# LOW SOIL TEMPERATURE AND EFFICACY OF ECTOMYCORRHIZAL FUNGI

by

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

FOREST SCIENCE DEPARTMENT

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

September 1991

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Date Oct. 2, 1991

DE-6 (2/88)

## ABSTRACT

The influence of root-zone temperature on the efficacy of various ectomycorrhizal fungi, i.e., their ability: (1) to colonize roots in a nursery environment, (2) to persist and colonize new roots in the field and (3) to improve the growth, nutrition, and physiology of white spruce (Picea glauca (Moench) Voss) seedlings, was examined in controlled environment experiments using water baths to regulate root-zone temperature.

Eight-week-old non-mycorrhizal seedlings were inoculated with 13 different inocula (1 forest floor inoculum, 12 specific fungi), then transplanted into 6, 16, or 26°C peat:vermiculite mixes for 8 weeks. Maximum root colonization occurred at 16°C for most inocula. The 6°C mix strongly reduced mycorrhiza formation with only 8 of the 13 inocula forming any mycorrhizae during the 8-week test period. Primary infection from ectomycorrhizal propagules (spores and hyphal fragments) was reduced more than was secondary infection from established mycorrhizae; once established, all inocula colonized new roots in 6°C forest soil.

Fall-lifted cold-stored seedlings infected with 8 inocula (forest floor, 7 specific fungi) were planted into 6 and 12°C forest soil mixtures with or without indigenous ectomycorrhiza inoculum. Survival and colonization of new roots by inoculant fungi was good (> 50%) for the 12-week test duration despite the significant potential for infection by indigenous inoculum. High persistence appeared to be due to successful (>75%) root colonization by the inoculant fungi in the nursery production phase, to the relative weakness of ectomycorrhizal propagules (spores and hyphal fragments) compared with live ectomycorrhizal attachments, and to the favorable pattern of lateral root egress from the container plug after planting.

Colonization of new roots by established mycorrhizae showed an effect of soil temperature in the presence, but not the absence, of indigenous inoculum. Percent new root colonization by inoculant fungi was lower in the 12°C forest soil. Rapid extension of lateral roots in the 12°C soil increased the likelihood that short roots initiated near the tips of elongating roots would be infected by indigenous fungi. There was no evidence of active or passive interactive replacement between inoculant and indigenous fungi. However, Hebeloma crustuliniforme appeared to inhibit mycorrhizal formation by indigenous fungi; roots not infected by this fungus remained non-mycorrhizal. Application of slow-release fertilizer reduced new root colonization by E-strain but had no effect on colonization by H. crustuliniforme or indigenous forest floor fungi.

Non-inoculated seedlings (controls) and seedlings inoculated with 5 different inocula (forest floor, 4 specific fungi) were planted in 6 and 12°C forest soil for 3 weeks. Inoculation influenced the rate at which seedlings acclimated to the 6°C soil with respect to resistance to water flow and net photosynthetic rate, but had no effect on pre-dawn stomatal conductance. Differences among inoculation treatments were related to the size and nutritional status of seedlings at the time of transplanting. Seedlings infected with Laccaria bicolor or E-strain exhibited the least decrease in resistance to water flow due to the relatively small size (dry weight, short root number) of their root systems at the time of transplanting. Net photosynthetic rate and new foliage production correlated positively with shoot N and P (% dry weight) and the proportion of total seedling N and P contained in shoot tissues at the time of planting.

Non-inoculated seedlings (controls) and seedlings inoculated with forest floor or 5 specific fungi were planted in 6 and 12°C forest soil for 12 weeks. The presence of "any" mycorrhiza at the time of transplanting did not improve seedling growth under the experimental conditions (i.e., cool, acidic soils with an indigenous ectomycorrhizal fungal population). On average, mycorrhizal infection increased N and P uptake at 12°C but not at 6°C. Growth response to specific fungi was very variable with some fungi depressing seedlings growth (e.g., E-strain and H. crustuliniforme) and others strongly promoting it (forest floor inoculum, L. bicolor, Thelephora terrestris). Seedling response to the various inocula was not related to the degree of mycorrhizal infection at the time of planting nor to the source of inocula; but was associated with differences in the content and distribution of nutrients at the time of transplanting and differences in total nutrient uptake, root efficiency, nutrient-use efficiency and net photosynthetic rate after transplanting. Root efficiency was not proportional to the number of short roots per unit root or to the amount of external mycelium attached to the various mycorrhizae. Implications for applied forestry and research are discussed in the final chapter.

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## ACKNOWLEDGEMENTS

This study was funded by Forestry Canada under the Canada-British Columbia Forest Resource Development Agreement (1985-1990), a National Science and Engineering Research of Canada scholarship and a Science Council of British Columbia G.R.E.A.T award. I would like to thank the Canadian taxpayers for supporting these programs.

Many people from Forestry Canada provided invaluable assistance during the research and analysis phase of the dissertation. I would like to acknowledge Clarence Simmons for his advice and programs for conducting non-parametric analyses; Rob Hagel for seedling culture advice; George Edwards for providing growth chamber space; Tony Trofymow, Tad Woods and Charles Dorworth for lending microscopes and other laboratory equipment. At U.B.C., Fred Lewis, Farida Bishay and Jeff Glaubitz completed hundreds of tedious root and leaf measurements with care and good humour. Chemical analyses were conducted by MacMillan Bloedel Limited, Woodlands Services Division, Nanaimo, B.C. Soilcon Laboratories, Richmond, B.C. provided soil water retention data for the various soil mixtures.

I owe a great deal to three dissertation advisors Drs. Tony Trofymow, Bob Danielson and Shannon Berch and to my thesis supervisor Denis Lavender. Tony Trofymow was not only very generous with advice but also provided the means (laboratory space and equipment) for me to conduct a major portion of my research in Victoria when my husband was transferred there in 1987. Bob Danielson and Shannon Berch introduced me to mycorrhizal fungi and the techniques for isolating and culturing specific fungi, identifying morphological types and assessing root colonization. While I still have much to learn in this regard, I am very grateful for the opportunity to share their enthusiasm and expertise. Denis Lavender shared his enthusiasm for silviculture and applied research and guided my selection and formulation of a research problem. The planning and writing of my thesis dissertation benefited from the ideas, suggestions and criticisms of all members of my thesis committee: Drs. I.E.P. Taylor, B. van der Kamp, T. Ballard and the above-mentioned advisors.

Finally, my family provided emotional support and a balanced perspective on life throughout my studies; thank you Eileen, Jack, Pauline, Tom, Matthew, Janice and Ron. And a special thanks to my husband, Terry Berscheid, for his constant encouragement and help with computer glitches, especially during the final year of my program.

# CHAPTER I

# INTRODUCTION

## The Problem

My thesis research was funded by the Canada-British Columbia Forest Agreement 1985-1990. This program was implemented to improve reforestation success, particularly on backlog sites (i.e., those logged more than 10 years ago and not reforested). The early growth of outplanted conifer seedlings on many northern sites is less than expected due in part to the cool soils of planting sites which are not mechanically site-prepared (Butt 1986). The average mid-summer temperature of northern planting sites at a soil depth of 10 cm is approximately 10°C; in spring and early summer it is closer to 4 or 5°C (Tyron and Chapin 1983, Binder et al. 1987). These soil temperatures are well below the optimal range (18-24°C) for most conifer species (Ritchie and Dunlap 1980).

Regardless of soil temperature, the degree to which nursery seedlings are preconditioned to the planting site has a great influence on seedling field performance (Burdett 1983). Preconditioning seedlings, by inoculating them with specific ectomycorrhizal (ECM) fungi, has been shown to improve field performance (Castellano and Trappe 1985, Kropp et al. 1985, Last et al. 1984, Molina and Trappe 1982, Wilson et al. 1987). Mycorrhizal seedlings may benefit from enhanced nutrient or water uptake, increased resistance to pathogens or greater root longevity (Harley and Smith 1983).

However, mycorrhizal inoculation does not always improve seedling performance (Bledsoe et al. 1982, Shaw et al. 1987b). Trofymow and van den Driessche (1991) summarized the results of 84 outplanting trials with artificially inoculated conifer seedlings. Inoculation increased seedling

performance on only 43% of the trials located on routine reforestation sites.

More research is needed to determine the conditions (host species, site and silvicultural system) under which benefits are most likely to occur. Use of mycorrhizal inoculation to improve seedling performance is severely limited by a lack of knowledge of the ecology of ECM fungi.

> "Mycorrhizal fungi occur in a large diversity of plant communities and their adaptation to extremes of environment is widely acknowledged; at the same time, we are still in the "Dark Ages" with regard to applying these features of ecological specialization to agriculture, forestry or in restoring native vegetation. In spite of enormous interest in "tailoring" host-fungus combinations for specific planting sites, we are limited by our understanding of the environmental characteristics of the site, the range of tolerance of the mycorrhizal symbiont, and the ability of the fungus to benefit the host under natural conditions" (Parke 1985, p. 107).

The applied forestry objective of the funding agency presented an opportunity to examine the influence of an environmental factor, soil temperature, on the growth and physiology of conifer seedlings; the modifications of these effects by ectomycorrhizal inoculation; and the influence of soil temperature on the efficacy of various ECM fungi.

Although it is known that temperature alters the growth of ECM fungi in culture (Dennis 1985, Hacskaylo et al. 1965, Marx et al. 1970, Samson and Fortin 1986, Theodorou and Bowen 1971) and in forest soils (Heninger and White 1974, Marais and Kotze 1978, Parke et al. 1983b), the influence of temperature on the efficacy of ECM fungi, in particular their ability to persist in forest soils and to promote seedling growth, is poorly understood (Perry et al. 1987). Trappe (1977) stressed the importance of soil temperature on the efficacy of various mycorrhizal fungi and the need for more knowledge of the adaptation of ECM isolates to the soil

temperatures at the planting site.

Inoculation with mycorrhizal fungi has been shown to reduce the adverse effects of high root-zone temperatures (Marx and Bryan 1971, Borges and Chaney 1989). Inoculation might have the reverse effect, however, in sub-optimal root temperatures. Research with vesicular-arbuscular mycorrhizae (VAM) has shown that cool temperatures eliminate the benefits normally derived from mycorrhizal infection (Moawad 1978, Smith and Roncadori 1986). The growth of VAM plants may even be less than that of non-mycorrhizal plants at low temperatures (Furlan and Fortin 1973, Hayman 1974, Schenck and Schroder 1974). Similar research has not been conducted for ectomycorrhizae.

Many studies have shown that some species or strains of mycorrhizal fungi are more efficient than others in promoting host plant growth (Benecke and Göbl 1974, Trappe 1977, Marx 1979). A fungus which increases growth of a host plant in one set of study conditions may not in another set (Trappe 1977). Much research on mycorrhizal efficacy has focussed on physiological attributes of ECM fungi, such as their ability to produce plant growth regulators or absorb nutrients from solutions. More research on the ecology of mycorrhizal fungi should also contribute to our understanding of differences in efficacy.

White spruce[1] seedlings were selected as host plants. The survival and growth of planted white spruce seedlings is less than satisfactory on many backlog reforestation sites in the northern interior of British Columbia (Butt 1986). Planting check, a period of slow shoot growth after planting, is common and results in reduced shoot growth (50% of expected)

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[1] The scientific name is Picea glauca (Moench) Voss. Common names of vascular plants are used in the dissertation with Appendix A listing both common and scientific names.

for several years after planting. This increases the susceptibility of spruce seedlings to brush competition.

The potential benefits of a nursery inoculation program are greatest when the growth of outplanted seedlings is limited by water or nutrient availability in the first growing season, the inoculum potential of indigenous fungi at the planting site is low, and the inoculant fungus is adapted to the planting site environment (Trappe 1977, Parke 1985).

Based on these criteria, mycorrhizal inoculation has good potential for improving the reforestation of white spruce on backlog sites. Planting check of white spruce results, at least in part, from poor root growth and reduced water or nutrient uptake in the first growing season (Burdett et al. 1984, Butt 1986). Furthermore, the native inoculum potential of backlog sites may be lower and less predictable compared to recently logged sites or more southern reforestation sites where most mycorrhizal research has been conducted. Danielson (1985) found that new roots formed outside the original root mass of bare-root seedlings planted on a backlog site near Prince George, B.C. were not colonized by indigenous fungi in the first growing season.

Finally, 70% of interior spruce seedlings are grown in southern nurseries. White spruce seedlings often become mycorrhizal in container nurseries. It is not known if the nursery ECM fungi persist or benefit seedling growth at northern planting sites. Fungi colonizing seedlings in warm (15 to 35$^{o}$C), fertile container growing mixes may not be adapted to the cool (5 to 10$^{o}$C), less fertile soils of northern planting sites.

Experimental Approach

The thesis research was conducted in a controlled environment chamber so that soil temperature could be varied independently of other environmental conditions, including soil fertility and indigenous ECM inoculum. Controlled environment studies have been criticized repeatedly, especially by foresters operating in the "real world" of fluctuating environments. This criticism is valid, especially if one wishes to calculate gains in growth due to various inoculation treatments. However, this was not the primary goal of the research.

Soil temperature effects on plant growth are influenced by many unrelated factors including soil fertility, water content, bulk density and air temperature (Sutton 1969, Nielson 1971, Ritchie and Dunlap 1980, Kuhns et al. 1985). In field experiments, it is very difficult to separate the effects of soil temperature from those of site preparation technique, organic matter content of the rooting zone and soil drainage (e.g, Binder et al. 1987, Brand and Janas 1988).

The formation and persistence of mycorrhizae depend to a significant degree on the physiology of the host plant which in turn is affected by its environment (Trappe 1977, Harley and Smith 1983). In a series of experiments examining the interactions between soil temperature, soybean growth, and mycorrhizal development, Schenck and Smith (1982) found that their results varied from year to year possibly due to uncontrolled variation in the shoot environment, i.e., photoperiod, light intensity, air temperature and humidity. Physiological measurements, such as the net photosynthetic rate and resistance to water flow, are also a function of many factors including air temperature, radiation, relative humidity, soil water content, temperature, and fertility. Given all these potential

interactions, in a "real world" environment, the effects of soil temperature on the mycorrhizal symbiosis may be confounded by many variable factors.

In a controlled environment, a constant shoot environment can be maintained for all inoculation and soil temperature treatments. The confounding of air-soil temperature differentials with soil temperature treatments was recognized, but not considered to be cause for concern. Examination of data presented by Lavender and Overton (1972) summarizing root and shoot growth of Douglas-fir seedlings over a range of soil, air and soil-air differentials verified this assumption. A degree change in soil temperature had a much greater effect on these growth data than did a degree change in air temperature over a wide range of shoot-air temperature differentials (2-20$^{o}$C). Similarly, data from Lawrence and Oechel (1983b) show a consistent soil temperature effect on the photosynthetic rate of four deciduous taiga trees over a 5 to 15$^{o}$C range in soil-air differentials.

Relationships developed in a controlled environment are not reliable predictors of absolute plant growth in natural environments; however, these have proven useful during the interpretation of field studies involving root pathogens (Salt 1979), water relations of plant communities (Jarvis 1976) and seedling physiology (Lavender 1988). In addition, the results of controlled environment studies can be used to develop models of field responses (Jarvis 1976) or to screen mycorrhizal fungi for particular field environments.

Scope of Thesis

Determining how to measure efficacy of ECM fungi was the first research question. According to Trappe (1977), the efficacy of a particular ECM fungus will depend on: (1) its aggressiveness in conifer nurseries (degree of root colonization), (2) its survival and colonization of new roots of outplanted seedlings and (3) its inherent ability to benefit the host plant.

It is the third aspect of Trappe's definition that is problematic. Although the benefits most often measured are host plant survival and growth, particularly shoot growth, there are numerous other equally valid measurements, e.g., nutrient uptake, drought tolerance, resistance to specific pathogens (Smith 1985). Parke (1985) stressed that efficacy should be defined by the factors most limiting to conifer seedling growth on particular reforestation sites. Improved nutrient uptake may not benefit seedlings growing on nutrient rich sites or on extremely droughty sites where nutrients are not the major factor limiting plant growth. A fungus which increases host plant drought resistance may not be effective in well-watered soils. Björkman (1962, cited in Kropp and Langlois 1990) suggested that alleviation of transplant shock is an important benefit of mycorrhizal infection to boreal spruce seedlings.

I decided to use four criteria to compare the efficacy of mycorrhizal fungi at different soil temperatures. The first and second were as defined by Trappe (1977), the third was the ability to reduce transplant shock of cold-stored spruce seedlings, and the fourth was the ability to improve their nutrient uptake and growth (both root and shoot) during a 12-week simulated growing season. Chapters II, III, IV, and V respectively address these four criteria. Finally Chapter VI discusses the implications of the

main results of this work for applied forestry and research.

# CHAPTER II

# EFFECT OF ROOT-ZONE TEMPERATURE ON ROOT COLONIZATION

## Introduction

The rate at which ECM fungi colonize root systems affects their ability to enhance host plant growth and physiology. The most effective fungi are often those that rapidly and extensively colonize seedling root systems (Abbott and Robson 1984, Perry et al. 1987). Temperature influences the growth of ECM fungi in pure culture (Hacskaylo et al. 1965, Marx et al. 1970, Theodorou and Bowen 1971, Dennis 1985, Samson and Fortin 1986) and the rate of mycorrhiza formation when seedlings are planted in forest soil (Heninger and White 1974, Marais and Kotze 1978, Parke et al. 1983b). These studies suggest that the optimum temperature for fungal growth in culture and ectomycorrhiza formation in forest soils lies between 18 and 35°C, i.e., temperatures that are well above the soil temperatures measured at northern planting sites.

However, significant intra-and interspecific variation in fungal response to temperature in the culture experiments has been reported (Dennis 1985, Samson and Fortin 1986) with some fungal isolates more tolerant of temperature extremes than others. Parke (1983) hypothesized that the response of ECM fungi to temperature was related to their geographic origin. Optimum temperatures for ECM fungi native to hot climates (e.g., southeastern U.S.A., Australia and Southern Africa) are higher, between 30 and 35°C (Marx et al. 1970, Theodorou and Bowen 1971, Marais and Kotze 1978), than optimum temperatures (between 18 and 24°C) for fungi native to the Pacific Northwest (Parke 1983).

In container nurseries, rapid colonization by selected inoculant

fungi is necessary if contamination by indigenous nursery fungi (e.g., Thelephora terrestris) is to be prevented. It would be useful to know if fungi indigenous to cool forest sites can form mycorrhizae rapidly in peat:vermiculite growing mixes that can reach temperatures as high as 35°C during the first 3 months after sowing (Husted and Barnes 1987).

In temperate and boreal forests, adaptation of ECM fungi to cool soil temperatures is more important than tolerance of high temperatures. Poor adaptation to cool soils could be a major cause of the observed inability of many mycorrhizal fungi established in container nurseries to persist and benefit outplanted conifer seedlings (Trappe 1977). In British Columbia, Pisolithus tinctorius, a fungus with a high optimum temperature for root infection (Marx and Bryan 1971) was the first ECM fungus tested in an outplanting trial[1]. Possibly because it was adapted to warmer forest soils than occur in British Columbia, P. tinctorius failed to persist on a southern Vancouver Island clear-cut, a relatively warm forest site for British Columbia.

The objective of the work reported in this chapter was to determine the effect of three growing mix temperatures, 6, 16, and 26°C on root colonization by a variety of fungal isolates collected from forest and container nursery environments. It was hypothesized that isolates collected from nursery environments (with temperatures reaching 35°C) would form mycorrhizae more rapidly at 26°C and more slowly at 6°C than isolates collected from Pacific Northwest forest sites. I originally planned to compare nursery and northern forest isolates of two or more fungal species, such as T. terrestris or Laccaria bicolor naturally found in both

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1 Personal communication with J. Dennis, Forestry Canada, Victoria, B.C.

environments. However, it was not possible with the time available to obtain this experimental material. British Columbia does not have a permanent culture collection of the mycorrhizal fungi commonly found in nursery and forest environments.

Approach

The inoculum potential of ECM fungal cultures varies with isolate, species, and culture conditions. To ensure that each test fungus had a high inoculum potential, I saturated the seedling growing medium with slurries containing higher concentrations of mycelium than reported in previous studies (Danielson et al. 1984b, Boyle et al. 1987).

Seedlings were grown for 8 weeks before inoculation. This delay increased the likelihood of contamination from air-borne spores of indigenous nursery fungi. In fact the first attempt at this experiment was a failure because of contamination. However, the delay had two important advantages. First, root colonization by mycorrhizal fungi is strongly influenced by the size and physiology of the host plant, especially its production of carbohydrates. During the 8-week growth period, the germinants were culled and thinned to minimize variation in seedling size. Second, the delay allowed some distinction between the effects of temperature on the availability of potential infection sites (i.e., short root number) and the effects of temperature on fungal growth and intensity of colonization.

## Methods

### Source of seed, fungi and forest soil inoculum

White spruce seed from a central interior (approx. 55°N, 123°W) seedlot (No. 29144) was obtained from the B.C. Ministry of Forests. Five ECM fungal isolates were collected from container nurseries; seven from forest sites. The origins of these twelve isolates are summarized in Table 2.1. T. terrestris is the most common mycorrhizal fungus identified on nursery seedlings in North America, but the others have also been reported in nurseries (Castellano and Molina 1989, Danielson and Visser 1990). Forest floor inoculum was obtained from three vigorous white spruce plantations established near Mackenzie, B.C (approx. 55°N).

### Production of tree seedlings

Seeds were surface sterilized 15 min in 30% $H_2O_2$, rinsed in distilled water and refrigerated for 24 h at 4-5°C; then sown, 3 per cavity, in 40 $cm^3$ Spencer-LeMaire Rootrainers filled with a pasteurized (70°C, 60 min) peat:vermiculite growing mix prepared from: 110 L (a bale) of Premier brand 'Forestry Peat', 57 L medium grade horticulture vermiculite, 175 g Micromax Micronutrients (Sierra Chemical Co., Milpitas, CA) and 750 g coarse dolomite lime.

Seedlings were grown in a controlled environment chamber for 8 weeks: 18 h photoperiod, 20°C day/night air temperature and 70-90% relative humidity. Three weeks after germination, the seedlings were thinned to one per cavity with the largest and smallest seedlings removed to minimize between seedling variation in size.

Table 2.1. Source of test fungi

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Amphinema byssoides (Fr.) J. Erikss. (0288)

Author. Isolated in 1988 from surface-sterilized ectomycorrhizae of container spruce seedlings grown at the Balco Canfor Reforestation Centre, Kamloops, B.C.

E-strain (or Complexipes moniliformis Walker) (947)

Dr. R.M. Danielson's collection at the University of Calgary. Isolated in 1978 from white spruce seedlings growing in a fertilized subalpine soil.

E-strain (0188)

Author. Isolated in 1988 from surface-sterilized ectomycorrhizae of container Douglas-fir seedlings grown at the Harrop Nursery, B.C.

Forest Floor

Author. Bulked sample collected in 1987 from three juvenile spruce plantations near Mackenzie, B.C.

Hebeloma crustuliniforme (Bull. ex St. Amans) Quelet (5247,5249)

University of Alberta, Microfungus Collection. Collected in 1985 from white spruce (Picea glauca) woods, Lac La Biche forest, AB.

Hebeloma crustuliniforme (Bull. ex St. Amans) (5)

University of Washington Collection (Dr. Bledsoe). Isolated in 1984 from a mixed conifer forest at 550 m in Wenatchee National Forest, WA.

Table 2.1. (continued)

Hebeloma crustuliniforme (Bull. ex St. Amans) (8)

University of Washington Collection (Dr. Bledsoe). Isolated in 1971 from a Douglas-fir forest, Benton County, Oregon.

Hebeloma crustuliniforme (Bull. ex St. Amans) (125)

From Dr. G. Hunt, Balco Canfor Reforestation Centre, Kamloops, B.C. Isolated in 1985 from a mixed spruce, fir and cedar forest near Barrier Lake, B.C. (1400m).

Laccaria bicolor (R. Mre.) Orton (5268)

University of Alberta Microfungus Collection. Isolated in 1985 from a pine-spruce forest, Slave Lake Forest, Alberta.

Laccaria bicolor (R. Mre.) Orton (5313)

University of Alberta Microfungus Collection. Isolated in 1976 from container seedlings in FSL greenhouses, Corvallis, Oregon.

Laccaria laccata (Scop. ex Fr.) Berk.& Br. (101C,D)

From Dr. G. Hunt, Balco Canfor Reforestation Centre, Kamloops, B.C. Isolated from sporocarps in the nursery.

Thelephora terrestris Ehrhart ex Fr. (2088)

Author. Isolated in 1988 from surface-sterilized ectomycorrhizae of spruce seedlings grown at CIP nursery, Saanichton, B.C.

Thelephora terrestris Ehrhart ex Fr.(Laval)

University of Laval CRBF Culture Collection. Isolated in 1980 from a young poplar stand near Quebec City.

Once a week, seedling blocks were rerandomized to minimize effects of block position within the growth chamber, and the seedlings were watered to saturation with a water-soluble fertilizer solution (100 mg/L N) containing micronutrients (Plant-Prod 20-20-20, Plant Products Co. Ltd., Bramalea, ON).

Production of inoculum

Twelve fungal symbionts were grown on modified Melin-Norkans (MMN) agar (Marx 1969) for 4-8 weeks until at least 50% of each Petri plate was covered by mycelium. Mycelial slurries (Danielson et al. 1984b, Boyle et al. 1987) were prepared by homogenizing mycelium and adhering agar from 10 Petri dishes for 15-20s in a Waring blender and then adding distilled water to give a final volume of 300 mL. Viability of mycelial fragments was tested by culturing 5 mL aliquots on MMN agar.

Inoculum for the control treatment was prepared by autoclaving mycelium and agar before preparing the slurry. Forest floor material from the three plantations was bulked, mixed thoroughly and passed through a 1 cm screen to remove large organic debris. It was stored at 2°C prior to use.

Inoculation of seedlings

Eight weeks after germination, a subsample of 60 seedlings was examined to ensure that there was no contamination by airborne spores. The Spencer-LeMaire books were subdivided into individual containers (using scissors and waterproof tape), and then the seedlings were inoculated with 4 mL of slurry or 4 $cm^3$ of forest floor inoculum. The slurry was applied with a syringe equipped with a stainless steel needle (2-mm-wide aperture).

The tip was inserted to the bottom of the plug and raised slowly while the slurry was ejected in order to distribute the slurry evenly through the root zone. Forest floor inoculum was placed along one side of the seedling plug before the sides of the containers were taped.

Soil temperature treatment

After inoculation, the containerized seedlings were transplanted into pots (4 L, 15 cm diameter, 17 cm high) filled with coarse gravel. The temperature of the gravel was maintained at either 6, 16 or 26°C by placing the pots in water baths. To minimize heat exchange between the gravel and air, the gravel surface of the pots was covered with styrofoam chips and the bath water level was maintained 2 cm above the gravel surface. The baths were located in a controlled environment chamber: 16-22°C day/night air temperature, 30-70% relative humidity, 18 h photoperiod, light intensity of 400 $\mu$mol/($m^2$s) (400-700 nm wavelength) from cool white fluorescent and incandescent bulbs. Seedlings were watered as necessary to maintain the growing mix water potential above field capacity. Once a week, 10 mL of a water-soluble fertilizer solution (100 mg/L N) containing micronutrients (Plant-Prod 20-20-20) was added to each seedling container and the pots were re-randomized within the water baths.

Assessment of mycorrhiza formation and root morphology

Eight weeks after inoculation, the seedlings were destructively sampled to assess the degree of mycorrhiza formation. A short root was considered mycorrhizal if a Hartig net was present. Intact unwashed root plugs were scanned at 12-40x magnification and whole mounts of short roots were examined at 1000x magnification as described by Danielson and Visser

(1984, 1990). This technique had several advantages for the dissertation research compared with methods which involve root washing, sectioning or staining, : (1) most short roots were found on the external surface of the plug and external hyphae which tend to be removed during root washing were easily detected, (2) early stages of infection (with only a portion of the short root occupied by a Hartig net) were more easily detected in whole mounts than in sections and (3) characteristics used to classify morphological types of mycorrhizae (e.g., colour, hyphal ornaments) were not lost by washing and staining.

Percent root colonization was recorded on a six-class scale: (1) 0% of short roots mycorrhizal, (2) 1-25% mycorrhizal, (3) 26-50% mycorrhizal, (4) 51-75% mycorrhizal, (5) 76-95% mycorrhizal, (6) > 95% mycorrhizal. Approximately 40 whole mounts of short roots were examined to classify each seedling. Accuracy of this subsampling system was checked by comparing the class ratings estimated from the subsample with those obtained from assessing all the short roots of 24 seedlings selected to represent infection levels from 0% to 100%.

After mycorrhizal development was assessed, the root systems of each seedling were washed and cut into 5-cm segments. The number of short roots per seedling was estimated from (1) a randomly-selected subsample of these segments comprising approximately 1/3 of the root system and (2) the oven-dry weight ratio between the subsample and total root system. Short root number at the time of inoculation was estimated from a destructive harvest of 12 seedlings per temperature treatment.

Experimental design and statistical analysis

The experiment was a completely randomized design with a factorial (3 x 14) arrangement of the treatments; there were 12 seedlings (six seedlings per pot) planted per treatment combination. Each seedling was an experimental unit. There were no significant differences ($P > 0.25$, randomization test) in root colonization between the two pots within each inoculation and treatment combination.

Mycorrhiza class data showed little or no variation within groups subjected to particular treatment combinations. The distribution of these data tended to be non-normal and heteroscedastic; thus it did not meet the assumptions for parametric statistical analysis (Eisenhart 1947). Therefore, statistical analysis of mycorrhizal class data was performed with various forms of the randomization test, one of the most powerful non-parametric statistical methods (Siegel 1956, Edgington, 1987).

The randomization test for two samples (Siegel 1956), as programmed in FORTRAN to run on a VAX computer by Dr. Clarence Simmons of Forestry Canada, Victoria, B.C., was used to find the probabilities of observed differences between two means, under the null hypothesis that the data were from the same population and occurred in the observed groups purely by chance of the sampling.

In the randomization test, a statistic, such as the difference between means of two groups of data, is compared with the same statistic derived from each of all possible permutations of their pooled data to the groups, as described by Siegel (1956). Applying the principles and broad definition of sampling randomization tests described by Edgington (1987), Dr. Simmons developed and programmed an extension of mean-based sampling randomization tests, going beyond using the simple difference of two means

as a test statistic to using orthogonal sets of linear contrasts on more than two means to obtain more complex linear combinations of means as test statistics, enabling significance of single-degree-of-freedom main effects and interactions to be estimated.

Where the sample sizes were too large to consider sampling all possible permutations, large with-replacement random samples of permutations were used instead, as described by Edgington (1987). This method called the "sample randomization test" by Edgington (1987) was programmed by Dr. Simmons in FORTRAN, using a library function to generate uniform distribution (pseudo-) random numbers in the process of deriving successive random permutations. The number of permutations or re-randomizations used in a particular sampling randomization test was generally 100,000. Following Edgington (1987), where the groups were subdivided by a cross-classification common to all, the re-randomization was done independently within each class of the cross-classification.

Analysis of variance (ANOVA) was used to (1) test the effects of temperature and inocula on 8-week root morphology and (2) the effect of inoculum source (field or nursery) on mycorrhiza formation. Mean mycorrhiza class data (for each inoculation and treatment combination) were used in the latter analysis; these data met the normal distribution and homogeneity of variance assumptions of ANOVA.

## Results

### 1. Root assessments

There was no evidence of mycorrhiza formation by contaminant fungi in the eight-week-old seedlings prior to inoculation. On average, there were more than 200 short roots per seedling available for colonization by ECM fungi (Table 2.2).

