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Scott L. Miles

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THE MYCORRHIZAL INOCULATIONS OF SEVERAL NORTHERN  
ROCKY MOUNTAIN CONIFERS; WITH SPECIAL REFERENCE  
TO INOCULATION PROBLEMS CONCERNING DOUGLAS-FIR

By

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B.A., University of Montana, 1983

Presented in partial fulfillment of the requirements  
for the degree of Master of Arts

University of Montana

1989

Approved by

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March 17, 1989  
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Miles, Scott L., Master of Arts, March 1989

Botany

The Mycorrhizal Inoculations of Several Northern Rocky Mountain  
Conifers; with Special Reference to Inoculation Problems Concerning  
Douglas-fir

Director: Dr. David E. Bilderback *DEB*

Previous inoculation attempts of Rocky Mountain Douglas-fir, Pseudotsuga menziesii (Mirb.) Franco var. glauca (Beissn.) Franco, had repeatedly failed to result in the formation of mycorrhizae on the seedling roots. Observations of the root development of the container-grown seedlings during those early studies indicated that short lateral roots developed late in the first growing season and were very limited in number. This late and limited short lateral root production was hypothesized to possibly affect the infectibility of the root system by the mycorrhizal fungi. Experiments were conducted to test the effects of cupric carbonate, 2-chloroethylphosphonic acid and various levels of nitrogen and phosphorus on short lateral root production and other seedling growth parameters. No treatment in the three experiments significantly increased the number of short lateral roots over the standard growth regime. Another experiment tested the temporal viability of Hebeloma crustuliniforme (Bull. ex Saint-Amans) Quel., a known Douglas-fir mycorrhizal associate. The viability, as tested, does not appear to overlap short lateral root production. Coastal Douglas-fir, P. menziesii var. menziesii, and Rocky Mountain Douglas-fir were grown under similar conditions and their development was compared. They differed in all growth parameters tested. Short lateral root production was approximately seven times higher in coastal Douglas-fir than in the Rocky Mountain variety. Finally, Douglas-fir and several other Rocky Mountain conifers were inoculated with spore and vegetative inocula to test for successful mycorrhizal formation. Douglas-fir did not form mycorrhizae with any fungi after either inoculation method, but mycorrhizae formed from several fungal/conifer combinations. H. crustuliniforme formed mycorrhizae with all other conifer species when applied as both spore and vegetative inocula. As spore inocula, a Lycoperdon species and Suillus grevillei (Kl.) Singer, infected ponderosa pine (Pinus ponderosa Dougl. ex Loud.) and S. tomentosus (Kauf.) Snell, Singer & Dick infected ponderosa and lodgepole pine (Pinus contorta Dougl. ex Loud. var. latifolia Engelm). In the vegetative inoculations, Laccaria laccata (Fr.) Berk. and Br. formed mycorrhizae with western larch (Larix occidentalis Nutt.), ponderosa and lodgepole pine, and L. bicolor (R. Maire) Orton infected both pine species.

### Acknowledgements

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Thanks to you all!

Scott

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Mycorrhizae are mutualistic associations between plant roots and certain fungi in which each partner in the symbiosis benefits. There are different morphological and physiological forms of mycorrhizal associations. In most, the fungus benefits by receiving sugars and other photosynthetic products from the plant. In exchange, the plant receives nutrients and water that the fungal hyphae has taken from the soil.

Many conifer tree roots have a particular association called ectomycorrhizae that is characterized by a changed appearance of the short lateral roots. Microscopic examination of these roots reveals: 1) usually, a fungal tissue layer around the outside of the outer root cells, 2) a net-like anastomosing growth of fungal hyphae among the outer few layers of cortical cells of the root, and 3) the lack of penetration of the plant cells by the fungal hyphae.

The ubiquitousness of this conifer/fungal association in nature and its known benefits to the conifer make mycorrhizal inoculation of seedlings prior to outplanting desirable to forest managers. Seedling inoculation followed by outplanting of the seedlings has been attempted in various regions of the United States with varying degrees

of inoculation success and growth enhancement. These programs have been most successful in the southeastern states, where mycorrhizal seedlings have survived and grown well on harsh sites, such as mine spoils, where nonmycorrhizal seedlings have difficulty surviving.

This study's original hypothesis was that mycorrhizal Rocky Mountain Douglas-fir, Pseudotsuga menziesii (Mirb.) Franco var. glauca (Beissn.) Franco, seedlings would survive and grow on hot, dry planting sites in western Montana better than nonmycorrhizal seedlings. The original study plan entailed growing mycorrhizal Douglas-fir seedlings and nonmycorrhizal control seedlings for one spring and summer, outplanting them in the fall, and measuring growth and survival the following spring and summer. The first summer's inoculation attempt was unsuccessful; none of the seedlings became mycorrhizal. I attributed the failure to an unfortunate choice of fungi. I started again the following year using local isolates of two fungal species that had been successfully used as inoculum with Douglas-fir from western Washington. Again, after a spring and summer growing season, no seedlings were mycorrhizal.

My observations on these failures to achieve colonization of the Douglas-fir seedlings, a review of the literature and personal communications with other mycorrhizal researchers lead me to the following generalizations:

-- successful inoculation of Douglas-fir is difficult to achieve.

-- Rocky Mountain Douglas-fir (*P. menziesii* var. *glauca*) seems more difficult to successfully inoculate than coastal Douglas-fir (*P. menziesii* (Mirb.) Franco var. *menziesii*). These varieties may have different susceptibilities to mycorrhizal colonization.

-- When grown in containers (65 ml Ray Leach growth tubes) Rocky Mountain Douglas-fir seedlings develop long, fibrous root systems. Short lateral roots, capable of colonization by mycorrhizal fungi, develop late in the first growing season and are not abundant.

From these generalizations, I developed the following questions:

1. Is the infectibility of Rocky Mountain Douglas-fir seedlings, the number of their short lateral roots and other seedling parameters affected by:
  - A. Varying the levels of nitrogen and phosphorus in the nutrient solution?
  - B. Applying a cupric carbonate treatment to the inside of the growth tubes?
  - C. Treating the seedlings with an ethylene-releasing compound in the nutrient solution?
2. Do coastal Douglas-fir and Rocky Mountain Douglas-fir differ in the number and time of formation of short



lateral roots and in other seedling parameters?

3. How long does fungal inoculum remain viable under standard greenhouse conditions?

4. Would either spore or vegetative inoculation methods result in the formation of mycorrhizae on Douglas-fir and other northern Rocky Mountain conifers?

5. Does mycorrhizal formation result in differential seedling growth?

The experimental investigations of these questions are the subject of this thesis.

The mycorrhizal symbiosis has been studied extensively during the last 50 years; a great deal of literature on the subject has accumulated. The purposes of this literature review are to: briefly discuss the classification and structure of ectomycorrhizae, discuss the biological benefits of the conifer mycorrhizal association, give a brief synopsis of its applied uses, and document the difficulties associated with Douglas-fir inoculations that have lead to this work.

CLASSIFICATION AND STRUCTURE. Mycorrhizal classification is based largely on the morphology of the plant/fungal interaction. Historically, mycorrhizal types have been divided into endomycorrhizae (or endotrophic) in which the fungus penetrates the root cell wall and ectomycorrhizae (or ectotrophic) in which the fungus does not penetrate the cell wall, but grows among the cells. This simple dichotomous classification has generally been abandoned. Researchers still use the 'ectomycorrhizal' grouping as before but now recognize several different types of endomycorrhizae in which fungal hyphae grow into the root cells

(intracellular). They also recognize an intermediate type, ectendomycorrhizae, with hyphae growing intracellularly but which has ectomycorrhizal-like structures (Mikola, 1966 and Harley and Smith, 1983). The mycorrhizae of the conifers discussed in this thesis are ectomycorrhizal.

Ectomycorrhizae are characterized by the presence of a fungal sheath and a Hartig net. The fungal tissue does not penetrate the root cell walls. The sheath, or mantle, is a matrix of fungal hyphae of varying thickness that surrounds the conifer short lateral roots and extends over the root cap. Inside the sheath and behind the areas of root cell division and elongation, fungal hyphae grow among the cells of the outer few layers of the root cortex. This anastomosing network of hyphae around the individual cortical cells is known as the Hartig net. It is thought that nutrient, water and sugar exchange takes place between the root cells and fungal tissue of the Hartig net.

A general description of ectomycorrhizal morphology is found in Harley and Smith (1983). Zak (1969 and 1971), Trappe (1967) and Molina and Trappe (1982) have given structural descriptions of mycorrhizae of coastal Douglas-fir associated with different fungi.

Though the diagnostic characteristics of ectomycorrhizae are fungal tissues, i.e. the sheath and Hartig net, the root cells and the rates of root development also are affected by the formation of the symbiosis.

Describing the amount of change in the root cells as 'great' or 'little' becomes a matter of semantics, but Harley and Smith (1983) after reviewing the literature agree with Clowes (1950, 1951, and 1981) that the basic architecture of mycorrhizal and nonmycorrhizal roots is the same. However, the lack of root hairs, the breakdown of epidermal cells and small differences in the diameters and orientation of the cortical cells are commonly observed differences in mycorrhizal roots.

Developmentally, mycorrhizal roots grow slower, resulting in compressed zones of root cell maturation behind the apex. There is also a tendency for mycorrhizal short roots to have more dichotomous branching and to live longer than noncolonized short roots (Harley and Smith, 1983).

The causes of these morphological changes are not well understood. Slankis (1950, 1958 and 1973) and Ulrich (1960) argue that fungal-produced auxins are the causative agents. This argument is based on two observations: 1) the fungi produce auxins in sufficient amounts, and 2) synthetic auxins applied to uninfected roots often cause similar changes in root structure. Harley and Smith (1983) agree to a fungal produced hormone influence on root development, but they present evidence for some genetic control by the plant. They also argue that acceptance of the primary importance of auxins may stifle research into other possible factors regulating ectomycorrhizal development.

BENEFITS TO THE PLANT AND FUNGI. The mycorrhizal symbiosis is considered to be mutually beneficial. The fungi benefit by obtaining sugars (carbohydrates) from the host plant. These sugars are the fungi's carbon source for both structural and energetic purposes (Melin and Nilsson, 1957 as cited in Harley and Smith, 1983).

The plant benefits in the relationship because the fungus seems to act as a part of, or an addition to, the plant's root system. The benefits arise because the fungal/plant absorption system, made of both mycorrhizal and nonmycorrhizal roots, is evidently more efficient than nonmycorrhizal roots alone.

The actual benefits to the plant and possible mechanisms by which these benefits arise are listed below:

1. Increased nutrient uptake. Three parameters have been used to demonstrate increased nutrient uptake by mycorrhizal plants: 1) increased nutrient concentrations in plant tissue, 2) increased total nutrients per plant, and 3) increased nutrient fluxes into mycorrhizal roots over nonmycorrhizal roots. Hatch (1937) reported significant increases in both of the first two parameters for N, P, and K in Pinus seedlings. Harley and McCready (1950), Rygiewicz and Bledsoe (1984), Rygiewicz et al. (1984a and b) and Bledsoe and Rygiewicz (1986) have shown greater fluxes of P, K, ammonium-N and nitrate-N into colonized roots than into noncolonized roots.

One important factor influencing nutrient uptake is the increased surface area provided for absorption by mycorrhizal roots when compared with nonmycorrhizal roots (Hatch, 1937). This increase in surface area is due to the longer life of mycorrhizal short roots, the increase in short root branching, the increase in short root diameter and the large amount of hyphae that emanates out into the soil. These hyphae, with a large surface area to volume ratio, are inexpensive (carbon cost to the plant) nutrient absorbing organs. Also because of their small diameter, fungal hyphae can penetrate smaller soil pores, gaining access to nutrients unavailable to the plant root (Bowen, 1973 and Parke et al. 1983).

Mycorrhizae may release compounds into the soil solution which free previously unavailable nutrients. This idea has been explored by many authors (Stone, 1950; Bowen and Theodorou, 1967 as cited in Harley and Smith, 1983; Alexander and Hardy, 1981 and Williamson and Alexander, 1975), but good experimental evidence of the importance of this phenomena is still lacking (Harley and Smith, 1983).

Mycorrhizal seedlings have some extended physiological ranges for nutrient uptake. Rygiewicz et al. (1984b) showed that mycorrhizal Douglas-fir seedlings take up nitrate-N better at high pH values. They speculate that this is correlated to higher pH optima for growth of the fungi than of noncolonized roots. Shemakhanova (1962) suggested that

fungal tissue has a higher tolerance to low osmotic potentials than does root tissue, especially young root tissue. In support of this suggestion, Mexal and Reid (1973) showed mycorrhizal fungi have differing abilities to withstand low osmotic potential in pure culture. Cenococcum graniforme grew at the lowest osmotic potential of the fungi tested. This same fungi will replace other fungal symbionts on Virginia pine (Pinus virginiana Mill.) during times of low soil water availability (Worley and Hacskaylo, 1959).

2. Increased transpiration and photosynthesis and decreased xylem resistance. Dixon et al. (1980) reported that inoculated seedlings demonstrated greater conductance values than uninoculated seedlings when both were water stressed. They also showed, as did Parke et al. (1983), that following water stress, mycorrhizal seedlings resumed normal levels of photosynthesis more rapidly following resumption of watering. Parke et al. (1983) list the possible mechanisms of these improvements as:

1. increased surface area for water absorption;
2. decreased resistance to water flow from the soil to the xylem tissues in the root;
3. the ability of hyphae to penetrate smaller soil pores than is possible for roots and root hairs; and
4. other factors related to improved nutrition of the plant.

3. Increased disease resistance. It is likely that improved general plant health, resulting from the synergistic effects of many mechanisms, is an important factor in disease resistance. However, there are other specific mechanisms, not mentioned elsewhere, that could affect disease resistance. Zak (1964) and Marx (1972) suggest five mechanisms leading to this resistance. The ectomycorrhizae may:

1. use the surplus carbohydrates exuded by the roots, reducing the availability of these carbohydrates to the pathogens;
2. provide an effective physical barrier, the sheath, to pathogen invasion;
3. secrete antibiotics which inhibit pathogen growth;
4. help support a protective microbial rhizosphere population; and
5. induce secretion of chemical inhibitors which restrict pathogen growth.

Pertinent articles, concerning increased disease resistance, dealing with Douglas-fir and fungi utilized in this study are Sylvia (1983), Sinclair et al. (1982), and Stack and Sinclair (1975).

4. Increased growth. This is probably the most commonly noted benefit attributed to the mycorrhizal condition. An example concerning afforestation (growing new forests where they had not previously existed) in Puerto



Rico, is described in a study by Vozzo and HacsKaylo (1971). Nonmycorrhizal Pinus caribaea Morelet seedlings, often did not survive, or at least grew appreciably less than those which were mycorrhizal. Many of the articles discussed in the section below document growth differences (Grossnickle and Reid, 1982; Marx et al. 1977a; Marx et al. 1979 and Marx and Artman, 1979). The increase in growth must result from one or many of the mechanisms discussed above.

APPLIED USES OF MYCORRHIZAL CONIFERS. Knowledge of the benefits of ectomycorrhizae to conifers and the potential cost effectiveness of increasing growth or survival have resulted in forest land managers attempting to induce the symbiosis on seedlings before outplanting. Programs experimenting with purposeful inoculations have been implemented throughout the United States and other parts of the world, with varying degrees of success. In this section, the applied literature of work done in the U.S. is briefly reviewed as it pertains to this research and to possible future work in the Northern Rocky Mountains. A regional, rather than historical, emphasis is presented.

In the United States, the practical use of ectomycorrhizae has been studied most extensively in the southeastern states. Workers at the USDA Institute for Mycorrhizal Research and Development in Athens, Georgia have been successful at inoculating seedlings and obtaining both

enhanced growth and survival after outplanting. Using primarily one species of fungus, Pisolithus tinctorius (Pers.) Coker and Couch, Marx and his associates have improved both of these parameters with a variety of Pinus species seedlings outplanted to many different sites throughout the southeastern states (Marx et al. 1977a; Marx and Artman, 1979; and Marx et al. 1979). In fact, Marx et al. (1977a) reported an increase of 450% in the plot volume index (PVI) of mycorrhizal sand pine (Pinus clausa var. immuginata Ward.) seedlings over nonmycorrhizal ones. PVI integrates survival and growth. The improvements were inversely related to site quality as measured by the amount of control seedling growth. It was on the poorest sites, with the lowest control seedling growth and survival, that the greatest improvements in PVI were observed with inoculated seedlings.

