Seedling bioassays were conducted in the greenhouse to determine if the inoculum potential of VA and ectomycorrhizal fungi in forest soils is affected by logging disturbance, soil temperature, drought, or removal of organic matter.

Inoculum potential of fungi forming ectomycorrhizae with Douglas-fir and ponderosa pine was less in soils from old clearcuts than in soils from adjacent undisturbed forest stands; it was further reduced in soils from clearcuts which had also been burned.

Root zone temperature affected mycorrhizal colonization on Douglas-fir, ponderosa pine and subterranean clover. Maximum formation of both VA and ectomycorrhizae occurred at 18.5 - 24°C; there were no significant qualitative or quantitative differences between mycorrhizae developing in soils from recently clearcut sites and undisturbed forest stands. Mycorrhiza formation was moderate even at the lowest temperature tested (7.5°C), but was greatly diminished or prevented at 29.5°C. Propagules of ectomycorrhizal
fungi tolerated prolonged treatment at 35°, but young mycorrhizae were injured by high temperature.

With net photosynthetic rate as an indicator of plant moisture stress, ectomycorrhizal Douglas-fir seedlings conditioned to cyclic drought tolerated and recovered from stress more quickly than nonmycorrhizal seedlings; net CO₂ fixation of mycorrhizal seedlings was 10x greater than that of nonmycorrhizal seedlings. Four mycorrhizal fungi were compared for their ability to improve host drought tolerance. Seedlings inoculated with *Rhizopogon vinicolor* were less affected by drought than other mycorrhizal or nonmycorrhizal treatments. *In vitro* growth of mycorrhizal fungi in nutrient solutions osmotically adjusted with polyethylene glycol was a poor indication of effectiveness in reducing plant moisture stress *in vivo*.

Western red cedar grown in soil from disturbed clearcut sites were highly mycorrhiza dependent (1400%), more so than in soil from adjacent nondisturbed forest sites. *Glomus tenuis* was the most abundant endophyte. Douglas-fir was less mycorrhiza dependent (145%).

Forest floor organic matter contained inoculum of VA and ectomycorrhizal fungi. Application of litter to seedlings resulted in growth enhancement beyond the effects of mycorrhizal inoculum or addition of nutrients, suggesting the presence of microorganisms stimulatory to plant growth and/or activity of mycorrhizal fungi.
Factors Affecting the Inoculum Potential of VA and Ectomycorrhizal Fungi in Forest Soils of Southwest Oregon and Northern California

by

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FACTORS AFFECTING THE INOCULUM POTENTIAL OF VA AND ECTOMYCORRHIZAL FUNGI IN FOREST SOILS OF SOUTHWEST OREGON AND NORTHERN CALIFORNIA

INTRODUCTION

An estimated 95% of the world's species of vascular plants belong to families which characteristically form mycorrhizae (Trappe, 1977). Vesicular-arbuscular (VA) mycorrhizae are formed by fungi belonging to the Endogonaceae (Zygomycetes); most plant species are VA mycorrhizal (Gerdemann, 1968). Ectomycorrhizae are formed mainly by Ascomycetes and Basidiomycetes, and are limited to plants belonging to the Pinaceae, Betulaceae, Fagaceae, and a few other families (Trappe, 1977). Mycorrhizal fungi are ubiquitous in soils of natural plant communities; they are associated with plant roots in a mutually beneficial relationship. In exchange for host photosynthate, mycorrhizal fungi enhance plant growth by increasing nutrient uptake, especially phosphorus (Mosse, 1973; Bowen, 1973), and they may also be important in deterring soilborne plant pathogens (Schenck and Kellam, 1978; Marx, 1972) and in improving host water relations (Reid, 1979). Mycorrhizal fungi have been shown to improve the growth and survival of plants on routine and adverse sites all over the world (Mikola, 1980).

In southern Oregon and northern California, first-year mortality of outplanted conifer seedlings can be very high, approaching 100% on the poorest sites (Hermann, 1965). Reasons for this mortality include temperature extremes, animal damage, and low water-holding
capacity of some soils, shallow root systems, and some infrequent summer precipitation, resulting in plant moisture stress (Williamson and Minore, 1978; Hobbs et al., 1980). As a result of repeated regeneration failures, some sites have lacked conifers for many years.

The effect of disturbance on inoculum potential of mycorrhizal fungi resulting from host removal, slash burning, loss of organic matter and subsequent changes in soil temperature and moisture conditions are unknown. Saprophytic capabilities of mycorrhizal fungi are considered to be low or non-existent (Meyer, 1974); the length of time these fungi can survive in the absence of a living host also is not known. Studies concerning the effects of disturbance and mycorrhizal fungi suggest that inoculation of denuded areas may be necessary to ensure adequate mycorrhizal colonization of plants (Trappe, 1977; Marx, 1980).

Because the inoculum potential of residual native mycorrhizal fungi in forest regeneration sites is unknown, considerable effort has been directed toward inoculation of conifer seedlings in forest nurseries prior to outplanting. Inconsistent success has prevented this practice from becoming operational in the Pacific Northwest, however. At the time of outplanting, most conifer seedlings are nonmycorrhizal or are colonized by ineffective mycorrhizal species adapted to the conditions of the nursery.

The objectives of this research were to assess the effects of logging disturbance, soil temperature, drought, and organic matter on
the development of mycorrhizae as they pertain to forest regeneration.
CHAPTER 1

EFFECTS OF LOGGING DISTURBANCE ON THE INOCULUM POTENTIAL OF ECTOMYCORRHIZAL FUNGI IN FOREST SOILS OF SOUTHWEST OREGON AND NORTHERN CALIFORNIA

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SUMMARY

As a result of repeated forest regeneration failures on poor sites in southwest Oregon and northern California, some clearcuts have remained without conifers for several years. A greenhouse bioassay with Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] and ponderosa pine (Pinus ponderosa Dougl. ex. P. Laws. & C. Laws.) as hosts, was conducted to compare abundance of ectomycorrhizal fungus propagules in soils from 36 such clearcuts and adjacent undisturbed forests. Mycorrhizal colonization after 14-16 wks was high for seedlings grown in soils from undisturbed forests. Approximately 20% fewer mycorrhizae formed on seedlings grown in soils from clearcuts
which had not been burned; there was a 40% reduction in mycorrhizal colonization of seedlings grown in clearcuts which had also been burned.

INTRODUCTION

Shallow, rocky soils and harsh climatic conditions in the interior valleys and slopes of the Klamath and Siskiyou Mountains in southern Oregon and northern California present foresters with a reforestation challenge. First-year mortality of outplanted conifer seedlings on poor sites can be severe due to a combination of factors such as seedling moisture stress, frost, and animal damage (Williamson and Minore, 1978; Hobbs et al., 1980). As a result of repeated regeneration failures, some sites have lacked of conifers for many years and have been invaded by vegetation dominated by grasses and shrubs.

Ectomycorrhizal fungi in symbiotic association with most tree species in this mixed conifer zone are ubiquitous in soils of undisturbed forests. Important in nutrient uptake and probably also water transport (Chapter 3), ectomycorrhizae have been shown to improve growth and survival of forest tree seedlings planted on both routine and adverse sites (Marx, 1980). Inoculation of seedlings with ectomycorrhizal fungi has been shown to be necessary when introducing non-native trees (especially pines) in the southern hemisphere, in prairie and steppe soils and in agricultural soils (Trappe, 1977). Nursery inoculation of planting stock with particular fungi is now operational in the southeastern United States, and considerable effort has been made to screen
ectomycorrhizal fungus species for their potential use as nursery inoculum in the Pacific Northwest. However, inconsistent success in inoculation trials and limitations in the mass production of vegetative mycelium have as yet restricted widespread application. Even if it were now practical to inoculate seedlings, fungi which can be successfully introduced in forest nurseries have been shown to compete poorly with native ectomycorrhizal fungi once the seedling has been outplanted (Benecke and Gobl, 1974; Marx, Bryan, and Cordell, 1977; Trappe, 1977). It therefore seemed important to assess the inoculum potential of native ectomycorrhizal fungi already present in forest regeneration sites.

It is not known how long propagules of ectomycorrhizal fungi can remain viable in the soil in the absence of a suitable living host; few studies have been conducted on the effects of disturbance on ectomycorrhizal fungi. Inoculum of ectomycorrhizal fungi can be in the form of spores, sclerotia, mycelia, mycorrhizae on living roots, and perhaps also in organic matter. It was necessary to employ a technique which could assess the overall inoculum potential of these combined sources. Since actual differences in inoculum levels could be obscured by variable soil temperature, light, and moisture conditions in the field, a greenhouse bioassay was used to compare the relative abundance of ectomycorrhizae occurring on Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] and ponderosa pine (Pinus ponderosa Dougl. ex P. Laws. & C. Laws.) seedlings grown in soils from clearcuts and undisturbed forests. Douglas-fir and ponderosa pine are the two most widely planted conifer species in the area.
MATERIALS AND METHODS

Soils from 36 "difficult to regenerate" sites chosen by foresters were sampled during July and August. Sites were located from Siskiyou County, California to Douglas County, Oregon; each consisted of a clearcut adjacent to an undisturbed forest stand. Sites were distributed over a wide range of elevations, soil types, slopes and aspects. Clearcuts ranged in age from 1-22 yrs (\(\bar{x} = 9.4\) yrs); of these, 20 clearcuts had been burned and 16 had not been burned. Vegetation ranged from very sparse grasses and herbs among recently cleared and burned sites to dense grass and shrub cover among older clearcuts. Conifers were absent or very rare. Hardwood species sprouting from buried roots were present occasionally.

At each site four 1 l soil samples were collected from the top 0-7 cm of mineral soil: three samples from within the clearcut and one sample from the forest stand. These were refrigerated and subsequently transported to Corvallis for processing. Individual soil samples were sieved through .5 cm mesh, mixed (1:1) with coarse vermiculite and seeded to Douglas-fir or ponderosa pine in 70 cc tubes (Ray Leach Cone-tainers, Inc.). There were three replications per soil sample for each host. Portions of the soil-vermiculite mix were autoclaved before planting to serve as mycorrhizae-free controls and to test for airborne contamination. Seedlings were maintained under greenhouse conditions and harvested 14 wks (ponderosa pine) or 16 wks (Douglas-fir) after germination. Root systems were then rinsed and microscopically examined for colonization by mycorrhizal fungi. The degree of colonization was visually estimated according
to a scale of 0-5: 0 = no mycorrhizae; 1 = 1-20% of root tips colonized; 2 = 21-40%; 3 = 41-60%; 4 = 61-80%; 5 = 81-100%. Root and shoot lengths and dry weights were also recorded.

RESULTS

Mycorrhiza formation was abundant on root systems of Douglas-fir (x rating = 4.28) and ponderosa pine (x rating = 4.31) grown in soil from undisturbed forest stands. For all sites combined, mycorrhizal development in soils from nonburned clearcuts was significantly (P = .05) less than that occurring in soils from undisturbed forests. For both Douglas-fir (x = 3.46) and ponderosa pine (x = 3.07) the reduction was approximately 20% (Figure 1.1). Among soils from clearcuts which had also been burned, mycorrhizal formation was significantly (P = .05) reduced by approximately 40% compared to soil from undisturbed forests. Douglas-fir mycorrhiza formation was weakly correlated (R = -.216) with clearcut age; no correlation was found between ponderosa pine mycorrizae and age of clearcut. Root and shoot lengths and dry weights were not significantly different among the mycorrhizal treatments or between mycorrhizal and nonmycorrhizal controls at this early age.

Less than 3% of the control tubes became contaminated, indicating that sources of ectomycorrhizal fungus inoculum other than soil were negligible.

Several types of ectomycorrhizae were encountered; the most prevalent was a white rhizomorphic type. Cenococcum geophilum Er. was also common but usually localized to a few root tips per seedling. Swollen, light brown and slender, dark brown mycorrhizae
were also abundant. Mycorrhiza types formed in soils from clearcuts did not differ strikingly from those formed in undisturbed forest soils.

DISCUSSION

The greenhouse bioassay technique was useful in assessing relative abundance of ectomycorrhizal fungus propagules in soils from clearcuts and forests and would seem to be a more meaningful measure of total inoculum potential than measurement of a single source of inoculum. Some of the mycorrhizal fungi which grew on seedlings under greenhouse conditions may not be effective or even viable under conditions within clearcuts but may compete strongly with site-adapted fungi in this artificial environment. Results presented in Chapters 2 and 3 indicate, however, that the relative composition of ectomycorrhizae by morphological type was not influenced significantly by temperature or soil moisture.

Our data suggest that soils from clearcuts may contain significantly fewer propagules than soils from undisturbed forest stands, and that propagule numbers may be reduced even more in areas which have been clearcut and burned. Whether this reduction constitutes a "mycorrhizal inoculum deficiency" for nonmycorrhizal seedlings outplanted on these sites remains to be determined. The difference in root colonization, although statistically significant, may not be biologically significant. Under favorable conditions ectomycorrhizal fungi spread rapidly from one location on a root to another. But, there may be a critical period for the establishment of ectomycorrhizae before the onset of environmental stresses and
cessation of root growth because of seasonal changes. More important than the percentage of root tips colonized may be the relative efficiency of the fungal symbionts in nutrient uptake and water transport and the extent of their hyphal network in soil.

Other researchers have observed the effects of clearcutting and burning on ectomycorrhizal fungi or on mycorrhizae. Harvey, Jurgensen, and Larsen (1980) found fewer active ectomycorrhizal root tips in soils from young clearcuts compared to soils from an undisturbed stand in western Montana. After clearcutting in October, low numbers of active mycorrhizal tips survived until the following July. Of clearcuts which also were burned, and sampled 2 yrs later, active mycorrhizal roots were lacking except for ingress 1.5 m into the clearcut from an adjacent forest stand. Other sources of ectomycorrhizal fungus inoculum (spores, hyphae, sclerotia, etc.) were not included in their study. Wright and Tarrant (1958) examined root systems of naturally-occurring Douglas-fir seedlings in unburned, lightly burned, and severely burned plots. Mycorrhizae were most abundant in unburned plots and least abundant in the severely burned plots. The first occurrence of mycorrhizae on seedling root systems was found to occur at a greater soil depth in severely burned areas, an observation also made by Mikola et al. (1964). Malajczuk and Hingston (as cited by Malajczuk et al., 1981) found that, compared with unburned areas, fewer eucalypt roots were ectomycorrhizal in areas in which fire had removed the litter layer. Meyer-Schoeneberger and Perry (1982), using the greenhouse bioassay technique, compared the quantity and type of ectomycorrhizae
developing on Douglas-fir and western hemlock from two old-growth sites, a recent clearcut, a recent clearcut which had been burned, a naturally burned-revegetated area, and a young regeneration site in the Oregon Cascades. Highest percentage of mycorrhizal colonization developed in soil from the recent clearcut. Fewer mycorrhizae developed in soil from the clearcut which had been burned.

