A recent concern in reforestation efforts has been that of the mycorrhizal status of container-grown seedlings that are outplanted on clear-cut sites. More specifically, research emphasis has been directed towards nursery management practices which influence mycorrhizal development. Past research has shown that high levels of soluble N and P inhibit mycorrhizal development. The objectives of this investigation were to determine the effect of fertilizer-P form and N-P-K rates on mycorrhizal development, total seedling weight and N and P nutrition.

A growth chamber experiment was conducted in which Douglas-fir (Pseudotsuga menziesii) and western hemlock (Tsuga heterophylla) seedlings were grown in Leach cells which were filled with a mixture of peatmoss and vermiculite to which inoculum of one of three mycorrhizal fungi (Hebeloma crustuliniforme, Laccaria laccata and Cenococcum graniforme) was added. An uninoculated control was also included. Fertilizer applications were made weekly for 20 weeks commencing the sixth week after sowing. Fertilizer treatments consisted of a High, Low and Low-High level of N-P-K and two treatments with a Low N-K level with rock phosphate (RP) or dicalcium phosphate (DCP) as the P-source. The High level rates were 1.0, 0.65 and 0.83 mg/seedling/week of N, P, and K, respectively. The Low level rates were
one half of the High rate. The Low-High treatment consisted of 13 weeks of Low level fertilization and 7 weeks of High level fertilization. For the treatments receiving rock phosphate or dicalcium phosphate, 8.6mg P was incorporated with the potting mix prior to sowing. These treatments received only N-K solution applications. The variables were arranged in a factorial split-plot design, with tree species as main plots and fungal X fertilizer treatments as subplots, with two replicates. Five seedlings from each subplot were destructively sampled at 60, 120 and 180 days after sowing.

The results show that percent mycorrhizal short roots (\%MSR) varied with fungal species as well as fertilizer treatments. As a general trend, H. crustuliniforme and L. laccata produced greater infection rates on both tree species than did C. graniforme. For Douglas-fir at sample period 1, seedlings under the RP and DCP treatments produced a greater \%MSR than did the High treatment seedlings. At sample periods 2 and 3, the Low, Low-High, RP and DCP treatments produced a greater \%MSR than did the High treatment. For western hemlock at sample periods 1 and 2, the Low, Low-High, RP and DCP treatments produced a greater \%MSR than did the High treatment. At sample period 3, the Low-High and DCP treatments produced a greater \%MSR than did the High treatment. Percent P (\%P) data show that Douglas-fir seedlings mycorrhizal with L. laccata had a greater \%P than did seedlings mycorrhizal with H. crustuliniforme. Hemlock seedlings mycorrhizal with H. crustuliniforme or L. laccata had a greater \%P than did the control seedlings. Seedlings of both tree species receiving the High, Low and Low-High treatments had a greater \%P than did the RP and DCP treatment seedlings. For Douglas-fir, seedlings receiving the DCP treatment had a greater \%P than did the RP treatment seedlings. Percent
N (%N) data show that Douglas-fir seedlings mycorrhizal with *L. laccata* or *C. graniforme* had a lower %N than did the control seedlings. Hemlock seedlings mycorrhizal with *H. crustuliniforme* had a greater %N than did seedlings mycorrhizal with *L. laccata* or *C. graniforme*. The control hemlock seedlings had a greater %N than did the seedlings mycorrhizal with *L. laccata*. Seedlings receiving the High and Low-High treatments had a greater %N than did seedlings receiving the Low, RP and DCP treatments. Total seedling weight (TSW) data show that at sample period 3, the control seedlings had a greater TSW than did the seedlings mycorrhizal with the three fungi. Seedlings mycorrhizal with *C. graniforme* had a greater TSW than did seedlings mycorrhizal with *L. laccata*, which in turn had a greater TSW than did seedlings mycorrhizal with *H. crustuliniforme*. At sample periods 2 and 3, seedlings receiving the High treatment had a greater TSW than did seedlings receiving the other four treatments. At sample period 3, seedlings receiving the RP treatment had a greater TSW than did seedlings receiving the Low, Low-High and DCP treatments.

The results suggest that additional studies testing similar treatments in both bare-root and container nurseries, as well as testing a variety of other phosphate fertilizer materials would be profitable.
EFFECT OF PHOSPHORUS FORMS AND FERTILIZER RATES ON MYCORRHIZAL DEVELOPMENT ON CONTAINERIZED SEEDLINGS

by

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As a result of increasing demands that are being placed on our forest resources, considerable attention is being given to afforestation and reforestation. The Forest Practice Acts that have been developed in the Pacific Northwest states require prompt restocking of timber harvest sites. Recently, research emphasis has been directed towards factors which influence the survival of outplanted nursery seedlings. One factor receiving considerable attention is that of the mycorrhizal condition of the seedling upon outplanting. The benefits of mycorrhizae to the survival and growth of forest trees are well documented in the literature.

It has been realized that some very attractive possibilities exist in the area of inoculating containerized seedlings with select mycorrhizal fungi to insure that the seedlings are equipped with mycorrhizal root systems prior to outplanting. Much of the mycorrhizal inoculation research being conducted is for the purpose of identifying those nursery conditions or treatments that influence mycorrhizal formation. Past research has shown that mycorrhizal infection in containerized seedlings is controlled to the greatest extent by 1) the occurrence and species of mycorrhizal fungi in the potting mixture and 2) fertility levels of the potting mixture, particularly nitrogen and phosphorus. The general practice in container seedling production is to partially sterilize the potting mixture. This procedure eliminates the mycorrhizal fungi. If mycorrhizal seedlings are to be produced, it is therefore necessary to
inoculate the potting mixture with a mycorrhizal fungus. Research has shown that different species of mycorrhizal fungi will produce different growth responses in different tree species. From the aspect of fertility levels, it can be generally concluded that mycorrhizal formation is inhibited by high levels of soluble nitrogen and phosphorus. It becomes evident then that a fertilizer schedule is needed which allows for adequate mycorrhizal infection as well as production of a seedling of physical stature and physiological quality satisfactory for outplanting.

Therefore, the objectives of this investigation were:

1) To determine the effect of fertilizer-P form on the development of mycorrhizae,

2) To determine the effect of rate and timing of fertilizer N-P-K application on the development of mycorrhizae,

3) To evaluate the effects of the treatments used in 1) and 2) above on seedling biomass production and N and P nutrition.
Mycorrhizae and the Forest Tree

Symbiotic Influence Under Natural Forest Conditions

The beneficial effects of mycorrhizae to forest tree survival and growth are well documented in the literature. As early as 1885, Frank proposed the occurrence of a symbiotic relationship between certain fungal organisms and the roots of forest trees. Though many of his hypotheses were based on rough field observations and intuition, he provided subsequent researchers with a fairly accurate description of the mycorrhizal habit. Early observations on the role of mycorrhizae were also made by McDougal (1922) and McArdle (1932), Hatch (1937), Björkman (1949, 1970), Melin (1953), Hacskaylo (1967), Harley (1969), Marks and Kozlowski (1973), Meyer (1974), and Kormanik et al. (1977) have provided excellent reviews and discussions on the various structures and physiological functions of mycorrhizae.

Trees with well developed mycorrhizal root systems have significantly larger and more physiologically active root surface area than do trees with non-mycorrhizal root systems. Hatch (1937) presented six reasons for the increase in absorptive surface area:

1) Continued elongation of the mycorrhizal structure,
2) an increase in the diameter of a mycorrhizal short root,
3) multiple tip development,
4) an increase in the life of the short root cortex by delaying suberization of the cortex and endodermis,
5) increased surface area of the fungal hyphae, and
6) the mycelium connected to the mycorrhizae by rhizomorphs. This increase in absorptive surface area provides the mycorrhizal root system with a greater capacity for water and nutrient absorption as compared to a non-mycorrhizal root system (Cromer 1935; Harley 1940; Routien and Dawson 1943; Bjorkman 1949; Wilde 1954; Melin 1959; Goss 1960). Considerable research attention has been given to the uptake of nitrogen and phosphorus by mycorrhizal short roots (Mitchell et al. 1937; McComb 1938, 1943; Rosendahl 1942; Finn 1943; McComb and Griffith 1946; Kramer and Wilbur 1949; Harley and McCready 1950; Melin and Nilsson 1950; Stone 1950; Stone and McAuliffe 1954; Morrison 1954, 1957, 1962; Jennings 1964; Rovira and Bowen 1966a; Bowen and Theodorou 1967; Bowen 1968; Mejstrik and Benecke 1969; Björkman 1970; Henderson and Stone 1970; Lundeberg 1970; Mejstrik 1970; Lamb and Richards 1971; Voight 1971; Malajczuk 1975). These studies show that nitrogen and phosphorus uptake is enhanced by mycorrhizae.

An additional benefit that mycorrhizae provide forest trees is protection from many root pathogens. Zak (1964) postulated five mechanisms by which mycorrhizae may provide protection:

1) The mycorrhizal fungus utilizes excess carbohydrates in the root, leaving no carbohydrates available for pathogens,

2) the fungal mantle acts as a mechanical barrier to pathogen penetration,

3) the mycorrhizal fungus may secrete antibiotics which inhibit pathogen growth,

4) the mycorrhizae may support a protective microbial rhizosphere population, and
5) the mycorrhizal fungus may induce the cortical cells of the host to exude growth inhibitors which limit pathogen growth.

