

**ECTOMYCORRHIZAL STATUS AND GROWTH OF
INTERIOR DOUGLAS-FIR ON DEGRADED
REFORESTATION SITES**

by

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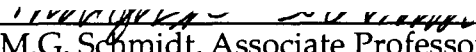
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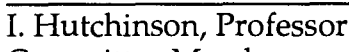
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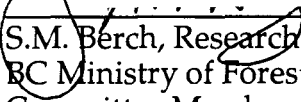
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ABSTRACT

Most conifers in the Pacific Northwest form ectomycorrhizae and the benefits of such symbiotic associations are increased nutrient and water uptake, resistance to root pathogens, and tolerance to environmental extremes. In this study, survival and growth of commercially inoculated Douglas-fir seedlings raised under current nursery conditions and outplanted on standard reforestation sites and degraded sites such as landings were evaluated. Landings were significantly more compacted and had lower levels of organic matter and mineralizable-N compared to adjacent clearcuts. Commercially available inoculants did not improve growth, yet it was found that at time of outplanting seedlings were not heavily colonized. On the same sites, the ectomycorrhizal status and growth of nonectomycorrhizal (at time of outplanting) Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco) seedlings outplanted on degraded and standard reforestation sites were also assessed. Chemical analysis revealed that seedlings grown on landings had potentially toxic levels of Fe and Al in their needles. In both the field and greenhouse, *Rhizopogon vinicolor*-like was the most abundant ectomycorrhizal type found in landing soil. On landings ectomycorrhizal colonization was significantly higher than on adjacent clearcuts however, seedling growth and the diversity of ectomycorrhizal types were significantly lower. Before drawing conclusions concerning the potential of commercial inoculants, nurseries should modify growing conditions if they wish to favor good ectomycorrhizal formation before outplanting. This research indicates that under the harsh conditions that are found on landings Douglas-fir can readily form ectomycorrhizae but with a limited number of symbionts.

Résumé

Les champignons ectomycorhiziens forment des symbioses mutualistiques avec les racines de la grande majorité des arbres de l'Ouest de l'Amérique du Nord. Ces associations intimes, appelés ectomycorhizes, sont responsables de l'augmentation de l'apport en éléments nutritifs et en eau. Ils assurent aussi une meilleure résistance contre certains pathogènes racinaires et protègent l'arbre des rigueurs du climat. La survivance et la croissance de semis de sapin de Douglas (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco) inoculés avec des champignons ectomycorhiziens produit commercialement, furent évalués après avoir été transplantés sur des sites forestiers dégradés. De plus, des semis non inoculés ont été transplantés dans le sol des aires de chargement et de coupes à blanc pour évaluer et comparer le status ectomycorhizien et la croissance. Les aires de chargement avaient des sols significativement plus compacts et pauvres en matières organiques et en azote minéralisable, comparés aux coupes à blanc. Les inoculants commerciaux n'ont pas augmentés significativement la survie et la croissance des semis. L'analyse chimique a démontré que les semis de sapin de Douglas avaient des concentrations toxiques en fer et en aluminium dans leurs feuillages. *Rhizopogon vinicolor* ou une espèce semblable a été trouvé en abondance dans les deux types de sols. D'autre part, lors de l'essai biologique en serre, les semis de sapin de Douglas poussant dans le sol des aires de chargement avaient un niveau de colonisation plus élevé que ceux poussant dans le sol des coupes à blanc. Par contre, l'établissement d'une microflore symbiotique diversifiée n'a pas eu lieu sur les aires de chargement comparativement aux coupes à blanc. Les semis de sapin de Douglas inoculés avaient des niveaux de colonisation peu élevés avant la transplantation, par conséquent leurs effets bénéfiques n'ont sûrement pas été complètement déterminés. Les pépinières intéressées à augmenter le niveau de colonisation des champignons ectomycorhiziens devraient modifier les conditions de culture des semis. La présente étude démontre que le sapin de Douglas peut former des ectomycorhizes sur des sites où les conditions de pousse sont défavorables.

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"Soil is a kind of placenta that enables living things to feed upon the earth."

Nathaniel Shaler



"A thing is right when it tends to preserve the integrity, stability, and beauty of the biotic community."

Aldo Leopold

To ∞ and this symbiotic planet



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INTRODUCTION

Symbiotic organisms are found on the roots of all plants. Fungi that form mutualistically symbiotic associations with plant roots are called mycorrhizae (*mycos* = fungus, *rhiza* = root). This intimate relationship is said to be mutual because the fungi exploits the surrounding soil therefore increasing the plants ability to uptake nutrients and water (Smith and Read 1997). In return the host plant provides a source of carbon that is required for the survival and growth of the fungi. Mycorrhizae that form a sheath around the root and penetrate the root tissue but stay between the root cells (Hartig net) are called ectomycorrhizae. Ectomycorrhizae are well known for their ability to increase nutrient and water uptake, and to enhance resistance to root pathogens and environmental extremes (Smith and Read 1997). Many different species of ectomycorrhizal fungi form on most conifer trees, such as Douglas-fir, in the Pacific Northwest. Trappe (1977) estimated that 2000 different species are potential ectomycorrhizal associates of Douglas-fir.

Available phosphorus (P) is often found in low concentrations in soil solution or is immobilized in the soil (Brady and Weil 2002). Under natural conditions, ectomycorrhizal trees benefit immensely from the symbiosis by increasing P uptake via soil penetrating extraradical hyphae and rhizomorphs (Finlay and Read 1986). Ectomycorrhizal plants tend to dominate forest ecosystems (Smith and Read 1997) where low availability of nitrogen (N) is characteristic (Fisher and Binkley 2000). Under these forest conditions, ectomycorrhizal seedlings take up significantly more N than nonectomycorrhizal seedlings

(Allen 1991; Bowen and Smith 1981; Smith et al. 1994). Some uncertainty remains about the uptake of other macronutrients and micronutrients but it seems that the uptake of potassium (K), calcium (Ca), iron (Fe) (Marschner and Dell 1994), and sulfur (S) (Rennenberg 1999) is enhanced when plants are ectomycorrhizal (Smith and Read 1997).

Under natural conditions ectomycorrhizae are considered an essential feature of a plant's stress-tolerance strategy (Jha et al. 1992). Seedling survival in forests is likely enhanced by ectomycorrhizal diversity, because different fungi do different jobs for different hosts in different environments (Perry et al. 1987). Since some ectomycorrhizal fungi benefit hosts in clearly identifiable ways (e.g. increase nutrient and water uptake or resistance to root pathogen), diversity probably acts like a buffer under a given stress.

Ectomycorrhizal seedlings have been shown to survive greater water stress than nonectomycorrhizal plants (Garbaye 2000). An increase in nutrient uptake may be responsible for better drought resistance (Kropp and Langlois 1990). It has been shown that ectomycorrhizal seedlings exposed to water stress have a significantly higher photosynthetic rate than nonectomycorrhizal seedlings (Parke et al. 1983).

Ectomycorrhizal fungi can also provide protection against root pathogens (Kropp and Langlois 1990; Marx 1973). Finally, mycorrhizal extraradical hyphae can increase soil aggregate stability (Tisdall 1994; Wright and Upadhyaya 1998), and increase the weathering of soil minerals (Van Breemen et al. 2000) benefiting the host plant indirectly.

Planted conifer seedling growth is sometimes slow, and may be very slow on landings. Landings are timber-harvesting constructions established for the loading and preliminary processing of cut trees. They are also busy traffic areas for large trucks and harvesting machinery. In British Columbia, landings are relatively small flat sites typically

with only B and C soil horizons remaining (Carr 1987). They are characterized by compacted soils, poor macropore structure, poor soil aeration, low organic matter content, poor water infiltration, and lower P concentrations than off-landing harvested areas (Carr 1987).

Compaction reduces soil aeration, which decreases root respiration and microorganism activity (Carr 1987; Page-Dumroese et al. 1998). High seedling mortality is common on severely compacted soils and the main cause seems to be poor soil aeration (Haselwandter and Bowen 1996; Kozlowski 1999). Detrimental effects of soil compaction on conifer seedlings can be observed at an early stage (Conlin and van den Driessche 1996b). One year after outplanting, Douglas-fir seedlings had smaller root volumes in compacted soils compared to uncompacted soils in northern Idaho (Page-Dumroese et al. 1998). Similar results were obtained in a soil compaction greenhouse study (Conlin and van den Driessche 1996a). Root development was poor because of high soil density (i.e. soil compaction) and high soil pH. Poor aeration and lower soil penetrability also result in decreases in ectomycorrhizal development (Skinner and Bowen 1974).

Compacted soils usually negatively affect ectomycorrhiza formation. Ectomycorrhizal root tips on Douglas-fir were reduced by 70 percent on compacted soils compared to soil with no compaction (Page-Dumroese et al. 1998). Amaranthus et al. (1996) found that Douglas-fir ectomycorrhizal diversity declined from 2.7 to 1 on severely compacted soils. Nadian et al. (1998), however discovered that certain species of ectomycorrhizal fungi are better adapted to compacted soils and thus can provide sufficient nutrients. In contrast, Simmons and Pope (1988) found that high levels of soil compaction precluded benefits of ectomycorrhizal association. They argued that alleviation of the

negative effects of soil compaction is required before ectomycorrhizal associations can benefit seedlings.

Forestry activities such as clearcutting and establishment of roads and landings can adversely affect ectomycorrhizal communities (Amaranthus et al. 1987; Amaranthus 1989; Hagerman 1999; Harvey et al. 1980; Parke et al. 1984; Perry et al. 1982). Seedlings outplanted on disturbed sites can become stressed because of poor soil physical and chemical properties. Adequate ectomycorrhizal development appears to be the first line of biological defense against abiotic and biotic stress (Marx et al. 1992). The diversity of fungal symbionts found on the roots of seedlings may also prove to be a critical factor for establishment on these disturbed sites (Perry et al. 1987). Usually ectomycorrhiza development primarily occurs in forest floor (Harvey et al. 1976), where an ideal habitat with adequate moisture, nutrients, and an abundance of fine roots can be found. Disturbed sites such as landings are often devoid of forest floor and are heavily compacted. To obtain adequate growth and ectomycorrhizal development under these conditions seedlings may need to be inoculated before outplanting (Kropp and Langlois 1990).

It has been argued that ectomycorrhizal seedlings survive and grow significantly better than nonectomycorrhizal seedlings on difficult sites (Kropp and Langlois 1990). However, ectomycorrhizal fungi may not always provide immediate benefits to the host tree if conditions are too harsh. Under these conditions, it is possible that the energy costs of maintaining fungal hyphae and a greater number of fine roots outweigh the benefits derived from increased nutrient or water uptake (Jurgensen et al. 1997). Nevertheless, many researchers have found survival and growth increases when seedlings were inoculated with ectomycorrhizal fungi before outplanting on disturbed sites (Cordell et al.

1987; Marx and Cordell 1987; Molina and Trappe 1982). Very little research has looked at the potential benefits of inoculating Douglas-fir with ectomycorrhizal fungi for improving survival and growth on landings in the interior of British Columbia.

In the interior of B.C., early successional species such as lodgepole pine (*Pinus contorta* var. *latifolia*) have been successfully established on disturbed sites (Plotnikoff et al. 2002) because they are well adapted to harsh conditions (Bulmer 1998). Lodgepole pine grows faster, is less susceptible to frost and adverse effects of soil compaction (Lotan and Perry 1983) and is often more heavily colonized by ectomycorrhizal fungi at time of outplanting than other conifers (Hunt 1992). Nonetheless, there is some interest in diversifying the disturbed landscape by successfully establishing Douglas-fir on degraded sites in the interior of B.C. To successfully establish Douglas-fir seedlings, ectomycorrhizae need to form and develop (Amaranthus 1992).

A high ectomycorrhizal colonization level should be associated with better seedling growth (Smith and Read 1997). Harvey et al. (1996) did not find that high growth rates corresponded to high ectomycorrhizal colonization levels with Douglas-fir on scraped clearcuts. However, good ectomycorrhizal colonization is only one factor that can positively affect growth rates. The number and diversity of fungal associates can also be important for survival and growth on harsh sites (Van der Heijden et al. 1998).

In a forest, the ectomycorrhizal inoculum potential (EIP) is defined as the ability of forest soil to maintain viable populations of ectomycorrhizal fungi (Amaranthus et al. 1990; Perry et al. 1987). The native EIP of disturbed soil is expected to be low. Thus outplanting noninoculated Douglas-fir seedlings on disturbed sites may result in poor growth. Douglas-fir is usually poorly colonized at time of outplanting (Hunt 1992). Thus

assessing the native EIP of landings can be valuable for forest companies interested in establishing Douglas-fir.

The EIP of a soil can be measured by calculating fungal species richness, diversity, and relative abundance that occurs on the roots of outplanted host plants (Jones et al. 1997; Parke et al. 1984; Perry et al. 1987). EIP is replenished by ectomycorrhizal fungi propagules (spores, mycelium, sclerotia, etc.) but survival of spores and mycelium is dependent on living host plants, which are often absent on disturbed sites (Allen et al. 1997). Three main factors that influence EIP following disturbance are: i) the balance of mortality and input of propagules; ii) recovery of host plants; and iii) diversity of fungal species (Perry et al. 1987).

The diversity of fungal species is important because it buffers the ectomycorrhizal community against environmental changes accompanying disturbance. Degraded forest sites usually have a low EIP compared to their undisturbed counterparts (Danielson 1988; Janos 1994; Reddell et al. 1999). Natural (i.e. native) inoculum is greatly reduced when topsoil is disturbed or removed (Amaranthus et al. 1990; Amaranthus and Trappe 1993). Humus, well decayed wood, and topsoil are the most biologically active parts of the soil and serve as temporary refuge for ectomycorrhizal fungi propagules. These are essential substrates for maintaining EIP and forest productivity (Amaranthus et al. 1999).

EIP is expected to decrease with the increasing severity of forest disturbance. In old-growth Douglas-fir stands Goodman and Trofymow (1998) found up to 100 different ectomycorrhizal types, while Kranabetter et al. (1999) found 52 on outplanted conifer seedlings in forest gaps. Roth and Berch (1992) found an average of 10 ectomycorrhizal types on Douglas-fir seedlings after one growing season on harsh clearcuts. Kranabetter et

al. (1997) discovered 9 ectomycorrhizal types on paper birch seedlings outplanted on a reclaimed skid road and on an old and hard to reforest clearcut in southwestern Oregon, Colinas et al. (1994) only described 3 ectomycorrhizal types on Douglas-fir seedlings.

In this study, inoculated and noninoculated Douglas-fir seedlings were outplanted on partially rehabilitated landings and standard reforestation sites where survival, growth, and foliar element content differences were assessed two years after outplanting. In addition a greenhouse bioassay was conducted with nonectomycorrhizal (at time of outplanting) Douglas-fir seedlings grown in landing and clearcut soils where growth, transpiration rates, foliar element content, and ectomycorrhizal status were measured after 20 weeks.

The main objectives of this research were to (1) assess soil and inoculation treatment effects on survival and growth of Douglas-fir; (2) to evaluate and compare the growth and ectomycorrhizal colonization levels of nonectomycorrhizal (at time of outplanting) Douglas-fir seedlings grown in landing and clearcut soils; (3) and to assess the native EIP of landing and clearcut soils.

CHAPTER 1

SURVIVAL AND GROWTH OF INOCULATED DOUGLAS-

FIR (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco)

SEEDLINGS OUTPLANTED ON PARTIALLY

REHABILITATED REFORESTATION SITES

Abstract

Commercial nurseries are often told that inoculating seedlings with appropriate ectomycorrhizal inoculants can substantially increase survival and growth of conifers on reforestation sites. To examine the possible usefulness of commercially available ectomycorrhizal inoculants, I studied their effects on degraded sites such as landings, burned piles, and clearcuts. Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco) seedlings were pre-inoculated with *Laccaria laccata* or *Rhizopogon parksii* (B.C. and Oregon sources) and then outplanted on shallow-tilled (15 cm depth) landings, deep-tilled (50 cm depth) landings, burned piles, and clearcuts. Shallow-tilled landing soils were significantly more compacted and drier than those in adjacent burned piles and clearcuts. Also, shallow- and deep-tilled landings had significantly lower levels of mineralizable-N content (forest floor + mineral soil) than adjacent clearcuts. No significant differences were observed in Douglas-fir growth between landing sites, or biological treatments. However, seedlings outplanted on deep-tilled landings were consistently taller compared to shallow-tilled landings and seedlings preinoculated with *Rhizopogon parksii* (Oregon) were consistently taller on all degraded sites. Survival was not affected by soil treatments or ectomycorrhizal inoculation treatments.

Introduction

Successful establishment of conifer tree seedlings on degraded sites depends on ectomycorrhizal development to capture scarce site resources (Amaranthus and Perry 1987; Danielson 1985; Perry et al. 1987). Ectomycorrhizal fungi can aid the seedlings in overcoming moisture and nutrient stress, and decrease transplant shock (Marx 1991) especially on disturbed sites (Perry et al. 1987). Seedlings must rapidly obtain the necessary nutrients and water not only to survive drought in the summer but also harsh winter conditions (Amaranthus et al. 1990; Amaranthus and Perry 1987).

On degraded sites such as burned piles, skid-roads and landings the EIP is often low (Perry et al. 1987) and pre-inoculated seedlings are expected to perform better compared to noninoculated seedlings (Bulmer 1998; Danielson 1985; Kropp and Langlois 1990). Hence there is a need for restoring the fungal component to the seedlings and/or to the soil (Amaranthus et al. 1990; Amaranthus and Perry 1987; Janos 1994). However, delayed growth is sometimes observed after outplanting inoculated seedlings (Rao et al. 1996; Smith and Read 1997). Ectomycorrhizal fungi can influence the carbon balance of tree seedlings via a number of normally interrelated processes, including net photosynthetic rate and mineral nutrition (Smith and Read 1997) and hence variable growth responses have been found on degraded sites (Castellano and Trappe 1991; Cram et al. 1999; Tosh et al. 1993). In a mature stand of Pacific silver fir (*Abies amabilis*) it was estimated that 15 % of net primary production was allocated to fungal structures excluding mycelium in the soil and turnover of the ectomycorrhizal rootlets (Vogt et al. 1982). In a young Douglas-fir stand, ectomycorrhizae biomass accounted for 7 – 8% of the total tree

standing crop and about 73% of total net primary production was invested in growth and maintenance of roots and ectomycorrhizae (Fogel and Hunt 1983).