The effect of burning may be direct, e.g., causing thermal death of ectomycorrhizal fungus propagules near the soil surface or in the litter layers during the fire. They may also be indirect, through physical changes in soils, changes in soil nutrients (Neal, Wright, and Bollen, 1965; Kraemer and Hermann, 1979), and subsequent increases in soil temperature (Viereck and Dyrness, 1979).

Loss of organic matter may be among the most important results of fire (Kraemer and Hermann, 1979). Activity of ectomycorrhizae has been correlated with organic matter by Harvey, Jurgensen, and Larsen (1978; 1979). In their studies soil humus was the main substrate for mycorrhizal tips except when moisture became limiting during July and August. Then, decayed soil wood became the most important substrate. Kropp (1982) reported that decaying wood can be a source of ectomycorrhizal inoculum for western hemlock; ectomycorrhizal fungi were present in logs suspended above soil before they were colonized by western hemlock seedlings. Forest floor organic matter has been shown to be a source of ectomycorrhizal inoculum for Douglas-fir (Chapter 4), but Alvarez, Rowney, and Cobb (1979) observed that organic matter impeded mycorrhiza formation on Abies concolor seedlings. The saprophytic capabilities of ectomycorrhizal
fungi in decaying wood and organic matter warrants further study. Moisture retention properties of decaying wood (Harvey, Jurgensen, and Larsen, 1978; 1979) may provide a niche for growth or survival of ectomycorrhizal fungus propagules and may provide a reservoir of inoculum on clearcut sites which have not been burned.

Small mammals are vectors of ectomycorrhizal fungus spores which may also be important to re-establishment of conifers (Maser, Trappe, and Nussbaum, 1978). Dead woody material remaining in clearcuts may serve a dual purpose by also providing habitats for these animals (Maser, Trappe, and Ure, 1978) for increased distribution of ectomycorrhizal fungus inoculum.

ACKNOWLEDGEMENTS

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two years on reforestation sites in North Carolina and Florida. Forest Science 23, 363-373.


Figure 1.1. Mycorrhizal colonization of Douglas-fir and ponderosa pine seedlings grown in soil from undisturbed forest sites (F), clearcut sites (C), or clearcut and burned sites (CB). Brackets indicate .95 confidence intervals. For each host, all treatments are significantly different, $P = .05$ (Scheffe Multiple Range Test). Scale: 0 = no mycorrhizae; 1 = 1-20% of root tips colonized; 2 = 21-40%; 3 = 41-60%; 4 = 61-80%; 5 = 81-100%.
CHAPTER 2

EFFECT OF ROOT ZONE TEMPERATURE ON VA AND ECTOMYCORRHIZA FORMATION IN DISTURBED AND UNDISTURBED FOREST SOILS OF SOUTHWEST OREGON

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SUMMARY

The presence of ecto- and VA mycorrhizal fungi in soils from five sites in a mixed conifer zone in southwest Oregon, each consisting of a 1-1.5 year-old clearcut adjacent to an undisturbed forest stand, was determined by bioassay with Pseudotsuga menziesii, Pinus ponderosa, and Trifolium subterraneum as hosts grown at root zone temperatures ranging from 7.5 to 35°C. Maximum formation of both ecto- and VA mycorrhizae occurred at 18.5-24°C in soils from all sites, and there were no significant qualitative or quantitative differences between disturbed (clearcut) or undisturbed (forest) soils. Mycorrhiza formation was moderate even at the lowest
temperature tested (7.5°C) but was greatly reduced or prevented at or above 29.5°C. Treatment of soil at 35°C for 1 week did not appear to adversely affect viability of ectomycorrhizal fungus propagules, but young mycorrhizae subjected to the same treatment appeared to be severely injured. Thus it appears that ectomycorrhizal fungi can tolerate high soil temperatures as dormant propagules, but perhaps not as young, actively growing mycelium close to the soil surface. More important, however, may be the ability of native mycorrhizal fungi to grow at low soil temperatures, especially as they may contribute to the survival of seedlings outplanted into climatic zones characterized by warm, dry summers following cool, wet winters and springs.

INTRODUCTION

Results from a previous study involving 36 sites in southwest Oregon and northern California indicated that the inoculum potential of ectomycorrhizal fungi in soil may be lower in clearcuts than in adjacent forest stands (Chapter 1). In that study, clearcuts of various ages, burn histories, and site modifications, distributed over a large geographical area, were chosen by foresters to represent a diversity of "difficult to regenerate" sites. In the present study, fewer sites (5) of clearcuts 1-1.5 yr old from a restricted geographical area (Douglas Co., Oregon) were assayed in more detail for soil populations of VA- and ectomycorrhizal fungi. Since many soil properties may be altered as a result of clearcutting and burning, including changes in nutrient availability, soil temperature, organic matter and associated soil microorganisms, soil
physical properties, and vegetation, it was not clear from the initial study if disturbance per se or the concomitant changes produced by disturbance were responsible for the apparent reduction in ectomycorrhizal inoculum potential. Of these factors, the effect of soil temperature on mycorrhiza development was chosen for the present study.

The climate in southwest Oregon is characterized by cool wet winters and warm dry summers, with most of the 82 cm average annual precipitation occurring as rain during October-March (Steerns, 1960; Waring and Franklin, 1979). Mean January temperature is 4°C and mean July temperature is 20°C. Conifer root growth begins in early spring when temperatures reach about 5.5°C. Root growth activity peaks in the weeks preceding bud burst in mid-spring and is reduced to a low level until fall rains initiate a second, smaller peak of activity (Heiner and Lavender, 1972; Cleary, Greaves, and Hermann, 1978). Under controlled conditions when moisture is not limiting, root growth of Douglas-fir from a southwest Oregon seed source is optimal at approximately 20°C (Lavender and Overton, 1972). Twenty-four hr temperature data recorded 20 cm below the soil surface in clearcuts during July ranged from pre-dawn minima of 13°C to maxima of 31°C (6:30-10:30 p.m.) (S. D. Hobbs, personal communication). The effect of soil temperature on the growth of mycorrhizal fungi native to forest soils in southwest Oregon is not known.

Researchers in Georgia and Missouri (U.S.A.), South Africa, and Australia have found optimal root colonization by ectomycorrhizal fungi to occur at relatively high soil temperatures. Marx, Bryan,
and Davey (1970) inoculated *Pinus taeda* seedlings with *Pisolithus tinctorius* and *Thelephora terrestris*, and found that percent root colonization by *P. tinctorius* was greatest at 34°C, while *T. terrestris* grew better at lower temperatures (14, 19, 24°C). These fungi were later shown to increase survival of pine seedlings grown at a root zone temperature of 40°C (Marx and Bryan, 1970). Theodorou and Bowen (1971) found that mycorrhizal growth along roots was optimal at 25°C and declined between 20°C and 15°C for some fungi. Differences in temperature response between isolates of the same species were observed. Marais and Kotze (1978) found greatest mycorrhizal colonization to occur at 35°C. *Pisolithus tinctorius* mycorrhizae formed on black oak only at the higher temperatures tested (28°C and 33°C) in a study by Dixon *et al.* (1980). In a subsequent experiment, growth of inoculated seedlings at high temperatures was found to be better than that of non-inoculated controls. In growth chamber experiments with the chamber programmed to simulate late fall and winter temperatures and photoperiods of western Oregon, Trappe (unpublished data) found that roots of Douglas-fir were dormant when the diurnal soil temperature maximum remained below 4 °C. Root elongation, mycorrhiza formation, and growth of hyphae from mycorrhizae into soil all occurred when soil temperature exceeded 4° for eight hours. The rate of these growth activities was higher with late fall and late winter maxima of 16° and 11°, respectively, than at the midwinter maximum of 8°. Sinclair (1974) observed an increase in Douglas-fir mycorrhizae in a Washington nursery during April-June which corresponded with a rise
in soil temperature from 6 to 18°C. Harvey, Jurgensen, and Larsen (1978) reported seasonal fluctuations in the number of mycorrhizal tips found in humus, decayed wood and mineral soil of a Douglas-fir/larch forest in Montana. Maximum number of mycorrhizal tips during May-June in humus and mineral soil coincided with increased soil temperatures (10-11.8°C) before soil moisture became limiting. Lobanow (1960) reported root growth and ectomycorrhiza formation in the Soviet Union in spring when soil temperatures reach 8°C. However, in 23- and 180-year-old Abies amabilis stands in western Washington, biomass of active mycorrhizae was lowest in summer and highest in fall and winter when soil temperatures under the snow pack were maintained at 1°C (Vogt et al., 1980). There seems to be little correlation between temperature response of ectomycorrhizal fungi in vitro and their response in association with a host (Marx et al., 1970; Theodorou and Bowen, 1971).

Clearcut areas are rapidly colonized by grasses, ferns, shrubs and hardwoods and other nonconifer plants. Many of these form VA mycorrhizae (Parke, unpublished observations), so the temperature response of VA fungi native to these soils was also investigated.

Researchers have examined the effects of temperature on spore germination, pre-colonization, root colonization, and spore production of VA mycorrhizal fungi. Furlan and Fortin (1973) observed maximum colonization of Allium cepa roots inoculated with Gigaspora calospora at their highest temperature regime (26°C day/21°C night) and the least at their lowest (16°C day/11°C night). High temperatures reduced the lag phase of the sigmoidal
curve of the root colonization process. Hayman (1974) reported that cool temperatures (14°C) did not reduce the percent onion roots colonized unless light was also limiting, but plant growth was enhanced only at the higher temperature regimes (23°C day/14°C night or 23°C). Schenck and Schroder (1974) found maximum mycelial development of Gigaspora calospora in soybean roots at 28-34°C with greatest arbuscular development at 30°C. Vesicle and spore production were favored by temperatures near 35°C. Spore germination of Gigaspora coralloidea and G. heterogama from Florida and Glomus mosseae from Washington was compared at 15°, 20°, 25°, and 34°C (Schenck, Graham, and Green, 1975). Glomus mosseae germinated best at 20°C; germination of the two Florida isolates improved with increasing temperature, indicating adaptation to the warm Florida climate. Smith and Bowen (1979) showed that temperature affected pre-colonization growth of naturally occurring VA mycorrhizal fungi. Formation of initial entry points along the roots was more rapid at higher soil temperatures (up to 25°C).

Objectives of our studies were:

1. To compare the temperature range and optima for root colonization by ecto- and VA mycorrhizal fungi native to southwest Oregon.

2. To compare the mycorrhizal colonization of roots grown in disturbed vs. undisturbed soils.

3. To determine if mycorrhizal fungi from disturbed vs. undisturbed soils respond differently to temperature.
4. To test survival of ectomycorrhizal propagules at sustained high soil temperature.

5. To test effects of high soil temperature on already established ectomycorrhizae.

MATERIALS AND METHODS

Experiment 1

Five sites, each a clearcut 1-1.5 yrs old adjacent to an undisturbed forest stand, were chosen in the Roseburg area (Douglas County) of southwest Oregon. Located in the Coast Range north of the Siskiyou Mountains, this area is characterized as a mixed-conifer zone (Franklin and Dyrness, 1973) with Douglas-fir (*Pseudotsuga menziesii*) the prevalent tree species. All clearcuts had been burned. Mean soil pH was 6.1 with 14 ppm Olsen available phosphorus, 7.5 ppm ammonium nitrogen, 11.1 ppm nitrate nitrogen, and 6.9% organic matter. The sparse vegetation in the clearcuts consisted mainly of grasses, herbs, and hardwoods sprouted from stumps. Each site is briefly identified below and vegetation of the undisturbed portion of each site described:

2. **Tom Taylor (T29S R8W S33):** Elev. 488 m, W aspect, 10% slope. Jory silty clay loam. *Pseudotsuga menziesii*, *Calocedrus decurrens*, *Pinus ponderosa*, *Arbutus menziesii*, *Abies grandis*, *Berberis nervosa*, *Vaccinium membranaceum*, *Gaultheria shallon*, *Arctostaphylos* sp.


4. **South Fork Middle Creek (T31S R6W S35):** Elev. 853 m, SE aspect, 70% slope. McGinnis very gravelly loam. *Pseudotsuga menziesii*, *Pinus lambertiana*, *Calocedrus decurrens*, *Arbutus menziesii*, *Castanopsis chrysophylla*, *Xerophyllum tenax*, *Ribes spp.*, *Gaultheria shallon*, *Berberis nervosa*.

5. **Boomer Hill (T29S R6W S33):** Elev. 610 m, N aspect, 35% slope. Beekman very gravelly loam. *Pseudotsuga menziesii*, *Calocedrus decurrens*, *Arbutus menziesii*, *Abies grandis*, *Gaultheria shallon*, *Polystichum* sp.
Soil was collected in January just prior to planting of conifer seedlings on the clearcuts. One soil sample was collected from the top 0-10 cm of mineral soil at 5 m intervals along three 20 m transects (15 samples total in each disturbed and each undisturbed area) for each of the five sites. Soil was sieved through .5 cm mesh to remove stones and large woody debris but permit passage of root fragments. The fifteen samples from each area were then combined, mixed (1:1) with coarse vermiculite and sown with surface-sterilized Douglas-fir, ponderosa pine, or subterranean clover (*Trifolium subterraneum*) seeds in 70 cc tubes (Ray Leach Cone-tainers, Inc.). Each soil had 15 replications per temperature treatment for each plant host. Clover plants were inoculated with *Rhizobium* suspension. Controls consisted of pasteurized (65°C/30 min aerated steam) soil blends. When plants had emerged, the replicates of each host-soil combination were randomly placed in controlled temperature "air bath" boxes which maintained root zone temperature at 7.5°, 13°, 18.5°, 24°, 29.5°, or 35°C ± .5°C for the remainder of the experiment. Temperatures were monitored every 15 min with a 12-point thermograph recorder. Plant shoots were exposed to ambient greenhouse temperatures (24°C day/18°C night) with a 16 hr photoperiod (240 uE m⁻² sec⁻¹). Beginning 6 wks after germination, plants were fertilized weekly with 3 ml Long-Ashton nutrient solution (Hewitt, 1966) at 1/4 strength phosphorus levels. Plants were harvested 14 wks (Douglas-fir, ponderosa pine) or 10 wks (clover) after germination. Conifer roots were washed and examined for ectomycorrhizal colonization. Total number of root tips, number and
percent of root tips which were mycorrhizal, total root length (using a map measurer), shoot height, shoot dry weight, and root dry weight were recorded for each seedlings. Mycorrhizal tips were classified according to morphological type. Clover roots were cleared and stained by a modification of the Philips and Hayman (1970) technique and percent root length colonized by VA fungi assessed (Biermann and Linderman, 1981). Dry weight of clover shoots was also recorded.