Zak felt that improved mineral nutrition was only a partial explanation for the benefit of mycorrhizae to forest trees. He cites the work of White (1941) and Briscoe (1959) where the application of nutrients alone did not produce healthy seedlings in nursery beds, but that healthy seedling growth was dependent on the presence of mycorrhizal fungi. Recent research has shown that mycorrhizae act as biological deterrents to organisms causing feeder root diseases (Sasek 1967; Sasek and Musilek 1967; Marx and Davey 1967, 1969a, 1969b; Marx 1969a, 1969b, 1970, 1973; Richard et al. 1972). Marx (1972) has provided an excellent review and discussion of the literature on mycorrhizae as barriers to root pathogens.

The final aspect of discussion on the beneficial effects of mycorrhizae is that of hormonal influence. Mycorrhizal fungi have been shown to produce gibberellic acid (Ho and Zak, unpublished data), cytokinins (Miller 1967, 1971) and auxins (Slankis 1958; Gruen 1959; Subba-Rao and Slankis 1959; Ulrich 1960a, 1960b; Meyer 1968; Slankis 1971). The chemical composition and physiological role of gibberellins, cytokinins and auxins in plant growth and development are presented respectively in Cleland (1969), Fox (1969) and Thimann (1969). Levisohn (1952, 1956) found that growth stimulation of non-endomycorrhizal Lawson cypress (Chamaecyparis lawsoniana [A. Murr.] Parl.) seedlings growing in pots occurred when the soil was infected with the ectomycorrhizal fungus Rhizopogon luteolus Fr. & Nordholm. It may be reasoned from these results that the fungus, even though in a non-mycorrhizal state, influenced seedling growth by influencing nutrient availability and or by releasing growth.
stimulating compounds. Turner (1962) tested 54 species of fungi and found that 92 percent produced exudates which stimulated plant root growth.

The documentation of the beneficial effects of mycorrhizae is certainly justification for continued research on the potential of mycorrhizae to enhance forest regeneration efforts.

**Symbiotic Influence On Forestation Success**

**Past Forestation Efforts**

The influence of mycorrhizae on forestation efforts has been dramatic. Hatch (1936) attempted to grow pine seedlings in a prairie soil and found that normal seedling growth could only be achieved when the soil was inoculated with a mycorrhizal fungus. Hatch emphasized the need for mycorrhizal inoculations in all forestation programs. Forestation attempts in west Australia (Kessell 1927), Rhodesia (Anonymous 1931), the Philippines (Oliveros 1932), Great Britain (Handley 1963) and Puerto Rico (Hacskaylo and Vozzo 1967; Vozzo 1971) have demonstrated convincingly the vital role of mycorrhizae in the establishment of forest trees. Björkman (1961) suggested that mycorrhizal seedlings have a greater potential for surviving the early critical stage, especially in dry soil or soil low in nutrients, than do non-mycorrhizal seedlings. Marx (1977) concluded that *Pisolithus tinctorius* (Pers.) Coker and Couch mycorrhizae significantly increase the survival and growth of southern pines out-planted on adverse as well as on better sites. Mikola (1969, 1970, 1973) has provided an excellent discussion on mycorrhizae, mycorrhizal inoculations and the promising future of mycorrhizae in forest tree production.
Adverse Environmental Conditions

In addition to the beneficial effects of mycorrhizae already discussed, it has been shown that mycorrhizae afford seedlings and trees with the ability to survive a variety of adverse soil related conditions. DeBell (1970) and McCalla (1971) discuss the plant growth problems associated with phytotoxic substances present in the soil. Bevege (1968) grew non-mycorrhizal pine seedlings which were watered with leachate from soils growing older trees of the same species. He found that the leachate contained phytotoxic substances which inhibited seedling growth. Rovira and Bowen (1966b) and Zak (1971) demonstrated that many fungi, including mycorrhizal fungi, possess the ability to detoxicate heat sterilized soils. Zak (1971) suggested that under actual soil conditions mycorrhizal fungi may aid tree growth by protecting the absorbing roots from soil phytotoxins.

In many cases, areas that are to be revegetated have one or more specific site conditions which are not conducive to vegetation establishment. Among these conditions may be extreme soil pH, extreme temperatures, moisture stress and low nutrient availability. Marx and Zak (1965) demonstrated that at pH 4.0 the average height of mycorrhizal seedlings was considerably greater than the average height of non-mycorrhizal seedlings. Dale et al. (1955) grew pine seedlings in calcareous soil at pH 8.2 and found that normal seedling growth could only be achieved if the seedlings were inoculated with forest litter known to contain mycorrhizal fungi. Seedlings in other treatments were non-mycorrhizal and did not respond to a standard N-P-K fertilizer. Marx et al. (1970) and Theodorou
and Bowen (1971) generally concluded that mycorrhizae offer some degree of protection to the host from above-optimum temperatures. They also pointed out that there are large differences between strains within fungal species and between fungal species in their ability to tolerate high soil temperatures. Marx and Bryan (1971) grew pine seedlings for five weeks at a root substrate temperature of 40°C. They found that only 45 percent of the non-mycorrhizal seedlings survived, while 70 percent and 95 percent of the seedlings survived that were mycorrhizal with *Thelephora terrestris* Ehrhart ex Fr. and *Pisolithus tinctorius*, respectively. From the aspect of water relations, Harley (1969) and Marks and Kozlowski (1973) pointed out that fungal hypae have the capacity to extract soil moisture at suctions considerably greater than non-mycorrhizal roots or root hairs. The positional availability of soil moisture to hypae is also greater than to a non-infected root by virtue of the rapid growth rate and extensibility of the hypae. Worley and Hacskaylo (1959) showed that different fungi will colonize a root system under different levels of moisture stress. Under moist conditions, the lighter pigmented mycorrhizae prevail, while the darker pigmented mycorrhizae prevail under extreme moisture stress conditions.

A topic of recent concern is the establishment of vegetation on disturbed land. Wilde and Iyer (1962), Schram (1966) and Marx (1975) concluded that mycorrhizal pine seedlings have a significantly greater potential for survival and growth on scalped or strip-mined sites than do non-mycorrhizal seedlings.
Mycorrhizae and the Forest Tree Nursery

Inoculation of Nursery Seedlings

It was discussed earlier in this review that mycorrhizae are essential to the survival and growth of most forest tree species. It is logical to assume that seedlings produced in a nursery must become mycorrhizal either in the nursery or once outplanted in the field. If mycorrhizae are to be formed in the field it is necessary that the soil at a particular site have an abundant population of mycorrhizal fungi, or that the seedlings be planted early enough in the season to allow for adequate root development. This would permit for subsequent mycorrhizal infection prior to the onset of natural environmental stresses (Trappe 1977). As is often the case in afforestation sites, strip-mined areas or sites that were severely disturbed by timber harvest, an adequate population of indigenous mycorrhizal fungi to insure infection is lacking. Under these conditions it is necessary to plant seedlings that became mycorrhizal in the nursery.

Early investigators realized the need for the mycorrhizal inoculation of nursery seedlings. Most of the early inoculations were accomplished by introducing soil that contained mycorrhizal fungi. As research on inoculation technology continued it was realized that some very attractive possibilities exist in the areas of inoculation methods, selection of specific fungi and host response.

Currently, the most practical means of inoculation are by fungal spores or vegetative fungal mycelium. Marx and Bryan (1975) provided a description of inoculum preparation and inoculation techniques that they
used with excellent results using the fungus *Pisolithus tinctorius*. Marx et al. (1976) found that infection rates were slightly better using vegetative mycelium as compared to spores. Recently, considerable attention has been directed towards the development of containerized seedlings. Göbl (1974), Marx and Barnett (1974), Landis and Gillman (1976) and Ruehle and Marx (1977) have provided excellent discussions on the inoculation of containerized seedlings with mycorrhizal fungi.

Several investigators have listed and discussed the variety of fungal associates of mycorrhizae (Trappe 1962, 1967; Hacskaylo and Bruchet 1972). Considering the diversity of mycorrhizal fungi in nature, some very interesting possibilities arise in the area of using specific fungi to inoculate seedlings. Theodorou and Bowen (1970) demonstrated in greenhouse and field studies that large differences existed between different fungi in their stimulation of pine growth. Levisohn (1957, 1959) and Göbl (1975) observed different seedling growth responses with different species and strains of mycorrhizal fungi. Donald (1975) found that pine seedlings inoculated with *Rhizopogon luteolus* increased in top height and stem diameter as compared to non-mycorrhizal seedlings; however, he speculated that *R. luteolus* may not be the most efficient fungus available for use under the specific experimental conditions. Marx (1976) discussed the use of specific mycorrhizal fungi for the forestation of disturbed sites. Trappe (1977) presented a comprehensive review of research directed to the selection of mycorrhizal fungi for nursery inoculation.

The variability of mycorrhizal relationships would not be complete without discussing host variability. Trappe (1964) listed the following
families as having representatives which are mycorrhizal hosts of *Cenococcum graniforme* (Sow.) Ferd. & Winge: Pinaceae, Betulaceae, Ericaceae, Fagaceae, Myricaceae, Rosaceae, Salicaceae and Tiliaceae. In pure culture synthesis tests, Marx and Bryan (1969) successfully cultured mycorrhizae of *P. tinctorius* and *T. terrestris* on 14 and 20 species of pine, respectively. Rayner and Levisohn (1941) also observed that host response varied with the species of fungal symbiont as well as host species.

The intent of this brief discussion on inoculation methods, fungal variability and host variability is to emphasize the biological feasibility of inoculating nursery seedlings with select mycorrhizal fungi. Sinclair (1974a) suggested that the potential exists for manipulation of fungi in fumigated soil during the first few weeks of seedling growth, before indigenous fungi colonize the root system. Marx (1977a) concluded that inoculation with *P. tinctorius* significantly improves the quality of pine seedlings, as compared to uninoculated seedlings, in both bare-root and container nurseries. Bowen (1965) suggested two microbiological questions that should be considered when selecting fungi for inoculation purposes: 1) what are the naturally occurring mycorrhizal fungi for a particular tree species, and 2) how will the fungi vary in their efficiency when associated with a certain tree species?