Temperature had a highly significant ($P < 0.001$, ANOVA) effect on short root production, accounting for approximately 40% of the total variability in short root data at 8 weeks. The mean number of short roots produced per seedling was reduced 80% and 20%, respectively in the 6 and 26°C mixes, compared with the 16°C mix during the 8-week test period (Table 2.2). Inoculation treatment also influenced short root production ($P < 0.05$, ANOVA) but accounted for only 8% of the total variability in 8-week short root data. There was no significant interaction between inoculation and temperature ($P = 0.20$) on short root production.

Table 2.2. Effect of growing mix temperature on mean number of short roots per seedling

| Week | Temperature °C | | |
|---|---|---|---|
| | 6 | 16 | 26 |
| 0 | 219 ( 60) | 214 ( 46) | 236 ( 61) |
| 8 | 471 (293) | 2311 (273) | 1868 (591) |

NOTE: Standard deviations are shown in parentheses.

2. Interaction of temperature and inoculation treatments on mycorrhiza formation

Orthogonal contrasts conducted by randomization tests showed there were three significantly ($P = 0.01$) different patterns of ECM mycorrhiza formation in response to soil temperature (Table 2.3). Percent root colonization for 8 of the 13 inocula, including the forest floor inoculum, was greatest at 16°C, decreasing at 6°C and 26°C (Fig. 2.1). In contrast, percent colonization decreased with temperature for L. bicolor 5313; and increased with temperature for the E-strain 947, T. terrestris (2088), T. terrestris (Laval) and L. laccata 101C inocula.

Averaged across all inoculation treatments, the percentage of mycorrhizal short roots was lower at 6°C ($P < 0.001$) and 26°C ($P = 0.13$) compared with 16°C. Mean mycorrhiza classes for the 6, 16 and 26°C root-zone temperatures were 1.9, 3.0, and 2.8, respectively.

3. Effect of inoculum source on mycorrhiza formation

ANOVA showed no effect ($P = 0.50$) of inoculum source (field versus nursery) on percent root colonization as estimated by mean mycorrhiza class; nor was there an interaction between temperature and inoculum source ($P = 0.87$) on mean mycorrhiza class. Several inocula originating from cool forest soil environments (e.g., E-strain 947; L. bicolor 5268 ) successfully colonized a significant percentage of the short roots in the 26°C peat:vermiculite mix; others (e.g., forest floor inoculum) formed significantly ($P < 0.001$) fewer mycorrhizae at 26°C compared to 16°C.

Table 2.3. Effect of inoculum and root-zone temperature on mean mycorrhiza class

| | | Growing mix temperature (°C) | | | |
|---|---|---|---|---|---|
| | | 6 | 16 | 26 | Mean |
| Inocula* | S** | Mycorrhiza class*** | | | |
| Control | | 1.0 (0.0) | 1.0 (0.0) | 1.0 (0.0) | 1.0 |
| Ab | N | 1.1 (0.3)c | 1.7 (0.7)c | 1.3 (0.5)d | 1.4d |
| E 0188 | N | 1.3 (0.5)c | 2.6 (1.5)c | 2.2 (1.2)d | 2.2c |
| E 947 | F | 2.8 (1.3)b | 5.3 (0.9)a | 6.0 (0.0)a | 4.5ab |
| Ff | F | 3.4 (1.7)a | 5.0 (1.1)ab | 2.7 (1.3)cd | 3.5b |
| Hc 5 | F | 1.0 (0.0)c | 2.5 (1.6)c | 1.3 (0.5) d | 1.4d |
| Hc 8 | F | 1.0 (0.0)c | 1.8 (1.0)c | 1.5 (0.5) d | 1.4d |
| Hc 5247 | F | 1.0 (0.0)c | 2.3 (1.1)c | 1.8 (0.8) d | 1.7cd |
| Hc 125 | F | 2.2 (0.7)b | 2.7 (0.9)c | 2.3 (0.5) d | 2.4c |
| Lb 5313 | N | 2.8 (1.3)b | 2.7 (1.4)c | 1.2 (0.4) d | 2.1c |
| Lb 5268 | F | 4.7 (0.5)a | 5.4 (0.5)a | 4.3 (1.1)b | 4.8a |
| Ll 101C | N | 1.5 (0.5)c | 3.2 (1.7)bc | 3.9 (1.0)bc | 2.8cb |

(continued on next page)

Table 2.3. (cont.)

| | | | | | |
|---|---|---|---|---|---|
| Tt 2088 | N | 1.0 (0.0) c | 4.8 (0.8)b | 5.9 (0.3)a | 3.9b |
| Tt Laval | F | 1.0 (0.0) c | 1.6 (1.2) c | 3.0 (2.3)bcd | 1.9cd |
| Mean | | 1.9 (1.2) | 3.0 (1.6) | 2.8 (1.8) | |

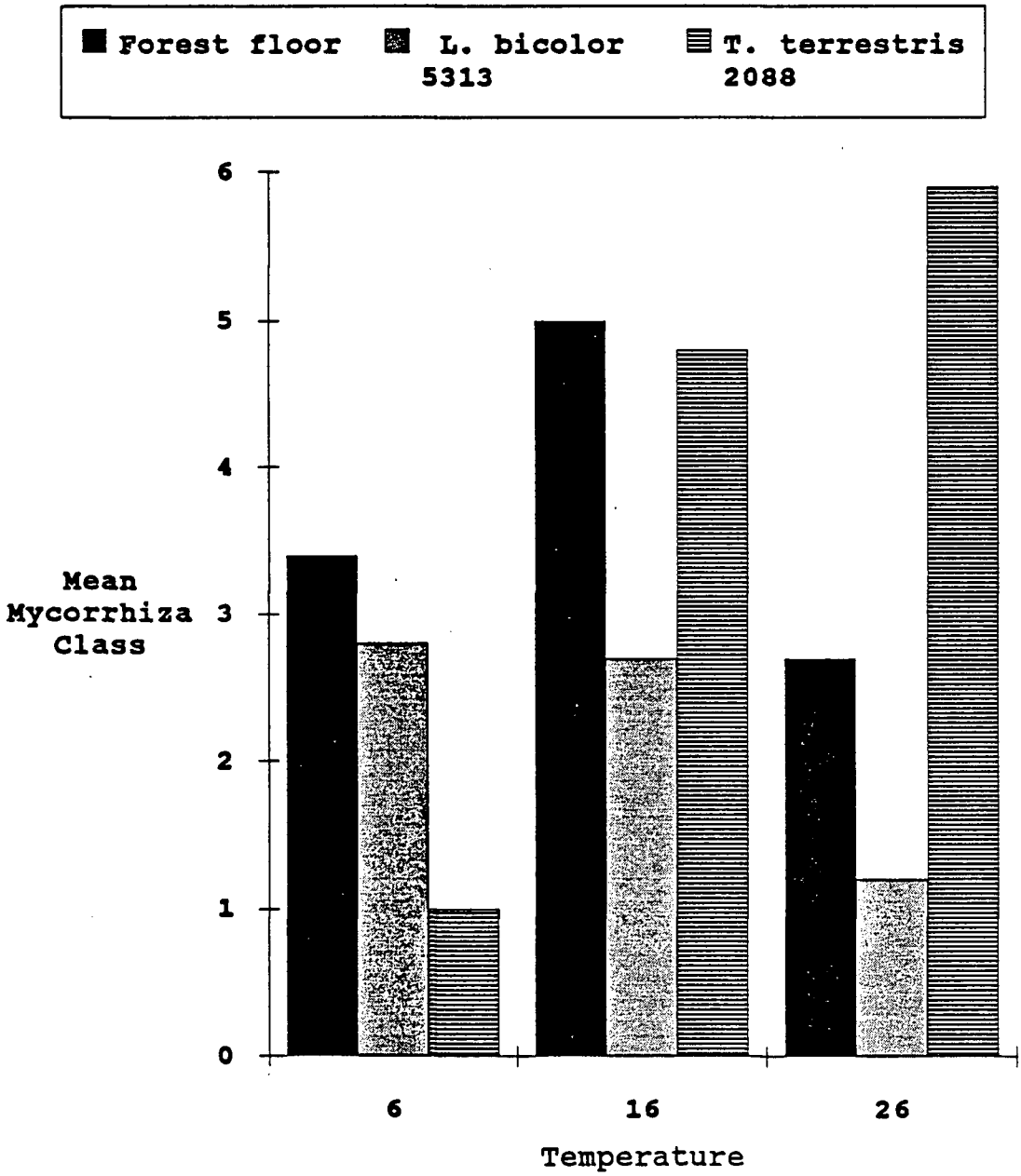
NOTE: For individual soil temperatures, N = 12. Means in columns with the same letter designation are not significantly different (P = 0.01, paired randomization tests). Standard deviation in parentheses.

*Abbreviations for inocula: Ab = A. byssoides, E = E-strain, Ff = forest floor, Hc = H. crustuliniforme, Lb = L. bicolor, Ll = L. laccata, Tt = T. terrestris

**S = source; either field (F) or nursery (N).

***Mean mycorrhiza classes: (1) 0% of short roots mycorrhizal, (2) 1-25% mycorrhizal, (3) 26-50% mycorrhizal, (4) 51-75% mycorrhizal, (5) 76-95% mycorrhizal, (6) > 95% mycorrhizal

Figure 2.1. Effect of root-zone temperature on mycorrhiza formation by three inocula: T. terrestris 2088, L. bicolor 5313 and forest floor.



In the 6°C mix, most mycorrhizae were formed by three field sources (L. bicolor 5268, forest floor, and E-strain 947) and one nursery source (L. bicolor 5313). Both field and nursery sources (e.g., H. crustuliniforme 5247, T. terrestris 2088) failed to form mycorrhizae in the 6°C mix. In the 16 and 26°C growing mixes, inocula that formed the most mycorrhizae were from both field (e.g., L. bicolor 5268) and nursery sources (e.g., T. terrestris 2088).

Averaged across all growing mix temperatures, two nursery isolates (L. laccata 101C and T. terrestris 2088) and three field sources of inoculum (forest floor, L. bicolor 5268 and E-strain 947) colonized the greatest percentage of short roots eight weeks after inoculation. Only four inocula (E-strain 947, L. bicolor 5268, H. crustuliniforme 125 and forest floor) tolerated the whole range of test temperatures, with mean mycorrhiza classes greater than 2.0 in 6, 16, and 26°C mixes.

There were significant ($P = 0.01$) differences in mycorrhiza formation among isolates of L. bicolor and E-strain at all growing mix temperatures; and for H. crustuliniforme isolates at 6°C and T. terrestris at 16 and 26°C.

## Discussion

Mycorrhiza formation occurred within eight weeks of inoculation at all three soil temperatures. Percent root colonization was greatest at 16°C for most inocula, confirming earlier observations that mycorrhiza formation is generally greatest at soil temperatures that are optimal for root growth (Parke 1983). The optimum temperature for spruce root growth is about 18°C (Heninger and White 1974, Ritchie and Dunlap 1980). High temperature limited percent colonization to a lesser degree than did cool

temperature. Thirteen of the fourteen inocula formed some mycorrhizae at 26°C; but only eight formed any mycorrhiza at 6°C.

The magnitude of the temperature effect was greater than appears from the percent colonization data because of the reduced production of short roots by seedlings in the 6°C soil compared with that in the 16 or 26°C soils. On the basis of total number of mycorrhizae formed per seedling, infection was severely reduced (by approx. 90%) at 6°C; and to a lesser degree (by 15%) at 26°C.

The formation of mycorrhizae at 6 and 16°C eight weeks after inoculation is not consistent with the results of an earlier study conducted by Heninger and White (1974). They grew spruce seedlings inoculated with forest soil ECM fungi for eight weeks at soil temperatures between 15 and 31°C. No mycorrhiza formation was observed at soil temperatures below 19°C. However, there are reports of fungal growth in culture (Dennis 1985, Samson and Fortin 1986) and mycorrhiza formation in soil (Orlov 1957, Parke et al. 1983b) at temperatures below 19°C. Orlov (1957, cited by Slankis 1974) observed mycorrhiza formation in spruce at soil temperatures between 3 and 9°C. Vogt et al. (1980) reported that the biomass of active mycorrhizae in amabilis fir stands was highest in fall and winter when soil temperatures averaged 1°C.

In a review of soil effects on mycorrhizae, Slankis (1974) states that although mycorrhiza formation occurs in cool soils, it is generally more rapid in warm soils. However, the rate of mycorrhiza formation at various soil temperatures is not well documented. Parke et al. (1983b) reported moderate mycorrhiza formation in Douglas-fir seedlings grown in 7.5°C soils fourteen weeks after inoculation. Coutts and Nicoll (1990a) observed mycorrhizal infection of Sitka spruce seed sown in 8-15°C soil

twelve weeks after inoculation. Mycorrhiza formation was more rapid in this study compared to their results.

Since the seedlings were non-mycorrhizal at the time of inoculation, the disparity between results of this and previous studies probably reflects differences in inoculation technique and, more important, in the method of assessing short roots. Eight-week-old seedlings were inoculated in this study; in the other studies (e.g., Heninger and White 1974, Parke et al. 1983b, Coutts and Nicoll 1990a) seeds were inoculated. Heninger and White (1974) assessed short roots "visually" for the presence or absence of mantles; Coutts and Nicoll used low power (40x) magnification and did not check for the initial stages of infection (e.g., hyphal penetration of the cortex and Hartig net formation) with high power magnification. Parke et al. (1983b) did not describe their method of assessment but probably did not use high power magnification.

Visual and low power methods have two important limitations. First, in this and other studies (e.g., Nylund and Unestam 1982) of the infection process in spruce seedlings, formation of the Hartig net, which is the diagnostic criterion for mycorrhiza formation, preceded mantle formation. At low power magnification, short roots with a Hartig net but lacking a well-developed mantle will likely be classified as non-mycorrhizal. The early stages of infection have been shown to influence host plant physiology (Nylund and Unestam 1982, Ekwebelam and Reid 1983, Daughtridge et al. 1986); therefore, their detection is important to understanding the mycorrhizal symbiosis. Second, gross characteristics (i.e., lack of root hairs, distinctive colours, branching and swelling) commonly used to identify mycorrhizae "visually" or under low power magnification can be found on non-mycorrhizal root systems (Nylund and Unestam 1982, Harley and

Smith and references therein) and, therefore, they are not reliable indicators of mycorrhiza formation.

Infection of roots from mycelial inoculum involves three phases (1) the growth of hyphae from the inoculum, (2) primary infection of roots from these "free" hyphae, and (3) secondary infections as hyphae from established mycorrhizae spread to other parts of the root system. Soil temperature affects these processes through its influence on fungal growth and root colonization. In addition, soil temperature influences many aspects of host plant growth and physiology which influence mycorrhiza formation including (1) rate of root growth (Hermann 1977, Ritchie and Dunlap 1980), (2) lateral branching and the production of short roots (Wilcox and Ganmore-Neumann 1975), (3) the maturation rate of roots (Nightingale 1935, Smit-Spinks 1983) and (4) the composition and quantity of root exudates (Rovira 1969, Slankis 1974). Changes in the growth, branching and maturation of roots alter the availability of suitable short roots for colonization by ECM fungi; changes in root physiology influence fungal growth in the rhizosphere (Slankis 1974, Wilcox and Ganmore-Neumann 1975).

It is difficult to separate the various effects of soil temperature on mycorrhiza formation. In the current study, spruce seedlings were grown for eight weeks in a warm growing medium (18$^{o}$C) before inoculation to ensure that availability of suitable colonization sites did not initially limit mycorrhiza formation at any temperature. The poor mycorrhiza formation observed in the 6$^{o}$C mix appeared to result from decreased viability of the inoculum. Few hyphae were observed adhering to the roots of these seedlings and many isolates produced no mycorrhizae. Low soil temperatures also depress the production of root exudates which are an

important nutrient source for "free" hyphae (Garbaye and Wilhelm 1985).

The 26°C temperature may have limited the growth of hyphae directly or altered root composition and hence primary or secondary infection. All but one isolate formed mycorrhizae suggesting that the growth of "free" hyphae may have been less affected by high soil temperature than the primary and secondary phases of infection. The susceptibility of short roots to infection may have been reduced by rapid rates of maturation or by lower sucrose concentrations. Suberization and the development of secondary tissues are more rapid at high soil temperature (Barney 1951, Nightingale 1935), reducing the amount of time roots are susceptible to infection. High sucrose concentrations are positively correlated with the susceptibility of loblolly pine roots to infection by P. tinctorius (Marx et al. 1977). Chalupa and Fraser (1968) compared sucrose concentrations of white spruce seedlings grown at soil temperatures ranging from 10 to 38°C with a constant air temperature of 21°C. They found that the level of root sucrose (% dry weight) was inversely proportional to root-zone temperature.

Differences among isolates in mycorrhiza formation at the various soil temperatures were not related to the origin of the fungal isolate (field or nursery). This conclusion must be considered with caution due to two important limitations of the study (1) the lack of two to three nursery and field isolates for each species of fungus, and (2) the differences in age and treatment of the various cultures used in the study.

The original intent of the study was to obtain at least two field and nursery isolates of E-strain, T. terrestris and L. laccata, three ECM fungi found in the nursery and field. Isolates for all three species were difficult to obtain. I was most successful locating L. laccata isolates in the University of Alberta Microfungus collection. However, during the

study, many of these isolates were reclassified as L. bicolor.

Not all the cultures were collected, isolated, cultured or stored in the same manner. Culture and storage conditions have been shown to affect the ability of fungi to form mycorrhizae (Marx 1981, Hung and Molina 1986). It is possible that the physiological activity of isolates forming few mycorrhizae at all growing mix temperatures (e.g., H. crustuliniforme 8) had been impaired during culture or storage.

Given the limitations of this study, the results are consistent with the results of in vitro studies of temperature response variation in ECM fungal isolates cultured from sporocarp tissue (Dennis 1985, Samson and Fortin 1986, Cline et al. 1987), i.e., the temperature response of isolates is not related to their geographic origin. Dennis (1985) reported differences as great as 10°C in the optimum temperature for mycelial growth; but there was no relationship between the origin of the fungus and optimum temperature. Samson and Fortin (1986) reported that the growth of 62 fungal isolates with similar collection, culture and storage histories was not related to the geographic origin of the isolate.

# CHAPTER III

# EFFECTS OF COOL SOIL TEMPERATURE AND SLOW-RELEASE FERTILIZER ON THE PERSISTENCE OF ESTABLISHED MYCORRHIZAE

## Introduction

Inoculant fungi may disappear in the first year after outplanting (Chu-Chou 1979, Bledsoe et al. 1982, Mason et al. 1983, Thomas et al. 1983, Castellano and Trappe 1985, Danielson and Visser 1989). Competition from indigenous ECM fungi and the inability of inoculant fungi to adapt to the field environment are considered to be the major reasons for poor persistence.

High soil temperature has been shown to reduce the persistence of established mycorrhizae. Parke et al. (1983b) reported that high soil temperatures (35°C for one week) reduced the persistence of mycorrhizae formed from fungi indigenous to Oregon and northern California. In container nurseries, the ability of established mycorrhizae to tolerate high temperatures could influence the success of artificial inoculation programs.

The ability of established mycorrhizae to survive and colonize new roots at cool temperatures (< 10°C) is a more important consideration for artificial inoculation of seedlings outplanted in the cool soils of northern British Columbia. If the inoculant fungus survives and colonizes new roots before the onset of warm, dry summer conditions, seedling stress during the first field season may be minimized.

There have been no studies of the effect of cool soil temperatures on the persistence of established ECM fungi although a study conducted by Riffle and Tinus (1982) suggests it is an important factor. They observed

that P. tinctorius (Pt), a fungus adapted to warm soils, did not persist on the root systems of pine seedlings planted in South Dakota and speculated that cool soils and a short growing season were responsible for its disappearance.

The objectives of the research reported in this chapter were (1) to study the ability of ECM fungi established in a nursery to persist in cool soils, (2) to determine if the presence of indigenous forest soil ECM fungi altered the effects of soil temperature on persistence, and (3) to study the effect of nutrient availability at the time of transplanting on the persistence of established mycorrhizae in a cool (12°C) soil.

The third objective was included because the application of slow-release fertilizer at the time of planting is one silvicultural option for improving early seedling growth on sites with low nutrient availability. Nutrient availability in boreal forests is strongly affected by soil temperature through its effect on the rate of decomposition (Nielsen and Humphries 1966, Van Cleve et al. 1990). ECM fungi vary in their response to fertilizer (Alexander and Fairley 1983, Hunt 1989). Two studies testing both artificial inoculation and fertilizer at the time of planting (Grossnickle and Reid 1983, Marx et al. 1985) were conducted on a mine reclamation site and an African savanna, respectively, and are not applicable to reforestation sites in northern B.C. It would be useful to know if slow-release fertilization at the time of planting influences the persistence of established mycorrhizae.

## Approach

Persistence was sub-divided into two components (1) survival on roots within the container root plug and (2) degree of new root colonization by inoculant fungi. The studies were conducted in pots with the recognition that persistence could be influenced by confinement of the roots to a restricted soil volume. The duration of the studies was limited to twelve weeks to minimize these effects.

Since mycorrhizal infection is influenced by the availability of photosynthate (Harley and Smith 1983 and references therein), variation in light intensity and the size of seedling shoots was minimized to reduce potential variation in photosynthetic efficiency and capacity. Non-mycorrhizal soil flora also affect root infection by ECM fungi (Brown and Sinclair 1981, Summerbell 1987, McAfee and Fortin 1988). Therefore, a soil slurry containing these microorganisms was added to the forest soil which was pasteurized to eliminate indigenous ECM fungal inoculum.

## Methods

### Production of inoculated tree seedlings

Eight-week-old white spruce seedlings (seedlot 29144) were inoculated as described in Chapter II with forest floor inoculum and mycelial slurries of seven ECM fungi typical of the early stages of mycorrhizal succession: (1) E-strain 947, (2) Hebeloma crustuliniforme 5249 (3) Laccaria laccata 101D (4) H. crustuliniforme 125, (5) Laccaria bicolor 5268, (6) Amphinema byssoides or (7) Thelephora terrestris 2088. Origins of the isolates are summarized in Table 2.1 in Chapter II.

After inoculation, the seedlings were grown in a controlled environment for 14 weeks under three cultural regimes: (1) 6 weeks with an 18 h photoperiod, 20°C air temperature and 50-80% relative humidity, (2) followed by 4 weeks of a short photoperiod treatment (8 h) to induce terminal bud formation, and (3) 4 weeks of cool conditions (14 h photoperiod, 9-14°C air temperatures) to harden the seedlings for cold storage. Once a week, seedling blocks were rerandomized to minimize possible position effects within the growth chamber; and the seedlings were watered to saturation with a water-soluble fertilizer solution (100 mg/L N) containing micronutrients (Plant-Prod 20-20-20, Plant Products Co. Ltd., Bramalea, ON). Every two weeks, the seedlings were watered to saturation with an insecticide (0.84 g/L Diazinon, Later Chemical Ltd., Richmond, B.C.) to minimize losses of fungal tissues.

The seedlings were cold-stored for 10 weeks at 3-4°C before the soil temperature treatments. Following cold-storage, approximately one-third of the seedlings were culled to reduce between-seedling variation in size; the largest and smallest seedlings were removed from each inoculation treatment.

Native ECM fungi and soil temperature treatments

Seedlings were removed from the Spencer-Lemaire containers and transplanted into pots (4 L, 15 cm in diameter) containing a forest soil mixture consisting of forest floor, mineral soil and perlite (1:1:2 by volume). The forest floor and mineral soil were collected from three vigorous spruce plantations established near Mackenzie, B.C. These soils were passed through a 1 $cm^2$ screen to remove gravel and large organic debris before the perlite was added. The mean values of pH (1:4 soil:water

suspension, Peech 1965), available phosphorus (Bray and Kurtz No1, Bray and Kurtz 1945) and mineralizable nitrogen (anaerobic incubation, Waring and Bremner 1964) for this mixture were respectively 4.9, 35 $\mu$g/g and 68 $\mu$g/g.

Five of the eight inoculation treatments were planted in forest soil with and without indigenous ECM fungal propagules at two soil temperatures, 6 and 12°C. Because of space limitations in the water baths, the remaining three inoculation treatments were planted only in forest soil with ECM fungal propagules (Table 3.1). Soil without viable indigenous ECM fungal propagules was prepared by pasteurizing it for 40 minutes at 65°C. An extract, prepared by passing a slurry of unpasteurized forest floor material and autoclaved sterile water through a 0.50 $\mu$m filter, was added to the cooled pasteurized soil to restore forest floor microbes. The extract comprised less than 1% of the total soil volume.

The ECM inoculum potential of pasteurized and unpasteurized forest soil mixture was assessed by transplanting 8-week-old non-inoculated control seedlings into each mixture. Three pots (6 seedlings/pot) were maintained at each soil temperature (6, 12°C). Mycorrhizal status of the seedlings was estimated 0, 5 and 12 weeks after transplanting by destructive sampling.

Water baths were used to maintain two soil temperatures (6, 12°C). Pots within each bath were randomized weekly to minimize possible position effects within the water baths. The pots were watered to field capacity as required but no fertilizer was applied to the forest soil mixture during the experiment.

Fertilizer treatment

Seedlings inoculated with (1) forest floor, (2) E-strain 947 or (3) H. crustuliniforme 5249 were transplanted into pots filled with either unfertilized or fertilized 12°C unpasteurized forest soil (1:1:2, by volume, forest floor, mineral soil and perlite). The forest soil was fertilized with a resin-coated slow-release fertilizer, Osmocote 14-14-14 (Sierra Chemical Co., Milpitas, CA) with a three month release time. Each fertilized pot was filled with 1.25 kg of the forest soil mixture thoroughly mixed with 1.3 g of Osmocote (100 kg/ha N).

Evaluation of seedling nutrition

Twelve weeks after transplanting and fertilizer application, newly-flushed foliage of seedlings in the fertilizer experiment were analyzed for nitrogen (N) and phosphorus (P) concentrations in the dry matter. Seedling foliage from each fertilizer-inoculation treatment was bulked and oven-dried (70°C, 48 h) for analysis. Dried needles were ground in a Wiley Mill to pass through a 20-mesh screen; and digested using a wet oxidation with $H_2SO_4$, Se, salts and $H_2O_2$ (Parkinson and Allen 1975). N and P concentrations in the digest were determined by a colorimetric (auto analyser) analysis using salicylate/nitroprusside for N and ascorbic acid/molybdate-antimony for P (Technicon Industrial Systems 1977). Accuracy and precision of these analyses were checked by including 3 samples of Standard Reference Material 1575 (pine needles) from the National Bureau of Standards[1] in each run of 40 samples.

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[1] Personal communication with A. Gammell, MacMillan Bloedel Ltd, Nanaimo, B.C.

Table 3.1. Fungal isolated and inoculum used to study the effects of soil temperature and the presence of native ECM fungal inoculum on persistence of inoculant fungi

| Forest soil with indigenous ECM fungi (not pasteurized) | Forest soil without indigenous ECM fungi (pasteurized) |
|---|---|
| Non-inoculated seedlings | Non-inoculated seedlings |
| E-strain 947* | E-strain 947 |
| H. crustuliniforme 5249* | H. crustuliniforme 5249 |
| L. laccata 101D | L. laccata 101D |
| Forest floor inoculum* | Forest floor inoculum |
| H. crustuliniforme 125 | H. crustuliniforme 125 |
| L. bicolor 5268 | |
| A. byssoides | |
| Thelephora terrestris 2088 | |

NOTE: Non-inoculated seedlings were used to test the inoculum potential of indigenous ECM fungi.

*Included in the fertilizer experiment.

Mycorrhizal assessment

Mycorrhizal infection was estimated on a six-class scale using low and high power magnification (as described in Chapter II). The mycorrhizal status of fifteen seedlings, selected randomly from each inoculation and soil temperature treatment combination after the largest and smallest seedlings were culled, was evaluated at the time of transplanting. All remaining seedlings were assessed 12 weeks after transplanting. The mycorrhizal status of roots within the original container root plug and new roots formed outside the plug were evaluated separately at 12 weeks.

Mycorrhizae formed by indigenous forest soil ECM fungi were classified using characteristics (1) of the mycorrhizae (e.g., morphology, colour, abundance of extramatrical (EM) hyphae, presence of cystidia or mycelial strands), (2) of the EM hyphae (e.g., diameter, presence of ornamentation, cell wall pigmentation, clamps) and (3) of the mantle structure in plan view (e.g, shape, size and orientation of hyphae), which are relatively stable for a particular fungal genus over a range of host plants (Trappe 1967, Zak 1973, Haug and Oberwinkler 1987). Mantle structure was described using the textura system (Eckblad 1968, Alexander 1981, Haug and Oberwinkler 1987).

Morphological types of indigenous mycorrhizae were grouped into several taxonomic types based on characteristics of fungal genera, for example Hebeloma-like, as recommended by Godbout and Fortin (1985) and Danielson and Pruden (1989). Descriptions of mycorrhizae formed by the pure cultures of inoculant fungi and forest soil ECM fungi are contained in Appendix B. The mycorrhizal types which occurred on non-inoculated seedlings transplanted into unpasteurized forest soil served as a basis for identifying indigenous ECM types. Morphological types formed by forest

soil fungi were generally quite distinct from those of the inoculant fungi.

Evaluation of seedling growth

Root collar caliper at the time of transplanting (0 week) was measured for each seedling. Root biomass, lateral root length, and number of short roots were measured at 0 and 12 weeks. The root system of each seedling was carefully extracted and cut lengthwise into two halves: one to assess the degree and type of mycorrhiza infection, and the other to estimate root length and short root number. Roots in the second sample were gently washed to remove the growing medium, cut into 3-5 cm long segments, thoroughly mixed, and a random subsample of fragments (approximately one-quarter of the total root system) was transferred to a Petri dish placed over a 1 cm grid. The number of short roots (sensu Sutton 1980) was counted in this subsample; the length of the long lateral roots was estimated using a modification (Tennant 1975) of a line intersect method (Newman 1966). Root biomass of each seedling was calculated by summing the oven-dry weights (70°C, 48 h) of the various subsamples. The total root length and number of short roots per seedling were estimated from dry weight ratio between the length subsample and whole root system.

Experimental Design and Statistical Analysis

Three completely randomized designs were used to test the various treatments on persistence:

(1) a 2 x 2 x 5 factorial with 12 seedlings per treatment combination to study the effects of soil temperature (6, 12°C) in the presence or absence of native ECM fungal inoculum on the persistence of 5 sources of inocula.

(2) a 2 x 8 factorial with 12 seedlings per treatment combination to study the effects of soil temperature (6, 12°C) on the persistence of 8 sources of inocula.

(3) a 2 x 3 factorial with 8 seedlings per treatment combination design to study the effects of fertilizer (none, 100 kg/ha N) on the persistence of 3 sources of inocula.

Seedlings in all experiments were grouped, four to a pot. Since variation between pots had an insignificant ($P > 0.30$) effect on mycorrhiza class or seedling root growth, individual seedlings were considered to be an experimental unit.

The distribution of mycorrhiza class data did not meet the assumptions of parametric tests (Eisenhart 1947). Therefore, statistical analysis was conducted using non-parametric randomization tests as described in Chapter II. To compensate for differences in mycorrhizal status at the beginning of the experiment, persistence on new roots was compared to persistence on roots within the original plug using a paired-sample randomization test (Siegel 1956).

The effects of soil temperature, presence of native ECM fungal inoculum and slow-release fertilizer on seedling root growth were tested by

least squares analysis of covariance (ANCOVA) using initial root collar caliper as the covariate; or by least squares analysis of variance (ANOVA) when caliper interacted significantly with treatments, invalidating the "homogeneity of slope" assumption of ANCOVA (Hicks 1973). Analyses were performed on log-transformed values for the short root data to meet the homogeneity of variance assumption of ANOVA and ANCOVA. All tests were conducted using a microcomputer statistical package, SYSTAT (Wilkinson 1988).