Experiment 2

Soil from site 2 (Tom Taylor) was mixed 1:1 with coarse vermiculite and incubated at 35° for 0, 4, 12, 24, 72 hrs and for 1 wk prior to sowing with Douglas-fir seed. At this time, tubes were placed in an 18.5° temperature box to maximize ectomycorrhizae formation. Seedlings were harvested 14 wks after germination and assessed for mycorrhizae and plant parameters as described for Experiment 1.

Experiment 3

Sixty tubes of the same soil mixture (Expt. 2) were sown with Douglas-fir seed and placed in an 18.5° temperature box. Fourteen wks following germination, 20 tubes were harvested and evaluated for mycorrhizae and plant growth. Twenty tubes were transferred to a 35° temperature box, and 20 tubes remained in the 18.5° box for an additional four weeks before both treatments were harvested and evaluated.
RESULTS

Experiment 1

Optimal temperature for Douglas-fir mycorrhizae was 18.5°; 24° was optimal for ponderosa pine mycorrhizae (Figs. 2.1 and 2.2). Colonization of both hosts was substantially reduced at 7.5°, 13°, 29.5°, and 35°. Clearcut and undisturbed forest soil sources did not differ significantly at any temperature for either host. Sites did not differ significantly from each other in response to temperature, so the data have been combined. Percent of roots which are mycorrhizal, number of mycorrhizae per cm root, and number of mycorrhizae per seedling showed parallel trends; therefore, only percent mycorrhizae are reported. Mycorrhizal plants were similar in size or smaller than non-mycorrhizal control plants except at 24°C for ponderosa pine (Figs. 2.6 and 2.7). Root and shoot growth of both hosts was largest at 18.5° or 24°. A smaller percentage of ponderosa pine short roots were mycorrhizal than of Douglas-fir except at 29.5 and 35°. Composition of mycorrhizae by morphological type was influenced by temperature (Figs. 2.4 and 2.5). A white rhizomorphic type predominated at all temperatures for Douglas-fir and was favored at 18.5-24°; this type was predominant only at 18.5-29.5° for ponderosa pine. A swollen brown type and to a lesser extent, *Cenococcum geophilum* mycorrhizae, were also found. Shoot/root ratios for all treatments showed similar trends with values near 1.0 occurring over the range 18.5-29° (Fig. 2.8).

Optimal temperature for VA mycorrhizal development on clover was 18.5° (65% of root length colonized) (Fig. 2.3). Colonization was
reduced to 35% at 7.5° and 10% at 29.5°. No colonization occurred at 35°. Infection levels did not differ significantly between clearcut and undisturbed forest soils at any temperature. Optimal temperature for clover shoot growth was 24° (Fig. 2.9). Mycorrhizal plants were significantly larger than nonmycorrhizal control plants at all but two temperatures (7.5°, 18.5°) where differences in growth were not significant (Fig. 2.9). Although percent root colonization was not significantly different between mycorrhizal plants grown at 18.5° and 24°, striking growth enhancement occurred at 24°, possibly indicating a temperature effect on external hyphae. Colonization of clover roots was by a fine endophyte, *Glomus tenuis* (Greenhall) Hall in all treatments over the range of temperatures tested. Other endophytes were rarely present (<10%) and spores were not found.

**Experiment 2**

Percent mycorrhizae did not differ significantly in response to the various durations of soil pre-treatments at 29.5°C (Table 2.1). Plant growth was also unaffected by soil pre-treatment.

**Experiment 3**

At 14 wks, 42% of the root tips were mycorrhizal (Table 2.2). This increased to 51% in the seedlings which remained at 18.5°C and were harvested 4 wks later. Only 21% of the root tips on seedlings transferred to 35°C were mycorrhizal. Root weight also declined for the seedlings transferred to the higher temperature. Mycorrhizae in the high temperature treatment appeared collapsed and moribund and lacked outgrowing hyphae and rhizomorphs. These mycorrhizae appeared physiologically inactive.
DISCUSSION

In this study, temperature optima for both ectomycorrhizae and VA mycorrhiza development were lower than reported previously by many researchers (Marx and Bryan, 1970; Marx et al., 1970; Theodorou and Bowen, 1971; Furlan and Fortin, 1973; Schenck and Schroder, 1974; Schenck et al., 1975; Marais and Kotze, 1978; Smith and Bowen, 1979; Dixon et al., 1980). Formation of ectomycorrhizae by fungi native to southwest Oregon forest soils was optimal at 18.5° (with Douglas-fir) or 24° (with ponderosa pine) and severely restricted at 29.5°, well below the 33-35° temperatures found to be optimal for Pisolithus tinctorius mycorrhiza development (Marx et al., 1970; Marais and Kotze, 1978; Dixon et al., 1980). In our study, mycorrhiza formation was moderate even at the lowest temperature tested (7.5°). Soilborne propagules of native ectomycorrhizal fungi survived an extended period of high soil temperature (1 wk/35°C) without any reduction in viability, but established, actively growing mycorrhizae were intolerant of high soil temperature. It is unlikely that soil temperatures under natural conditions become high enough to kill "dormant" mycorrhizal propagules, but newly established mycorrhizae on the shallow root systems of conifer seedlings may be vulnerable to high soil temperature during the first summer after planting. After this time, roots would probably extend below the zone where high temperature could restrict mycorrhizal activity. In a study of a Coast Range (Oregon) Douglas-fir stand, mycorrhizae were found as deep as 30-50 cm (Fogel and Hunt, 1979). A reservoir of other propagules (spores, hyphae, sclerotia) is likely to be sustained deep
in the soil profile, and air- or mammal-borne (Maser, Trappe, and Nussbaum, 1978) spores may be deposited once or twice annually. More important than heat tolerance may be the ability of mycorrhizal fungi to grow at cool soil temperatures (Theodorou and Bowen, 1971). It is during the cool, wet season that most conifer root growth occurs and thus most infection sites are formed. Since conifer seedlings are planted during the winter and early spring, rapid colonization by mycorrhizal fungi could enhance plant growth and survival through increased water and nutrient uptake before the onset of environmental stress in summer. Mycorrhiza establishment and rhizomorph formation may be critical to the survival of newly planted seedlings subjected to moisture stress (Chapter 3).

Temperature optima for mycorrhiza development seems to reflect adaptation to climate and coincide with temperature optima for active root growth. Studies with Pisolithus tinctorius isolates from the southeast U.S. show maximal development at a high soil temperature. The Georgia climate is characterized by warm summers with frequent precipitation and mild winters. Oregon's summer drought limits root growth to cooler, wet periods of the year. Temperature response of conifer root growth has been shown to vary according to seed source (Lavender and Overton, 1972). Just as provenance is an important consideration in selecting seed for particular environmental conditions, so, too, could ecotypes of ectomycorrhizal fungi adapted to growth at cool soil temperatures be selected for nursery inoculations and subsequent outplanting on harsh sites.
Interestingly, neither the degree of root colonization nor the composition of morphologically distinguishable types of ectomycorrhizae differed significantly between clearcut and undisturbed forest soils. This contrasts with a previous study in which the ectomycorrhiza inoculum potential of disturbed sites was less than in adjacent forest stands (Chapter 1). However, in the present study only recently disturbed areas, more representative of sites currently being replanted with conifers, were sampled as contrasted with our earlier study where many older clearcuts were examined. These results also differ from those described by Harvey et al. (1980) in which the number of active residual ectomycorrhizal tips was significantly reduced by August after clearcutting the previous fall. However, ectomycorrhizal tips are probably only one of many sources of inoculum, and structures likely to be better suited to long-term survival in the absence of a living host, such as spores and sclerotia, were not measured in their study. Our seedlings were harvested at an early enough age that degree of root colonization probably would have reflected differences in propagule numbers, but more importantly for our purposes, indicated that inoculum of ectomycorrhizal fungi does not appear to be limiting up to 1.5 yrs after disturbance, at least not during the winter-spring when conifer seedlings are normally planted in this area.

It is possible that differences in mycorrhiza composition were obscured because of limitations in visually identifying and classifying ectomycorrhizae, especially at the early stages of infection. However, numerous isolations of fungi from mycorrhizae
all yielded a single fungus from the predominant "white type". _Cenococcum geophilum_ was the only ectomycorrhizal symbiont encountered which was identifiable. Fruiting bodies of several ectomycorrhizal fungus species were collected from the undisturbed stands, indicating that although inoculum of several fungi was probably present, our bioassay conditions could have selected for fewer mycorrhizal fungi than might naturally occur.

The response of VA mycorrhizal fungi was similar to that of the ectomycorrizal fungi; again, the temperature optima and range reported here are cooler than that reported by many researchers and seem to reflect adaption to climate as proposed by Schenck _et al._ (1975).

Our results differ from a number of studies on VA mycorrhizal fungi and disturbance in natural systems. Moorman and Reeves (1979) bioassayed an old roadbed and adjacent undisturbed area of an arid mid-elevation sagebrush community and determined that number of propagules in the roadbed was 1/40 that of the undisturbed area. Using an end-point dilution technique, Powell (1980) found that eroded soils contained fewer VA propagules than mature pasture soils. However, in a newly cleared Florida woodland, root colonization by VA fungi was initially high for the first year or two, declining thereafter for 3 yrs and then eventually increasing in the fifth year of crop plant monoculture (Schenck and Kinloch, 1980). Reeves _et al._ (1979) observed a gradual increase over time in the number of non-mycorrhizal plant species which invaded an old roadbed, substantiating their premise that a lack of VA fungi in
these soils prevented recolonization by mycorrhizal plant species. In our study, virtually all plants invading clearcuts belong to mycorrhizal families (Parke, unpublished observations). Although a single harvest at a single soil dilution may have obscured differences in number of propagules between clearcut and undisturbed forest soils, our main interest was to determine the likelihood that plant growth in the clearcuts would be limited by the availability of mycorrhizae. It does not seem likely that VA inoculum is limiting in clearcuts less than 1.5 yrs old. However, the diversity of VAM fungi is definitely limited.

The absence of VA spores in these forest soils, even after 1.5 yrs of pot-culturing, is consistent with other investigations of natural systems in which spore numbers were low even though root colonization was high (Mosse and Bowen, 1968; Gerdemann and Trappe, 1974; Read, Koucheki, and Hodgson, 1976; Redhead, 1977; Schenck and Kinlock, 1980) and suggests that other forms of inoculum may be more important. Colonized roots and hyphae are important sources of inoculum, particularly in communities of perennial plants (Mosse and Bowen, 1968; Read et al., 1976; Johnson, 1977). It has also recently been proposed that VA fungi are capable of limited saprophytic growth on moribund roots of hosts and non-hosts or on organic debris (Hirrel, Mehravan, and Gerdemann, 1978; Parke and Linderman, 1980; Ocampo and Hayman, 1981; Warner and Mosse, 1982). This may account for the survival of VA fungi in the absence of living host roots immediately following disturbance.
**Glomus tenuis**, by far the predominant VA endophyte encountered in our study, has a world-wide distribution, occurring in forests (Johnson, 1977), grasslands (Crush, 1973a, 1973b; Molina, Trappe, and Strickler, 1978), and an alpine community (Read and Haselwandter, 1981). It appears to be an important pioneer (Johnson, 1977) and is capable of greatly increasing plant growth in soils of low phosphorus availability (Crush, 1973; Powell, 1979). Soils used in our study were low in available phosphorus, and growth of *Thuja plicata* (western red cedar) was severely limited in the absence of *G. tenuis* inoculum (Appendix). Propagules of this endophyte were abundant in litter (decomposing needles, etc.) from the undisturbed mixed conifer stands (Chapter 4) in addition to soils of clearcut and undisturbed forest areas.

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REFERENCES


Table 2.1. Effect of 35°C soil incubation of different durations on subsequent root colonization by ectomycorrhizal fungi.

<table>
<thead>
<tr>
<th>Incubation period</th>
<th>Percent mycorrhizal tips</th>
</tr>
</thead>
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<tr>
<td>0 hr</td>
<td>38.2 ± 5.0</td>
</tr>
<tr>
<td>4 hr</td>
<td>50.4 ± 2.1</td>
</tr>
<tr>
<td>12 hr</td>
<td>40.9 ± 9.8</td>
</tr>
<tr>
<td>24 hr</td>
<td>53.0 ± 8.2</td>
</tr>
<tr>
<td>72 hr</td>
<td>56.4 ± 8.5</td>
</tr>
<tr>
<td>1 wk</td>
<td>27.9 ± 6.4</td>
</tr>
</tbody>
</table>

Values are means of 20 seedlings and ± s.e. (mean).

Values not significantly different, P = .10
Table 2.2. Effect of high temperature on established ectomycorrhizae.

<table>
<thead>
<tr>
<th></th>
<th>Number of mycorrhizae</th>
<th>Percent mycorrhizae</th>
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<td>18.5°, 18 wks</td>
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<td>110b (7)</td>
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<td>18.5° → 35°, 18 wks</td>
<td>8.6b 91.9)</td>
<td>1.0b (4.7)</td>
<td>100ab (5)</td>
<td>76a (6)</td>
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Values are means of 17 seedlings and ± s.e. (mean).