The primary point of this discussion on mycorrhizal inoculation is to emphasize the need to investigate the specific interaction of fungal species, tree species and the environmental conditions of the forestation site.
Nursery Factors Affecting Mycorrhizal Infection

**Standard Nursery Practices**

It is necessary at this point to discuss separately bare-root and container nursery conditions which influence mycorrhizal formation. In the bare-root nursery, any treatment which affects soil or seedling growth properties may ultimately affect the mycorrhizal association. Factors such as soil pH, temperature and moisture tension affect fungal growth and tree root development. Two treatments of particular importance with respect to mycorrhizal formation are 1) soil fumigation and 2) adjustment of fertility levels. Soil fertility levels will be discussed in the final section of this review. The objective of soil fumigation is to reduce or eliminate feeder root pathogens. Reports on the effects of soil fumigants on soil microflora, especially mycorrhizal fungi, are varied. Sinclair (1974b) found that 12 week old Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco.) seedlings growing in fumigated soil had more mycorrhizae than seedlings growing in non-fumigated soil. Apparently, mycorrhizal fungi propagules survived in the soil below the depth of effective fumigation and were able to rapidly colonize the soil that had been affected by the treatment. Ridge and Theodorou (1972) found that fungal populations in two forest soils were greatly reduced by fumigation with methyl bromide or dazomet, and although recolonization was rapid, original population levels had not developed 7 months after treatment. Laiho and Mikola (1964) tested methyl bromide and three other fumigants and found no harmful influence on mycorrhizal formation. They point out that mycorrhizal formation occurs 6-7 weeks after seeding and that most fumigants
evaporate or disintegrate within a few days or weeks. They felt that the effect of fumigation on mycorrhizal fungi depends on how completely the fungi are destroyed and what chances the fungi, either naturally or artificially introduced, have to invade the soil and infect the roots. This in turn depends upon the strength of the treatment and soil properties.

Laiho and Mikola (1964) suggest that as new chemicals are introduced into nursery practice the possible harmful effects on soil fungi should be taken into consideration.

The methodology of containerized seedling production is discussed in a variety of articles collectively edited by Tinus et al. (1974). Many container seedling operations use a non-soil potting mix, such as peat moss and vermiculite. Microbial populations in this type of mixture are fairly low compared to those of soil. Trappe et al. (unpublished data) have shown that partial sterilization of peat moss-vermiculite potting medium with methyl bromide or steam and subsequent incorporation of vegetative mycorrhizal inoculum increases the mycorrhizal infection rate as compared to untreated potting medium.

As a result of limited space and medium for root growth within individual containers and nutrient deficient medium, container operations must incorporate a schedule of weekly nutrient applications. It has been shown that the two most significant factors which inhibit mycorrhizal formation in containers are 1) high levels of soluble nutrients, especially nitrogen and phosphorus, and 2) a sterile medium free of mycorrhizal fungi. The latter factor affords greater flexibility for the inoculation of select mycorrhizal fungi as compared to a nursery soil situation. Bunt (1976) provided an excellent
description of the physical and chemical properties of various potting mixtures.

**Fertility Levels**

A variety of research has been conducted to determine the most suitable fertility levels for the soil or artificial media culture of forest tree seedlings. The mineral nutrition aspect of seedling production techniques in bare-root and container operations is discussed by Cleary et al. (1978) and Brix and van den Dreissche (1974), respectively.

Many factors influence the formation of mycorrhizae on the roots of trees (Rayner and Neilson Jones 1944; Slankis 1959; Wright 1959; Levishon 1960; Harley 1969; Marks and Kozlowski 1973; Slankis 1974). These authors have shown that nutritional levels are of major importance to development of mycorrhizae on nursery seedlings. Correlation between the levels of major nutrients, particularly nitrogen and phosphorus, and the formation of mycorrhizae has been reported by several investigators (Hatch 1937; Björkman 1956; Fowells and Krauss 1959; Macskaylo and Snow 1959; Richards and Wilson 1963; Richards 1965; Dumbroff 1968; Lister et al. 1968; Theodorou and Bowen 1969; Menge et al. 1977). These investigators observed that an inverse correlation exists between nitrogen and phosphorus levels, and mycorrhizal infection. In many cases, if nitrogen and phosphorus were extremely deficient, both seedling growth and mycorrhizal infection were severely inhibited. Lamb and Richards (1974) found that mycorrhizal infection was inhibited in an Australian soil severely deficient in phosphorus and that an application of 40 kg P/ha of superphosphate greatly increased mycorrhizal formation. If nutrients were
present in luxurient amounts, tree growth would be excellent but mycorrhizae would not form. Optimum mycorrhizal development was achieved when nitrogen and phosphorus were present at intermediate levels.

Considering the physiology of the fungal symbiont and the host, it is reasonable to expect that available nutrient levels will affect the type and amounts of compounds in the roots. Hatch (1937) suggested that mycorrhizal development was largely influenced by the internal concentrations of major nutrients in the roots. Pursuing this idea, Björkman (1942, 1944) found that nitrogen and phosphorus levels in the soil did strongly affect mycorrhizal infection, and that the internal concentration of soluble carbohydrates in the root was the dominant factor controlling infection. Björkman's theory was that a high level of soluble carbohydrates in the root system was necessary for mycorrhizal infection and that low levels decreased infection. More specifically, under conditions of high nutrient availability all of the carbohydrates produced by photosynthesis are rapidly converted to protein for tree growth, leaving little or no carbohydrates available in the root system for fungal infection. Handley and Sanders (1962) grew non-mycorrhizal pine seedlings under various light and nutrient schedules and analyzed the roots for soluble carbohydrates. They concluded from their results that, contrary to Björkman's theory, a high level of soluble carbohydrates in the roots was the result of mycorrhizal infection rather than the cause of infection. Marx et al. (1977) found a negative correlation between high levels of nitrogen and phosphorus, and the soluble sugar content of the short roots. They identified sucrose to be the primary carbohydrate present.
In light of the preceding discussion on the inverse relation between nitrogen and phosphorus levels and mycorrhizal infection it seems logical to consider the possible influence on infection by varying the availability of the nutrients rather than absolute levels. Phosphorus, as compared to nitrogen, would allow for the greatest flexibility in testing this theory due to the variety of fertilizer materials with a wide range of phosphorus availability. The idea behind this theory is that if phosphorus were present in a form unavailable to the tree but to some extent available to a mycorrhizal fungus, conditions would be conducive to early formation of mycorrhizae. In a container nursery operation, this may allow for greater flexibility in post-mycorrhization fertility schedules.

A key factor in tests of this theory would be that microorganisms, particularly mycorrhizal fungi, could utilize phosphorus that was present in forms unavailable to the tree seedling. Rosendahl (1942) demonstrated in chemical analyses of sand cultures that both mycorrhizal and non-mycorrhizal fungi tested increased the solubility of phosphorus in apatite. Sperber (1958a, 1958b) demonstrated the existence of apatite-solubilizing rhizosphere organisms. She tested two species of fungi, one species of actinomycete and two bacterial species and found that all solubilized apatite to some degree by formation of various organic acid metabolites. Murdoch et al. (1967) found that mycorrhizal and non-mycorrhizal maize plants grew equally as well when fertilized with superphosphate. When fertilized with rock phosphate, the mycorrhizal maize had a much higher phosphorus content and growth rate than non-mycorrhizal maize. Several investigators have evaluated the plant growth effectiveness of various sources of phosphorus (Wilde 1946; Armiger and Fried 1957; Pritchett and
Llewellyn 1966; Ensminger et al. 1967). Most studies compared the effectiveness of rock phosphate and superphosphate and found that rock phosphate was poorer or no better than superphosphate as a phosphorus source for plants.

In conclusion to this review on the influence of fertility levels on mycorrhizal development it is necessary to point out that large, healthy, non-mycorrhizal forest tree seedlings may be produced in a relatively short period of time under an intense fertilization schedule. The potential for improved survival and growth of mycorrhizal seedlings as compared to non-mycorrhizal seedlings is of practical significance. Future research and management decisions must be made to determine if it is most important to produce large uninfected seedlings or to sacrifice the large size to be certain of achieving mycorrhizal infection.
MATERIALS AND METHODS

Tree and Fungal Species Tested

The tree species tested were Douglas-fir (*Pseudotsuga menziesii*) and western hemlock (*Tsuga heterophylla* Raf. Sarg.). Prestratified seeds of both species were obtained from Crown Zellerbach's Aurora nursery on 14 June 1978. Douglas-fir seeds were collected in Clatsop county, Oregon, at an elevation between 150 and 300 meters in 1976. Western hemlock seeds were collected in Clatsop county, Oregon, at an elevation between 0 and 150 meters in 1973.

The fungi tested were *Hebeloma crustuliniforme* (Bull. ex St. Ann.), *Laccaria laccata* (Scop. ex Fr.) Bk. & Br. and *Cenococcum graniforme*. An uninoculated control was also tested. The isolate of *H. crustuliniforme* was collected in October 1976 under a lodgepole pine (*Pinus contorta* Dougl.)-Douglas-fir canopy in Union county, Oregon. The isolate of *L. laccata* was collected in September 1976 under a mountain hemlock (*Tsuga mertensiana* Bong. Carr.) canopy in Crater Lake National Park, Oregon. The isolate of *C. graniforme* was collected in December 1974 under a Douglas-fir canopy in the H.J. Andrews Experimental Forest, Oregon.