## Results

### 1. Inoculum potential of forest soil mixtures

Root systems of 8-week-old non-inoculated seedlings transplanted into the unpasteurized soil mixture were colonized rapidly by forest soil ECM fungi. At five weeks, 66% of these seedlings had some mycorrhizae; at twelve weeks, more than 95% of the seedlings had been infected by forest soil mycorrhizal fungi. At 12 weeks, mean mycorrhiza formation for individual seedlings exceeded 37% and 50%, respectively, in the 6 and 12°C soils.

At five weeks, no mycorrhizae were found in 8-week-old seedlings transplanted into pasteurized 6 and 12°C soils. However, at twelve weeks, a light infection of T. terrestris was observed at both temperatures on approximately 20% of the seedlings. T. terrestris inoculum may have survived pasteurization of the forest soil; it is more likely, however, that this infection resulted from air-borne spores. The growth chambers were located outdoors adjacent to a conifer nursery.

2. Initial mycorrhizal status of seedlings

Mean mycorrhiza class for the various inoculation treatments ranged from 4.2 to 6.0 at the time of transplanting. Mycorrhiza formation was lower ($P < 0.001$) in the A. byssoides and T. terrestris treatments (mean mycorrhiza classes of 4.3 and 4.7, respectively) compared with the other isolates (mean classes 5.9 to 6.0).

T. terrestris mycorrhizae were identified on 15% of the seedlings inoculated with A. byssoides. This infection was weak with (1) fewer than 25% of the short roots of these seedlings infected, (2) extramatrical (EM) hyphae, mycelial strands and cystidia uncommon and (3) mantle formation discontinuous. Some seedlings inoculated with forest floor were also colonized by T. terrestris; 15% had 50% or more of the short roots colonized weakly by T. terrestris. It was not clear whether these infections originated from forest soil propagules or from air-borne spores in the nursery.

Seedlings inoculated with T. terrestris 2088 may also have been contaminated by air-borne spores of T. terrestris. However, most mycorrhizae observed on seedlings inoculated with T. terrestris 2088 were well-developed (i.e., mantles covered most of the infected short roots, numerous cystidia and mycelial strands), strongly suggesting that they developed from the inoculant fungus and not from spores. Mycorrhizae formed from air-borne spores in seedlings inoculated with forest floor inoculum or A. byssoides were not well-developed (i.e., exhibited weak mantle development, few mycelial strands).

3. Seedling root growth and morphology

Lateral roots egressed horizontally from the top to bottom of the root plug. Root biomass, lateral root length, and number of short roots per seedling measured at 12 weeks increased ($P < 0.01$) with soil temperature (Table 3.2). Pasteurization of the forest soil mixture had no effect ($P > 0.20$) on these root parameters. Seedlings assigned to each temperature treatment were not significantly different ($P > 0.25$) at the time of transplanting. Slow-release fertilizer increased root biomass ($P < 0.001$) and lateral root length ($P = 0.02$) at 12 weeks; but had no effect ($P = 0.22$) on the number of short roots per seedling (Table 3.3).

Table 3.2. Effect of soil temperature on mean seedling root biomass (oven-dry weight), root length and number of short roots for seedlings at 0 and 12 weeks

| Root parameter | Duration of temperature treatment | | |
|---|---|---|---|
| | 0 weeks | 12 weeks | |
| | | 6°C | 12°C |
| biomass (mg) | 90 ( 26) | 190 ( 14) | 260 (036) |
| length (cm) | 256 (122) | 465 (197) | 606 (306) |
| short roots/seedling | 863 (392) | 1394 | 1772 |

NOTE: Standard deviations are shown in parentheses. Data were averaged across 8 inocula; N = 96. Short root data for the 12 week measurement were log-transformed to meet the assumptions of ANOVA; means are shown in the original scale.

4. Survival of inoculant fungi within the original root mass

Survival of inoculant fungi was high on roots within the container root mass with mean mycorrhiza class ranging from 4.7 to 6.0. Neither the presence of native inoculum ($P > 0.50$) nor soil temperature ($P > 0.40$) had an effect on survival within the original root mass. With one exception, mycorrhizae appeared turgid and healthy. E-strain mycorrhizae, particularly those in the 6°C soil, were frequently shrivelled and dark-brown or black.

Table 3.3. Effect of slow-release fertilizer (NPK) at 12°C soil temperature on root parameters at 0 and 12 weeks

| Root parameters | Duration of fertilizer treatment | | |
|---|---|---|---|
| | 0 weeks | 12 weeks | |
| | | -NPK | +NPK |
| biomass (mg) | 90 (22) | 231 (51) | 388 (84) |
| length (cm) | 233 (122) | 550 (224) | 752 (335) |
| short roots/seedling | 887 (352) | 1808 | 2122 |

NOTE: Data averaged across 3 inocula; N = 24. Short root data for the 12 week measurement were log-transformed before ANOVA; means are shown in original units (i.e., back-transformed means of logarithms). Except for these transformed data, standard deviations are shown in parentheses. It is inappropriate to back-transform standard deviations (Steel and Torrie 1980, p. 236).

5. Effects of temperature and indigenous inoculum on the colonization of new short roots

Infection of new short roots formed outside the original root mass by inoculant fungi was good with more than 75% of short roots infected (Table 3.4) even though the potential for mycorrhiza formation by indigenous ECM fungi was significant. Non-inoculated seedlings transplanted into unpasteurized forest soil were rapidly infected by indigenous ECM fungi. EM hyphae of the inoculant fungi extended along lateral roots as they egressed from the container plugs, colonizing newly-initiated short roots.

The intensity of new root colonization was highly correlated with the degree of infection of the original root mass ($P = 0.85$, $P < 0.001$). At the time of transplanting, percent root colonization by A. byssoides and T. terrestris was lower compared with other fungal symbionts. To compensate for this difference, persistence of the various test fungi on newly-formed roots was compared to that on roots within the container plug, i.e., essentially using initial infection as a covariate.

In unpasteurized soil with all eight inocula, percent colonization of new roots was lower in the 12°C soil than in the 6°C soil (Table 3.4). At 6°C, only one fungal isolate, H. crustuliniforme 5249, exhibited reduced ($P = 0.03$) persistence on new roots compared to old roots; at 12°C, three isolates, L. laccata 101D ($P = 0.001$), H. crustuliniforme 5249 ($P = 0.06$), and A. byssoides ($P = 0.06$) exhibited reduced persistence on new roots. Indigenous ECM fungi colonized short roots not infected by L. laccata or A. byssoides. In contrast, most short roots not colonized by H. crustuliniforme 5249 remained non-mycorrhizal.

Table 3.4. Mean mycorrhiza class for roots within original container plug (old roots) and for new roots formed outside the container plug

| | Old roots | | New roots | | | |
|---|---|---|---|---|---|---|
| | | | pasteurized | | unpasteurized | |
| | 6°C | 12°C | 6°C | 12°C | 6°C | 12°C |
| Inocula* | Mean mycorrhiza class** | | | | | |
| E | 6.0 | 6.0 | 6.0 | 6.0 | 5.9 | 6.0 |
| Hc 5249 | 5.9 | 5.8 | 5.7 | 6.0 | 5.4 | 5.3 |
| Hc 125 | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 |
| Ll | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 | 4.3 |
| Ff | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 |
| Lb | 6.0 | 6.0 | --- | --- | 6.0 | 6.0 |
| Ab | 4.4 | 4.2 | --- | --- | 4.3 | 3.8 |
| Tt | 4.3 | 5.0 | --- | --- | 4.2 | 4.8 |

NOTE: N = 15 and 12, respectively for old and new roots.

*Abbreviations for inocula: Ab = A. byssoides, E = E-strain, Ff = forest floor, Hc = H. crustuliniforme, Lb = L. bicolor, Ll = L. laccata, Tt = T. terrestris

**Mean mycorrhiza classes: (1) 0% of short roots mycorrhizal, (2) 1-25% mycorrhizal, (3) 26-50% mycorrhizal, (4) 51-75% mycorrhizal, (5) 76-95% mycorrhizal, (6) > 95% mycorrhizal.

For the five inocula in both soils and temperatures, the presence of indigenous fungi reduced new root colonization ($P = 0.001$) with significant interactions between the presence of indigenous native fungi, soil temperature and inoculation treatments on new root colonization. Soil temperature had no effect on new root colonization in the absence of indigenous ECM fungi, but did affect new root colonization in the presence of indigenous inoculum. The presence of indigenous inoculum reduced ($P = 0.04$) new root colonization by H. crustuliniforme 5249 and L. laccata 101D, but had no effect on new root colonization by other inocula. The presence of indigenous ECM fungal inoculum reduced new root infection by H. crustuliniforme 5249 at both soil temperatures, but reduced new root infection by L. laccata 101D only at 12°C.

6. Effect of nutrient addition (Osmocote) on persistence

At the time of transplanting, mean mycorrhiza class for roots within the container plug averaged 6.0, 5.9, and 6.0 respectively for the E-strain, H. crustuliniforme and forest floor seedlings. Slow-release fertilizer had no effect ($P > 0.40$) on survival of inoculant fungi within the original root plug but did affect colonization of new roots formed outside the plug (Table 3.5). In the unfertilized forest soil, colonization of new root growth was greater than 75% for all inoculant fungi. Fertilization had no effect on new root colonization by forest floor, but reduced ($P = 0.05$) that by E-strain by approximately 20% While new root colonization by H. crustuliniforme 5249 was lower than that in old roots, nutrient addition had no effect ($P > 0.05$) on new root colonization. Short roots not infected with E-strain were colonized by forest floor fungi; those not infected by H. crustuliniforme 5249 remained non-mycorrhizal.

Table 3.5. Degree of short root colonization by inoculant fungi at the time of slow-release fertilizer (Osmocote 14-14-14 NPK) application and at 12 weeks after application

| | Roots at the time of fertilizer application | New roots formed after fertilization | |
|---|---|---|---|
| | | -NPK | +NPK |
| Inocula* | Mean mycorrhiza class** | | |
| E | 6.0 | 6.0 | 4.6 |
| Hc 5249 | 5.9 | 5.3 | 5.6 |
| Ff | 6.0 | 6.0 | 6.0 |

NOTE: N = 16 at the time of fertilizer application; N = 8 for both fertilizer treatments at 12 weeks.

*Abbreviations for inocula: E = E-strain, Ff = forest floor, Hc = H. crustuliniforme.

**Mean mycorrhiza classes: (1) 0% of short roots mycorrhizal, (2) 1-25% mycorrhizal, (3) 26-50% mycorrhizal, (4) 51-75% mycorrhizal, (5) 76-95% mycorrhizal, (6) > 95% mycorrhizal.

7. Foliar nutrition

Slow-release fertilization appeared to increase the levels of foliar N of seedlings inoculated with forest floor; but had little effect on foliar N and P of the other seedlings (Table 3.6). Levels of foliar P (% dry weight) decreased slightly in all inoculation treatments probably due to growth dilution. Fertilized seedlings had more than twice the P content (mg/seedling) in new foliage than did unfertilized seedlings.

**Table 3.6.** Effect of slow-release fertilizer (NPK) and inoculation treatments on N and P concentrations (% oven-dry weight) in new foliage at 12 weeks

| Inoculation treatment | -NPK | | +NPK | |
|---|---|---|---|---|
| | N% | P% | N% | P% |
| Forest floor | 1.03 | 0.21 | 1.73 | 0.19 |
| E-strain | 1.67 | 0.22 | 1.80 | 0.19 |
| *H. crustuliniforme* | 0.93 | 0.20 | 0.90 | 0.15 |

NOTE: N = 1; each sample consists of pooled foliage from 8 seedlings.

## Discussion

In general, spread of inoculant fungi to new roots produced outside the original container plug was considered good (> 50% infection) in the experimental conditions (cool, acidic soils) of the study. This result suggests mycorrhizae established in the nursery have the potential to significantly influence mycorrhiza development, seedling nutrition, growth and physiology at least in the first growing season after planting. A similar conclusion was reached by Dahlberg (1990) for lodgepole and Scots pine seedlings outplanted in Sweden and by Fleming et al. (1984) for silver birch seedlings outplanted in Great Britain. In the study conducted by Fleming et al. (1984) a short period (8 weeks) of mycorrhizal inoculation in the nursery influenced mycorrhiza formation in the field for at least 45 months.

Soil temperature and the presence of indigenous ECM fungi interacted to influence the infection by inoculant fungi of short roots initiated outside the root plug. When native ECM fungi inoculum was present, inoculant fungi colonized a greater percentage of new roots in the 6°C soil than in the 12°C soil. Inoculant fungi may persist and influence seedling growth and physiology for a longer period on routine reforestation sites in boreal forest regions compared with sites in warm forest regions where most mycorrhizal research has been conducted. In the absence of native ECM inoculum, soil temperature had no effect on the percentage of new roots colonized by inoculant fungi. Infection of old and new roots by inoculant fungi exceeded 95% in 6 and 12°C pasteurized soils.

Several factors may be responsible for the reduced persistence of the test fungi in unpasteurized 12°C soil. Extramatrical (EM) hyphae of the inoculant fungi were observed spreading along lateral roots as they

egressed from the container root plug, colonizing short roots near established mycorrhizae. Indigenous mycorrhizae when present were found near the tips of elongating lateral roots. This pattern of infection by inoculant and indigenous fungi was also observed by McAfee and Fortin (1986) on jack pine seedlings two months after planting in various field environments. In the 6°C soil, short roots tended to emerge into forest soil containing extramatrical hyphae of the inoculant fungi. In the 12°C soil, the more rapid rate of root elongation increased the probability that short root apices would contact indigenous sources of inoculum before being colonized by EM hyphae of the inoculant fungi. Warming of the soil from 6 to 12°C appeared to increase the rate of root extension more than the rate of hyphal extension. Short root production and root elongation was 70% greater in the 12°C soil.

Second, cool soil temperatures may have slowed primary infection from "free" indigenous propagules (hyphal fragments, germinating spores) more than secondary infection from hyphae attached to established mycorrhizae. Established mycorrhizae have access to host plant photosynthate for mycelial respiration and growth. Whereas established infections of T. terrestris 2088 and A. byssoides survived and colonized new roots in 6°C soils, these isolates did not form mycorrhizae from "free" mycelial inoculum at 6°C (see Chapter II).

Significant differences in persistence were evident for the various fungal isolates. Averaged across all treatments (temperature, +/- native inoculum), established L. bicolor, T. terrestris, E-strain, H. crustuliniforme 125 inocula were the most successful colonizers of roots outside the plug in the study conditions, i.e., cool, acidic, wet forest soils. L. laccata, H. crustuliniforme 5249, and A. byssoides inocula were

the least successful.

In field studies, Laccaria species have been shown to persist and compete successfully in a variety of soil types during the first growing season (McAfee and Fortin 1986, Wilson et al. 1987, Danielson and Visser 1989). L. bicolor competed successfully with native ECM fungi in acidic (pH 3.7-6.1) disturbed forest soils of the boreal forest zone (McAfee and Fortin 1986).

Long-term persistence of Laccaria spp. is more variable and may depend on soil type. Danielson and Visser (1989) reported that L. proxima persisted only one growing season in neutral (pH 6.3-7.5) oil sands amended with peat in a cool, continental climate. In contrast, Wilson et al. (1987) found that L. proxima persisted for at least four growing seasons in a poorly-drained acidic peat soil. Shaw et al. (1987b) also reported good persistence (> 50% of short roots) of L. laccata for two years on Sitka spruce seedlings planted in variety of organic and mineral soil microsites in a cool, moist maritime climate. Long-term persistence of Laccaria spp. also may depend on the overwinter survival of EM hyphae. Coutts and Nicoll (1990a) monitored mycelial growth of established T. terrestris and L. proxima infections on potted Sitka spruce seedlings for a summer and winter. They found that EM hyphae of L. proxima disappeared during the winter as soil temperatures dropped below 6$^{o}$C, decreasing the likelihood that short roots emerging the following spring would contact L. proxima inoculum.

T. terrestris is one of the most common fungi in forest nurseries throughout the world. Although it is adapted to the high fertility and moisture conditions of nurseries (Marx et al. 1984, Hunt 1989), T. terrestris established in a nursery can successfully persist after

outplanting (Cruz 1974, Ruehle 1982, Thomas et al. 1983, Danielson and Visser 1989). Cruz (1974) found that T. terrestris persisted on slash pine seedlings planted in poorly-drained, acidic soils, whereas, Pisolithus tinctorius was replaced by native fungi. Extramatrical hyphae of T. terrestris have been shown to overwinter successfully at soil temperatures below 6°C (Coutts and Nicoll 1990a), increasing the probability that short roots formed the following year would emerge into forest soil containing T. terrestris inoculum.

E-strain has been shown to survive for several years after outplanting, particularly in dry, neutral to moderately alkaline soils (Danielson and Pruden 1989, Danielson and Visser 1989) or in burnt, clear-cut soils (Mikola 1965). Whether E-strain will persist for several years in cool, wet, acidic soils has not been determined. Its ability to survive and compete at cool temperatures may be the deciding factor. E-strain is known to tolerate a wide range of soil pH values (Mikola 1965) and poor soil drainage (Levisohn 1954).

The high degree of new root colonization (> 75% of short roots) for both isolates of H. crustuliniforme in the acidic soils of the study was not expected. Previous studies have suggested that the persistence of Hebeloma spp. would be low (Lamb 1979, Chu-Chou and Grace 1981, Mason et al. 1983), especially in acidic soils. Mason et al. (1983) observed that native Hebeloma fungi colonized birch seedlings planted in mineral soils but not those planted in peat soils; and that H. sacchariolens established on nursery birch seedlings did not persist in an acid peat soil. In vitro studies (Hung and Trappe 1983, Dennis 1985) indicate Hebeloma spp. are adapted to neutral to alkaline soils. Optimum growth in culture occurs at pH 7 (Hung and Trappe 1983), with some growth even at pH 9 (Dennis 1985).

Danielson and Pruden (1989) reported that Hebeloma-like native soil fungi persisted for a long period on urban spruce growing in dry, neutral to moderately alkaline soils.

Data on field persistence of established A. byssoides infections is not available in the mycorrhiza literature. However, the common occurrence of this species in coniferous forests suggests established infections may compete successfully with native ECM fungi. Based on fruit-body distribution, Eriksson and Ryvarden (1973, cited in Danielson et al. 1984d) consider A. byssoides to be a characteristic species of European coniferous forests. In North America, A. byssoides has been collected from mature stands of jack pine (Danielson 1984b) and white spruce (Danielson et al. 1984d). The optimum soil pH, moisture, and temperature conditions for persistence of established A. byssoides infections is not known. The poor persistence of A. byssoides in the forest soil mixture used in this study suggests its ability to compete with native ECM fungi may be low in cool, wet, acid soils. However, the A. byssoides isolate used in the study originated from a container nursery environment; and may have been better adapted to a more fertile, less acidic growing medium. More isolates need to be tested to determine the ecological characteristics of this species.

The three inocula sources varied in their response to nutrient addition at the time of transplanting. Fertilization reduced colonization of roots outside the plug by E-strain; but not colonization by H. crustuliniforme or by the forest soil inoculum. High rates of NPK application have been shown to inhibit mycorrhiza formation in nursery stock (Crowley et al. 1986, Gagnon et al. 1987, Hunt 1989). Inhibition may be due to a build-up of fertilizer salts in the growing medium (Crowley et al. 1986) or to a reduction in root sucrose concentrations (Marx et al.

1977). E-strain is known to be sensitive to high rates of soluble (Danielson and Visser 1988) and slow-release (Gagnon et al. 1987, Hunt 1989) NPK fertilizer in container growing medium. However, the application rate used in this study was not high and should not have reduced mycorrhiza formation by E-strain; the amount of slow-release fertilizer applied was less than half the rate found to be inhibitory to mycorrhiza formation by other researchers, e.g., Crowley et al. (1986), Gagnon et al. (1987) and Hunt (1989). Shaw et al. (1987a) reported that E-strain could successfully infect conifer roots at application rates much higher than those used in this study. Also levels of foliar N and P (1.90% and 0.22%, respectively) were not excessively high. E-strain can successfully colonize roots of seedlings with similar levels of N and P (Danielson and Visser 1988). Possibly, fertilizer-induced changes in root growth, host physiology (e.g., composition or quantity of root exudates, hormones or root content of sucrose) or in the population of rhizosphere microorganisms decreased the ability of E-strain hyphae to contact and colonize short roots before they were occupied by indigenous inoculum. Alternatively, E-strain may be more sensitive to an increase in fertilizer salts or to a lowering of root sucrose concentrations in cool moist soils than in warm nursery mixes.

Danielson and Visser (1989) noted that competition from indigenous ECM fungi is the most frequent explanation for lack of persistence by inoculant fungi. Active replacement of inoculant by indigenous fungi has been observed in the field. However, in their study of field persistence of nine ECM fungi on jack pine seedlings planted on a mine reclamation site, they observed that replacement of inoculant fungi by indigenous fungi was often passive, i.e., the inoculant fungus infecting a short root died and was subsequently replaced by an indigenous fungus with no apparent

physical contact between the two fungi. Lack of persistence with non-interactive replacement is more likely due to poor adaptation of the inoculant fungus to the host plant or environment. Danielson and Visser (1989) hypothesized that ill-adapted mycorrhizae lose vigour because they do not function effectively as sinks for host plant carbohydrate.

Neither active nor passive replacement of inoculant fungi was observed during this study. Twelve weeks may be too short a time for active or passive replacement to occur. In addition, an interruption in root growth may be necessary before one fungus is able to replace another (Marks and Foster 1967). Root growth was continuous in the controlled environment, possibly because soil moisture was not limiting. The unhealthy appearance of many E-strain mycorrhizae suggests this fungus may be replaced in a longer-term study. Alternatively, the lack of replacement in the study may reflect the "free" nature of the indigenous inoculum, i.e., hyphal fragments or dormant propagules. In field environments, the infecting hyphae of indigenous ECM fungi may be connected to a carbohydrate reserve (living plant root) which supports faster growth and greater competitiveness with inoculant fungi.

Except in the H. crustuliniforme treatment, short roots not infected by inoculant fungi were colonized by native ECM fungi. Most short roots not infected by H. crustuliniforme remained non-mycorrhizal, suggesting that this fungus may inhibit colonization of the root system by other fungi. Other researchers have reported similar inhibitory effects in species of Laccaria, Hebeloma and Rhizopogon. Gagnon et al. (1987) noted that L. bicolor or H. cylindrosporum infections on container-grown pine seedlings appeared to inhibit root colonization by fungi indigenous to container nurseries (e.g., T. terrestris). McAfee and Fortin (1986)

reported that Rhizopogon rubescens prevented infection by ECM fungi native to boreal forests. They hypothesized that R. rubescens might secrete chemicals which inhibit root colonization by competing ECM fungi. Cultures of Rhizopogon have been shown to secrete chemicals that inhibit other ECM fungi[1].

The initial mycorrhizal status of the seedlings and the pattern of lateral root egress from the container plug may have contributed to the high persistence of inoculant fungi in this study. Inoculum density is a major factor determining the ability of fungal isolates to form mycorrhizae in a nursery environment (Garbaye 1983, Egli and Kälin 1985); and may have a significant influence on field persistence of inoculant fungi. Colonization of roots outside the plug was positively correlated with the percentage of container plug roots infected by the inoculant fungi prior to transplanting. Analysis of data presented by Danielson and Visser (1989) for nine ECM fungi showed that percent colonization at the time of planting was also positively correlated ($P = 0.006$) with the percentage of new roots infected by inoculant fungi one year after planting.

Egress of lateral roots from the entire root plug, as occurred in this study, improves field persistence of inoculant fungi (Ruehle 1983, 1985). Ruehle concluded that persistence of P. tinctorius was greater on outplanted bare-root loblolly pine seedlings than on container-grown seedlings because lateral roots egressed horizontally from the entire root system of the bare-root stock. In contrast, lateral roots elongated vertically from the bottom of the container root plugs. P. tinctorius did not colonize these vertical laterals, apparently due to inhibitory

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[1] Castellano 1987, unpublished data cited in Castellano and Molina 1989.

biological factors (e.g., native ECM symbionts, microbes or pathogenic fungi). P. tinctorius was able to successfully colonize vertically egressing roots in sterilized soil.

The possible effects of inoculum potential and lateral root egression on field persistence should be considered when interpreting the results of outplanting trials. Although Bledsoe et al. (1982) cited competition as the major reason for poor field persistence of L. laccata and H. crustuliniforme inoculum on the root systems of outplanted container-grown Douglas-fir seedlings, three results of their study suggest that inoculum density and lateral root egression patterns affected persistence: (1) fewer than 25% of the roots formed outside the plug were colonized by native forest soil ECM fungi five months after planting, (2) new lateral roots egressed vertically from the bottom of the root plugs, and (3) the inoculant fungi had not colonized the root plug extensively prior to outplanting. Inoculum distribution within the plug was not described. It is possible that low infection levels in the bottom of the container plugs reduced the potential for colonization of vertically egressing lateral roots.

## Summary

Fungal symbionts established in a warm container nursery environment persisted within the original root mass and colonized new roots initiated outside the container plug when white spruce seedlings were tranplanted into cool soils (6, 12°C). Even the common indigenous nursery fungus, T. terrestris, considered to be poorly adapted to field environments, rapidly colonized new roots. Soil temperature and the presence of indigenous ECM fungi interacted to influence new root colonization by inoculant fungi.

Indigenous forest fungi (from "free" inocula propagules) were poor root colonizers at 6°C, and their presence reduced percent root colonization by inoculant fungi only at 12°C. Factors which contributed to successful colonization of new roots by inoculant fungi, included (1) degree of root colonization by inoculant fungi in the nursery, (2) the pattern of new root growth, and (3) the relative weakness of ectomycorrhizal propagules (spores and hyphal fragments) compared with live ectomycorrhizal attachments. The negative effect of NPK fertilizer at the time of transplanting on new root colonization by E-strain fungi suggests that interactions between inoculant fungi and silvicultural practices need further investigation.

# CHAPTER IV

# EFFECT OF VARIOUS MYCORRHIZAL FUNGI ON THE ACCLIMATION OF WHITE SPRUCE SEEDLINGS TO LOW SOIL TEMPERATURES

## Introduction

In cold soils, planted seedlings are subject to water stress even when soil water content is high. Low root-zone temperatures reduce the permeability of root cell membranes and increase the viscosity of water resulting in high plant resistance to water flow and low water absorption (Kramer 1940, Kaufmann 1975, Running and Reid 1980, Teskey et al. 1984, Orlander and Due 1986). In 7°C soil, for example, water absorption by Scots pine is approximately 30% of that in 25°C soil (Orlander and Due 1986). If seedlings transpire more water than the amount absorbed under these conditions, they may develop shoot water deficits despite adequate soil moisture (Goldstein et al. 1985, Lopushinsky and Kaufmann 1984).

Cold soils also decrease root and shoot growth of newly planted stock (Barney 1951, Lopushinsky and Kaufmann 1984). Decreased metabolic activity or turgor would influence the growth of root tissues. In addition, carbon assimilation may be reduced in cool soils (Babalola et al. 1968, Lawrence and Oechel 1983b, Delucia 1986), limiting the availability of current photosynthate for new shoot and root growth. Any reduction in root growth after planting increases the susceptibility of seedlings to summer drought (Lopushinsky and Kaufmann 1984).

When water uptake is reduced by high root resistances, the development of severe water stress is prevented in the short-term by delayed flushing of new shoot tissue (Blake 1983) and by stomatal closure (Lopushinsky and Kaufmann 1984). The delay in flushing is important

because newly-flushed needles of spruce seedlings transpire at 4-5 times the rate of mature needles (Blake 1983). Cold-stored white spruce seedlings, however, may not be able to regulate stomata of mature needles immediately after transplanting. Grossnickle and Blake (1985) observed that the stomata of white spruce seedlings, in contrast to those of jack pine seedlings, did not close fully at night for two to three weeks after they were removed from cold storage. Consequently, the white spruce seedlings were unable to control water loss even when photosynthesis was not occurring.

Resistance to water flow, or its inverse hydraulic conductance, is affected by many plant characteristics including (1) total volume or surface area of the root system (Carlson 1986), (2) total plant size and shoot/root ratio (Levy et al. 1983, Koide 1985), (3) amount of new root growth or the proportion of unsuberized to suberized roots (Kramer and Bullock 1966, Sands et al. 1982), (4) nitrogen and phosphorus nutrition (Safir et al. 1972, Graham and Syvertsen 1984, Radin and Eidenbock 1984), and (5) presence of root disease (Marschner 1986).

Ectomycorrhizal infection influences all of these plant characteristics (Harley and Smith 1983 and references therein) suggesting that it would alter root resistance to water flow as has been shown for vesicular-arbuscular (VA) infections in crop plants. These effects have been attributed to improved phosphorus nutrition (Nelsen and Safir 1982, Graham and Syvertsen 1984, Koide 1985), changes in plant root/shoot ratios (Andersen et al. 1988) or in the balance of plant growth regulators (Levy and Krikun 1980, Allen et al. 1981). VA infections also have been shown to influence stomatal conductance (Levy and Krikun 1980), possibly by altering plant nutrient status or the balance of plant growth regulators (e.g.,

cytokinins or abscisic acid) that affect stomatal opening and closing.

If cold-stored spruce seedlings do not quickly acclimate to the environment of the planting site, transplant stress may develop (Grossnickle and Blake 1985). The objectives of the work reported in this chapter were (1) to describe the effects of inoculation on seedling response to low soil temperature during the transplant stress period, i.e., the first 6 weeks after planting, and (2) to test the hypothesis that inoculation with specific fungi could speed seedling acclimation.

Five variables were chosen to compare the acclimation of cold-stored inoculated and non-inoculated seedlings: flushing date, amount of new root growth, resistance to water flow, pre-dawn stomatal conductance, and the rate of net photosynthesis. Resistance to water uptake was measured using a whole plant rather than a root pressurization technique. Root pressurization measurements are subject to artifacts (Passioura 1988) and may underestimate the resistance of seedlings (Grossnickle and Russell 1990).

These parameters were measured for three weeks after transplanting, the time required for cold-stored spruce and pine seedlings to acclimate physiologically to cool soils (Grossnickle and Blake 1985). Two soil temperatures (6, 12°C) were chosen to bracket the soil temperature (7-8°C) below which root resistance of lodgepole pine (Running and Reid 1980) and Engelmann spruce (Kaufmann 1975) rises exponentially, and the soil temperature (10°C) at which root growth of spruce sharply declines (Tyron and Chapin 1983).

Methods

Eight-week-old seedlings sown in 40 $cm^3$ Spencer-LeMaire Rootrainers were inoculated, grown and cold-stored as described in Chapter II. They were then transplanted into pots containing a mixture of forest floor, mineral soil and perlite (1:1:2 by volume) maintained at either 6°C or 12°C. The surface of the soil was covered with a 2 cm layer of white Styrofoam chips in order to minimize heat exchange between the soil and air. A preliminary study showed that soil temperature varied vertically by 1.5°C and horizontally by less than 1.0°C.

Six inoculation treatments were included in this experiment: (1) 4 $cm^3$ of forest floor from vigorous spruce plantations, (2) 4 mL of sterilized agar slurry (control), or 4 mL of a mycelial slurry prepared from pure cultures of (3) Hebeloma crustuliniforme 5249, (4) Laccaria bicolor 5268, (5) E-strain 947, and (6) Thelephora terrestris (Laval). All inocula were collected from forest sites (Table 2.1).

Seedlings were not fertilized after transplanting but were watered with tap water of the appropriate temperature (6 or 12°C) to maintain soil moisture potential at -0.01 MPa, i.e., the soils were both cool and moist. The three week study was conducted in a controlled environment chamber: 30 to 50% relative humidity, respective day/night air temperatures of 20/12°C, and an 18-h photoperiod with a photosynthetic photon flux density of 400 $\mu mol/(m^2 s)$ from a combination of incandescent and cool white fluorescent lights.