Organic matter is important for establishment and growth of most young conifer seedlings and it also promotes good ectomycorrhizal development (Jurgensen et al. 1997). On degraded sites without organic matter, ectomycorrhizal fungi are stressed and utilize more of the seedling's photosynthates than usual (Rao et al. 1996). Roldán and Albaladejo (1994) found that growth of inoculated conifer seedlings was the greatest when small doses of organic matter were applied to planting holes. Colinas et al. (1994) noted that similar soil additions increased ectomycorrhizal formation but did not change the ectomycorrhizal types present. Pilz and Perry (1984) argued that inoculation of seedlings with forest or plantation site soil is more vital where soil organic matter is low and soil compaction is high. On a reclaimed skid road, small increases in root and shoot growth, and an increase in fungal species diversity were observed after seedlings were provided with fresh soil inoculum (Kranabetter et al. 1997).

The most widely used and most successful ectomycorrhizal inoculation practices have employed *Pisolithus tinctorius* (Pers.) Coker et Couch (Smith and Read 1997). Its wide geographic distribution, broad host range, and ability to increase growth of trees on disturbed sites, have made it a choice fungal inoculant for reclamation projects in the Southeastern United States and in countries where pines are exotics (Marx et al. 1992). In the Pacific Northwest, inoculation of seedlings with *Pisolithus tinctorius* have not always resulted in increased survival and growth (Castellano 1996) and a similar fungal inoculant has yet to be discovered (Amaranthus et al. 1999; Castellano and Trappe 1991; Marx et al. 1992).

Douglas-fir seedling responses to fungal inoculants are variable. Inoculation trials using *Laccaria laccata* increased early survival and growth of Douglas-fir seedlings on certain plantation sites (Berch and Hunt 1988; Hunt 1992; Perry et al. 1987), while on other sites no beneficial responses were observed (Hunt, unpublished data). However, *Laccaria bicolor* increased total volume of wood produced by 60%, 8 years after outplanting on clearcuts in France (Selosse et al. 2000). Perhaps the most promising results in the Pacific Northwest have been observed when inoculating Douglas-fir seedlings with *Rhizopogon parksii* and *Rhizopogon vinicolor* (Castellano 1996). *Rhizopogon* spp. are common and often dominant on Douglas-fir seedlings grown in disturbed forest soils (Molina and Trappe 1994). They show strong host specificity with Douglas-fir (Molina and Trappe 1982b) and they possess rhizomorphs (Trappe 1965) which can provide increased drought resistance to Douglas-fir seedlings (Parke et al. 1983). In Oregon, *Rhizopogon parksii* and *Rhizopogon vinicolor* were used to inoculate Douglas-fir and, five years after outplanting, survival, height, diameter, and biomass were significantly increased compared to controls (Castellano 1996). Factors such as site characteristics and fungal physiology were important in determining the outcome of inoculation trials. For instance, *Pisolithus tinctorius* consistently performs poorly on wet sites however significant benefits have been observed on droughty sites (Castellano 1996). Bledsoe et al. (1982) concluded that sources of fungal inoculum are very important and should be matched to planting site conditions.

The objectives of this study were: to assess the effect of soil treatments (tillage at two depths and burning) on soil physical and chemical properties and on survival and growth of Douglas-fir seedlings; and to determine if inoculating commercially-grown

Douglas-fir seedlings with various commercially available and native ectomycorrhizal inoculants (*Laccaria laccata* and *Rhizopogon parksii*) increases seedling survival and growth two years after outplanting on degraded forest sites in the southern interior of British Columbia.

Materials and methods

Study site description and experimental layout

This study is part of a Forest Renewal British Columbia- (FRBC) funded project of Xiao et al. (1996-2002) where the suitability of various ectomycorrhizal inoculants for conifer seedlings was assessed on a range of different reclamation and standard reforestation sites. The study was carried out at Miriam Creek (50°24'N, 118°57'W) which is approximately 40 km east of Vernon in the southern interior of British Columbia (Fig. 1). The study area is in the Interior Cedar-Hemlock (ICH) biogeoclimatic zone and part of the moist warm (mw) subzone, where winters are cool and wet, and summers are warm and dry (Meidinger and Pojar 1991). Miriam Creek was chosen because it had representative sites in the part of the ICH that were disturbed as a result of harvesting operation. The mean annual temperature in the study area is 6.5°C and precipitation is 420 mm with 34% falling as snow as measured at the nearest climate station (Environment Canada 1982). The dominant soil type is a Brunisolic Grey Luvisol (Soil Classification Working Group 1998; Xiao et al. 1996-2002) with a Mor humus form and the parent material is glacial till. Soil texture ranges from sandy loam to loamy sand.

Three replicate sites were selected in the study area using the following criteria (Bulmer, pers. comm.): mesic sites; uniform site conditions; adequate land area for

treatment to be applied (upper and middle harvested areas were roughly 47 ha, while the lower harvested area was 7 ha); typical examples of sites that were disturbed as a result of harvesting operation; and within geographic area specified as appropriate for the Douglas-fir seedlot that was available, based on seed transfer guidelines. The three study sites were selected to be at a range of elevation between about 800 and 1100 meters above sea level, are in different clearcuts, and are approximately 1500 m apart (Table 1).

The study sites were harvested in 1998 by ground skidding (Bulmer, pers. comm.). Soil rehabilitation treatments on the landings involved shallow (15 cm) and deep tilling (50 cm) using an excavator (Hitachi EX200) with a five tooth site preparation rake and bucket, respectively. The soil rehabilitation on the landings was carried out in the fall of 1999, and Douglas-fir seedlings were outplanted in the spring of 2000. At each of the three study sites, four plots were established: a plot on a shallow- and deep-tilled landing; a plot on a burned pile; and a plot in the adjacent clearcut. Available burned piles were used and their location on each block varied. The clearcut plots were randomly located near the landings. Plot size was approximately 0.33 ha with an average of 200, 40, and 153 seedlings on the shallow- and deep-tilled landing, burned pile, and clearcut plots, respectively. A small number of naturally regenerated seedlings of the following species were found on the sites: paper birch (*Betula papyrifera*), trembling aspen (*Populus tremuloides*), black cottonwood (*Populus trichocarpa*), lodgepole pine, western white pine (*Pinus monticola*), western larch (*Larix occidentalis*), Douglas-fir and western hemlock (*Tsuga heterophylla*). Lodgepole pine, western larch, Douglas-fir and western hemlock were the only species found regenerating naturally on the landings.

In the spring of 2000, inoculated and control Douglas-fir seedlings were outplanted in rows on each soil treatment plot. On each of the 3 study sites (i.e. blocks) shallow- and deep-tilled landing, clearcut, and burned pile plots were planted with seedlings from 4, 3, and 2 ectomycorrhizal inoculation treatments, respectively. Each row was made up, on average, of 8 seedlings inoculated with the same inoculant. On average 21, 11, and 9 rows were laid out on each landing (shallow and deep-tilled), clearcut, and burned pile plot, respectively. Rows of inoculated seedlings were not randomly assigned, however they were systematically dispersed across each plot.

Ectomycorrhizal inoculant treatments

In June of 1999, 10 week-old Douglas-fir seedlings were inoculated with commercially available and native ectomycorrhizal fungi and then grown in styroblocs at Nursery Extension Services (Surrey, B.C.). Styroblocs of seedlings were inoculated with one of the following three ectomycorrhizal inoculants: *Laccaria laccata* (Scop.:Fr.) Berk. & Br. by mycelial slurry; *Rhizopogon parksii* Smith from Oregon (OR) by spore suspension; and *Rhizopogon parksii* Smith from British Columbia (BC) by spore suspension. *Laccaria laccata* was collected by Mikro-Tek, Inc. near Jasper National Park, AB. in 1986 and was isolated from a fruiting body growing near Douglas-fir. *Rhizopogon parksii* (OR) sporocarps were collected by Mycorrhizal Applications Ltd. near Detroit Lake, Oregon in a 120 year old Douglas-fir stand at 2400 feet above sea level and put into spore suspension. *Rhizopogon parksii* (BC) was collected by Bill Chapman near Williams Lake, B.C. in 1998. Noninoculated seedlings served as controls but were not prevented from forming ectomycorrhizae (i.e. colonization by air-borne spores or hyphae from greenhouse soil mix).

Soil sampling and analysis

Soil samples were collected in June of 2001, using the excavation method (Blake and Hartge 1986), from the top of the mineral layer to a depth of 20 cm at 6 locations within each plot (following a grid pattern) to determine bulk density (Db), gravimetric moisture content (θ_m), pH, and nutrient concentrations. For clearcut plots, forest floor was sampled and placed in separate bags. No forest floor was found on landings.

Soil analyses were carried out by Dr. Chuck Bulmer (Ministry of Forests Research Branch), and by the Analytical Laboratory in Victoria, B.C. (Ministry of Forests Research Branch) using the following methods. Mineral soil samples were air dried and sieved through a 2 mm sieve. Soil pH in H₂O was determined using an electrometric pH meter. Total C and N concentrations were measured by high temperature combustion with a Fisons NA-1500 NCS Analyzer (McGill and Figueiredo 1993; Tiessen and Moir 1993). Mineralizable-N (min-N) was measured from ammonium-N in a KCl extract of soil after a 2 week anaerobic incubation at 30°C (Keeney 1982). Available P was determined using a UV/Visible spectrophotometer (Milton Roy Specronic 1201) at 880 nm following the Bray 1 (Dilute Acid-Fluoride) procedure (Kalra and Maynard 1991). Exchangeable K, Ca, Mg, Fe, Mn were measured by Inductively Coupled Argon Plasma (ICAP) spectrometer (ARL Model 3560) following a strong acid bath heated with a microwave (Kalra and Maynard 1991) using the Hendershot and Duquette 1986 method (Hendershot et al. 1993).

Initial seedling growth and ectomycorrhizal assessment

At time of lifting from the nursery (i.e. before outplanting), 20 Douglas-fir seedlings per inoculation treatment had been subsampled to measure height, root-collar diameter, and biomass (Xiao and Berch, pers. comm.). Percent ectomycorrhiza

colonization was also assessed for seedlings at time of lifting (Xiao, pers. comm.) after roots were cleared and stained since this is the only practical means by which minimally developed ectomycorrhizae can be detected (Roth and Berch 1992). Determination of percent ectomycorrhizal colonization was based on the gridline intercept method (Giovannetti and Mosse 1980). Survival and height were assessed for all seedlings in the fall of 2001 at the end of two growing seasons in the field.

Data analysis

The soil data were analyzed as a one-way randomized block design (Sit 1995). Two-factor ANOVA was used to test the following model:

$$Y_{ijk} = \mu + B_i + S_j + e_{ijk}$$

where Y is a measure for the k th experimental unit in the i th block and j th soil treatment; μ is the mean; B is block (site; $i = 1, 2, 3$); S is soil treatment (shallow- or deep-tilled landing; burn pile or clearcut; $j = 1, 2, 3, 4$); and e is random error within block \times soil treatment combination. The block and soil treatment effects were set as random and fixed factors, respectively. Groups of soil samples within block \times soil treatment combination were treated as the experimental units. Soil treatment effects on soil physical and chemical properties were analyzed.

The initial growth and ectomycorrhizal colonization (i.e. at time of lifting) data were analyzed as a complete randomized design. One-factor ANOVA was used to test the following model:

$$Y_{jk} = \mu + I_j + e_{jk}$$

where Y is a measure for the k th experimental unit in the j th inoculation treatment; μ is the mean; I is inoculation treatment (*Laccaria laccata*; *Rhizopogon parksii* (OR); *Rhizopogon parksii* (BC); control; $j = 1, 2, 3, 4$); and e is the unexplained residual error. Initial seedling height, diameter, shoot biomass, and percent ectomycorrhizal colonization were analyzed.

The survival and growth data were analyzed as a randomized block split-plot design (Sit 1995). Three-factor analysis of variance (ANOVA) (Zar 1999) was used to test the following model:

$$Y_{ijkh} = \mu + B_i + S_j + (BS)_{ij} + I_k + (BI)_{ik} + SI_{jk} + (BSI)_{ijk}$$

where Y is a measure for the h th experimental unit in the i th block, j th soil treatment, and k th inoculation treatment; μ is the mean; B_i , S_j , and $(BS)_{ij}$ represent the main-plot and correspond respectively to blocks (site; $i = 1, 2, 3$); main soil treatments (shallow- or deep-tilled landing, burn pile, clearcut; $j = 1, 2, 3, 4$), and main-plot error (block \times soil treatment combination); and I_k , $(BI)_{ik}$, SI_{jk} , and $(BSI)_{ijk}$ represent the split-plot and correspond respectively to the split-plot inoculation treatment (*L. laccata*; *R. parksii* (OR); *R. parksii* (BC); control; $k = 1, 2, 3, 4$), the block \times inoculation and soil \times inoculation interactions, and the split-plot error. Note that the main-plot error is the block \times soil interaction and the split-plot error is the three-factor interaction block \times soil \times inoculation. The block was set as a random factor while the soil and inoculation treatment

effects were set as fixed factors. Rows of seedlings within the block \times soil \times inoculation combination were treated as experimental units. Soil, inoculation, and soil \times inoculation effects on survival and growth were analyzed.

The Tukey HSD multiple comparison test was subsequently performed to differentiate significant differences ($p < 0.05$) for variables among soil and inoculation treatments. SYSTAT (version 10.2, SAS Institute Inc.) was used to accomplish all statistical tests.

Results

Soil physical and chemical properties

Soil from shallow-tilled landings had significantly higher bulk density and lower moisture content (in June 2000) than soils from burned piles and clearcuts (Table 2). Soil from burned piles was slightly alkaline and had a significantly higher pH than landings (shallow and deep-tilled) and clearcut soils.

Total C content was found in significantly greater amounts on clearcuts as compared to all other treatments (Table 3). However, shallow- and deep-tilled landings were devoid of forest floor but still averaged 40000 and 34000 kg ha⁻¹ of total C, respectively. Shallow- and deep-tilled landings and burned plots had significantly lower levels of mineralizable-N than clearcuts. Clearcuts had approximately 12 times greater amounts of mineralizable-N than other treatments. Exchangeable K concentrations and contents were significantly lower on shallow-tilled landings compared to burned piles and clearcuts, respectively. Also a trend for lower available P, although not significant ($p =$

0.20), was found on landings. Exchangeable Ca, Mg, and Fe concentrations and contents in the mineral soil were similar for all soil treatments (Table 3).

Seedling growth and ectomycorrhizal status at time of lifting

Before outplanting inoculated and control seedlings did not differ in height, diameter, or shoot biomass (Table 4). Percent ectomycorrhizal colonization of control seedlings was very low (1%) and was significantly lower ($p < 0.01$) than inoculated seedlings (36%) (Table 4). None of the inoculants provided significantly greater percent ectomycorrhizal colonization.

Growth and survival responses

After two growing seasons, seedlings growing on clearcuts and burned piles had significantly greater height increment than did seedlings growing on shallow or deep-tilled landings (Figure 2). Both shallow and deep-tilled landings produced very little height increment. Differences between shallow and deep-tilled soil rehabilitation treatments were not significant but deep-tilled landings, on average, showed larger height increments. Seedling survival did not differ among soil treatments. There were no significant differences in the height increment or survival between any of the inoculation treatments (Figure 2). Nevertheless, *Rhizopogon parksii* (OR) tended to show the greatest height increases on all soil treatments. The ANOVA showed that there were no interactions between the soil treatments and ectomycorrhizal inoculants for survival and height increment data.

Discussion

Influences of soil treatments on soil properties and seedling survival and growth

Shallow- and deep-tilled landings had quite similar physical and chemical soil properties. However, clearcuts and burned piles were clearly less compacted and held more water than shallow- and deep-tilled landings. Soil bulk densities and moisture content were in the same range of those found by Carr (1987) and Plotnikoff et al. (2002), respectively for landings in the interior of B.C. High compaction and low moisture content can inhibit conifer seedling root development (Conlin and van den Driessche 1996a) and ectomycorrhizal growth (Skinner and Bowen 1974). High levels of compaction and low moisture content may have reduced seedling growth on landings. Shallow-tilled landings could be expected to have fewer large pores and, in times of heavy rain, soils in these areas may become waterlogged.

Total N, available P, and K contents on landings were in the same range reported by Carr (1987). It was not surprising to find total C and mineralisable-N significantly lower on both shallow- and deep-tilled landings than in clearcuts. Low levels of organic matter and woody debris on landings were likely responsible. Microbial activity was probably low in mineral soils on landings. Microbial activity is positively correlated with total C and mineralisable-N (Ballard and Carter 1985; Myrold 1987). Low levels of available P were found on shallow- and deep-tilled landings although these values were not significantly different than controls, perhaps due to insufficient sampling. Unfavorable soil conditions likely created much stress for outplanted Douglas-fir seedlings. Seedlings were raised under ideal growing conditions in the nurseries thus, transplant shock was probably quite pronounced.

Clearcut and burned pile-grown seedlings had much greater height increments than both shallow- and deep-tilled landing-grown seedlings. Douglas-fir seedlings and ectomycorrhizal fungi usually encounter growth problems in compacted soil (Skinner and Bowen 1974; Wert and Thomas 1981). I found that seedlings growing on deep-tilled landings showed slightly, but not significantly, greater height increments amongst all inoculation treatments, compared to shallow-tilled landings. Early growth of Douglas-fir may be impaired on landings because of remaining compaction, deficiencies in mineralisable-N, and harsh summer climatic conditions. I observed that Douglas-fir seedlings growing on both shallow- and deep-tilled landings showed signs of stress.