Inoculum Preparation

Cultures of each fungus were grown at 20°C for 2 weeks on potato-dextrose agar slants. Mycelium of each fungus was then transferred to prescription bottles containing a small amount of crushed glass and 125 ml of sterile Melin-Norkrans nutrient solution. The cultures were grown at 20°C for 3 weeks with periodic shaking. The inoculum medium was
prepared with the following components per 2-liter Erlynmeyer flask: 1450 ml grade 2 vermiculite, 100 ml peatmoss and 800 ml Melin-Norkrans nutrient solution. The mouth of each flask was covered with an inverted drinking glass, into the bottom of which was glued a 2.5 cm layer of cotton covered with cheesecloth. The flasks were then autoclaved for 75 minutes, allowed to cool to room temperature, and each aseptically inoculated with 20 ml of liquid mycelial slurry. Uninoculated control flasks were also prepared. The flasks were stored at room temperature. Once a week each flask was shaken gently to distribute the colonized vermiculite particles and hasten complete medium colonization. After 12 weeks the medium was well colonized with mycelium. Prior to incorporation into the potting mix, the inoculum was removed from the flasks, placed in a large vegetable strainer lined with two layers of cheesecloth and leached with cool tap water. Excess water was removed by gentle squeezing. The leaching process is necessary to remove all non-assimilated nutrients to insure against a flush of saprophitic microorganisms. The inoculum was used within 2 hours after leaching.

Inoculation and Seeding Procedure

The potting mix consisted of a 1:1 ratio of sphagnum peatmoss and grade 2 vermiculite partially sterilized with aerated steam at 80°C for 30 minutes in a Lindig apparatus. The initial pH of the mix was 5.1. The Ray Leach single cell container system was used for the experiment. Each cell measures 2.5 cm top diameter and 16.5 cm in length with an approximate volume of 65.6 cm$^3$. Tray dimensions are 30 cm by 60 cm. Each tray has a 200 cell capacity.

The final potting mixture was prepared by combining the leached
inoculum with the partially sterilized peatmoss-vermiculite at a 1:5 ratio. Mixing was done in a large plastic bag with 16.2 liters mixed per batch. Each batch filled 200 cells. Two batches were required for each fungus. New plastic bags were used for each fungal species.

The phosphate treatments consisted of powdered rock phosphate or dicalcium phosphate incorporated with the potting mix. These treatments were prepared in 80-cell batches. After preparation of the final potting mix (inoculum plus peatmoss-vermiculite), 6.3 liters were placed in a plastic bag. Due to medium compaction, it was necessary to use approximately 20 percent excess of potting mix. Into the 6.3 liters of final potting mix was combined 5.0 gm of rock phosphate or 3.2 gm of dicalcium phosphate. This mixture is enough to fill 80 cells. Four batches of 80 cells were needed for each phosphate treatment.

Three to four seeds were sown in each cell and covered with approximately 5 mm of grade 2 chicken grit on 27 July 1978. Cells were thinned to one seedling each, one week after germination. The total number of cells in the experiment was 1600. In addition to weekly fertilizer applications, all cells were watered 2-3 times per week. To avoid nutrient leaching, cells were not watered to the point of saturation.

Seedlings were grown in a Sherer-Gillett model number CEL 511-38 growth chamber. Inside horizontal dimensions of the chamber are 258 cm by 140 cm. Trays were held on a movable platform 95 cm from the fluorescent and incandescent light source. Factory specifications for the light intensity under the given conditions are 2700-3000 foot candles. Daylength throughout the experiment was 15.5 hours. Day and night
temperatures were 21°C and 15°C, respectively.

Fertilizer Treatments

Table 1 presents the nutrient treatments tested in this experiment. The High and Low nutrient sources and treatments were selected according to a standard nursery schedule for containerized seedlings. The values are given on a mg of nutrient/seedling/week basis. Table 2 presents the total nutrients received by each seedling per treatment for the duration of the study. Fertilizer solutions were applied once a week beginning the sixth week following sowing. The rock phosphate and dicalcium phosphate treatment seedlings received only N+K solution applications subsequent to the initial phosphate application. Nutrient solution was

TABLE 1. Nutrient application rates for container seedlings inoculated with mycorrhizal fungi, on a mg/seedling/week basis. 1/

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>High N-P-K</td>
<td>1.00</td>
<td>0.65</td>
<td>0.83</td>
<td>0.18</td>
</tr>
<tr>
<td>Low N-P-K</td>
<td>0.50</td>
<td>0.32</td>
<td>0.42</td>
<td>0.18</td>
</tr>
<tr>
<td>Low-High N-P-K 3/</td>
<td>0.50-1.00</td>
<td>0.32-0.65</td>
<td>0.42-0.83</td>
<td>0.18</td>
</tr>
<tr>
<td>Low N-RP-K</td>
<td>0.50</td>
<td>8.60 4/</td>
<td>0.42</td>
<td>0.18</td>
</tr>
<tr>
<td>Low N-DCP-K</td>
<td>0.50</td>
<td>8.60 5/</td>
<td>0.42</td>
<td>0.18</td>
</tr>
</tbody>
</table>

1/ Applications began in week 6 and concluded in week 25. Total applications = 20.
2/ Fe applied on 12 consecutive weeks, between week 7 and week 18.
3/ Low level for the first 13 applications, then High level for the final seven applications.
4/ Rock phosphate as the P-source; one initial application mixed with the potting mix.
5/ Dicalcium phosphate as the P-source; one initial application mixed with the potting mix.
dispensed to the medium surface of each cell individually using a
Brinkman adjustable "Dispensette." The necessary N-P-K-Fe or N-K-Fe nutrients were delivered in 5 ml of water at each application. Table 3 presents the fertilizer materials used in this experiment.

**TABLE 2. Total nutrients applied to container seedlings inoculated with mycorrhizal fungi, as of final sample. Values are in mg/seedling.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>High N-P-K</td>
<td>20.0</td>
<td>12.9</td>
<td>16.6</td>
<td>2.16</td>
</tr>
<tr>
<td>Low N-P-K</td>
<td>10.0</td>
<td>6.45</td>
<td>8.30</td>
<td>2.16</td>
</tr>
<tr>
<td>Low-High N-P-K</td>
<td>13.5</td>
<td>8.71</td>
<td>11.2</td>
<td>2.16</td>
</tr>
<tr>
<td>Low N-RP-K</td>
<td>10.0</td>
<td>8.60 /</td>
<td>8.30</td>
<td>2.16</td>
</tr>
<tr>
<td>Low N-DCP-K</td>
<td>10.0</td>
<td>8.60 /</td>
<td>8.30</td>
<td>2.16</td>
</tr>
</tbody>
</table>

/ Rock phosphate or dicalcium phosphate was initially mixed with the peatmoss-vermiculite medium.

**TABLE 3. Nutrient sources.**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>(NH₄)₂SO₄, KNO₃</td>
</tr>
<tr>
<td>P</td>
<td>H₃PO₄, CaHPO₄, rock phosphate /</td>
</tr>
<tr>
<td>K</td>
<td>KNO₃</td>
</tr>
<tr>
<td>Fe</td>
<td>Sequestrene 138</td>
</tr>
</tbody>
</table>

/ Idaho rock phosphate, 14% P.
Experimental Design

The containers were arranged in the growth chamber in a factorial split-plot design with tree species as main plots and fungi X fertilizer treatments as subplots, with two replicates. With three fungi plus a control and five fertilizer treatments, there were 20 subplots per main plot. With two main plots and two replicates, there were a total of 80 subplots. Twenty seedlings were raised in each subplot. Figure 1 presents a diagram of the experimental plot design.

Sampling and Measurement Procedure

Five seedlings were sampled from each subplot at 60, 120 and 180 days after the seeds were sown. Although only 15 seedlings per subplot were needed to meet the sampling requirement, 20 seedlings were raised per subplot to buffer against possible individual seedling mortality.

Prior to each sample period a random sampling pattern was established. This pattern was used consistently in each subplot. A new pattern was used for each sample period. Therefore, in any one sample period, seedlings were sampled from the same relative locations within each subplot. Samples were stored at 5°C pending analysis.

The following measurements were made on each seedling:

1. Top height
2. Stem diameter
3. Percent mycorrhizal short roots
4. Oven dry weight of the top and roots.

Top height was measured using a millimeter scale from the point on the stem 1 cm above the first lateral root to the tip of the shoot.
FIGURE 1. Diagram of the experimental plot design within the growth chamber. Twenty seedlings per subplot, 80 subplots, total seedlings= 1600. Letter and number code described below:

**Fungi**
- A = H. crustuliniforme
- B = L. laccata
- C = C. graniforme
- D = Uninoculated Control

**Fertilizer Treatments**
- 1 = High N-P-K
- 2 = Low N-P-K
- 3 = Low-High N-P-K
- 4 = Low N-RP-K
- 5 = Low N-DCP-K
Measurements were recorded to the tenth of a centimeter. Stem diameter was measured using a vernier caliper scaled in millimeters. The measurement was taken at the point on the stem 1 cm above the first lateral root, and recorded in millimeters. Percent mycorrhizal short roots was measured by counting all mycorrhizal and non-mycorrhizal short roots and dividing the number of mycorrhizal short roots by the total number of short roots. Absolute counts of all short roots were made for seedlings in the first and second sample period. By the third sample period, root development had advanced to the stage that, from a time efficiency standpoint, it was not practical to make absolute short root counts. Therefore, a 25% sample of each root system was taken. The sampling was facilitated by the fairly uniform root plug produced by the restricted volume of the individual cells. A plexiglass jig was constructed to allow for uniform sampling. The jig consisted of two identical pieces of 3 mm thick plexiglass with dimensions of 15 cm by 10 cm. Three 1.25 cm slots were cut in both pieces of plexiglass along one 15 cm edge. The two pieces of plexiglass were hinged together on the opposing 15 cm edge. To take a sample, a root system was positioned in the jig and the portions of the roots exposed in the slots were removed with a razor blade. The slots were spaced such that a portion of the top third, middle third and lower third of the root system was sampled. These samples were placed in a petri dish for the short root count. To arrive at a final estimate of mycorrhizal and non-mycorrhizal short roots, the numbers of short roots found in the sample were multiplied by four. Counting of all short roots was done at 6X magnification with the aid of a Wild M7A binocular dissecting scope. To measure oven dry weight, a seedling was
separated into top and roots at a point 1 cm above the first lateral root. The tops and roots were placed in labeled coin envelopes and dried in a forced air oven at 70°C for 48 hours. After drying, the samples were placed in a dessicator, allowed to cool to room temperature and weighed to the fourth decimal place on a Mettler H10Tw balance.