Two bulked samples of root and shoot tissue (8 seedlings per sample) from each inoculation treatment were analyzed for nitrogen (N) and phosphorus (P) at the time of transplanting. After oven-drying (70°C, 48 h), milling and wet oxidation with $H_2SO_4$, Se, salts and $H_2O_2$ (Parkinson and

Allen 1975), tissues were analyzed for N and P by colorimetric analysis using salicylate/nitroprusside for N and ascorbic acid/ molybdate-antimony for P (Technicon Industrial Systems 1977).

Stem diameter, shoot biomass, root biomass and morphology (lateral root length and short root number), pre-dawn stomatal conductance, net photosynthesis and resistance to water flow were measured 2, 9 and 21 days after transplanting on 8 seedlings per temperature and inoculation treatment. Individual seedlings were carefully removed from the pots to minimize disturbance to the remaining seedlings; cavities were filled with forest soil mixture. Disturbance to the remainder of the seedlings in a pot was negligible due to the coarse friable nature of the forest soil mixture and to the lack of significant root elongation during the experiment. Measurements of biomass and root morphology at day 2 were assumed to be representative of these parameters at the time of transplanting (day 0). Flushing was recorded 9, 16 and 21 days after transplanting. A seedling was considered flushed if the terminal bud had broken through the bud scales.

The root system of each seedling was cut lengthwise with one half examined for mycorrhizal infection. Mycorrhiza formation by inoculant and nursery contaminant fungi was assessed at low and high power as described in Chapter III. The second half was carefully washed and cut into 2-3 cm long segments. No attempt was made to remove all extramatrical hyphae from mycorrhizae. Approximately a third of these segments were selected randomly to estimate short root numbers and root length. Root length was estimated using Newman's line intersect method (1966) as modified by Tennant (1975). Total seedling root length and short root number were derived from the dry weight (70$^{o}$C for 48 h) ratio between sample segments

and the whole root system.

Stomatal conductance, net photosynthesis and internal needle $CO_2$ were measured using a portable gas exchange system (LI-6200, Li-Cor Ltd.). Measurements were conducted 4 to 6 hours into the light period. A preliminary study showed that net photosynthesis and stomatal conductance of individual seedlings were not affected ($P > 0.25$) by the time of measurement during this period. All measurements were made on mature needles; any newly flushed needles were removed carefully with tweezers prior to measurement. Incorporation of newly flushed needles causes errors in physiological measurements (e.g., Lippu and Puttonen 1989) since their photosynthetic rates are low in comparison to those of mature needles (Troeng and Linder 1982). Net photosynthetic rates were calculated on the basis of needle dry weight and surface area which was estimated from needle length and displaced volume (Brand 1987). Photosynthetic nitrogen-use efficiency or PNUE, the rate of net photosynthesis per unit of shoot nitrogen, was derived from net photosynthesis and shoot foliar N data.

Growth, conductance and net photosynthesis data were tested by least-squares analysis of variance (ANOVA) using SYSTAT (Wilkinson 1988). Data were analyzed using a completely randomized three-way factorial design with fixed treatments: soil temperature (2 levels), inoculation (6 types) and time (3 levels) with 4 pots (each containing 6 seedlings) nested within the soil temperature and inoculation treatments. When pot-to-pot variability was insignificant ($P < 0.25$, Bancroft 1964) compared to seedling-to-seedling variability, these two sources of variability were combined as the error term to test main effects and interactions. Otherwise pot-to-pot variability was used as the error term. Data were transformed when necessary to meet the normality and homogeneity of variance assumptions of

ANOVA (Eisenhart 1947). Linear contrasts were used to compare inoculation treatments. The error rate for multiple comparisons was controlled using the Bonferroni procedure, i.e., for an overall rate of p, k comparisons were conducted at p/k (Wilkinson 1988). Correlation analysis was used to examine relationships between seedling physiology and seedling morphology and nutrition.

Root resistance to water uptake was estimated by measuring "soil-to-xylem" resistance (RSX) to water flow. Root resistance is considered to be the major resistance along this pathway unless soils are very dry (Passioura 1982), with soil water potentials less than -0.1 MPa (Gardner and Ehlig 1962). The root resistance of lodgepole pine seedlings is 67% and 93%, respectively, of total plant resistance at 7°C and 0°C (Running and Reid 1980). RSX was measured indirectly using a modification of Elfving et al.'s (1972) model of plant water relations in which, needle water potential ($\Psi_{needle}$) is considered a function of soil water potential ($\Psi_{soil}$), resistance to water flow through the soil-plant-air-continuum (RSPAC) and transpirational flux density (TFD):

$$\Psi_{needle} = \Psi_{soil} - RSPAC*(TFD)$$

Assuming (1) that experimental seedlings had a negligible capacity for water storage, (2) that $\Psi_{xylem}$ approximated $\Psi_{needle}$ and (3) that the water potential of the soil was zero, this model was reduced to:

$$-RSX = (\Psi_{xylem})/TFD$$

The first two assumptions are reasonable given the small size of the seedlings used in this study. The third was met by maintaining soil water content above field capacity.

Three seedlings per inoculation-temperature treatment combination were measured to determine pre-dawn $\Psi_{xylem}$ using a pressure chamber and a

hand-held lens (Ritchie and Hinckley 1975). Five seedlings were measured to determine mid-day (4 hours into the light period) $\Psi_{xylem}$ and transpirational flux density (TFD) using a portable gas exchange system (LI-6200). RSX for each treatment combination was calculated from the slope of the regression of $U_{xylem}$ on log-transformed values of TFD. This transformation was necessary to meet the requirement for residual homogeneity of variance in least squares regression analysis (Steel and Torrie 1980, p. 248). $R^2$ values for these regressions ranged from 0.60 to 0.90. Equality of regression slopes (RSX) was examined by t-tests (Neter and Wasserman 1974).

## Results

### 1. Mycorrhizal status

Sixty percent of the non-inoculated control seedlings were non-mycorrhizal at the time of transplanting; the remainder had a weak infection of T. terrestris, i.e., less than 25% of the short roots exhibiting a Hartig net, negligible mantle development and rare occurrence of cystidia, external hyphae and mycelial strands. Colonization by inoculant fungi was successful with all plants showing some infection. More than sixty percent of the short roots of seedlings inoculated with H. crustuliniforme (5249), E-strain (947) and L. bicolor (5268) were infected by the test fungus; other short roots were predominantly non-mycorrhizal. Hebeloma mycorrhizae produced abundant extramatrical (EM) hyphae; E-strain mycorrhizae produced few EM hyphae.

Three-quarters of the seedlings inoculated with T. terrestris had at least 50% of the short roots colonized by this fungus; other short roots remained non-mycorrhizal or were colonized by E-strain fungi. These

Thelephora mycorrhizae were well-developed (i.e., mantles covered more than 75% of the short roots, cystidia and mycelial strands were common) indicating that infection resulted from the inoculum and not from air-borne spores of Thelephora.

Eighty-five percent of the seedlings inoculated with forest floor were colonized primarily by forest floor fungi; 15% of these seedlings were infected by forest floor fungi and by T. terrestris.

2. Seedling size and morphology at the time of transplanting

At the time of transplanting, seedling morphology (Table 4.1) and nutrition (Tables 4.2) differed by inoculation treatments ($P < 0.001$). Seedlings assigned to each temperature treatment were not significantly different ($P > 0.60$) at the time of transplanting. Root length and short root number were least for seedlings inoculated with L. bicolor; and greatest for those inoculated with T. terrestris. Root biomass ranged from a low of 68 mg for seedlings inoculated with L. bicolor to a high of 105 mg for those inoculated with T. terrestris.

Comparison of foliar N and P concentrations with published foliar analyses of spruce seedlings (e.g., Leyton 1948, Ingestad 1959, Swan 1962, Beaton et al. 1965, Swan 1971, Benzian and Smith 1973, Morrison 1974, Farr et al. 1977, Ballard and Carter 1986) suggested that nitrogen was very deficient in seedlings inoculated with E-strain, H. crustuliniforme and T. terrestris (Table 4.2); and that P levels were adequate for spruce seedling growth in all inoculation treatments.

Table 4.1. Effect of inoculation treatment on seedling morphology at the time of transplanting

| Inocula* | Shoot/ root ratio | # short roots/ seedling | Root length (cm) | # short roots/cm of root |
|---|---|---|---|---|
| Control | 2.7 (0.7) | 936 (373) | 347 (130) | 2.8 (0.8) |
| E | 2.0 (0.4) | 695 (263) | 170 ( 82) | 4.4 (1.3) |
| Ff | 2.9 (0.8) | 1010 (396) | 290 (118) | 3.6 (0.7) |
| Hc | 2.7 (0.7) | 958 (320) | 240 ( 76) | 4.2 (1.2) |
| Lb | 3.0 (0.8) | 491 (130) | 159 ( 64) | 3.5 (1.5) |
| Tt | 2.3 (0.6) | 1061 (480) | 328 (113) | 3.2 (0.9) |

NOTE: N = 16; standard deviations are shown in parentheses. Short roots were not included in the estimate of root length.

*Abbreviations for inocula: E = E-strain, Ff = forest floor, Hc = H. crustuliniforme, Lb = L. bicolor, Tt = T. terrestris.

Table 4.2. Effect of inoculation treatment on seedling N and P concentrations (% oven-dry weight) at the time of transplanting

| Inocula* | Shoot | | Root | |
|---|---|---|---|---|
| | N% | P% | N% | P% |
| Control | 1.2(0.06) | 0.25(0.01) | 1.4(0.24) | 0.26(0.04) |
| E | 0.9(0.03) | 0.27(0.02) | 1.9(0.11) | 0.42(0.09) |
| Ff | 1.4(0.05) | 0.36(0.01) | 1.6(0.03) | 0.35(0.01) |
| Hc | 0.9(0.09) | 0.24(0.05) | 1.4(0.06) | 0.25(0.01) |
| Lb | 1.1(0.06) | 0.37(0.04) | 1.3(0.19) | 0.29(0.06) |
| Tt | 0.8(0.07) | 0.23(0.02) | 1.2(0.11) | 0.24(0.01) |

NOTE: N = 2; each sample consisted of pooled root or shoot tissues from 8 seedlings. One standard deviation in parentheses.

*Abbreviations for inocula: E = E-strain, Ff = forest floor, Hc = H. crustuliniforme, Lb = L. bicolor, Tt = T. terrestris.

3. Resistance to water flow from soil to xylem (RSX)

RSX decreased over time in both soil temperatures (Figure 4.1). Two days after transplanting, resistance to water flow was greater ($P < 0.01$) in the 6°C soil; at 9 and 21 days, there was no appreciable difference in RSX ($P > 0.11$) between the 6 and 12°C soils. Xylem pressure potential increased (became less negative) from day 2 to 21 in the 6°C soil (Table 4.3) even though transpiration rates more than doubled, increasing from 0.3 to 0.8 $\mu g\ H_2O/(cm^2 s)$.

There were significant ($P = 0.05$) differences in RSX among inoculation treatments 2 and 9 days at both soil temperatures; at 21 days, these differences were significant only in the 6°C soil (Table 4.4). RSX with several exceptions declined in all inoculation treatments and temperature combinations. In the 6°C soil, RSX of seedlings inoculated with E-strain or L. bicolor did not decrease significantly from 2 to 21 days; in the 12°C soil, RSX of non-inoculated seedlings was consistently low from 2 to 21 days.

**Table 4.3.** Range in mean mid-day mean xylem pressure potentials (-MPa) of the various inocula at 2, 9 and 21 days

| Soil temperature | 2 | 9 | 21 |
|---|---|---|---|
| 6°C | 1.1-1.5 | 0.8-1.0 | 0.5-0.8 |
| 12°C | 0.5-1.0 | 0.7-0.9 | 0.5-0.6 |

Figure 4.1. Effect of soil temperature on RSX [MPa/log ($\mu$g $H_2O/cm^2s$)] at 2, 9 and 21 days. Vertical bars represent one standard error of the estimate.

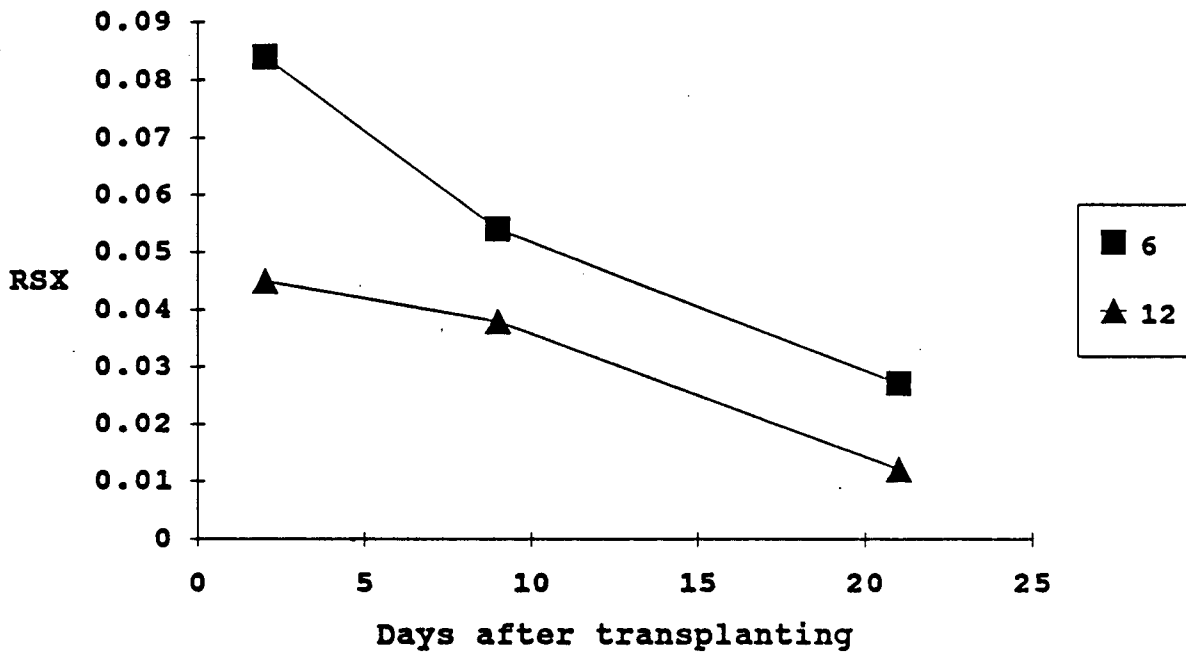

Table 4.4. Summary of soil-to-xylem resistance (RSX) estimates at 2, 9 and 21 days

| Inoculation | 6°C soil | 12°C soil |
|---|---|---|
| | 2 days after transplanting | |
| Control | 0.113 (0.011) | 0.016 (0.008) |
| E-strain | 0.041 (0.013) | 0.063 (0.009) |
| Forest Floor | 0.107 (0.013) | 0.039 (0.013) |
| H. crustuliniforme | 0.112 (0.038) | 0.057 (0.018) |
| L. bicolor | 0.073 (0.029) | 0.027 (0.007) |
| T. terrestris | 0.046 (0.022) | 0.074 (0.027) |
| | 9 days after transplanting | |
| Control | 0.060 (0.004) | 0.015 (0.014) |
| E-strain | 0.068 (0.013) | 0.033 (0.011) |
| Forest Floor | 0.051 (0.009) | 0.065 (0.015) |
| H. crustuliniforme | 0.069 (0.009) | 0.049 (0.018) |
| L. bicolor | 0.034 (0.008) | 0.021 (0.009) |
| T. terrestris | 0.046 (0.006) | 0.034 (0.010) |
| | 21 days after transplanting | |
| Control | 0.011 (0.007) | 0.016 (0.009) |
| E-strain | 0.053 (0.004) | 0.012 (0.009) |
| Forest Floor | 0.016 (0.006) | 0.011 (0.005) |
| H. crustuliniforme | 0.032 (0.008) | 0.006 (0.004) |
| L. bicolor | 0.060 (0.014) | 0.020 (0.010) |
| T. terrestris | 0.008 (0.006) | 0.009 (0.005) |

NOTE: Units for RSX are MPa/log ($\mu$g $H_2O/cm^2s$); standard error of regression estimate (N = 8) in parentheses.

At 21 days, RSX for seedlings inoculated with E-strain, H. crustuliniforme and L. bicolor were influenced by soil temperature (respective P values of 0.05, 0.10, and 0.05); suggesting that inoculation with these fungi slowed the rate of seedling acclimation to the 6°C soil.

Differences among inoculation treatments in RSX values were not related to the presence or absence of root pathogens; symptoms of root disease (e.g.,lesions, necrosis) were not evident in any of the inoculation treatments. Nor were they correlated ($P > 0.30$) with (1) root growth (length, dry weight and number of short roots) after transplanting, (2) with seedling size and shoot/root ratio or (3) shoot N and P nutrition.

RSX values at 21 days were related to root biomass, root form and nutrition at the time of transplanting (Table 4.5). However, there were no significant correlations ($P > 0.10$) between these parameters and RSX estimates at 2 and 9 days. RSX at 21 days was inversely proportional to lateral root length, number of short roots per seedling, root biomass and root length per unit root dry weight. Figure 4.2 shows RSX at 21 days as a function of initial root biomass. These relationships were strongest in the 6°C soil (Table 4.5). Seedlings inoculated with E-strain or L. bicolor, with the highest RSX at 21 days, had approximately 50% fewer short roots and 50% less lateral root length per seedling at the time of transplanting (Table 4.1) than non-inoculated control seedlings or those inoculated with forest soil, T. terrestris or H. crustuliniforme.

Root %P and RSX were weakly and positively correlated ($P = 0.07$) due to the coincidence of high values of RSX and root %P (Table 4.2) in E-strain seedlings. There was no association ($P > 0.35$) between RSX and root P when E-strain data were excluded from the correlation analysis.

Figure 4.2. Relationship between seedling resistance to water flow (RSX) at 21 days and initial root biomass; units for RSX are MPa/log ($\mu$g $H_2O/cm^2s$).

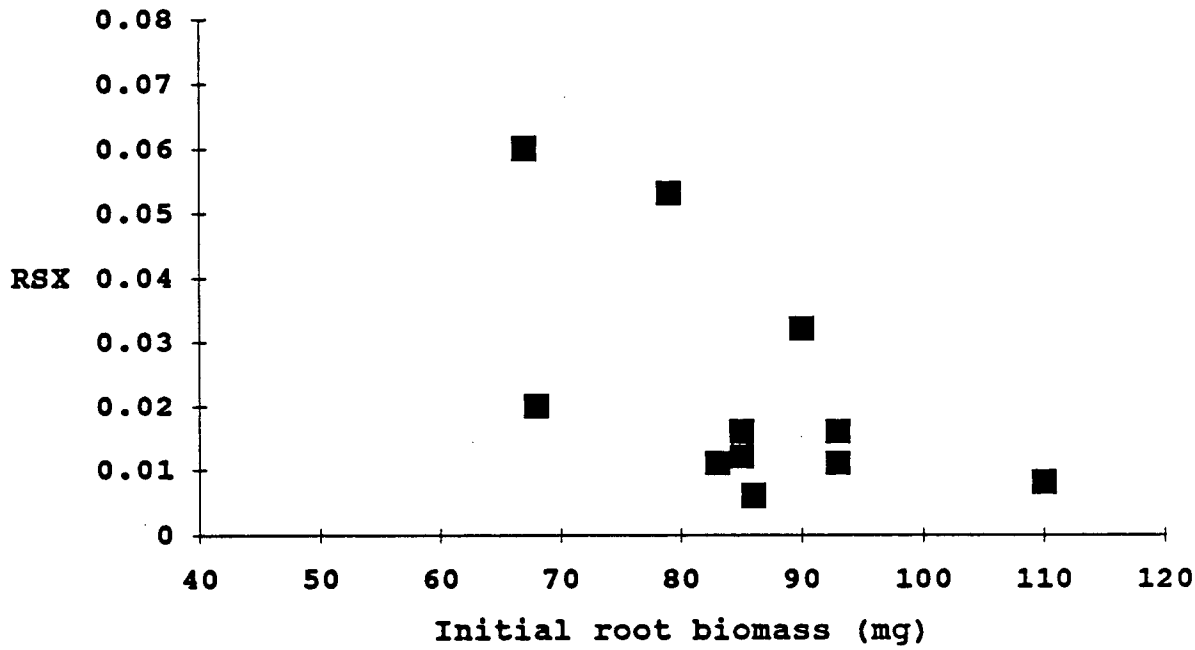

Table 4.5. Pearson correlation coefficients (r) between 21-day RSX and root morphology and nutrition at the time of transplanting

| Variable | Soil temperature | | |
|---|---|---|---|
| | 6°C | 12°C | 6 & 12°C |
| # short roots per seedling | -0.93 (0.01) | -0.70 (0.12) | -0.65 (0.02) |
| root length (cm) per seedling | -0.94 (0.01) | -0.07 (0.91) | -0.60 (0.04) |
| # short roots/ cm of root | 0.41 (0.43) | -0.32 (0.53) | 0.32 (0.31) |
| length/unit root biomass (cm/g) | -0.90 (0.01) | 0.10 (0.85) | -0.56 (0.06) |
| root biomass | -0.79 (0.06) | -0.65 (0.17) | -0.65 (0.02) |
| root %P | 0.61 (0.20) | -0.12 (0.83) | 0.54 (0.07) |

NOTE: Probability values for the Pearson correlation coefficients are shown in parentheses. N = 6 for individual soil temperatures.

4. Root and Shoot Growth after transplanting

New root growth was evident at the 9 day sample. Inoculation treatments accounted for 40% of the variability in final (21 day) root biomass data; whereas soil temperature treatments accounted for only 8%. Inoculation effects were due in large part to differences in root biomass at the time of planting. When covariance analysis was used to compensate for initial differences in seedling size, inoculation accounted for only 9% of the variability in the final root biomass data. Increases in root biomass during the test period ranged from less than 10% for some inocula (e.g., E-strain and forest soil) to greater than 35% for others (e.g., H. crustuliniforme). Mean root dry weight increased by 10 and 33%, respectively, in the 6 and 12°C soils.

Terminal buds began flushing after day 9. Soil temperature had no effect on the incidence of flushing at 16 or 21 days. Sixteen days after transplanting, a greater ($P < 0.01$) percentage (69%) of the non-inoculated seedlings had flushed compared to inoculated seedlings (25% averaged across the 5 inocula). Seedlings inoculated with forest soil flushed earlier than those inoculated with other inocula (Table 4.6).

5. Pre-dawn Stomatal Conductance

Analysis of variance showed no effect of inoculation ($P = 0.04$) on pre-dawn conductance (Table 4.7). Values of pre-dawn stomatal conductance values were very low (averaging less than 0.04 cm/s) indicating that stomata were fully closing at night. Two days after transplanting, pre-dawn conductance was lower ($P = 0.05$) in the 6°C soil (0.02 cm/s) than in 12°C soil (0.04 cm/s). Soil temperature had no effect on pre-dawn stomatal conductance at 9 and 21 days.

Table 4.6. Effect of inoculation treatment on terminal bud break

| Inoculation treatment | % of seedlings flushed | |
|---|---|---|
| | 16 days | 21 days |
| Non-inoculated | 69 | 100 |
| E-strain | 19 | 94 |
| Forest soil | 56 | 100 |
| H. crustuliniforme | 6 | 88 |
| L. bicolor | 19 | 88 |
| T. terrestris | 25 | 94 |

NOTE: N = 16 for each inoculation treatment.

Table 4.7. ANOVA of pre-dawn stomatal data

| Source of variation | df | Mean-square | F-ratio | Prob. |
|---|---|---|---|---|
| Soil temperature (ST) | 1 | 2.45 | 7.0 | 0.01 |
| Inocula (I) | 5 | 0.61 | 1.7 | 0.14 |
| Time (T) | 2 | 1.23 | 3.5 | 0.03 |
| ST x I | 5 | 0.24 | 0.7 | 0.64 |
| ST x T | 2 | 1.69 | 4.8 | 0.01 |
| I x T | 10 | 0.20 | 0.6 | 0.83 |
| ST x T x I | 10 | 0.51 | 1.5 | 0.18 |
| Error | 72 | 0.35 | | |

6. Rate of Net Photosynthesis (Pn)

Data for each measurement time were analyzed separately by two-way analysis of variance because pot-to-pot variation was significant at 9 and 21 days but not at 2 days. Parallel results were obtained for Pn calculated on a per unit shoot weight and unit leaf area basis. Therefore only the results for Pn on a leaf area basis are presented.

Inoculation treatments had more influence on net photosynthesis (Pn) than did soil temperature (Table 4.8). Soil temperature had the greatest effect ($P = 0.06$) at 9 days with net photosynthetic rate reduced by 13% in the 6°C soil. In comparison, Pn varied by 40 to 60% across inoculation treatments 2 and 21 days after transplanting.

Two days after transplanting, there was a significant interaction between inoculation and temperature treatments on Pn. In the 6°C soil, Pn of control seedlings and those inoculated with either L. bicolor or forest floor exceeded that of seedlings inoculated with other fungi (Table 4.9); in the 12°C soil, the Pn of seedlings inoculated with T. terrestris was lower ($P < 0.01$) compared with all other seedlings. At 21 days, there was no interaction between temperature and inoculation treatments, therefore only the mean values for inoculation treatment are presented (Table 4.10). Regardless of soil temperature, Pn was higher ($P = 0.01$) in control, forest floor and L. bicolor treatments compared to E-strain, H. crustuliniforme and T. terrestris treatments. Photosynthetic nitrogen-use efficiency (PNUE) showed no effect of soil temperature ($P > 0.21$) or inoculation treatment ($P > 0.12$).

Table 4.8. ANOVA of log-transformed Pn ($\mu$moles $CO_2/m^2s$) data

| Source of variation | df | Mean-square | F-ratio | Prob. |
|---|---|---|---|---|
| | | Two days after transplanting | | |
| Soil temp (ST) | 1 | 0.17 | 3.0 | 0.09 |
| Inocula (I) | 5 | 0.49 | 8.7 | 0.00 |
| ST x I | 5 | 0.18 | 3.1 | 0.02 |
| Error | 48 | 0.06 | | |
| | | Nine days after transplanting | | |
| Soil temp (ST) | 1 | 0.48 | 3.8 | 0.06 |
| Inocula (I) | 5 | 0.14 | 1.1 | 0.37 |
| ST x I | 5 | 0.07 | 0.6 | 0.71 |
| Pots (ST x I) | 36 | 0.13 | | |
| Error | 12 | 0.06 | | |
| | | Twenty-one days after transplanting | | |
| Soil temp (ST) | 1 | 0.09 | 1.2 | 0.29 |
| Inocula (I) | 5 | 0.45 | 6.0 | 0.00 |
| ST x I | 5 | 0.12 | 1.6 | 0.18 |
| Pots (ST x I) | 36 | 0.07 | | |
| Error | 12 | | | |

**Table. 4.9.** Interaction of soil temperature and inoculation on mean values of net photosynthetic rate 2 days after transplanting

| Inoculation treatment | Net PS (umoles $CO_2/m^2s$) | |
|---|---|---|
| | 6°C soil | 12°C soil |
| Forest Floor | 1.3 | 1.1 |
| Control | 1.1 | 1.1 |
| *L. bicolor* | 1.0 | 1.1 |
| *T. terrestris* | 0.7 | 0.7 |
| E-strain | 0.7 | 1.1 |
| *H. crustuliniforme* | 0.6 | 0.9 |

NOTE: Log-transformed Pn data were analyzed; values shown are in the original units; N = 5.

Table 4.10. Comparison of net photosynthesis ($\mu$moles $CO_2/m^2s$) 21 days after transplanting with shoot N (% oven-dry weight), seedling N (mg/seedling) and the ratio of shoot N to root N at the time of transplanting

| Inocula* | Pn | Shoot %N | Seedling mg N | Shoot N/ root N |
|---|---|---|---|---|
| Ff | 1.4 | 1.4 (0.05) | 4.7 (0.4) | 2.4 (0.1) |
| Control | 1.3 | 1.2 (0.05) | 3.7 (0.1) | 2.2 (0.3) |
| Lb | 1.2 | 1.1 (0.06) | 3.8 (0.3) | 2.7 (0.2) |
| Tt | 1.0 | 0.8 (0.07) | 3.1 (0.1) | 1.5 (0.3) |
| E | 0.9 | 0.9 (0.03) | 3.6 (0.3) | 1.1 (0.2) |
| Hc | 0.8 | 0.9 (0.09) | 3.2 (0.2) | 1.7 (0.1) |

NOTE: Log-transformed Pn data (N = 10) were analyzed; values shown are in the original units. N = 2 for nutrient parameters; standard deviations shown in parentheses.

*Abbreviations for inocula: Ff = forest floor, Lb = L. bicolor, Tt = T. terrestris, E = E-strain and Hc = H. crustuliniforme.

Pn at 21 days correlated positively with initial seedling N nutrition (Table 4.10) including shoot N (% oven-dry weight) ($r = 0.78$, $N = 12$, $P = 0.003$), total seedling N ($r = 0.66$, $N = 12$, $P = 0.02$) and the ratio of shoot N to total seedling N ($r = 0.70$, $N = 12$, $P = 0.01$); and to a lesser degree with shoot %P ($r = 0.50$, $N = 12$, $P = 0.10$) and the ratio of shoot to total seedling P ($r = 0.57$, $N = 12$, $P = 0.05$). Inoculation treatments significantly influenced ($P = 0.01$) all of these nutrient parameters.

Except at day 2, there was no correlation ($P > 0.20$) between Pn and xylem pressure potential (XPP). At day 2, net photosynthetic rate was inversely related ($P = 0.01$) to XPP, i.e., seedlings with high rates of Pn tended to have lower values (more negative) of XPP.

Discussion

Inoculation with E-strain, H. crustuliniforme, T. terrestris and L. bicolor delayed terminal bud break. The delay was not greater than 6 days, and the significance for seedling acclimation to cool soils was not clear. Shoot nutrition may have influenced the rate of flushing. Levels of shoot nitrogen were highest in the two treatments (control, forest floor) with the most rapid rate of bud break. Late-season nitrogen fertilizer applications accelerate bud break of Sitka spruce seedlings (Benzian et al. 1974).

The lack of an appreciable difference (only 0.1%) in shoot nitrogen between certain treatments (e.g., L. bicolor and control) with significantly different rates of flushing, suggests, however, that non-nutritional factors also influenced bud break. Spring shoot growth may be stimulated by a plant growth substance exported from roots (Atkin et al. 1973, Lavender et al. 1973). In cultures, ECM fungi have been shown to synthesize some of these substances, for example auxins and cytokinins (Ek et al. 1983, Harley and Smith 1983 and references therein), which are thought to influence the timing of bud break in trees (Kramer and Kozlowski 1979). Garbaye (1986) found that some ectomycorrhizal fungi (e.g., H. crustuliniforme, L. laccata) promoted earlier bud break in oak and beech seedlings than others (e.g., T. terrestris). Mycorrhizal-induced variation in time of bud break was attributed to differences in the ability of ECM fungi to synthesize plant growth regulators; however, Garbaye did not compare the nutritional status of seedlings infected with different fungi.

Averaged across inoculation treatments, seedling resistance to water flow, RSX, decreased from 2 to 21 days at both soil temperatures. This result is in agreement with other long-term studies of soil temperature effects on cold-stored white spruce seedlings (Grossnickle and Blake 1985, Grossnickle 1987). In contrast to these studies, however, seedling acclimation with respect to resistance to water flow was more rapid; occurring at 9 days compared to 14 days (Grossnickle and Blake 1985) or more than 21 days (Grossnickle 1987). Averaged across all inocula, resistance to water flow decreased as root biomass increased over time ($N = 6$, $r = -0.86$, $P = 0.03$), suggesting that new root growth contributed to the decrease in resistance from 2 to 21 days observed at both soil temperatures. Previous studies have shown positive correlations between new root growth and the inverse of resistance, hydraulic conductivity of spruce root systems (Colombo and Asselstine 1989). The hydraulic conductivity of new unsuberized roots may be 3-to 4-fold greater than that of suberized roots (Sands et al. 1982, Carlson 1986). Therefore a relatively small increase in root biomass can have a significant impact on root system conductivity. The statistically insignificant 10% increase in root biomass in the 6°C soil would be sufficient to increase whole root system conductance by 30 to 40%.