Chlorosis (see appendix A) was very common and many seedlings had very few live buds. Foliar analysis revealed that seedlings growing on deep-tilled landings had low P, K, Ca, and Cu contents and potentially toxic levels of Fe and Al (see chapter 2). In these conditions Douglas-fir may not overcome transplant shock, and is perhaps more sensitive than other commercial species such as lodgepole pine to compacted and nutrient deficient soils found on landings (Plotnikoff et al. 2002). Survival and growth of inoculated Douglas-fir is variable on reforestation sites (Castellano 1996). Significant increases in survival and growth of inoculated Douglas-fir in bare-root nursery and on plantation sites in Spain have been found (Castellano and Trappe 1985; Pera et al. 1999). However, on Vancouver Island clearcuts and on harsh dry sites in eastern Washington similar to the clearcuts and landings, respectively in this study, no significant increases in survival and growth of inoculated Douglas-fir were reported (Berch and Roth 1993; Bledsoe et al. 1982). It seems evident that compacted (Page-Dumroese et al. 1998; Wert

and Thomas 1981) and organic poor sites (Page-Dumroese et al. 1990) negatively affect growth of Douglas-fir regardless of ectomycorrhizal status.

Influences of ectomycorrhizal inoculants on seedling survival and growth

Overall, ectomycorrhizal inoculation did not significantly improve early survival and growth of Douglas-fir seedlings on degraded sites. In general, *Rhizopogon parksii* (OR) tended to promote greater seedling height increment on all soil treatments but differences were not significant as compared to controls. In the Pacific Northwest, Douglas-fir has been inoculated with various ectomycorrhizal fungi species using different inoculation methods. Variable results were obtained when Douglas-fir was inoculated with *Laccaria laccata*. Morgan (1985) and Sinclair et al. (1982) reported increases in survival and height while Hunt (unpublished data) found no positive growth effect. Furthermore, Bledsoe et al. (1982) assessed survival and growth of Douglas-fir seedlings inoculated with *Laccaria laccata*. They found that *Laccaria laccata* did not compete well after outplanting and seedlings did not perform significantly better than controls. They emphasized the importance of matching fungal inoculum source with the planting site conditions.

Some reforestation sites have a relatively high EIP where native ectomycorrhizal fungi are very competitive for newly-forming roots of outplanted seedlings (Bledsoe et al. 1982). Since both *Rhizopogon parksii* inoculants failed to increase survival and growth I think inoculating with *Rhizopogon vinicolor* which has consistently increased performance (Castellano 1996; Castellano and Trappe 1985) merits further research. Inoculating seedlings at time of planting or in the nursery with native ectomycorrhizal fungi from the adjacent plantations is also worth further investigation. In Oregon and California,

Amaranthus and Perry (1987 and 1989) reported increased survival and ectomycorrhiza formation of bare-root Douglas-fir by adding small amounts (150 ml) of plantation soil to planting holes. If this alternative were to be successful, nurseries could consider implementing this practice for seedlings destined to be outplanted on degraded or difficult sites (Amaranthus and Perry 1989). To avoid dissemination of unwanted organisms throughout the nursery (i.e. on the regular stock) this practice should be done in separate or isolated areas of the nursery. It would also be useful to conduct a cost-benefit analysis before attempting this practice. Having a diverse ectomycorrhizal community is probably more beneficial for seedlings since ectomycorrhizal fungi provide different benefits for different hosts in different environments (Perry et al. 1987).

In my study, at time of lifting, roots of inoculated seedlings were colonized at 36% compared to 1% for control seedlings. Similarly Hunt (1992) found inoculated and control Douglas-fir seedlings colonized at 52% and 0%, respectively at time of planting. Other conifers raised under similar nursery conditions in the Pacific Northwest typically have much better colonization at time of planting. For instance, inoculated and control Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) and lodgepole pine were both colonized in the 90% range (Hunt 1992; Xiao et al. 1996-2002). Both Xiao et al. (1996-2002) and I (Fig. 4) showed that Douglas-fir readily forms abundant ectomycorrhizae within months. In this study, I suspect nursery conditions did not favor high colonization levels on Douglas-fir seedlings. Current nursery cultural conditions could be modified to permit higher colonization at time of planting. Maintaining soil pH between 4.0 and 6.0, keeping N and P fertilization rates around 55 and 100 kg ha⁻¹, respectively, promoting timely irrigation and allowing adequate substrate aeration (Amaranthus et al. 1996), and

using selected fungicides such as ferimate, captan, and benomyl (i.e. which stimulate ectomycorrhizal development) could result in improved seedling and ectomycorrhizal development (Linderman 1987; Marx and Cordell 1987).

Conclusions

Commercially available ectomycorrhizal inoculants did not increase survival or growth of Douglas-fir seedlings on landings, burned piles, or clearcuts. I found that landings remained compacted and poor in mineralizable-N regardless of soil tilling treatments. Soil rehabilitation treatments conducted on landings, such as deep tilling did not significantly increase survival and height, compared to shallow tilling. Beyond allowing for planting to occur, deep tilling probably does not provide an immediate benefit for seedlings. In this study, ectomycorrhizal inoculants on Douglas-fir seedlings did not produce highly colonized seedlings in the nursery therefore I cannot advocate commercial inoculation as a tool for improving survival and growth under current nursery cultural conditions. In that respect, more research is needed to determine the nursery conditions that can be favorable to both ectomycorrhizal development and growth of inoculated Douglas-fir seedlings. Persistence of inoculated fungi is a critical factor determining seedling survival and growth responses on disturbed soils. Before valid conclusions on the effectiveness of ectomycorrhizal inoculants can be made, the rate and level of colonization after outplanting should be determined in future research.

Table 1. Characteristics for the Miriam Creek study site

| Site Characteristics | Upper block | Middle block | Lower block |
|----------------------|-------------------------|-------------------------|-------------------------|
| Slope (%) | 10 | 10 | 10 |
| Aspect (°) | NE | NE | NE |
| Elevation (m) | 1050 | 950 | 790 |
| Soil classification | Brunisolic Grey Luvisol | Brunisolic Grey Luvisol | Brunisolic Grey Luvisol |
| Soil texture | Sandy loam | Sandy loam | Sandy loam |
| Forest humus form | Mor | Mor | Mor |

Table 2. Bulk density, forest floor depth, moisture content and pH of soils from landings tilled to a depth of 15cm (L15cm), landings tilled to a depth of 50 cm (L50cm), clearcut and burn plots at Miriam Creek.

| Soil treatment | Bulk density (g cm ⁻³) | Forest floor depth (cm) | Moisture content (Θ _m [†]) | pH |
|----------------|------------------------------------|-------------------------|---|-----------------|
| L15cm | 1.59a (0.15) | 0.00a (0) | 0.12a (0.02) | 6.07a (0.31) |
| L50cm | 1.26ab (0.15) | 0.00a (0) | 0.14a (0.02) | 6.00a (0.35) |
| Clearcut | 0.82bc (0.13) | 5.36b (0.8) | 0.28b (0.003) | 5.27a (0.06) |
| Burn | 0.74c (0.16) | 0.00a (0) | 0.28b (0.06) | 7.72b (0.24) |
| <i>p</i> | 0.003*** | 0.009*** | 0.0001*** | 0.004*** |

Note: Values are means with standard errors in parentheses.

*, **, *** Significant difference at $p < 0.1$, 0.05 , and 0.01 , respectively.

Means in the same column followed by the same letter are not significantly different (a $p < 0.05$ was used for Tukey's HSD multiple comparison test).

[†]Θ_m: kg H₂O/kg soil

Table 3. Concentrations and contents of soils from landings tilled to a depth of 15cm (L15cm), landings tilled to a depth of 50 cm (L50cm), clearcut and burn plots at Miriam Creek. Concentrations are for mineral soils (0-20 cm depth) while total element contents are for the mineral soils (0-20 cm depth) plus the forest floor.

| Soil treatment | Exchangeable | | | | | | | | |
|---|-----------------------|------------------------|------------------------|------------------------|------------------------|-----------------------|------------------------|------------------------|------------------------|
| | Total C | Total N | Min. N | Avail. P | K | Ca | Mg | Fe | Mn |
| <i>Concentrations</i> (g kg ⁻¹) | (g kg ⁻¹) | (mg kg ⁻¹) | (mg kg ⁻¹) | (mg kg ⁻¹) | (mg kg ⁻¹) | (g kg ⁻¹) | (mg kg ⁻¹) | (mg kg ⁻¹) | (mg kg ⁻¹) |
| L15cm | 14.7a (6.2) | 0.49a (0.17) | 1.3a (0.1) | 22a (8) | 51a (11) | 2.05a (0.11) | 269a (60) | 1.1a (0.8) | 59a (28) |
| L50cm | 17.4a (10.2) | 0.54a (0.24) | 1.9a (1.0) | 23a (12) | 65ab (25) | 2.08a (0.18) | 271a (76) | 2.8a (1.4) | 42a (8) |
| Clearcut | 27.9a (7.6) | 1.08a (0.35) | 21.7b (7.6) | 75a (37) | 108ab (25) | 2.20a (0.44) | 137a (12) | 4.2a (1.6) | 38a (12) |
| Burn | 27.9a (10.7) | 0.68a (0.14) | 3.6ab (1.56) | 68a (18) | 142b (9) | 2.65a (0.71) | 282a (59) | 0.0a (0.0) | 6a (2) |
| <i>p</i> | 0.47 | 0.21 | 0.03** | 0.20 | 0.03** | 0.77 | 0.37 | 0.05* | 0.13 |
| <i>Contents</i> (kg ha ⁻¹) | | | | | | | | | |
| L15cm | 40000a (14000) | 1420a (350) | 4a (1) | 63a (27) | 160a (30) | 6340a (450) | 890a (280) | 2.5a (1.6) | 170a (64) |
| L50cm | 34000a (16000) | 1150a (360) | 4a (1) | 50a (23) | 150a (40) | 4950a (310) | 690a (260) | 6.7a (3.9) | 91ab (8) |
| Clearcut | 98000b (23000) | 2270a (540) | 50b (12) | 125a (49) | 2480b (750) | 4550a (610) | 1650a (470) | 7.5a (1.2) | 101ab (18) |
| Burn | 35000a (11000) | 930a (140) | 5a (2) | 107a (45) | 210a (40) | 3640a (760) | 380a (20) | 0.0a (0.0) | 9b (3) |
| <i>p</i> | 0.02** | 0.07* | 0.006*** | 0.26 | 0.01** | 0.07* | 0.16 | 0.08* | 0.04** |

Note: Values are means with standard errors in parentheses. *, **, *** Significant difference at p < 0.1, 0.05, and 0.01, respectively. Means in the same column followed by the same letter are not significantly different (a p < 0.05 was used for Tukey's HSD multiple comparison test).

†Θ_m: kg H₂O/kg soil

Table 4. Inoculated Douglas-fir seedling growth and percent ectomycorrhizal colonization at time of lifting from the nursery (1999).

| Inoculation treatment | Height (cm) | Diameter (mm) | Shoot dry biomass (g) | Ectomycorrhizal colonization (%) |
|------------------------|----------------|------------------|-----------------------------|--|
| Control | 21.0a (0.7) | 4.04a (0.16) | 1.66a (0.10) | 1a (0) |
| <i>L. laccata</i> | 20.1a (0.6) | 3.87a (0.13) | 1.62a (0.08) | 38b (3) |
| <i>R. parksii</i> (BC) | 21.1a (0.6) | 4.21a (0.16) | 1.82a (0.07) | 33b (2) |
| <i>R.parksii</i> (OR) | 21.5a (0.7) | 3.89a (0.09) | 1.70a (0.09) | 38b (2) |
| <i>p</i> | 0.46 | 0.27 | 0.37 | < 0.01 |

Note: Values are means with standard errors in parentheses. Means in the same column followed by the same letter are not significantly different ($p < 0.05$).

Fig. 1. Map of the study area and the layout. Miriam Creek's location is marked by the dot in the ICH biogeoclimatic zone (shaded area) on the map of British Columbia. The installation scheme shows the arrangements of treatment plots for a typical block.

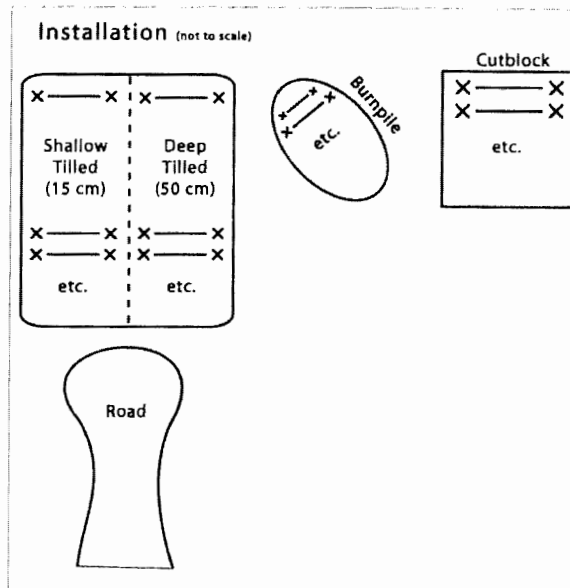
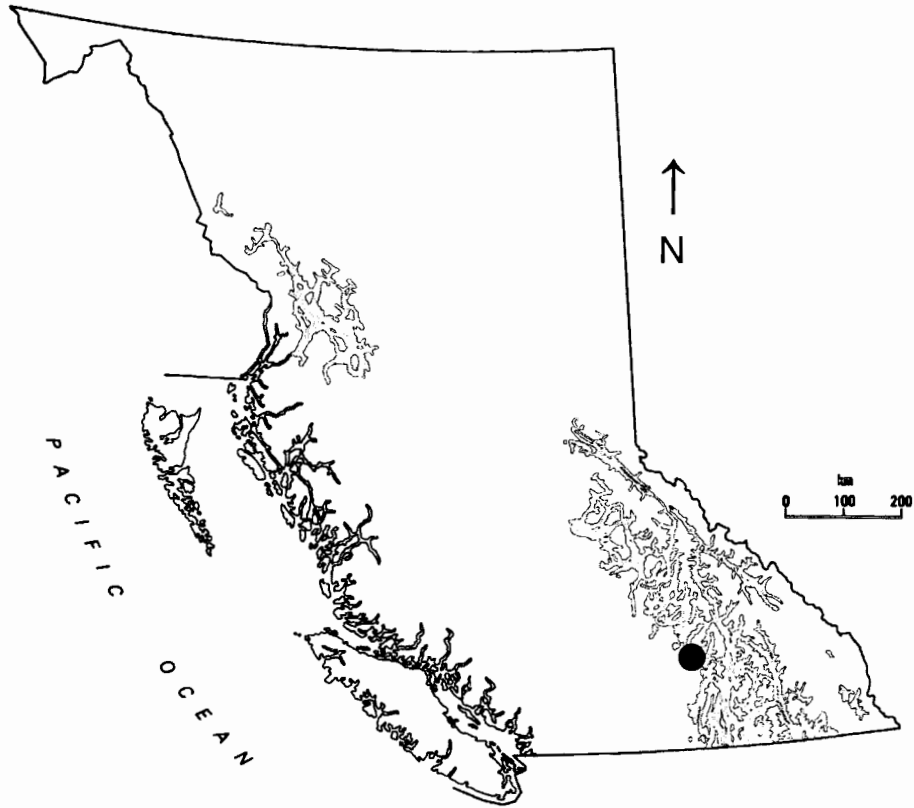
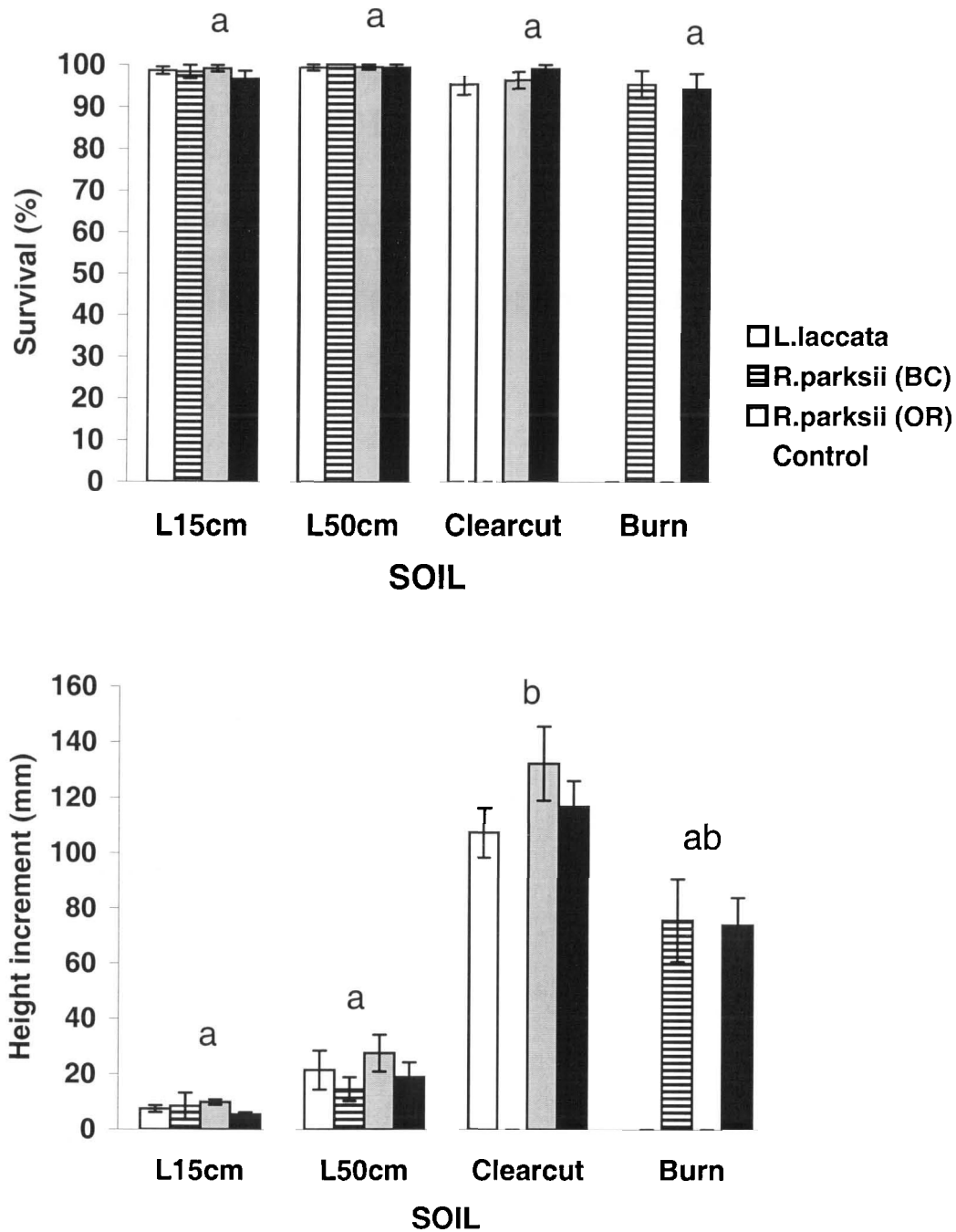


Fig. 2. Early survival and growth of Douglas-fir seedlings at Miriam Creek after two growing seasons. Significant soil treatment effects (height increment $p = 0.001$; survival $p = 0.10$) are designated by different letters and inoculation treatment (height increment $p = 0.47$; survival $p = 0.67$) effects by asterisks, where *, **, and *** indicates significant differences at $p < 0.1$, < 0.05 , and < 0.01 level, respectively.