Medium pH Differences Among Treatments

The pH of the medium of each subplot was measured at the third sample period. The potting mix from each of the five seedlings sampled within each subplot was combined and saved in a small plastic bag. Samples were stored at 5°C pending pH measurement. Samples were prepared by combining 95 ml of potting mix with 95 ml distilled water. Samples were stirred initially, then at 15 minutes and again at 30 minutes. The pH measurements were made after the final stirring, on a Corning model 7 pH meter.

Tissue Analysis

The oven dry tops of the seedlings from the third sample period were ground to 20 mesh in a Wiley mill. The five seedling tops from each subplot were combined for tissue analysis for nitrogen and phosphorus. Nitrogen was determined by the micro-Kjeldahl method. Phosphorus was determined colorimetrically after standard perchloric acid digestion.

Computer Analysis of Data

Data were subjected to an Analysis of Variance on the Oregon State University Statistical Interactive Programming System (Rowe and Barnes 1976). The significance among mean values was determined using the Studentized Range Test at the 0.05 level.
RESULTS

The analysis of variance performed on the data showed that a variety of significant interactions existed among treatments, both within sample periods and between sample periods. In light of these interactions, it is necessary, for reasons of clarity, to present the seedling variables evaluated which specifically address the questions of this investigation. For this purpose, data on percent mycorrhizal short roots, percent P, percent N and total seedling weight will be presented. Data for each variable will be presented individually.

Percent Mycorrhizal Short Roots (%MSR)

The analysis of variance test on the %MSR data showed that the interactions significant at the .05 level were a) sample period X tree species X fungi, b) sample period X tree species X fertilizer, c) sample period X fungi X fertilizer and d) tree species X fungi X fertilizer.

Table 4 presents the mean values for %MSR for the sample period X tree specie X fungi interaction. Figures 1 and 2 graphically present the mean values listed in Table 4 for Douglas-fir and western hemlock, respectively. The results show that for Douglas-fir at sample period 1, L. laccata produced a greater %MSR than did H. crustuliniforme, which produced a greater %MSR than did C. graniforme. All uninoculated Douglas-fir seedlings were non-mycorrhizal. For Douglas-fir at sample periods 2 and 3, no difference in %MSR was observed between H. crustuliniforme and L. laccata, however, both produced a greater %MSR than did C. graniforme. For western hemlock at sample periods 1 and 2, L. laccata produced a
TABLE 4. Mean values of percent mycorrhizal short roots (%MSR) for the sample period X tree species X fungi interaction. D value = 3.3.

<table>
<thead>
<tr>
<th>Tree Species</th>
<th>Fungus</th>
<th>Sample Period 2/</th>
<th>%MSR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1/</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H.c.</td>
<td>83.5 a</td>
</tr>
<tr>
<td>Douglas-fir</td>
<td>L.l.</td>
<td>95.8 b</td>
<td>93.5 ab</td>
</tr>
<tr>
<td></td>
<td>C.g.</td>
<td>12.5 c</td>
<td>62.1 c</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0 d</td>
<td>0 d</td>
</tr>
<tr>
<td>Western</td>
<td>H.c.</td>
<td>48.2 a</td>
<td>73.9 a</td>
</tr>
<tr>
<td>Hemlock</td>
<td>L.l.</td>
<td>83.1 b</td>
<td>93.1 b</td>
</tr>
<tr>
<td></td>
<td>C.g.</td>
<td>22.5 c</td>
<td>61.4 c</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0 d</td>
<td>1.4 d</td>
</tr>
</tbody>
</table>

H.c.= Hebeloma crustuliniforme  
L.l.= Laccaria laccata  
C.g.= Cenococcum graniforme  
Control = Uninoculated  

1/ Values with the same letter are not statistically different at the 0.05 level of probability.

greater %MSR than did H. crustuliniforme, which produced a greater %MSR than did C. graniforme. Of the uninoculated hemlock seedlings, all but two were non-mycorrhizal. The two that were mycorrhizal had only a few short roots that were infected with L. laccata. For western hemlock at sample period 3, no difference in %MSR was observed between H. crustuliniforme and L. laccata, however, both produced a greater %MSR than did C. graniforme.

Table 5 presents the mean values for %MSR for the sample period X tree species X fertilizer interaction. Figures 3 and 4 graphically present
FIGURE 1. Mean values of percent mycorrhizal short roots on Douglas-fir by sample period within fungal treatments, averaged over fertilizer treatments.

H.c. = Hebeloma crustuliniforme  L.l. = Laccaria laccata  C.g. = Cenococcum graniforme
Control = Uninoculated.
FIGURE 2. Mean values of percent mycorrhizal short roots on western hemlock by sample period within fungal treatments, averaged over fertilizer treatments. H.c. = Hebeloma crustuliniforme L.l. = Laccaria laccata C.g. = Cenococcum graniforme Control = Uninoculated.
TABLE 5. Mean values of percent mycorrhizal short roots (%MSR) for the sample period x tree species x fertilizer interaction. D value = 4.5.

<table>
<thead>
<tr>
<th>Tree Species</th>
<th>Fertilizer Treatment</th>
<th>Sample Period 1</th>
<th>Sample Period 2</th>
<th>Sample Period 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Douglas-fir</td>
<td>High</td>
<td>59.5 a</td>
<td>69.1 a</td>
<td>70.5 a</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>63.8 ab</td>
<td>84.2 b</td>
<td>83.4 b</td>
</tr>
<tr>
<td></td>
<td>Low-High</td>
<td>63.8 abc</td>
<td>85.4 bc</td>
<td>88.8 c</td>
</tr>
<tr>
<td></td>
<td>RP 2/</td>
<td>67.7 bcd</td>
<td>87.8 bcd</td>
<td>90.5 cd</td>
</tr>
<tr>
<td></td>
<td>DCP 3/</td>
<td>64.9 bcd</td>
<td>87.2 bcd</td>
<td>89.0 cd</td>
</tr>
<tr>
<td>Western</td>
<td>High</td>
<td>34.3 a</td>
<td>53.5 a</td>
<td>80.6 a</td>
</tr>
<tr>
<td>Hemlock</td>
<td>Low</td>
<td>43.8 b</td>
<td>81.4 b</td>
<td>84.2 ab</td>
</tr>
<tr>
<td></td>
<td>Low-High</td>
<td>50.1 c</td>
<td>78.5 bc</td>
<td>85.5 bc</td>
</tr>
<tr>
<td></td>
<td>RP 2/</td>
<td>65.6 d</td>
<td>84.9 bd</td>
<td>85.1 abc d</td>
</tr>
<tr>
<td></td>
<td>DCP 3/</td>
<td>62.8 d</td>
<td>83.8 bd</td>
<td>88.6 bcd</td>
</tr>
</tbody>
</table>

1/ Values with the same letter are not statistically different at the 0.05 level of probability.
2/ RP = rock phosphate
3/ DCP = dicalcium phosphate

the mean values listed in Table 5 for Douglas-fir and western hemlock, respectively. For Douglas-fir at sample period 1, no differences were observed in the %MSR produced by the Low, Low-High, rock phosphate (RP) and dicalcium phosphate (DCP) treatments, however, seedlings receiving the RP and DCP treatments produced a greater %MSR than did the High treatment seedlings. For Douglas-fir at sample periods 2 and 3, the Low, Low-High, RP and DCP treatments produced a greater %MSR than did the High treatment. At sample period 2, no differences in %MSR were found among the Low,
FIGURE 3. Mean values of percent mycorrhizal short roots on Douglas-fir by sample period within fertilizer treatments, averaged over fungal treatments. H = High, L = Low, L-H = Low-High, RP = rock phosphate, DCP = dicalcium phosphate.
FIGURE 4. Mean values of percent mycorrhizal short roots on western hemlock by sample period within fertilizer treatments, averaged over fungal treatments. 
H = High, L = Low, L-H = Low-High, RP = rock phosphate, DCP = dicalcium phosphate.
Low-High, RP and DCP treatments. At sample period 3, no differences in %MSR were observed among the Low-High, RP and DCP treatments, however, the three treatments produced a greater %MSR than did the Low treatment. For western hemlock at sample periods 1 and 2, the Low, Low-High, RP and DCP treatments produced a greater %MSR than did the High treatment. Also, at sample period 1, the Low-High, RP and DCP treatments produced a greater %MSR than did the Low treatment. At sample periods 1 and 2, no difference in %MSR was found between the RP and DCP treatments, although both treatments produced a greater %MSR than did the Low-High treatment. At sample period 2, no differences in %MSR were observed between the Low treatment and the Low-High, RP and DCP treatments. For western hemlock at sample period 3, the Low-High and DCP treatments produced a greater %MSR than did the High treatment, although no differences in %MSR were found among the Low, Low-High, RP and DCP treatments.