These reported successes using P. tinctorius prompted researchers in other regions of the country to use it and other local fungi as inocula for different conifers. Baer and Otta (1981) used P. tinctorius to increase survival of outplanted pine seedlings in South Dakota prairie soils. Grossnickle and Reid (1982) used the same fungus in an attempt to revegetate a high-elevation mine site in Colorado, but without increases in either growth or survival of three conifer species. Another fungus, Suillus granulatus (L. ex Fr.) Kuntze, isolated in Colorado,

improved growth over seedlings inoculated with P. tinctorius and Cenococcum geophilum Fr., both isolated in Georgia. The authors speculated the ineffectiveness of these two fungi may have been because they were not ecologically adapted to the growing site.

In the far west, the regional center for conifer mycorrhizal research has been in Oregon. B. Zak, James Trappe, Randy Molina, and others associated with Oregon State University (Corvallis, Oregon) and the USDA Forest Service, Pacific Northwest Forest and Range Experiment Station, have concentrated on testing for mycorrhizal formation between various conifer and fungal species (Trappe, 1967; Molina, 1979; Molina, 1980; Graham and Linderman, 1981a; Molina and Trappe, 1982 and Hung and Molina, 1986a & b). They sometimes grew the two putative symbionts together under aseptic, controlled conditions, to test for successful mycorrhizal formation (Trappe, 1967 and Molina and Trappe, 1982). In the other studies, the inoculated seedlings were grown in greenhouse containers under less controlled conditions. These latter studies showed colonization and growth are affected by different fungi. Unfortunately, these studies have not included outplanting trials.

In Washington state, Bledsoe et al. (1982) grew mycorrhizal and nonmycorrhizal, two-year old eastern Washington Douglas-fir seedlings in containers and then

planted them on dry, burned sites on the eastern slope of the Cascade Mountains. The mycorrhizal seedlings did not exhibit increased growth or survival over the nonmycorrhizal ones during the first 17 months following outplanting.

In a greenhouse study at Lewiston, Idaho, Kidd et al. (1983) of Potlatch Corporation grew three different conifer species in combination with eight fungi. Though they achieved significant increases in colonization over the seemingly airborne-caused colonization of their controls, no significant differences were observed in height, caliper, or weight. The outplanting results of this study were unavailable.

Two studies on growing mycorrhizal seedlings in Montana have been completed. In 1984, Terry Peterson, at Champion International, Plains, Montana, inoculated four species of conifers with three fungal isolates. After a growth season under regular greenhouse conditions, three of the conifers, western larch (Larix occidentalis Nutt.), ponderosa pine (Pinus ponderosa Dougl. ex Loud.) and lodgepole pine (Pinus contorta Dougl. ex Loud. var. latifolia Engelm.) were colonized; Douglas-fir was not. Growth and survival differences during the greenhouse growing season were not adequately measured on these seedlings. Also, an outplanting study was not done.

In 1978, on the University of Montana campus, Charles Loeb grew ectomycorrhizal ponderosa pine seedlings as a

senior thesis project for his undergraduate degree in forestry. He was successful in achieving colonization with three different fungal species. He reported the results of analyses of root tissue for different nutrients, but not for the effects of the mycorrhizal infections on the growth of the seedlings. Again, no outplanting was reported.

PROBLEMS ADDRESSED IN THIS THESIS. The purpose of this section is to more fully state and document the difficulties associated with the establishment of Douglas-fir mycorrhizae.

Besides my previous two years of attempts to inoculate first year, Rocky Mountain Douglas-fir seedlings, I know of only two other such endeavors. These are the Champion International and Potlatch Corporation experiments listed above. Champion used commercially produced vegetative inoculum in their experiment. The three fungal isolates were from western Oregon sources. I examined their seedlings for mycorrhizae and found, as I had with my own, that the Douglas-fir were uninfected.

In contrast, Potlatch successfully inoculated northern Idaho Douglas-fir using western Oregon fungi. The infection rates of the Douglas-fir were generally less than those for ponderosa pine and western white pine. Their Douglas-fir mycorrhizae confound possible explanations for the lack of mycorrhizal formation in Champion's and my work.

Bledsoe et al. (1982) inoculated two-year-old eastern Washington Douglas-fir and planted them on the eastern slopes of the Cascade Mountains in Washington state. They used western Oregon fungi and were successful in obtaining mycorrhizal short roots. The age of their seedlings before inoculation makes it difficult to draw many conclusions when comparing their results with Champion's, Potlatch's or my experiences.

Bledsoe (personal communication, 1986) has stated that she and her colleagues find it difficult to successfully inoculate western Washington Douglas-fir seedlings. In their physiological studies, as well as in the study described above, they often use older seedlings that have well established root systems to insure mycorrhizal formation. Graham and Linderman (1981a) demonstrated this point when they found that coastal Douglas-fir seedlings inoculated 1, 2 and 3 months after seed sowing developed more mycorrhizae than did seedlings inoculated prior to seed sowing. The greatest mycorrhizal development was on seedlings inoculated 2 months after seed sowing. Graham and Linderman (1981a) indicated that 2 months was the approximate seedling age when short lateral roots developed. They hypothesized that the temporal matching of inoculation and short lateral root development caused the maximizing of mycorrhizal development at 2 months.

These findings reaffirm my observation that only late in the first growing season do short lateral roots develop on Douglas-fir. The delay in short root formation may be more pronounced in Rocky Mountain Douglas-fir than in coastal Douglas-fir.

The typical root system development that I have observed, but not systematically analyzed, on container-grown Douglas-fir seedlings is:

- 1) the primary root emerges from the embryo and grows to the bottom of the growth tube,
- 2) on approximately 30-40% of the seedlings, this root will have one or two long lateral roots develop from it before the primary root reaches the bottom of the tube,
- 3) once the primary root reaches the bottom and stops growing, lateral roots develop off of it near its terminal end and grow down to the bottom of the tube,
- 4) more laterals come off the main root further up from the bottom. These too usually become long roots and grow to the bottom of the tube,
- 5) this process continues until the tube is quite full of long roots with only a few short laterals.

Trappe (1971) in studying the regrowth of Douglas-fir root systems after severe, purposeful pruning described similar root development for Rocky Mountain Douglas-fir and a different development, with more branching and less long lateral root growth for coastal Douglas-fir.

Throughout the various experiments conducted for this thesis, certain common procedures were performed. Those procedures will be described here and will not be repeated in the chapters devoted to the individual experiments. The Materials and Methods sections of each experimental chapter contain information on any procedures specific to that experiment. These general procedures will be discussed in the following order: isolation and maintenance of fungal cultures; growing the inoculum; seed selection, sterilization, and stratification; inoculation and seed sowing; the growth regime; harvesting and data collection; and lastly, statistical analysis.

ISOLATION AND MAINTENANCE OF FUNGAL CULTURES. Except for one commercially obtained batch of inoculum used in the Regional Varieties experiment and one fungal culture used in the Inoculations experiment, all of the utilized fungi were isolated locally by myself or Dr. R.K. Antibus. Isolation generally was accomplished by aseptically removing a piece of inner cap tissue of a young, fresh fruiting body (mushroom) and transferring this to a petri dish of solid



nutrient agar medium (Molina and Palmer, 1982). Isolation was performed in the laboratory rather than in the field, as they describe. The fungi used in these experiments are listed in the Appendix (Table 12).

A different isolation procedure was used for one fungus. This is worth noting as the method has rarely been discussed in the literature outside the publications by the original author (Fries, 1977) and because the fungus, Laccaria laccatta (Fr.) Berk and Br., is a commonly used ectomycorrhizal symbiont. At least locally, this fungus is difficult to isolate because the fruiting body tissue is usually contaminated with other microorganisms. In this procedure, the fungal spores are deposited on a select medium to which antibiotics and activated charcoal have been added. The antibiotics reduce bacterial growth; the charcoal evidently inhibits the activity of some chemical in the medium that restricts spore germination. This method was not performed quantitatively; the culture was isolated and used and there was no attempt to assess the reasons or rates of success. Positive identification of the culture and the fruiting bodies yielding the spores was made by Dr. Greg Mueller, Chicago Natural History Museum.

After isolation, all fungal cultures were grown in petri dishes on solid nutrient agar medium called MMN by Marx (1969). Each culture was transferred aseptically once a month to maintain young, actively growing fungi.

GROWING INOCULUM. The inoculum used in all experiments, except the spore inoculation experiment, is identified as vegetative inoculum and consisted of vegetative hyphae grown on a solid carrier that had been previously soaked in nutrient solution and sterilized. The solid carrier was a 20:80 mixture of peat and vermiculite (PV). Both constituents of the carrier were sieved to eliminate pieces larger than 3.35 mm and smaller than 1.0 mm. The nutrient solution was MMN without the malt extract. The nutrient solution and PV were put in canning jars, 60:100 by volume, and autoclaved. Culture starts of the various fungi were added aseptically and were grown approximately 5-6 weeks at room temperature before use. By that time the fungal hyphae had generally permeated most of the jar's volume.

SEED STERILIZATION AND STRATIFICATION. The seeds used were obtained from: the Montana State Forest Nursery, Missoula, Montana; Champion International, Timberlands Division, Bonner, Montana and Silva Seed Company, Roy, Washington. Seed batch numbers and other pertinent information are given in Appendix Table 13.

Approximately 45 days before sowing, the seeds were soaked overnight in running tap water to permit imbibition needed for stratification. They were surface sterilized for 10 minutes in 30% hydrogen peroxide (Trappe, 1961), rinsed twice in sterile, distilled water and put on trays of

autoclaved, moist perlite. The trays were covered and placed in a refrigerator at 4° C and allowed to stratify for 45 days before sowing. These procedures for seed handling generally follow guidelines developed by B. Zak as cited by Molina and Palmer (1982).

SEED SOWING AND INOCULATION. The seedlings were grown in a basic mix to which the inoculum carrier or a control carrier was added. The basic mix was a 50:50 mixture of peat and vermiculite. The control carrier was a 20:80 mixture of peat and vermiculite, the same as the inoculum carrier. The control carrier was soaked in nutrient solution to approximate the nutrient content of the inoculum carrier. Prior to mixing with the basic mix, the inoculum and control carriers were rinsed with tap water for two minutes to remove excess nutrients. The appropriate carrier was then mixed with the basic mix at a 20:80 ratio, yielding the potting mix in which the seedlings were grown. This potting mix was then 44:56 peat to vermiculite.

The potting mix was used to fill 65 ml Ray Leach Growth Tubes. On top of the mix 2 or 3 conifer seeds were added. The seeds were covered with a thin layer of crushed rock (#2 poultry grit). This rock prevents the splashing out of the seeds and the potting mix, and prohibits the growth of algal mats which inhibit water uptake by the potting mix (Tinus and McDonald, 1979).

GROWTH REGIME. The newly sown seeds were watered twice daily and kept at room temperature during germination. The seedlings were then transferred to either the University of Montana Forestry Greenhouse or the University of Montana Botany Department walk-in growth chamber located in McGill Hall. At these locations they were watered daily for 15 days with distilled water; they were then watered with the appropriate nutrient solutions. In most experiments, a solution called NPK was used. It and the other solutions used in the Nutrients experiment are described in Chapter 7 and their formulas are given in Appendix Tables 9 & 10. Another nutrient solution was used in the Inoculations experiments (Chapter X). The formula for it is given in Appendix Tables 10 & 11. Nutrient solutions were applied by hand, using a sprinkling can. The tubes were soaked to saturation with each watering.

Twenty days after sowing, the seeds were thinned to one per tube. A subjective evaluation of height, caliper and general appearance was used to choose the healthiest/largest seedling. The other seedlings were removed from the tube.

The growth regimes for the Forestry greenhouse and the growth chamber were different and will be described separately.

Due to the condition, age and style of construction of the Forestry greenhouse, the growing environment was hard to precisely control. Both temperature and light fluctuated

somewhat with external conditions. Night temperatures usually stabilized between 50° and 60° F. Day temperatures were variable, usually being close to or just above the outside temperature. The cooling fans came on around 80° F, but since the swamp cooling system was not functioning, high outside temperatures would result in similarly high inside temperatures. One afternoon, it was 96° F in the greenhouse.

At night the light environment was stable for the seedlings. Three banks of eight-foot fluorescent lights were on continuously. These provided sufficient light, 25-45 uE (microEinsteins) PHAR (photosynthetically active radiation), to prevent dormancy induction (Tinus and McDonald, 1979). Appreciably less light is needed for this purpose than for photosynthesis.

During the day these fluorescent lights, their supportive hardware, the greenhouse structure, and the uneven paint on the glass roof resulted in partial shading that caused slightly uneven lighting of the seedlings. This variation in light intensity was compensated by the continuous change of the sun angle and by rearrangement of the seedling trays approximately every two weeks. On heavy cloud cover days, 100 uE PHAR would reach all seedlings throughout the day. On cloudless days, this level was approximately 450 uE, with some seedlings being illuminated part of the time to 650 uE.

The growth chamber environment was more stable. The 24 hour light regime consisted of 18 hours of 400  $\mu$ E PHAR provided by a combination of fluorescent and incandescent lights, and 6 hours of darkness. The 'daytime' temperature was 72-78° F, the 'night' was 60-65° F.

HARVESTING AND DATA COLLECTION. The seedlings were harvested, except where noted in the individual experiments, when they were 50, 75, and 100 days old. On the respective harvest dates, ten seedlings were randomly chosen from the different treatments. The seedlings were taken to the laboratory, where the root ball and potting mix were pulled from the growth tubes. The potting mix was gently shaken and washed from the seedling roots. The roots were examined under the stereomicroscope for mycorrhizae. A limited number of short lateral roots were removed and small sections of these were examined under a compound scope. Total root length (RtL), excluding short lateral roots (less than 1 cm) was measured, and the short lateral roots (SLR) were counted. The shoot was removed from the root system and both parts were dried at 80° C for 24 hours. The two parts were then removed from the oven and weighed immediately on an analytical balance, yielding the other two measured parameters, root weight (RW) and shoot weight (SW).

STATISTICAL ANALYSES. Many of the statistical analyses for the different experiments were similar. In general, a multiple analysis of variance (MANOVA) procedure was used to test for the differences between means of groups.

The usual protocol for analysis was as follows: For most of the experiments, two-way MANOVA's were performed, testing for the effects of the unique treatments of the particular experiment and for the effects of the fungal verses control inocula. Each of the measured parameters (RtL, RW, SW, and SLR) was tested, as were three derived parameters, root-to-shoot ratio (RSR), short lateral roots per cm root length (SLR/cm) and root weight per cm root length (RW/cm).

Often the original data did not meet the assumptions upon which analyses of variance procedures are based. Sokal and Rohlf (1981) discuss these five underlying assumptions, which are: random sampling, homogeneity of variance, independence of the variance terms from the means, normality, and additivity. To test the homogeneity of variance assumption, two tests, Cochran's C and Bartlett-Box F were run. These tests also partially measure independence of the means and their variances, and the normality of the data. These last two assumptions were not measured further, probably without consequence as the MANOVA procedure readily accommodates nonideal data for these assumptions (Sokal and Rohlf, 1981 and Ott, 1984).

If Cochran's and/or Bartlett's tests indicated significant (0.05) heteroscedasticity (unequal variances) for any parameter, the data were transformed to their natural logarithms. This is a logical transformation to use as conifer seedlings, like many plants, exhibit exponential growth (Tinus and McDonald, 1979). Exponential growth may cause size differences in very young seedlings to be magnified over time, resulting in large differences in the means and variances of growth parameters at later harvests. These differences may result in a skewed distribution of the data and will often disallow the use of analysis of variance procedures.