CHAPTER 2

ECTOMYCORRHIZAL STATUS AND GROWTH OF OUTPLANTED DOUGLAS-FIR (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco) SEEDLINGS ON PARTIALLY REHABILITATED LANDINGS AND CLEARCUTS

Abstract

Under natural conditions Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco) forms ectomycorrhizae. Ectomycorrhizal formation and growth of Douglas-fir is usually reduced on degraded sites such as landings. In this study, foliar nutrient concentration, ectomycorrhizal status, and growth of Douglas-fir were determined after two growing seasons on partially rehabilitated landings and clearcuts in the southern interior of British Columbia, and after one growing season in a greenhouse. The field and greenhouse bioassays were laid out as randomized block designs with 90 and 126 Douglas-fir seedlings, respectively. Seedlings were grown on deep-tilled (50 cm depth) landings and clearcuts (field bioassay) and in soil collected from the landings and clearcuts (greenhouse bioassay). A total of 13 and 6 morphologically distinct ectomycorrhizae were characterized in the field and greenhouse, respectively. Douglas-fir seedlings in both the field and greenhouse bioassays were heavily colonized by *Rhizopogon vinicolor*-like fungus in landing soil. These results demonstrate that on degraded sites, Douglas-fir seedlings can readily form ectomycorrhizae with well-adapted fungi. In the greenhouse, Douglas-fir seedlings had a higher percent colonization when grown in landing soil compared to clearcut soil. In both the field and greenhouse bioassays, seedlings had greater height, diameter and dry root biomass when grown in the clearcut soil compared to landing soil. This suggests that amending landings with organic matter coupled with deep-tilling may be required to obtain adequate growth of Douglas-fir seedlings. The types of ectomycorrhizae and growth of Douglas-fir seedlings that occurred in the field were similar to those in the greenhouse. This confirms that greenhouse bioassays can be used to predict growth response and dominant types of ectomycorrhizae that can form on degraded sites.

Introduction

Conifer trees in North America are practically all ectomycorrhizal (Molina and Trappe 1982b). Ectomycorrhizae are important for seedling survival and growth since they are well known for their ability to increase nutrient and water uptake, resistance to root pathogens, and tolerance to environmental extremes (Smith and Read 1997). However, under harsh conditions with high levels of soil compaction and low levels of organic matter, ectomycorrhiza and seedling growth may be adversely affected (Amaranthus 1992; Skinner and Bowen 1974). Formation of ectomycorrhizae is variable (Amaranthus et al. 1996; Page-Dumroese et al. 1990) but reduced inoculum potential of native ectomycorrhizae is often associated with such degraded soil conditions (Perry et al. 1987; Pilz and Perry 1984).

In the Pacific Northwest, very little research has addressed ectomycorrhizal development on degraded sites such as landings. Poor growth and reduced native ectomycorrhizal fungi of paper birch seedlings were reported on skid roads (Kranabetter et al. 1997). However, no known studies have been conducted on the effects of harsh conditions found on landings on the growth and ectomycorrhizal development of Douglas-fir seedlings. Most research on Douglas-fir has been conducted on burned and undisturbed clearcuts where a relationship is often found between the severity of disturbance and poor ectomycorrhizal development (Amaranthus et al. 1999). On 27 year-old unsuccessfully reforested clearcuts at high elevation, Douglas-fir seedlings formed very few ectomycorrhizal root tips (Amaranthus and Perry 1987). On these sites, inoculating Douglas-fir with native ectomycorrhizae increased seedling survival by 50%.

Ectomycorrhizal formation on Douglas-fir may be more sensitive to compacted soil than other conifers such as lodgepole pine and western white pine (Page-Dumroese et al. 1998).

In the interior of B.C., early successional species such as lodgepole pine have been favored for silvicultural planting on disturbed sites because they are well adapted to harsh conditions (Bulmer 1998). There is some interest in diversifying the disturbed landscape by successfully establishing Douglas-fir on degraded sites in the interior of B.C. However, in order to successfully establish Douglas-fir seedlings, ectomycorrhizae need to form and develop (Amaranthus 1992).

It is uncertain how nonectomycorrhizal Douglas-fir at time of outplanting respond to conditions found on landings. On some harsh sites, Amaranthus et al. (1987) found that ectomycorrhizal formation and growth of Douglas-fir was significantly reduced however, other studies have found the opposite with other conifers (Alvarez et al. 1979; Amaranthus et al. 1996). Research of this type may help forest companies and nurseries decide if investing in commercial ectomycorrhizal inoculants is worthwhile. Research is needed to assess the effects of soil conditions found on landings, on Douglas-fir growth, ectomycorrhizal status, and the inoculum potential of native ectomycorrhizal fungi.

The objective of this research was to evaluate and compare ectomycorrhizal percent colonization and percent occurrence of Douglas-fir seedlings; the EIP (relative abundance, richness, diversity, and evenness of ectomycorrhizal fungi); and growth of Douglas-fir seedlings on partially rehabilitated landings and adjacent clearcuts. Secondly, I investigated relationships among percent ectomycorrhizal colonization, seedling growth variables and foliar element concentrations. Thirdly, I assessed the usefulness of performing a greenhouse bioassay by evaluating the similarity between morphotypes found

in the greenhouse and in the field. Finally, I used the greenhouse bioassay to investigate Douglas-fir seedling transpiration rates under drought conditions.

Materials and methods

Study site description and layout

See chapter 1 for a description of the study site and the experimental layout.

Field bioassay – Experimental design

Douglas-fir seedlings were outplanted on deep-tilled landings and on adjacent clearcut in the spring of 2000 (Chuck Bulmer, pers. comm.). At each of three harvested sites, two plots were established: a plot on the landing and a plot in the adjacent clearcut. The clearcut plot was randomly located near the landing. Plot size was approximately 0.33 ha with an average of 153 and 202 seedlings on the clearcut and landing plots, respectively.

In September 2001, 15 Douglas-fir seedlings were randomly selected and destructively sampled from each treatment plot (i.e. 15 seedlings were sampled from each deep-tilled landing and each clearcut; 15 seedlings x 2 treatments (landing, clearcut) x 3 blocks (lower, middle, upper) = 90 seedlings). Those seedlings had been dipped in dilute solution of a plant growth promoting bacteria (PGPR) (Chanway 1997) at time of outplanting (spring of 2000). The PGPR inoculant *Bacillus polymyxa* L6-16-R which is a rifamycin-resistant derivative of strain L6 (Shishido et al. 1996) was originally isolated from rhizosphere soil containing roots of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) in a 45-year-old pasture (Chanway et al. 1990). These seedlings were treated as controls since PGPR effects are of short duration (i.e. 3 to 6

months) and are often confounded in the field where a very complex and diverse community of rhizosphere bacteria is present (Chris Chanway, pers. comm.). Furthermore, Shishido et al. (1996) demonstrated that this PGPR strain did not influence the ectomycorrhizal status of seedlings. At the sampling date, seedlings had been growing in the field for 2 growing seasons (16 months).

Greenhouse bioassay – Experimental design

Bulk mineral soil was collected from each of the 3 landings and 3 adjacent clearcuts in October 2000. Mineral soil was collected with a shovel to a depth of approximately 20 cm at random locations in each plot. Surface organic layers were excluded from the soil samples. The soils were sieved using a large screen (1 cm mesh size) excluding coarse materials (coarse fragments and coarse woody debris) to produce a homogeneous growing medium. The soil samples were then placed in black plastic bags and stored for 18 weeks in a shed at cool temperatures (5°C or colder) at the Kalamalka Research Station in Vernon.

Douglas-fir seedlings (seedlot 31851 for the greenhouse bioassay) were grown in styroblocks by Riverside Nursery in Vernon for one growing season. Originally the seedlings were to be used to assess the effectiveness of various commercially available ectomycorrhizal inoculants (Shannon Berch, pers. comm.). In June 2000 the seedlings had been inoculated in Riverside Nursery with: *Laccaria laccata* (Scop.:Fr.) Berk. & Br. (Mikrotek Inc., Timmins ON) by mycelial slurry; *Rhizopogon parksii* Smith from Oregon (OR) (Mycorrhizal Applications, Grants Pass OR); and *Rhizopogon parksii* Smith from British Columbia (BC) by spore suspension. Seedlings had been kept for 12 weeks in cold storage (- 2 °C) and then were permitted to thaw to enable root examination. Ten seedlings

per inoculation treatment had been randomly selected to verify ectomycorrhizal colonization and it was determined that all seedlings were nonectomycorrhizal. The seedlings were thus available to be used in the present study.

In March 2001, 21 seedlings by inoculation treatment were randomly selected and randomly assigned to a soil treatment plot (i.e. 21 seedlings \times 2 soil treatments (landing, clearcut) \times 3 blocks (lower, middle, upper) = 126 seedlings). An equal number of seedlings per inoculation treatment were selected just in case inoculation treatment had some effect on the seedlings. The seedlings were potted in black plastic 3.2 l (175 mm \times 180 mm) pots (Listo Products Ltd.) with 6 leaching holes per pot and randomly placed on greenhouse benches. Pots had been cleaned and sterilized by letting them soak in Javex (1:10) for 30 minutes. Perlite (Micronise Ultratech) mixed with collected soil samples (1:1) was used as the potting medium. Re-randomization of pot location on greenhouse benches was performed once a month.

The seedlings were grown in a greenhouse at Simon Fraser University (49°17'N, 123°55'E), Burnaby, B.C at 350 meters above sea level (Crampton 1979). Abiotic conditions were controlled, therefore the greenhouse provided ideal growing conditions for Douglas-fir seedlings to form ectomycorrhizae. High-pressure sodium lamps were used with a photoperiod of 15 hours per day until the native photoperiod surpassed this value in mid June. Day and night temperatures were set at 20 °C (\pm 5 °C). The seedlings were watered when volumetric soil moisture was < 0.1 v/v, determined using a theta probe (Delta-T Theta Probe Meter type HH1). After 20 weeks, seedlings were destructively sampled and stored at 3.5°C (roots) and at -20°C (tops).

Foliar analysis

All of the needles were removed from each seedling from both the field (90 seedlings) and greenhouse (126 seedlings) bioassays, dried overnight at 70°C and then weighed. Needles from each sample were then placed in paper bags and later ground with a mortar and pestle. Foliar analyses were carried out by the Analytical Laboratory in Victoria, B.C. (Ministry of Forests Research Branch). Total N, P, K, Ca, Mg, S, Fe, Mn, B, Zn, Cu, and Al were measured for each sample with an ICAP spectrometer following a strong acid bath heated by microwave (Kalra and Maynard 1991).

Ectomycorrhizae morphotyping

Fifteen and five seedlings were randomly selected per treatment × block combination from the field (90 seedlings) and greenhouse (30 seedlings), respectively for morphotyping. Entire root systems were carefully washed under cold running water. All lateral and egressed roots were examined in a large dish with water using a dissecting microscope (Wild Heerbugg Switzerland, 6-50X magnification). Each unique ectomycorrhizal type or suspected ectomycorrhiza was placed in a Petri dish with water for further characterization. To determine abundance by morphotype, a subsample was examined. Roots were cut into approximately 2 cm segments and placed over a grid of 21 cells (5 cm²). The 2 cm root segments were randomly placed in small bunches into the cells and then one bunch was randomly selected. Subsequent bunches were randomly chosen until 300 tips were selected.

Counts of active ectomycorrhizal root tips were performed. An active ectomycorrhiza was one that had the following characteristics: turgid, generally smooth-surfaced, and possessed surface structures such as emanating hyphae or rhizomorphs

(Harvey et al. 1976). No distinction or count was made between inactive ectomycorrhizal and dead root tips. However, a descriptive account was made when large quantities of inactive and/or dead root tips were encountered. Only ectomycorrhizal root tips with a length at least 1.5X greater than its width were counted. Root tip size criterion was similar to that used by Roth and Berch (1992). Squashed and cross-sectioned ectomycorrhizal tips were prepared and observed using a compound microscope (Wild Heerbugg Switzerland, 40-1000X magnification). Subsamples of all morphologically distinct ectomycorrhizae were then kept in frozen water until further characterization with Shannon Berch at the Analytical Laboratory in Victoria, B.C. (Ministry of Forests Research Branch) where longitudinal sections were observed.

Ectomycorrhizal roots were classified following the procedure described by Goodman et al. (1996). Ectomycorrhizal roots were categorized as ectomycorrhizal, ectomycorrhizal but undifferentiated, and nonectomycorrhizal. Morphological descriptions were mostly made with reference to Goodman et al. (1996), Goodman and Trofymow (1998), and Roth (1990) and occasionally with Agerer (1985-1998) and Ingleby et al. (1990). Some morphotypes were not readily identifiable to genus or species and were thus classified as unknown (e.g. unknown (flaky yellow)). Photographs of the most common morphotypes can be found in appendix A.

Extraction, amplification, restriction endonuclease digestion, and gel electrophoresis of ectomycorrhizal DNA

A subsample of three root tips was randomly collected for each distinct morphotype encountered. The subsamples were placed in 1-ml micro-vials, and stored at -80°C until DNA analysis was carried out. All molecular work was carried out in the lab

with the assistance of Dr. Keith Egger, UNBC. I used the procedure outlined in Mah et al. (2001) except for the following modifications.

For DNA extraction, one randomly selected frozen root tip for each distinct morphotype encountered was placed in micromortars and then was refrozen at -20°C for 15-30 min, and then crushed with micropestles (Mandel Scientific). A slurry was made in 1.5 ml Eppendorf tubes by adding $175\mu\text{L}$ 2X CTAB buffer (1.4 M NaCl, 100 mM Tris-HCl (pH 8.0), 20mM ethylenediaminetetraacetic acid (EDTA) (pH 8.0), 2 CTAB, 0.2% β -mercaptoethanol), and incubated in a 60°C water bath for 1 hour, followed by addition of $350\mu\text{L}$ chloroform- isoamyl alcohol (24:1), and vortexed briefly. The slurry was then centrifuged at 13 000 X g for 10 minutes at room temperature. The top aqueous phase was transferred to new 1.5mL Eppendorf tubes and $350\mu\text{L}$ of cold (-20°C) absolute isopropanol was added to precipitate DNA. Tubes were inverted repeatedly for 1 minute, let sit at -10°C for 5 minutes, inverted a few more times, and then centrifuged at 13 000 X g for 10 minutes at room temperature. The top aqueous phase (isopropanol) was gently poured out leaving approximately $50\text{-}70\mu\text{L}$ at the bottom of the tube. A repeated DNA pellet washing was performed by adding $175\mu\text{L}$ of cold 70% ethanol, then gently vortexing tubes, and centrifuging at 13 000 X g for 3 minutes at room temperature. Finally, DNA was dried overnight in a desiccator. DNA pellet was resuspended in $50\mu\text{L}$ TE buffer and stored at -20°C for further use.

Polymerase Chain Reaction (PCR) amplification was accomplished with a PTC-100 Programmable Thermal Controller (MJ Research, Inc.). DNA was denatured at 94°C for 30 s, annealing at 50°C for 53 s, and extension at 72°C for 300 s increasing 5 s per

cycle for 35 cycles. Pure UltraTherm™ DNA polymerase (BIO/CAN Scientific), with primers ITS1 (White et al. 1990) and NL6Bmun (Egger 1995) were used.

For digestion of PCR products a 1 Kb DNA ladder from Invitrogen (Cat. # 15615-016, lot # 1111573) was used to estimate RFLP band sizes. RFLP patterns were analyzed manually and with the Gene Profiler v.4.03 software (Scanalytics, Inc.). Log piecewise linear curve fitting was used to calibrate fragment sizes. Comparisons were done to differentiate or group various morphotypes together. RFLPs previously generated from other studies were used to attempt genus or species identification.

Ectomycorrhizal status (colonization, abundance, occurrence, richness, diversity and evenness)

Following morphotyping of the 90 field and 30 greenhouse seedlings, percent ectomycorrhizal colonization was determined based on 300 randomly subsampled root tips. In addition, ten greenhouse seedlings per treatment × block combination (60 seedlings) were subsampled to determine percent ectomycorrhizal colonization by clearing and staining the roots. A modification of Phillips and Hayman (1970) clearing and staining method was used: washed roots were cleared by heating in 10% KOH at 75-90°C for 1-2 hours, retrieved on a 63-µm sieve, rinsed in water, bleached using H₂O₂ solution (30ml 10% H₂O₂ + 3ml NH₄OH + 567ml dH₂O) for 1 hour, stained with 0.1% (w/v) Trypan Blue in 85% lactic acid – glycerol – dH₂O (1:1:1).

The number of morphotypes and their relative abundance were determined.