Table 5a presents the mean values for %MSR for the tree species X fertilizer X fungi interaction. The fertilizer X fungus combinations which resulted in the greatest %MSR for Douglas-fir and western hemlock were rock phosphate + H. crustuliniforme or L. laccata, and rock phosphate + L. laccata, respectively.

Percent Phosphorus (%P)

The analysis of variance test on the %P data showed that the interactions significant at the 0.05 level were a) tree species X fungi, b) tree species X fertilizer and c) tree species X fungi X fertilizer. Percent P analysis was conducted only on the tops of the seedlings from the third sample period.
TABLE 5a. Mean values of percent mycorrhizal short roots (%MSR) for the tree species X fungi X fertilizer interaction. D value for comparing means within tree species = 5.9.

<table>
<thead>
<tr>
<th>Fertilizer Treatment</th>
<th>Fungus/1/</th>
<th>Tree Species</th>
<th>Douglas-fir</th>
<th>Western Hemlock</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>H.c.</td>
<td>73.4</td>
<td>42.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L.1.</td>
<td>87.7</td>
<td>81.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C.g.</td>
<td>38.0</td>
<td>45.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>H.c.</td>
<td>87.2</td>
<td>67.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L.1.</td>
<td>90.4</td>
<td>88.7</td>
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</tr>
<tr>
<td></td>
<td>C.g.</td>
<td>53.8</td>
<td>53.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Low-High</td>
<td>H.c.</td>
<td>89.3</td>
<td>66.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L.1.</td>
<td>93.3</td>
<td>90.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C.g.</td>
<td>55.5</td>
<td>57.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Rock phosphate</td>
<td>H.c.</td>
<td>96.6</td>
<td>90.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L.1.</td>
<td>95.6</td>
<td>95.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C.g.</td>
<td>53.7</td>
<td>50.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>H.c.</td>
<td>93.9</td>
<td>86.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L.1.</td>
<td>93.1</td>
<td>92.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C.g.</td>
<td>54.1</td>
<td>56.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Table 6 presents the mean values for %P for the tree species X fungi interaction. Figure 5 graphically presents the mean values listed in Table 6.

**TABLE 6. Mean values of percent phosphorus for the tree species X fungi interaction. D value = 0.008.**

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Tree Species 1/</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Douglas-fir</td>
<td>Western Hemlock</td>
<td></td>
</tr>
<tr>
<td></td>
<td>%P</td>
<td>%P</td>
<td></td>
</tr>
<tr>
<td>Hebeloma</td>
<td>0.219 a</td>
<td>0.228 a</td>
<td></td>
</tr>
<tr>
<td>crustuliniforme</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laccaria</td>
<td>0.253 b</td>
<td>0.249 ab</td>
<td></td>
</tr>
<tr>
<td>laccata</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cenococcum</td>
<td>0.228 abc</td>
<td>0.202 ac</td>
<td></td>
</tr>
<tr>
<td>graniforme</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.236 abc</td>
<td>0.192 c</td>
<td></td>
</tr>
</tbody>
</table>

1/ Values with the same letter are not statistically different at the 0.05 level of probability.

The results show that Douglas-fir seedlings mycorrhizal with L. laccata had a greater %P than did seedlings mycorrhizal with H. crustuliniforme. For Douglas-fir seedlings, no differences in %P were observed between H. crustuliniforme, C. graniforme and the control.

Western hemlock seedlings mycorrhizal with H. crustuliniforme and L. laccata had a greater %P than did the control seedlings. Hemlock seedlings mycorrhizal with L. laccata had a greater %P than did seedlings mycorrhizal with C. graniforme. For hemlock seedlings, no differences in %P were observed between seedlings mycorrhizal with H. crustuliniforme and seedlings mycorrhizal with L. laccata or C. graniforme, or between seedlings mycorrhizal with C. graniforme and the controls.
FIGURE 5. Mean values of percent phosphorus by tree species within fungal treatments at sample period 3, averaged over fertilizer treatments.

H.c. = Hebeloma crustuliniforme  L.l. = Laccaria laccata  C.g. = Cenococcum graniforme
Control = Uninoculated.
Table 7 presents the mean values for %P for the tree species X fertilizer interaction. Figure 6 graphically presents the mean values listed in Table 7.

**TABLE 7. Mean values of percent phosphorus (%P) for the tree species X fertilizer interaction. D value = 0.008.**

<table>
<thead>
<tr>
<th>Fertilizer Treatment</th>
<th>Tree Species 1/</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Douglas-fir</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>0.317 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>0.284 ab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-High</td>
<td>0.335 ac</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RP 2/</td>
<td>0.092 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCP 3/</td>
<td>0.141 e</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Western Hemlock</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.292 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.300 ab</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.322 abc</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.084 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.090 d</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1/ Values with the same letter are not statistically different at the 0.05 level of probability.

2/ RP = rock phosphate

3/ DCP = dicalcium phosphate

The results show that Douglas-fir seedlings receiving the High, Low and Low-High treatments had a greater %P than did the RP or DCP treatment seedlings. No difference in %P was observed between the High treatment seedlings and the Low or Low-High treatment seedlings, however, the seedlings receiving the Low-High treatment had a greater %P than did the Low treatment seedlings. The Douglas-fir seedlings receiving the DCP treatment had a greater %P than did the RP treatment seedlings. For western hemlock seedlings, no differences in %P were found among the High, Low or Low-High treatments, or between the RP and DCP treatments. Hemlock seedlings receiving the High, Low and Low-High treatments had a greater %P than did the RP or DCP treatment seedlings.
FIGURE 6. Mean values of percent phosphorus by tree species within fertilizer treatments at sample period 3, averaged over fungal treatments.

H = High, L = Low, L-H = Low-High, RP = rock phosphate, DCP = dicalcium phosphate.
Table 8 presents the mean values for %P for the tree species X fungi X fertilizer interaction. Figures 7 and 8 graphically present the mean values listed in Table 8 for Douglas-fir and western hemlock, respectively.

TABLE 8. Mean values of percent phosphorus (%P) for the tree species X fungi X fertilizer interaction. D value = 0.065.

<table>
<thead>
<tr>
<th>Tree Species</th>
<th>Fungus 1/</th>
<th>Fertilizer Treatments 3/</th>
<th>%P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Douglas-fir</td>
<td>H.c.</td>
<td>0.310 a</td>
<td>0.250 a</td>
</tr>
<tr>
<td></td>
<td>L.1.</td>
<td>0.305 a</td>
<td>0.270 ab</td>
</tr>
<tr>
<td></td>
<td>C.g.</td>
<td>0.295 a</td>
<td>0.280 ab</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.360 a</td>
<td>0.335 b</td>
</tr>
<tr>
<td>Western Hemlock</td>
<td>H.c.</td>
<td>0.295 a</td>
<td>0.330 a</td>
</tr>
<tr>
<td></td>
<td>L.1.</td>
<td>0.335 ab</td>
<td>0.310 a</td>
</tr>
<tr>
<td></td>
<td>C.g.</td>
<td>0.290 abc</td>
<td>0.275 a</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.250 ac</td>
<td>0.285 a</td>
</tr>
</tbody>
</table>

1/ H.c. = Hebeloma crustuliniforme  
L.1. = Laccaria laccata  
C.g. = Cenococcum graniforme  
Control = uninoculated  
2/ RP = rock phosphate  
DCP = dicalcium phosphate  
3/ Values with the same letter are not statistically different at the 0.05 level of probability.

For Douglas-fir, there were no differences in %P observed in seedlings receiving the High fertilizer treatment. For seedlings receiving the Low treatment, the control seedlings had a greater %P than did the seedlings mycorrhizal with H. crustuliniforme. Within the Low fertility treatment, no differences in %P were found among seedlings mycorrhizal with H.
FIGURE 7. Mean values of percent phosphorus in Douglas-fir seedling tops at sample period 3, by fungal treatments within fertilizer treatments.

H.c. = Hebeloma crustuliniforme  L.l. = Laccaria laccata  C.g. = Cenococcum graniforme
Control = Uninoculated.
H = High, L = Low, L-H = Low-High, RP = rock phosphate, DCP = dicalcium phosphate.
FIGURE 8. Mean values of percent phosphorus in western hemlock seedling tops at sample period 3, by fungal treatments within fertilizer treatments.

H.c. = *Hebeloma crustuliniforme*  
L.l. = *Laccaria laccata*  
C.g. = *Cenococcum graniforme*

Control = Uninoculated.

H = High, L = Low, L-H = Low-High, RP = rock phosphate, DCP = dicalcium phosphate.
crustuliniforme, L. laccata and C. graniforme, or among seedlings mycorrhizal with L. laccata, C. graniforme and the uninoculated controls. In the Low-High treatment, seedlings mycorrhizal with L. laccata had a greater %P than did the seedlings mycorrhizal with H. crustuliniforme. Also, in the Low-High treatment, no differences in %P were found among seedlings mycorrhizal with H. crustuliniforme, C. graniforme and the controls, or among seedlings mycorrhizal with L. laccata, C. graniforme and the controls. For Douglas-fir receiving the RP treatment, seedlings mycorrhizal with L. laccata had a greater %P than did the seedlings mycorrhizal with H. crustuliniforme. However, no differences in %P were found among seedlings mycorrhizal with H. crustuliniforme, C. graniforme and the controls, or among seedlings mycorrhizal with L. laccata, C. graniforme and the controls. For Douglas-fir receiving the DCP treatment, no differences in %P were found among seedlings mycorrhizal with H. crustuliniforme, L. laccata and C. graniforme, however, all mycorrhizal seedlings had a greater %P than did the non-mycorrhizal control seedlings.