Also, if any of the parameters showed significant (0.05) additive effects, the data were transformed. Additive effects show interactions between the factors influencing growth; analyses of variance procedures are inefficient and possibly misleading if the interactions of the two factors are large (Ott, 1984).

Transformed data were reexamined for heteroscedasticity. If the inequalities of the variances had been corrected, a MANOVA was ran on the transformed data. If the inequalities had not been corrected, the original data were analyzed with the Kruskal-Wallis nonparametric test. This method uses numerical ranking to judge the probability (significance) of observed differences in groups. It is commonly used to avoid the rigid



underlying assumptions inherent in the parametric MANOVA procedure. The reason for not using it originally in preference to a MANOVA is that it is not as accurate at discerning differences if the assumptions of the MANOVA procedure are met.

When the MANOVA tests on the original or transformed data or the Kruskal-Wallis test showed significant differences due to the treatments, simultaneous t-tests were run between the means of the various treatments. These t-tests showed which pairs of means were significantly different. The t-test error rate was adjusted to give an experimentwise alpha value of 0.05. This resulted in the individual t-test being very conservative, so that large differences between means had to exist before those means would be declared different. The interpretations of probability values for all of the statistical analyses are discussed in the individual experiments.

In several of the experiments, the fungal inoculum enhanced seedling growth over the controls. Showing and discussing a pooled mean of the fungal and control groups in those cases would be misleading. Instead both of the means or just the control group means are shown. When shown separately, a one-way analysis of variance (ANOVA) was run on the fungal and/or control groups. When the data did not meet the underlying assumptions, the same sequence of alternate analyses was made as described above for the

MANOVA. Similarly, t-tests were run to determine significantly different groups when the respective analysis of variance technique showed significant effects due to the treatment.

## Chapter IV The Effects of The Fungal Inoculations

In all of the experiments except Inoculum Viability (Chapter IX), the seedlings were divided into groups that were subject to fungal or to control inoculum. Within these groups of inoculated and control seedlings, subgroups were subject to the treatments unique to that experiment, e.g. different nutrient levels. In only one experiment, Inoculations (Chapter X), was mycorrhizal formation observed using the stereomicroscopic observations outlined in the General Procedures. The apparent lack of mycorrhizae in the other experiments is contradicted by the values of many of the measured and derived parameters. Often in those experiments the fungal inoculated groups were significantly larger than the uninoculated groups (Table 1). This growth enhancement, without apparent mycorrhizal development, will be called the 'fungal effect' throughout the thesis.

Appendix Tables 1 to 8 show separate control and fungal group means for four of the experiments. The tables within the body of the text generally include the combined means, except when a parameter showed a significant fungal effect. In those situations the control group means are given.

In the Ethephon experiment (Chapter V), the fungal

effect was less apparent than in the other experiments; only one parameter in one harvest (RtL, Harvest 2) showed a statistically significant effect. Additionally, the fungal and control groups showed nearly equal numbers of times of having higher means for the parameters in the experiment.

In the  $\text{CuCO}_3$ , Nutrients and Regional Varieties experiments (Chapters VI, VII and VIII) fungal inoculated seedling groups were significantly larger than the controls for the three basic seedling growth parameters, RtL, RW and SW, 16 of the possible 27 times in the three harvests (Table 1). The pattern of larger means associated with fungal inoculated seedlings generally was followed in the remaining

Table 1. Analyses showing significant (0.05) fungal effects

Experiment	Parameter	Harvest*
Ethephon	RtL	2
$\text{CuCO}_3$	RW	2,3
$\text{CuCO}_3$	SW	2,3
$\text{CuCO}_3$	RSR	2
$\text{CuCO}_3$	RW/cm	1
Nutrients	RtL	1,2,3
Nutrients	RW	2,3
Nutrients	SW	1,2,3
Nutrients	SLR/cm	1
Nutrients	RW/cm	1
Regional Varieties	RtL	2
Regional Varieties	RW	2
Regional Varieties	SW	1,2
Regional Varieties	SLR	1
Regional Varieties	RW/cm	1

\* 1 is the 50 day harvest, 2 the 75 day and 3 the 100 day

11 comparisons, but these comparisons were not significant at the 0.05 level as shown by MANOVAs.

This factor of a fungal effect without apparent mycorrhizal formation is biologically interesting; possible explanations will be presented in the Discussion section of the Nutrients experiment (Chapter VII). The fungal effect also required special treatment statistically and in the presentation of results. Those changes were previously discussed in the General Procedures (Chapter III).



1-naphthaleneacetic acid, a synthetic auxin, but did not get significant increases using 3-indolebutyric acid.

Ethylene seems to act as an intermediary in auxin related root development (Abeles, 1973). In testing a possible auxin-independent role for ethylene, Wilson and Field (1984) showed that the ethylene releasing compound, 2-chloroethylphosphonic acid (ethephon) increased short lateral root branching in pines in a manner similar to mycorrhizal infection and auxin application.

Graham and Linderman (1981b) used the same substance on coastal Douglas-fir and showed that certain levels of ethylene in the soil increased short lateral root production in the absence of mycorrhizae. In view of their results, a decision was made to test the effects of ethephon on the number of short lateral roots and other growth parameters of Rocky Mountain Douglas-fir.

#### Materials and Methods

Douglas-fir seeds were sown in 300 Ray Leach tubes according to the methods outlined in the General Procedures. Of these tubes, 150 were inoculated with a local isolate of Hebeloma crustuliniforme (Bull. ex St-Amans) Quel., (this fungus was used as vegetative inoculum in most of the reported experiments and will be identified as Heb 181 throughout the thesis -- see Appendix Table 11 for more information on Heb 181). The other half were subject to the

control inoculum (uninoculated). At 10 days, each group of 150 was subdivided into three subgroups of 50. Each of the three subgroups in the inoculated and uninoculated larger groups was then watered with the NPK nutrient solution. Ten days later the ethephon treatments were begun. The treatments were 0, 1, and 5 ppm 2-chloroethylphosphonic acid in the NPK nutrient solution. The 1 and 5 ppm additions were chosen to approximate two application rates used by Graham and Linderman (1981b). Unfortunately, they reported their data in respect to soil ethylene levels and gave only the range of their application rates. Interpolation was used to approximate two of their application rates.

The seedlings were watered every other day to saturation with their respective ethephon treatment in NPK for the duration of the experiment. This follows a 2-day depletion cycle of ethylene release from ethephon as reported by Graham and Linderman (1981b). At 50, 75, and 100 days, 10 seedlings were randomly selected and harvested from each treatment. The usual parameters were measured and derived at each harvest.

## Results

No mycorrhizae formed in this experiment; additionally there was only one case of a significant effect due to the fungal inoculation. Root length, root weight, and shoot weight, the three most basic parameters of seedling growth,



Table 2. Means of the measured parameters at the 50, 75 and 100 day harvests resulting from three ethephon treatments.

HARVEST days after sowing	ETHEPHON TREATMENT ppm	ROOT LENGTH cm	ROOT WEIGHT g#100	SHOOT WEIGHT g#100	SHORT LATERAL ROOTS number
50	0	30.9	1.73	3.53	1.6
50	1	34.4	1.86	3.45	1.2
50	5	29.0	1.98	3.59	1.5
-----					
75	0	57.6	3.92	7.92	5.3 a
75	1	52.4	4.53	9.11	2.2
75	5	50.7	4.34	8.35	1.9 LN
-----					
100	0	78.5 a	7.47	12.32	7.5 a
100	1	59.7 ab	7.42	14.51	2.3
100	5	55.9 b LN	8.26	13.93	2.3 LN

Within a vertical group of three means for one harvest, means not followed by a common letter are significantly different at a 0.05 level.

Groups underlined are means for the control groups uninoculated with fungi.

Groups not underlined are combined means of the fungal and control groups.

Letters under groups of three indicate the type of statistical analysis.

ORIG indicates the original data was subject to a MANOVA, LN indicates a logarithmic transformation followed by a MANOVA, and NPAR indicates the original data was subject to the nonparametric analysis. No letters indicate that statistical differences were not found.

were largely unaffected by the various levels of ethephon throughout the experiment, the lone exception being RtL which at 100 days was inhibited by the 5 ppm treatment in

Table 3. Means of the derived parameters at the 50, 75 and 100 day harvests resulting from three ethephon treatments.

HARVEST days after sowing	ETHEPHON TREATMENT ppm	ROOT-TO- SHOOT RATIO	SHORT LATERAL ROOTS per cm ROOT LENGTH (#/cm)*100	ROOT WEIGHT per cm ROOT LENGTH (g/cm)*10000
50	0	0.487	5.6	5.8 ‡
50	1	0.539	4.3	5.7 ‡
50	5	0.556	5.1	7.0 ‡ LN
75	0	0.503	10.1	8.4 b
75	1	0.505	4.4	9.6 ab
75	5	0.523	5.3	11.0 a DRIB
100	0	0.642 a	10.1 ‡	10.0 b
100	1	0.520 b	4.3 ‡	13.5 ab
100	5	0.596 ab NPAR	5.4 ‡ NPAR	17.3 a LN

Within a vertical group of three means for one harvest, means not followed by a common letter are significantly different at a 0.05 level.

‡'s indicate that the MANOVA for this group indicated significant differences exist between the means, but the more conservative t-tests did not.

Groups underlined are means for the control groups uninoculated with fungi.

Groups not underlined are combined means of the fungal and control groups.

Letters under groups of three indicate the type of statistical analysis.

DRIB indicates the original data was subject to a MANOVA, LN indicates a logarithmic transformation followed by a MANOVA, and NPAR indicates the original data was subject to the nonparametric analysis. No letters indicate that statistical differences were not found.

comparison to the control (Table 2). The four other parameters all showed significant differences at one of the three harvests (Tables 2 & 3).

Of primary interest, SLR was decreased by the 1 and 5 ppm ethephon treatments compared to the control at the 75 and 100 day harvests. Likewise, SLR/cm was less in the 1 and 5 ppm treatments than in the control. The MANOVA procedure found the differences significant in the 100 day harvest, but the more conservative t-tests found the groups to have nonsignificant differences.

Although root length (RtL) and root weight (RW) differences showed little effect of the ethephon treatments, RW/cm, a parameter derived from RtL and RW showed a decreasing trend with increasing ethephon levels. This tendency was significant in all three harvests according to the MANOVAs, but the t-tests showed differences only at the 75 and 100 day harvests. In each case the 5 ppm treatment resulted in larger RW/cm means than did the control.

RSR showed a significant difference at the third harvest, but the relative positions of the means of the three treatments changed throughout the experiment and an overall trend was not discernable.

The ethephon additions also resulted in hypertrophy (increased cell size) of the roots at some lenticels and at some root branch points.

## Discussion

Graham and Linderman (1981b) reported an increase in short lateral roots of coastal Douglas-fir at low levels,

0.02 and 0.04 ppm, of ethylene in the soil. At their next experimental level, 0.10 ppm, short lateral root numbers were equal to those found on seedlings grown with no ethephon addition. At two higher levels, short lateral root production was reduced significantly.

Assuming similar ethylene release rates (from ethephon), similar seedling reactions in the two experiments and a linear relationship between ethephon in solution and ethylene release in the potting mix, the levels of ethephon used in this experiment should have both increased (1 ppm) and decreased (5 ppm) short lateral root production. In contrast, both levels, 1 and 5 ppm, decreased the number of short lateral roots. In the 100 day harvest, SLR and SLR/cm for the control seedlings were approximately twice those for seedlings tested with 1 and 5 ppm ethephon. Graham and Linderman (1981b) reported only half that amount of decrease for their highest, 12.5 ppm, application of ethephon. The two simplest explanations for these seemingly different results are; 1) the two P. menziesii varieties, var. glauca and var. menziesii, react differently under similar situations, or 2) the experimental conditions were different and account for the discrepancies. Both explanations call for a combined comparative study.

Graham and Linderman (1981b) reported increasing root weights and decreasing shoot weights with increasing concentrations of ethephon. This contrasts to no

significant differences in either parameter in this study. Their increasing RSR with increasing ethylene contrasts to a significantly higher control RSR than that of seedlings treated with 1 ppm in this study.

There was agreement of some root responses between the two studies. Graham and Linderman (1981b) documented hypertrophy (cell enlargement) at root lenticels and root branch points. This hypertrophy increased with increasing concentrations of ethephon. The seedlings in this study also showed similar reactions that increased with increasing ethephon applications.

Also, the increase in RW/cm with increasing concentrations of ethephon, seems to correspond to observations made by Graham and Linderman. Although they do not give root length data, they report "root system stunting" with increased concentrations of ethephon. Since the root weights of their seedlings increased, this stunting must mean decreases in root length. If so, they also then demonstrated increases of root weight per unit of root length as was shown in this experiment.

In conclusion, the two ethephon additions, in this study, decreased both SLR and SLR/cm, rather than increasing them. The additions also affected several other root parameters differently than those reported by Graham and Linderman (1981b) for their study on the effects of ethephon on root systems of coastal Douglas-fir. A combined study,

with common conditions, involving the two Douglas-fir varieties is needed to confirm the divergent results.

Chapter VI        The Effects of Cupric Carbonate  
                    Treatments on the Seedlings

Introduction

The paucity of short lateral roots on Rocky Mountain Douglas-fir lead to the testing of cupric carbonate ( $\text{CuCO}_3$ ) in a procedure that originally was designed to increase the mechanical stability of outplanted container-grown seedlings by changing seedling root architecture in the containers (Burdett, 1978). After its original development, this procedure was shown to not only increase the number of short lateral roots on pine seedlings, but also to increase the percentage of short lateral roots that were mycorrhizal (McDonald, 1981 and Ruehle, 1985).

Burdett (1978) tested the effect of a  $\text{CuCO}_3$  and paint mixture applied to the inside of seedling growth tubes on container-grown seedling root morphology. He showed that lateral roots, after contact with the  $\text{CuCO}_3$ -laden paint, would stop growing and higher-order laterals roots would emerge from them. These new laterals also would contact the wall and stop growing. Burdett proposed that the retardation of long lateral growth would improve outplanted stability by increasing the number of roots which would grow horizontally from the root ball. He found that the short

laterals, inhibited in the tube, would quickly resume growth after outplanting, resulting in the root system acquiring the basic form of a natural root system, rather than the cylindrical form often associated with planted seedlings.

McDonald (1981) tested the effect of tubes treated with paint containing 50 g/l  $\text{CuCO}_3$  on ponderosa pine inoculated with different mycorrhizal fungi. In combination with Pisolithus tinctorius (Pers.) Coker and Couch, the pine showed a three-fold increase of short lateral roots. With Suillus granulatus (L. ex. Fr.) there was a doubling of the percentage of mycorrhizal short lateral roots.

Ruehle (1985) also tested the effect of 50 g/l  $\text{CuCO}_3$  in paint on the root growth and mycorrhizal infection of four southeastern pine species inoculated with Pisolithus tinctorius. The treatment effectively decreased long lateral root growth to the bottoms of the containers without affecting other seedling growth parameters. In one species, mycorrhizal infection was increased, in one it was decreased, and in the other two species the  $\text{CuCO}_3$  treatment had no effect.

Burdett and Martin (1982) tested the root pruning (growth inhibition at the painted wall) effect of  $\text{CuCO}_3$  on 10 conifer species. It was effective on many of the species at a concentration of 500 g/l, but was highly toxic to Douglas-fir at that level. A concentration of 100 g/l was not toxic to Douglas-fir, but in large (300 ml) growth tubes



the roots did not stop growing at the container wall as desired. In smaller (30 ml) containers with a 100 g/l treatment, root pruning occurred and no toxic effects were seen, but the pruned roots had low root growth capacity and few resumed growth after transplantation from the container. Further testing of Douglas-fir with different sized containers and different potting media resulted in successful root pruning, without toxic effects or low root growth capacity.

In this experiment, the effects of  $\text{CuCO}_3$  on short lateral root production and growth parameters of Rocky Mountain Douglas-fir were investigated.

