-fir seedlings as soon as their roots grew deeply enough to make contact.

Several biocides are highly toxic to mycorrhizal fungi; depending on how extensively biocidal application reduces fungal populations, reasonably uniform reinvasion of the soil may require one or more growing seasons (Palmer and Hacskaylo 1958, Laiho and Mikola 1964). This delay can be disastrous for tree species which require early mycorrhiza formation for adequate nutrition during the first year.

Elimination of mycorrhizal fungi may also deprive seedling roots of protection from pathogens as Zak (1964) has suggested. Marx (1966) has demonstrated that *Phytophthora cinnamomi* Rands. will not infect mycorrhizal portions of pine seedling root systems. If a fumigated nursery soil were reinvaded by a rapidly

growing pathogen, the resulting damage to root systems left unprotected by mycorrhizal fungi could seriously hinder seedling development and survival.

Clearly, fumigation or other soil treatments that deplete populations of mycorrhizal fungi should be applied only after careful evaluation of the seedlings' mycorrhizal needs and related factors of soil biology that may be important. Unless adequate caution is observed, the results of such treatments may be more vexing than the problems for which treatment was originally prescribed.

Literature Cited

ALBAN, L. A., and MILDRED KELLOGG. 1959. Methods of soil analysis as used in the OSC Soil Testing Laboratory. Oreg. State Col Misc Pap 65, 9 p.

BOWEN, G. D. 1965. Mycorrhiza inoculation in forestry practice. Aust Forest 29:231-237.

BRYAN, W. C., and B. ZAK. 1961. Synthetic culture of mycorrhizae of southern pines. Forest Sci 7:123-129.

BURGES, ALAN. 1958. Micro-organisms in the soil. Hutchison & Co., Ltd., London. 188 p.

HACSKAYLO, EDWARD. 1965. Thelephora terrestris and mycorrhizae of Virginia pine. Forest Sci 11:401-404.

HACSKAYLO, E., and JOHN G. PALMER. 1957. Effects of several biocides on growth of seedling pines and incidence of mycorrhizae in field plots. Plant Dis Rep 41:354-358.

HARLEY, J. L. 1959. The biology of mycorrhiza. Leonard Hill (Books) Ltd., London. 233 p.

LAIHO, OLAVI, and PEITSA MIKOLA. 1964. Studies on the effect of some eradicants on mycorrhizal development in forest nurseries. Acta Forest Fenn 77(2):1-34.

MARX, DONALD HENRY. 1966. The role of ectotrophic mycorrhizal fungi in the resistance of pine roots to infection by *Phytophthora cinnamomi* Rands. Ph.D. Diss, NC State Univ, Raleigh. 179 p. (Diss Abstr 27:1692B).

MCCOMB, A. L., and JOHN E. GRIFFITH. 1946. Growth stimulation and phosphorus absorption of mycorrhizal and non-mycorrhizal northern white pine and Douglas-fir seedlings in relation to fertilizer treatment. Plant Physiol 21:11-17.

MITCHELL, H. L., R. F. FINN, and R. O. ROSENDAHL. 1937. The relationship between mycorrhizae and the growth and nutrient absorption of coniferous seedlings in nursery beds. Black Rock Forest Pap 1:57-73.

PALMER, JOHN G., and EDWARD HACSKAYLO. 1958. Additional findings as to the effect of several biocides on growth of seedling pines and incidence of mycorrhizae in field plots. Plant Dis Rep 42:536-537.

SCHRAMM, J. R. 1966. Plant colonization studies on black wastes from anthracite mining in Pennsylvania. Trans Amer Phil Soc 56 (Pt. 1):1-194.

TRAPPE, JAMES M. 1962. Fungus associates of ectotrophic mycorrhizae. Bot Rev 28:538-606.

TRAPPE, JAMES M. 1967. Pure culture synthesis of Douglas-fir mycorrhizae with species of *Hebeloma, Suillus, Rhizopogon,* and *Astraeus*. Forest Sci 13(2):121-130.

ZAK, BRATISLAV. 1964. Role of mycorrhizae in root disease. Annu Rev Phytopath 2:377-392.