Values in columns not followed by same letters are significantly different, P = .05 (Duncan's Multiple Range Comparison).
Figure 2.1. Effect of root zone temperature on percent mycorrhizal colonization of Douglas-fir roots (--- undisturbed forest soil; --- clearcut soil).
Figure 2.2. Effect of root zone temperature on percent mycorrhizal colonization of ponderosa pine roots (--- undisturbed forest soil; - - - - clearcut soil).
Figure 2.3. Effect of root zone temperature on percent clover root length colonized by VA fungi (— undisturbed forest soil; —— clearcut soil).
Figure 2.4. Effect of temperature and disturbance on composition of Douglas-fir ectomycorrhizae by morphological type (white rhizomorphic, brown, Cenococcum geophilum). Left-hand column of each pair = undisturbed forest soil; right-hand column of each pair = clearcut soil.
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CHAPTER 3

THE ROLE OF ECTOMYCORGHAZAE IN DROUGHT TOLERANCE OF DOUGLAS-FIR SEEDLINGS

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SUMMARY

Experiments were conducted to test the relative ability of mycorrhizal and nonmycorrhizal Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] seedlings to tolerate and recover from drought conditions, using reduction in CO₂ fixation as an overall indicator of plant moisture stress. Mycorrhizal and nonmycorrhizal seedlings, either watered daily or conditioned to cyclic drying and rewetting of the soil, were compared. Net photosynthetic rates of mycorrhizal and nonmycorrhizal seedlings watered daily did not differ significantly; however, drought stressed mycorrhizal seedlings fixed CO₂ at a rate 10x that of nonmycorrhizal seedlings. Although total leaf water potentials of mycorrhizal treatments were more negative than for
nonmycorrhizal treatments, mycorrhizal seedlings recovered more rapidly than nonmycorrhizal seedlings.

Nonmycorrhizal seedlings and seedlings inoculated with four ectomycorrhizal fungus species were allowed to become desiccated, then rewatered and compared for their ability to tolerate and recover from drought. Seedlings inoculated with *Rhizopogon vinicolor* were less affected by drought than any of the other mycorrhizal or nonmycorrhizal treatments. Net photosynthetic rate of *Rhizopogon*-inoculated seedlings 24 hr following rewatering was 7x that of nonmycorrhizal seedlings. The transpiration rate of *Rhizopogon*-inoculated seedlings was high during periods of soil water availability but declined rapidly during the drought period, and after rewatering quickly resumed a transpiration rate higher than for other treatments.

*In vitro* growth of these ectomycorrhizal fungi over a range of nutrient solutions osmotically adjusted with polyethylene glycol was a poor indication of effectiveness in reducing plant moisture stress *in vivo*.

**INTRODUCTION**

In southern Oregon and northern California, container-grown or bare-root conifer seedlings are planted on reforestation sites in the winter and spring. First-year mortality of these seedlings can approach 100% on harsh sites (Hermann, 1965). Plant moisture stress is probably the single most important cause of first-year mortality in southern Oregon (Hermann, 1965; Cleary, 1971; Heiner and Lavender, 1972; Williamson and Minore, 1978; Gratkowski, Jaszowski, and
Armstrong, 1979; Hobbs et al., 1980). This is due to low water-holding capacity of some soils, shallow root systems, infrequent summer precipitation and warm temperatures (Hobbs et al., 1980). Considerable effort and expense has been directed toward reducing plant competition for limited soil water through elimination of non-conifer species using herbicides and manual brush control. At the time of outplanting, conifer seedlings are generally nonmycorrhizal or colonized by fungi not necessarily adapted to forest soils. Inoculum density and viability of native ectomycorrhizal fungi may be reduced as a result of timber harvest and soil disturbance (Chapter 1).

Although ectomycorrhizae are commonly assumed to enhance water uptake by their hosts (Trappe, 1977; Trappe and Fogel, 1977; Ruehle and Marx, 1979), few researchers have actually addressed this experimentally (Reid, 1979). Some mycorrhizal fungi grown in vitro have been shown to grow or at least survive at water potentials below the permanent wilting point of their host (Theodorou, 1978) although tolerance to low water potentials varies widely among ectomycorrhizal fungus species (Mexal and Reid, 1973; Theodorou, 1978). Cromer (1935) found that Pinus radiata mycorrhizae resumed growth more quickly than did nonmycorrhizal roots following rewatering of drought-stressed trees. Theodorou and Bowen (1970) observed that outplanted P. radiata seedlings inoculated with Suillus granulatus or Rhizopogon luteolus survived a particularly dry summer better than uninoculated seedlings. In a pot experiment in which water was withheld from mycorrhizal and nonmycorrhizal P. radiata seedlings,
Sands and Theodorou (1978) determined that resistance to water flow from the soil through mycorrhizal plants was greater than for nonmycorrhizal plants, due largely to differences in root geometry. Transpiration rates of the two treatments did not differ significantly, but using leaf potential as a measure of plant water stress they determined that mycorrhizal seedlings became more stressed than nonmycorrhizal seedlings under their conditions. D. M. Maronek and J. W. Hendrix (unpublished data as cited in Maronek, Hendrix, and Kiernan, 1981) reported that greenhouse-grown nonmycorrhizal oak seedlings wilted more readily than seedlings inoculated with *Pisolithus tinctorius*. Dixon et al. (1980) showed that although *Quercus alba* seedlings inoculated with *P. tinctorius* and subjected to drought conditions had xylem potentials more negative than nonmycorrhizal seedlings, root growth during the drying period was greater for mycorrhizal seedlings. Following rewatering, xylem potential recovered to normal levels more rapidly among mycorrhizal seedlings.

It is well established that net photosynthesis declines in response to plant water stress, mainly due to stomatal closure that accompanies loss of turgor (Boyer, 1976). Experiments were conducted to test the relative ability of mycorrhizal and nonmycorrhizal container-grown Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] seedlings to tolerate and recover from drought conditions. *In vitro* growth of these fungi in osmotically adjusted nutrient solutions was also compared.
MATERIALS AND METHODS

Experiment 1: Effect of native ectomycorrhizal fungi on growth and tolerance of Douglas-fir seedlings to periodic desiccation.

In this experiment, net CO₂ fixation by mycorrhizal and nonmycorrhizal Douglas-fir seedlings conditioned to cyclic drying and rewetting of the soil was compared to net CO₂ fixation of mycorrhizal and nonmycorrhizal seedlings watered daily.

Forest soil known to contain an abundance of ectomycorrhizal propagules was collected near Roseburg, Oregon (Boomer Hill study site, Chapter 2). The site is an old-growth mixed conifer stand dominated by *Pseudotsuga menziesii* (Douglas-fir). Soil was sieved through 1 cm² mesh to remove pebbles and large organic debris and mixed 1:1 (volume basis) with coarse grade vermiculite. A portion of soil-vermiculite mix was left untreated; the remaining portion of the soil-vermiculite mix was treated with aerated steam (65°C/30 min) to eliminate mycorrhizal propagules without altering soil nutrient status. Both treatments were sown with surface-sterilized (30% H₂O₂/30 min) Roseburg area Douglas-fir seed in 70 cc tubes (Ray Leach Cone-tainers, Inc.). Plants were maintained in the greenhouse under high pressure sodium vapor lamps (16 hr photoperiod) with a 22°C day/18°C night temperature. All seedlings were watered daily for four months and received 3 ml Long-Ashton nutrient solution (Hewitt, 1966) at 1/4 phosphorus levels (11 ppm) every five days to supplement the low P availability in the soil-vermiculite mixture (7 ppm Olsen available P). Four months after planting, randomly selected seedlings from each soil treatment were harvested and examined for
mycorrhizal fungus colonization. Seedling shoot length, root and shoot dry weight were also recorded. The remaining seedlings were randomly assigned to one of two watering regimes: watered daily or watered every fifth day. At age seven months, data on net photosynthetic rates, leaf water potential, seedling growth and percent mycorrhizal colonization were obtained.

Net CO$_2$ fixation was used as an overall indicator of physiological responses associated with plant water stress and recovery (Odening, Strain, and Oechel, 1974). A portable plexiglass chamber modified from Cary (1977) was constructed to enclose individual Douglas-fir shoots in situ (Figure 1). Syringes inserted into the sealed chamber enabled collection of air samples at the beginning and end of 2.5 min fixation periods. Air samples were analyzed for CO$_2$ concentration using an infrared gas analyzer (Clegg, Sullivan, and Eastin, 1978) calibrated daily with CO$_2$ of a known concentration. Differences in the two CO$_2$ concentrations were considered to represent net photosynthetic fixation and were expressed on a needle area basis as mg CO$_2$ dm$^{-2}$ needle area hr$^{-1}$. In this experiment photosynthetic rates of plants watered daily were taken on five successive days in the greenhouse (light intensity 220-260 uEm$^{-2}$s$^{-1}$). Plants watered every fifth day were allowed to reach maximum stress of the drought cycle and then rewatered daily during the five day fixation period. Temperature in the chamber during the sampling periods varied less than ±1°C. Photosynthetic rates were averaged over the 5-day period to compensate for variability in greenhouse light intensity.
Leaf potential was measured at times representing maximum and minimum water stress. An isopiestic thermocouple psychrometer was used for leaf potential determination (Boyer and Knipling, 1965); five needles per seedling were sacrificed for each measurement.

At the time of harvest, seedling root systems were rinsed free of soil and examined for percent roots colonized by ectomycorrhizal fungi. Needles were removed and the two-dimensional surface measured using a LiCor Portable Area Meter. Needles, shoots and roots were dried 72 hrs at 70°C and weighed.

Experiment 2: Effect of four ectomycorrhizal fungi on drought tolerance and recovery of Douglas-fir seedlings.

In this experiment, nonmycorrhizal seedlings and seedlings inoculated with one of four species of ectomycorrhizal fungi were allowed to become desiccated and then rewatered. Treatments were compared for their ability to tolerate and recover from drought.

The same source of forest soil was used as for Experiment 1. All soil was sieved and treated with aerated steam (95°C/30 min) to eliminate mycorrhizal propagules and most other soil microorganisms. Soil was mixed 1:1 with modified Melin-Norkrans medium (MMN), vermiculite mycelial cultures of ectomycorrhizal fungi, or with autoclaved cultures for nonmycorrhizal controls and the treatment involving spore inoculum. Mycelial inocula included *Laccaria laccata* (L1) S238A, *Pisolithus tinctorius* (Pt) S471, and an unidentified native fungus (Na) isolated from mycorrhizal roots grown in Boomer Hill soil (Chapter 2). Three sporocarps of *Rhizopogon vinicolor* (Rv) from Mary's Peak near Corvallis, Oregon, collected and
identified by Dr. James M. Trappe, were macerated in a Waring blender in sterile distilled water to make 1 l spore suspension and stored at 4°C for 12 wks. Soil-vermiculite mixtures were placed in 70 cc tubes (Ray Leach Cone-tainers, Canby, Oregon) and sown with surface-sterilized (30% H₂O₂, 30 min) Douglas-fir seed. Three ml spore suspension was pipetted into tubes of the *Rhizopogon vinicolor* treatment. In an attempt to partially restore soil microbial populations, an extract free of ectomycorrhizal propagules was prepared from natural soil and added back to all treatments. This was made by mixing 500 cc soil with 2 l water, allowing the suspension to sit overnight, and filtering through a series of mesh sizes and ultimately through a nucleopore membrane (3 um). Three ml of extract were pipetted onto each tube of steam-treated soil.

Seedlings were grown in the greenhouse for 6 months under the same conditions as in Experiment 1. At this time they were moved to a growth chamber (16 hr photoperiod, light intensity 240 uEm⁻²s⁻¹, 22°C day/18°C night temperature, 35% relative humidity). Two weeks later seedlings were divided into two groups: those to be watered daily as before, and those allowed to dry out for seven days before being rewatered. Day 1 was the last day in which all seedlings were watered. The stressed seedlings were not rewatered until Day 8 but were watered daily thereafter during the recovery period (Days 9-13).

Photosynthetic rates were determined for all seedlings on Day 1, Day 8, and Day 13. Photosynthetic rates of stressed plants were also measured daily during the recovery period (Days 9-13). The technique
was the same as that described for Experiment 1. Daily means were determined for each experiment.

Transpiration rates, calculated by measuring hourly change in weight due to plant transpirational water loss, were measured daily after sealing the tops of tubes around stems with Permagum and covering basal drainage holes with plastic film. Plants in sealed containers were weighed hourly for three hours each day (10 am - 1 pm) to yield a mean rate of water loss (mg H₂O hr⁻¹). This rate was no different than hourly rates determined during a single 3 hr period, so seedling transpiration was not significantly changed by manipulations during weighing and handling.

Needle areas, shoot dry weight, root dry weight, and percent mycorrhizal roots were measured following plant harvest.

Experiment 3: In vitro growth of ectomycorrhizal fungi in solutions osmotically adjusted with polyethylene glycol.

Five ectomycorrhizal fungi, Laccaria laccata 238A, Pisolithus tinctorius S216, Cenococcum geophilum A145, Rhizopogon vinicolor A153, and the native mycorrhizal fungus described above, were grown in sterile liquid shake cultures of MMN solution amended with increasing concentrations of polyethylene glycol (PEG) 4000 (Baker) (average mol. wt. 3000-3700) to achieve osmoticas of -0.53, -2, -5, -10, -15, and -20 bars. Our source of PEG was found to be free of contamination by phosphorus or other elements at significant levels (Reid, Bowen, and McCleod, 1978) as analyzed by mass spectroscopy (Oregon State Univ. Plant Analysis Lab, Dept. of Horticulture). An isopiestic thermocouple psychrometer was used to measure osmotic
potential of PEG-amended solutions, and a standard curve for increasing concentrations of PEG in MMN was plotted using sucrose standards and tabular values for comparison (Boyer and Knipling, 1965). Table 3.4 shows the concentration of PEG required for a given osmoticum.

Sterile 125 ml flasks containing 40 ml concentrated PEG-MMN solutions were inoculated with 10 ml macerated (Waring blender 10-20 sec) ectomycorrhizal mycelia (19-26 mg oven dry wt per flask) in MMN. Random aliquots of fungus macerate were oven-dried and weighed to establish initial biomass of fungal inocula. Cultures were maintained on a rotary shaker (100 rpm) at room temperature (25°C) for 8 wks. Osmotic potential of the nutrient solutions varied less than ±.5 bar during the course of the experiment. At harvest, mycelia were gently rinsed on a 30 um nylon mesh sieve with distilled water to remove media, oven-dried and weighed. Net weight gain was determined for each culture.