For comparison with published RSX values, 21-day RSX values were estimated without log transforming the transpiration data. The values of RSX calculated in this manner (0.14 and 0.06 MPa/[ug $H_2O/cm^2s$], respectively in the 6 and 12°C soils) were considerably lower than the 21-day values of RSX for non-flooded 10°C soil (1.02 MPa/[ug $H_2O/cm^2s$]) reported by Grossnickle (1987) and the 18-day value of RSX (0.48 MPa/[ug $H_2O/cm^2s$]) for 10°C soil calculated from a regression of RSX on time

developed by Grossnickle and Blake (1985). In this study, transpiration rates increased and plant moisture status improved from day 2 to 21. In contrast, during the three weeks of the study conducted by Grossnickle and Blake, transpiration rates did not show any detectable increase over time. RSX values in the present study were more similar to those measured by Running and Reid (1980) for cold-stored lodgepole pine exposed for two days to cool temperatures. Estimates of RSX obtained from their regression of RSX on root temperature were 0.37 and 0.12 MPa/(ug $H_2O/m^2s$), respectively, for 6 and 12°C soil temperatures.

A comparison of methods for these various studies suggests that lifting date and cold-storage conditions (i.e., duration and temperature) contribute to the resistance to water uptake after transplanting. Cold-storage conditions increased in severity in the same order as RSX values: this study (fall-lifted, 2 months, 4°C), Grossnickle and Blake (spring-lifted, 2 months, -2°C) and Grossnickle (fall-lifted, at least 6 months, -2°C). The lodgepole pine seedlings used by Running and Reid (1980) were only cold-stored 2 months at 2°C. Cold-storing (-5°C) white spruce seedlings for more than 18 weeks reduces their root growth capacity at low soil temperatures (Camm and Harper [1991]). New root growth potential, an indicator of overall seedling vigor (Lavender 1988), was higher in this study with mean root biomass increasing by 9 mg and 28 mg, respectively, in the 6 and 12°C soils. In the same time period, Grossnickle and coworkers found root biomass increased by only 4-6 mg in 10°C soil even though their seedlings were much larger; 6 mm in stem diameter compared to 1.2 mm.

There were significant differences among inoculation treatments in resistance to water flow in both soils at 2 and 9 days; but only in the 6°C soil at 21 days. Differences at 21 days correlated with root size, length and short root number at the time of transplanting. These root parameters varied significantly with inoculation treatment but not soil temperature treatment. Although inoculation treatments significantly influenced new root growth during the 21 day test period, differences among inoculation treatments in RSX at 21 days were not correlated to the amount of new root growth (dry weight, short roots). Again the initial inoculation effects on roots outweighed the effects of inocula on incremental changes in roots.

Seedlings inoculated with E-strain and L. bicolor which had the smallest root biomass, length and short root number at the time of transplanting exhibited negligible decreases in resistance to water flow over time. This result emphasizes the importance of root size and morphology at the time of planting. In a review of seedling characteristics which correlate with field performance, Lavender (1988) notes that growth of conifer seedling after planting is often more closely related to root mass or volume at the time of planting than to root growth capacity, especially when new root growth is limited by droughty or cool soils. The growth of outplanted white and black spruce seedlings has been shown to increase with seedling size at the time of planting (Dobbs 1976, Sutherland and Day 1988).

In controlled environment studies, ectomycorrhizal infection has been found to reduce the hydraulic conductance of whole root systems of radiata pine (Sands and Theodorou 1978) and Douglas-fir seedlings (Coleman et al. 1987, 1990). Coleman et al. (1990) concluded that the smaller size (length, dry weight) of mycorrhizal root systems compared to non-

mycorrhizal control seedlings was the major factor responsible for the reduction in root conductivity. Sands and Theodorou (1978) also attributed at least part of the mycorrhizal effect to the smaller size of mycorrhizal seedlings compared with non-mycorrhizal seedlings. In a later study, Sands et al. (1982) concluded that mycorrhizal infection had no effect on hydraulic conductivity per unit root length.

Based on previous studies an inverse relationship between root phosphorus and resistance was expected. VA-induced decreases in root resistance have been attributed, directly or indirectly, to improved root phosphorus nutrition (Graham and Syvertsen 1984, Koide 1985). In greenhouse-grown Douglas-fir seedlings, Coleman et al. (1990) found that root phosphorus and conductance were positively correlated. Nitrogen and phosphorus deficiencies in crop plants have been shown to increase root resistance per unit root dry weight or length (Radin and Boyer 1982, Radin and Eidenbock 1984). The mechanism involved is not known; although phosphorus deficiencies are known to alter membrane permeability (Ratnayake et al. 1978).

In this study, root phosphorus and 21-day RSX were weakly and positively correlated ($P = 0.07$), the inverse of published studies; seedling resistance increased with root %P. The coincidence of high values of RSX and root phosphorus in E-strain seedlings accounted for this unexpected relationship. RSX was not correlated with root phosphorus when E-strain data were excluded from the analysis.

Levels of root phosphorus for all inoculation treatments in the present study were 2 to 3-fold greater than those reported (< 0.10%.) by Coleman et al. (1990). If plants are not deficient in phosphorus, root resistance may be independent of this nutrient.

Two days after transplanting, resistance to water flow was lower in non-inoculated seedlings in 12°C soil, but higher in the 6°C soil, compared with average inoculated seedlings. This suggested that inoculation, on average, decreased the temperature sensitivity of seedling root systems. Root resistance increases rapidly below a threshold soil temperature of approximately 7°C for spruce and pine seedlings (Kaufmann 1975, Running and Reid 1980). The sharp increase in resistance has been attributed to a phase transition in root cell membrane lipids (Kaufmann 1975, Running and Reid 1980). It is possible that fungal membrane lipids have a different threshold temperature for phase transition or that inoculation alters the balance of plant growth substances affecting root conductivity. Exogenously applied ABA has been shown to increase root hydraulic conductance and reduce the effect of cool soil temperatures on root resistance (Fiscus 1981, Markhart 1984).

Pn values were 10 to 20% of the maximum values reported for spruce seedlings (e.g., Brix 1979, Beadle et al. 1981, Binder et al. 1987). The low values reflect two factors: (1) Pn was measured at non-saturating light intensities, and (2) surface area rather than projected leaf area was used in the calculations. On a dry weight basis, the mean photosynthetic rate of 4.4 mg $CO_2$/g/h was about 50% of the maximum photosynthetic rate reported by Brix (1979).

Inoculation treatments had a much greater effect on the rate of net photosynthesis than did soil temperature. A decrease in soil temperature from 12 to 6°C reduced the rate of net photosynthesis at 9 days by 13%. Similar or smaller temperature differentials have been shown to reduce the net photosynthetic rate by 15 to 20% in short-term (Delucia 1986) and long-term studies (Lippu and Puttonen 1989). There was no significant effect of soil temperature at day 2 or 21. At 21 days, there was a 46% difference in the rate of net photosynthesis among the six inoculation treatments; compared to a 6% difference between the two soil temperatures.

In both soil temperatures, inoculation with T. terrestris, E-strain or H. crustuliniforme reduced the net photosynthetic rate of seedlings both on an area and dry weight basis compared with non-inoculated control seedlings or those inoculated with forest soil or L. bicolor. In previous research, mycorrhizal inoculation has been shown to have no effect (Dosskey et al. 1990) or a positive effect on net photosynthesis per unit needle area (Parke et al. 1983a, Reid et al. 1983, Dosskey et al. 1990). Positive effects have been attributed to (1) enhancement of the nutrient or water status of seedlings (Benecke and Göbl 1974, Parke et al. 1983a), (2) changes in the balance of plant growth regulators (Slankis 1973) or (3) increased demand for carbohydrates to produce fungal tissues such as extramatrical hyphae (Reid et al. 1983, Dosskey et al. 1990). Reid et al. (1983) reported that mycorrhizal infection of pine seedling root systems increased respiration of $^{14}CO_2$ about 4-fold. Dosskey et al. (1990) observed that some mycorrhizal fungi increased net photosynthetic rate in Douglas-fir seedlings, but others had no effect. They hypothesized that fungi which formed mycorrhiza with abundant external hyphae (e.g., Rhizopogon vinicolor) were more likely to create a carbon demand and

stimulate net photosynthetic rate than those species with weak extramatrical development (e.g., L. laccata).

According to the "carbon demand" hypothesis, the increased photosynthetic sink created by mycorrhizal fungi stimulates the rate of net photosynthesis. A substantial portion of current photosynthate (as much as 38%) may be incorporated into ectomycorrhizae (Tranquillini 1964). Carbon demand of the various mycorrhizal root systems was not measured. However, several observations suggest that it was unlikely that differences in carbon demand were responsible for the inoculation-induced changes in net photosynthetic rate. First, soil temperature had a negligible effect on net photosynthetic rate, even though new root growth (dry weight) was 3-fold greater in the 12°C soil. Carbon lost through root respiration also increases with soil temperature. Graphical data presented by Lawrence and Oechel (1983a) shows about a 60% increase in root respiration as soil temperature rises from 6 to 12°C. Second, the rate of net photosynthesis was not associated with the amount of extramatrical (EM) hyphae produced by different fungi. Compared with non-inoculated controls, the rate of net photosynthesis was depressed both in seedlings with mycorrhizae producing abundant amounts of EM hyphae (e.g., H. crustuliniforme) and in those with mycorrhizae producing few EM hyphae (e.g., E-strain).

The results also suggest inoculation effects were independent of seedling water status. Net photosynthesis and stomatal conductance of many plant species is relatively constant over a wide range of xylem pressure potentials (Hsiao 1973); decreasing significantly for a variety of conifer species only at potentials in the range of -1.5 to -2.0 MPa (Turner and Jarvis 1975, Running 1976, Beadle et al. 1981). The levels of xylem pressure potential measured in this study were not indicative of severe

moisture stress. The lowest value of xylem pressure potential for any seedling measured in this experiment was -1.7 MPa two days after transplanting; mean values for the inoculation treatments were greater than -1.5 MPa at all sample dates for both soil temperatures.

Nitrogen deficiency increases the sensitivity of plants to water deficits (Marschner 1986). Consequently, the rate of net photosynthesis of seedlings used in this study may have been more sensitive to a moderate plant moisture stress than those used in other studies (e.g., Beadle et al. 1981). If this were the case, values of xylem pressure potential and net photosynthesis should decrease in parallel. Except for 2-day data, however, these data were not correlated. At this time, seedlings with low (the most negative) values of xylem pressure potential tended to have the highest photosynthetic rates. If plant moisture stress negatively affected net photosynthetic rates, the opposite trend would have occurred.

Net photosynthetic rate correlated positively with shoot nitrogen and phosphorus (% dry weight). Levels of shoot nitrogen and phosphorus varied significantly with inoculation treatment; but not with soil temperature suggesting that inoculation effects on net photosynthetic rate involved changes in the nutritional status of seedlings. A high percentage (>80%) of the nitrogen in conifer foliage is contained in proteins used to maintain photosynthesis (Small 1972, Chapin and Kedrowski 1983). Numerous studies have shown positive correlations between foliar nitrogen and the rate of net photosynthesis in conifers (Brix 1981, Smolander and Oker-Blom 1989) with the net photosynthetic rate of Douglas-fir increasing with foliar nitrogen, N, to an optimum of 1.74% (Brix 1981). Levels of shoot nitrogen were below this optimum, ranging from 0.08 to 1.4 %. Reid et al. (1983) demonstrated that mycorrhizal infection of pine seedlings increased

net photosynthesis per unit leaf area for nine months after inoculation. Mycorrhizal seedlings had consistently higher foliar nitrogen and phosphorus concentrations suggesting enhancement of net photosynthetic rate was due to nutrition. However, they were not able to statistically test difference in foliar nutrition between mycorrhizal and non-mycorrhizal seedlings.

Net photosynthetic rate correlated positively with the proportion of total seedling nitrogen in shoot tissue. Inoculation treatments affected nutrient distribution as well as nutrient content. The low proportion of total nitrogen contained in the shoot tissues of E-strain seedlings may account for their low rate of net photosynthesis compared with seedlings in other inoculation treatments (e.g., control, forest floor and L. bicolor seedlings) with a similar total nitrogen content.

Black (1986) reported that mycorrhizal infection by L. laccata increased the proportion of nitrogen and phosphorus retained in the root tissues of nursery-grown Douglas-fir seedlings. The presence of sporocarps at the time of sampling for nutrient analysis suggested to him that L. laccata had sequestered nutrients and diverted them to reproductive tissues instead of releasing them to the host plant. Unfortunately, I did not look for reproductive structures in this study.

The cold storage effect on pre-dawn stomatal conductance reported by Grossnickle and Blake (1985) (i.e., cold-stored seedlings were not able to fully close their stomata at night) was not observed in this study, possibly because cold storage duration and temperature were less stressful. It would be useful to know if inoculation affected pre-dawn stomatal conductance when seedlings are stored for longer periods at freezing temperatures.

Summary

The various inocula influenced the rate at which white spruce seedlings acclimated to low soil temperatures with respect to resistance to water flow and net photosynthetic rate, but had no effect on pre-dawn stomatal conductance. Differences among inoculation treatments were related to root size (dry weight, short root number) and nutritional status (shoot N and P) of the seedlings at the time of transplanting.

# CHAPTER V

# EFFECT OF VARIOUS MYCORRHIZAL FUNGI ON THE GROWTH AND NUTRIENT STATUS OF WHITE SPRUCE SEEDLINGS TRANSPLANTED INTO COOL SOILS

## Introduction

The ability to improve the growth and nutrition of spruce seedlings planted in cold soils was the final criterion used to assess the potential efficacy of ectomycorrhizal (ECM) fungi. Several studies (e.g., Marx 1977, Last et al. 1984, Wilson et al. 1987) have reported that artificial inoculation of nursery seedlings with specific ECM fungi improves field performance. However, not all studies (e.g., Bledsoe et al. 1982, Shaw et al. 1987b) have shown that mycorrhizal inoculation benefits seedling growth or nutrition.

Although rarely reported, ECM fungal inoculation may even depress the growth of conifer seedlings (e.g., Bledsoe et al. 1982, Sands and Theodorou 1978). Negative growth effects have not been given much attention in the mycorrhizal research literature. Perhaps this reflects the common view that the ECM fungus-host plant relationship is a symbiosis in the narrowest sense of the term, i.e., mutualistic relationship between two living organisms in which both partners benefit. The host plant benefits from improved nutrient uptake and the fungus benefits from host-derived carbohydrates. The lack of attention to negative host plant response may also reflect the practical objective of much ECM research, i.e., to improve reforestation success. Funding for this objective is not based on negative growth results.

> "Most research on inoculation with ectomycorrhizal fungi has been based on two premises. First, any ectomycorrhizal association on roots of tree seedlings is far better than none.....Second, some species of ectomycorrhizal fungi on certain sites are more beneficial to trees than other fungal species that naturally occur on such sites." (Marx and Kenney 1982, p. 131)

Wilcox (1983) recommends examining the mycorrhizal-host plant relationship using the broader (and original) definition of a symbiotic relationship. In addition to mutualistic partnerships, this definition encompasses (1) relationships in which one partner benefits but the other neither benefits or loses (commensalism), and (2) those in which one partner benefits at the expense of the other (parasitism).

Environmental conditions likely play a important role in determining the type of symbiotic relationship which develops between ECM fungi and host plants (Wilcox 1983, Harley and Smith 1983). Cool soil temperatures may shift the balance of the mycorrhizal symbiosis in favor of the fungi. Research with VA-infected seedlings has shown that cool temperatures eliminate the benefits normally derived from mycorrhizal infection (Moawad 1978, Smith and Roncadori 1986). The growth of VAM plants may even be reduced compared to that of non-mycorrhizal plants at low temperatures (Furlan and Fortin 1973, Hayman 1974, Schenck and Schroder 1974).

The lack of host plant response to infection at sub-optimum temperatures may be due to the inability of established fungi to absorb or transport phosphorus while still utilizing host carbon. Smith and Roncadori (1986) reported that mycorrhizae increased the phosphorus and copper uptake of cotton plants at soil temperatures of 24°C and 36°C; but mycorrhizae had no effect on nutrient uptake at a soil temperature of 18°C. Comparable research has not been conducted with ectomycorrhizal conifer seedlings.

The objectives of the research reported in this chapter were to study the effect of ECM inoculation on the relative growth and nutrition of spruce seedlings grown in cool forest soils to determine:

(1) if the presence of "any" ECM fungus is better than "none" when seedlings are planted into cool soils,
(2) if some ECM fungi are more effective than others in cool soils,
(3) if there are interactions between temperature and inoculation effects on seedling growth and physiology.
(4) if inoculation-induced changes in whole seedling response to cold soils are related to the uptake, distribution or use of nutrients.

Approach

Both root and shoot growth and nutrition were measured so that changes in dry matter, nutrient distribution and nutrient use could be described. Soil temperature (Davidson 1969) and mycorrhizal inoculation (Harley and Smith 1983, Black 1986) have been shown to alter dry matter and/or nutrient distribution between root and shoot. Bowen (1973) suggested that mycorrhizal plants use limited nutrients more efficiently than non-mycorrhizal plants.

Nutrient analyses were limited to four nutrients, nitrogen (N), phosphorus (P), iron (Fe) and calcium (Ca). P was analysed because mycorrhizal benefits are often attributed to improved P uptake; N and Fe because these nutrients are often seriously deficient in young spruce plantations (Ballard 1985). Ca was included in the study because its uptake is related to seedling transpiration (Barber 1984). Compared to N

and P, the level of Ca in newly flushed foliage is more dependent on the mass flow of water and less dependent on redistribution from older plant tissues (Marschner 1986).

Since the experiment was conducted in containers, growth and nutrition of the seedlings were measured several times during the experiment. Periodic sampling is particularly important for container experiments because of the limited amount of available nutrients and water (Parke 1985). Seedlings inoculated with different ECM fungi may deplete the limited resources of containers at different rates. Mycorrhizal treatments which result in rapid depletion of water or nutrients may not show a benefit to the host plant unless growth is measured before these resources are depleted. Periodic sampling also allowed for the calculation of growth analysis indices (Hunt 1982) which are more independent of initial seedling size and nutritional status than are absolute measures of growth.

Most studies of mycorrhizal efficacy are confounded by differences in initial seedling size. Three approaches were used to compensate for size differences: (1) after cold-storage, the largest and smallest seedlings were culled to minimize the differences, (2) the initial caliper of all seedlings was measured and used in covariance analysis to adjust treatment means for differences in initial size, and (3) the relative rather than absolute growth rates of seedlings were calculated.

An alternative approach would have been to produce seedlings with equivalent dry weights, nutrient contents and patterns of nutrient and dry matter distribution using different levels of nutrients for each inoculation treatment. In practice this is difficult to achieve because different aspects of the host growth and physiology respond differently to mycorrhizal infection and fertilization (Pacovsky et al. 1986) with mycorrhizal and fertilizer treatments interacting to influence seedling growth and nutrition.

Growth analysis techniques (Hunt 1982) were used to examine whole plant response to the stress of cold soils. Relative growth rate was used as a whole plant index of physiological vigor (Margolis and Brand 1990). Three components of relative growth rate (root efficiency, nutrient-use efficiency and biomass allocation) were used to compare the effect of inoculation treatments on acquisition and use of nutrients.

Root efficiency was estimated by calculating the rate of mineral uptake per unit root weight per unit time, termed the specific absorption rate by Welbank (1962). Root dry weight was assumed to be a good index of the absorption capacity of roots. Hackett (1969) studied relationships between dry weight, volume, surface area and length of barley root systems. He found that these relationships were relatively independent of nutrient regime, plant age and variety; consequently, for comparative purposes, nutrient uptake could be expressed on the basis of any of these root dimensions. In a previous study (Chapter IV), root dry weight, length and number of short roots per seedlings were highly correlated ($P < 0.001$).

On the premise that ECM fungi predominantly infect short roots, the efficiency of mycorrhizal and non-mycorrhizal root systems has been compared on the basis of nutrient uptake per short root (Alexander and Fairley 1986, Högberg 1989). Since many ECM fungi (e.g., E-strain) infect long lateral roots (Robertson 1954), nutrient uptake per unit root weight was considered a better index of root efficiency than uptake per short root. Ideally, nutrient uptake should be based on the surface area of roots, including fungal structures such as external hyphae. However, measurement of surface area was not feasible.

## Methods

Inoculation treatments included: (1) a non-inoculated control (sterilized agar medium), (2) forest floor inoculum, and pure cultures of (3) Hebeloma crustuliniforme 5249, (4) Laccaria bicolor 5268, (5) Amphinema byssoides 0288, (6) Thelephora terrestris 2088, (7) E-strain 947. Except for the Amphinema and Thelephora isolates, the test inocula were obtained from northern B.C. or Alberta forest sites (as described in Table 2.1). T. terrestris 2088 and A. byssoides 0288 were isolated in 1988 from surface-sterilized ectomycorrhizae of containerized spruce seedlings grown respectively at the Canadian Forest Products nursery in Saanichton, B.C. and at the Balco Canfor Reforestation Centre in Kamloops, B.C.

Seedlings were inoculated, grown, cold-stored and culled as described previously (Chapters II, III, and IV). Mean seedling caliper, after culling, was 1.2 mm. Twenty-four seedlings, selected to represent the full range of caliper values, were destructively sampled to develop regressions of shoot and root dry weight on initial caliper. The remaining seedlings were transplanted into pots (4 litres, 15 cm diameter) containing a mixture

of unpasteurized forest floor, mineral soil and perlite (1:1:2 by volume). Each pot held 6 seedlings from one inoculation treatment; 8 pots were filled for each inoculation treatment; and 4 pots were randomly assigned to each of 2 water baths (i.e., soil temperature treatments, 6 and 12°C).

The forest soil mixture contained propagules of indigenous ECM fungi and non-mycorrhizal microflora. The mean values of pH (1:4 soil:water suspension, Peech 1965), available phosphorus (Bray and Kurtz No 1, Bray and Kurtz 1945) and mineralizable nitrogen (anaerobic incubation, Waring and Bremner 1964) for this mixture were respectively 4.9, 35 μg/g and 68 μg/g.

After transplanting, the seedlings were grown for 12 weeks with an 18 h photoperiod, relative humidity of 30 to 50%, air temperature of 20°C day/12°C, and light intensity of 400 μmol/($m^2$s) in the 400-700 nm wavelength. No fertilizer was applied during the experiment. An insecticide (0.84 g/L Diazinon, Later Chemical Ltd., Richmond, B.C.) was applied every 2 weeks to minimize insect grazing on fungal mycelium. The pots were re-randomized once a week to minimize the effects of position within the water baths and to a lesser extent within the growth chamber.

A soil water retention curve was developed to estimate soil water tension from the weight of a pot and its forest soil mixture. The weight of container-grown seedlings at various water tensions was estimated from a water retention curve developed for the plug medium (peat:vermiculite) and the fresh/dry weight ratio of seedlings at the time of planting. For the first 5 weeks, the pots were watered as necessary to maintain a high soil moisture potential (above -0.03 MPa). During the 5 to 10 week period, watering was reduced in frequency with the objective of gradually drying the 6 and 12°C soils to a soil moisture potential less than -0.06 MPa to

compare the response of the various inoculation treatments to a mild soil water deficit as would occur in the field. Between 10 and 12 weeks the pots were watered to maintain a high soil water content.

Measurements of seedling growth and physiology were made at 5, 10 and 12 weeks on 8 seedlings per treatment combination. Seedlings were carefully extracted to minimize root loss and disturbance to the remaining seedlings. The loose nature of the forest soil:perlite mixture facilitated seedling extraction. Root growth was slow and roots of neighbouring seedlings did not intermingle or reach the bottom of the containers until the final sample in the 12°C soil. Growth measurements included: root, mature shoot and new foliage biomass, lateral root length, short root number and caliper. Total root length and short root number were estimated from dry weight ratios between subsamples and the total root system as described in Chapter IV. At the same time, roots within the original container plug and new roots formed outside the plug were assessed for mycorrhizal infection as described in Chapter III.

Shoot and root tissues from 8 seedlings per treatment combination were bulked separately for chemical analysis of nitrogen (N), phosphorus (P) and calcium (Ca) at 0, 5, and 12 weeks. Only shoot tissues were analyzed for "active" iron (Fe) concentrations. New and mature shoot tissue were separated for chemical analysis; the mature shoot sample included stem tissue. Wet oxidation of milled, oven-dried (70°C, 48 h) tissue with $H_2SO_4$, Se, salts and $H_2O_2$ (Parkinson and Allen 1975), was followed by colorimetric analysis for N (salicylate/nitroprusside) and P (ascorbic acid/molybdate-antimony) and atomic absorption spectrophotometry for Ca (Technicon Industrial Systems 1977). Active Fe was extracted with 1 M HCl (Oserkosky 1933, cited in Ballard and Carter 1986) and analyzed by

atomic absorption spectrophotometry. It was impossible to remove all external hyphae from mycorrhizal root systems. Therefore, root biomass and nutrient data include undetermined amounts of fungal mycelium, especially in the H. crustuliniforme and A. byssoides treatments.

Percent caliper growth was calculated from measurements of initial (Ci) and final caliper (Cf) using the equation: [100% x (Cf-Ci)/Ci]. The allometric constant (k) for shoot and root growth was calculated by dividing shoot relative growth rate (RGR) by root RGR (Huxley 1932). Initial shoot and root dry weights were estimated from the regressions of dry weight on caliper conducted at the time of transplanting. Initial shoot/root ratio was used as a covariate in the analysis of the shoot/root allometric constant.

Physiological measurements (i.e., net photosynthesis, transpiration and xylem pressure potential) are described in Chapter IV. Two indices of potential resource-use efficiency were derived from the nutritional and physiological data: (1) instantaneous nitrogen- and phosphorus-use efficiency (respectively, INUE and IPUE) defined as the ratio of photosynthetic capacity to shoot N or P content, and (2) water-use efficiency (WUE) defined as the ratio of carbon assimilated (net photosynthetic rate) to water evaporated (transpiration rate).

Relative growth rate, RGR, was subdivided into an algebraically equivalent set of four indices (1) relative root weight or RWR, representing the proportion of total seedling biomass allocated to the nutrient absorbing system, (2) specific absorption rate (A), representing the rate of nutrient uptake per unit root dry weight or root efficiency, (3) specific utilization rate or NUE, representing the rate of dry weight increment per unit of absorbed nutrient and (4) the relative increase in

nutrient content (R). The equation for this relationship (Hunt 1982) for nitrogen is presented below:

$$RGR = (RWR \times A \times NUE) / (R) \quad or$$

$$\frac{\frac{1}{W}\frac{dW}{dt}}{\frac{1}{N}\frac{dN}{dt}} = \frac{RW}{W} \times \frac{1}{RW}\frac{dN}{dt} \times \frac{1}{N}\frac{dW}{dt}$$

where RW, W, N and t are respectively root dry weight, seedling dry weight, seedling nitrogen content, and time. Mean values of these parameters were used to calculate the growth indices as shown in Table 5.1. The calculation of these indices assumes a linear relationship exists between root weight and nutrient content; and between root and shoot dry weights (Welbank 1962, Hunt 1973, 1982). Plots of the data showed these assumptions were met.

Table 5.1. Equations used to calculate growth analysis indices

| Index | Equation |
|---|---|
| relative growth rate (RGR) | [g growth/(g seedling/week)] $(\ln W_2 - \ln W_1)/(t_2 - t_1)$ |
| root weight ratio (RWR) | (g root/g seedling) $[(RW_1/W_1)+(RW_2/W_2)]/2$ |
| specific absorption for N (AN) | [g N/(g root/week] $[(N_2-N_1)/(t_2-t_1)][(\ln RW_2 - \ln RW_1)/(RW_2-RW_1)]$ |
| nitrogen-use efficiency (NUE) | [g growth/(g N/week)] $[(W_2-W_1)/(t_2-t_1)][(\ln N_2 - \ln N_1)/(N_2-N_1)$ |
| Relative increase in N (RN) | [g N/(g N seedling/week] $(\ln N_2 - \ln N_1)/(t_2-t_1)$ |

NOTE: RW, W, N and t are respectively root dry weight, seedling dry weight, seedling nitrogen content, and time. Subscripts indicate the time of measurement (i.e., 0, 5, 12 weeks).

The experiment was a completely randomized design with a factorial arrangement of the 7 inoculation treatments and 2 soil temperatures; 24 seedlings per treatment combination. Data were tested by least squares analysis of variance (ANOVA) or analysis of covariance (ANCOVA), if the assumption of parallel slopes was met (Hicks 1973), using a microcomputer statistical program, SYSTAT (Wilkinson 1988). The ANOVA model consisted of three fixed treatments (soil temperature, inoculation and time treatments) with the 4 pots nested within the soil temperature and inoculation treatments. The mean square for between-pot error was tested for significance against the residual error. If it were insignificant ($P > 0.25$, Bancroft 1964), these two errors were pooled, with all treatments and interactions tested against the pooled error term. Otherwise, the between-pot error was used to test main effects and interactions.

Some parameters were log-transformed to meet the assumptions of normality and homoscedasticity for ANOVA and ANCOVA (Eisenhart 1947). Variability of these parameters was proportional to the value of their mean. Tabulated means of these parameters when shown in the original units are geometric means of the original data. Data which were log-transformed are identified in the tables.

Preplanned and post-hoc tests of means were conducted using linear contrasts (SYSTAT, Wilkinson 1988). A preplanned linear contrast of the control treatment (no inoculation) versus the 6 inoculation treatments was conducted on the entire data set when interactions between soil temperature and inoculation treatments were not statistically significant. Otherwise this contrast was conducted separately for each soil temperature. A similar comparison of mycorrhizal and non-mycorrhizal (at the time of transplanting) seedlings was also conducted excluding control seedlings

with T. terrestris contamination and inoculated seedlings with poor (less than 50% of short roots colonized) nursery infection by inoculant fungi. This reduced the total sample size from 336 to 314 seedlings; with the sample for non-inoculated seedlings decreasing from 48 to 36.

Relationships among nutrition, growth and physiological parameters were examined by linear correlation analysis using either individual seedling data (growth versus physiological parameters) or mean values for the 14 treatment combinations (nutrition versus growth and physiological parameters). Cause and effect relationships cannot not be inferred from correlation analysis but the degree of association between two parameters can be described. Furthermore, lack of significant correlation negates a cause and effect relationship between two parameters.

## Results

### 1. Mycorrhizal status

Mycorrhizal infection of new roots formed outside the container plugs was rapid in the 6 and 12°C soils (Table 5.2). Most new roots (> 75%) of seedlings inoculated with E-strain, T. terrestris, H. crustuliniforme, L. bicolor were infected by the inoculant fungi. New roots of seedlings inoculated with A. byssoides were infected (> 30% of short roots) with other ECM fungi, mainly those native to the forest soil during. The degree of infection on new roots by A. byssoides correlated positively ($r = 0.80$, $P < 0.001$) with the degree of A. byssoides infection on roots within the original root mass.