For western hemlock receiving the High fertility treatment, seedlings mycorrhizal with L. laccata had a greater %P than did the control seedlings. However, no differences in %P were found among seedlings mycorrhizal with H. crustuliniforme, L. laccata and C. graniforme, or among seedlings mycorrhizal with H. crustuliniforme, C. graniforme and the controls. For hemlock receiving the Low treatment, no differences in %P were found among any of the fungal treatments or the control. For hemlock receiving the Low-High treatment, seedlings mycorrhizal with H. crustuliniforme or L. laccata had a greater %P than did the control
seedlings. However, no difference in %P was found between seedlings mycorrhizal with H. crustuliniforme and seedlings mycorrhizal with L. laccata or C. graniforme. Seedlings mycorrhizal with L. laccata had a greater %P than did seedlings mycorrhizal with C. graniforme. No differences in %P were found between the control seedlings and those mycorrhizal with C. graniforme. No differences in %P were found in the seedlings receiving the RP treatment or the DCP treatment, comparing fungal treatments.

Percent Nitrogen (%N)

The analysis of variance test on the %N data showed that the fertilizer variable and the tree species X fungi interaction were significant at the 0.05 level of probability. Percent N analysis was conducted only on the tops of the seedlings from the third sample period.

Table 9 presents the mean values for %N for the tree species X fungi interaction. Figure 9 graphically presents the mean values listed in Table 9. The results show that for Douglas-fir, no difference in %N was found between the seedlings mycorrhizal with H. crustuliniforme and those mycorrhizal with L. laccata, C. graniforme and the control. However, seedlings mycorrhizal with L. laccata and C. graniforme had a greater %N than did the control seedlings. No difference in %N was found between Douglas-fir seedlings mycorrhizal with L. laccata and C. graniforme.

For western hemlock, the seedlings mycorrhizal with H. crustuliniforme had a greater %N than did seedlings mycorrhizal with L. laccata or C. graniforme. However, no difference in %N was found between seedlings mycorrhizal with H. crustuliniforme or C. graniforme and the control.
TABLE 9. Mean values of percent nitrogen (%N) for the tree species X fungi interaction. D value = 0.098.

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Tree Species 1/</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Douglas-fir</td>
<td>Western Hemlock</td>
<td></td>
</tr>
<tr>
<td>Hebeloma</td>
<td>0.687 a</td>
<td>0.822 a</td>
<td></td>
</tr>
<tr>
<td>crustuliniforme</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laccaria</td>
<td>0.593 ab</td>
<td>0.571 b</td>
<td></td>
</tr>
<tr>
<td>laccata</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cenococcum</td>
<td>0.637 abc</td>
<td>0.666 bc</td>
<td></td>
</tr>
<tr>
<td>graniforme</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.735 ad</td>
<td>0.735 ac</td>
<td></td>
</tr>
</tbody>
</table>

1/ Values with the same letter are not statistically different at the 0.05 level of probability.

seedlings. The control hemlock seedlings had a greater %N than did the seedlings mycorrhizal with L. laccata. No difference in %N was found between seedlings mycorrhizal with L. laccata and those mycorrhizal with C. graniforme.

Table 10 presents the mean values for %N for the fertilizer treatments, averaged over tree species and fungi. Figure 10 graphically presents the mean values listed in Table 10. The results show that no difference in %N was found between High and Low-High treatment seedlings, or among Low, RP and DCP treatment seedlings. Seedlings receiving the High and Low-High treatments had a greater %N than did seedlings receiving the Low, RP and DCP treatments.
FIGURE 9. Mean values of percent nitrogen by tree species within fungal treatments at sample period 3, averaged over fertilizer treatments. H.c. = Hebeloma crustuliniforme  L.l. = Laccaria laccata  C.g. = Cenococcum graniforme  Control = Uninoculated.
TABLE 10. Mean values of percent nitrogen (%N) for the fertilizer treatments, averaged over tree species and fungi. D value = 0.082.

<table>
<thead>
<tr>
<th>Fertilizer Treatment</th>
<th>Mean Values 3/</th>
<th>%N</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>0.770 a</td>
<td>0.770</td>
</tr>
<tr>
<td>Low</td>
<td>0.639 b</td>
<td>0.639</td>
</tr>
<tr>
<td>Low-High</td>
<td>0.732 ac</td>
<td>0.732</td>
</tr>
<tr>
<td>RP 1/</td>
<td>0.621 bd</td>
<td>0.621</td>
</tr>
<tr>
<td>DCP 2/</td>
<td>0.641 bd</td>
<td>0.641</td>
</tr>
</tbody>
</table>

1/ RP = rock phosphate  
2/ DCP = dicalcium phosphate  
3/ Values with the same letter are not statistically different at the 0.05 level of probability.

Total Seedling Weight (TSW)

The analysis of variance test on the TSW data showed that the significant interactions at the 0.05 level were a) sample period X fungi, b) sample period X fertilizer and c) sample period X tree species.

Table 11 presents the mean values for TSW for the sample period X fungi interaction. Figure 11 graphically presents the mean values listed in Table 11. The results from sample period 1 show that no difference in TSW was found among fungi treatments. At sample period 2, seedlings that were mycorrhizal with L. laccata, C. graniforme and the controls had a greater TSW than did seedlings mycorrhizal with H. crustuliniforme. Seedlings that were mycorrhizal with C. graniforme and the controls had a greater TSW than did seedlings mycorrhizal with L. laccata. No difference in TSW was found between the seedlings mycorrhizal with C.
FIGURE 10. Mean values of percent nitrogen in seedling tops at sample period 3 within fertilizer treatments, averaged over tree species and fungal treatments. $H =$ High, $L =$ Low, $L-H =$ Low-High, RP = rock phosphate, DCP = dicalcium phosphate.
TABLE 11. Mean values for total seedling weight (TSW), in grams, for the sample period X fungi interaction. D value = 0.029.

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Sample Period</th>
<th>1/</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Hebeloma crustuliniforme</td>
<td>0.070 a</td>
<td>0.292 a</td>
</tr>
<tr>
<td>Laccaria laccata</td>
<td>0.076 a</td>
<td>0.414 b</td>
</tr>
<tr>
<td>Cenococcum graniforme</td>
<td>0.071 a</td>
<td>0.449 c</td>
</tr>
<tr>
<td>Control</td>
<td>0.074 a</td>
<td>0.447 c</td>
</tr>
</tbody>
</table>

1/ Values with the same letter are not statistically different at the 0.05 level of probability.

graniforme and the control seedlings. At sample period 3, the control seedlings had a greater TSW than did the seedlings mycorrhizal with the three fungi. Seedlings mycorrhizal with C. graniforme had a greater TSW than did seedlings mycorrhizal with L. laccata, which in turn had a greater TSW than did seedlings mycorrhizal with H. crustuliniforme.

Table 12 presents the mean values for TSW for the sample period X fertilizer interaction. Figure 12 graphically presents the mean values listed in Table 12. The results from sample period 1 show that TSW did not differ among the five fertilizer treatments. At sample period 2, seedlings under the High treatment had a greater TSW than did seedlings under the Low, Low-High, RP and DCP treatments. No difference in TSW was found between the Low and Low-High treatment seedlings, and between the
FIGURE 11. Mean values for total seedling weight by sample period within fungal treatments, averaged over tree species and fertilizer treatments.
TABLE 12. Mean values for total seedling weight (TSW), in grams, for the sample period X fertilizer interaction. D value = 0.035.

<table>
<thead>
<tr>
<th>Fertilizer Treatments</th>
<th>Sample Period</th>
<th>3/</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>High</td>
<td>0.074 a</td>
<td>0.547 a</td>
</tr>
<tr>
<td>Low</td>
<td>0.071 a</td>
<td>0.346 b</td>
</tr>
<tr>
<td>Low-High</td>
<td>0.072 a</td>
<td>0.345 bc</td>
</tr>
<tr>
<td>RP 1/</td>
<td>0.073 a</td>
<td>0.395 d</td>
</tr>
<tr>
<td>DCP 2/</td>
<td>0.074 a</td>
<td>0.368 bcd</td>
</tr>
</tbody>
</table>

1/ RP = rock phosphate
2/ DCP = dicalcium phosphate
3/ Values with the same letter are not statistically different at the 0.05 level of probability.

Low-High and DCP treatment seedlings. Seedlings produced with the RP treatment had a greater TSW than did seedlings produced with the Low or Low-High treatments. No difference in TSW was found between the RP and DCP treatment seedlings. At sample period 3, seedlings receiving the High fertilizer treatment had a greater TSW than did seedlings receiving the other four treatments. Seedlings produced with the RP treatment had a greater TSW than did seedlings in the Low, Low-High and DCP treatments. Seedlings receiving the Low-High and DCP treatments had a greater TSW than did the Low treatment seedlings. No difference in TSW was found between the Low-High and DCP treatment seedlings.

Table 13 presents the mean values for TSW for the sample period X tree species interaction. Figure 13 graphically presents the mean values listed in Table 13. The results simply show that TSW for both Douglas-fir
FIGURE 12. Mean values for total seedling weight by sample period within fertilizer treatments, averaged over tree species.

H = High, L = Low, L-H = Low-High, RP = rock phosphate, DCP = dicalcium phosphate.
and western hemlock seedlings increased significantly from sample period 1, to sample period 2, to sample period 3.

TABLE 13. Mean values for total seedling weight (TSW), in grams, for the sample period X tree species interaction. D value = 0.018.

<table>
<thead>
<tr>
<th>Sample Period</th>
<th>Tree Species 1/</th>
<th>Douglas-fir</th>
<th>Western Hemlock</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TSW, gm.</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>0.123 a</td>
<td>0.022 a</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.495 b</td>
<td>0.306 b</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>0.970 c</td>
<td>0.932 c</td>
</tr>
</tbody>
</table>

1/ Values with the same letter are not statistically different at the 0.05 level of probability.
FIGURE 13. Mean values for total seedling weight by tree species within sample periods, averaged over fungal and fertilizer treatments.
DISCUSSION

A general discussion of the results of this project is complicated by the variety of significant interactions that occurred among sample periods, tree species, fungal species and fertilizer treatments. This discussion will therefore be organized around the project objectives as presented in the Introduction.