RESULTS

Experiment 1

Results of Experiment 1 are summarized in Tables 3.1 and 3.2. Growth data for the early harvest (16 wks), before any plants were subjected to water stress, indicate no significant differences in shoot length, shoot or root dry wt between seedlings grown in natural vs. pasteurized soil. Percent mycorrhizal colonization was 66% for seedlings grown in natural soil and 0% for seedlings grown in pasteurized soil. At seven months, mycorrhizal and nonmycorrhizal seedlings watered daily showed no significant differences in growth
parameters; however, mycorrhizal and nonmycorrhizal seedlings conditioned to cyclic drought were smaller than seedlings watered daily. Stressed mycorrhizal plants had greater needle areas, root dry weight, and shoot dry weight than stressed nonmycorrhizal plants.

Net photosynthetic rate did not differ significantly between mycorrhizal and nonmycorrhizal seedlings watered daily. However, net photosynthetic rate of stressed mycorrhizal seedlings was approximately 10x greater than that of stressed nonmycorrhizal seedlings. Photosynthetic rate of stressed mycorrhizal seedlings did not differ significantly from rates for plants watered daily.

Minimum leaf potential of mycorrhizal and nonmycorrhizal seedlings watered daily did not differ significantly. For stressed treatments, however, minimum leaf potential measured at the peak of the drying cycle was more negative for mycorrhizal (-19.8 bars) than for non-mycorrhizal seedlings (-12.8 bars). Leaf potentials measured after rewatering of stressed seedlings did not differ significantly between treatments.

Mycorrhizal treatments were colonized at high levels (>90% of root tips colonized), predominantly (>85%) by an unidentified fungus forming thick white mantles and large ropey dark rhizomorphs. Present at much lower levels (<5%) were brown, smooth, swollen mycorrhizae. _Cenococcum geophilum_ mycorrhizae occurred only occasionally (<1%). Among the mycorrhizal seedlings subjected to periodic drought, a black, slender mycorrhiza was common (20% of tips) although the white rhizomorphic fungus still predominated.
Experiment 2

Comparison of size parameters between nonstressed Douglas-fir seedlings and seedlings suddenly subjected to drought stress shows that needle area, total dry wt, and shoot dry wt:root dry wt ratios were similar for all treatments (Table 3.3).

Rates of net CO$_2$ fixation and transpiration were similar for nonstressed seedlings and for stressed seedlings during initial stages of the drying period although this varied according to fungus inoculation treatment.

When water was not limiting, seedlings inoculated with Pt fixed CO$_2$ at a rate higher than that of other treatments (Figure 3.2). However, after seedlings were allowed to dry for a week before being rewatered on Day 8, Rv-inoculated seedlings fixed CO$_2$ at the highest rate (Figure 3.3). On Day 8, all treatments showed a negative net CO$_2$ fixation rate, e.g., respiration exceeded photosynthesis, but Rv-inoculated seedlings were least affected by drought and resumed a positive net CO$_2$ fixation rate more quickly and at significantly higher levels ($P = .05$, Student-Newman-Keuls' Test) than other treatments during the recovery period (Days 9-13). Net photosynthesis by Rv-inoculated seedlings 24 hrs after rewatering was approximately 7x greater than for nonmycorrhizal seedlings. The slight downward trend in net CO$_2$ fixation among well watered (non-stressed) seedlings during the course of the experiment (Figure 3.2) probably reflects growth (increase in needle area) during the 13-day period; since needles were destructively sampled and measured only at
the end of the experiment, CO₂-fixation rates for Day 1 are probably somewhat higher than actual rates.

Figure 3.4 shows that seedlings inoculated with Rv transpire at rates higher than for other treatments when water is not limiting, indicating a reduced whole-plant resistance to water flow. However, with initiation of drying, transpiration by Rv seedlings rapidly declined to low levels. Nonmycorrhizal seedlings were the slowest to respond to drought and continued to lose water at rates higher than for mycorrhizal seedlings. When stressed seedlings were rewatered on Day 8, Rv-inoculated seedlings significantly resumed transpiration rates (P = .05 Student-Newman-Keuls' Test) sooner than other treatments. Twenty-four hours after rewatering, the transpiration rate of Rv-inoculated seedlings was approximately 3.5x that of other treatments.

**Experiment 3**

Net weight gain of ectomycorrhizal fungi grown in vitro over the range of PEG concentrations is shown in Table 3.5. With the exception of *Pisolithus tinctorius*, for which growth is positively correlated ($R = .8577$) to increasing osmotica and which exhibited maximum growth at -20 bars, fungi grew best at the lower osmotica (-.53 and -2 bars). Growth was strongly, negatively correlated with increasing concentration of PEG for both *Cenococcum geophilum* ($R = -.8991$) and *Laccaria laccata* ($R = -.9562$). Unlike the other fungi which exhibited linear growth to form a filamentous mass of mycelium, *Rhizopogon* cultures produced balls of mycelium varying in diameter from .3-2 cm. Large balls eventually fractionated into smaller
balls. Since growth of this fungus was probably nonlinear and PEG concentrations and O₂ diffusion in the center of large balls was probably different than that of the ambient medium, response of *Rhizopogon* should probably be excluded from these results.

**DISCUSSION**

The effects of water stress on plant growth include loss of turgor resulting in stomatal closure, reduction of photosynthetic surface, decreased biomass, reduction in photosynthetic rates, and diversion of photosynthate to roots at the expense of shoot growth (Kramer, 1969). Results from Experiments 1 and 2 strongly indicate that certain ectomycorrhizal fungi can help plants to tolerate and recover from conditions of soil water deficits.

Net photosynthetic rate as measured here provided a sensitive and rapid assessment of the overall effects of plant moisture stress (PMS) in intact Douglas-fir seedlings. Optimal photosynthetic rates of nonstressed seedlings correspond to those reported elsewhere for Douglas-fir (Brix, 1979; Doehlert and Walker, 1981). The increase in photosynthetic rate among mycorrhizal seedlings is similar to that reported by Allen et al. (1981) for the VA-mycorrhizal grass *Bouteloua gracilis*.

In Experiment 1, during drought imposed on seedlings conditioned to cyclic stress, mycorrhizal seedlings had a net CO₂ fixation rate (on a unit area basis) ten times that of nonmycorrhizal seedlings. This occurred even though mycorrhizal seedlings had a larger transpirational surface and might have been expected to become more desiccated than nonmycorrhizal seedlings. Rate of net photosynthesis
of stressed mycorrhizal seedlings did not differ significantly from rates for seedlings watered daily. In addition to a reduction in photosynthetic rates, cyclic drought reduced root and shoot dry weight, needle area, and shoot:root ratio of nonmycorrhizal seedlings more than for mycorrhizal seedlings. In Experiment 2, in which nonmycorrhizal Douglas-fir seedlings and seedlings inoculated with one of four ectomycorrhizal fungi were suddenly subjected to drought, one fungus (*Rhizopogon vinicolor*) in particular appeared to lessen the severity of drought effects on its host. Rates of net CO₂ fixation during drought were reduced less and recovery to near-normal CO₂ fixation levels occurred more rapidly after rewatering among *Rv*-inoculated seedlings than among other treatments. Interestingly, the native ectomycorrhizal fungus in this experiment did not enhance host drought tolerance as it did in Experiment 1; perhaps conditioning to stress is necessary for this fungus to develop water-absorption or water-transport mechanisms beneficial to its host during drought.

In Experiment 1, leaf water potential of stressed mycorrhizal seedlings became more negative than nonmycorrhizal seedlings; however, total leaf water potential alone can be a misleading measure of prolonged PMS. Nonmycorrhizal seedlings had such a low mean net photosynthetic rate for the recovery period that sugar reserves may have been depleted, leading to a reduction in osmotic potential and less negative total leaf potential. Osmotic potential measurements, in addition to total leaf potential, would have been helpful in evaluating these effects. The probable depleted state of leaf solutes might help to explain the results of others (Sands and
Theodorou, 1978; Dixon et al., 1980) who have found mycorrhizal treatments with total leaf or xylem potential lower than nonmycorrhizal treatments and accepted this as an indicator of increased PMS.

Transpiration rates of drought-stressed seedlings (Experiment 3) indicate that nonmycorrhizal seedlings may continue to transpire water later into the drying cycle than mycorrhizal seedlings. Interestingly, transpiration rates of seedlings inoculated with \textit{Rhizopogon vinicolor} were higher than other treatments when soil water was available but declined rapidly when water was withheld. This could be a result of several phenomenae including more rapid depletion of available soil water and/or stomatal closure as a result of lower leaf turgor. Once water again became available however, seedlings inoculated with \textit{Rhizopogon vinicolor} quickly regained their photosynthetic capacity and transpiration also increased.

Mycorrhizae may help to reduce PMS by increased water uptake through a) decreased resistance to water flow from soil to roots, b) increased absorptive surface, or c) potential for fungal hyphae to penetrate smaller soil pores than root hairs (Reid, 1979). Additional mechanisms proposed for the role of VA mycorrhizae in host water relations are indirect benefits as a result of improved host nutrition (Safir, Boyer, and Gerdemann, 1972), perhaps through changes in membrane permeability as a result of increased phosphorus availability (Nelsen and Safir, 1982), or altered hormonal regulation of stomatal closure (Levy and Krikun, 1980). Hardie and Leyton (1981) found that larger water flow rates through roots of
mycorrhizal clover could not be explained by P nutrition alone. Mycorrhizal roots were longer and larger in diameter which together led to a 26-86% increase in absorptive surface compared to nonmycorrhizal root systems. When soil water was not limiting, transpiration rates of mycorrhizal plants were higher than for nonmycorrhizal plants, indicating lower root and possibly also lower leaf diffusion resistance. When soil water became limiting, however, mycorrhizal plants developed a higher leaf diffusion resistance and transpiration rates lower than that for nonmycorrhizal plants. They concluded that VA mycorrhizae somehow enabled plants to develop a lower leaf potential under stress conditions through osmoregulation. Lower leaf potential and a root system with higher conductivity would explain how mycorrhizal plants recover from stress more quickly than nonmycorrhizal plants.

It is quite possible that VA- and ectomycorrhizae have similar modes of action in host water relations. In our study, growth of mycorrhizal and nonmycorrhizal treatments was similar except under prolonged conditions of cyclic drought (Experiment 1), indicating host nutrition was not a factor. Increased water uptake would seem to be the best explanation for our results.

Rhizomorphs, or mycelial strands, were well developed among seedlings inoculated with *Rhizopogon vinicolor* or the native ectomycorrhizal fungus, and may be important in water conductance. Foster (1981) has recently described the morphology of these rhizomorphs. In mature strands, two types of thin-walled cells, one type containing cytoplasm and the other type ("vessels") devoid of
cell contents, are surrounded by a rind of small thick-walled hyphae. A polysaccharide gel is found between and surrounding the hyphal strands. Rhizomorphs have been shown to transport phosphorus and zinc (Skinner and Bowen, 1974a and 1974b); mechanisms for water transport are thought to function similarly (Sanders and Tinker, 1973). Reid (1979) states that it is unlikely that mycorrhiza sheaths themselves function in water absorption, but it is conceivable that hydration of spongy fungus mantles, mycelial strands and gels could prevent rapid water loss through soil as after a sudden summer rainfall. It is interesting that mycorrhizal fungi convey host benefits even when soil volume is artificially limited and hyphae growth is restricted; this suggests that absorption and/or transport by fungi is more effective than by roots.

There is no evidence to suggest that in vitro growth of ectomycorrhizal fungi over a range of osmotic potentials is a valid indicator of ability to grow at low soil water potential or alleviate PMS. This is in agreement with Theodorou and Bowen (1970), Theodorou (1978), and Mexal and Reid (1973). In vitro growth of ectomycorrhizal fungi may be impaired nutritionally or by low O₂ diffusion rates (Mexal et al., 1975), and host-mediated effects are eliminated. Although growth in liquid culture is probably quite different from growth in soil, our standard curve shows that third order interactions do occur in PEG-amended solutions, indicating that PEG may be more accurately considered a matricum rather than an osmoticum (Steuter, Mozafar, and Goodin, 1981). No "toxic" substances were found (Reid et al., 1978).
Tolerance to and recovery from drought stress would be valuable attributes for young conifers outplanted on dry sites. Root growth cessation in spring limits uptake to the upper soil regions which are rapidly depleted of moisture in the summer. A functioning mycelial network already in place would assure a competitive advantage over deep-rooted vegetation. Ability to photosynthesize during the summer would lead to increased seedling growth and more rapid biomass accumulation. Colonization by mycorrhizal fungi can be viewed as a kind of "drought avoidance" in that dry soil conditions are avoided spatially through hyphal penetration of deeper zones. Since in the Pacific Northwestern climate root growth is largely limited to spring and fall when soil moisture is available and temperatures may be cool, seasonal drought avoidance could be achieved by mycorrhizal fungi able to grow and colonize roots at cool soil temperatures (Chapter 2). It is of interest that growth of mycorrhizal treatments differed from nonmycorrhizal only when seedlings were subjected to water stress, indicating that when water is available, photosynthate is diverted to mycorrhizae at the expense of root and shoot growth; this is compensated for by increased absorptive capacity, more rapid recovery of photosynthetic activity, and increased chances for survival during the drought. Differences in host response to inoculation with various ectomycorrhizal fungi suggest that drought tolerance should be considered one of the more important criteria for selection of fungus species and ecotypes suitable for nursery inoculation.
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REFERENCES


Table 3.1. Experiment 1: Growth data for 16-wk-old mycorrhizal (M) and non-mycorrhizal (NM) Douglas-fir seedlings. All seedlings were watered daily.