#### Materials and Methods

To implement the  $\text{CuCO}_3$  treatments, cupric carbonate powder was added to a grey acrylic latex paint and the mixture was applied to the inside of the Ray Leach Growth tubes. One hundred tubes each were treated as follows:

- 1) 100 g/l  $\text{CuCO}_3$  in paint (100 g/l treatment)
- 2) 50 g/l  $\text{CuCO}_3$  in paint (50 g/l treatment)
- 3) paint only (0 g/l treatment or paint control)
- 4) no  $\text{CuCO}_3$  or paint (no paint control)

For each treatment fifty tubes were inoculated with Heb 181 and 50 were inoculated with the control inoculum. Seed sowing, the growth regime, harvesting, data collection and analysis followed as outlined in the General Procedures.

## Results

No mycorrhizae formed in this experiment. There was no statistically significant change in SLR over the no paint control by any of the paint treatments (Table 4), but the 100 g/l treatment had a significantly larger SLR than did the 0 g/l treatment at 100 days. Paint alone significantly inhibited the three basic growth parameters, RtL, RW and SW. RtL is the extreme example. At each harvest, seedlings in untreated tubes (no paint control) had significantly longer roots than those growing in all three treatments with painted tubes. Although no discernable difference existed among the 0, 50 and 100 g/l treatments, there was a trend of decreasing RtL with increasing  $\text{CuCO}_3$  concentration. At the 50 and 75 day harvests SW did not vary among the painted treatments and by the third harvest, the 100 g/l treatment resulted in a SW mean that was not significantly different than the SW mean of the no paint control. The RW means do not show such strong trends. At the 50 and 75 day harvests though, the paint alone (the paint control) decreased the average RW in comparison to the no paint control.

Among the derived parameters, no obvious trend is seen with RSR (Table 5). At the 100 day harvest, SLR/cm was significantly larger for the 50 g/l treatment than for the two controls, and the 100 g/l treatment mean was larger than the means of all other groups. Also at the 100 day harvest,

Table 4. Means of the measured parameters at the 50, 75 and 100 day harvests resulting from four CuCO<sub>3</sub> treatments.

HARVEST days after sowing	CuCO <sub>3</sub> /PAINT TREATMENT g/l	ROOT LENGTH cm	ROOT WEIGHT g#100	SHOOT WEIGHT g#100	SHORT LATERAL ROOTS number
50	No Paint	37.1 a	1.89 a	4.45 a	2.7
50	0	24.5	1.50 b	2.94	3.9
50	50	22.9	1.53 ab	3.18	4.9
50	100	19.7	1.42 b	3.03	4.1
		LN	ORIG	NPAR	
75	No Paint	54.3 a	4.21 a	9.13 a	4.9
75	0	33.9	2.58	5.82	5.7
75	50	25.3	2.76	5.41	5.0
75	100	25.6	3.13	5.87	6.3
		LN	ORIG	ORIG	
100	No Paint	65.0 a	5.20	13.50 a	6.1 ab
100	0	49.6	3.91	8.67 b	3.6 b
100	50	40.3	5.15	9.27 b	9.2 a
100	100	34.8	5.36	11.00 ab	11.5 a
		ORIG		ORIG	LN

Within a vertical group of four means for one harvest, means not followed by a common letter are significantly different at a 0.05 level.

Groups underlined are means for the control groups uninoculated with fungi.

Groups not underlined are combined means of the fungal and control groups.

Letters under groups of four indicate the type of statistical analysis.

ORIG indicates the original data was subject to a MANOVA, LN indicates a logarithmic transformation followed by a MANOVA, and NPAR indicates the original data was subject to the nonparametric analysis. No letters indicate statistical differences were not found.

Table 5. Means of derived parameters resulting from four CuCO<sub>3</sub> treatments at the 50, 75 and 100 day harvests.

HARVEST days after sowing	TREATMENT CuCO <sub>3</sub> /PAINT g/l	ROOT-TO- SHOOT RATIO	SHORT LATERAL ROOTS per cm ROOT LENGTH (#/cm)*100	ROOT WEIGHT per cm ROOT LENGTH (g/cm)*10000
50	No Paint	0.423 b	7.3	6.1
50	0	0.514 a	16.0	6.3
50	50	0.488 ab	20.6	7.3
50	100	0.468 ab	20.1	7.8
		ORIG		
75	No Paint	0.482	10.0 b	9.0
75	0	0.450	17.2 ab	8.7
75	50	0.510	19.6 ab	12.2 a
75	100	0.536	25.0 a	13.3 a
			NPAR	ORIG
100	No Paint	0.422 b	9.5	10.6
100	0	0.480 ab	6.3	9.6
100	50	0.551 a	21.2 b	13.6 a
100	100	0.492 ab	34.5 a	16.9 a
		ORIG	NPAR	LN

Within a vertical group of four means for one harvest, means not followed by a common letter are significantly different at a 0.05 level.

Groups underlined are means for the control groups uninoculated with fungi.

Groups not underlined are combined means of the fungal and control groups.

Letters under groups of four indicate the type of statistical analysis.

ORIG indicates the original data was subject to a MANOVA, LN indicates a logarithmic transformation followed by a MANOVA, and NPAR indicates the original data was subject to the nonparametric analysis. No letters indicate that statistical differences were not found.

RW/cm was larger in the 50 and 100 g/l treatments than in the two control groups.

#### Discussion

Burdett and Martin (1982) found that the root pruning effectiveness of  $\text{CuCO}_3$  paint treatments depends on species, container size, growing medium and concentration of  $\text{CuCO}_3$  in the container wall paint. In a series of tests with Douglas-fir they had to manipulate the container size, the growing medium and the  $\text{CuCO}_3$  concentration before finding a combination that effectively pruned the roots without being toxic or without lowering the root growth capacity of the seedlings. Unfortunately, their descriptions of the tests with Douglas-fir are all qualitative. Consequently, no known investigations exist that could be used in quantitative comparisons with the Rocky Mountain Douglas-fir results obtained during this experiment.

McDonald (1981) reported an increase in ponderosa pine short lateral roots with a 50 g/l treatment compared to a no paint control. A similar trend was observed in this study, however the increases were not statistically significant due to the large amount of variance among the seedlings. Consequently, the treatments do not appear to be useful methods of increasing the number of short lateral roots on container-grown Rocky Mountain Douglas-fir. In this experiment, the general trend of decreasing RtL, RW and SW

with any paint treatment indicates that the paint alone has a restrictive effect on seedling growth. Burdett and Martin (1982) found that certain experimental conditions which resulted in effective and nonharmful root pruning by  $\text{CuCO}_3$  treatments on many other conifer seedlings, were toxic to Douglas-fir seedlings. It is possible that the decreases in the basic growth parameters in Rocky Mountain Douglas-fir are early signs of a toxic reaction caused by the paint, or at the higher concentration of  $\text{CuCO}_3$ , the paint and  $\text{CuCO}_3$  in combination.

In summary, there does not seem to be justification for, and there appears to be reasons against, the use of  $\text{CuCO}_3$  treatments with Rocky Mountain Douglas-fir to induce short lateral root formation.

## Chapter VII     The Effects of Various Nutrient                     Treatments on the Seedlings

### Introduction

Fertilizing seedlings with high levels of nutrients decreases the level of mycorrhizal infection in some studies (Marx et al. 1977b; Crowley et al. 1981 and Ruehle and Wells, 1984). Marx et al. (1977b), seeking a possible mechanism for this phenomenon, found that high levels of nitrogen and phosphorus in the soil decreased the sucrose concentration in loblolly pine roots. The amount of sucrose in the roots had a high positive correlation to the level of mycorrhizal infection. Consequently, high fertility resulted in low infection levels. In the same study, fertilizing with low levels of nutrients yielded higher infection levels, but did not increase the number of short lateral roots (Marx et al. 1977b). No other known studies report the effects of fertilization on the number of short lateral roots.

There are several problems concerning the design of a study of the effects of different nutrient levels on container seedlings. Knowledge of the specific nutritional requirements of forest tree seedlings is generally poor (Tinus and McDonald, 1979). Raising seedlings for maximum

growth in small containers requires frequent replacement of the major nutrients because the volume of soil for nutrient storage is low (Brix and van den Driessche, 1974). On the other hand, care must be taken not to overfertilize. Many researchers (Brix and van den Driessche, 1974; Ingestad, 1979 and Van den Burg, 1971, as reported by Ingestad, 1979) have stressed the importance of balancing the relative amounts of the major nutrients for maximum growth. They often do not present the absolute amounts of nutrients needed for maximum growth or the minimum for 'average' growth. Also, their recommendations are often contradictory, largely leaving the choice of both absolute and relative amounts of specific nutrients to the designers of specific experiments. For this study, high and low levels of nitrogen and phosphorus were chosen according to information found in the literature just mentioned (Brix and van den Driessche, 1974; Ingestad, 1979 and Van den Burg, 1971, as reported by Ingestad, 1979). These high and low levels of nitrogen and phosphorus were tested in all possible combinations to determine their effects on the numbers of short lateral roots and other growth parameters of Rocky Mountain Douglas-fir.

#### Materials and Methods

Five hundred growth tubes were sown with Rocky Mountain Douglas-fir seeds, after 250 tubes had been inoculated with



Heb 181 and the other 250 treated to be uninoculated controls. Following germination and two weeks of watering with distilled water, five fertilization regimes were implemented on subgroups of 50 seedlings within both the inoculated and uninoculated groups. Fertilization took place with each watering throughout the experiment. The varying levels of nutrients and the names of the treatments are given in Table 6. The K in the treatment names is used to help the reader distinguish between upper and lower case letters and to indicate that the levels of all other nutrients besides nitrogen and phosphorus were held constant.

Table 6. Nitrogen and phosphorus amounts, plus the names for the various nutrient treatments.

Nitrogen (ppm)	Phosphorus (ppm)	Treatment Name*
70	16	NPK
70	2	NpK
15	16	nPK
15	2	npK
distilled water		dH2O

\* N and P indicate high levels of the nitrogen and phosphorus; n and p indicate low levels.

The other macronutrients were provided in each solution at the following levels: Potassium - 50 ppm; Calcium - 10 ppm; Sulfur - 13 ppm; Magnesium - 10 ppm. The chemical sources of these nutrients, their mixing levels and similar

information for the micronutrients are shown in Appendix Tables 9 & 10. The seedlings were grown in the Forestry greenhouse until harvests at 50, 75 and 100 days.

## Results

As discussed in Chapter IV, the fungal inoculum, despite the lack of apparent mycorrhizal formation, generally enhanced the growth of the seedlings. This enhancement was especially evident in this experiment. Consequently, the Results and Discussion sections will each be divided into two sections: the first generally dealing with the effects of the nutrient treatments and the second with this 'fungal effect'.

Nutrient Treatments Table 7 presents the means of the measured and derived parameters for the 100 day harvest with each mean representing a combined mean of the fungal and control groups within that particular nutrient treatment for that parameter. Table 8 gives separate 100 day harvest means for the fungal and control groups within the nutrient treatments for RtL, RW and SW. The statistical significance of the groupings indicating differences between means of the various treatments in Table 7 were obtained by a MANOVA and subsequent t-tests on all possible pairs of the combined means. Likewise the groupings in Table 8 were determined by one-way analyses of variance and subsequent t-tests on the

means of the separate fungal and control groups. Similar patterns of differences among the groups are evident.

SLR was significantly affected by the nutrient treatments, with low levels of nitrogen (nPK and npK) resulting in fewer short lateral roots than the two treatments with high nitrogen (Table 7).

The nutrient treatments also affected the three basic growth parameters, RtL, RW and SW. In general, high nitrogen and/or high phosphorus resulted in larger means for all three parameters. Similar relationships occur for the means of the separate fungal and control groups (Table 8).

Fungal Effect At 100 days, each inoculated group mean for the three basic growth parameters exceeded the uninoculated control mean for each treatment (Table 8). For these parameters, a MANOVA indicated that this fungal effect on seedling growth was significant. This fungal effect was most pronounced with nutrient treatments containing high nitrogen and/or high phosphorus. The relative size of this fungal effect for the various nutrient treatments in relation to its size for distilled water is more easily seen in Figure 1. For all treatments with high nitrogen and/or high phosphorus as well as the npK treatment for SW, the fungus enhanced seedling growth over the effects of the fungus in the distilled water treatment. The fungal-nutrient enhancement is most pronounced on SW, with the NpK

Table 7. Means of the measured and derived parameters at the 100 day harvest resulting from five nutrient treatments.

Means of Measured Parameters					
HARVEST days after sowing	NUTRIENT TREATMENT	ROOT LENGTH cm	ROOT WEIGHT g#100	SHOOT WEIGHT g#100	SHORT LATERAL ROOTS number
100	NPK	65.0 a	6.80 a	15.91 a	6.1 ab
100	NpK	72.5 a	6.33 a	14.02 ab	11.1 a
100	nPK	66.4 a	6.01 a	11.87 b	3.1 c
100	npK	38.1	3.16	7.73 c	2.8 c
100	dH2O	40.8	4.57 b	5.34	3.7 bc
		LN	NPAR	LN	LN

Means of Derived Parameters				
HARVEST days after sowing	NUTRIENT TREATMENT	ROOT-TO- SHOOT RATIO	SHORT LATERAL ROOTS per cm ROOT LENGTH (#/cm)#100	ROOT WEIGHT per cm ROOT LENGTH (g/cm)#10000
100	NPK	0.422 bc	9.5 ab	10.6
100	NpK	0.474 bc	13.2 a	9.0
100	nPK	0.535 b	5.5 b	10.6
100	npK	0.418 c	6.7 ab	8.4
100	dH2O	0.856 a	9.5 ab	12.5
		NPAR	NPAR	

Within a vertical group of five means, means not followed by a common letter are significantly different at a 0.05 level.

Letters under groups of five indicate the type of statistical analysis.

ORIG indicates the original data was subject to a MANOVA, LN indicates a logarithmic transformation followed by a MANOVA, and NPAR indicates the original data was subject to the nonparametric analysis.

No letters indicate statistical differences were not found.

Table 8. Fungal and control group means for root length, root weight and shoot weight at the 100 day harvest resulting from five nutrient treatments.

NUTRIENT TREATMENT formula	ROOT LENGTH		ROOT WEIGHT		SHOOT WEIGHT	
	cm fungus	cm control	g#100 fungus	g#100 control	g#100 fungus	g#100 control
NPK	76.1 a	53.9 ab	8.41 a	5.20 a	18.32 a	13.50 a
NpK	83.7 a	61.3 a	7.87 a	4.80 a	18.20 a	9.84 ab
nPK	84.8 a	48.0 ab	7.33 a	4.70 ab	14.88 a	8.87 bc
npK	39.1	37.0 ab	3.47	2.85 b	9.30 b	6.15 cd
dH2O	45.4	36.2 b	5.26	3.88 ab	6.05	4.63 d
	LN	LN	ORIG	LN	LN	LN

Within a vertical group of five means for one harvest, means not followed by a common letter are significantly different at a 0.05 level.

Letters under groups of five indicate the type of statistical analysis.

ORIG indicates the original data was subject to a MANOVA, LN indicates a logarithmic transformation followed by a MANOVA, and NPAR indicates the original data was subject to the nonparametric analysis.

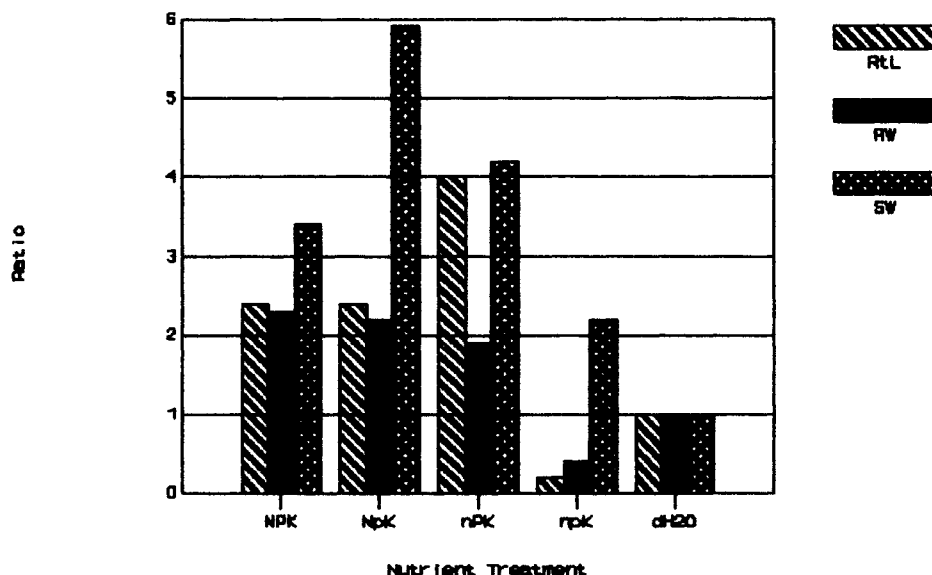
fungal effect being almost six times that of the fungal effect in the distilled water treatment.