Effect of P-form on Mycorrhizal Development

The treatments used to test the effect of P-form on mycorrhizal development were a) Low N-P-K, with \( \text{H}_3\text{PO}_4 \) as the P-source, b) RP, with rock phosphate as the P-source and c) DCP, with dicalcium phosphate as the P-source. Levels of N and K were the same for the three treatments.

The three P treatments had no differential effect on mycorrhizal development on Douglas-fir at sample periods 1 and 2. However, by sample period 3, both the RP and DCP treatment seedlings had a greater percentage of mycorrhizal short roots than did the Low treatment seedlings. This suggests that on Douglas-fir, mycorrhizal formation is enhanced by the use of rock phosphate or dicalcium phosphate, as compared to a low level of P in a readily available form. This appears to be fairly consistent with the findings of Bowen and Theodorou (1967).

The same trend was apparent for western hemlock only in sample period 1. By sample periods 2 and 3, no differences in percent mycorrhizal short roots were observed among the three P treatments.

The P and N nutrition and total seedling weight data are provided to supplement the mycorrhizae data. As initially expected, the %P in the
tops of both Douglas-fir and western hemlock was lower for the RP and DCP treatments as compared to the Low treatment. Even though the %P levels are lower for the RP and DCP treatments, apparently they are still above the critical level for the two tree species. This observation is supported by the TSW data, which show that the TSW of the RP and DCP treatment seedlings is greater than the TSW of the Low treatment seedlings. In addition, the RP treatment seedlings had a greater TSW than did the DCP treatment seedlings. The %N in both tree species did not differ among the Low, RP and DCP treatments.

To qualitatively summarize the results on the effects of P-form on mycorrhizal development, it appears that the RP and DCP treatments produced a greater percentage of mycorrhizal short roots than did the Low treatment with $\text{H}_3\text{PO}_4$ as the P-source. Finally, the P and N nutrition of the seedlings did not appear to have been negatively affected by the RP and DCP treatments. This is supported by the data showing that RP and DCP produced seedlings having a greater TSW than those receiving the Low Treatment.

Effect of N-P-K Rate and Timing of Application on Mycorrhizal Development

The treatments used to test the effect of N-P-K rates and timing of application on mycorrhizal development were a) High N-P-K, b) Low N-P-K and c) Low-High N-P-K. All nutrients were in a readily available form (refer to the Methods section for a quantitative description of the treatment rates).

The percent mycorrhizal infection on Douglas-fir was not different among the three treatments at sample period 1. However, for western
hemlock at sample period 1, mycorrhizal infection was greatest at the Low-High level, followed by the Low level, and then by the High level. It was expected that no differences would be found at the first sample date since the fertilizer solution had only been applied twice prior to sampling and at the time of sampling the Low level and Low-High level were receiving the same nutrient levels. Douglas-fir proved consistent with expectations. Western hemlock may perhaps be fairly sensitive to nutrient rates and the effect on mycorrhizal formation, as suggested by the lower rate of infection at the High fertility level. No explanation can be presented as to the difference in percent mycorrhizal infection between the Low and Low-High treatments. By sample period 2, infection in both tree species was suppressed by the High fertility level, although infection was still high (60% plus).

The Low-High treatment received the High fertility rates following the second sample period. Although the Low-High level had a greater rate of infection than did the High level at the third sample period, the effect of the High level on infection on hemlock was less apparent as compared to the earlier sample periods. The difference was more dramatic on Douglas-fir. The Low-High level had a greater rate of infection as compared to the Low level, which had a greater rate of infection than the High level.

No differences in %P were found among the three treatments for either tree species. This suggests that lower rates of P applied to enhance mycorrhizal formation may not have had a negative effect on the P nutrition of the seedling. No difference was found in %N in either species between
the High and Low-High levels, however, both had a greater %N than did the Low level.

Total seedling weights followed an expected trend. No differences were found at sample period 1, but by sample period 2 the High level seedlings had a greater TSW than did the other two treatments. At sample period 3, the High level seedlings had a greater TSW than did the Low-High level seedlings, which had a greater TSW than did the Low level seedlings.

In summary, the intent of the High, Low and Low-High treatments was to add supportive evidence that mycorrhizal development is inhibited by high fertility levels, and that perhaps it is possible to grow seedlings under a low fertility level to insure mycorrhizal development, then fertilize at higher rates to improve the physical stature of the seedling. The data show that mycorrhizal infection was inhibited at high fertility levels. Also, the Low-High level results suggest that perhaps a well-developed mycorrhizal root system may be developed under a low fertility level, and then maintained when higher rates of nutrients are applied. Furthermore, total seedling weight began to increase due to the higher nutrient levels. One can only speculate as to the time required for the Low-High seedlings to achieve a TSW comparable to the High level seedlings.

**Fungal Species Effect on %Mycorrhizae, %P, %N and Seedling Weight**

An additional aspect that deserves attention is that of fungal species effect on mycorrhizal infection. At sample period 1, a greater percentage of mycorrhizal short roots was formed on both tree species by
L. laccata, followed by H. crustuliniforme and finally by C. graniforme. By sample period 2, there was no difference in mycorrhizal formation on Douglas-fir by H. crustuliniforme and L. laccata, however, on hemlock L. laccata had produced more mycorrhizae than had H. crustuliniforme, followed by C. graniforme. By sample period 3, no difference in percent mycorrhizal short roots was found on either tree species between the H. crustuliniforme and L. laccata treatments, but both produced more mycorrhizal short roots than C. graniforme.

An interesting example of fungal growth rates was apparent in a comparison of the change in percent mycorrhizal short roots over time for each fungus. H. crustuliniforme and L. laccata have fairly rapid growth rates and can colonize the medium in a container rapidly (perhaps in 4 to 6 weeks). Consequently, both fungal species had high rates of infection at all sample periods. In contrast, C. graniforme has a relatively slow growth rate, suggesting that a longer period of time is needed for medium colonization. This is exemplified by the increase in percent mycorrhizae over time by C. graniforme on both tree species. If a sample had been taken at 32 weeks, the percent mycorrhizae by C. graniforme may have equaled that of the other two fungi.

An evaluation of the fungal species performance shows that seedlings mycorrhizal with L. laccata had a greater %P than did the other seedlings, however, the difference was only significant for western hemlock, and for H. crustuliniforme on Douglas-fir. A general trend shows that both tree species had a lower %N when mycorrhizal with L. laccata as compared to the uninoculated control. At sample period 3, the TSW of the control seedlings was significantly greater than any of the mycorrhizal seedlings.
Chemical vs. Positional Availability of Nutrients

It has been suggested in the past that the fungal symbiont of a mycorrhizal association improves nutrient uptake by two possible mechanisms: 1) by increasing the solubility of nutrients from less available forms and 2) by virtue of the hyphal extensibility, to increase the soil volume available for nutrient extraction. Several investigators have shown that mycorrhizal fungi produce various organic acids which may contribute to the chemical weathering of soil minerals. However, evidence to support either theory has not been conclusive.

Figure 7 presents the %P values for the fungal treatments within fertilizer treatments for Douglas-fir. The portion of the graph of particular interest is the data on the RP and DCP treatments. In the case of the RP treatment, only *L. laccata* increased P uptake as compared to the control. It is important to point out that the difference between *L. laccata* and *C. graniforme* or the controls was 0.065%, which was also the D value for testing significance. This suggests that *L. laccata* was able to utilize more P from the rock phosphate source than did the other two fungi. At the third sample period, all species of fungi had colonized the medium in the plugs. This suggests that although fungal hypae had completely colonized the medium, *H. crustuliniforme* and *C. graniforme* could not utilize the P from the rock phosphate. An assumption here is that the %P in the tops of the seedlings is an indication of the amount of P removed from the medium by the fungi. It appears then that the increase in %P that occurred in the seedlings mycorrhizal with *L. laccata* was due to an increase in chemical availability induced by *L. laccata*. 
By contrast, the DCP treatment, which had approximately 50% available P, produced strikingly different results. In this case, seedlings that were mycorrhizal with any one of the three fungi had a significantly greater V than did the control seedlings. This suggests that due to hypal extension by the mycorrhizae, the plant root-medium contact was increased. It appears then that because C. graniforme and H. crustuliniforme did not affect P uptake from rock phosphate, but did increase P uptake from dicalcium phosphate that these two fungal species increased the positional availability of P.

Implications For Future Mycorrhizal Research

This project was undertaken to collect information on how the mycorrhizal habit of Douglas-fir and western hemlock seedlings may be influenced by nutrient applications. The results suggest that some very attractive possibilities exist for the use of rock phosphate as a P-source, together with a moderate level of N and K fertility to allow for adequate mycorrhizal development. This treatment may then be followed by a higher fertility level to allow for adequate seedling development.

This suggests that additional studies testing similar treatments in both bare-root and container nurseries would be profitable. A variety of other phosphate fertilizer materials may be formulated so as to permit adequate mycorrhizal development as well as seedling growth. In light of the well developed mycorrhizal root systems on the High fertility seedlings, an interesting treatment combination may be to use rock phosphate together with a high level of N and K.
Ultimately, it will be necessary for nursery managers to decide whether to produce large, non-mycorrhizal seedlings or somewhat smaller, mycorrhizal seedlings. From the practical standpoint, if outplanting survival is a key objective, perhaps the decision should be in favor of the mycorrhizal seedlings.


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