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<td>1.14a</td>
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<tr>
<td>NM</td>
<td>0b (0)</td>
<td>.111a (.007)</td>
<td>.096a (.007)</td>
<td>.86a</td>
</tr>
</tbody>
</table>

Values are means ± s.e. Values not followed by the same letter are significantly different at the P = .05 level.
Table 3.2. Response of 7-month-old mycorrhizal (M) and non-mycorrhizal (NM) Douglas-fir seedlings watered daily or subjected to cyclic drought (watered every fifth day).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Net Photosynthesis (mgCO₂dm⁻²hr⁻¹)</th>
<th>Leaf Potential (bars)</th>
<th>Needle Area (cm²)</th>
<th>Root Dry Wt (g)</th>
<th>Shoot Dry Wt (g)</th>
<th>Shoot: Root</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>max</td>
<td>min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M, watered daily</td>
<td>11.07a (.95)</td>
<td>-10.7a (.6)</td>
<td>10.24a (1.32)</td>
<td>.408a</td>
<td>.287a (.020)</td>
<td>.72ab (.058)</td>
</tr>
<tr>
<td>NM, watered daily</td>
<td>7.55a (1.07)</td>
<td>-10.6a (.6)</td>
<td>11.23a (2.04)</td>
<td>.337ab</td>
<td>.268a (.044)</td>
<td>.76ab (.075)</td>
</tr>
<tr>
<td>M, watered every 5th day</td>
<td>8.95a (1.48)</td>
<td>-12.0a (.50)</td>
<td>8.40a (1.87)</td>
<td>.253bc</td>
<td>.237ab (.027)</td>
<td>.96b (.041)</td>
</tr>
<tr>
<td>NM, watered every 5th day</td>
<td>.87b (1.02)</td>
<td>-12.3a (.31)</td>
<td>3.30b (.31)</td>
<td>.182c</td>
<td>.118b (.014)</td>
<td>.65a (.025)</td>
</tr>
</tbody>
</table>

Values are means of five daily readings for 11 (watered daily) or 7 (watered every fifth day) seedlings. ± s.e. Values not followed by the same letter differ significantly at the P = .05 level (Scheffe's Multiple Range Comparison).
Table 3.3. Experiment 2: Comparison of size parameters for stressed and non-stressed seedlings inoculated with ectomycorrhizal fungi or non-mycorrhizal (see text for explanation of treatments).

<table>
<thead>
<tr>
<th></th>
<th>Needle area (cm²)</th>
<th>Total dry wt (g)</th>
<th>Shoot dry wt: root dry wt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Stressed</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rv</td>
<td>17.4abc</td>
<td>.582a</td>
<td>1.25a</td>
</tr>
<tr>
<td></td>
<td>(2.0)</td>
<td>(.048)</td>
<td>(.21)</td>
</tr>
<tr>
<td>Ll</td>
<td>14.5abc</td>
<td>.988b</td>
<td>.84a</td>
</tr>
<tr>
<td></td>
<td>(2.5)</td>
<td>(.047)</td>
<td>(.13)</td>
</tr>
<tr>
<td>Na</td>
<td>13.9abc</td>
<td>.681a</td>
<td>.98a</td>
</tr>
<tr>
<td></td>
<td>(2.5)</td>
<td>(.051)</td>
<td>(.18)</td>
</tr>
<tr>
<td>Pt</td>
<td>8.5a</td>
<td>.640a</td>
<td>.73a</td>
</tr>
<tr>
<td></td>
<td>(.5)</td>
<td>(.057)</td>
<td>(.06)</td>
</tr>
<tr>
<td>Nm</td>
<td>9.5ab</td>
<td>.607a</td>
<td>.90a</td>
</tr>
<tr>
<td></td>
<td>(1.3)</td>
<td>(.036)</td>
<td>(.20)</td>
</tr>
<tr>
<td><strong>Non-stressed</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rv</td>
<td>16.2abc</td>
<td>.629a</td>
<td>1.31a</td>
</tr>
<tr>
<td></td>
<td>(1.4)</td>
<td>(.033)</td>
<td>(.10)</td>
</tr>
<tr>
<td>Ll</td>
<td>14.9abc</td>
<td>.930b</td>
<td>.81a</td>
</tr>
<tr>
<td></td>
<td>(.6)</td>
<td>(.049)</td>
<td>(.03)</td>
</tr>
<tr>
<td>Na</td>
<td>20.6c</td>
<td>.925b</td>
<td>1.12a</td>
</tr>
<tr>
<td></td>
<td>(3.6)</td>
<td>(.133)</td>
<td>(.12)</td>
</tr>
<tr>
<td>Pt</td>
<td>13.8abc</td>
<td>.909b</td>
<td>.73a</td>
</tr>
<tr>
<td></td>
<td>(2.0)</td>
<td>(.082)</td>
<td>(.07)</td>
</tr>
<tr>
<td>Nm</td>
<td>11.7ab</td>
<td>.675a</td>
<td>.83a</td>
</tr>
<tr>
<td></td>
<td>(.8)</td>
<td>(.044)</td>
<td>(.07)</td>
</tr>
</tbody>
</table>

Values are means ± s.e. Values within a column not followed by the same letter are significantly different at the P = .05 level (Student-Newman-Keuls' Test).
Table 3.4. Concentration of polyethylene glycol (PEG 4000) used to achieve a range of osmotica.

<table>
<thead>
<tr>
<th>g PEG per liter MMN</th>
<th>Molarity PEG</th>
<th>-bars</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>-.53</td>
</tr>
<tr>
<td>80</td>
<td>2.40 \times 10^{-2}</td>
<td>-2</td>
</tr>
<tr>
<td>135</td>
<td>4.05 \times 10^{-2}</td>
<td>-5</td>
</tr>
<tr>
<td>205</td>
<td>6.15 \times 10^{-2}</td>
<td>-10</td>
</tr>
<tr>
<td>255</td>
<td>7.65 \times 10^{-2}</td>
<td>-15</td>
</tr>
<tr>
<td>300</td>
<td>9.00 \times 10^{-2}</td>
<td>-20</td>
</tr>
</tbody>
</table>
Table 3.5. Net gain (g) in dry weight among five ectomycorrhizal fungi grown in vitro in nutrient solutions amended with polyethylene glycol.

<table>
<thead>
<tr>
<th>bars</th>
<th>Rhizopogon vinicolor</th>
<th>Laccaria laccata</th>
<th>Native</th>
<th>Pisolithus tinctorius</th>
<th>Cenococcum geophilum</th>
</tr>
</thead>
<tbody>
<tr>
<td>-.53</td>
<td>.378b (.035)</td>
<td>.269a (.008)</td>
<td>.235a (.003)</td>
<td>.207a (.005)</td>
<td>.614a (.008)</td>
</tr>
<tr>
<td>-2</td>
<td>.859c (.058)</td>
<td>.244ab (.008)</td>
<td>.344c (.008)</td>
<td>.374ab (.024)</td>
<td>.601a (.010)</td>
</tr>
<tr>
<td>-5</td>
<td>.328ab (.068)</td>
<td>.202bc (.014)</td>
<td>.319c (.012)</td>
<td>.378ab (.008)</td>
<td>.516b (.021)</td>
</tr>
<tr>
<td>-10</td>
<td>.265ab (.077)</td>
<td>.192bc (.022)</td>
<td>.294bc (.014)</td>
<td>.349ab (.013)</td>
<td>.538b (.024)</td>
</tr>
<tr>
<td>-15</td>
<td>.126a (.024)</td>
<td>.153c (.013)</td>
<td>.264ab (.024)</td>
<td>.423b (.022)</td>
<td>.518b (.012)</td>
</tr>
<tr>
<td>-20</td>
<td>.162ab (.020)</td>
<td>.144c (.021)</td>
<td>.256ab (.016)</td>
<td>.614c (.111)</td>
<td>.437c (.019)</td>
</tr>
</tbody>
</table>

Regression coefficients: -.7072, -.9562, -.3543, .8577, -.8991

Values within a column not followed by the same letter are significantly different at the P = .05 level (Student-Newman-Keuls' Test). Values are means ± s.e.
Figure 3.1. Hand-held plexiglass photosynthesis chamber for measuring in situ CO$_2$ fixation rates of Douglas-fir seedlings. (a) Battery-operated, (b) fan circulates air during fixation periods.
Figure 3.2. Comparison of net CO$_2$ fixation by (a) non-stressed and (b) stressed Douglas-fir seedlings either non- mycorrhizal or inoculated with one of four ectomycorrhizal fungi. Arrow indicates time of rewatering for stressed seedlings.

--- O --- Rv, --- ■ --- Ll, --- △ --- Na, --- * --- Pt, --- .... --- Nm.
Figure 3.3. Comparison of net CO$_2$ fixation by mycorrhizal and non-mycorrhizal seedlings during the recovery period following drought. Arrow indicates time of rewatering.

- - - - Rv, ----- Ll, ----- Na, ---- Pt, ......... Nm.
Figure 3.4. Transpiration rate of mycorrhizal and non-mycorrhizal Douglas-fir seedlings subjected to drought. Arrow indicates time of rewatering. —— Rv, —— L1, —— Δ Na, —— Pt, —— Nm.
Figure 3.5. Root colonization by *Rhizopogon vinicolor* showing mycorrhizae and rhizomorphs.
CHAPTER 4

EFFECTS OF FOREST LITTER AND HUMUS ON MYCORRHIZA
DEVELOPMENT AND SEEDLING GROWTH OF DOUGLAS-FIR
AND WESTERN RED CEDAR

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SUMMARY

Preparation of forest regeneration sites prior to conifer
planting often includes slash burning or physical removal of soil
organic matter. Experiments were conducted to determine if loss of
organic matter could account for observed reduction in mycorrhizal
fungus inoculum potential in soils from clearcut and burned areas as
compared to soils from undisturbed forest sites, and to compare the
growth of Douglas-fir and western red cedar seedlings with and
without litter. Litter and humus was found to include inoculum of
both VA and ectomycorrhizal fungi. Litter amendment usually enhanced
growth of host seedlings, but growth enhancement could not be fully
attributed to addition of mycorrhizal inoculum or nutrients provided by litter. These findings suggested that other biological factors stimulated the growth of conifer seedlings and/or activity of mycorrhizal fungi.

INTRODUCTION

Forest regeneration sites are usually cleared and often slash-burned before conifer seedlings are outplanted. Particularly adverse sites may sometimes warrant more intensive site preparation, including removal or windrowing of forest floor litter and organic debris ("scalping"), extensive terracing of steep slopes, and mechanical "ripping" to loosen compacted soil in rows where seedlings are to be planted (Stewart, 1978). Although the long-term effects of organic matter removal are not known, many foresters report better initial growth and survival of seedlings planted in mineral soil.

Litter is a substantial source of nutrients in Douglas-fir ecosystems (Youngberg, 1966; Fogel and Cromack, 1977) responsible for up to 72% of aboveground nutrient return in an old growth stand (Abee and Lavender, 1972). In addition to nutrients, litter and soil organic matter are important in maintaining soil physical properties as well (Kraemer and Hermann, 1979). Slash burning can remove up to 75% of soil organic matter (Austin and Baisinger, 1955) resulting in nitrogen volatilization (Kraemer and Hermann, 1979), but can cause large increases in available phosphorus, potassium, calcium and magnesium (Grier and Cole, 1971) subject to rapid loss by leaching (Grier, 1975). Once organic matter is removed, it may take several years to accumulate (Kraemer and Hermann, 1979).
Although an apparent allelopathic response of ectomycorrhizae to forest litter has been reported for seedlings (Alvarez, Rowney, and Cobb, 1979; Meyer-Schoeneberger and Perry, 1982), ectomycorrhizae of older trees develop better in humus and litter layers than in to mineral soil (Meyer, 1973; Mikola, 1973; Harvey, Jurgensen, and Larsen, 1978, 1979; Fogel, 1980). It is not known if this reflects differences in aeration, moisture relations, pH, availability of nutrients, or activity of microorganisms in the litter layer. Mycorrhizal fungi present in litter layers have been implicated in nutrient cycling, thereby preventing nutrient leaching (Went and Stark, 1968; Fogel and Hunt, 1979; Fogel, 1980), although most of these fungi are considered incapable of saprophytic growth and lack enzymes necessary for utilization of complex carbohydrates such as cellulose and lignin (Hacskaylo, 1973; Meyer, 1974). Ectomycorrhizal fungus-containing litter has been exploited as inoculum for over a century (Trappe, 1977) and is probably the most widely used source of ectomycorrhizal inoculum for forest nurseries worldwide (Marx, 1980). Advantages to using litter include reliability and relative ease of obtaining large amounts of viable inoculum of fungi presumably adapted to the forest sites from which they were taken; disadvantages include the potential for introducing pathogens into a nursery and the inability to select particular fungus species with specific desirable attributes such as tolerance to drought, high temperatures, or low pH soils (Trappe, 1977).

In an earlier study (Chapter 1) fewer ectomycorrhizae were found to develop in soils from clearcut and burned areas than from
undisturbed forests. The present study was undertaken to determine if a loss of organic matter could account for the differences in mycorrhizal development in soils from disturbed vs. undisturbed sites, to determine if forest floor material suppresses growth of conifer seedlings, and to determine if these effects are biological or chemical. To test if forest floor material affects VA and ectomycorrhizal fungi differently, both western red cedar (Thuja plicata J. Donn ex D. Donn) and Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] were used in greenhouse bioassays.

MATERIALS AND METHODS

Forest litter and humus was collected in August 1981 from the Boomer Hill study site southwest of Roseburg, Oregon (Chapter 2). The forest floor consisted of a layer approximately 4 cm deep and included a range of materials from recently fallen needles on the surface to well decomposed humus overlying the mineral soil. Litter and humus was sieved through .5 cm mesh to remove large woody material and was used fresh or pasteurized (65 °C/30 min, aerated steam). An additional control treatment consisted of coarse vermiculite substituted for the litter and humus component. Vermiculite was chosen for its properties of aeration and moisture retention and because it is relatively inert, biologically and chemically.

Soil collected from the undisturbed forest and 4-yr-old cleared and burned area of the Boomer Hill site was similarly sieved and used fresh or pasteurized (65 °C/30 min, aerated steam). Each of three litter treatments was mixed 1:1 (volume basis) with each of four soil
treatments to yield a total of twelve soil-litter combinations. These were either sown to surface-sterilized (30% H$_2$O$_2$/30 min) Douglas-fir seed or planted with 1-month-old western red cedar seedlings in 70 cc tubes (Ray Leach Cone-tainers, Inc.). Each soil-litter treatment per host had ten replicates.

Seedlings were maintained under greenhouse conditions (24 °C day/18 °C night) with a 16 hr photoperiod (240 uE m$^{-2}$sec$^{-1}$). All plants were fertilized weekly with 3 ml Long-Ashton nutrient solution (Hewitt, 1966) at 1/4 strength phosphorus (11 ppm P).