Relative abundance (RA) was calculated as follows:

$$RA = \frac{Sm}{\sum_{i=1}^n Sm_i} \times 100$$

where S_m is the number of seedlings with the morphotype m

n is the number of morphotypes

When a morphotype was seen during the initial root system examination but not found in the random 300 root tip count, a value of 0 was assigned (i.e. indicating it was present but with negligible colonization).

Five seedlings per treatment \times block combination were randomly selected to determine the percent occurrence of specific morphotypes on the field and greenhouse-grown seedlings. Percent morphotype occurrence (POM) was calculated as follows:

$$\text{POM} = \frac{S_m}{T} \times 100$$

where S_m is the number of seedlings with the morphotype m

T is the total number of seedlings.

Richness (i.e. number of morphotypes per seedling), diversity (Shannon diversity index,

$$H' = - \sum_{i=1}^M p_i \ln p_i, \text{ where } p_i \text{ is the proportion of all individuals on the seedling that belong to}$$

morphotype i and M is the total number of morphotypes on the seedling), and evenness

(Shannon evenness index, $E = \frac{H'}{\ln M}$) were calculated for each treatment (Magurran 1988).

A diversity index sensitive to rare morphotypes was needed so I chose the Shannon diversity index (Peet 1974).

Seedling growth measurement

At the beginning of the field bioassay (spring 2000), 20 seedlings were randomly selected and height, root-collar diameter, and dry biomass (root and shoot) were measured (Berch and Xiao, pers. comm.). At the end of the field bioassay (fall 2001) seedling height and root-collar diameter were recorded in the field for the 90 seedlings that were

destructively sampled. Root systems from all 90 seedlings with surrounding soil were placed in plastic bags and stored (up to 4 months) at 3.5°C until ectomycorrhizal description (i.e. morphotyping). After morphotyping, root and shoot biomass were assessed by drying the samples overnight at 70°C in a Despatch drying oven and weighing immediately after with an analytical scale (MC1 Analytic AC 120S Sartorius).

For the greenhouse bioassay, height, root-collar diameter, and dry biomass (root and shoot), were measured at the start and end of the greenhouse bioassay to determine responses to soil treatments. At the start of the greenhouse bioassay, a subsample of 20 seedlings was randomly selected and destructively sampled to measure initial dry biomass.

Transpiration

In the greenhouse bioassay, a subsample of 15 seedlings per treatment × block combination (90 seedlings) were randomly selected to measure transpiration rates ($\text{mg H}_2\text{O}\cdot\text{dm}^{-2}\cdot\text{h}^{-1}$) during a simulated 14-day drought in June 2001. Transpiration rates were calculated from daily changes in pot weight during the drought and total needle surface area (Parke et al. 1983). Pots were enveloped in thick plastic bags (LDPE co.) and were sealed around the stems of seedlings with heavy duty double stick tape (Scotch™) to stop soil surface water evaporation. Sealed pots were weighed with an Ohaus® Precision Plus Digital Scale. To measure total needle surface area, a subsample (by weight) of needles was surface-scanned on a flatbed scanner. The scanned images were then analyzed with Scion Image Beta 4.02 (Scion Corporation) to determine the total surface area.

Data analysis

The data from the field and greenhouse bioassays were analyzed as a one-way randomized block design (Sit 1995). Two-factor analysis of variance (ANOVA) (Zar 1999) was used to test the following model:

$$Y_{ijk} = \mu + B_i + S_j + e_{ijk}$$

where Y is a measure for the k th experimental unit in the i th block and j th soil treatment; μ is the mean; B is block (site; $i = 1,2,3$); S is soil treatment (landed or clearcut; $j = 1,2$); and e is random error within block \times soil treatment combination. The block and soil treatment effects were set as random and fixed factors, respectively. Groups of seedlings and soil samples within soil treatment \times block combination were treated as the experimental units. Treatment effects on foliar element concentrations, seedling ectomycorrhizal status, growth, and transpiration were analyzed.

Spearman's rank correlations were used to investigate the association between percent occurrence of morphotypes in the field and greenhouse bioassays. Testing the significance of the Spearman rank correlation coefficient (r_s) was done with a one-tailed t -test at the $p < 0.01$ level (Siegel 1956; Sokal and Rohlf 1995). Pearson correlations were carried out on percent ectomycorrhizal colonization and seedling growth variables, and on percent ectomycorrhizal colonization and foliar element concentrations. These correlations were run one set at a time with Bonferonni probabilities. SYSTAT (version 10.2, SAS Institute Inc.) was used to accomplish all statistical tests.

Results

Foliar analysis

In the greenhouse bioassay, seedlings grown in the landing soil showed significantly lower N, S, B, and Zn concentrations and lower S and B contents than those in the clearcut (Table 5). Lower concentrations of P ($p = 0.15$), K ($p = 0.12$), Ca ($p = 0.11$), Mg ($p = 0.11$), and Cu ($p = 0.19$) were found in seedlings grown in landing soils than clearcuts but differences were not significant.

In the field bioassay, landing grown seedlings had significantly greater concentrations of P, Fe, and Al (Table 5). Except for Mg, S, B, and Zn, other foliar nutrients, such as N ($p = 0.37$), K ($p = 0.25$), and Cu ($p = 0.38$) had lower (insignificant) concentrations in seedlings grown in landing soils than clearcuts. Seedlings grown in clearcut soils had significantly higher P, K, Ca, and Cu contents than those in landings.

Pearson correlations did not suggest any strong association between percent ectomycorrhizal colonization, growth variables, and foliar nutrient concentrations (Table 6). Nevertheless, in the field ectomycorrhizal colonization did have a moderate positive relationship with N, P, K, S, Fe, Mn, Zn, and Cu in foliage for seedlings grown on landings (Table 5).

Ectomycorrhizae morphotype and RFLP type characterization

Fifteen clearly different morphotypes were observed and described (Table 7). Six morphotypes could not be matched to descriptions in the literature. One of these had few morphological characters to observe and was labeled “undifferentiated”. Thirteen and six morphotypes were found in the field and greenhouse, respectively.

Overall nineteen different RFLP types were found. Each morphotype produced distinct RFLP patterns which may suggest that at least fifteen different species were observed (Table 8). However, certain of the same morphotypes produced different RFLP patterns most likely indicating genotypic variation (Mah et al. 2001). Greenhouse *Thelephora terrestris*-like and field unknown (flaky yellow) produced similar band sizes with all three restriction endonucleases. *Thelephora terrestris*-like found in the field and greenhouse produced six different RFLP patterns. RFLP patterns produced by *Rhizopogon vinicolor*-like, *Thelephora terrestris*-like, *Cenococcum geophilum*, *Amphinema*-like, and E-strain roughly matched those of Goodman et al. (1996), Hagerman et al. (1999b), Mah et al. (2001), and Wurzbürger et al. (2001).

Ectomycorrhizal status

In the greenhouse bioassay, seedlings grown in landing soil had significantly higher ectomycorrhizal colonization (14 % higher determined by the clearing and staining method) than seedlings grown in the clearcut soil (Fig. 3). In the field there was a similar weak tendency ($p = 0.32$) for higher ectomycorrhizal colonization on landing soils as compared to clearcut soil. Relative abundance of morphotypes was greater on seedlings grown on clearcut soil than on landing soil (Fig. 4) in both the greenhouse and field studies. For comparison, 5 naturally regenerated seedlings were collected from landings and ectomycorrhizal status was determined. Naturally regenerated seedlings growing on the landings had on average 37 % of their fine root tips colonized by ectomycorrhizal fungi (data not shown). Naturally regenerated seedlings were mostly colonized by *Rhizopogon vinicolor*-like and *Thelephora terrestris*-like. These results are very similar to what I found with outplanted seedlings (see Fig. 3 and Fig. 4). One of the five naturally

regenerated seedlings that were studied was heavily colonized (77 %) by Unknown (dark brown SFU004).

Rhizopogon vinicolor-like, *Thelephora terrestris*-like, *Cenococcum geophilum*, and Unknown (flaky yellow) were the four most abundant morphotypes in both the field and greenhouse. *Rhizopogon vinicolor*-like and *Thelephora terrestris*-like were significantly more abundant on landing soils while *Cenococcum geophilum* was clearly more abundant on clearcut soils. In the greenhouse, *Rhizopogon vinicolor*-like dominated (100 %) roots of seedlings grown in landing soil. Similarly, in the field *Rhizopogon vinicolor*-like was the most abundant (42 %) morphotype found on landings.

In the field, morphotype richness was marginally higher on roots of seedlings grown in clearcut soil ($p = 0.13$) than landing soil (Fig. 5). Root systems from the field had significantly higher morphotype evenness on clearcut soil than on landing soil. Morphotype diversity was higher on clearcut soil in the field but the difference was not statistically significant ($p = 0.12$).

Similarities between morphotypes on greenhouse and field seedlings

The same morphotypes were dominant on both greenhouse and field seedlings. Out of the fifteen morphotypes characterized (Table 7), four were found to occur in both the greenhouse and field and 3 of these were among the most abundant in both treatments. A positive correlation ($r_s = 0.74$; $p = 0.002$) was found between the greenhouse and field-occurring morphotypes (Fig. 6).

Seedling growth

In both the field and greenhouse, height and diameter increment were significantly greater for seedlings grown in clearcut soils than for those on landings (Fig. 7). In the

field, seedlings grown in clearcut soil had significantly greater shoot and root biomass gain and a greater shoot to root ratio ($p = 0.06$) (data not shown) compared to seedlings grown in landing soil.

In the greenhouse, shoot biomass was the same for seedlings grown in clearcut and landing soil while root biomass was greater for seedlings grown in the clearcut soil. However, shoot to root ratios for seedlings grown in clearcut soil were significantly ($p = 0.02$) lower (data not shown).

Transpiration rates

In the greenhouse, transpiration rates were significantly higher when seedlings were grown in clearcut soils (Fig 8). Transpiration rates started declining consistently with seedlings grown in both the landing and clearcut soils after nine days into the drought.

Discussion

Foliage properties

Higher concentrations of foliar N, S, B, and Zn in the clearcut-grown seedlings from the greenhouse may reflect both a larger root system and greater nutrient availability within the clearcut soil. In the field, seedlings grown on landings were not significantly more colonized by ectomycorrhizal fungi than seedlings grown on clearcuts. Nevertheless, higher P, Fe, and Al concentrations in landing-grown seedlings may be related to more efficient uptake via ectomycorrhizae. Regardless that both landing and clearcut seedlings were abundantly colonized by the same fungi, efficient uptake of P and Fe may have been greater on landings, since it is well known that fungi do not aid the host plant in the same way in different soil environments (Allen 1991; Perry et al. 1987). Seedlings colonized by

ectomycorrhizae are more efficient at P uptake than nonectomycorrhizal seedlings (Smith and Read 1997).

Fe-chelating agents, such as the siderophores produced by practically all aerobic and facultative aerobic rhizosphere organisms (Neilands 1977), including ectomycorrhizae (Bossier 1988; Powell et al. 1980; Powell et al. 1982) could have contributed to higher foliar Fe levels by enhancing Fe availability in landing soils. However, Perry et al. (1984) showed that Douglas-fir seedlings were Fe-limited on clearcuts where siderophore production was reduced compared to undisturbed forests. They suggested that a reduction in siderophore-producing soil organisms was mostly responsible for Fe limitations. Since my clearcuts probably harbored more siderophore-producing soil organisms, I cannot explain why Fe concentration was higher with landing-grown seedlings compared to clearcut-grown seedlings.

Al concentrations and contents in seedlings grown on landing soil were significantly higher than in clearcuts. With red spruce (*Picea rubens*) toxic effects such as increased dark respiration and reduced photosynthesis were associated with foliar Al concentrations of approximately 65 mg kg⁻¹ (McLaughlin et al. 1991). van den Drissche (1989) reported toxic effects associated with foliar Fe concentrations of 330 mg kg⁻¹. Height and weight of Douglas-fir seedlings were significantly reduced compared to seedlings with Fe concentrations of 79 mg kg⁻¹. In my study, the high concentrations of foliar Fe (630 mg kg⁻¹) and Al (660 mg kg⁻¹) may have been toxic to Douglas-fir seedlings. Many of the seedlings grown on landing soil had pronounced chlorosis (see appendix A) which can be indicative of the toxic effects of high levels of foliar Fe and Al (Macdonald et al. 1998). Concentrations and contents of exchangeable Fe in landing soil were not

significantly different than clearcut soil (Table 3). Surprisingly these findings suggest that uptake of Fe was significantly greater for seedlings growing on the landing soil. I cannot fully explain why foliar Fe was greater in seedlings growing on landing soil however, good ectomycorrhizal colonization (Fig. 3) may have been partly responsible since mycorrhizae have been shown to increase uptake of heavy metals such as Fe to toxic levels (El-Kherbawy et al. 1989; Gadd 1993).

In this study, pH differences do not account for the differences in Fe and Al availability since Fe and Al availability usually decline with rising pH (Brady and Weil 2002). Also landing and clearcut soils respectively had a pH of 6.00 and 5.27, which were not significantly different ($p = 0.77$) (Table 2). However, landing soil was mineral soil with very little organic matter (see chapter 1). Macdonald et al. (1998) suggested that seedlings rooting in mineral soil could be more at risk of toxic levels of Al than seedlings on clearcuts where some rooting took place in the forest floor and organic horizons. Interestingly, some species of ectomycorrhizal fungi have been involved in increased metal uptake such as Al (Wilkins and Hodson 1989). I suspect that the harmful effects of high concentrations of foliar Fe and Al, such as stunted root growth (Nosko 1988) and a decrease in photosynthesis may have been one of the factors responsible for the poor growth response of seedlings growing on landings.

Ectomycorrhizal status

Out of the fifteen morphotypes found on both clearcuts and landings, six were not identifiable to the genus level. Seedlings may have harbored more than fifteen morphotypes since I may have mistaken dark immature ectomycorrhizal tips and E-strain for nonectomycorrhizal or dead tips. As expected, considerable amounts of

undifferentiated ectomycorrhizae (i.e. very young ectomycorrhizae) were found. Berch and Roth (1993) reported eight morphotypes on Douglas-fir seedlings after one growing season on clearcuts on eastern Vancouver Island. After two years, Kranabetter et al. (1997) found thirteen morphotypes on paper birch seedlings growing on old reclaimed skid roads. The ectomycorrhizal community in my study was slightly larger in number but still remains within the range of what is commonly observed on disturbed sites.

It was not surprising to find such a relatively small community of ectomycorrhizal fungi on these Douglas-fir seedlings two years after outplanting. It is well known that environmental factors affect the abundance of ectomycorrhizae forming on seedlings (Allen 1991; Parke et al. 1984; Pilz and Perry 1984; Schoenberger and Perry 1982; Skinner and Bowen 1974; Slankis 1974; Smith and Read 1997). Soil factors such as low organic matter, high compaction, very high or low moisture, and high summer temperature on landings (see chapter 1) and poor seedling growth were likely unfavorable factors influencing the development of many ectomycorrhizal species.

All fifteen morphotypes produced nineteen distinct RFLP types. Therefore, either more than fifteen different taxa were present (since certain morphotypes produced two or more different RFLP patterns) or certain morphotypes exhibited genotypic variation. Such discrepancies are often observed (Hagerman et al. 2001; Mah et al. 2001; Wurzbürger et al. 2001) and indicate that morphological overlap across ectomycorrhizal fungal taxa and variation within morphotypes exist.

Although, most ectomycorrhizae occur in soils with surface organic layers (Harvey et al. 1976; Slankis 1974), some conifers in the Pacific Northwest have been found to have a higher percent ectomycorrhizal colonization when grown in mineral soil (Alvarez et al.

1979). Similarly, I observed better percent colonization of Douglas-fir root tips by ectomycorrhizae on mineral landing soil than in organic matter-rich soil on clearcuts but root biomass was lower. Since no counts of the total number of ectomycorrhizal root tips were made it is difficult to say if overall ectomycorrhiza formation was better on landing soil.

In previous studies, *Rhizopogon vinicolor*-like was predominantly found in the humus layer and forest floor woody debris (Trappe 1965; Zak 1971) where it can rapidly form ectomycorrhizae on new emerging outplanted Douglas-fir roots can become dominant on the root system (Castellano 1987). I found *Rhizopogon vinicolor*-like readily forming ectomycorrhizae and dominating Douglas-fir seedling roots grown on landing mineral soil. I suggest that *Rhizopogon vinicolor*-like or a related species can occur and may be well-adapted under a wider set of environmental conditions than previously observed.

In the Pacific Northwest nurseries, *Thelephora terrestris* is often abundantly found on container-grown coastal Douglas-fir (Molina and Trappe 1982a; Morgan 1985; Roth and Berch 1992). *Thelephora terrestris* is very tolerant of frequent watering and high fertility levels and is an aggressive colonizer under nursery conditions (Hunt 1991; Molina and Trappe 1982a; Morgan 1985). However, it is apparently not common on interior Douglas-fir (Hunt 1991) in B.C. container nurseries. It is found on Douglas-fir seedlings after outplanting, although in much lower abundance (Berch and Roth 1993; Jones et al. 1997). I found that *Thelephora terrestris*-like was relatively abundant in both landing and clearcut soils. Since it was not recorded on roots of Douglas-fir seedlings in an early assessment before the start of the greenhouse bioassay, *Thelephora terrestris*-like was

likely not present at time of outplanting unless it was present in nursery soil only as dormant propagules. Therefore, it seems likely that *Thelephora terrestris*-like occurred naturally and was present in both landing and clearcut soils and not as dormant propagules in the nursery soil.

High percent colonization ($\approx 90\%$) of Douglas-fir seedlings outplanted in clearcut soils has been reported after one growing season (Borchers and Perry 1990; Roth and Berch 1992). Two years after outplanting, I found that Douglas-fir seedlings had a relatively low percent colonization (in the 30 – 50% range). The seedlings in the previous studies were colonized at time of outplanting while those in my study were not. A qualitative analysis revealed high fine root mortality on both landing and clearcut-grown seedlings in my study. I suspect that a certain portion of the root systems died off to give rise to entirely new fine roots. Under this scenario, ectomycorrhiza formation would be limited until new roots were established. Also, such a process would present a high carbon demand in the roots. Douglas-fir seedlings growing on harsh landings may be at a disadvantage compared to other conifer species such as lodgepole pine (*Pinus contorta*) that are ectomycorrhizal at time of outplanting (Xiao et al. 1996-2002) and apparently do not go through this process. Lodgepole pine is commonly outplanted on degraded sites (Bulmer, pers. comm.) and does reasonably well on rehabilitated landings (Plotnikoff et al. 2002). It is quite possible that high percent ectomycorrhizal colonization of lodgepole pine at time of outplanting plays a role in the success of this tree species on degraded sites. Nevertheless, physiological traits such as faster early growth, more plastic response to light and soil conditions are also likely responsible (Lotan and Perry 1983).