## Discussion

Nutrient Treatments With no observed mycorrhizal development, the differences in fertilization regimes did not overcome the conditions restricting mycorrhizal infection.

Only one known study has reported the effect of differing nutrient regimes on the number of short lateral roots of container-grown seedlings (Marx et al. 1977). They

Figure 1. The relative effects, at 100 days, of the fungal inoculum as a ratio of the fungal effects for the various nutrient treatments in comparison to the fungal effect for distilled water for the three primary growth parameters - root length, root weight and shoot weight. The ratios are the quotients of the fungal effect for the parameter and nutrient treatment divided by the fungal effect for distilled water for that parameter.



found no short lateral root differences resulting from ten fertilization treatments. However, the low nitrogen treatments (nPK and npK) in this study both resulted in lower SLR means than did the high nitrogen (NPK and Npk) treatments. The distilled water treatment resulted in an intermediate SLR mean. The highest SLR value was in the Npk treated seedlings, specifically those subject to the fungal

inoculum (fungal group mean = 16.1; control mean = 6 and combined mean = 11.1). These figures are anomalous, as no other set of fungal/control means for any nutrient treatment, in any of the three harvests indicate this trend. Due to this anomaly, their value is questionable.

The differences in the three growth parameters due to the nutrient treatments are not unexpected. In general, higher nutrient treatments resulted in larger parameter means. Comparisons with literature values are difficult due to the lack of closely similar research. The container size, species tested, application method, parameters measured, and the presentation of nutrient levels in relative versus absolute amounts, are all variables that make direct comparisons difficult, if not impossible.

Despite these problems, one interesting result is the similarity of the means of the nPK and NPK groups, for RtL and RW. The lack of balance between the nutrients (relative amounts) and the seemingly low amount of nitrogen in nPK (15 ppm) in comparison to NPK (70 ppm) make this similarity surprising (Brix and van den Driessche, 1974 and Ingestad, 1979). Possibly, 15 ppm nitrogen is not restrictively low.

Fungal Effect The fungal effect on seedling growth is evident in the Ethephon,  $\text{CuCO}_3$ , Regional Varieties and the Nutrients experiments. Although not always statistically significant, it is discernable in all experiments by the

third harvest. The effect is often large and seems to be enhanced in this experiment by the presence of any combination of high nitrogen and/or phosphorus (Figure 1). In the Regional Varieties study (Chapter VIII) both fungal inoculations, Heb 181 and SS166, exerted fungal effects on both coastal Douglas-fir and Rocky Mountain Douglas-fir. The resulting group means were not statistically significant as often as in this experiment (Nutrients), but the means generally showed the same trends.

Possible explanations for this growth enhancement by the fungal inoculations will be divided into two main groups, physical and biological, and discussed separately.

#### Physical

Seedling tubes inoculated with fungi may have been treated differently. This seems highly unlikely because once in the greenhouse, the seedlings were handled very little, except during the periodic randomization of the growth trays on the greenhouse bench.

The inoculation procedure may have resulted in a nutritional boost for the fungal-inoculated seedlings. Both types of inocula were rinsed with water to remove excess nutrients before the inoculum was placed in the growth tubes, but this process could have removed different amounts of nutrients from the two types of inocula. With fungal hyphae having colonized most of the peat/vermiculite and



absorbed nutrients that had originally been placed in the jars, it is probable that those nutrients were not removed by rinsing and consequently were transferred to the growth tubes. In comparison, the control inoculum was peat/vermiculite soaked in the same original nutrient solution, but without the fungi. No 'trapping' of the inorganic nutrients would occur, except for binding to the peat/vermiculite surfaces, so nutrient transfers into the growth tubes should have been minimal after the rinsing. Consequently, the amount of nutrient transfer probably was quite different between the two types of inoculation. This explanation, on first thought, is appealing, but the increased growth of the well fertilized seedlings over the poorly fertilized ones makes this idea seem unlikely. Also, the amount of the possible nutrients added with the fungi is small when compared to those added by the fertilization regimes throughout the experiments.

#### Biological

The fungi in the inoculum may have survived in the growth tubes as free-living hyphae on the root surfaces or in the potting mix. Some relatively old papers (Burges, 1936 and Levisohn, 1956) contain reports of growth enhancements without apparent mycorrhizal formation on seedlings in bare root nursery beds. Also, Stack and Sinclair (1975) reported a protective effect towards coastal

Douglas-fir against a root rotting fungi (Fusarium oxysporum Schlect.) in certain inoculation situations by the ectomycorrhizal fungus Laccaria laccata (Scop. ex Fr.) Berk. and Br., without mycorrhizal formation.

Concerning the growth enhancement without mycorrhizal formation Levisohn (1956) proposed that the fungi were free-living and were freeing bound nutrients from organic matter. Since the seedlings in this experiment were fertilized with each watering, the regular influx of a balanced nutrient supply makes Levisohn's proposal seem unlikely in this case. Also, peat/vermiculite potting mixes are commonly used in research and greenhouse operations; the literature does not caution against nutrient binding which could restrict plant growth, so it is unlikely to be a factor.

Stack and Sinclair (1975) briefly discuss several mycorrhizal fungus/pathogen interactions as possible causes for the protection given Douglas-fir by the apparently free-living mycorrhizal fungus against Fusarium oxysporum. As there was no evidence of pathogenic root problems throughout the experiments in this thesis, their possible explanations are generally not applicable. The exception is that they mention the chance that mycorrhizal fungi may effect rhizosphere populations of other organisms. Since interactions between the multitude of organisms in the region of root surfaces are poorly understood (Harley and Smith, 1983), it is possible that free-living mycorrhizal

fungi may impart a beneficial effect directly on the plant or on other rhizosphere organisms and consequently, on the plant. The current literature though, does not yield much evidence for this, except that in the few cases mentioned it is a possible, but unexplored, explanation for the documented benefits.

Another possible explanation for the fungal effect is that an undetected mycorrhizal infection could have been present. Infections not having sheath hyphae and which did not restrict root hair formation could have possibly gone unnoticed. In the first two years of examining Douglas-fir root systems, I periodically embedded in plastic, pieces of short lateral roots, cut thin cross sections and examined them under a compound microscope for Hartig nets, typical of ectomycorrhizae. These procedures confirmed the lack of mycorrhizal infection on Douglas-fir roots that appeared nonmycorrhizal using stereoscopic examinations. Consequently, during the experiments reported in this thesis, I used stereomicroscopic examinations and occasionally squashed and stained small sections of short lateral roots and examined them under a compound microscope. No embedding and sectioning was done though. With these techniques it is possible, but doubtful, that infections without sheaths and with only very light Hartig nets could have continually escaped detection.

Two types of mycorrhizae exist that could match this description. The first is simply ectomycorrhizae without the usual sheath. In these, a functional Hartig net is present, but the sheath or mantle is lacking or greatly reduced. Bogar and Smith (1975) and Laiho (1966) both reported roots of coastal Douglas-fir seedlings as matching this description. The second type of mycorrhizae possibly involved is the so-called ectendomycorrhizae, in which both ecto- and endomycorrhizal structures are seen. A sheath is usually not found in these, but a Hartig net is. Similar to endomycorrhizae, intracellular penetration by the hyphae is characteristic and root hair suppression usually does not occur.

Ectendomycorrhizae are often found on nursery seedlings of members of the Pinaceae (Laiho, 1966 and Harley and Smith, 1983). Their occurrence on Douglas-fir seedlings is not known to have been reported. Also, despite some identification problems, the fungi involved in conifer ectendomycorrhizae seem to be ascomycetes (Harley and Smith, 1983) rather than basidiomycetes, the taxonomic group to which Hebeloma crustuliniforme belongs.

Given the possibility that one of the two types of mentioned mycorrhizae could have gone undetected, this explanation does have one major detraction. Following the conventional wisdom that mycorrhizae are most beneficial to their hosts in situations of low to moderate, but not

severely deficient, nutrient availability (Hatch, 1937; Bjorkman, 1942 - as reported in Harley and Smith, 1983), then the largest growth enhancements should have resulted from the lower nutrient treatments in this experiment. The evidence is against this suggestion, as the fungal effect for the npK treatments (low nutrient availability) is small in comparison to the distilled water treatment (severely deficient in nutrients) and to the NPK, NpK and nPK treatments (high nutrient availability in nitrogen, phosphorus or both).

Overall, the explanation of an undetected mycorrhizal infection for the fungal effect seems at least possible, but improbable as 1) the examination techniques were probably adequate and 2) the relative size of the fungal effects for the various nutrient treatments are different than is usually expected with functioning mycorrhizal associations.

Since the explanations discussed are improbable or not specific (an influence on other rhizosphere populations) the ultimate explanation of the growth enhancement by the fungi must be subject to further testing.

Chapter VIII      Comparisons of Seedlings of  
Coastal and Rocky Mountain  
Douglas-fir

Introduction

Two geographic varieties of Douglas-fir, Pseudotsuga menziesii (Mirb.) Franco var. menziesii and P. menziesii var. glauca (Beissn.) Franco, are recognized in North America (Hitchcock et al. 1969). Coastal Douglas-fir (var. menziesii) is faster growing, longer lived, and usually taller, with a height of over 300 feet for some mature individuals. Rocky Mountain Douglas-fir (var. glauca) is slower growing, shorter lived and rarely exceeds a height of 130 feet (Owston and Stein, 1972).

Purposeful mycorrhizal inoculations of coastal Douglas-fir (cDf) have been performed in a multitude of studies (see documentation throughout this thesis), but inoculations of first year Rocky Mountain Douglas-fir (RMDf) seedlings are limited to two known instances (Kidd et al. 1983 and Peterson, personal communication, 1984).

Wright and Ching (1962) included one group of RMDf seedlings while investigating the amounts of natural mycorrhizal formation in a western Oregon nursery seedbed on Douglas-fir seedlings from different seed sources. Other

comparative studies concerning the mycorrhizal associations of the two varieties are unknown.

The need to match specific fungi with specific conifers for the best outplanting results has been demonstrated (Grossnickle and Reid, 1982). Also, differing infection rates on seedlings of the same seed source have been demonstrated when different isolates of one fungal species were used as inocula (Molina, 1979).

A few comparative studies of factors other than the mycorrhizal associations of seedlings of the two Douglas-fir varieties have been performed. Sorenson and Ferrell (1973) compared photosynthetic rates and growth under two temperature regimes for cDf and RMDf seedlings. Other physiological differences of the two varieties were studied by Krueger and Ferrell (1965), Pharis and Ferrell (1966) and Zavitkovski and Ferrell (1968).

The purposes of the present experiment were to: 1) compare the differences in mycorrhizal infection of cDf and RMDf after inoculation with western Oregon and western Montana isolates of Hebeloma crustuliniforme and 2) compare the differences in the number of short lateral roots and other parameters between the two varieties.

#### Materials and Methods

Three groups of 100 tubes each were inoculated with Heb 181 (Western Montana isolate), SS166 (commercially obtained

inoculum of a Western Oregon isolate of H. crustuliniforme - see the appendix for more information on this inoculum) or the nonfungal control inoculum before seed sowing. Seeds for 50 cDf and 50 RMDf were sown into each group of 100 tubes. The seedlings were raised in the Forestry greenhouse until harvests at 50, 75 and 100 days.

## Results

No mycorrhizae formed in any of the seedling/fungal crosses, but the two varieties showed many differences in the measured and derived parameters (Tables 9 & 10). At the 75 and 100 day harvests, the RMDf and cDf means for each of the parameters were significantly different.

Coastal Douglas-fir had significantly more short lateral roots than RMDf at all three harvests (Table 9). The mean SLR of cDf and RMDf at 50 and 100 days were 8.7 and 2.4, and 50.6 and 7.7, respectively. SLR/cm reflected these SLR figures and was significantly higher for cDf than RMDf at all three harvests.

For cDf the means for RtL and SW were over twice as large as those for RMDf at the third harvest and the RW difference was nearly as large. However, the RMDf seedlings had larger RSR at all three harvests and their roots were heavier per unit root length (RW/cm) in the 75 and 100 day harvests (Table 10).



Table 9. Means of the measured parameters at the 50, 75 and 100 day harvests of coastal and Rocky Mountain Douglas-fir seedlings.

HARVEST days after sowing	DOUGLAS-FIR VARIETY	ROOT LENGTH cm	ROOT WEIGHT g#100	SHOOT WEIGHT g#100	SHORT LATERAL ROOTS number
50	<u>cDf</u>	31.7	1.81	4.63	6.2
50	RMDf	30.1	1.71	4.10	1.4 LN
75	<u>cDf</u>	79.0	5.19	13.82	26.4
75	RMDf	29.0 LN	3.38 ORIG	7.11 ORIG	3.2 LN
100	<u>cDf</u>	135.7	13.93	32.30	50.6
100	RMDf	56.3 LN	7.61 NPAR	15.17 ORIG	7.7 LN

Groups underlined are means for the control groups uninoculated with fungi.

Groups not underlined are combined means of the two fungal and one control groups.

Letters under groups of two indicate that the two means are statistically different at the 0.05 level and also, the type of statistical analysis performed on the groups. ORIG indicates the original data was subject to a MANOVA, LN indicates a logarithmic transformation followed by a MANOVA, and NPAR indicates the original data was subject to the nonparametric analysis.

## Discussion

The lack of mycorrhizal formation between Rocky Mountain Douglas-fir and the western Oregon isolate of Hebeloma crustuliniforme contrasts with the successful inoculation of RMDf (northern Idaho) seedlings by Kidd et al. (1983) using this same fungal species from a western

Table 10. Means of the derived parameters at the 50, 75 and 100 day harvests of coastal and Rocky Mountain Douglas-fir seedlings.

HARVEST days after sowing	DOUGLAS-FIR VARIETY	ROOT-TO- SHOOT RATIO	SHORT LATERAL ROOTS per cm ROOT LENGTH (#/cm)*100	ROOT WEIGHT per cm ROOT LENGTH (g/cm)*10000
<u>50</u>	<u>cDf</u>	<u>0.369</u>	<u>30.7</u>	<u>4.6</u>
<u>50</u>	<u>RMDf</u>	<u>0.408</u>	<u>8.5</u>	<u>6.0</u>
		NPf	NPf	
<u>75</u>	<u>cDf</u>	<u>0.422</u>	<u>33.3</u>	<u>7.6</u>
<u>75</u>	<u>RMDf</u>	<u>0.465</u>	<u>8.3</u>	<u>11.4</u>
		NPf	NPf	ORIG
<u>100</u>	<u>cDf</u>	<u>0.439</u>	<u>38.8</u>	<u>10.9</u>
<u>100</u>	<u>RMDf</u>	<u>0.515</u>	<u>14.0</u>	<u>14.4</u>
		NPf	ORIG	ORIG

Groups underlined are means for the control groups uninoculated with fungi.

Groups not underlined are combined means of the two fungal and one control groups.

Letters under groups of two indicate that the two means are statistically different at the 0.05 level and also, the type of statistical analysis performed on the groups. ORIG indicates the original data was subject to a MANOVA, LN indicates a logarithmic transformation followed by a MANOVA, and NPA indicates the original data was subject to the nonparametric analysis.

Oregon source. Also, the lack of infection between the cDf and the western Oregon fungal isolate contrasts with Hung and Molina (1986b). The differences between the results of this study and those just mentioned are interesting; they may indicate an inhibitive influence on mycorrhizal formation of the growth regime in the Forestry greenhouse during the experiments conducted for this thesis.