Douglas-fir seedlings were harvested 16 wks after germination and their root systems examined for percent of total feeder root tips which were ectomycorrhizal. Shoot length, shoot and root dry weight were recorded. Western red cedar seedlings were harvested 12 wks after transplanting. Root systems were excised, chopped into 1 cm segments, bleached in 3% H$_2$O$_2$ for 1 hr and cleared and stained (Phillips and Hayman, 1970). Percent root length colonized by VA fungi was estimated using the technique described by Biermann and Linderman (1981).

Litter and soil components were analyzed for pH, Olsen available phosphorus, and total nitrogen by the Oregon State University Soil Testing Laboratory.

RESULTS

Douglas-fir

Ectomycorrhizae were more abundant on Douglas-fir seedlings grown in undisturbed forest soil ($\bar{x} = 92.5%$) than on seedlings grown in clearcut soil ($\bar{x} = 75.3%$), regardless of litter treatment,
although these differences in mean response were not statistically significant (P = .05) (Table 4.1). Mycorrhizae did not develop in pasteurized soil treatments unless litter was added; in these treatments addition of litter restored mycorrhizal colonization to levels comparable to non-pasteurized soils. The addition of litter had no effect on mycorrhiza formation on seedlings grown in nonpasteurized soils. Litter, pasteurized litter or vermiculite treatments did not differ significantly for these soils.

Shoot height and total weight were greatest for seedlings grown in soils to which litter had been added, and values were larger among undisturbed forest soil treatments than among clearcut soil treatments. Undisturbed forest soil mixed with vermiculite also yielded seedlings with large shoot height and total weight values, but pasteurized litter and vermiculite did not differ significantly in mycorrhiza colonization, shoot height or total weight. Shoot height and total weight of mycorrhizal Douglas-fir were approximately 20% larger than nonmycorrhizal seedlings for comparable treatments.

**Western red cedar**

VA mycorrhiza colonization occurred in undisturbed forest and clearcut soils or in pasteurized soils to which litter had been added (Table 4.2). Percent root length colonized was slightly higher for seedlings planted in disturbed soil ($\bar{x} = 31.3\%$) compared to seedlings planted in undisturbed soil ($\bar{x} = 26.7\%$) although this difference was not statistically significant (P = .05). The addition of litter to pasteurized soils restored mycorrhiza colonization to levels comparable with nonpasteurized soil treatments. For non-pasteurized
soils, litter did not significantly affect levels of VA mycorrhizal colonization when compared to pasteurized litter or vermiculite treatments, nor did pasteurized litter and vermiculite treatment result in significant differences in mycorrhiza colonization.

Shoot height and shoot weight were largest for seedlings grown in clearcut soil regardless of litter treatment and in other soil treatments to which litter had been added. An exception to this was the undisturbed forest soil + litter treatment, for which shoot weight and shoot height were significantly less than for undisturbed forest soil + pasteurized litter or undisturbed forest soil + vermiculite. Shoot height of mycorrhizal seedlings averaged approximately 80% larger than for non-mycorrhizal seedlings of comparable treatments; shoot weight of mycorrhizal western red cedar seedlings was approximately 4.3x greater than for non-mycorrhizal seedlings.

*Glomus tenuis* (Greenhall) Hall was the only endophyte found in western red cedar roots.

Soil and litter nutrient analyses are summarized in Table 4.3.

**DISCUSSION**

Results indicate that litter plus humus from the forest floor contains inoculum of both VA and ectomycorrhizal fungi. In contrast to the work by Alvarez, Rowney, and Cobb (1979) which showed litter to have an apparent allelopathic effect on *Abies concolor* growth and ectomycorrhizae formation, and research by Meyer-Schoeneberger and Perry (1982) on litter and Douglas-fir mycorrhizae, the addition of litter in our study did not affect mycorrhizal colonization of
Douglas-fir or western red cedar except when mycorrhizal inoculum, otherwise lacking, was provided by litter. Compared to treatments including either pasteurized litter or vermiculite, the addition of litter generally enhanced seedling growth of western red cedar and Douglas-fir. The stimulatory effect of litter even when soil inoculum of mycorrhizal fungi was abundant suggests that litter contributes more than added mycorrhizal propagules. The difference in growth response between seedlings grown in soil amended with litter vs. soil amended with pasteurized litter when mycorrhizal inoculum is provided in the soil indicates further that these differences are biological rather than nutritional, suggesting that litter may contain microorganisms stimulatory to seedling growth or mycorrhizal fungus metabolic activity and nutrient uptake (Meyer, 1974; Bowen and Theodorou, 1979).

Another possibility is that pasteurization of litter results in formation or release of compounds deleterious to plant growth and/or mycorrhizal fungus activity. It is unlikely that this would have occurred at the temperature and duration of pasteurization used (65 °C/30 min), but pasteurization of litter and, to a lesser extent, undisturbed forest soil did result in slight increases in phosphorus availability (Table 4.3). It is doubtful that these increases could have resulted in P levels inhibitory to mycorrhizal colonization, however, since mycorrhizal dependency experiments (Appendix) suggest that mycorrhizal colonization is not impeded even by fertilization with 43 ppm P nutrient solution.
Peuss (1958) observed that addition of peat to soil reduced colonization and growth enhancement by VA mycorrhizal fungi. Biermann (1982) found that peat decreased the growth response of mycorrhizal plants only when the planting medium lacked soil; solution P increased to a high level if not removed from solution by soil. Thus, the apparent inhibition of mycorrhizal fungi by organic matter (and other soilless media) was actually a phosphorus effect.

Mycorrhizal fungi are generally considered to be parasites that are unable to complete their life cycles in the absence of a suitable living host, and many have little or no growth potential on non-living substrates. The ability of ectomycorrhizal fungi to utilize complex carbohydrates in axenic culture is restricted to a few genera (Meyer, 1973, 1974; Hacskaylo, 1973) although ectomycorrhizal fungi isolated from rotten wood have formed ectomycorrhizae with conifer hosts in pure culture and in the field (Kropp, 1982), and decayed soil wood and humus can be the major substrates for ectomycorrhizal fungi (Harvey, Jurgensen, and Larsen, 1978; 1979). VA mycorrhizal fungi have been shown to colonize senescent roots of nonhost plants (Hirrel, Mehravaran, and Gerdemann, 1978; Parke and Linderman, 1980; Ocampo and Hayman, 1981) and to spread independently through soil in the absence of host roots (Warner and Mosse, 1980). *Glomus tenuis* has been found in association with bryophytes distant from soil or roots of higher plants (Johnson, 1977). The potential for mild saprophytism by VA mycorrhizal fungi in general, and *Glomus tenuis* in particular, should be further investigated. Even if active saprophytism does not occur, it is quite possible that mycelium or
spores of mycorrhizal fungi associated with plant roots in the litter layer may survive as dormant propagules in this substrate.

Mycorrhizal fungi have been identified as important components in nutrient cycling in Douglas-fir ecosystems (Fogel and Hunt, 1979; Fogel, 1980). In the absence of a litter layer and fungi to mobilize and transfer these nutrients to plant roots, nutrients may be leached deep in the soil, becoming thereby unavailable to young outplanted conifer seedlings. Loss of this organic matter through burning or scalping may result in initial decreases in mycorrhizal inoculum, loss of microorganisms stimulatory to seedling growth and/or mycorrhizal activity, and decreased availability of nutrients to growing seedlings.

ACKNOWLEDGEMENTS

REFERENCES


Table 4.1. Effect of litter on ectomycorrhiza formation and growth of Douglas-fir seedlings.

<table>
<thead>
<tr>
<th>Soil Treatment</th>
<th>Litter Treatment</th>
<th>Pasteurized litter</th>
<th>Litter</th>
<th>Vermiculite</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undisturbed forest</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% mycorrhizal tips</td>
<td>92 b</td>
<td>94 b</td>
<td>92 b</td>
<td></td>
</tr>
<tr>
<td>Shoot ht</td>
<td>8.11 a</td>
<td>11.01 cd</td>
<td>10.58 bcd</td>
<td></td>
</tr>
<tr>
<td>Total wt</td>
<td>.402 bc</td>
<td>.607 e</td>
<td>.538 de</td>
<td></td>
</tr>
<tr>
<td>Undisturbed forest, pasteurized</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% mycorrhizal tips</td>
<td>0 a</td>
<td>66 b</td>
<td>0 a</td>
<td></td>
</tr>
<tr>
<td>Shoot ht</td>
<td>6.50 a</td>
<td>11.70 d</td>
<td>6.80 a</td>
<td></td>
</tr>
<tr>
<td>Total wt</td>
<td>.282 a</td>
<td>.552 de</td>
<td>.370 abc</td>
<td></td>
</tr>
<tr>
<td>Clearcut</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% mycorrhizal tips</td>
<td>79 b</td>
<td>68 b</td>
<td>79 b</td>
<td></td>
</tr>
<tr>
<td>Shoot ht</td>
<td>7.58 a</td>
<td>10.16 bc</td>
<td>6.96 a</td>
<td></td>
</tr>
<tr>
<td>Total wt</td>
<td>.340 ab</td>
<td>.506 d</td>
<td>.300 a</td>
<td></td>
</tr>
<tr>
<td>Clearcut, pasteurized</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% mycorrhizal tips</td>
<td>0 a</td>
<td>79 b</td>
<td>0 a</td>
<td></td>
</tr>
<tr>
<td>Shoot ht</td>
<td>6.87 a</td>
<td>9.52 b</td>
<td>6.64 a</td>
<td></td>
</tr>
<tr>
<td>Total wt</td>
<td>.315 ab</td>
<td>.430 c</td>
<td>.348 ab</td>
<td></td>
</tr>
</tbody>
</table>

Values are means of 10 replicates. For each parameter, values not followed by the same letter are significantly different, P = .05 (Student-Newman-Keuls' Test) among the 12 soil x litter treatments.
Table 4.2. Effect of litter on VA mycorrhiza formation and growth of western red cedar seedlings.

<table>
<thead>
<tr>
<th>Soil Treatment</th>
<th>Litter Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pasteurized litter</td>
</tr>
<tr>
<td>Undisturbed forest</td>
<td></td>
</tr>
<tr>
<td>% root length mycorrhizal</td>
<td>25 b</td>
</tr>
<tr>
<td>Shoot ht</td>
<td>4.94 de</td>
</tr>
<tr>
<td>Shoot wt</td>
<td>.109 c</td>
</tr>
<tr>
<td>Undisturbed forest, pasteurized</td>
<td></td>
</tr>
<tr>
<td>% root length mycorrhizal</td>
<td>0 a</td>
</tr>
<tr>
<td>Shoot ht</td>
<td>3.15 bc</td>
</tr>
<tr>
<td>Shoot wt</td>
<td>.049 ab</td>
</tr>
<tr>
<td>Disturbed</td>
<td></td>
</tr>
<tr>
<td>% root length mycorrhizal</td>
<td>30 b</td>
</tr>
<tr>
<td>Shoot ht</td>
<td>5.29 de</td>
</tr>
<tr>
<td>Shoot wt</td>
<td>.135 ce</td>
</tr>
<tr>
<td>Disturbed, pasteurized</td>
<td></td>
</tr>
<tr>
<td>% root length mycorrhizal</td>
<td>0 a</td>
</tr>
<tr>
<td>Shoot ht</td>
<td>3.38 c</td>
</tr>
<tr>
<td>Shoot wt</td>
<td>.038 ab</td>
</tr>
</tbody>
</table>

Values are means of 10 replicates for each parameter. Values not followed by the same letter are significantly different, $P = .05$ (Student-Newman-Keuls' Test) among the 12 soil x litter treatments.
Table 4.3. Nutrient analysis of soil and litter treatment combinations.

<table>
<thead>
<tr>
<th>Soil + litter treatment</th>
<th>pH</th>
<th>P  (ppm)</th>
<th>Total N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forest + pasteurized litter</td>
<td>6.8</td>
<td>26</td>
<td>.32</td>
</tr>
<tr>
<td>+ litter</td>
<td>6.8</td>
<td>23</td>
<td>.33</td>
</tr>
<tr>
<td>+ vermiculite</td>
<td>6.9</td>
<td>20</td>
<td>.24</td>
</tr>
<tr>
<td>Forest, pasteurized + litter</td>
<td>6.9</td>
<td>28</td>
<td>.31</td>
</tr>
<tr>
<td>+ litter</td>
<td>6.8</td>
<td>25</td>
<td>.32</td>
</tr>
<tr>
<td>+ vermiculite</td>
<td>7.0</td>
<td>22</td>
<td>.23</td>
</tr>
<tr>
<td>Clearcut + pasteurized litter</td>
<td>6.3</td>
<td>23</td>
<td>.25</td>
</tr>
<tr>
<td>+ litter</td>
<td>6.3</td>
<td>21</td>
<td>.26</td>
</tr>
<tr>
<td>+ vermiculite</td>
<td>6.5</td>
<td>18</td>
<td>.17</td>
</tr>
<tr>
<td>Clearcut, pasteurized + litter</td>
<td>6.3</td>
<td>23</td>
<td>.25</td>
</tr>
<tr>
<td>+ litter</td>
<td>6.3</td>
<td>21</td>
<td>.26</td>
</tr>
<tr>
<td>+ vermiculite</td>
<td>6.5</td>
<td>18</td>
<td>.17</td>
</tr>
</tbody>
</table>
SUMMARY AND CONCLUSIONS

Results from these studies indicate that inoculum of native VA and ectomycorrhizal fungi is present in soils from recent clearcuts but that ectomycorrhizal inoculum potential of older clearcuts may be substantially reduced, especially if the clearcut has also been burned.

Propagules of ectomycorrhizal fungi are tolerant of high soil temperatures while in a dormant state, but young mycorrhizae appear to be intolerant of prolonged high temperatures. It is unlikely that clearcut soils reach temperatures high enough to kill all mycorrhizal fungi except, perhaps, near the soil surface. As important as tolerance to high soil temperatures may be the ability of mycorrhizal fungi to grow at low soil temperatures so as to coincide with periods of soil moisture availability and active root growth. The ability to grow at low temperatures would ensure more rapid colonization of outplanted conifers early in the season before environmental stresses.