On degraded sites, high percent ectomycorrhizal colonization of outplanted seedlings is only one factor amongst many that can contribute to successful establishment and growth. Another factor is the EIP of soils on degraded sites (Perry et al. 1987). The EIP can be defined as the ability of forest soil to maintain viable populations of ectomycorrhizal fungi (Perry et al. 1987). Previous studies have indicated that the EIP is low on degraded sites (Parke et al. 1984; Perry et al. 1982). Not surprisingly in my study, landing soils had a lower EIP compared to clearcuts. Interestingly, I found that landing soils had a higher percent ectomycorrhizal colonization than clearcut soils similar to what Amaranthus et al. (1996) and Alvarez et al. (1979) observed with both Douglas-fir, western white pine and white fir (*Abies concolor*) on relatively similar sites. However, seedlings grown on landing soils were predominantly colonized by *Rhizopogon vinicolor*-like, which has been reported to be dominant and persistent on Douglas-fir seedlings outplanted on burned clearcuts (Castellano et al. 1985). Castellano (1987) reported *Rhizopogon vinicolor*-like was able to colonize feeder roots rapidly and maintain a position of dominance on Douglas-fir. I think *Rhizopogon vinicolor*-like was well adapted to soil conditions on the landings and had a competitive advantage over other ectomycorrhizal fungi. *Rhizopogon vinicolor*-like's competitiveness and rapid growth (Molina and Trappe 1994) may have permitted higher percent colonization of Douglas-fir roots on landings and helped seedlings to survive. It is also possible that certain morphotypes encountered on clearcut-grown seedlings were mistakenly noted as dead tips. This mistake would consequently decrease the overall percent colonization values recorded.

Landing-grown seedlings had insignificantly lower morphotype richness ($p = 0.13$) and diversity ($p = 0.12$) than clearcut grown seedlings. Morphotype evenness was

significantly lower on landings indicating a low EIP. I did not find this surprising since landings had no organic layers or woody debris where much of the ectomycorrhizal community is found in the form of propagules (including preexisting ectomycorrhizae on root systems).

Further colonization of surviving seedlings will undoubtedly occur on both landings and clearcuts with time. Much of the remaining ectomycorrhizal community is still present under clearcut soils in forms such as fungal spores, hyphal fragments, sclerotia, and attached to suitable host plants (Amaranthus et al. 1989; Grove and Malajczuk 1994; Harvey et al. 1976). As for landing soils, much of the ectomycorrhizal community is absent because the forest floor and organic rich soil horizons have been removed. However, landings are relatively small sites where epigeous and hypogeous ectomycorrhizal fungi spores may immigrate quite rapidly with wind, wind-blown topsoil, mammal feces, birds, and insects (Malajczuk et al. 1987; Maser et al. 1978).

Comparison of greenhouse and field bioassay

This study has shown that greenhouse bioassays can be useful to simulate what happens in the field. Ectomycorrhizal status (except for non-dominant morphotypes) and growth (except for shoot dry biomass) of Douglas-fir under greenhouse conditions were similar to outplanted seedlings in the field. Greenhouse bioassays produce comparable results on a relative scale and can help to answer questions about the inherent influences of soil from the field. In this study the greenhouse bioassay permitted environmental variables to be controlled (e.g. temperature and moisture) while focusing on the inherent effects of the landing and clearcut soils on ectomycorrhiza formation and seedling growth.

Comparing greenhouse and field results also allowed me to gain some insight into the environmental differences found on landings and clearcuts.

Seedling growth and transpiration

Douglas-fir seedlings were found to grow better (greater height and diameter increment in the field and greenhouse bioassay and greater shoot and root biomass gain in the field bioassay) in clearcut soil compared to landing soil. Seedlings growing in landing soil in both the field and greenhouse bioassay had significantly lower root biomass gain than seedlings growing in clearcut soil. It is possible that landing soil was inherently unfavorable for normal root development. The inherent physical and chemical properties, such as higher bulk density (in the field) and lower mineralisable-N of the mineral landing soil (see chapter 1) probably had the greatest influence on overall seedling growth. On landings, it is likely that seedlings were concentrating their carbon allocation to shoot and ectomycorrhiza development. Though seedlings on landings were more heavily colonized with ectomycorrhizal fungi, growth did not appear to improve. High demands of photosynthate by certain species of ectomycorrhizal fungi such as *Rhizopogon vinicolor* have been observed on Douglas-fir seedlings (Dosskey et al. 1991; Dosskey et al. 1990). These ectomycorrhizas can increase rate of photosynthesis and establish a sink for photosynthate by generating extensive extramatrical hyphae and/or rhizomorphs. My results seem consistent with this photosynthate source-sink concept. Nevertheless, ectomycorrhizal fungi appears to have helped seedlings to survive on landings.

Rhizopogon spp. are well known for their production of rhizomorphs.

Rhizomorphs can rapidly exploit surrounding soil for water and nutrients (Pilz and Perry 1984) and are capable of exploring soil several meters away from host plants (Allen 1992).

Prolific rhizomorph production is normally observed after outplanting of seedlings and such structures can present a considerable carbon cost. Since rhizomorph functioning can be impaired on compacted soils (Pilz and Perry 1984) benefits of such structures probably did not occur.

In the greenhouse, seedlings growing on landing soil had lower transpiration rates during drought conditions. Parke et al. (1983) observed low transpiration rates on Douglas-fir seedlings inoculated with *Rhizopogon vinicolor* grown in soil from a Douglas-fir dominated old-growth forest. They concluded that Douglas-fir seedlings inoculated with *Rhizopogon vinicolor* were less affected by drought than other ectomycorrhizal or nonectomycorrhizal seedlings. In my study, I think that ectomycorrhizal influences were not the dominating factors contributing to different transpiration rates between clearcut and landing grown seedlings. First, clearcut grown seedlings in the greenhouse had lower shoot to root ratios than landing grown seedlings enabling better water acquisition and thus, allowing more transpiration to occur. This may not be the case in the field where shoot to root ratios were greater for clearcut grown seedlings compared to landing grown seedlings. Secondly, clearcut soils possessed more organic matter and had better water-holding capacity than mineral landing soil. These factors may have enabled the seedlings to keep stomata open for longer periods of time in clearcut soil, leading to higher transpiration rates. Higher transpiration rates would allow more photosynthesis to occur. Since seedlings growing in clearcut soil could photosynthesize more, higher amounts of carbon could be fixed and stored by seedlings grown in clearcut soils compared to landing soil, which may have allowed more growth.

Conclusions

Douglas-fir seedlings and native ectomycorrhizal fungi performed poorly on partially rehabilitated landings. In this study, *Rhizopogon vinicolor*-like was found in abundance on landings. Seedlings had higher ectomycorrhizal colonization but lower root biomass on landings compared on clearcuts, however poor soil chemical and physical conditions probably restricted normal root and ectomycorrhizal development. I found that on clearcuts, Douglas-fir seedlings grew better and formed a more diverse (as measured by evenness) community of ectomycorrhizae. These results indicate that Douglas-fir can readily form ectomycorrhizae under harsh conditions such as on landings, however benefits such as increased growth sometimes associated with these symbionts may be impaired. Despite poor seedling growth, I found foliar P concentrations significantly higher on landings. Nevertheless since landing soil showed a trend for lower available P than clearcut soil (see chapter 1) native ectomycorrhizal fungi may have been providing adequate P to seedlings. An important factor contributing to poor growth may have been Fe and Al toxicity effects indicated by high concentrations in needles.

Douglas-fir seedlings and their ectomycorrhizal fungi growing on landings were probably stressed by a combination of factors such as inadequate porosity, high soil temperatures and drought during the summer, possible deficiencies in N (see chapter 1), and Fe and Al toxicity. Soil compaction, low organic matter contents, and Fe and Al toxicity could be addressed by combining deep-tilling with nearby organic matter amendments.

Table 5. Mean concentrations and contents of elements in foliage of Douglas-fir seedlings grown in landing and clearcut soils after one growing season in the greenhouse and after two years in the field.

| Soil source | Foliar biomass | | | | | | | | | | | | |
|-----------------------|-----------------------------|--|-----------------------|-----------------------|-----------------------|-----------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|--------------------|
| | N | P | K | Ca | Mg | S | Fe | Mn | B | Zn | Cu | Al | |
| <i>Greenhouse</i> | | | | | | | | | | | | | |
| <i>Concentrations</i> | (g kg ⁻¹) | (g kg ⁻¹) | (g kg ⁻¹) | (g kg ⁻¹) | (g kg ⁻¹) | (g kg ⁻¹) | (mg kg ⁻¹) | (mg kg ⁻¹) | (mg kg ⁻¹) | (mg kg ⁻¹) | (mg kg ⁻¹) | (mg kg ⁻¹) | |
| Landing | 5.55 (0.88) | 0.663 (0.35) | 5.57 (1.82) | 1.45 (1.25) | 1.561 (0.21) | 0.448 (0.17) | 148.6 (8.4) | 348 (25) | 22.3 (2.1) | 23.3 (1.5) | 2.91 (0.051) | 118.6 (15.6) | |
| Clearcut | 6.66 (0.20) | 0.832 (0.42) | 6.10 (0.38) | 1.97 (0.67) | 1.848 (1.06) | 0.588 (0.22) | 152.2 (10.6) | 422 (35) | 29.0 (1.4) | 33.8 (1.5) | 3.43 (0.22) | 122.6 (1.0) | |
| <i>p</i> | 0.004*** | 0.15 | 0.12 | 0.11 | 0.11 | 0.006*** | 0.86 | 0.34 | 0.047** | 0.06* | 0.19 | 0.82 | |
| <i>Field</i> | | | | | | | | | | | | | |
| <i>Concentrations</i> | (g seedling ⁻¹) | <i>Contents (mg seedling⁻¹)</i> | | | | | | | | | | | |
| Landing | 4.286 (0.084) | 23.7 (0.4) | 2.80 (0.15) | 23.65 (0.52) | 6.14 (0.60) | 6.65 (0.09) | 1.90 (0.10) | 0.632 (0.027) | 1.466 (0.125) | 0.0949 (0.0087) | 0.099 (0.007) | 0.01252 (0.00047) | 0.5020 (0.0549) |
| Clearcut | 4.118 (0.143) | 27.0 (0.9) | 3.39 (0.22) | 24.99 (1.03) | 8.00 (0.50) | 7.50 (0.45) | 2.38 (0.01) | 0.602 (0.024) | 1.720 (0.206) | 0.1172 (0.0041) | 0.137 (0.011) | 0.01409 (0.00150) | 0.4946 (0.0230) |
| <i>p</i> | 0.53 | 0.12 | 0.25 | 0.42 | 0.23 | 0.18 | 0.04** | 0.59 | 0.52 | 0.04** | 0.15 | 0.50 | 0.87 |
| <i>Field</i> | | | | | | | | | | | | | |
| <i>Concentrations</i> | (g kg ⁻¹) | (g kg ⁻¹) | (g kg ⁻¹) | (g kg ⁻¹) | (g kg ⁻¹) | (g kg ⁻¹) | (g kg ⁻¹) | (mg kg ⁻¹) | (mg kg ⁻¹) | (mg kg ⁻¹) | (mg kg ⁻¹) | (mg kg ⁻¹) | |
| Landing | 10.4 (3.0) | 1.83 (0.10) | 7.32 (1.05) | 3.294 (0.449) | 2.298 (0.221) | 1.205 (0.174) | 630 (172) | 366 (3) | 49 (5) | 40.1 (13.0) | 2.96 (0.52) | 660 (134) | |
| Clearcut | 14.3 (0.4) | 1.31 (0.07) | 9.19 (0.13) | 3.296 (0.056) | 2.134 (0.046) | 1.196 (0.076) | 80 (9) | 313 (62) | 41 (1) | 49.2 (2.9) | 3.50 (0.07) | 90 (9) | |
| <i>p</i> | 0.37 | 0.036** | 0.25 | 0.99 | 0.57 | 0.97 | 0.09* | 0.46 | 0.24 | 0.61 | 0.38 | 0.06* | |
| <i>Concentrations</i> | (g seedling ⁻¹) | <i>Contents (mg seedling⁻¹)</i> | | | | | | | | | | | |
| Landing | 2.4 (0.7) | 29 (16) | 4.1 (1.0) | 19 (8) | 8.1 (3.0) | 5.7 (2.0) | 3.2 (1.4) | 1.21 (0.09) | 0.849 (0.228) | 0.119 (0.045) | 0.121 (0.072) | 0.0079 (0.0038) | 1.34 (0.03) |
| Clearcut | 6.1 (0.2) | 86 (4) | 7.8 (0.5) | 56 (3) | 20.5 (1.4) | 12.5 (0.5) | 7.5 (0.8) | 0.45 (0.05) | 1.789 (0.503) | 0.240 (0.008) | 0.305 (0.026) | 0.0214 (0.0007) | 0.51 (0.04) |
| <i>p</i> | 0.05** | 0.11 | 0.097* | 0.07* | 0.07* | 0.11 | 0.14 | 0.01*** | 0.27 | 0.15 | 0.20 | 0.08* | 0.001*** |

Note: Values are means with standard errors in parentheses. *, **, *** Significant difference at p < 0.1, 0.05, and 0.01, respectively.

Table 6. Pearson correlations of percent ectomycorrhizal colonization (PEC) with height and diameter increment at time of planting, dry biomass, and foliar element concentrations for each seedling.

| | Height increment | Diameter increment | Biomass* | | Foliar element concentrations | | | | | | | | | | | | | |
|-------------------|------------------|--------------------|----------|-------|-------------------------------|-------------|-------------|-------|-------|-------|-------------|-------------|-------------|------|------|-------------|-------------|-------|
| | | | Root | Shoot | N | P | K | Ca | Mg | S | Fe | Mn | B | Zn | Cu | Al | | |
| <i>Greenhouse</i> | | | | | | | | | | | | | | | | | | |
| PEC | 0.11 | -0.10 | -0.25 | 0.06 | -0.28 | -0.20 | 0.16 | 0.16 | 0.16 | 0.16 | -0.16 | 0.18 | 0.02 | 0.18 | 0.18 | 0.12 | 0.16 | 0.19 |
| <i>p</i> (n = 30) | 0.58 | 0.58 | 0.19 | 0.74 | 0.14 | 0.29 | 0.39 | 0.39 | 0.39 | 0.39 | 0.40 | 0.35 | 0.90 | 0.34 | 0.34 | 0.54 | 0.40 | 0.30 |
| PEC on landings | 0.44 | 0.02 | -0.27 | -0.22 | 0.23 | 0.14 | 0.26 | 0.26 | 0.26 | 0.26 | 0.18 | 0.17 | 0.02 | 0.26 | 0.26 | 0.00 | 0.25 | 0.22 |
| <i>p</i> (n = 15) | 0.10 | 0.93 | 0.32 | 0.44 | 0.42 | 0.63 | 0.36 | 0.36 | 0.36 | 0.36 | 0.52 | 0.54 | 0.95 | 0.36 | 0.36 | 0.99 | 0.36 | 0.44 |
| PEC on clearcuts | 0.14 | 0.10 | 0.15 | 0.21 | -0.12 | -0.14 | 0.14 | 0.14 | 0.14 | 0.14 | -0.28 | 0.38 | 0.38 | 0.19 | 0.19 | 0.35 | 0.15 | 0.37 |
| <i>p</i> (n = 15) | 0.62 | 0.73 | 0.59 | 0.44 | 0.68 | 0.61 | 0.61 | 0.61 | 0.61 | 0.61 | 0.31 | 0.16 | 0.16 | 0.50 | 0.50 | 0.20 | 0.60 | 0.17 |
| <i>Field</i> | | | | | | | | | | | | | | | | | | |
| PEC | -0.20 | -0.19 | -0.03 | -0.13 | <u>0.36</u> | <u>0.33</u> | 0.24 | 0.04 | 0.03 | 0.03 | <u>0.32</u> | <u>0.37</u> | <u>0.32</u> | 0.10 | 0.10 | <u>0.33</u> | <u>0.36</u> | 0.01 |
| <i>p</i> (n = 90) | 0.06 | 0.07 | 0.80 | 0.22 | 0.0005 | 0.001 | 0.02 | 0.69 | 0.75 | 0.75 | 0.002 | 0.0004 | 0.002 | 0.37 | 0.37 | 0.001 | 0.0005 | 0.90 |
| PEC on landings | 0.10 | 0.08 | 0.22 | 0.23 | <u>0.53</u> | <u>0.47</u> | <u>0.54</u> | 0.28 | -0.22 | -0.22 | <u>0.53</u> | <u>0.43</u> | <u>0.53</u> | 0.25 | 0.25 | <u>0.47</u> | <u>0.53</u> | -0.01 |
| <i>p</i> (n = 45) | 0.53 | 0.60 | 0.14 | 0.13 | 0.001 | 0.004 | 0.001 | 0.07 | 0.16 | 0.16 | 0.001 | 0.01 | 0.001 | 0.11 | 0.11 | 0.004 | 0.001 | 0.97 |
| PEC on clearcuts | -0.15 | -0.11 | 0.09 | -0.07 | 0.16 | 0.31 | 0.14 | -0.01 | -0.04 | -0.04 | 0.12 | 0.27 | 0.12 | 0.06 | 0.06 | 0.31 | 0.08 | 0.04 |
| <i>p</i> (n = 45) | 0.32 | 0.47 | 0.55 | 0.64 | 0.30 | 0.05 | 0.37 | 0.94 | 0.81 | 0.81 | 0.44 | 0.08 | 0.44 | 0.69 | 0.69 | 0.05 | 0.62 | 0.81 |

Note: Coefficients > 0.25 with *p* < 0.05 are in underlined.