In this experiment, the difference in short lateral root production at the three harvests indicates a probable increased infectibility of cDf over RMDf. In contrast, Bledsoe (personal communication, 1986) reported that coastal Douglas-fir is relatively difficult to successfully inoculate in comparison to other conifers. The difficulties with both varieties, may be related to their low short lateral root production; in another experiment in this thesis (Chapter X), lodgepole and ponderosa pine at times had greater than 500 short lateral roots per seedling at 100 days.

Sorenson and Ferrell (1973) showed that coastal Douglas-fir had a significantly higher dry weight than Rocky Mountain Douglas-fir when both were grown under 'warm' temperatures (36° C day, 21° C night). Their 'cool' temperatures (18° C day, 4° C night) resulted in seedlings with similar dry weights. The Forestry greenhouse conditions more closely matched their 'warm' temperatures, and similar to their results (Sorenson and Ferrell, 1973), the dry weights were significantly different with the coastal Douglas-fir being considerably higher than the RMDf.

RMDf seedlings had slightly higher RSR than the cDf seedlings throughout the study, and the RMDf roots were heavier per centimeter length.

In summary, the Rocky Mountain seedlings had smaller shoots and root systems, but allocated a larger percentage

of their carbon resources to the root system than did the coastal seedlings. Little of this allocation was directed toward short lateral roots, but instead it went toward relatively heavy long roots.

Chapter IX                    The Inoculum Viability of  
Hebeloma crustuliniforme,  
a Douglas-fir Symbiont

Introduction

In succeeding chapters of Methods and Principles of Mycorrhizal Research, Marx and Kenney (1982) and Riffle and Maronek (1982) emphasize many factors and problems influencing successful ectomycorrhizal inoculations. Inoculum type, age and density, inoculation timing and method, growth regimes and a variety of interactions among the plant, fungus and other organisms, all may contribute to the success or failure of any inoculation.

Certainly possible reasons for the consistent failures of attempts to inoculate RMDf could be that the vegetative inoculum loses its viability before short lateral root production or the inoculum is for some reason not vigorous enough to penetrate the seedling roots. The viability of Heb 181 (one of the two fungi unsuccessfully used in the 1985 greenhouse inoculations and in 1986 for the experiments reported here), was demonstrated in the winter of 1985/1986 when it formed mycorrhizae with lodgepole pine grown in a growth chamber experiment. However, that test did not indicate its vigor with other species, nor did it indicate

the length of time it remained viable after inoculation.

A minimum of 4 to 6 weeks of inoculum survival is needed between inoculation and short lateral root development when inoculation is performed before seed sowing (Marx and Kenney, 1982). Graham and Linderman (1981a) demonstrated that coastal Douglas-fir developed more mycorrhizae when inoculated 1, 2 or 3 months after seed sowing, than when inoculated at seed sowing. The possibility exists that with RMDf, Heb 181 totally loses its viability before short lateral roots develop.

In this experiment, lodgepole pine was used as a bioassay of the temporal viability of the Heb 181 inoculum. The formation of mycorrhizae between the two, after transplanting of lodgepole seedlings into inoculated RMDf potting mix, was used as an indication of this viability.

#### Materials and Methods

One hundred tubes were inoculated with Heb 181 and sown with RMDf. Beginning at seed sowing, every ten days the RMDf seeds or seedlings were removed from 10 tubes and 30 day old lodgepole pine seedlings were then transplanted into the inoculated potting mix. These seedlings were grown under the standard growth chamber regime for fifty days before harvest and examination. Rt1, SLR and infected SLR were measured or counted; SLR/cm and percent infection were derived.

## Results

The lodgepole pine seedlings formed mycorrhizae only when transplanted into the Heb 181 inoculated potting mix at 0, 10 and 20 days (Table 11). The first two transplants, 0 and 10 days, resulted in 100% of the seedlings developing mycorrhizae. At 20 days, only 60% of the seedlings were mycorrhizal. Similarly, 34% and 39% of all of the short lateral roots were infected on seedlings of the 0 and 10 day transplants, while only 12% were infected when transplanting occurred at 20 days. RtL and SLR of the seedlings transplanted at 10 days were generally larger than for the 0 and 20 day transplants, but SLR/cm did not significantly change in the three transplanting events.

Table 11. Inoculum viability of Heb 181 as shown by the percent of lodgepole pine seedlings infected and percent of short lateral roots infected after transplanting the lodgepole into successively older inocula. The first transplant was at the time of sowing of Douglas-fir; subsequent transplants were performed every 10 days.

Transplant: days after inoculation	Seedlings surviving the transplant	Percent of seedlings infected	Percent of SLR infected	Short lateral roots	Root length cm	SLR/cm
0	6	100	34	40.0	72.8	0.60
10	6	100	39	62.1	107.8	0.57
20	10	60	12	38.7	84.5	0.48
30	10	0	0	‡	‡	‡

‡ Parameters not measured

## Discussion

By the 20 day transplant, the vigor or aggressiveness, of the Heb 181 inoculum, as shown by the percent of seedlings with mycorrhizae and by the percent of infected short lateral roots had significantly dropped. By the 30 day transplant (approximately 4 weeks) the viability of the inoculum, as tested, was completely lost. Marx and Kenney (1982) stated that inoculum must remain viable 4 to 6 weeks to be effective due to the time required for short lateral root production. Graham and Linderman (1981a) indicated short lateral root production occurs at approximately 2 months for coastal Douglas-fir. They demonstrated the link between the timing of short lateral root production and mycorrhizal infection, when seedlings inoculated at 2 months developed more mycorrhizae than those inoculated at sowing, 1 or 3 months.

Data from other parts of this thesis (Regional Varieties, Nutrients, Ethephon and  $\text{CuCO}_3$  experiments) as well as untold numbers of personal observations suggest that the average container-grown RMDf seedling at 4 weeks lacks roots destined to be short laterals. At two and three months the number of short lateral roots is still very limited (Figure 2).

The 30 day loss of Heb 181 viability and the late formation of short lateral roots on RMDf, seem to provide at least a partial explanation for the continual failure of



mycorrhizal formation during previous inoculation attempts. Many other factors such as inoculation method, host/fungus incompatibility, and other biological interactions also may be contributing factors. Further testing may reveal the importance of these factors on mycorrhizal formation.

Chapter X                    The Inoculations of Several  
Northern Rocky Mountain Conifer Species  
using Regional Fungi

Introduction

A wide variety of specific procedures for purposeful seedling inoculations have been developed (Molina and Palmer, 1982; Marx and Kenney, 1982 and Riffle and Maronek, 1982). The choice of a procedure by a researcher or nurseryman depends on many factors, such as the seedling use (research or reforestation), economics, and the facilities available. These inoculation procedures can be grouped into two main types: those using fungal spores and those using vegetative hyphae. Within these groups are the variety of specific procedures, each of which require the exposure of sufficient quantities of viable spores or hyphae to the roots of the growing seedlings.

As discussed elsewhere (Chapter IX), many other factors besides the placement of viable inocula near the root will determine success at obtaining infection.

Thorough discussions of the collection, production, use and resulting effects of spore and vegetative inoculum can be found in Methods and Principles of Mycorrhizal Research (N.C. Schenck, ed., 1982) by the three pairs of authors

cited above. Two procedures, one utilizing spores and one vegetative hyphae, were chosen to investigate mycorrhizal formation between regional conifer seedlings and fungi. These investigations will be discussed separately below. Unfortunately, due to the timing of the experiments and to the difficulties in obtaining spores and fungal cultures, none of the sources of inoculum in the two experiments were the same. Two fungal species, Hebeloma crustuliniforme and Suillus tomentosus, were used in both experiments, but in each case the fungi were different isolates.

### Spore Inoculations

#### Materials and Methods

Seeds of Douglas-fir, ponderosa pine and lodgepole pine were germinated and grown in the growth chamber according to the methods outlined in the General Procedures.

The spores were collected using a variety of methods. For three Suillus species, S. lakei (Murr.) Smith and Thiers, S. grevillei (Kl.) Singer, and S. tomentosus (Kauf.) Snell, Singer & Dick, microscopic slides and larger glass plates were placed around and under fruiting bodies in the field. After two days, the slides and plates were collected with the naturally deposited spores and brought to the laboratory. The spores were allowed to air dry one more day and were then scraped from the glass with a razor blade, put in clean, dry vials and stored at 4° C.

Hebeloma crustuliniforme fruiting bodies were brought to the laboratory from the field. The stipes were cut off squarely, at about one half their length. The fruiting bodies were stood on the stipe ends on glass plates and covered. After two days the covers and fruiting bodies were removed. The deposited spores were allowed to air dry at room temperature for two days and were then placed in vials and stored at 4° C.

This last procedure was attempted with Amanita muscaria (Fr.) S. F. Gray, without successful spore discharge. The fruiting bodies were then allowed to dry slowly for three days on a drying rack. The dry gills were cut from the sporocarps, crushed, sieved and put in vials for cold storage. Microscopic examination of the spore/gill mixture suspended in water revealed mature appearing spores.

A Lycoperdon species was the final source of spore inoculum. These fungi fruit as small puffballs. The fruiting bodies were dried on the drying racks and stored intact. At inoculation the outer cover was removed and the gleba crushed to release the spores.

The seedlings were inoculated 30 days after sowing. The spores were suspended in water and diluted to a concentration yielding  $2 \times 10^6$  spores/seedling. The Amanita spores were diluted to yield  $1 \times 10^6$  spores/seedling. Each seedling was inoculated by discharging a three ml aliquot of spore suspension into the potting mix. Three ml aliquots of

water were used as controls.

The seedlings were grown on the standard growth regime until the 100 day harvest. The infected and uninfected short lateral roots on the seedlings with mycorrhizae were counted. The short lateral roots of seedlings without mycorrhizae were not counted (some of the pines had more than 500 short lateral roots).

## Results

Table 12 presents the results of the spore inoculations. No Douglas-fir became infected. The

Table 12. Results of spore inoculations of three conifer species seedlings by six fungi.  
See text for procedure.

Fungi	Lodgepole pine		Ponderosa pine		Douglas-fir
	Number of mycorrhizal seedlings out of 10	SLR's infected percent	Number of mycorrhizal seedlings out of 10	SLR's infected percent	Number of mycorrhizal seedlings out of 10
<u>Amanita muscaria</u>	0		0		0
<u>Suillus grevillei</u>	0		2	1.8	0
<u>S. tomentosus</u>	5	4	4	15.2	0
<u>S. lakei</u>	0		0		0
<u>Lycoperdon sp.</u>	0		1	16.9	0
<u>Hebeloma crustuliniforme</u>	7	12.2	3	4.2	0
Control	0		0		0

lodgepole pine and 2 of the 6 fungi and the ponderosa pine and 4 of the 6 fungi formed mycorrhizae. All of the mycorrhizal pine/fungal combinations resulted in less than 20% of the short lateral roots being infected.

#### Discussion

The stated purpose of this investigation was to test for mycorrhizal infection with the various conifer/fungal combinations. The results from this experiment are discussed in comparison to what may have been expected from the crosses according to known conifer/fungal affinities.

Mushroom field guides such as that by Miller (1981) often report associations of the fruiting bodies of known mycorrhizal fungi with certain host species or larger taxonomic groups. Trappe also reported this type of information from his personal field observations (Molina and Trappe, 1982). These reports indicate a biologically important interaction between host and fungus, but it is generally thought that many more fungi will inhabit the roots of trees than will fruit in these associations. Consequently, fruiting body/host associations should not be the only criteria for the selection of fungi for inoculation attempts.

Another type of research has focused on the pure culture synthesis of mycorrhizae. In this research, procedures are used to insure the presence of only the

specific host and fungus in a sealed vessel. A type of compatibility is demonstrated by these syntheses, but natural associations may not be indicated as other factors of the environment may prohibit the formation of these mycorrhizae under any conditions other than the most controlled. Finally, other research, using more natural conditions, ascertains the potential for purposefully developing mycorrhizae from certain crosses and assesses their potential for use in practical applications. Of primary concern here is work with container-grown conifer seedlings.

Amanita muscaria is known to have associations with a wide range of hardwood and conifer hosts (Trappe, 1962 and Miller, 1981). As vegetative inoculum in pure culture synthesis trials, it developed extensive mycorrhizae with all seven conifer species tested, including the three species used in this study (Molina and Trappe, 1982). The lack of mycorrhizae in this experiment could have been due to the spore collection method. Since the spores were not released naturally from the fruiting bodies, they may not have matured before drying.

Suillus lakei is a common associate of Douglas-fir in the Pacific Northwest (Miller, 1981). The spores used were collected from fruiting bodies under Douglas-fir and ponderosa pine. Molina and Trappe (1982) found this species to readily form mycorrhizae with coastal Douglas-fir and

lodgepole pine, and to a lesser extent with ponderosa pine, in pure culture.

S. grevillei is found fruiting near larch throughout much of the U.S. (Miller, 1981 and Molina and Trappe, 1982). Molina and Trappe (1982) reported observing no other fruiting body/host associations involving this fungus in 15 years of personal collections. In pure culture syntheses trials, it formed mycorrhizae with all three conifers used in this study (Molina and Trappe, 1982). Interestingly, here it formed mycorrhizae with ponderosa pine, but not lodgepole pine, while in the pure culture syntheses, it formed more mycorrhizae with lodgepole than with ponderosa pine (Molina and Trappe, 1982).

S. tomentosus is often found fruiting under lodgepole and other two-needle pines (Miller, 1981). Its mycorrhizal formation with both pines in this study indicates it may be a good candidate for further practical applications. Pure culture synthesis attempts and other inoculations of container-grown seedlings using S. tomentosus are unknown.

The Lycoperdon species formed mycorrhizae with one seedling of ponderosa pine. If this collection is L. perlatum as it appears, it is often found with various hardwoods and conifers (Miller, 1981). Its puffball fruiting body type, which yields a large number of spores, makes it a desirable species for practical applications if infection rates could be increased.



Hebeloma crustuliniforme formed mycorrhizae with lodgepole and ponderosa pine, but not with Douglas-fir. This collection was made from a nearly pure stand of Douglas-fir. This species was used as vegetative inoculum by Bledsoe et al. (1982) and Hung and Molina (1986b) to successfully inoculate coastal Douglas-fir. H. crustuliniforme also formed extensive mycorrhizae with three of four conifers in the Vegetative Inoculations section of this chapter. Consequently, it appears to be an excellent candidate for research and reforestation use.

Besides testing for successful crosses between the conifers and fungi, the spore collection methods, inoculation procedures and growth regime were of interest in this investigation. Direct testing of these factors against other possible choices was not performed, and discussions can only be generalized.

It is logical to collect spores as naturally as possible. Glass plates under fruiting bodies in the field is ideal and subsequent inoculations resulted in two of the three Suillus species forming mycorrhizae with lodgepole and/or ponderosa pine. Unfortunately, the irregularity of fruiting, the weather, logistics and other factors eliminate the chance for regular use of this method. Bringing mature, or nearly mature, fruiting bodies to the laboratory and allowing spore deposition is a similar procedure to field collection; it worked with Hebeloma and Lycoperdon, but not

with Amanita.

Injecting a water-based spore suspension into the growth tubes was used successfully in this study and by Castellano et al. (1985). In testing the effects of various spore concentrations, they also used an application rate of  $10^6$  spores/seedling, but they showed that a concentration 1000 times less ( $10^3$  spores seedling) was just as effective.

The infection levels in this study are quite low. In comparison, Castellano et al. (1985) achieved infection levels of up to 99% with coastal Douglas-fir. High fertility regimes have been shown to decrease mycorrhizal infection (Ruehle and Wells, 1984; Crowley et al. 1981 and Marx et al. 1977). This appears to be a possible explanation for the low levels, as the ample growth period (100 days between inoculation and harvest) should have allowed extensive mycorrhizal development in the absence of inhibitory factors. Evidence against this explanation are: 1) seedlings in the Vegetative Inoculations section of this chapter developed much more mycorrhizae under the same growth regime, and 2) Castellano et al. (1985) achieved high infection levels of spore inoculated coastal Douglas-fir under a high fertility regime. The reasons for the low rates are undeterminable without further testing.