Early colonization of outplanted seedlings would also allow development of an extensive hyphal network deep in the soil and, for some fungal symbionts, formation of rhizomorphs, which appear to be extremely beneficial in alleviating plant moisture stress. The ability of some mycorrhizal fungi to increase drought tolerance and hasten the recovery of stressed seedlings, as demonstrated in this study, could be important in reducing first-year mortality of conifer seedlings on droughty sites.
Growth of Douglas-fir and western red cedar in forest and clearcut soils is significantly enhanced by native mycorrhizal fungi. Forest organic matter is a source of VA and ectomycorrhizal fungus inoculum and of microorganisms stimulatory to conifer growth and/or mycorrhizal fungus activity. Slash burning or mechanical removal of the litter layer from a clearcut site may result in decreased numbers of mycorrhizal propagules, loss of nutrients, and less favorable microbiological conditions for mycorrhiza development.

Considerations for management of regeneration sites to maximize the inoculum potential of native soilborne mycorrhizal fungi should include the following:

1. If slash burning is necessary, burn lightly to leave soil humus and woody material on the site as survival niches for mycorrhizal fungi.

2. Remove only small, localized patches of the litter layer to expose just enough mineral soil for planting. Do not remove the entire litter layer from the site.

3. Restock clearcuts as soon as possible, and plant as early in the season as feasible to ensure good mycorrhiza development before the onset of summer.

   In some areas, fall planting may be desirable.

   If native inoculum of mycorrhizal fungi is limited, nursery inoculation of conifer species that have a high mycorrhizal dependency should receive top priority; less dependent species, and
seedlings to be outplanted on sites where native mycorrhizal fungi are plentiful should have a lower priority.

Mycorrhizal fungi with potential for nursery inoculations should be compared with native fungi for their ability to grow at low soil temperatures, improve plant water relations, and enhance growth. To circumvent limitations in mass production of pure mycelial cultures, the use of spore inoculum of *Rhizopogon* spp. and other fungi should be exploited. Microorganisms from forest litter or from forest soils with an abundance of mycorrhizal fungi could be added to nursery beds to stimulate mycorrhizal activity. Inoculation at the time of outplanting with spores or forest litter, first tested for pests and pathogens, may be of practical value until problems in nursery inoculation are overcome.


APPENDIX

MYCORRHIZAL DEPENDENCY OF DOUGLAS-FIR AND WESTERN RED CEDAR: A NOTE

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APPENDIX

SUMMARY

An experiment was conducted to test the mycorrhizal dependencies of young Douglas-fir and western red cedar seedlings on native VA and ectomycorrhizal fungi in soil from a clearcut and an adjacent undisturbed forest at two phosphorus levels. Western red cedar was found to be highly dependent on VA mycorrhizae, especially in the clearcut soil at the low P level tested; shoot dry weight of mycorrhizal seedlings was 14x greater than for nonmycorrhizal seedlings at the same phosphorus level. Douglas-fir was dependent on ectomycorrhizal fungi but to a lesser extent; where growth enhancement was maximal, total weight of mycorrhizal seedlings was 1.45x that of nonmycorrhizal controls. Results are discussed with regard to forest nursery inoculation priorities.

APPENDIX

INTRODUCTION

It is estimated that 95% of the world's vascular plant species belong to families which are characteristically mycorrhizal (Trappe,
Mycorrhizal fungi have been shown to be important in the mineral nutrition of their hosts, particularly with regard to phosphorus uptake from soils low in available P (Bowen, 1973; Mosse, 1973); other benefits may include protection against soilborne plant pathogens (Marx, 1972; Schenk and Kellam, 1978) and increased drought tolerance (Theodorou and Bowen, 1970; Hardie and Leyton, 1981). The degree to which an individual plant requires a mycorrhizal symbiont, however, depends on the plant species and other factors such as soil fertility (Gerdemann, 1975). Often the need for mycorrhizal fungi can be eliminated by fertilizing with soluble phosphorus, a practice widespread in agriculture and in the production of container-grown plants. Until mass production of mycorrhizal inoculum is possible or our understanding of mycorrhizal fungus ecology allows management of these fungi to maximize their potential usefulness, the addition of phosphorus fertilizer will continue to be a cost-effective practice for achieving optimal crop yields.

In contrast, regeneration of forests does not permit the degree of intensive management possible in agriculture. It is here, perhaps, that manipulation of mycorrhizal fungi is of immediate value. Although forest nurseries are inoculated with mycorrhizal fungi in some parts of the United States (Marx, 1980), limitations in the availability of inoculum and inconsistent success in introducing effective species and ecotypes have delayed operational use of these fungi in the Pacific Northwest. Conifer seedlings outplanted on regeneration sites are often nonmycorrhizal or are colonized with ineffective symbionts prevalent in the nursery. Inoculum of
mycorrhizal fungi remaining in deforested areas may be limited, especially if the area has also been burned (Chapter 1).

The term "mycorrhizal dependency" was used by Gerdemann (1975) to express and compare the degree of growth enhancement of various plant species caused by mycorrhizal fungi. Mycorrhizal dependency is defined as "the degree to which a plant is dependent on the mycorrhizal condition to produce its maximum growth or yield at a given level of soil fertility." Mycorrhizal dependency can be determined by expressing the dry weight of a mycorrhizal plant as a percentage of the dry weight of a nonmycorrhizal plant grown under the same conditions (Menge, Johnson, and Platt, 1978). Mycorrhizal dependencies ranging from 74-2600% have been reported for citrus cultivars (Menge, Johnson, and Platt, 1978) and from 100-207% for wheat cultivars (Azcon and Ocampo, 1981).

An experiment was conducted with an ectomycorrhizal forest tree species, Douglas-fir *Pseudotsuga menziesii* (Mirb.) Franco and a VA mycorrhizal forest species, western red cedar (*Thuja plicata* J. Donn ex D. Donn) to determine the degree of mycorrhizal dependency of each grown in soil from an undisturbed forest or from an adjacent 4-year-old burned clearcut at two levels of phosphorus fertilization.

APPENDIX

MATERIALS AND METHODS

Soil was collected in August 1981 from an old growth Douglas-fir stand and from an adjacent 4-year-old burned clearcut at the Boomer Hill study site (Chapter 2). Soil was sieved through .5 cm mesh and used fresh or pasteurized (65 °C/30 min) to eliminate propagules of
mycorrhizal fungi. Soils were mixed 1:1 (volume basis) with pasteurized sand (11 ppm P) and sown to surface-sterilized (30% \( \text{H}_2\text{O}_2/30 \text{ min} \)) Douglas-fir seed or planted with 1-month-old western red cedar seedlings in 70 cc tubes (Ray Leach Cone-tainers, Inc.). Plants were maintained in the greenhouse and fertilized weekly (3 ml/tube) with Long-Ashton nutrient solution (Hewitt, 1966) at full-strength phosphorus (= 43 ppm P) or at 1/4-strength phosphorus (= 11 ppm P). Each soil-phosphorus treatment per host had ten replicates.

Plants were harvested 16 wks later. Douglas-fir was assessed for percent mycorrhizal root tips, shoot height, and total dry weight. Roots of western red cedar were chopped into 1 cm segments, bleached for 1 hr (3% \( \text{H}_2\text{O}_2 \)), cleared and stained (Phillips and Hayman, 1970) and assessed for percent root length colonized by VA mycorrhizal fungi. Shoot height and shoot weight were also recorded.

**APPENDIX**

**RESULTS**

Results of this experiment are summarized in Tables A.1 and A.2. Ectomycorrhiza formation and growth of Douglas-fir were best at the high rate of P fertilization in undisturbed forest soil, and growth was lowest in clearcut soil which had been pasteurized and fertilized at the lower rate. When expressed as mycorrhizal dependency (dry weight of mycorrhizal plant as a percentage of dry weight of a nonmycorrhizal plant grown under the same conditions of soil fertility), growth of mycorrhizal Douglas-fir in undisturbed soil at the low rate of P was 145% that of nonmycorrhizal controls. At the high rate of P fertilizer, this value was 127%. In clearcut
soil, mycorrhizal dependency was 106% and 110% for seedlings grown at low and high P, respectively.

For western red cedar, maximum growth occurred in clearcut soil. Percent root length colonized and growth were not correlated. Mycorrhizal dependency was 1400% in clearcut soil at the low P level, 1286% at the high P level, and 404% or 177% in low or high P levels in undisturbed forest soil.

APPENDIX

DISCUSSION

Western red cedar seedlings appear to be highly dependent on VA mycorrhizal fungi for growth in forest soils. This dependence occurs in spite of high levels of available phosphorus added to the medium. In clearcut soil, shoot weight of mycorrhizal western red cedar was 14x larger than shoot weight of the comparable non-mycorrhizal treatment.

The lack of correlation between root length colonization and seedling growth among the mycorrhiza treatments suggests that the amount of external hyphae may be more important than the extent of structures within the roots.

It is not clear why mycorrhizal dependence is reduced so significantly in the undisturbed forest soils. This difference cannot be attributed to increased P availability, because the undisturbed forest soil contains only slightly more available P than the clearcut soil (20 ppm and 17 ppm, respectively). Possibly another nutrient or soil factor limiting to mycorrhizal activity
and/or seedling growth in the clearcut area is not limiting in the undisturbed forest soil.

Douglas-fir is also dependent on mycorrhizal fungi but to a lesser extent. Seedling growth and mycorrhizal colonization were correlated for this host. Maximum growth enhancement occurred in undisturbed forest soil at the low level of P; total dry weight of mycorrhizal seedlings was 1.45x that of nonmycorrhizal seedlings. In the clearcut soil treatments, some other factor may have limited the growth or activity of ectomycorrhizae. This factor may be the absence of beneficial microorganisms normally present in the litter layer (Chapter 4), resulting in reduced growth enhancement in clearcut soil at both P levels.

The concept of mycorrhizal dependency is useful in evaluating growth response as a result of the mycorrhizal condition at a variety of soil fertility levels, or in comparing the relative benefits of native vs. introduced species of mycorrhizal fungi. These experiments were conducted on seedlings 16 wks (Douglas-fir) or 20 wks (western red cedar) old, therefore numerical values of mycorrhizal dependency might be expected to be quite different at other stages of growth. However, host physiology of phosphorus uptake is not likely to change; young seedlings with a high mycorrhizal dependency are probably also highly dependent when mature. Since quantities of mycorrhizal inoculum are often limited, nursery inoculation of highly dependent hosts should receive top priority if inoculum levels are low at the outplanting site. Conversely, inoculation of plants not highly dependent on
mycorrhizae, or plants for which effective on-site inoculum is present should be a lower priority.
APPENDIX

REFERENCES


Table A.1. Mycorrhizal dependency of Douglas-fir seedlings in forest soils fertilized at two phosphorus levels.

<table>
<thead>
<tr>
<th>Soil Treatment</th>
<th>Fertilizer Phosphorus</th>
<th>Fertilizer Phosphorus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11 ppm P</td>
<td>43 ppm P</td>
</tr>
<tr>
<td>Undisturbed forest</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% mycorrhizal tips</td>
<td>89 b</td>
<td>100 b</td>
</tr>
<tr>
<td>Shoot ht</td>
<td>9.12 c</td>
<td>9.79 c</td>
</tr>
<tr>
<td>Total wt</td>
<td>.446 bc</td>
<td>.476 c</td>
</tr>
<tr>
<td>Mycorrhizal dependency (%)</td>
<td>145</td>
<td>127</td>
</tr>
<tr>
<td>Undisturbed forest, pasteurized</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% mycorrhizal tips</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Shoot ht</td>
<td>6.87 ab</td>
<td>7.79 b</td>
</tr>
<tr>
<td>Total wt</td>
<td>.308 a</td>
<td>.375 ab</td>
</tr>
<tr>
<td>Mycorrhizal dependency (%)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Clearcut</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% mycorrhizal tips</td>
<td>70 ab</td>
<td>71 ab</td>
</tr>
<tr>
<td>Shoot ht</td>
<td>6.67 ab</td>
<td>7.17 ab</td>
</tr>
<tr>
<td>Total wt</td>
<td>.305 a</td>
<td>.325 a</td>
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<tr>
<td>Mycorrhizal dependency (%)</td>
<td>109</td>
<td>110</td>
</tr>
<tr>
<td>Clearcut, pasteurized</td>
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<td></td>
</tr>
<tr>
<td>% mycorrhizal tips</td>
<td>0</td>
<td>0</td>
</tr>
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<td>Shoot ht</td>
<td>6.08 a</td>
<td>6.51 ab</td>
</tr>
<tr>
<td>Total wt</td>
<td>.288 a</td>
<td>.294 a</td>
</tr>
<tr>
<td>Mycorrhizal dependency (%)</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

For each parameter among the 8 soil x P treatments, values not followed by the same letter are significantly different at the P = .05 level (Student-Newman-Keuls' Test).
Table A.2. Mycorrhizal dependency of western red cedar seedlings in forest soils fertilized at two phosphorus levels.

<table>
<thead>
<tr>
<th>Soil Treatment</th>
<th>Fertilizer Phosphorus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11 ppm P</td>
</tr>
<tr>
<td>Undisturbed forest</td>
<td></td>
</tr>
<tr>
<td>% root length mycorrhizal</td>
<td>28.2 a</td>
</tr>
<tr>
<td>Shoot ht</td>
<td>4.86 b</td>
</tr>
<tr>
<td>Total wt</td>
<td>.097 bc</td>
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<tr>
<td>Mycorrhizal dependency (%)</td>
<td>404</td>
</tr>
<tr>
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<tr>
<td>% root length mycorrhizal</td>
<td>0</td>
</tr>
<tr>
<td>Shoot ht</td>
<td>2.40 a</td>
</tr>
<tr>
<td>Total wt</td>
<td>.024 a</td>
</tr>
<tr>
<td>Mycorrhizal dependency (%)</td>
<td>100</td>
</tr>
<tr>
<td>Clearcut</td>
<td></td>
</tr>
<tr>
<td>% root length mycorrhizal</td>
<td>44.6 b</td>
</tr>
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<td>6.26 c</td>
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<td>.182 d</td>
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<td>1400</td>
</tr>
<tr>
<td>Clearcut, pasteurized</td>
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</tr>
<tr>
<td>% root length mycorrhizal</td>
<td>0</td>
</tr>
<tr>
<td>Shoot ht</td>
<td>1.94 a</td>
</tr>
<tr>
<td>Total wt</td>
<td>.013 a</td>
</tr>
<tr>
<td>Mycorrhizal dependency (%)</td>
<td>100</td>
</tr>
</tbody>
</table>

For each parameter among the 8 soil x P treatments, values not followed by the same letter are significantly different at the P = .05 level (Student-Newman-Keuls’ Test).