Table 5.2. Mycorrhizal infection of new roots formed outside the container plug in 6°C and 12°C soil

| Inocula | Percent mycorrhizal infection | | | | | |
|---|---|---|---|---|---|---|
| | 6°C | | | 12°C | | |
| | 5wk | 10wk | 12wk | 5wk | 10wk | 12wk |
| Control | 16 | 82 | 88 | 42 | 88 | 88 |
| Forest soil | 88 | 88 | 88 | 88 | 88 | 88 |
| E-strain | 88 | 84 | 84 | 88 | 80 | 81 |
| *L. bicolor* | 88 | 88 | 88 | 88 | 88 | 88 |
| *H. crust.** | 77 | 77 | 85 | 85 | 77 | 77 |
| *A. byssoides* | 70 | 57 | 63 | 60 | 50 | 52 |
| *T. terrestris* | 69 | 78 | 82 | 88 | 88 | 88 |

NOTE: All control seedlings showed some new root infection by 10 weeks; new roots of all inoculated seedlings were infected by 5 weeks. N = 8 seedlings for each time-inoculation-temperature combination. Percent mycorrhizal infection for each seedling was estimated on a six-class scale: 0, 1-25, 26-50, 51-75, 76-95, >95; the midpoint of each class was used to calculate average infection per seedling.

*H. crust. = H. crustuliniforme

Infection of non-inoculated seedlings by indigenous fungi was rapid. All non-inoculated seedlings were infected by mycorrhizal fungi 10 weeks after transplanting. Mycorrhizae formed from forest soil ECM dominated in the new root system of 80% of the non-inoculated seedlings; Thelephora-like mycorrhizae dominated in the other 20%. These latter mycorrhizae probably resulted from contamination by air-borne spores of T. terrestris prior to transplanting. At the time of transplanting, approximately 35% of the control seedlings showed some mycorrhiza formation by T. terrestris.

2. Phenology

Seedlings began to flush 10 to 14 days after transplanting. About 65% , 40% and 50%, respectively, of new foliage, root and caliper growth was completed 5 weeks after transplanting. Shoot elongation ceased and terminal buds formed between 5 and 10 weeks. Early in the 5 week drying cycle, I realized it was impossible to decrease the soil water potential of the 6°C soil to -0.06 MPa, a level which would significantly reduce root growth (Day and MacGillivray 1975). The lowest potential reached was -0.02 MPa which was above field capacity -0.03 MPa. Poor seedling growth and low transpiration rates combined with condensation of water from the surrounding air (15-20°C) on the cool soil surface slowed net water loss from the pots. Therefore, I abandoned the idea of comparing the response of the various inoculation treatments to a drying cycle and watered both soils to maintain soil water tension above field capacity.

3. Total Seedling Biomass

Seedling biomass at the time of transplanting was influenced by inoculation treatment ($P = 0.01$) but not by soil temperature ($P > 0.60$). Mean values for the seven inoculation treatments ranged from 0.20 to 0.24 g/seedling. After transplanting, seedling biomass was strongly influenced by inoculation treatment, time and soil temperature (Table 5.3). There was also a significant but weak interaction between soil temperature and time. Seedling biomass was greater in the 12°C soil at 10 (0.67 g at 12°C versus 0.73 g at 6°C) and 12 weeks (0.69 g versus 0.73 g); however, at 5 weeks soil temperature had no effect on total seedling biomass (0.64 versus 0.63 g).

Inoculation treatments accounted for a much greater percentage (22%) of the variation in biomass data than did time (3.5%), soil temperature (<1%) or the time-temperature interaction (<1%). There were no significant interactions between the three main effects (i.e., inoculation, temperature and time). Therefore, mean values for inocula, averaged across time and temperature, are presented in Table 5.4. Forest soil, L. bicolor or T. terrestris inoculation increased seedling biomass compared with non-inoculated seedlings; in contrast, E-strain or H. crustuliniforme inoculation decreased total biomass, and A. byssoides inoculation had no effect.

Table 5.3. Analyses of covariance for total, shoot and root biomass

| Source of variation | df | Mean square | F-ratio | prob. |
|---|---|---|---|---|
| | | Total seedling biomass | | |
| Time of sample (T) | 2 | 0.49 | 17.9 | <0.001 |
| Soil temperature (ST) | 1 | 0.15 | 5.6 | 0.02 |
| Inocula (I) | 6 | 1.00 | 36.9 | <0.001 |
| T x ST | 2 | 0.08 | 2.9 | 0.05 |
| T x I | 12 | 0.03 | 1.1 | 0.36 |
| ST x I | 6 | 0.04 | 1.4 | 0.20 |
| T x ST x I | 12 | 0.03 | 1.1 | 0.34 |
| initial caliper | 1 | 11.26 | 414.8 | <0.001 |
| Error | 291 | 0.04 | | |
| | | Mature shoot biomass (MSB) | | |
| Time of sample (T) | 2 | 0.71 | 17.8 | <0.001 |
| Soil temperature (ST) | 1 | 1.36 | 34.2 | <0.001 |
| Inocula (I) | 6 | 0.70 | 17.5 | <0.001 |
| T x ST | 2 | 0.16 | 3.9 | 0.02 |
| T x I | 12 | 0.03 | 0.7 | 0.73 |
| ST x I | 6 | 0.01 | 0.1 | 0.99 |
| T x ST x I | 12 | 0.05 | 1.3 | 0.22 |
| initial caliper | 1 | 16.02 | 403.5 | <0.001 |
| Error | 293 | 0.04 | | |

Table 5.3. (continued)

| | | | | |
|---|---|---|---|---|
| | New foliage biomass (NFB) | | | |
| Time of sample (T) | 2 | 10.24 | 43.9 | <0.001 |
| Soil temperature (ST) | 1 | 3.92 | 16.8 | <0.001 |
| Inocula (I) | 6 | 8.26 | 35.4 | <0.001 |
| T x ST | 2 | 0.29 | 1.3 | 0.29 |
| T x I | 12 | 0.16 | 0.7 | 0.78 |
| ST x I | 6 | 0.34 | 1.5 | 0.19 |
| T x ST x I | 12 | 0.36 | 1.5 | 0.11 |
| initial caliper | 1 | 7.29 | 31.3 | <0.001 |
| Error | 293 | 0.23 | | |
| | Root biomass | | | |
| Time of sample (T) | 2 | 0.120 | 69.4 | <0.001 |
| Soil temperature (ST) | 1 | 0.180 | 104.3 | <0.001 |
| Inocula (I) | 6 | 0.014 | 8.4 | <0.001 |
| T x ST | 2 | 0.016 | 9.2 | <0.001 |
| T x I | 12 | 0.003 | 1.6 | 0.10 |
| ST x I | 6 | 0.007 | 4.2 | <0.001 |
| T x ST x I | 12 | 0.002 | 1.3 | 0.22 |
| initial caliper | 1 | 0.342 | 198.1 | <0.001 |
| Error | 291 | 0.002 | | |

4. Mature shoot biomass

Mature shoot biomass (MSB) varied with time ($P < 0.001$), soil temperature ($P < 0.001$), inoculation treatment ($P < 0.001$) and to a lesser degree with a second-order interaction between temperature and time ($P = 0.02$) (Table 5.3). MSB increased during the first five weeks after transplanting in both soil temperatures but showed different trends with time for the 5 to 12 week period. In the 6°C soil, MSB decreased gradually from 5 to 12 weeks; in the 12°C soil, MSB was essentially constant from 5 to 10 weeks and then decreased from 10 to 12 weeks (Fig. 5.1). The decrease coincided with an increase in root biomass, suggesting carbohydrate stored in mature shoot tissue was allocated to new root growth. Mean MSB, averaged across time and soil temperature treatments (Table 5.4), ranged from 0.40 g in the forest soil treatment to 0.29 g in the H. crustuliniforme and A. byssoides treatments.

5. New foliage biomass and caliper growth

New foliage biomass (NFB) increased with time ($P < 0.001$) and soil temperature ($P < 0.001$) and varied with inoculation treatment ($P < 0.001$) (Table 5.3). Inoculation with forest floor and L. bicolor increased NFB ($P < 0.001$) compared with control seedlings or those inoculated with other fungi; inoculation with E-strain or H. crustuliniforme reduced NFB ($P < 0.001$) relative to other treatments (Table 5.4); and inoculation with A. byssoides and T. terrestris had no effect on NFB ($P > 0.10$).

Figure 5.1. Mean biomass of mature shoot, new foliage and root tissues at 0, 5, 10 and 12 weeks at 6 and 12°C soil temperatures.

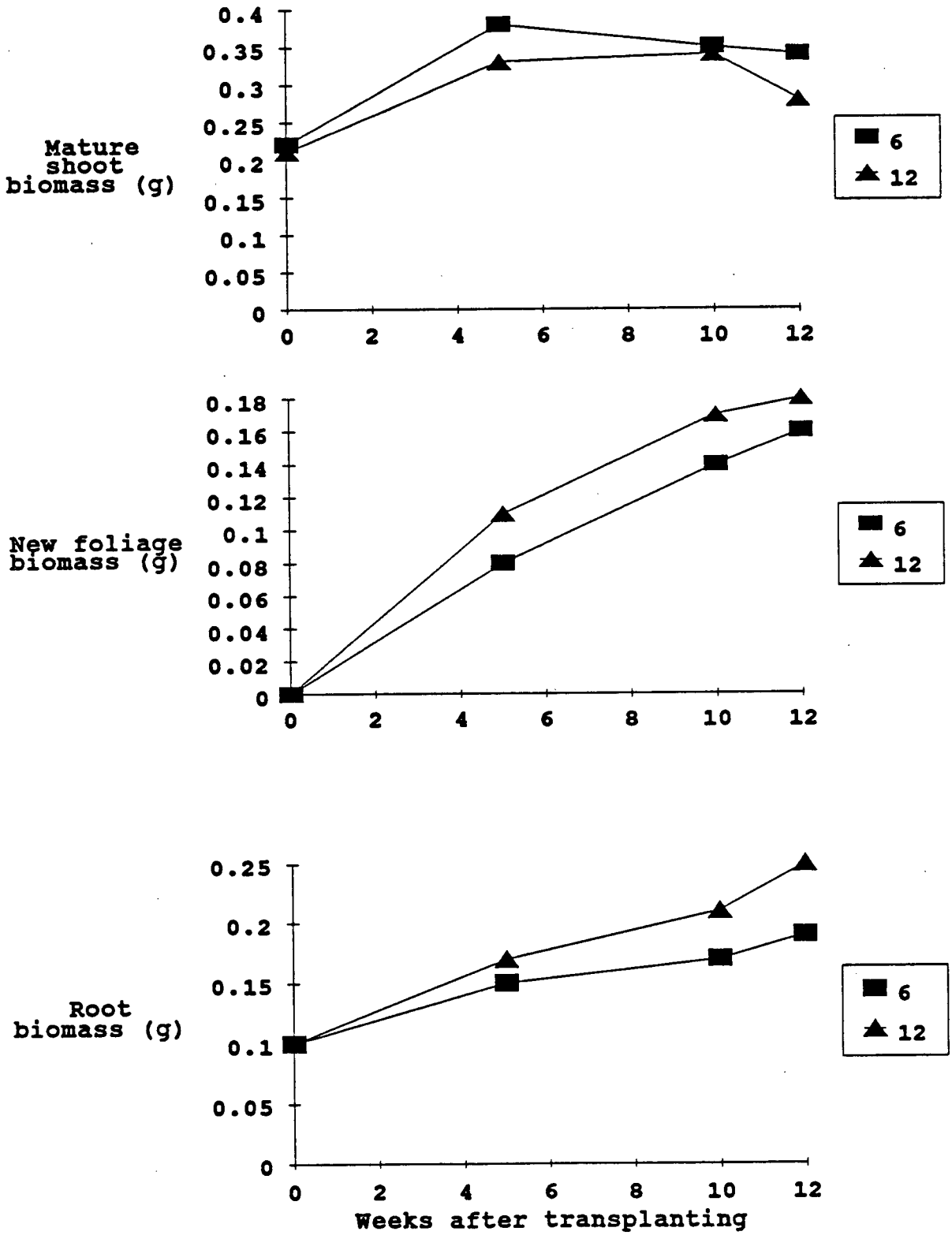

Table 5.4. Total seedling, mature shoot (MS) and new foliage (NF) biomass (oven-dry weight) averaged across time and temperature compared with seedling nitrogen status at the time of transplanting

| Inocula | Biomass (g) | | | Initial N | |
|---|---|---|---|---|---|
| | Total | MS | NF | mg/plant | shoot % |
| Control | 0.68 | 0.33 | 0.12 | 3.8 | 1.2 |
| Forest floor | 0.86 | 0.40 | 0.22 | 4.9 | 1.4 |
| E-strain | 0.65 | 0.36 | 0.08 | 3.8 | 0.9 |
| *L. bicolor* | 0.75 | 0.34 | 0.21 | 4.0 | 1.1 |
| *H. crust.** | 0.54 | 0.29 | 0.08 | 3.4 | 0.9 |
| *A. byssoides* | 0.64 | 0.29 | 0.14 | 3.9 | 1.1 |
| *T. terrestris* | 0.73 | 0.36 | 0.14 | 3.3 | 0.8 |

NOTE: Biomass data were log-transformed for analysis of covariance. All biomass values shown are adjusted means using initial caliper as a covariate; biomass data were averaged across time and soil temperature (N = 48). Caliper data analyzed using initial caliper as a covariate; adjusted means (N = 16) are shown. Nitrogen nutrition data (N = 2) were averaged across temperature. N content calculated as mg/seedling; shoot N as % oven-dry weight. Each sample consisted of tissue pooled from 8 seedlings.

*H. crust. = H. crustuliniforme

Analysis of covariance showed no significant ($P > 0.21$) interaction between soil temperature and inoculation treatments on percent caliper growth. Averaged across inocula, caliper growth increased ($P < 0.001$) from 54% in the 6°C soil to 72% in the 12°C soil. Stem growth was similar for non-inoculated (56%) and inoculated seedlings (64%, averaged across 6 inocula). However, there was considerable difference in caliper growth among inoculation treatments. Inoculation with forest soil (79%), L. bicolor (82%) or T. terrestris (71%) increased stem growth compared with non-inoculated seedlings (56%). Inoculation with H. crustuliniforme reduced stem growth (46%); and inoculation with E-strain or A. byssoides had no effect on stem growth (53%).

6. Root biomass

Root biomass varied ($P < 0.001$) with time, soil temperature and inocula (Table 5.3). Soil temperature effects interacted with those of time and inoculation. In both soils, root biomass increased during the 10 weeks following transplanting. From 10 to 12 weeks the rate of root growth remained unchanged in the 6°C soil, whereas it accelerated in the 12°C soil (Fig. 5.1). Mean root biomass data averaged across time are shown in Table 5.5. In contrast to other seedlings, root growth of seedlings infected with E-strain or H. crustuliniforme did not respond to increasing soil temperature.

7. Root morphology

Both soil temperature and inocula influenced the number of short roots per seedling (Table 5.6). There was a significant interaction between these main effects with a different ranking of inoculation

treatments for the two soil temperatures (Table 5.7). In the 6°C soil, seedlings inoculated with H. crustuliniforme or A. byssoides produced the greatest number of short roots; in the 12°C soil, control seedlings and those inoculated with forest floor and A. byssoides produced the greatest number.

**Table 5.5.** Interaction of soil temperature and inocula on mean root biomass (g oven-dry weight/seedling)

| Inocula | Soil temperature | |
|---|---|---|
| | 6°C | 12°C |
| Control | 0.19 | 0.25 |
| Forest floor | 0.18 | 0.25 |
| E-strain | 0.20 | 0.21 |
| L. bicolor | 0.16 | 0.23 |
| H. crustuliniforme | 0.16 | 0.17 |
| A. byssoides | 0.18 | 0.21 |
| T. terrestris | 0.18 | 0.24 |

NOTE: Biomass data were log-transformed for analysis of covariance; adjusted means are shown in the original units. Means are averaged across time (N = 24).

Table 5.6. Analysis of (co)variance for root morphology data

| Source of variation | df | Mean-square | F-ratio | Prob. |
|---|---|---|---|---|
| | Number of short roots/seedling | | | |
| Time of sample (T) | 2 | 3.35 | 27.2 | <0.001 |
| Soil temperature (ST) | 1 | 3.39 | 27.6 | <0.001 |
| Inocula (I) | 6 | 3.03 | 24.6 | <0.001 |
| T x ST | 2 | 0.05 | 0.4 | 0.19 |
| T x I | 12 | 0.05 | 0.5 | 0.94 |
| ST x I | 6 | 0.26 | 2.1 | 0.05 |
| T x ST x I | 12 | 0.14 | 1.2 | 0.31 |
| caliper | 1 | 9.92 | 80.5 | <0.001 |
| Error | 289 | 0.12 | | |
| | Short roots per unit root weight | | | |
| Time of sample (T) | 2 | 61.5 | 9.7 | <0.001 |
| Soil temperature (ST) | 1 | 4.6 | 0.7 | 0.39 |
| Inocula (I) | 6 | 230.6 | 36.2 | <0.001 |
| T x ST | 2 | 3.5 | 0.6 | 0.58 |
| T x I | 12 | 3.9 | 0.6 | 0.83 |
| ST x I | 6 | 8.0 | 1.3 | 0.28 |
| T x ST x I | 12 | 5.8 | 0.9 | 0.54 |
| Error | 290 | 6.4 | | |

In contrast to other treatments (including the control), short root formation in the H. crustuliniforme treatment showed no response to increasing soil temperature.

The number of short roots per unit root weight was strongly influenced by inoculation but not by soil temperature treatments (Table 5.6). On average, inoculation decreased ($P < 0.001$) the ratio of short roots per unit of root biomass. This effect was due to low short root ratios in seedlings infected with either T. terrestris or L. bicolor (Table 5.7).

8. Dry matter allocation

Soil temperature ($P < 0.001$) and inoculation treatments ($P < 0.001$) influenced shoot-to-root allometry; but there was no interaction between these two factors ($P > 0.20$). Mean values for the allometric coefficient (k) and shoot/root ratios are shown in Table 5.8. Allometric data paralleled the seedling biomass data. Shoot growth relative to root growth was highest for the forest soil and L. bicolor treatments and lowest for the H. crustuliniforme and E-strain treatments. New root and shoot growth correlated positively ($r = 0.86$, $P = 0.012$) in the 12°C but not the 6°C soil. At 6°C, the ratio of current foliage produced per unit of new root biomass was twice that at 12°C; this resulted in higher shoot-to-root ratios and allometric coefficients in the 6°C soil.

**Table 5.7.** Effect of inocula on root morphology

| Inocula | # of short roots per seedling | | per mg root |
|---|---|---|---|
| | 6°C | 12°C | 6 and 12°C |
| Non-inoculated | 1652 | 2253 | 9.9 (2.6) |
| Forest floor | 1525 | 2080 | 9.3 (3.2) |
| E-strain | 1249 | 1541 | 7.4 (1.9) |
| *L. bicolor* | 854 | 1188 | 5.8 (1.6) |
| *H. crustuliniforme* | 1702 | 1588 | 10.8 (3.0) |
| *A. byssoides* | 1845 | 2018 | 10.6 (3.4) |
| *T. terrestris* | 934 | 1200 | 5.6 (1.6) |

NOTE: Means are averaged across time. Adjusted means (N = 24) are shown for short root number; these data were log-transformed before analysis of covariance. Data for the ratio of short roots to root biomass were not transformed or adjusted by covariance; standard deviations for these means (N = 48) are shown in parentheses.

**Table 5.8.** Effect of soil temperature and inoculation treatment on the allometric coefficient (k) and shoot/root ratios

| | K | | Shoot/root ratio | |
|---|---|---|---|---|
| | 0-5 wk | 0-12 wk | 0 wk | 12 wk |
| Inocula | | | | |
| Non-inoculated | 1.1 | 0.8 | 2.7 (0.2) | 2.2 (0.6) |
| Forest floor | 1.8 | 1.2 | 2.6 (0.2) | 3.1 (0.9) |
| E-strain | 1.2 | 0.6 | 2.4 (0.3) | 1.9 (0.7) |
| *L. bicolor* | 2.2 | 1.1 | 2.4 (0.2) | 2.7 (0.8) |
| *H. crustuliniforme* | 1.0 | 0.7 | 2.5 (0.2) | 2.2 (0.5) |
| *A. byssoides* | 1.2 | 0.8 | 2.6 (0.2) | 2.2 (0.5) |
| *T. terrestris* | 1.6 | 0.8 | 2.5 (0.2) | 2.1 (0.8) |
| Soil temperature | | | | |
| 6°C | 1.7 | 1.0 | 2.5 (0.2) | 2.8 (0.7) |
| 12°C | 1.1 | 0.7 | 2.5 (0.2) | 1.9 (0.5) |

NOTE: Analysis of covariance for the shoot-to-root allometric constant (K) was conducted on log-transformed data using shoot/root ratio at the time of transplanting as a covariate. Adjusted means are shown in the original scale. Standard deviations for shoot/root data are shown in parentheses. N = 16 and 56, respectively for the inoculation and soil temperature data.

9. Effects of inoculation and mycorrhizal infection on seedling growth and morphology

The hypothesis that "any" mycorrhizal symbiont is better than "none" at the time of transplanting was tested on the whole data set (inoculation versus no inoculation) and a subset (mycorrhizal infection versus none) which excluded non-inoculated seedlings exhibiting any mycorrhizal development at the time of transplanting and inoculated seedlings with less than 50% root colonization by the inoculant fungus. The probabilities associated with these comparisons for seedling growth and morphology are shown Table 5.9. Neither inoculation nor mycorrhizal infection had a significant impact on shoot growth or dry matter distribution. Mycorrhizal infection had no statistically significant effect on root biomass. In contrast, the root biomass of non-inoculated seedlings was greater than that of the average inoculated seedling. Contamination by T. terrestris appears to have increased the mean root weight of the control (non-inoculated) seedlings. Both inoculation and mycorrhizal infection altered the form of the root system; by decreasing the number of short roots per seedling and per unit root dry weight.

**Table 5.9.** Probabilities that inoculation or mycorrhizal infection present at the time of transplanting influenced the growth and morphology of transplanted seedlings

| Parameter | Inoculation | Mycorrhizal infection |
|---|---|---|
| total seedling biomass | 0.96 | 0.68 |
| old shoot biomass | 0.65 | 0.81 |
| new foliage biomass | 0.28 | 0.50 |
| caliper growth | 0.20 | 0.48 |
| root biomass at 12 weeks | | |
| 6°C soil | 0.05 | 0.16 |
| 12°C soil | 0.02 | 0.10 |
| short roots/seedling | 0.001 | <0.001 |
| short roots/mg root | <0.001 | 0.03 |
| biomass distribution | | |
| 0-5 weeks | 0.24 | 0.34 |
| 0-12 weeks | 0.91 | 0.92 |

NOTE: Probabilities were determined from linear contrasts. Unless otherwise indicated, data were averaged across time and soil temperature.

10. Effect of soil temperature on nutrient concentrations

Complete shoot and root N, P, Ca, and Fe concentration data are presented in Appendix C. Shoot nutrient concentrations grouped by soil temperature and time are summarized in Table 5.10. Shoot N, P, Ca and Fe concentrations showed no effect of soil temperature at the time of transplanting (0 weeks, $P > 0.30$); but 12 weeks later, Ca, N and P concentrations were higher ($P < 0.01$) in the 12°C soil; this effect was weaker for Fe ($P = 0.10$). Root N, P, and Ca concentrations were not influenced by soil temperature.

Mature shoot data were compared to published foliar analyses for spruce seedlings and trees (e.g., Leyton 1948, Ingestad 1959, Swan 1962, Beaton et al. 1965, Swan 1971, Benzian and Smith 1973, Morrison 1974, Farr et al. 1977, Ballard and Carter 1986) to determine which nutrients might limit spruce seedling growth. At the time of transplanting, levels of shoot P, Ca and Fe concentrations were within the range considered adequate for spruce; but N levels were low, suggesting this nutrient was mildly to severely deficient. At 12 weeks, after terminal bud formation, levels of P and Ca in newly-flushed needles were well above 0.15%, the level associated with deficiencies in spruce. Foliar N levels were indicative of a slight deficiency and very severe deficiency, respectively, in the 12°C (1.10% N) and 6°C soils (0.64% N).

At 5 weeks, newly-flushed needles showed visual symptoms of nitrogen deficiency (e.g., needle chlorosis and reddish-purple tips) as described by Morrison (1974) in both soils. These symptoms persisted in the 6°C soil during the 12-week test duration, but disappeared in the 12°C soil between 5 and 10 weeks.

Table 5.10. Effect of soil temperature on shoot concentrations of N, P, Ca and Fe (oven-dry weight basis)

| Week | Mature shoot tissue | | New foliage | |
|---|---|---|---|---|
| | 6°C | 12°C | 6°C | 12°C |
| | | ----------Ca%---------- | | |
| 0 | 0.46 (.03) | 0.49 (.06) | --- | --- |
| 5 | 0.30 (.03) | 0.36 (.04) | 0.11 (.04) | 0.17 (.01) |
| 12 | 0.31 (.03) | 0.47 (.03) | 0.24 (.02) | 0.46 (.05) |
| | | ----------N%---------- | | |
| 0 | 1.02 (.21) | 1.07 (.21) | --- | --- |
| 5 | 0.50 (.05) | 0.55 (.07) | 0.79 (.04) | 0.74 (.05) |
| 12 | 0.49 (.08) | 0.78 (.10) | 0.64 (.12) | 1.10 (.27) |
| | | ----------P%---------- | | |
| 0 | 0.28 (.07) | 0.29 (.05) | --- | --- |
| 5 | 0.14 (.02) | 0.17 (.04) | 0.21 (.02) | 0.18 (.01) |
| 12 | 0.14 (.03) | 0.23 (.04) | 0.17 (.03) | 0.23 (.02) |
| | | ----------Fe μg/g---------- | | |
| 0 | 61 (14) | 65 ( 6) | --- | --- |
| 5 | 38 (11) | 36 ( 4) | 23 (2) | 22 (2) |
| 12 | 38 ( 9) | 52 (18) | 26 (9) | 35 (4) |

NOTE: N = 7 for N, P, and Ca; N = 5 for Fe. Values averaged across inoculation treatments. Standard deviations in parentheses.

Active Fe in new foliage averaged 26 μg/g for the 6°C soil, suggesting Fe uptake may also have limited shoot growth. Concentrations below 30 μg/g in current fall foliage indicate a possible Fe deficiency (Ballard and Carter 1986).

11. Effect of inoculation treatments on nutrient concentrations

Inoculation treatment influenced initial shoot and root N, P, Ca, and Fe concentrations. The lack of replicate analyses within each temperature and inoculation treatment combination at 5 and 12 weeks meant that (1) interactions between soil temperature and inoculation treatments could not be examined statistically and (2) the effect of inocula had to be averaged across the 2 soil temperatures. Averaged across both temperatures, neither mature or new shoot nutrient concentrations showed any significant effect of inoculation at 5 or 12 weeks. However, comparison of 12-week new foliage %P data (Table 5.11) with published analyses indicated that, in the 6°C soil, P as well as N may have limited the growth of seedlings inoculated with either E-strain or H. crustuliniforme.

Table 5.11. Concentration (% oven-dry weight) and content (mg) of N and P in new foliage biomass at 12 weeks

| Inocula | Temp | N% | P% | mg N | mg P |
|---|---|---|---|---|---|
| Control | 6 | 0.65 | 0.17 | 0.82 | 0.21 |
| | 12 | 0.87 | 0.22 | 1.50 | 0.38 |
| Forest floor | 6 | 0.62 | 0.15 | 1.51 | 0.37 |
| | 12 | 1.03 | 0.21 | 3.33 | 0.68 |
| E-strain | 6 | 0.51 | 0.13 | 0.56 | 0.14 |
| | 12 | 1.67 | 0.22 | 1.57 | 0.21 |
| L. bicolor | 6 | 0.66 | 0.20 | 1.49 | 0.45 |
| | 12 | 0.93 | 0.26 | 2.64 | 0.74 |
| H. crustuliniforme | 6 | 0.47 | 0.14 | 0.62 | 0.18 |
| | 12 | 0.93 | 0.20 | 0.94 | 0.20 |
| A. byssoides | 6 | 0.82 | 0.19 | 1.14 | 0.26 |
| | 12 | 1.04 | 0.23 | 2.22 | 0.49 |
| T. terrestris | 6 | 0.75 | 0.18 | 1.16 | 0.28 |
| | 12 | 1.23 | 0.25 | 1.93 | 0.39 |
| Mean | 6 | 0.64 | 0.17 | 1.04 | 0.27 |
| | 12 | 1.10 | 0.23 | 2.02 | 0.44 |

NOTE: Each sample includes pooled tissues of 8 seedlings per inoculation-temperature combination.

Averaged across both temperatures, root N and P varied significantly ($P < 0.01$) among inoculation treatments at 5 weeks. Inoculation (averaged across 6 inocula) increased root N and P concentrations. Root N of inoculated seedlings ranged from 1.28% to 1.93% in the 6°C soil and from 1.34% to 1.67% in the 12°C soils; equivalent values for control seedlings were 1.13% and 1.25%. The level of root P for inoculated seedlings ranged from 0.19 to 0.30% in the two soils; root P of control seedlings was 0.18% in both soils. Root N levels were higher than those reported by Farr et al. (1977) for naturally regenerated Sitka spruce seedlings and by Benzian and Smith (1973) for nursery Sitka spruce seedlings; root P concentrations were similar to their published values.

12. Nutrient Uptake and distribution

The uptake of N, P and Ca after transplanting was independent ($P > 0.40$) of seedling nutrition at the time of transplanting. Total seedling N and P uptake data during the 12-week experiment are presented in Table 5.12. These data suggest that inoculation, on average increased N uptake in the 12°C soil; but had no effect on N and P uptake in the 6°C soil.

There was considerable difference in uptake among the various inoculation treatments. Regardless of soil temperature, N and P uptake was greatest for seedlings inoculated with T. terrestris and least for those inoculated with H. crustuliniforme. T. terrestris seedlings absorbed 150% and 60% more N, respectively at 6 and 12°C, than did non-inoculated seedlings. Nutrient uptake was inversely related ($P < 0.05$) to the number of short roots per seedling and to the number of short roots per unit root weight.

Table 5.12. Initial seedling N and P content and N and P uptake between 0 and 12 weeks

| | Initial content | | Uptake 6°C | | Uptake 12°C | |
|---|---|---|---|---|---|---|
| | N | P | N | P | N | P |
| Inocula | .....mg/seedling..... | | | | | |
| Control | 3.8 | 0.78 | 1.8 | 0.27 | 2.6 | 0.62 |
| Forest floor | 4.9 | 1.19 | 1.2 | 0.22 | 4.6 | 0.78 |
| E-strain | 3.8 | 0.99 | 1.0 | 0.15 | 3.9 | 0.38 |
| *L. bicolor* | 4.0 | 1.16 | 2.3 | 0.31 | 5.4 | 1.12 |
| *H. crust.** | 3.4 | 0.81 | 0.5 | 0.05 | 1.9 | 0.19 |
| *A. byssoides* | 3.9 | 0.90 | 2.0 | 0.27 | 3.6 | 0.50 |
| *T. terrestris* | 3.3 | 0.88 | 2.9 | 0.45 | 6.5 | 1.16 |
| Mean of inoculated seedlings | 3.9 | 0.99 | 1.7 | 0.24 | 4.3 | 0.69 |

**H. crust.* = *H. crustuliniforme*.

The distribution of N, P, and Ca between shoot and root tissues was strongly influenced by inoculation at 0 and 5 weeks ($P < 0.02$); but inoculation effects were weak by the 12-week sample (P values ranged from 0.11 for calcium to 0.05 for the nitrogen). Soil temperature had no effect on nutrient distribution at any sample time ($P > 0.25$). Nitrogen distribution data are shown in Table 5.13; P and Ca distribution data followed similar trends. The proportion of nutrient in shoot tissues was greatest for seedlings inoculated with forest soil and L. bicolor; and least for seedlings inoculated with E-strain or H. crustuliniforme; paralleling the effects of inoculation treatments on total seedling growth.