## Vegetative Inoculations

### Materials and Methods

Six isolates of fungi were raised as vegetative inoculum according to the General Procedures. The fungi were: Suillus brevipes (Pk.) Kuntze, S. tomentosus (Kauf.) Snell, Singer & Dick, Hebeloma crustuliniforme (Bull. ex St-Amans) Quel., Laccaria laccata (Fr.) Berk. and Br., L. bicolor (R. Maire) Orton and a Rhizopogon sp. These fungi, plus a control inoculum, were used to inoculate 20 seedlings each of RMDf, ponderosa pine, lodgepole pine and western larch. The seedlings were raised under standard conditions in the growth chamber. Harvest was at 85 days after inoculation. After cleaning the potting mix from the root systems, the relative amounts of mycorrhizal infection of the short lateral roots was visually estimated. The infection categories used were zero, light (0-10% of the short lateral roots infected), moderate (11-40% infection) and heavy (>40% infection).

### Results

The results of the vegetative inoculations are presented in Table 13. No Douglas-fir became infected; however, the infection of the other three conifer species was more extensive than resulted from the spore inoculations. In all combinations where there was infection, all of the seedlings were mycorrhizal. Western

larch and both of the pine species developed heavy (>40%) infections with Hebeloma crustuliniforme. The two pines developed a heavy infection in combination with Laccaria bicolor. L. laccata inoculation resulted in light to moderate infection (<10%, 11-40%) on the three species.

#### Discussion

The genus Rhizopogon contains fungi whose tough, puffball-like fruiting bodies are usually found in the litter layer or top soil layer. Most Rhizopogon species are thought to be mycorrhizal and are found with a variety of hosts (Miller, 1981 and Molina and Trappe, 1982). In pure culture, Molina and Trappe (1982) crossed four Rhizopogon species with seven conifers. Infection rates were mixed, ranging from 0 to greater than 75% of the short lateral roots being mycorrhizal. Rhizopogon species appear to be good candidates for vegetative inoculations due to their ease of isolation, fast growth in culture and success as spore inocula (Lamb and Richards, 1974a & b; Parke et al. 1983; Ivory and Munga, 1983; Castellano et al. 1985 and Castellano and Trappe, 1985), but no known successful vegetative inoculations of container-grown seedlings have occurred with species of this genus (Molina, 1980).

Species of Suillus also appear to be good candidates for use as vegetative inoculations due to their ease of isolation, fast growth in culture and success at mycorrhizal

Table 13. Infection amounts resulting from the vegetative inoculations of four conifers by six fungi. Infection categories: 0 = no infection, light = 1-10% of short lateral roots infected, moderate = 11-40% infection and heavy = >40% infection.

Fungi	Lodgepole pine	Ponderosa pine	Western larch	Douglas-fir
<u>Rhizopogon sp.</u>	0	0	0	0
<u>Suillus brevipes</u>	0	0	0	0
<u>S. tomentosus</u>	0	0	0	0
<u>Hebeloma crustuliniforme</u>	heavy	heavy	heavy	0
<u>Laccaria laccata</u>	light	moderate	light	0
<u>L. bicolor</u>	heavy	heavy	0	0
Control	0	0	0	0

syntheses in pure culture (Molina and Trappe, 1982). Only limited success though, has resulted from their use to inoculate container-grown seedlings (Molina, 1980; McDonald, 1981 and Grossnickle and Reid, 1982). Two species were tested in this study, S. brevipes and S. tomentosus, no infection resulted. Molina and Trappe (1982) reported that S. brevipes formed mycorrhizae with all four conifer species used in this experiment during pure culture syntheses. Evidently, the container environment usually prohibits infection with Suillus vegetative inoculum, but not necessarily with Suillus spore inoculum (Lamb and Richards, 1974a & b and Spore Inoculations, this chapter).

Hebeloma crustuliniforme has a broad host range (Trappe, 1962 and Miller, 1981) and has been successfully used to inoculate coastal Douglas-fir (Bledsoe et al. 1982). In this study, it formed mycorrhizae with ponderosa pine, lodgepole pine and western larch. Its ability to form mycorrhizae as both a vegetative and spore inoculum make it a good choice for further experimentation.

The Laccaria species, L. laccata and L. bicolor, both formed mycorrhizae with the two pine species. L. laccata was also successful with western larch. Reports on the host range of L. bicolor and its use in other inoculation attempts are unknown. L. laccata has a broad host range (Trappe, 1962 and Molina and Trappe, 1982). In pure culture synthesis trials, it formed extensive mycorrhizae with all seven conifer species tested (Molina and Trappe, 1982). Additionally, it has been used as vegetative inoculum to form mycorrhizae with Douglas-fir (Brown and Sinclair, 1981; Sinclair et al. 1982; Sylvia, 1983; Hung and Molina, 1986a and Bledsoe et al. 1982). Like Hebeloma crustuliniforme, L. laccata seems to be a good choice for further inoculation use.

Setting aside the repeated unsuccessful inoculations of Rocky Mountain Douglas-fir and the general lack of success that researchers have had with Rhizopogon and Suillus species, the vegetative inoculation method used in this study worked well. H. crustuliniforme and the two Laccaria

species formed mycorrhizae on all seedlings in eight of the nine combinations with the two pines and western larch. Infection was heavy in many of those cases, indicating that the growth regime (specifically the fertilization regime) was probably not a major factor limiting the spread of mycorrhizae on the root systems.

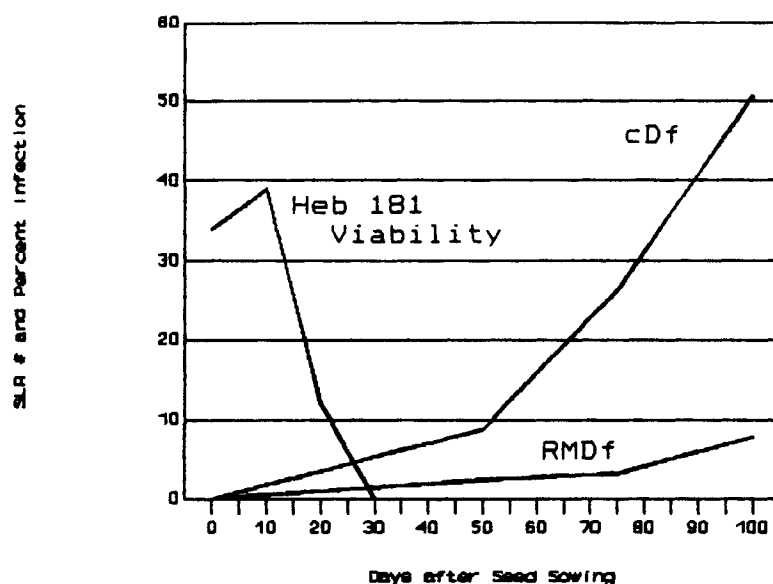
This chapter includes: 1) a brief summary and interpretation of the experimental results and 2) a list of questions and comments that have arisen from this work.

A central hypothesis tested by many of the experiments was that the low number and late production of short lateral roots on container-grown Rocky Mountain Douglas-fir limits the formation of mycorrhizae on the seedlings. The viability of the fungal inocula was hypothesized to have been reduced or lost by the time of short lateral root production by the seedlings.

Figure 2 shows the temporal viability of Hebeloma crustuliniforme and the production of short lateral roots of the two varieties of Douglas-fir. Not shown, but relevant to the discussion, is the fact that seedlings of some pines that are considered easy to inoculate had greater than 500 short lateral roots by 100 days, whereas coastal Douglas-fir had 51 and Rocky Mountain Douglas-fir had less than 10. The conclusion is that the low number of short lateral roots and the lack of overlap in the timing between having viable inoculum and short lateral root production are at least partial reasons for the repeated inoculation failures



Figure 2. Inoculum viability of Heb 181 and short lateral root production of coastal and Rocky Mountain Douglas-fir. The viability of Heb 181 is shown as percent of short lateral roots of lodgepole pine infected in the 10 day transplants beginning as seed sowing (Chapter IX). Short lateral root production by the two Douglas-fir varieties are the SLR data from the 50, 75 and 100 harvests (Chapter VIII).



experienced with Rocky Mountain Douglas-fir.

Besides higher short lateral root production, coastal Douglas-fir seedlings had mean values for root length, root weight and shoot weight that were approximately twice those of Rocky Mountain Douglas-fir.

The  $\text{CuCO}_3$ , Ethephon and Nutrient treatments failed to significantly increase Douglas-fir short lateral root

production, and actually decreased production by at least one treatment in each experiment.

Regional isolates of a variety of fungal species were successfully used as inocula with seedlings of several northern Rocky Mountain conifers. The vegetative and spore inoculation methods both resulted in mycorrhizal formation with certain conifer/fungal crosses.

These experiments have raised as many questions as they have answered. Some questions and comments about them follow:

1. What is responsible for the so called 'fungal effect'? Arguments were presented against several simple explanations. A direct effect on the seedlings, or an indirect effect through an influence on rhizosphere populations of other organisms, by free-living mycorrhizal fungi seems the best explanation. Discussion of such an effect in the current literature is unknown.

2. Will mycorrhizae form on Rocky Mountain Douglas-fir in pure culture syntheses? The container environment may be a factor in the continued difficulties with inoculation prior to seed sowing.

3. How does Douglas-fir root development differ when the seedlings are grown in containers than when grown naturally in forest soils? How does it differ when grown in larger containers? Phenotypic plasticity may result in many

developmental patterns, depending on a variety of environmental factors.

4. While transplanting successively older Douglas-fir into fresh inoculum, when (if at all) will Douglas-fir first become infected? It could be that the fungi used in these experiments are not common associates of very young Douglas-fir. Possibly the seedlings do not normally develop mycorrhizae their first year.

## APPENDIX and BIBLIOGRAPHY

Pages 96 - 103. Tables containing the means for the uninoculated (Control) and inoculated (Heb 181 & SS166) groups for the Ethephon, Cupric Carbonate, Nutrient and Regional Varieties experiments.

Pages 104 - 106. Tables containing information on the nutrient solutions used in the thesis.

Pages 107 - 108. Tables containing information on the fungi and conifers used in the thesis.

Pages 109 - 117. Bibliography.

Appendix Table 1. Uninoculated (Control) and inoculated (Heb 181) group means of the measured parameters at the 50, 75 and 100 day harvests, resulting from three ethephon treatments.

HARVEST days after sowing	ETHEPHON TREATMENT ppm	FUNGAL TREATMENT	ROOT LENGTH cm	ROOT WEIGHT g#100	SHOOT WEIGHT g#100	SHORT LATERAL ROOTS number
50	0	Control	33.4	1.81	3.57	1.4
50	0	Heb 181	28.4	1.64	3.49	1.7
50	1	Control	31.5	1.83	3.26	1.2
50	1	Heb 181	37.2	1.88	3.65	1.1
50	5	Control	31.4	2.19	3.69	1.8
50	5	Heb 181	26.6	1.77	3.49	1.1
75	0	Control	57.6	4.08	8.77	4.7
75	0	Heb 181	45.8	3.76	7.08	5.8
75	1	Control	52.4	4.32	8.48	3.0
75	1	Heb 181	46.5	4.73	9.75	1.3
75	5	Control	50.7	5.03	9.35	2.1
75	5	Heb 181	34.7	3.65	7.35	1.7
100	0	Control	76.0	7.06	11.68	7.6
100	0	Heb 181	81.0	7.87	12.95	7.4
100	1	Control	59.3	6.18	13.02	3.2
100	1	Heb 181	60.0	8.67	16.01	1.3
100	5	Control	65.9	9.02	14.68	1.7
100	5	Heb 181	45.8	7.51	13.17	2.9

Appendix Table 2. Uninoculated (Control) and inoculated (Heb 181) group means of the derived parameters at the 50, 75 and 100 day harvests, resulting from three ethephon treatments.

HARVEST days after sowing	ETHEPHON TREATMENT ppm	FUNGAL TREATMENT	ROOT-TO- SHOOT RATIO	SHORT LATERAL ROOTS per cm ROOT LENGTH (#/cm)*100	ROOT WEIGHT per cm ROOT LENGTH (g/cm)*10000
50	0	Control	0.498	5.0	5.8
50	0	Heb 181	0.473	6.3	5.8
50	1	Control	0.560	5.4	6.0
50	1	Heb 181	0.518	3.1	5.3
50	5	Control	0.605	5.1	6.9
50	5	Heb 181	0.508	5.2	7.1
75	0	Control	0.475	7.5	7.7
75	0	Heb 181	0.532	12.7	9.1
75	1	Control	0.531	5.6	8.8
75	1	Heb 181	0.480	3.1	10.4
75	5	Control	0.548	4.7	10.7
75	5	Heb 181	0.498	5.9	11.2
100	0	Control	0.615	11.5	9.9
100	0	Heb 181	0.670	8.8	10.0
100	1	Control	0.486	6.1	11.8
100	1	Heb 181	0.554	2.5	15.2
100	5	Control	0.616	2.5	17.2
100	5	Heb 181	0.576	8.4	17.4

Appendix Table 3. Uninoculated (Control) and inoculated (Heb 181) group means of the measured parameters at the 50, 75 and 100 day harvests, resulting from four cupric carbonate/paint treatments.

HARVEST days after sowing	CuCO <sub>3</sub> /PAINT TREATMENT q/l	FUNGAL TREATMENT	ROOT LENGTH cm	ROOT WEIGHT g#100	SHOOT WEIGHT g#100	SHORT LATERAL ROOTS number
50	No Paint	Control	36.5	2.04	4.62	3.5
50	No Paint	Heb 181	37.6	1.74	4.28	1.8
50	0	Control	27.5	1.72	3.13	4.4
50	0	Heb 181	21.5	1.29	2.75	3.3
50	50	Control	20.4	1.44	2.71	4.8
50	50	Heb 181	25.3	1.61	3.66	5.0
50	100	Control	19.2	1.35	2.82	4.3
50	100	Heb 181	20.1	1.49	3.25	3.9
75	No Paint	Control	46.2	4.21	9.13	4.9
75	No Paint	Heb 181	62.3	4.78	12.31	4.9
75	0	Control	34.0	2.58	5.82	5.6
75	0	Heb 181	33.7	2.89	6.43	5.8
75	50	Control	25.3	2.76	5.41	4.8
75	50	Heb 181	25.2	3.35	7.04	5.2
75	100	Control	24.2	3.13	5.87	5.7
75	100	Heb 181	27.0	3.31	7.72	6.8
100	No Paint	Control	53.9	5.2	13.50	6.5
100	No Paint	Heb 181	76.1	8.41	18.32	5.7
100	0	Control	52.3	3.91	8.67	4.7
100	0	Heb 181	46.9	4.79	9.73	2.4
100	50	Control	38.4	5.15	9.27	10.0
100	50	Heb 181	42.2	5.37	9.55	8.4
100	100	Control	31.1	5.36	11.00	10.1
100	100	Heb 181	38.4	5.62	12.19	12.8

Appendix Table 4. Uninoculated (Control) and inoculated (Heb 181) group means of the derived parameters at the 50, 75 and 100 day harvests, resulting from four cupric carbonate/paint treatments.

HARVEST days after sowing	CuCO <sub>3</sub> /PAINT TREATMENT g/l	FUNGAL TREATMENT	ROOT-TO- SHOOT RATIO	SHORT LATERAL ROOTS per cm ROOT LENGTH (#/cm)#100	ROOT WEIGHT per cm ROOT LENGTH (g/cm)#10000
50	No Paint	Control	0.442	10.1	6.1
50	No Paint	Heb 181	0.403	4.5	4.8
50	0	Control	0.543	18.5	6.3
50	0	Heb 181	0.486	13.5	6.0
50	50	Control	0.542	22.1	7.3
50	50	Heb 181	0.433	19.0	6.3
50	100	Control	0.476	21.5	7.8
50	100	Heb 181	0.460	18.7	7.4
75	No Paint	Control	0.482	11.7	9.4
75	No Paint	Heb 181	0.389	8.4	8.6
75	0	Control	0.450	18.2	8.3
75	0	Heb 181	0.453	16.3	9.0
75	50	Control	0.510	19.0	11.0
75	50	Heb 181	0.496	20.3	13.3
75	100	Control	0.536	26.2	13.9
75	100	Heb 181	0.437	23.7	12.6
100	No Paint	Control	0.383	11.5	9.8
100	No Paint	Heb 181	0.460	7.5	11.3
100	0	Control	0.460	8.2	8.6
100	0	Heb 181	0.500	4.4	10.5
100	50	Control	0.554	23.5	13.5
100	50	Heb 181	0.548	19.0	13.6
100	100	Control	0.493	35.5	17.6
100	100	Heb 181	0.491	33.4	16.1



Appendix Table 5. Uninoculated (Control) and inoculated (Heb 181) group means of the measured parameters at the 50, 75 and 100 day harvests, resulting from five nutrient treatments.