*Dry biomass gain.

Table 7. Description of morphological characteristics of greenhouse (G) and field (F) grown Douglas-fir ectomycorrhizal types.

| Identification No. | Morphotype | G | F | Brief morphotype description* |
|--------------------|---|---|---|--|
| SFU01 | <i>Rhizopogon vinicolor</i> -like (Rv) | + | + | Irregular to subterculate silvery white mycorrhiza; hairy brown mycelial strands; felt prosenchyma outer mantle; emanating hyphae wide with elbow-like bends |
| SFU02 | <i>Thelephora terrestris</i> -like (Tt) | + | + | Brown or orange thin and sometimes wrinkled mycorrhiza; net synenchyma outer mantle; long cystidia with basal clamp |
| SFU03 | <i>Cenococcum geophilum</i> (Cg) | + | + | Pure black mycorrhiza; net synenchyma in a stellate pattern; large (5 µm wide) black emanating hyphae at low magnification |
| SFU04 | Unknown (dark brown) (Db) | + | - | Dark brown felty mycorrhiza; net prosenchyma outer mantle; large (4.5 µm) yellow emanating hyphae |
| SFU05 | Unknown (silver-amorphous) (Sa) | - | + | Smooth silver mycorrhiza; interlocking irregular synenchyma outer mantle with mucilaginous matrix |
| SFU06 | <i>Hydnellum peckii</i> -like (Hp) | + | - | Whitish mycorrhiza with flat cottony rhizomorphs; mantle pattern unclear; loose emanating hyphae occasionally with adhering debris |
| SFU07 | <i>Amphinema byssoides</i> -like (Ab) | - | + | Orange-yellow stringy mycorrhiza; yellow branched mycelial strands; felt prosenchyma outer mantle; emanating hyphae with keyhole clamps |
| SFU08 | Unknown (flaky yellow) (Fy) | - | + | Cream yellow flaky mycorrhiza; net prosenchyma outer mantle with adhering debris |
| SFU09 | E-strain (<i>Wilcoxina</i> sp.) (E-s) | - | + | Brownish-orange mycorrhiza; mantle patchy and not distinct; large emanating hyphae (6 µm) |
| SFU10 | Unknown (caramel jigsaw) (Cj) | - | + | Smooth brown thick mycorrhiza; interlocking irregular synenchyma outer mantle resembling a jigsaw puzzle |
| SFU11 | CDE5-like (C5) | - | + | Light brown cottony mycorrhiza; variable outer mantle resembling an interlocking irregular synenchyma; coarsely ornamented emanating hyphae |
| SFU12 | <i>Lactarius</i> -like (Lac) | - | + | Smooth brown mycorrhiza with occasional green patches on surface of outer mantle; no mycelial strands or emanating hyphae seen |
| SFU13 | <i>Amphinema</i> -like (Aw) | - | + | Very similar to <i>Amphinema byssoides</i> described above except for white colored ectomycorrhiza and mycelial strands |
| SFU14 | Unknown (spiny brown) (Sb) | - | + | Brown spiny mycorrhiza; felt prosenchyma outer mantle; common tortuous light brown emanating hyphae |
| SFU15 | Undifferentiated (Undif) | + | + | Very young mycorrhiza without any noticeable distinct characters except for Hartig net |

Note: Presence and absence are indicated by plus (+) and minus (-) signs respectively.

*A more complete morphotype description can be obtained by contacting the corresponding author.

Table 8. Douglas-fir ectomycorrhizal RFLP types found in the greenhouse (G) and field (F).

| Morphotype | Site | RFLP fragment sizes (bp) | | | | RFLP type |
|------------------------------------|------|--------------------------|-----------------------------------|------------------------------|----|-----------|
| | | <i>AluI</i> | <i>HinfI</i> | <i>RsaI</i> | | |
| <i>Amphinema byssoides</i> | F | nd* | nd | nd | nd | nd |
| <i>Amphinema</i> -like | F | nd | 325, 305, 170, 155 | 930, 780, 670 | 18 | |
| CDE5-like | F | 735 | 345, 295, 170, 150 | 755, 665, 555, 195 | 16 | |
| <i>Cenococcum geophilum</i> | G | nd | nd | 910 | 9 | |
| <i>Cenococcum geophilum</i> | F | 445, 150, 115 | 295, 170, 125 | 915 | 10 | |
| E-strain (<i>Wilcoxina</i> sp.) | F | nd | 530, 190, 170, 130 | 910, 720 | 14 | |
| <i>Hydnellum peckii</i> -like | F | nd | nd | nd | nd | |
| <i>Lactarius</i> -like | F | 525, 285, 190, 110 | 425, 365, 170, 150 | 1025, 740 | 17 | |
| <i>Rhizopogon vinicolor</i> -like | G | 970, 795, 185, 115 | 225, 175, 140, 120, 100 | 1015 | 1 | |
| <i>Rhizopogon vinicolor</i> -like | F | 500, 275 | 225, 175, 140, 120, 100 | 1000 | 2 | |
| <i>Thelephora terrestris</i> -like | G | 610, 195, 125, 115 | 335, 280, 170, 155, 100 | 1005 | 3 | |
| <i>Thelephora terrestris</i> -like | F | nd | 335, 280, 170, 155, 100 | 995, 830, 210 | 4 | |
| <i>Thelephora terrestris</i> -like | F | 600, 190, 120, 105 | 335, 280, 170, 155, 100 | 1000, 815, 695, 565, 205 | 5 | |
| <i>Thelephora terrestris</i> -like | F | 615, 185, 110 | 465, 335, 315, 275, 170, 155, 100 | 995, 785, 560, 460, 265, 205 | 6 | |
| <i>Thelephora terrestris</i> -like | F | 420, 235 | 335, 300, 170, 155 | 920, 640, 295, 235 | 7 | |
| <i>Thelephora terrestris</i> -like | F | nd | 330, 270, 170, 150 | 980, 800, 565, 205 | 8 | |
| Undifferentiated | F | nd | 450, 170 | 780 | 19 | |
| Unknown (caramel jigsaw) | F | 590 | 345, 320, 180, 125 | 360, 305, 260 | 15 | |
| Unknown (dark brown) | G | nd | nd | nd | nd | |
| Unknown (flaky yellow) | G | nd | nd | 890 | 11 | |
| Unknown (flaky yellow) | F | 95 | 340, 300, 170, 155 | 965 | 12 | |
| Unknown (flaky yellow) | F | 625, 195, 125, 110 | 335, 275, 160, 150, 100 | 1005, 785, 550, 205 | 13 | |
| Unknown (silver-amorphous) | F | nd | nd | nd | nd | |
| Unknown (spiny brown) | F | nd | nd | nd | nd | |

Note: Restriction fragment sizes (bp) approximated by Gene Profiler v4.03 (Scanalytics, Inc.).

*nd, not determined.

Fig. 3. Ectomycorrhizal colonization of Douglas-fir grown in landing and clearcut soils after two (field) and one (greenhouse) growing seasons using the root tip count method. Percent colonization of the greenhouse seedlings was also determined using the clearing and staining method. Error bars indicate one standard error of the mean. *, **, *** indicate that means differ significantly within the particular bioassay at $p < 0.1$, 0.05, 0.01 respectively.

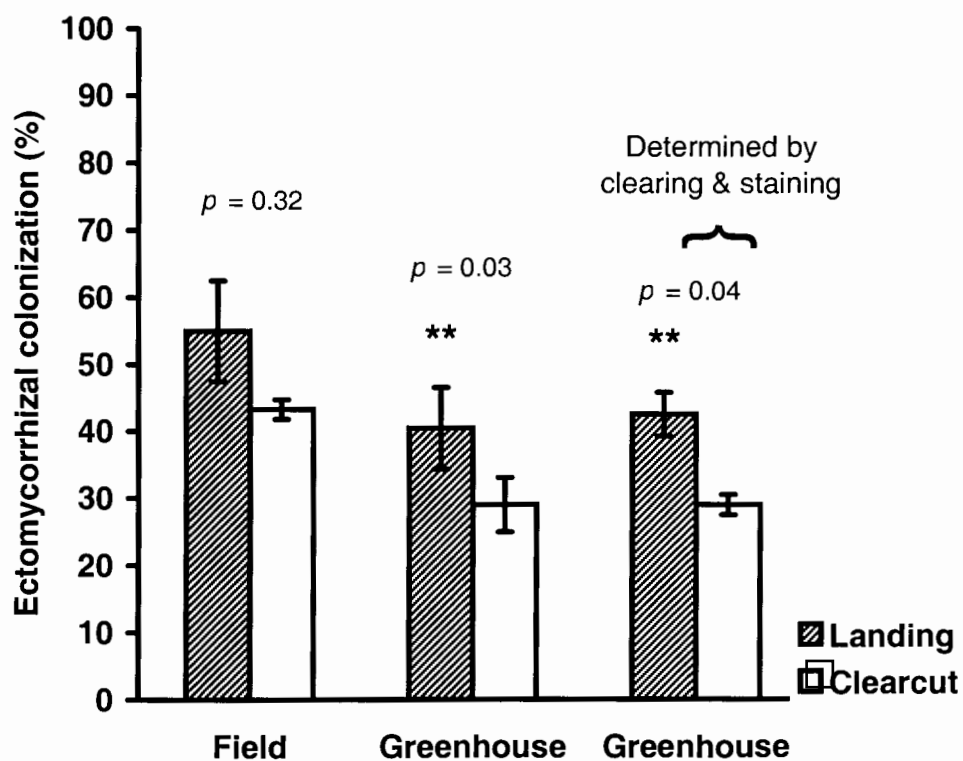


Fig. 4. Relative abundance of common (>5%) morphotypes (*Rhizopogon vinicolor*-like (Rv); *Thelephora terrestris*-like (Tt); *Cenococcum geophilum* (Cg); Unknown-dark brown (Db); Unknown-flaky yellow (Fy); E-strain (E-s); Unknown-caramel jigsaw (Cj); *Amphinema*-like (Aw); Unknown-spiny brown (Sb); and Undifferentiated (undif) found on Douglas-fir roots grown in landing and clearcut soils.

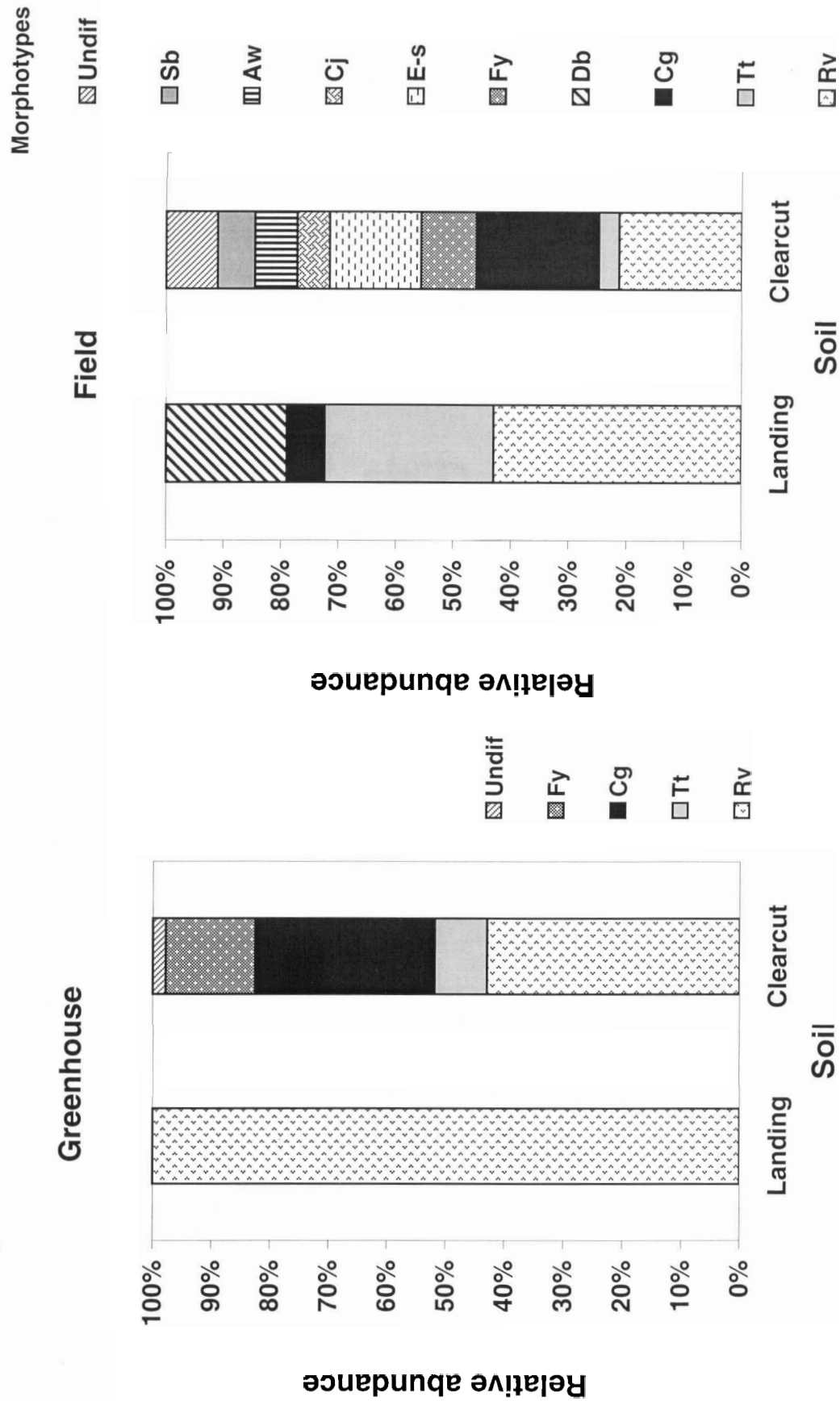


Fig. 5. Richness, Shannon's diversity index, and evenness of the ectomycorrhizal communities of Douglas-fir grown in landing and clearcut soils after two (field) and one (greenhouse) growing seasons. Error bars indicate one standard error of the mean. *, **, *** indicate that means differ significantly within the particular bioassay at $p < 0.1$, 0.05, 0.01 respectively.

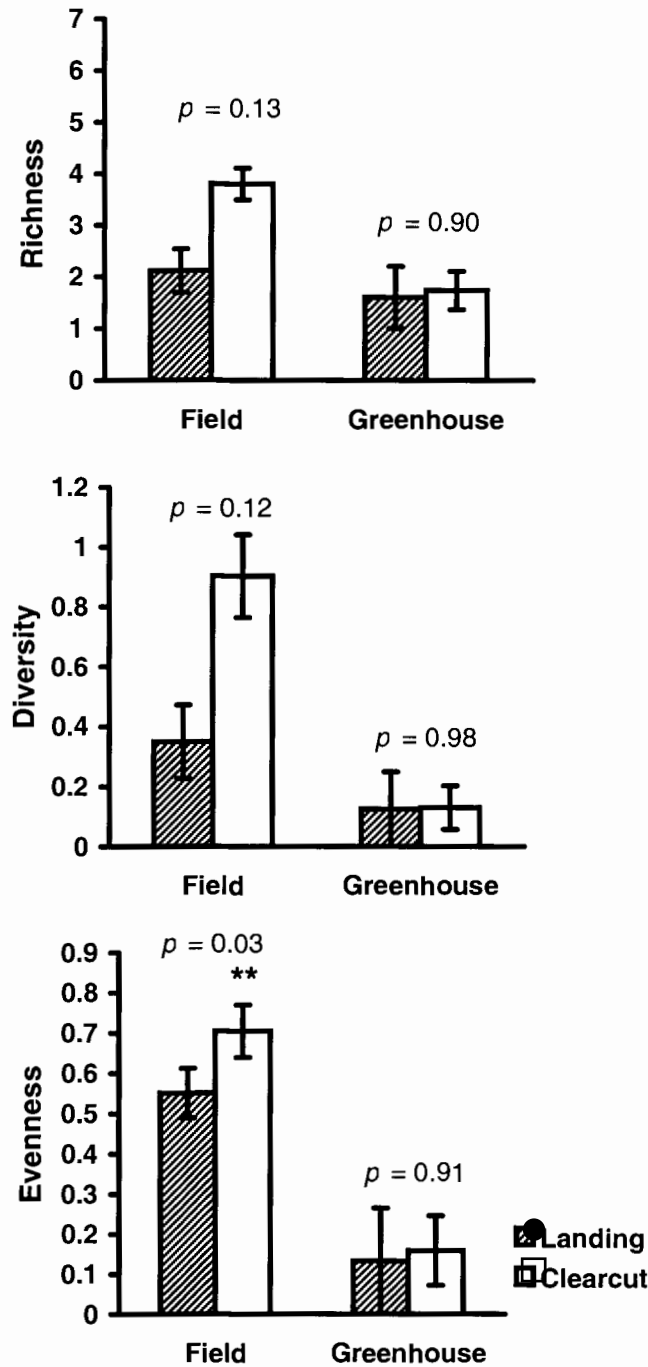


Fig. 6. Degree of similarity between field and greenhouse occurring morphotypes. The significance of Spearman's rank correlation coefficient (r_s) was tested with a one-tailed t-test.