The distribution of N and Ca was similar ($P > 0.25$) for Ca and N in non-inoculated and inoculated seedlings (averaged across 6 inocula); but the proportion of P (2.3) in shoot tissues of inoculated seedlings, on average was higher ($P = 0.02$) compared with that of non-inoculated seedlings (2.1).

Table 5.13. Effect of inocula on the ratio of shoot N to root N

| | N shoot/root | |
|---|---|---|
| | 5 weeks | 12 weeks |
| | .....mg/mg..... | |
| Control | 1.1 (0.06) | 0.9 (0.09) |
| Forest floor | 1.6 (0.03) | 1.6 (0.20) |
| E-strain | 0.9 (0.02) | 0.9 (0.15) |
| *L. bicolor* | 1.3 (0.23) | 1.2 (0.09) |
| *H. crustuliniforme* | 0.9 (0.03) | 0.9 (0.21) |
| *A. byssoides* | 1.1 (0.15) | 1.1 (0.33) |
| *T. terrestris* | 1.2 (0.26) | 1.0 (0.15) |

NOTE: N = 2; standard deviations in parentheses.

13. Relationship of Nutrition to Growth

There were significant positive correlations between mean values of seedling biomass and nutrition (Table 5.14). However the various growth parameters correlated with different aspects of seedling nutrition. Final new foliage biomass (12 week data) correlated strongly with initial shoot nutrient concentrations. To a lesser extent, so did the relative growth of shoot to roots (allometric constant). In contrast, caliper growth and 12-week root biomass were positively related to nutrient uptake but not to initial shoot nutrition. New foliage biomass was not correlated with nutrient uptake even though both of these parameters increased, 20% and 140% respectively, with soil temperature. A possible explanation for the lack of correlation was the timing of the temperature effects. Most new foliage growth occurred in the 0-5 week period; whereas nutrient uptake was most strongly influenced by soil temperature in the 5-12 week period.

When data within each soil temperature were analyzed separately, mean allometric constant data correlated positively with the N and P content of shoot tissues at the time of transplanting ($r > 0.88$, $N = 7$, $P < 0.01$); and to a lesser extent with total seedling N and P content ($P > 0.03$). There were no significant correlations with total N or P uptake nor with root efficiency after transplanting.

Table 5.14. Pearson correlation coefficients (r) relating seedling initial shoot nutrition and nutrient uptake to caliper growth (0-12 weeks), shoot-to-root allometry (k), 12-week new foliage and root biomass

| Nutrition parameter | Caliper growth | Root biomass | New foliage biomass | Allometry (k) |
|---|---|---|---|---|
| Shoot/0 week | | | | |
| N% | 0.43(0.13) | 0.26(0.36) | 0.76(0.002) | 0.60(0.02) |
| P% | 0.64(0.01) | 0.17(0.56) | 0.85(0.001) | 0.68(0.01) |
| Ca% | 0.35(0.23) | 0.27(0.36) | 0.63(0.02) | 0.39(0.17) |
| Fe μg/g | 0.09(0.80) | 0.17(0.56) | 0.06(0.83) | 0.18(0.53) |
| Uptake/0-5 weeks | | | | |
| N | 0.64(0.01) | 0.43(0.13) | 0.34(0.24) | 0.13(0.67) |
| P | 0.63(0.01) | 0.56(0.04) | 0.24(0.41) | 0.07(0.81) |
| Ca | 0.39(0.16) | 0.72(0.004) | 0.20(0.50) | 0.43(0.12) |
| Uptake/5-12 weeks | | | | |
| N | 0.71(0.01) | 0.87(<0.001) | 0.34(0.24) | 0.43(0.13) |
| P | 0.81(0.001) | 0.94(<0.001) | 0.59(0.15) | 0.21(0.47) |
| Ca | 0.59(0.02) | 0.74(0.002) | 0.41(0.14) | 0.41(0.15) |
| Fe | 0.70(0.01) | 0.72(0.009) | 0.35(0.26) | 0.27(0.40) |

NOTE: N = 14 except for Fe data (N = 12); probability values in parentheses; concentration data are based on oven-dry weight; uptake unit is mg/seedling for N, P and Ca and mg/shoot for Fe.

14. Growth Analysis

Relative growth rate (RGR) was expressed as 4 indices (1) relative root ratio (RWR) representing the proportion of biomass allocated to the nutrient absorbing organ, (2) specific absorption rate (A) representing the rate of nutrient uptake per unit root weight, (3) relative rate of nutrient accumulation (R) and (4) nutrient-use efficiency (NUE), representing the amount of biomass produced per unit of absorbed nutrient. These components were calculated for N, P and Ca. N, P and Ca indices showed parallel trends across temperature and inoculation treatments, and therefore, only the N indices are presented in detail.

Nitrogen-use efficiency (NUE) accounted for the greatest amount of variability (>80%) in the relative growth rate data. Neither NUE or RGR was related to initial seedling biomass ($P > 0.40$). However, RGR calculated for the 0-5 week period correlated positively ($r = 0.74$, $N = 14$, $P < 0.01$) with total seedling N content and shoot N concentration at the time of transplanting. RGR (5-12 weeks) was not related ($P > 0.70$) to the nutritional status of seedlings at either 5 or 12 weeks.

Root weight ratio did not account for a significant ($P > 0.50$) proportion of the variability in the RGR data, indicating the proportion of biomass allocated to the absorbing system was not an important determinant of RGR. RGR (0-5 weeks) did not vary ($P > 0.30$) with root efficiency (specific absorption rate or A) but RGR (5-12 weeks) did vary significantly ($P < 0.05$) with root efficiency.

15. Effect of soil temperature on growth indices

Soil temperature had no effect ($P > 0.25$) on relative growth rate (RGR) or its components in the 0-5 week period. During the 5-12 week period, absorption of N per unit root weight, AN, ($P < 0.001$), root weight ratio, RWR, ($P = 0.01$) and growth per unit of nitrogen, NUE, ($P = 0.04$) were greater in the 12°C soil (Table 5.15). Results for P uptake paralleled those for N. Absorption of calcium (ACa and RCa ) was higher ($P = 0.03$) in the 12°C compared to the 6°C soil during the 0-5 week period; this difference increased in the 5-12 week period ($P = 0.003$).

Table 5.15. Effect of soil temperature on growth analysis indices in the 5 to 12 week period

| Index | 6°C | 12°C |
|---|---|---|
| RGR | 0.02 (0.01) | 0.03 (0.01) |
| RWR | 0.26 (0.03) | 0.31 (0.02) |
| AN | 0.50 (0.10) | 1.80 (0.20) |
| RN | 0.02 (0.01) | 0.06 (0.01) |
| NUE | 2.30 (0.70) | 3.50 (1.30) |

NOTE: Standard deviations in parentheses. Units for RGR, RWR, AN, RN and NUE are respectively: [g growth/(g seedling/week)], (g root/g seedling), [mg N/(g root/week)], [g N/(N seedling/week)], and [g growth/(g N/week)].

16. Effect of Inoculation on Growth Analysis Indices

For the first 5 weeks after transplanting, RGR was influenced by inoculation treatment ($P < 0.001$, ANOVA). Averaged across both soil temperatures, RGR was greatest for seedlings inoculated with forest soil and least for those inoculated with H. crustuliniforme (Table 5.16). In the 5-12 week period, inoculation treatment, averaged across both soil temperatures, had no effect ($P = 0.88$) on RGR. However, the data suggest that there was an interaction between the effects of soil temperature and inocula on RGR. Unfortunately there were not enough replicates to test the statistical significance of these trends. Ranking of inocula differed for the two soil temperatures. In the 6°C soil, RGR of the L. bicolor and A. byssoides treatments was lowest; in the 12°C soil, RGR of the E-strain treatment was lowest. RGR of seedlings infected with L. bicolor, T. terrestris or A. byssoides exhibited the greatest increase with soil temperature.

RWR showed an effect of inoculation treatment ($P < 0.04$) for both time periods (Table 5.17). In seedlings inoculated with forest soil and L. bicolor, a smaller proportion of total biomass ($P < 0.001$) was allocated to the absorbing system than in other treatments.

**Table 5.16.** Relative growth rate, RGR, [(g growth/(g seedling/week)] by inoculation treatment for the 0-5 and 5-12 week periods

| Inocula | 0-5 weeks | 5-12 weeks | | |
|---|---|---|---|---|
| | 6 & 12°C | 6°C | 12°C | 6 & 12°C |
| Control | 0.15 (0.01) | 0.024 | 0.031 | 0.027 (0.005) |
| Forest soil | 0.18 (<0.01) | 0.016 | 0.033 | 0.024 (0.012) |
| E-strain | 0.15 (<0.01) | 0.017 | 0.009 | 0.013 (0.006) |
| L. bicolor | 0.13 (0.01) | 0.012 | 0.052 | 0.032 (0.028) |
| H. crust.* | 0.09 (0.01) | 0.020 | 0.034 | 0.027 (0.009) |
| A. byssoides | 0.13 (0.01) | 0.013 | 0.028 | 0.021 (0.011) |
| T. terrestris | 0.13 (<0.01) | 0.016 | 0.037 | 0.027 (0.015) |

NOTE: Standard deviations in parentheses; N = 2 when data averaged across soil temperature.

*H. crust. = H. crustuliniforme

Table 5.17. Effect of inoculation treatment on relative root ratio, RWR, (g root/g seedling)

| | 0-5 weeks | 5-12 weeks |
|---|---|---|
| Control | 0.30 (0.01) | 0.30 (0.03) |
| Forest soil | 0.25 (0.01) | 0.24 (0.04) |
| E-strain | 0.30 (0.01) | 0.30 (0.03) |
| *L. bicolor* | 0.24 (0.01) | 0.25 (0.03) |
| *H. crustuliniforme* | 0.30 (0.01) | 0.30 (0.02) |
| *A. byssoides* | 0.29 (0.02) | 0.30 (0.03) |
| *T. terrestris* | 0.29 (0.03) | 0.28 (0.05) |

NOTE: N = 2; standard deviations in parentheses.

During the 0-5 week period, specific absorption of nitrogen, AN, ($P = 0.008$) and relative increase in nitrogen, RN, ($P = 0.004$) varied with inoculation treatments. AN (Table 5.18) and RN (Table 5.19) were greatest for seedlings inoculated with L. bicolor and T. terrestris; and least for control seedlings and those inoculated with H. crustuliniforme (Table 5.18, 5.19). AP and RP showed less response to inoculation treatments ($P = 0.03$ and 0.05, respectively) for the 0-5 week period; ACa and showed no effect of inoculation ($P > 0.60$).

During the 5-12 week period, AN and RN showed no effect of inoculation treatment but the data suggest there was an interaction between soil temperature and inoculation effects on AN and RN. In the 6°C soil, non-inoculated seedlings had higher rates of N absorption (AN and RN) than did inoculated seedlings (averaged across 6 inocula); in the 12°C soil this pattern was reversed. AN and RN more than doubled in the warmer soil for all the inoculated seedlings; in contrast values for non-inoculated (control) seedlings were similar for both soil temperatures. Inoculation with E-strain or H. crustuliniforme appeared to reduce N absorption efficiency in the 6°C soil; but not in the 12°C soil. Neither AN nor RN correlated with the number of short roots per unit root dry weight ($P < 0.02$).

**Table 5.18.** Specific absorption rate for nitrogen, AN, for the 0-5 and 5-12 week periods

| Inocula | 0-5 weeks | 5-12 weeks | | |
|---|---|---|---|---|
| | 6 & 12°C | 6°C | 12°C | 6 & 12°C |
| Control | 0.9 ( 0.2) | 1.0 | 1.1 | 1.1 (0.1) |
| Forest soil | 1.5 ( 0.9) | 0.7 | 1.8 | 1.2 (0.6) |
| E-strain | 1.5 ( 0.1) | 0.1 | 1.9 | 1.0 (0.9) |
| L. bicolor | 3.2 ( 0.1) | 0.4 | 2.5 | 1.4 (1.0) |
| H. crust.* | 0.7 (<0.01) | 0.1 | 1.2 | 0.6 (0.5) |
| A. byssoides | 1.9 ( 0.2) | 0.5 | 1.6 | 1.0 (0.6) |
| T. terrestris | 3.1 ( 0.1) | 0.7 | 2.5 | 1.6 (0.5) |

NOTE: N = 2 when data averaged across temperature; standard deviations in parentheses. AN units are [mg N/(g root/week)].

*H. crust.= H. crustuliniforme.

Table 5.19. Effect of inocula on the relative increase in nitrogen, RN, [g N/(g N seedling/week] for the 0-5 and 5-12 week periods

| Inocula | 0-5 weeks | 5-12 weeks | | |
|---|---|---|---|---|
| | 6 & 12°C | 6°C | 12°C | 6 & 12°C |
| Control | 0.03 ( 0.01) | 0.040 | 0.048 | 0.04 (.01) |
| Forest soil | 0.04 ( 0.02) | 0.021 | 0.054 | 0.04 (.02) |
| E-strain | 0.05 (<0.01) | 0.002 | 0.065 | 0.03 (.05) |
| L. bicolor | 0.08 (<0.01) | 0.010 | 0.068 | 0.04 (.04) |
| H. crust.* | 0.02 (<0.01) | 0.005 | 0.044 | 0.03 (.03) |
| A. byssoides | 0.06 (<0.01) | 0.016 | 0.056 | 0.04 (.03) |
| T. terrestris | 0.10 (<0.01) | 0.022 | 0.080 | 0.05 (.04) |

NOTE: N = 2 when data averaged across temperature; standard deviations in parentheses.

*H. crust.= H. crustuliniforme.

Nitrogen-use efficiency, calculated on the basis of absorbed nitrogen, for the 0-5 week period correlated weakly ($P = 0.07$) with initial seedling nutrition. Therefore, a second estimate of nitrogen-use efficiency (growth per unit of initial N) which did not vary with initial N content ( $P > 0.25$) was calculated. Inoculation treatment influenced ($P < 0.001$) both estimates of NUE in the first 5 weeks after transplanting. NUE was greatest in the control and forest soil treatments and least in the H. crustuliniforme treatment (Table 5.20). In the 5-12 week period, NUE did not vary significantly ($P = 0.75$) with inoculation treatment. With the exception of the E-strain treatment, NUE increased with soil temperature. The poor NUE of E-strain seedlings in 12°C soil was associated with a low relative growth rate even though root efficiency (AN and RN) indices were similar to those of other inoculation treatments. NUE values for the 5-12 week period were not correlated ($P > 0.80$) with seedling nitrogen (content or concentration) at 5 weeks.

Table 5.20. Effect of inocula on nitrogen-use efficiency estimated on the basis of absorbed N (NUEa) and on the basis of initial N content (NUEi) for the 0-5 week period; and on the basis of absorbed N for the 5-12 week period

| Inocula | 0-5 weeks (6,12°C) | | NUEa 5-12 weeks | | |
|---|---|---|---|---|---|
| | NUEa | NUEi | 6°C | 12°C | 6,12°C |
| Control | 16.0(1.2) | 40(3.5) | 3.2 | 4.2 | 3.7(0.7) |
| Forest soil | 16.8(1.0) | 37(0.1) | 2.3 | 3.6 | 2.9(1.0) |
| E-strain | 15.2(0.1) | 39(0.7) | 2.4 | 0.9 | 1.7(1.1) |
| L. bicolor | 12.9(1.4) | 33(2.9) | 1.4 | 5.2 | 3.3(2.7) |
| H. crust.* | 9.7(1.6) | 25(3.4) | 2.9 | 4.1 | 3.5(0.8) |
| A. byssoides | 12.4(0.8) | 32(1.8) | 1.6 | 3.0 | 2.3(1.0) |
| T. terrestris | 14.9(0.3) | 40(0.6) | 2.0 | 3.8 | 2.9(1.3) |

NOTE: N = 2 when data averaged across temperature; standard deviations in parentheses. Unit for NUEa are [g growth/(g absorbed N/week)]; units for NUEi are [g growth/(g initial N/week)].

*H. crust.= H. crustuliniforme.

17. Rate of Net Photosynthesis (Pn)

Values of Pn were 50 - 75% lower compared to values reported by other researchers (e.g., Beadle et al. 1981, Binder et al. 1987), probably because needle surface area rather than projected needle area was used to calculate Pn. Values calculated on an oven-dry needle weight basis, were comparable to those reported by Brix (1979) for white spruce seedlings grown in a growth chamber; the maximum value of Pn reported was 9.1 mg $CO_2$/g/h dry weight of needles. On a dry weight basis, mean values of Pn in the 6°C soil ranged from a low of 1.5 at 5 weeks to a high of 2.9 mg $CO_2$/g/h at 10 weeks; and from 3.0 at 5 weeks to 7.3 mg $CO_2$/g/h at 12 weeks in the 12°C soil.

Data collected at 5, 10, and 12 weeks were analyzed separately to meet the homogeneity of variance assumption of ANOVA (Table 5.21). At 5 weeks, there was a significant interaction between soil temperature and inoculation treatments on the rate of Pn. Averaged across all inocula, Pn increased with soil temperature. At 5 weeks, however, Pn showed no response ($P > 0.20$) to temperature in the control and L. bicolor treatments (Table 5.22). The Pn of seedlings inoculated with forest soil exhibited the greatest response to soil temperature; increasing from 0.5 to 1.4 $\mu$mol $CO_2/m^2s$ in the 12°C soil. At 10 and 12 weeks, interactions between soil temperature and inoculation treatments were not significant ($P > 0.07$).

Table 5.21. Analysis of variance for Pn data at 5, 10 and 12 weeks

| Source of variation | df | Mean square | F-ratio | prob. |
|---|---|---|---|---|
| | | 5 weeks | | |
| Soil temperature (ST) | 1 | 4.574 | 54.0 | <0.001 |
| Inoculation (I) | 6 | 0.429 | 5.1 | <0.001 |
| ST x I | 6 | 0.270 | 3.2 | 0.009 |
| Error | 56 | 0.095 | | |
| | | 10 weeks | | |
| Soil temperature (ST) | 1 | 1.906 | 20.4 | <0.001 |
| Inoculation (I) | 6 | 0.223 | 2.4 | 0.04 |
| ST x I | 6 | 0.192 | 2.1 | 0.07 |
| Error | 56 | 0.093 | | |
| | | 12 weeks | | |
| Soil temperature (ST) | 1 | 28.568 | 171.2 | <0.001 |
| Inoculation (I) | 6 | 0.061 | 0.4 | 0.90 |
| ST x I | 6 | 0.276 | 1.7 | 0.15 |
| Error | 56 | 0.167 | | |

NOTE: Pn data were log-transformed for ANOVA.

**Table 5.22.** Effect of inoculation treatments on the rate of Pn ($\mu$mol $CO_2/m^2s$) and WUE ($\mu$mol $CO_2$/mmol $H_2O$)

| | Pn 5 wk | | Pn 10 wk | WUE |
|---|---|---|---|---|
| Inocula | 6°C | 12°C | 6/12°C | 6/12°C |
| Control | 0.51 | 0.61 | 0.89 | 3.2 (1.3) |
| Forest soil | 0.49 | 1.39 | 1.01 | 4.1 (1.8) |
| E-strain | 0.43 | 0.88 | 1.00 | 3.6 (1.5) |
| L. bicolor | 0.84 | 0.94 | 1.26 | 4.1 (1.2) |
| H. crust. | 0.39 | 0.73 | 0.81 | 3.5 (1.5) |
| A. byssoides | 0.70 | 0.97 | 0.98 | 4.0 (1.6) |
| T. terrestris | 0.48 | 0.89 | 1.16 | 4.5 (1.8) |
| Mean | 0.53 | 0.89 | 1.00 | 3.8 |

NOTE: Pn data were log-transformed prior to analysis. Means in original units are shown. N = 5 and 10, respectively, for the 5 and 10 week data. 10-week Pn data averaged across soil temperature. N = 30 for WUE; means averaged across time and temperature.

Inoculation treatments influenced Pn at 10 weeks (Table 5.22) but not at 12 weeks. At 10 weeks Pn was highest in the L. bicolor and T. terrestris treatments and least in the control and H. crustuliniforme treatments. In contrast, soil temperature effects on Pn increased with time. In the 6°C soil, values for Pn at 5, 10 and 12 weeks were 0.53, 0.85 and 0.57 $\mu$mol $CO_2/m^2s$; equivalent values for the 12°C soil were 0.89, 1.19, and 1.85 $\mu$mol $CO_2/m^2s$.

At 5 weeks, the rate of Pn correlated positively with mature shoot N ($N = 14$, $r = 0.70$, $P = <0.01$) and Ca ($N = 14$, $r = 0.80$, $P < 0.001$) concentrations; but not with shoot P or Fe concentrations. Pn data were not correlated ($P > 0.40$) with the nutritional status of shoot tissues at the 10 and 12 week measurements. Pn correlated positively with new foliage biomass (NFB) at 5 weeks ($N = 70$, $r = 0.53$, $P < 0.001$); but not with NFB at 10 and 12 weeks. Caliper growth (0 to 12 weeks) correlated positively with the rate of Pn at 5 weeks ($N = 14$, $r = 0.76$, $P = 0.002$) and weakly with the rate of Pn at 12 weeks ($N = 14$, $r = 0.46$, $P = 0.10$).

In order to make inferences about the role of carbohydrate accumulation or drain on the rate of net photosynthesis (Herold 1980), specific leaf dry weight (mg needle mass/$cm^2$ all-sided surface area) or SLDW, was calculated from the dry weight and surface area of the needles used to measure Pn. This index which estimates the status of leaf carbohydrate (Ehret and Jolliffe 1985) was strongly affected by soil temperature (ANOVA $P < 0.001$), time ($P <0.001$), an interaction between time and temperature ($P <0.001$) and to a lesser degree by inoculation treatment ($P = 0.02$) and an interaction between inoculation and temperature treatments ($P = 0.01$). Mean values of SLDW by temperature and time treatments are shown in Table 5.23.

Table 5.23. Effect of soil temperature and time on specific leaf dry weight ($mg/cm^2$)

| Temperature | Week | | |
|---|---|---|---|
| | 5 | 10 | 12 |
| 6°C | 5.4 (0.7) | 4.8 (0.7) | 5.4 (0.8) |
| 12°C | 4.7 (1.0) | 4.2 (0.7) | 3.9 (0.7) |

NOTE: N = 56, standard deviations in parentheses.

18. Instantaneous values of nitrogen- (INUE) and phosphorus-(IPUE) use efficiency

INUE and IPUE showed no effect of inoculation treatment ($P > 0.75$). Soil temperature had a significant effect on both NUE and PUE at 5 and 12 weeks ($P < 0.001$). PUE and NUE values followed the same trend; therefore only NUE values are presented. At 5 weeks, NUE doubled in the warmer soil, increasing from 28 to 52 μmol $CO_2$/mol N; comparable values at 12 weeks were 30 and 86 μmol CO2/mol N. Values of NUE were similar to those estimated by Field et al. (1983) for 5 species of California evergreens, i.e., 28 to 68 μmol CO2/mol N.

19. WUE (instantaneous water-use efficiency)

WUE showed significant effects of soil temperature ($P < 0.001$), inoculation treatment ($P = 0.006$), time ($P < 0.001$), and an interaction between soil temperature and time ($P < 0.001$). Mean WUE (averaged across time and temperature) for the various inoculation treatments ranged from 3.2 to 4.5 $\mu$mol $CO_2$/mmol $H_2O$ (Table 5.22). The mean value for non-inoculated seedlings (3.2) was lower ($P = 0.01$) than the mean for inoculated seedlings (4.0, averaged across 6 inocula).

Seedlings inoculated with H. crustuliniforme or E-strain had lower ($P = 0.01$) mean values of WUE (3.5 and 3.6, respectively than did seedlings inoculated with T. terrestris (4.5), forest soil (4.1), L. bicolor (4.1), and A. byssoides (4.0). The values of WUE were similar to those reported by Field et al. (1983) for 5 California evergreen shrubs but higher than those reported by MacDonald and Lieffers (1990) for current foliage of black spruce trees. In the latter study, WUE ranged between 1.5 and 3.0 $\mu$mol $CO_2$/mmol $H_2O$.

Averaged across time and temperature, mean values of WUE for the inoculation treatments correlated positively ($P < 0.03$) with mean values of mature shoot %P and %N. Mean WUE values correlated weakly with new foliage production ($r = 0.31$, $N = 70$, $P = 0.01$) and caliper growth ($r = 0.36$, $N = 35$, $P = 0.03$) in the 6°C soil; but were not correlated ($P > 0.50$) with these growth parameters in the 12°C soil.

20. Plant Moisture Stress

At 5 weeks, plant moisture stress estimated from xylem pressure potential (XPP) varied with inocula ($P < 0.001$) and soil temperature ($P < 0.001$). These effects were modified by a significant interaction between

inocula and temperature (P = 0.01). Averaged across all inocula, plant moisture stress was lower in the 12°C soil. Ranking of inocula, however, differed for the two soil temperatures (Table 5.24). XPP showed no effect of inoculation at either soil temperature at 10 and 12 weeks; and XPP data did not correlate with growth or nutrient data.

**Table 5.24.** Effect of inoculation treatment on mean xylem pressure potential (-MPa) 5 weeks after transplanting

| | Soil temperature | |
|---|---|---|
| | 6°C | 12°C |
| Control | 0.63 | 0.33 |
| Forest soil | 0.68 | 0.53 |
| E-strain | 0.53 | 0.30 |
| L. bicolor | 0.63 | 0.41 |
| H. crustuliniforme | 0.61 | 0.57 |
| A. byssoides | 0.53 | 0.46 |
| T. terrestris | 0.58 | 0.39 |
| Mean | 0.52 | 0.36 |

NOTE: N = 5. Data transformed prior to analysis; means in original units (MPa) are shown.

## Discussion

I emphasize that the results of this study apply to specific edaphic conditions (i.e., acidic, cool moist soils with a good potential for indigenous ECM infection) and specific plant material (i.e., cold-stored white spruce seedlings with below optimum shoot concentrations of nitrogen). In addition, comparisons of soil temperature and inoculation treatments were made in a controlled environment to minimize fluctuations in environmental conditions other than soil temperature.

### 1. Interactions between Soil Temperature and Inoculation

With the exception of root growth, growth and physiology of white spruce seedlings were not appreciably altered by interactions between soil temperature and inoculation treatments. Averaged across all inocula, root growth (dry weight, short root production) was greater, as expected, in the 12°C soil. The optimum soil temperature for root growth of many conifer species lies between 18 and 24°C (Heninger and White 1974, Nambiar *et al.* 1979, Ritchie and Dunlap 1980, Andersen *et al.* 1986). However, seedlings inoculated with E-strain or *H. crustuliniforme* showed no detectable increase in root growth in the 12°C soil. In a study of white spruce seedling response to soil temperature, Dobbs and McMinn (1977) found root function declined at a threshold temperature between 10 and 15°C. E-strain and *H. crustuliniforme* inoculation appeared to raise this threshold temperature compared with other inocula. Alternatively, these inocula may lower the root growth capacity of white spruce seedlings regardless of soil temperature.

The nutrient data suggest that important interactions occurred between soil temperature and inoculation effects on total nutrient uptake

and the rate of uptake per unit root per unit time, particularly in the 5 to 12 week period. Two inocula, T. terrestris and L. bicolor, improved root efficiency or specific absorption of N at both soil temperatures compared with non-inoculated seedlings; but their effect on specific absorption was much greater in the 12°C soil. In the 6°C soil, the ranking of inoculation treatments in descending order of root efficiency was: T. terrestris > L. bicolor > A. byssoides and non-inoculated > forest floor > E-strain > H. crustuliniforme. For the 12°C soil the equivalent ranking was T. terrestris > L. bicolor > forest floor and E-strain > A. byssoides > non-inoculated > H. crustuliniforme. Unfortunately, the lack of replicate nutrient data for the different inoculation and temperature treatment combinations precluded testing the statistical significance of these interactions.

2. Effects of Soil Temperature on Seedling Growth and Physiology

The reductions in shoot growth and the rate of net photosynthesis observed in the cooler soil (6°C) are in agreement with the numerous studies (Nielsen 1971, Lavender and Overton 1972, Rook and Hobbs 1975, Lopushinsky and Kaufmann 1984, Delucia 1986) that have demonstrated a positive correlation between soil temperature, shoot growth and photosynthesis. Reduced shoot growth in cool soils has been attributed to various factors including (1) decreased availability, uptake and translocation of nutrients (Power et al. 1963, Bowen 1970, Cooper 1973), (2) increased plant moisture stress resulting from increases in water viscosity and root resistance to water flow (Kramer 1940, Sutton 1969, Running and Reid 1980, Grossnickle and Blake 1985), and (3) reduced synthesis and transport of plant growth regulators such as cytokinin and

gibberellin from root to shoot tissues (Ritchie and Dunlap 1980, Atkin et al. 1973).

In the first 5 weeks after transplanting, differences in shoot growth at the two soil temperatures was not related to nutrient availability and uptake. A significant reduction (about 40%) in the rate of net photosynthesis and new foliage production occurred in the 6°C soil even though there was no detectable effect of soil temperature on N and P uptake or content. Several observations suggest that water stress contributed to poor shoot growth during this period. Mean values of xylem pressure potential, water-use efficiency and calcium absorption, which is related to the mass flow of water (Marschner 1986), were lower in the 6°C soil. The level of plant moisture stress (0.7 MPa) measured in the 6°C soil is considered sufficient to inhibit turgor-mediated processes such as root or leaf area growth in many plant species (Hsiao 1973).

During the 5-12 week period, seedling moisture status showed no appreciable effect of soil temperature. In contrast, foliar nutrient concentrations, total nutrient uptake, and root efficiency (specific absorption of nutrients) were strongly influenced by soil temperature. Newly-flushed foliage of seedlings grown in the 6°C soil was chlorotic, a visual symptom of nutrient deficiency commonly exhibited by plant species grown at sub-optimum soil temperatures (Cooper 1973). Foliar analysis indicated very severe and mild deficiencies, respectively of N and Fe. The specific absorption of nitrogen (uptake per unit root per time) increased by 72% in the 12°C soil. In addition, root biomass was approximately 20% greater in the 12°C soil.

These results indicate that the availability, absorption or translocation of nutrients limited shoot growth in the 6°C soil either

directly or indirectly, e.g., through alterations in the balance of plant growth substances. The nutritional status of plants, particularly that of nitrogen, alters the quantity and composition of plant growth regulators synthesized in root tissues (Marschner 1986 and references therein).

Because of the parallel decline in stomatal conductance and net photosynthesis with decreasing soil temperature, many researchers (e.g., Anderson and McNaughton 1973, Kaufmann 1977) assume that plant water stress, through its effect on $CO_2$ availability, is the major factor limiting net photosynthesis in cool soil. However, three observations suggest that net photosynthesis in the 6°C soil was not limited by water stress. First, the level of plant moisture stress measured in the 6°C soil (xylem pressure potential of -0.7 MPa) was not sufficient to reduce net photosynthesis significantly; the rate of net photosynthesis is relatively constant over a wide range of xylem pressure potentials above -1.5 MPa for many plants, including spruce seedlings (Hsiao 1973, Turner and Jarvis 1975, Beadle et al. 1981). Second, mean xylem pressure potential differed by less than 0.2 MPa between 6 and 12°C soil. Third, internal concentrations of $CO_2$ were not lower in the 6°C soil as would be expected if stomatal conductance limited $CO_2$ availability. Internal $CO_2$ concentrations were actually higher ($P < 0.001$) in the 6°C soil; averaging 283 and 253 $\mu g/g$, respectively for the 6 and 12°C soils.