HARVEST days after sowing	NUTRIENT TREATMENT formula	FUNGAL TREATMENT	ROOT LENGTH cm	ROOT WEIGHT g#100	SHOOT WEIGHT g#100	SHORT LATERAL ROOTS number
50	NPK	Control	36.5	2.04	4.62	3.5
50	NPK	Heb 181	37.6	1.74	4.28	1.8
50	NpK	Control	26.5	1.75	3.77	3.6
50	NpK	Heb 181	36.7	2.06	4.59	3.6
50	nPK	Control	28.8	1.74	4.11	2.6
50	nPK	Heb 181	32.5	1.69	4.23	1.7
50	npK	Control	19.7	1.17	3.05	1.5
50	npK	Heb 181	26.2	1.35	4.01	0.9
50	dH2O	Control	22.3	1.53	3.05	2.2
50	dH2O	Heb 181	40.1	2.19	3.39	3.0
75	NPK	Control	46.2	4.21	9.13	4.9
75	NPK	Heb 181	62.3	4.78	12.31	4.9
75	NpK	Control	39.6	3.18	6.91	5.7
75	NpK	Heb 181	51.3	3.85	8.41	5.0
75	nPK	Control	41.3	3.65	6.58	4.0
75	nPK	Heb 181	63.9	4.21	9.13	5.0
75	npK	Control	24.1	1.47	4.08	2.0
75	npK	Heb 181	33.0	1.88	6.55	2.0
75	dH2O	Control	28.5	2.42	3.72	4.6
75	dH2O	Heb 181	44.5	3.62	5.04	5.3
100	NPK	Control	53.9	5.20	13.50	6.5
100	NPK	Heb 181	76.1	8.41	18.32	5.7
100	NpK	Control	61.3	4.80	9.84	6.0
100	NpK	Heb 181	83.7	7.87	18.20	16.1
100	nPK	Control	48.0	4.70	8.87	2.9
100	nPK	Heb 181	84.8	7.33	14.88	3.3
100	npK	Control	37.0	2.85	6.15	2.5
100	npK	Heb 181	39.1	3.47	9.30	3.0
100	dH2O	Control	36.2	3.88	4.63	3.6
100	dH2O	Heb 181	45.4	5.26	6.05	3.7

Appendix Table 6. Uninoculated (Control) and inoculated (Heb 181) group means of the derived parameters at the 50, 75 and 100 day harvests, resulting from five nutrient treatments.

HARVEST days after sowing	NUTRIENT TREATMENT formula	FUNGAL TREATMENT	ROOT-TO- SHOOT RATIO	SHORT LATERAL ROOTS per cm ROOT LENGTH (#/cm)*100	ROOT WEIGHT per cm ROOT LENGTH (g/cm)*10000
50	NPK	Control	0.442	10.1	6.1
50	NPK	Heb 181	0.403	4.5	4.8
50	NpK	Control	0.472	12.8	6.7
50	NpK	Heb 181	0.443	10.5	6.0
50	nPK	Control	0.421	10.1	6.3
50	nPK	Heb 181	0.416	5.7	5.6
50	npK	Control	0.383	7.2	6.0
50	npK	Heb 181	0.336	3.5	5.2
50	dH2O	Control	0.506	9.0	7.1
50	dH2O	Heb 181	0.647	7.0	5.6
75	NPK	Control	0.482	11.7	9.4
75	NPK	Heb 181	0.389	8.4	8.6
75	NpK	Control	0.465	13.1	8.4
75	NpK	Heb 181	0.460	9.2	7.7
75	nPK	Control	0.567	9.0	10.2
75	nPK	Heb 181	0.464	7.6	7.4
75	npK	Control	0.360	7.7	6.3
75	npK	Heb 181	0.285	5.2	6.1
75	dH2O	Control	0.648	14.5	8.6
75	dH2O	Heb 181	0.712	12.2	8.4
100	NPK	Control	0.383	11.5	9.8
100	NPK	Heb 181	0.460	7.5	11.3
100	NpK	Control	0.504	9.8	7.9
100	NpK	Heb 181	0.443	16.5	10.1
100	nPK	Control	0.558	7.2	10.9
100	nPK	Heb 181	0.513	3.9	10.2
100	npK	Control	0.475	6.0	7.9
100	npK	Heb 181	0.360	7.3	8.9
100	dH2O	Control	0.835	11.4	11.5
100	dH2O	Heb 181	0.877	7.5	13.4

Appendix Table 7. Uninoculated (Control) and inoculated (Heb 181 and SS166) group means of the measured parameters at the 50, 75 and 100 day harvests.

HARVEST days after sowing	DOUGLAS-FIR VARIETY	FUNGAL TREATMENT	ROOT LENGTH cm	ROOT WEIGHT g#100	SHOOT WEIGHT g#100	SHORT LATERAL ROOTS number
50	cDf	Control	33.0	1.49	4.63	6.2
50	cDf	Heb 181	29.7	1.67	4.44	8.6
50	cDf	SS166	32.5	2.26	5.64	11.2
50	RMDf	Control	29.4	1.68	4.10	1.4
50	RMDf	Heb 181	29.9	1.73	4.10	2.8
50	RMDf	SS166	31.1	1.72	4.54	2.9
75	cDf	Control	79.0	5.19	13.82	19.0
75	cDf	Heb 181	86.9	6.82	14.56	34.4
75	cDf	SS166	85.1	6.62	15.69	25.8
75	RMDf	Control	29.0	3.38	7.11	2.1
75	RMDf	Heb 181	44.6	4.53	10.30	2.1
75	RMDf	SS166	45.5	5.15	10.66	5.5
100	cDf	Control	137.3	13.45	30.07	46.3
100	cDf	Heb 181	156.6	14.70	31.86	55.2
100	cDf	SS166	113.3	13.65	34.98	50.2
100	RMDf	Control	52.0	6.87	12.81	10.5
100	RMDf	Heb 181	58.1	7.88	16.01	5.2
100	RMDf	SS166	58.7	8.07	16.70	7.3

Appendix Table 8. Uninoculated (Control) and inoculated (Heb 181 and SS166) group means of the derived parameters at the 50, 75 and 100 day harvests.

HARVEST days after sowing	DOUGLAS-FIR VARIETY	FUNGAL TREATMENT	ROOT-TO- SHOOT RATIO	SHORT LATERAL ROOTS per cm ROOT LENGTH (#/cm)*100	ROOT WEIGHT per cm ROOT LENGTH (g/cm)*10000
50	cDf	Control	0.317	19.3	4.6
50	cDf	Heb 181	0.383	34.7	6.1
50	cDf	SS166	0.407	38.1	7.7
50	RMDf	Control	0.414	3.9	6.0
50	RMDf	Heb 181	0.429	10.8	6.2
50	RMDf	SS166	0.379	10.6	5.8
75	cDf	Control	0.376	24.2	6.8
75	cDf	Heb 181	0.463	43.4	8.1
75	cDf	SS166	0.427	32.4	8.0
75	RMDf	Control	0.467	7.4	11.8
75	RMDf	Heb 181	0.441	4.3	10.6
75	RMDf	SS166	0.486	13.1	11.9
100	cDf	Control	0.447	35.1	9.9
100	cDf	Heb 181	0.472	34.7	10.3
100	cDf	SS166	0.398	46.5	12.4
100	RMDf	Control	0.554	20.8	14.3
100	RMDf	Heb 181	0.492	8.7	14.3
100	RMDf	SS166	0.499	12.5	14.7

Appendix Table 9. Chemical sources, their mixing concentrations and resulting levels of the nutrients, nitrogen, phosphorus, potassium and calcium in the NPK, NpK, nPK and npK solutions. Similar information for the other nutrients can be found in Appendix Table 10.

Chemical Source	SOLUTIONS			
	NPK	NpK	nPK	npK
	mg/l ----- nutrients- ppm	mg/l ----- nutrients- ppm	mg/l ----- nutrients- ppm	mg/l ----- nutrients- ppm
NH <sub>4</sub> NO <sub>3</sub>	129	129	23	23
	----- N - 45	----- N - 45	----- N - 8	----- N - 8
NaH <sub>2</sub> (PO <sub>4</sub> )H <sub>2</sub> O	71	9	71	9
	----- P - 16	----- P - 2	----- P - 16	----- P - 2
KNO <sub>3</sub>	129	129	0	0
	----- N - 18 K - 50	----- N - 18 K - 50	-----	-----
K <sub>2</sub> CO <sub>3</sub>	0	0	89	89
	-----	-----	----- K - 50	----- K - 50
Ca(NO <sub>3</sub> ) <sub>2</sub> (4H <sub>2</sub> O)	59	59	59	59
	----- N - 7 Ca - 10	----- N - 7 Ca - 10	----- N - 7 Ca - 10	----- N - 7 Ca - 10
Totals	ppm	ppm	ppm	ppm
	N - 70	N - 70	N - 15	N - 15
	P - 16	P - 2	P - 16	P - 2
	K - 50	K - 50	K - 50	K - 50
	Ca - 10	Ca - 10	Ca - 10	Ca - 10

Appendix Table 10. Chemical sources, their mixing concentrations and resulting levels of sulfur, magnesium, chlorine, manganese, boron, iron, zinc, copper, and molybdenum in the NPK, NpK, nPK and npK solutions. The mixing concentrations and nutrient levels were the same in all four solutions. Similar information for four other nutrients used in these solutions can be found in Appendix Table 9.

Chemical Source	mg/l ----- nutrients- ppm	Chemical Source	mg/l ----- nutrients- ppm
MgSO <sub>4</sub> (7H <sub>2</sub> O)	99 ----- S - 13 Mg - 10	ZnCl <sub>2</sub>	0.07 ----- Zn - 0.03 Cl - 0.04
NaCl	5.8 ----- Cl - 3.5	H <sub>3</sub> BO <sub>3</sub>	1.24 ----- B - 0.2
MnCl <sub>2</sub> (4H <sub>2</sub> O)	1.4 ----- Mn - 0.4 Cl - 0.3	FeSO <sub>4</sub> (7H <sub>2</sub> O)	0.5 ----- Fe - 0.1 S - 0.06
CuCl <sub>2</sub> (H <sub>2</sub> O)	0.07 ----- Cu - 0.03 Cl - 0.02	Na <sub>2</sub> MoO <sub>4</sub> (2H <sub>2</sub> O)	0.002 ----- Mo - 0.001
Totals	ppm S - 13.1 Mg - 10 Cl - 3.9 Mn - 0.4 B - 0.2		ppm Fe - 0.1 Zn - 0.03 Cu - 0.03 Mo - 0.001

Appendix Table 11. Chemical sources, their mixing concentrations and resulting levels of nitrogen, phosphorus, potassium, calcium, sulfur and magnesium in the solution used in the Spore and Vegetative Inoculations (Chapter X). Similar information for chlorine, manganese, copper, zinc, boron, molybdenum, and iron can be found in Appendix Table 10 and is the same as in the other nutrient solutions in the thesis.

Chemical Source	mg/l ----- nutrients- ppm	Chemical Source	mg/l ----- nutrients- ppm
NH <sub>4</sub> NO <sub>3</sub>	143 ----- N - 50	CaSO <sub>4</sub> (2H <sub>2</sub> O)	108 ----- Ca - 25 S - 20
KH <sub>2</sub> PO <sub>4</sub>	53 ----- P - 12 K - 15	MgSO <sub>4</sub> (7H <sub>2</sub> O)	99 ----- Mg - 10 S - 13
Totals	ppm  N - 50 P - 12 K - 15		ppm  Ca - 25 S - 33 Mg - 10

Appendix Table 12. Identification and information on the fungi used in the thesis.

Fungal Species	Collection Number	Isolation location and date.
-----		
From the collection of Scott L. Miles		
<u>Laccaria laccata</u> (Fr.) Berk. and Br.	9	Printz Gulch, west of Victor, Ravalli County, MT T7N R21W Sec. 5 10/30/84
<u>Suillus grevillei</u> (Kl.) Singer	37	beside Pattee Canyon Rd. 0.1 mile below Crazy Canyon Rd. Missoula County, MT T12N R19W Sec. 1 8/29/85
<u>Suillus tomentosus</u> (Kauf.) Snell, Singer and Dick	38	beside Pattee Canyon Rd. 0.1 mile below Crazy Canyon Rd. Missoula County, MT T12N R19W Sec. 1 8/29/85
<u>Suillus lakei</u> (Murr.) Smith and Thiers	39	beside Pattee Canyon Rd. 0.1 mile below Crazy Canyon Rd. Missoula County, MT T12N R19W Sec. 1 8/29/85
<u>Lycoperdon</u> sp.	43	S. Fork Lolo Ck. near parking lot Missoula County, MT T11N R21W Sec. 6 8/29/85
<u>Amanita muscaria</u> (Fr.) S. F. Gray	44	West Fork Butte Ck. 0.5 mile up from Elk Meadows Rd. Missoula County, MT T12N R22W Sec. 35 8/30/85
<u>Suillus brevipes</u> (Pk.) Kuntze	48	West Fork Butte Ck. 0.5 mile up from Elk Meadows Rd. Missoula County, MT T12N R22W Sec. 35 8/30/85
<u>Hebeloma crustuliniforme</u> (Bull. ex St.-Amans) Quel.	53	near confluence of Welcome and Rock Cks. Granite County, MT T9N R17W Sec. 2 10/6/85
From the collection of Dr. R.K. Antibus Clarkson University Potsdam, NY		
<u>Hebeloma crustuliniforme</u> (Bull. ex St.-Amans) Quel.	181	(= Heb 181) Charles Waters Memorial Recreation Area Ravalli County, MT 10/3/81
<u>Rhizopogon</u> sp.	377	Garnet Ridge Rd. Lubrecht Experimental Forest Missoula County, MT 7/22/83
<u>Suillus tomentosus</u> (Kauf.) Snell, Singer and Dick	435	Cash Ck. Rd. Ravalli County, MT 9/30/84
From the collection of Dr. Greg Mueller Museum of Natural History Chicago, IL		
<u>Laccaria bicolor</u> (R. Maire) Orton	1230	Binarch Ck. Rd. #639 near Priest Lake Bonner County, ID 10/26/81
From Sylvan Spawn Laboratory, Inc. Kittanning, PA 16201		
<u>Hebeloma crustuliniforme</u> (Bull. ex St.-Amans) Quel.	SS166	(= SS166) Inoculum bought from Sylvan Spawn. Western Oregon isolate



Appendix Table 13. Seed sources of the conifers used in the thesis.

Tree Species	Batch Number	Elevation and collection location.	
-----			
From Champion International Timberlands Division Bonner, Montana			
<u>Pseudotsuga menziesii</u> var. <u>glauca</u> (Beissn.) Franco	80\36-14-17	Elevation - 3800 ft.	Western Montana
<u>Pinus contorta</u> Dougl. ex Loud. var. <u>latifolia</u> Engelm.	S - 31	Elevation - 4000 ft.	Western Montana
From the Montana State Forest Nursery Missoula, Montana			
<u>Larix occidentalis</u> Nutt.	818	Elevation - 5000 ft.	Western Montana
<u>Pinus ponderosa</u> Dougl. ex Loud.	629	Elevation - 4000 ft.	Western Montana
From Silva Seed Co. Roy, WA			
<u>Pseudotsuga menziesii</u> (Mirb.) Franco var. <u>menziesii</u>		Elevation - 500 ft.	Western Washington
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