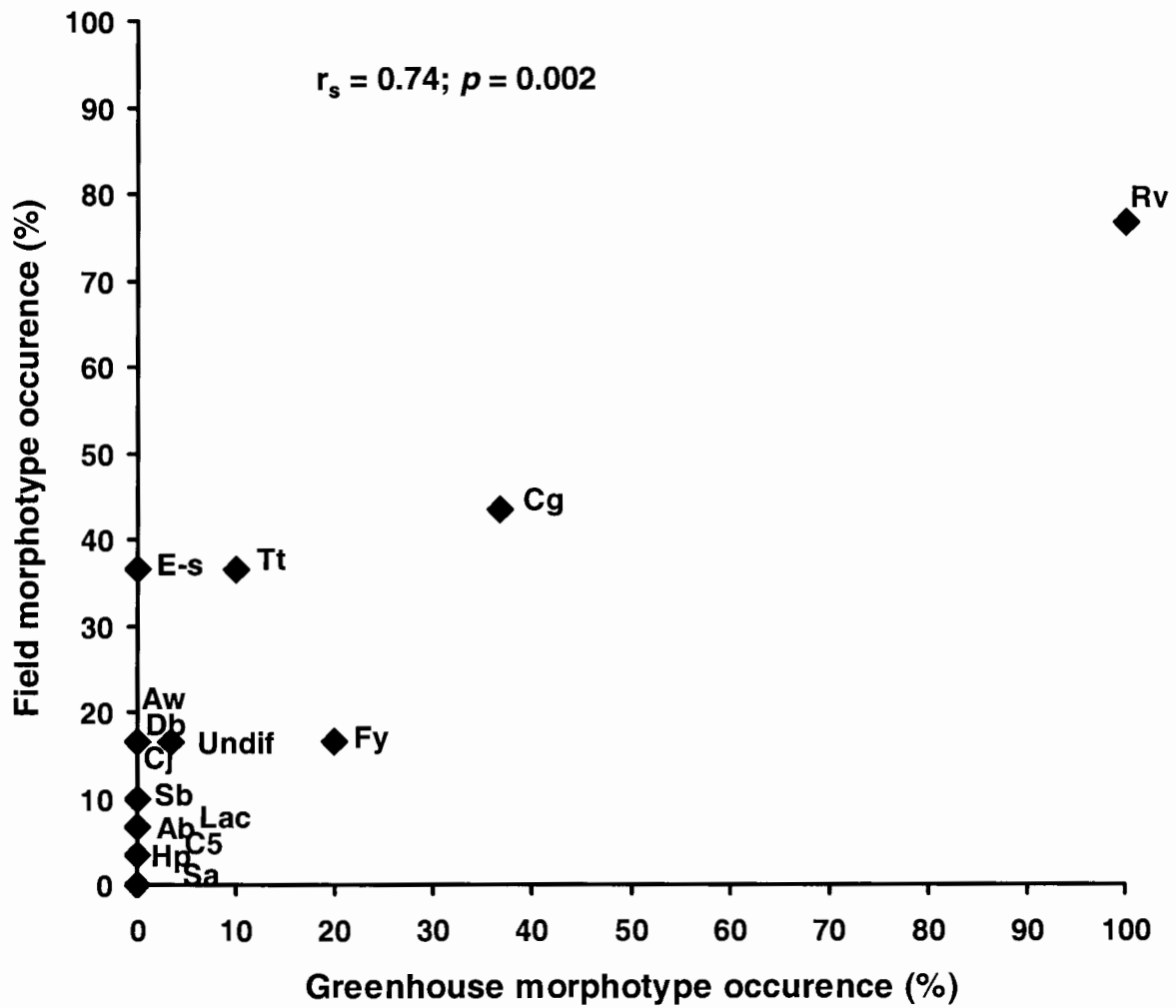


Fig. 7. Height and diameter increment, and shoot and root dry biomass gain of Douglas-fir seedlings grown in landing and clearcut soils after two (field) and one (greenhouse) growing seasons. Error bars indicate one standard error of the mean. *, **, *** indicate that means differ significantly within the particular bioassay at $p < 0.1$, 0.05, and 0.01 respectively.

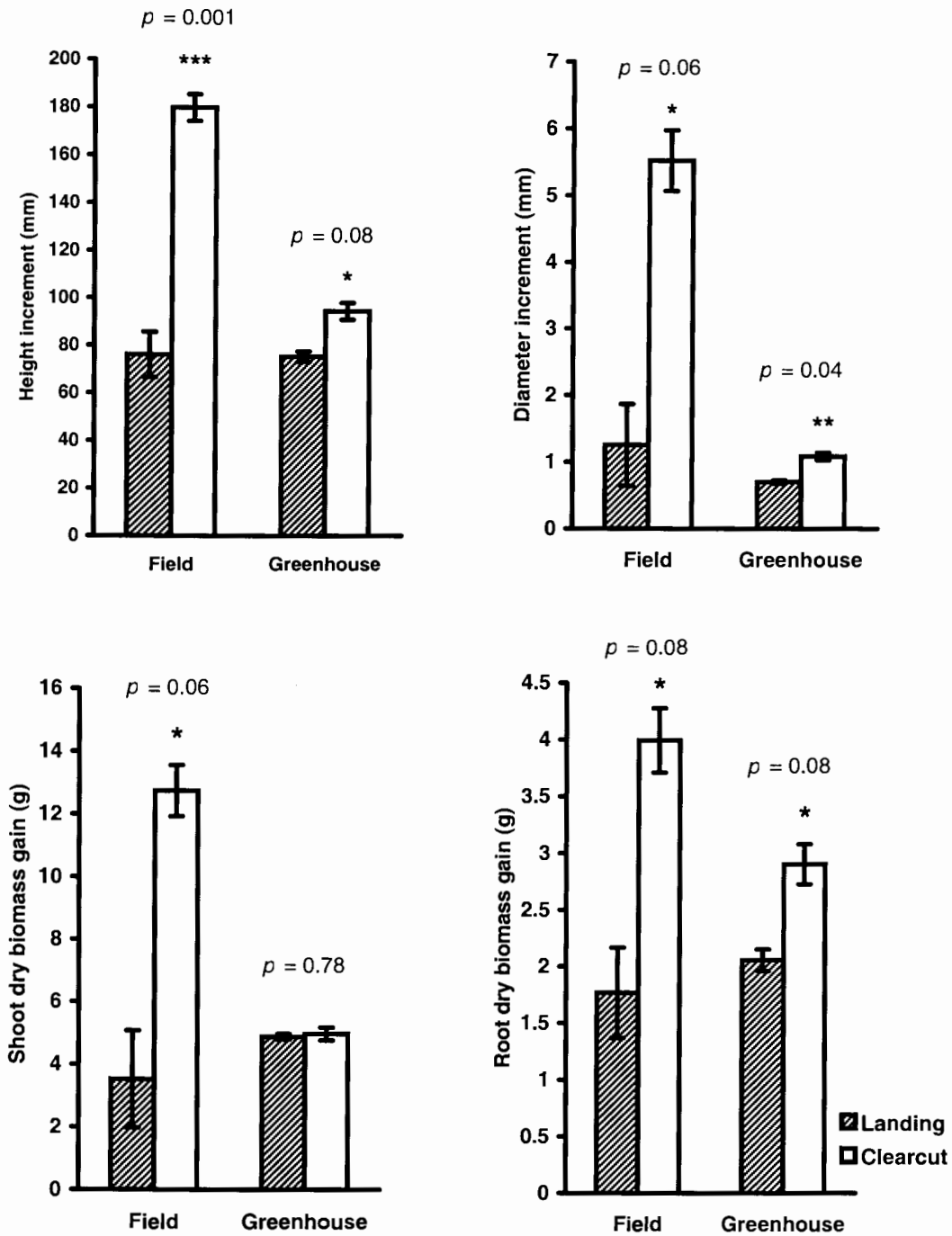
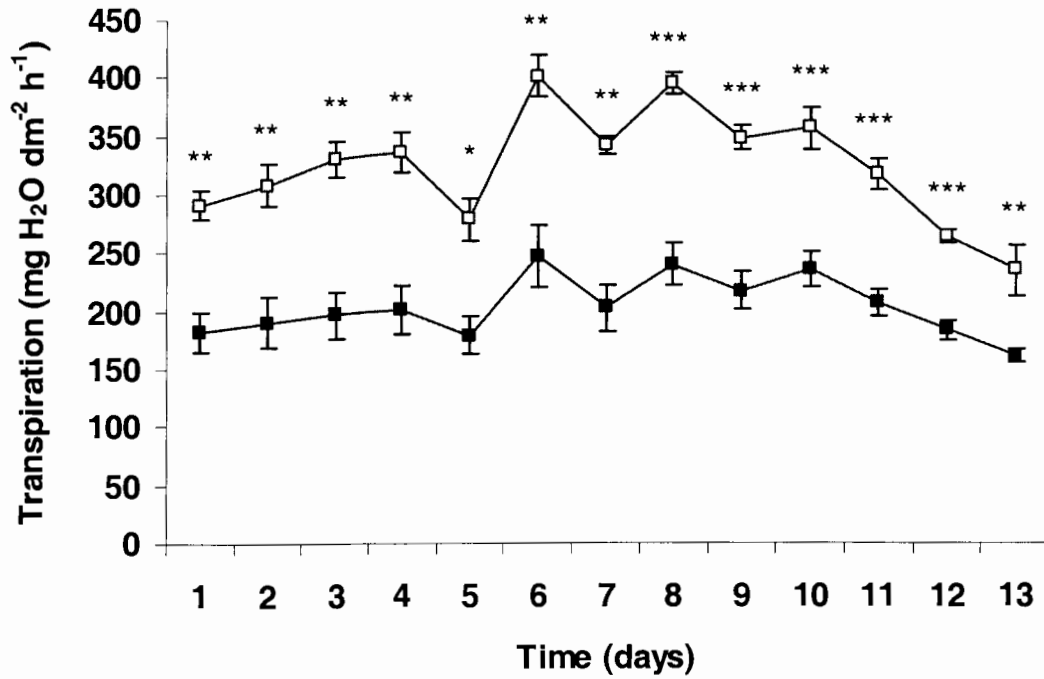


Fig. 8. Transpiration rate of Douglas-fir seedlings subjected to drought while growing in clearcut (□) and landing (■) soils in the greenhouse. Error bars indicate one standard error of the mean. *, **, *** indicate that means differ significantly at $p < 0.1, 0.05, 0.01$ respectively.



CONCLUSION

The objectives of the first part of this study were mainly to determine if commercial ectomycorrhizal inoculants could increase survival and growth of Douglas-fir seedlings outplanted on partially rehabilitated landings, clearcuts, and burned piles. Two years after outplanting inoculated seedlings showed little survival and height differences on all of the sites. The greatest height differences were found between soil treatments where seedlings grown on clearcuts were significantly taller than seedlings grown on both shallow- and deep-tilled landings.

The potential for a fungal inoculant to successfully colonize and benefit Douglas-fir seedlings is great because Douglas-fir is usually very poorly colonized by ectomycorrhizal fungi when seedlings are grown under present cultural conditions found in B.C. nurseries. Yet, at time of outplanting poor colonization levels (mean of 36%) of inoculated Douglas-fir seedlings were observed in this study. These levels may be too low to determine the potential of commercial inoculants. I suggest that nursery cultural conditions should be adjusted to favor high levels of ectomycorrhizal colonization by inoculated fungi. More research should be conducted with seedlings heavily colonized by inoculated fungi at time of outplanting.

However, altering nursery conditions to favor an increase in colonization levels of inoculated fungi may only be part of the solution. Contaminating and opportunistic nursery fungi may also find such conditions favorable and hence compete by colonizing new emerging root tips. Research is needed to verify this and how a more diverse community of ectomycorrhizal fungi would affect growth performance in the field. If

performance does increase with seedlings harboring a diverse ectomycorrhizal community at time of planting more research should also be conducted with seedlings inoculated with soil from the field sites. This soil transfer technique would allow native pioneering fungi to colonize seedlings in the nursery. However, further research should be carried out to verify the usefulness of such inoculations.

In this study, regardless of the partial soil rehabilitation that was conducted (shallow or deep till, burned), harsh conditions remained present on the landings. Even with higher levels of ectomycorrhizal colonization on the landings compared to the clearcuts, noninoculated seedling height was lower on the landings. This is probably because landings were more compacted, drier, and had lower levels of mineralizable-N than clearcuts.

The primary goal of the second part of this study was to evaluate and compare ectomycorrhizal status, and growth of Douglas-fir and the native EIP on landing and clearcut soil. Field and greenhouse bioassays were conducted using noninoculated and nonectomycorrhizal seedlings, respectively. In the field, seedlings grown in clearcut soil had significantly better growth than seedlings grown in landing soil. In the greenhouse similar results were obtained except that shoot biomass did not differ between landing and clearcut soil. These results are important for forest companies that may want to use Douglas-fir to reclaim landings in the ICH since Douglas-fir seedlings performed poorly and it may not be the ideal conifer candidate for reclamation of landings. Previous studies have shown relatively good results using lodgepole pine and this may in part be because lodgepole pine is better adapted to harsh conditions and is usually heavily colonized by ectomycorrhizal fungi at time of outplanting (Hunt 1992).

In the field and greenhouse, *Rhizopogon vinicolor*-like was the most abundant ectomycorrhizal type found in both the landing and clearcut soil. As expected clearcuts had more ectomycorrhizal types than landings and had a significantly higher evenness index. The native EIP of the landings was lower than on the clearcuts. This is not surprising, since landings were poor in organic matter and did not have any forest floor. Inoculating seedlings may be important for obtaining adequate growth on harsh sites such as landings where the native inoculum is low.

In the greenhouse, seedlings grown in the landing soil had significantly higher percent ectomycorrhizal colonization than seedlings grown in the clearcut soil and I found that *Rhizopogon vinicolor*-like was well adapted to the harsh conditions found on the landing soil and could be a potential candidate as an inoculant. Results found in the greenhouse resembled the ones from the field, thus this research demonstrates that greenhouse bioassays can be used to assess the ectomycorrhizal status of seedlings growing on degraded forest sites.

Further research on degraded sites such as landings should concentrate on assessing the survival and growth responses of inoculated Douglas-fir seedlings heavily colonized at time of outplanting. Field and greenhouse bioassays could be conducted with seedlings permitted to form ectomycorrhizae at various percent colonization levels (including no colonization) in order to determine some sort of beneficial threshold and to demonstrate the importance of such symbiotic associations. Also, more research should be done on landings that have been partially rehabilitated with nearby organic matter amendments coupled with deep-tilling to determine if nutrient deficiency and toxicity, seedling growth, and EIP can be addressed and to allow adequate Douglas-fir seedling growth.

APPENDIX A - Photo CD-ROM

Please see the attached CD-ROM.

Study site in the fall 2000:

1. Upper landing.
2. Upper shallow-tilled landing.
3. Upper deep-tilled landing.
4. Upper burn pile.
5. Upper cutblock.
6. Rows of outplanted Douglas-fir seedlings on upper landing.
7. Row of outplanted Douglas-fir seedlings on upper burn pile.
8. Landing (left) and cutblock (right) soil.
9. Sieving the middle landing soil for the greenhouse bioassay.
10. Sieving and collecting soil from the middle landing for the greenhouse bioassay.

Soil and bulk density sampling using the excavation method:

11. Bulk density sampling at the upper landing.
12. Excavating the landing soil.
13. Collecting the excavated soil in a plastic bag.
14. Determining the volume of the excavated hole.

Greenhouse bioassay:

15. Douglas-fir seedlings in pots.
16. *Laccaria* sp. sporocarp found in a pot during the greenhouse bioassay.
17. *Rhizopogon vinicolor*-like with rhizomorphs (arrows) at the bottom of a pot.
18. *Rhizopogon vinicolor*-like (arrows) colonizing root tips at the bottom of a pot.
19. *Rhizopogon vinicolor*-like clusters.

Study site in the fall 2001:

20. Upper landing with shallow-till (left) and deep-till (right) treatment.
21. Middle landing with shallow-till (right) and deep-till (left) treatment.
22. Lower landing.
23. Chlorosis of outplanted Douglas-fir seedling on shallow-tilled upper landing.
24. Outplanted Douglas-fir seedling on deep-tilled upper landing.
25. Outplanted Douglas-fir seedling on upper burn pile.
26. Outplanted Douglas-fir seedling on upper cutblock.
27. Barely surviving outplanted Douglas-fir seedling on shallow-tilled middle landing.
28. Chlorosis of outplanted Douglas-fir seedling on shallow-tilled middle landing.
29. Relatively healthy outplanted Douglas-fir seedling on shallow-tilled middle landing.
30. Naturally regenerating Douglas-fir seedling on lower landing.
31. Naturally regenerating Western larch seedling on lower landing.
32. Root system of naturally regenerated Douglas-fir seedling on lower landing.

Features of some ectomycorrhizal types under a dissecting or/and compound microscope:

33. *Rhizopogon vinicolor*-like (SFU 001) monopodial pinnate form with thick rhizomorphs (arrow).
34. *Rhizopogon vinicolor*-like subtuberculate form with thick rhizomorphs (arrows).
35. *Rhizopogon vinicolor*-like with elbow-like emanating hyphae (arrow).
36. *Rhizopogon vinicolor*-like with a net prosenchyma outer mantle.
37. *Rhizopogon vinicolor*-like intercellular colonization (labyrinthic Hartig net) of Douglas-fir roots.
38. *Thelephora terrestris*-like (SFU 002) not branched form.
39. *Thelephora terrestris*-like irregular form.
40. *Thelephora terrestris*-like orange tint irregular form.
41. *Thelephora terrestris*-like with cystidias.
42. *Thelephora terrestris*-like with basal clamped cystidia (arrow).
43. *Cenococcum geophilum* (SFU 003) pure black not branched form.
44. *Cenococcum geophilum* with thick brown emanating hyphae.
45. *Cenococcum geophilum* with a characteristic stellate patterned net synenchyma.
46. Unknown silver-amorphous (SFU 005).
47. Unknown silver-amorphous with an interlocking irregular synenchyma outer mantle.
48. *Amphinema byssoides*-like (SFU 007) with yellow rhizomorphs (arrow).
49. *A. byssoides*-like emanating hyphae with keyhole clamps (arrows) and 90° junction (arrowhead).
50. Unknown flaky yellow (SFU 008).
51. E-strain (*Wilcoxina* sp.) (SFU 009) patchy outer mantle.
52. Unknown caramel jigsaw (SFU 010).
53. Unknown caramel jigsaw outer mantle an interlocking irregular synenchyma resembling a jigsaw puzzle.
54. CDE5-like (SFU 011) coarsely ornamented emanating hyphae.
55. *Amphinema*-like (SFU 013) white form with white rhizomorphs (arrows).
56. Nonectomycorrhizal root with root hairs.
57. Dead necrotic root tips.

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