In most studies, soil or root-zone temperatures are reduced rapidly and plants are exposed to different temperatures for very brief periods, generally less than 24 hours. This exposure time is too short to study the acclimation response of plants to cool soils. The mechanisms underlying short-term and long-term plant response to soil temperatures may differ considerably (Delucia 1986, Setter and Greenway 1988). After exposing

Englemann spruce seedlings to seven days of cold soil temperatures, Delucia (1986) concluded that net photosynthesis was limited mainly by non-stomatal factors, such as carbohydrate accumulation in needle tissues when root sink activity was reduced by cool temperatures. The rate of net photosynthesis may be regulated by the demand for carbohydrates created in non-photosynthetic tissue (Herold 1980). Alternatively, in cool soils the export of plant growth regulators, such as cytokinins, from root to shoot tissues may be reduced. These substances are thought to influence net photosynthetic rates by altering enzyme synthesis, membrane permeabilities and translocation patterns (Wareing et al. 1968).

A nutritional explanation for the temperature-induced changes in photosynthesis during the 5-12 week period is supported by the positive correlation between net photosynthetic rate and mature shoot N or P. However, it is also possible that sink activity influenced the rate of net photosynthesis (Herold 1980) as proposed by Delucia (1986). The specific dry weight (leaf dry weight/leaf area) of mature needle tissues was higher in the cooler soil; perhaps reflecting an increased accumulation of needle carbohydrates (Ehret and Jolliffe 1985) or a lowering of root sink activity (Harley and Smith 1983 and references therein).

Nutrient-use efficiency estimated as net photosynthesis per unit shoot N and P (PNUE) was 2- to 3-fold greater in the 12°C soil. Because PNUE was derived from the ratio of net carbon assimilation to shoot nutrient content, increases in PNUE may reflect either (1) a decrease in shoot N or P, or (2) an increase in net photosynthetic efficiency at a particular level of shoot N or P. The temperature-induced increase in PNUE observed in this study was not accompanied by a decrease in shoot N or P. Concentrations of shoot N and P in the 12°C soil were similar (at 5 weeks)

or higher (at 12 weeks; 0.5% N at 6°C versus 0.8% at 12°C) than in the 6°C soil. Moreover, PNUE increased as shoot N and P concentrations increased. The poor nutritional status of seedlings in 6°C soil probably contributed to the low PNUE of these seedlings. Needles with low nitrogen concentrations may have low nutrient-use efficiencies because a high proportion of their nitrogen is bound to non-photosynthetic compounds involved in cell regulation and respiration (Chapin et al. 1987)

Decreasing soil temperature from 12 to 6°C reduced root growth more than shoot growth; consequently shoot/root ratios were higher in the 6°C soil. A similar response was reported by Barney (1951) for pine germinants grown at soil temperatures ranging from 5 to 20°C. However, the opposite trend, i.e., shoot/root ratios increasing with soil temperature, is more commonly reported for many plant species (Davis and Lingle 1961, Davidson 1969, Lavender and Overton 1972).

The variable results of soil temperature studies probably reflect the use of different growing environments, soil temperatures and plant material. In a study of Douglas-fir seedlings in their first growing season, Lavender and Overton (1972) observed that root growth of Douglas-fir seedlings was relatively unaffected by soil temperatures between 10 and 20°C. However, a sharp decline in root growth could alter shoot/root allometry in soils below 10°C. Root function and growth of many conifer species declines sharply at soil temperatures below 10°C (Kramer 1940, Babalola et al. 1968, Kaufmann 1975, Running and Reid 1980, Delucia 1986).

The low light intensities (approx. 10% full sunlight) common to soil temperature studies conducted in growth chambers may also influence shoot/root ratios. Under these conditions, root growth is limited by photosynthate availability and may be less responsive to changes in soil

temperature. Light intensity was higher, approximately 25% of full sunlight, in this study.

The interaction between root and shoot growth is often viewed as a competitive source-sink relationship with root growth limited by carbohydrates from the shoot and shoot growth limited by nutrients from the root (Loomis 1953). According to this hypothesis, when nutrient availability and the efficiency of nutrient absorption are limited by infertile, cold or droughty soils, shoot growth is reduced more than root growth and shoot/root ratios decrease. The pattern of plant growth is adjusted to maximize nutrient uptake. The results of this study do not support this hypothesis since shoot/root ratios increased even though seedling nutrient uptake and root efficiency (nutrient absorption per unit root dry weight) decreased in the cooler soil. In most soil temperature studies, experimental plants are annuals or perennials in their first growing season. Shoot growth of perennial cold-stored seedlings is less likely to be depend on current root activity or growing environment. Shoot tissue formed in previous years can supply considerable amounts of nutrients (Fife and Nambiar 1984) and carbohydrates (Gordon and Larson 1968, Webb 1977) to new shoot growth of conifer seedlings. During the first 5 weeks, the N and P content of mature shoot tissue decreased by about 20%. Some of these nutrients were probably translocated to new foliage as described for pine seedlings by Fife and Nambiar (1984).

The coincidence of higher shoot/root ratios and lower foliar nutrient concentrations is not in agreement with a growth analysis of soil temperature effects reported by Margolis and Brand (1990). In their study, both shoot/root ratios and foliar nutrient concentrations of outplanted eastern white pine seedlings were inversely related to soil temperature.

They attributed this effect to an increase in root efficiency (nutrient uptake per unit root) with decreasing soil temperature. However, numerous studies have shown that root efficiency is maximum at soil temperatures in the 20 and 30°C range and that it decreases at soil temperatures below this range (Nielsen and Humphries 1966, Bowen 1970, Cooper 1973).

Alternatively, their results may reflect (1) the confounding of soil temperature treatments with soil fertility, or (2) the inadequacy of the particular index they used to estimate root efficiency. Warm microsites were created by removing organic soil horizons and surface debris; a practice which also reduces soil fertility. Margolis and Brand (1990) used the ratio of foliar (not total) N to root weight (N/RW) as an indicator of the ability of a given amount of root to supply nitrogen. N/RW calculated from data in the present study, showed no effect of soil temperature ($P > 0.30$). Moreover, this index was closely related ($r > 0.80$, $P < 0.001$) to the nutritional status of seedling at the beginning of the measurement period.

The distribution of dry matter between shoots and roots varies with environmental conditions. These changes may be interpreted as adaptive, with the plant maintaining an optimum balance between the nutrient or water absorbing capacity of the root and the photosynthetic capacity of the shoot in a particular environment (Davidson 1969, Troughton 1980, Chapin et al 1987). However, the decrease in shoot/root ratio observed in the 6°C soil would seem to have limited or no adaptive value. The potential for seedling moisture or nutrient deficits would be increased by the high proportion of shoot to root growth in an environment which limits root efficiency. It is more common for the shoot/root ratios of spruce seedlings to decrease after their first growing season (Hermann 1977).

Alternatively, the inverse relationship observed during the 12-week study may not be an adaptive response. It may simply be the result of soil temperatures that are sub-optimum for root growth; air temperatures that are optimum for shoot growth; and the ability, at least in the short-term, for new shoot growth of determinate species to be sustained by nutrients translocated from mature shoot tissues.

3. Seedling response to "any" mycorrhizae at the time of transplanting

Averaged across all inocula, the presence of mycorrhizae at the time of transplanting, did not improve spruce seedling growth. This result does not support the premise that the presence of "any" fungus is better than "no" fungus for the particular study conditions, i.e., cool moist acidic forest soil with good potential for root colonization by indigenous ECM fungi. Mycorrhizal infection, however, did alter root morphology by reducing the number of short roots per seedling and per unit root weight.

On average, mycorrhizal infection increased the water-use efficiency of mature white spruce needles by approximately 25%. Inoculated seedlings tended to have higher rates of net photosynthesis but lower rates of transpiration. Water-use efficiency was not associated with improved growth in the particular study conditions. However, the improved water-use efficiency of mycorrhizal seedlings suggests that "any" fungus might be better than "none" in cool, droughty soils.

Water-use efficiency was related positively with shoot N and P indicating improved nutrition was in part responsible for the effect of mycorrhizal infection on water-use efficiency. Averaged across temperature and time, inoculated seedlings had higher mean values of P and N in mature shoot tissues (respectively, 0.18 and 0.60%) compared to non-inoculated

seedlings (respectively, 0.14 and 0.51%). Nutritionally-induced changes in the rate of net photosynthesis, the hydraulic conductivity of the root system, the sensitivity of stomata to abscisic acid (ABA), or the balance between ABA and cytokinins in needle tissue (Marschner 1986) would also affect water-use efficiency.

Guehl et al. (1990) also found that inoculation with a variety of ECM fungi increased the water-use efficiency of Italian stone pine seedlings grown in moist soils. Although they attributed this effect to the improved nutritional status of inoculated seedlings, the nutritional status of non-inoculated and inoculated seedlings was not compared. However, a positive relationship between the water-use efficiency of conifer seedlings and foliar N and P have been reported by Sheriff et al. (1986).

Due to insufficient replication, it was not possible to test statistically the effect of inoculation on nutrient uptake by white spruce seedlings. The raw data suggest that inoculation increased nutrient uptake and efficiency of uptake (rate of uptake per unit root per unit time) in the 12°C but not the 6°C soil, even though the inoculant fungi grew and infected new roots in the 6°C soil. This conclusion is supported by experiments conducted by Mejstrík (1970). He found that differences in $^{32}P$ uptake between excised non-mycorrhizal and ectomycorrhizal root segments increased with solution temperature in the range of 5 to 30°C.

Inoculation, on average, had the greatest influence on nitrogen uptake, which was the most limiting of the analyzed nutrients. Nitrogen uptake by inoculated seedlings (averaged across all inocula) was 65% greater than that of non-inoculated seedlings in the 12°C soil. There was no appreciable difference in P or Ca uptake between non-inoculated or inoculated seedlings at either soil temperature. Fe data were incomplete

and could not be used to compare uptake by inoculated and non-inoculated seedlings. However, inoculation (averaged across 4 inocula) increased the Fe content of new foliage by about 55% at both soil temperatures, suggesting comparison of Fe uptake by mycorrhizal and non-mycorrhizal seedlings warrants further study.

The results are in agreement with studies conducted with VA mycorrhizae (Smith and Roncadori 1986) and excised ectomycorrhizae (Harley and Wilson 1959, Edmonds et al. 1976) showing that the benefits of the mycorrhizal symbiosis in terms of nutrient uptake decrease in cool soils. Smith and Roncadori (1986) reported that VA mycorrhizae increased the phosphorus and copper uptake of cotton plants at soil temperatures of 24°C and 36°C; but mycorrhizae had no effect on nutrient uptake at sub-optimum soil temperatures of 18°C. Harley and Smith (1983) speculated that the optimum temperature for nutrient uptake by mycorrhizae is lower than the optimum for fungal growth.

The distribution of N and Ca between shoot and root tissues was not affected by inoculation; but the proportion of P in shoot tissues was increased, on average, by inoculation. Studies of VA (Powell 1975, Smith and Daft 1978) and ECM infections (Black 1986) have demonstrated that mycorrhizal symbioses alter the distribution of nutrients within the host plant, especially when plant nutrients are deficient. In contrast to this study, Black (1986) found that mycorrhizal infection increased the proportion of N and P in root tissues of Douglas-fir seedlings. His nutrient analyses coincided with sporocarp production by the inoculant fungus (L. laccata) suggesting to him that nutrients stored in fungal tissues were preferentially used by the mycorrhizal fungi during its reproductive phase. Unfortunately, in this study, no attempt was made to

monitor spore production by the inoculant fungi.

4. Seedling response to specific fungi

The presence of specific mycorrhizae (e.g., forest soil, L. bicolor or T. terrestris) at the time of transplanting did improve spruce seedling growth and/or nutrition; supporting the second premise of artificial inoculation programs, i.e., some ECM fungi are more beneficial to host plants than others in cool soils. These benefits occurred even though most non-inoculated seedlings were infected with indigenous ECM fungi by the fifth week after transplanting.

Of the inocula tested, forest soil was one of the most effective in enhancing spruce seedling growth. Seedlings inoculated with forest soil grew better than non-inoculated seedlings even though (1) non-inoculated seedlings were rapidly infected with forest fungi 5 weeks after they were transplanted into forest soil and (2) the size and nutritional status of non-inoculated seedlings and those inoculated with forest soil were similar at the time of transplanting. Soil inoculum has been used successfully around the world (Mikola 1970); and in some cases, it has proved more effective than pure culture inoculum (Riffle and Tinus 1982). The greater diversity of mycorrhizae (more than 3 morphological types) infecting the root systems of seedlings inoculated with forest floor may have contributed to the efficacy of this inoculum (Perry et al. 1987). Naturally-regenerated seedlings typically have more than one fungal partner (Trappe 1977). Sinclair (1974) reported that Douglas-fir seedlings grew better in bare-root nursery soil when infected with more than one type of ECM fungus. It is also possible that microorganisms associated with the mycorrhizosphere contributed to the growth promoting effect of the forest soil inoculum. Soil collected from an arbutus stand was shown to increase

$N_2$ fixation in the rhizosphere of planted Douglas-fir seedlings (Amaranthus et al. 1990).

L. bicolor was an effective pure culture inoculum, increasing all aspects of seedling growth. Under nursery conditions, Laccaria spp. have been shown to depress seedling growth (Molina 1982, Shaw et al. 1982, Danielson et al. 1984c). In outplanting studies, response to inoculation with Laccaria sp. has been variable. Shaw et al. (1987b) and Loopstra et al. (1988) reported no growth response to L. laccata; others (e.g., Wilson et al. 1987, Danielson and Visser 1989) reported positive responses with L. proxima infection. Also Richter and Bruhn (1989) found that L. bicolor infection increased the survival of container-grown red and jack pine seedlings planted in sandy, xeric soil.

Laccaria spp. may be especially tolerant of acidic soils. Gagnon et al. (1987, as cited by Kropp and Langlois 1990) successfully inoculated conifer seedlings with L. bicolor in peat-based growing media, with pH values as low as 3.5. L. laccata inoculation has been shown to increase the growth of Sitka spruce seedlings on acidic organic and mineral soils (Thomas and Jackson 1983, Mason et al. 1983).

T. terrestris was effective especially in promoting nutrient uptake and caliper growth. In North America, this fungus is considered a "weed fungus" which contaminates inoculation experiments and naturally infects conifer seedlings in both bare-root and container nurseries (Mikola 1989, Castellano and Molina 1989, Danielson and Visser 1990). T. terrestris had proven less effective than Pisolithus tinctorius on planting sites with a soil moisture deficit (Marx and Cordell 1987); thus T. terrestris is assumed to be adapted to the fertile, moist conditions of nurseries and not to the more stressful environments of planting sites (Marx et al. 1984).

However, seedling stress and poor growth can also result from an excess, rather than a lack of soil moisture. T. terrestris is better adapted to acidic poorly-drained forest soils than are other ECM fungi including P. tinctorius (Cruz 1974, Thomas and Jackson 1983, Wilson et al. 1987). Coutts and Nicoll (1990b) noted that mycelial strands of T. terrestris are extremely tolerant of flooding, surviving 149 days of waterlogged soils.

Inoculation with E-strain reduced new foliage production relative to non-inoculated seedlings and most other ECM fungi. A similar response was reported by Danielson et al. (1984a) when jack pine seedlings were inoculated with E-strain and grown over a wide range of fertilizer regimes in a container nursery environment. The thin mantle and coarse Hartig net development characteristic of E-strain mycorrhizae and the coincidence of E-strain infection with poor seedling growth, suggested to Levisohn (1954) and Björkman (1949, cited in Mikola 1965) that E-strain infections were parasitic on conifer seedlings. In contrast, Mikola (1965) found there was no correlation between seedling growth and E-strain infection.

Published results of conifer response to E-strain inoculation suggest that the efficacy of this fungus may vary with soil acidity. On a reclamation site in northern Alberta, shoot growth of Jack pine seedlings inoculated with E-strain was more than double that of non-inoculated seedlings (Danielson and Visser 1989) two years after planting. The seedlings were planted in peat-amended oil sands with pH values ranging from 6.3-7.5. In contrast, Holden et al. (1983) observed that E-strain was less effective in promoting the growth of Sitka spruce seedlings than were other ECM fungi, including T. terrestris and Laccaria spp. Their experiment was conducted in a greenhouse with an acidic humus-type forest soil (pH 4.8). Similarly, in greenhouse and field experiments conducted by

Thomas and Jackson (1983), E-strain was found to be less effective in an acidic forest soil compared to T. terrestris and Laccaria laccata. Mikola (1965) noted that bare-root nurseries located on acidic soils (pH 4.0-6.2) have less E-strain infection compared with those located on more neutral soils (pH 5.1-6.6). Danielson and Pruden (1989) reported that E-strain was the most common fungal symbiont of young and mature urban spruce growing in moderately alkaline soils (pH 7.3-8.1).

H. crustuliniforme significantly depressed the growth of white spruce seedlings in this study. Previous studies have shown the efficacy of H. crustuliniforme in promoting host growth is poor relative to other fungi (Chu-Chou 1985, Tyminska et al. 1986, Danielson and Visser 1989). Its efficacy may be greater in soils that are dryer or less acidic than those used in this study. Stenström et al. (1990) noted that nursery seedlings inoculated with H. crustuliniforme appeared to tolerate drought better than non-inoculated seedlings. In culture, H. crustuliniforme mycelium tolerates pH values as high as 9 (Dennis 1985); and in soils, H. crustuliniforme mycorrhizae tolerate dry, moderately alkaline soils (Danielson and Pruden 1989). Soil type may also strongly influence the efficacy of H. crustuliniforme. Mason et al. (1983) observed that birch trees were naturally infected with H. crustuliniforme in mineral soils but not in organic soils.

Inoculation with A. byssoides had negligible effects on the growth of seedlings under the conditions of this study. I am not aware of any other studies of A. byssoides efficacy. Inoculation with A. byssoides in previous studies has not resulted in successful root infection (Danielson et al. 1984c, Danielson and Visser 1989). Like E-strain and H. crustuliniforme, Amphinema mycorrhizae tolerate dry, alkaline soil

conditions (Danielson and Pruden 1989) and it may be a more effective symbiont in these conditions.

5. Possible reasons for differences in Mycorrhizal Efficacy

The lack of a positive host response to mycorrhizal inoculation can result from low levels of root infection by the inoculant fungi (Ruehle et al. 1981, Lee and Koo 1983, McAfee and Fortin 1986, Wilson et al. 1987, Last et al. 1990). Ruehle et al. (1981) and Last et al. (1990) found that the response of conifer seedlings to inoculation was negligible unless more than 50% of short roots were infected by the inoculant fungus. However, in this study, variable spruce seedling response to inoculation could not be attributed to the degree of root colonization by the inoculant fungi at the time of transplanting. With the exception of the A. byssoides treatment, inoculant fungi colonized more than 95% of the short roots of inoculated seedlings during the nursery production phase. Furthermore, the growth of seedlings inoculated with A. byssoides was not related ($P > 0.25$) to percent infection at the time of transplanting (estimated from percent of original root plug colonized by A. byssoides).

Nor was seedling growth related to the source of inocula. The least (H. crustuliniforme) and most effective (L. bicolor) inocula tested in this study were collected from northern field sites. Of the two nursery isolates, one (A. byssoides) had a negligible effect on seedling growth; the other (T. terrestris) improved seedling growth.

Variable host response to specific fungi was related to inocula-induced differences in four parameters: (1) the nutritional status of seedlings at the time of planting, (2) rate of net photosynthesis, (3) nutrient uptake after planting and (4) nutrient-use efficiency. These factors are discussed below in four sections.

Initial nutritional status of seedlings

New foliage biomass, 65% of which occurred in the first 5 weeks after transplanting, was strongly influenced by seedling nutrition at the time of transplanting. The amount of new foliage biomass in spruce seedlings is to a large extent predetermined and, therefore, can be expected to be more responsive than root or caliper growth to inocula-induced changes in seedling nutrition during the nursery production phase. However, other factors may also have been involved since T. terrestris seedlings with an initial nutrient status similar (i.e., low) to H. crustuliniforme seedlings produced much more new foliage.

New foliage production was also related to the proportion of total seedling N and P contained in shoot tissues. However, E-strain seedlings produced much less new foliage than seedlings with similar total N and P contents (e.g., A. byssoides and control seedlings) at the time of transplanting. The smaller proportion of total seedling N and P contained in the shoot tissues of E-strain seedlings likely contributed to their inferior production of new foliage.

Regardless of soil temperature, inoculant fungi (e.g., forest floor, T. terrestris and L. bicolor) which increased total seedling dry weight also increased the proportion of dry matter allocated to shoot relative to root tissues. Ectomycorrhizal infection has been previously shown to

increase the rate of shoot growth relative to root growth (Hatch 1937, Cline and Reid 1982, Black 1986), an effect which is usually attributed to an improvement in the nutritional status of the host plant. Mycorrhizal infection is considered to increase the efficiency (nutrient uptake/unit root) and therefore, to reduce the amount of root biomass required for nutrient absorption (Cline and Reid 1982, Black 1986). In this study, shoot/root allometry was not correlated with N and P uptake or with root efficiency, but was influenced by the initial nutrient content of shoot tissues and to a lesser degree by total seedling nutrient content.

Rate of net photosynthesis

Inoculation with forest floor, L. bicolor and T. terrestris increased seedling net photosynthetic rates at 5 and 10 weeks. This increase was accompanied by greater new foliage production and percent caliper growth. Enhanced rates of net photosynthesis in response to mycorrhizal infection have been observed in previous studies (Ekwebelam and Reid 1983, Harley and Smith 1983, Reid et al. 1983). Numerous studies have shown that net photosynthetic rate increases with increasing levels of foliar N and P (Marschner 1986) and that mycorrhizal infection increases nutrient uptake (Harley and Smith 1983). Improved nutrition probably explains the differences among inoculated seedlings in net photosynthesis at the 5-week measurement. At 5 weeks, the rate of net photosynthesis correlated positively with the level of N and P in mature shoot tissue; and initial and 5-week shoot N and P were influenced by inoculation treatments.

However, net photosynthetic rate at 10 weeks showed no relationship to the nutrient status of mature shoot tissues at 0, 5, or 12 weeks. Possibly, a nutrient other than N, P, Ca or Fe limited the rate of net

photosynthesis. Alternatively, the various inocula may have altered source-sink relationships or the balance of plant growth regulators affecting carbon assimilation. Reid et al. (1983) speculated that the carbon cost of mycorrhizal infections might stimulate the rate of net photosynthesis in the host plant. If this is the case, one might expect 10-week net photosynthetic rate to be inversely related to specific leaf dry weight (i.e., needle dry weight/surface leaf area). Specific leaf dry weight has been shown to increase when leaf carbohydrates accumulate (Ehret and Jolliffe 1985). However, there was no ($P > 0.70$) relationship between these two parameters.

Nutrient uptake after transplanting

Seedlings inoculated with different fungi varied widely in their ability to absorb nutrients, particularly N at low soil temperatures. Root and caliper growth, 50% or more of which occurred in the 5-12 week period, correlated positively with nutrient uptake but not with the initial nutritional status of seedlings. Inoculation with T. terrestris, L. bicolor and forest soil significantly improved seedling caliper growth and nutrient uptake. New foliage production, the majority of which occurred in the first 6 weeks after transplanting, was not correlated with nutrient uptake.

T. terrestris and H. crustuliniforme seedlings which were nutritionally inferior to other inoculated and control seedlings at the time of transplanting absorbed, respectively, the most and least N for 12 weeks after transplanting. Averaged across the two soil temperatures, there was a 3-fold difference in N uptake between seedlings infected with T. terrestris and those infected with H. crustuliniforme. Large

differences (as great as 10-fold) in nutrient uptake by various kinds of excised mycorrhizae in solution culture have been recorded (Mejstrík 1970, Langlois and Fortin 1978). Harley and Smith (1983) suggest that nutrient uptake by different kinds of mycorrhizae is strongly influenced by the amount of external hyphae (i.e., absorbing surface area) extending into the rooting medium. The relatively low nutrient uptake by E-strain and H. crustuliniforme mycorrhizae does not support this hypothesis. These two types of mycorrhizae had, respectively, sparse and very abundant development of external hyphae.

Chu-Chou (1985) also observed that H. crustuliniforme was less effective in enhancing N and P uptake of radiata pine compared with other ECM fungi. Although H. crustuliniforme may be less effective than other fungi in promoting nutrient uptake, seedlings inoculated with H. crustuliniforme have been found to absorb more ammonium- and nitrate-nitrogen in solution culture than non-mycorrhizal conifer seedlings (Rygiewicz et al. 1984a,b). It is important to note that most control seedlings in this study were infected with mycorrhizal fungi 5 weeks after transplanting; therefore they were not non-mycorrhizal.

Washing of root samples prior to oven-drying for chemical analysis removed external hyphae attached to mycorrhizae, therefore total nutrient uptake for the mycorrhizal symbiosis was underestimated particularly for the H. crustuliniforme infection with its abundant external mycelium. The low content of nutrients in H. crustuliniforme seedlings may have resulted, in part, from the retention of nutrients in external hyphae. Low temperatures are thought to depress the ability of vesicular-arbuscular mycorrhizae to absorb phosphorus and also to transport phosphorus to the host plant (Harley and Smith 1983 and references therein).

Nutrient-use efficiency

Nutrient-use efficiency, or the relative gain in dry weight per unit of seedling N or P, accounted for the greatest proportion of variability in relative growth rates among inoculation treatments. This variation was not due to inoculation effects on nutrition during the nursery production phase, nor to effects on seedling water status (e.g, water-use efficiency, xylem pressure potential, calcium uptake) after transplanting. Nor were there differences among inocula in net photosynthetic rate per unit foliar N or P, as reported by Bethlenfalvay et al. (1987) for mycorrhizal soybean plants.

The variation may have resulted from nutrients which were not analyzed or from non-nutritional factors, e.g., alterations in carbon metabolism or in the balance of plant growth regulators. The amount of carbon lost through root respiration or the development of fungal tissues (external mycelium , fruit bodies) may have varied among inocula. ECM infection has been shown to increase root respiration and the translocation of photosynthate to root systems (Tranquillini 1964, Reid et al. 1983, Ingestad et al. 1986). Root respiration losses can be significant with as much as 90% of the assimilated carbon lost through root respiration, particularly when plants are nitrogen-deficient (Hermann 1977). Significant quantities of host photosynthate can also be incorporated into fungal structures. Inhibition of conifer seedlings in nursery environments has been attributed to use of host photosynthate for external hyphae (Langlois 1983, cited in Gagnon et al. 1987) and sporocarp production (Shaw et al. 1982). Various plant growth regulators, e.g., cytokinins and auxins, are produced by ECM fungi in culture (Ek et al. 1983, Ho 1987).

Although there is no direct evidence for transfer of these substances to the host plant, they may affect the host plant or rhizosphere population. Alternatively, ECM fungi may stimulate or inhibit host plant production of certain plant growth regulators.

The low nutrient-use efficiency of seedlings inoculated with H. crustuliniforme supports a non-nutritional explanation for the differences among inocula. In previous studies the poor growth response of seedlings to infection by H. crustuliniforme has been attributed to the carbon cost of its extensive mycelium (Tyminska et al. 1986), its relative inability to synthesize plant growth regulators (Tyminska et al. 1986), and its high respiration rate (Marshall and Perry 1987). Reid et al. (1983) and Ingestad et al. (1986) proposed that the higher carbon cost of mycorrhizal systems was compensated for by increased rates of net photosynthesis since the growth of mycorrhizal seedlings was equal to or greater than that of non-mycorrhizal seedlings. In this regard, if H. crustuliniforme infection represented a high carbon cost to spruce seedlings, there was no compensatory increase in the rate of net photosynthesis.

Differences in the timing of bud break whether hormone- or nutrient-induced may also have contributed to inocula-induced differences in nutrient-use efficiency. The percentage of seedlings (data from Chapter IV for all inocula except A. byssoides) flushed at 21 days correlated positively with mean nitrogen-use efficiency ($r = 0.88$, $N = 6$, $P = 0.02$). Seedlings with high values of nutrient-use efficiency (control, forest floor) also flushed earlier than other seedlings. Differences in the speed of bud break may be due to inocula-mediated alterations in the availability of carbohydrates and nutrients for bud expansion or to shifts in the balance of plant growth regulators. Fungal infections alter the

translocation patterns of host plants (Smith et al. 1969). Ingestad et al. (1986) estimated that 30-40% more carbon was translocated to ectomycorrhizal root systems than to non-mycorrhizal systems when nitrogen was deficient (shoot N ranged from 0.7 to 0.9%) as in this study.

During the 5-12 week period, the nitrogen-use efficiency of L. bicolor seedlings increased five-fold in the warmer soil (12°C), while the nitrogen-use efficiency of E-strain seedlings showed no response to soil temperature. The reason for the low nutrient-use efficiency of this fungus are not clear. Nitrogen uptake during the 5-12 week period was similar to that of inoculation treatments (e.g., control, A. byssoides) with higher values of nutrient-use efficiency. The low nitrogen-use efficiency of E-strain seedlings was reflected in the elevated levels of nitrogen in new foliage (1.7%). Phosphorus uptake was relatively low in comparison to N uptake and it is possible that an imbalance of N and P decreased nitrogen-use efficiency. Alternatively, at 12°C, the E-strain fungus may have been stimulated to produce spores, increasing carbon translocation to fungal tissues.

Summary

Seedling growth and net photosynthetic rates were lower at 6°C than at 12°C. Root growth was reduced proportionally more than shoot growth with the result that seedlings grown at 6°C had higher shoot/root ratios. Reductions in net photosynthetic rate were attributed to plant moisture stress in the short-term and to nutrient stress in the long-term.

White spruce seedlings showed no response in the particular study conditions (i.e., cool, wet, acidic forest soils) to the presence of "any" mycorrhizae at the time of transplanting. In contrast, they responded positively to inoculation by specific inocula (e.g., T. terrestris, L. bicolor and forest floor); and negatively to others (e.g., H. crustuliniforme and E-strain). Positive responses to inoculation included increased growth, nutrient uptake, nutrient-use efficiency and allocation of biomass to shoot tissues. Since plant growth rate is highly correlated with the proportion of carbon invested in leaf area (Potter and Jones 1977), the increased partitioning of dry matter to shoot tissues suggests that the inoculation effects observed in this study would persist into another growing season. Bowen (1973) and Black (1986) emphasize that nutrient relations of plants involve not only the uptake, but also the distribution and utilization of nutrients. The results support further investigation of mycorrhizal effects on nutrient distribution and nutrient-use efficiency.

# CHAPTER VI

## IMPLICATIONS FOR APPLIED FORESTRY AND RESEARCH

The dissertation began by underscoring the need to improve our knowledge of the efficacy of ectomycorrhizal fungi in different soil environments. Past research has shown that ectomycorrhizal inoculation has variable success in promoting conifer seedling field performance (Bledsoe et al. 1982, Shaw et al. 1987b, Trofymow and van den Driessche 1991). The overall objective of the dissertation was to examine the effects of cool soil temperature on mycorrhizal efficacy. Trappe (1977) stressed the importance of soil temperature to the efficacy of mycorrhizal fungi and the need for more knowledge of the adaptation of specific ectomycorrhizal fungi to soil temperatures at the planting site. Cool temperatures have been shown to eliminate the benefits normally derived from infection by vesicular-arbuscular mycorrhizal fungi (Moawad 1978, Smith and Roncadori 1986). Similar research has not been conducted for the ectomycorrhizal symbiosis.

White spruce seedlings were chosen as host plants. It is well known that the growth of planted white spruce in the sub-boreal forest region is often poor for several years after planting (Mullin 1963, Vyse 1981, Burdett et al. 1984, Butt 1986), and that cool soil temperatures contribute to the poor growth of spruce seedlings (Butt 1986, Binder et al. 1987). Artificial inoculation with specific ectomycorrhizal fungi has the potential to promote growth in white spruce planted in cool soils during this period. Research conducted by McAfee and Fortin (1989) suggests that artificial inoculation with selected fungi will benefit spruce seedlings more than pine seedlings. They planted 6-week-old non-mycorrhizal black

spruce and jack pine seedlings together on ten diverse reforestation sites in Quebec. Two months after planting, percent colonization of spruce roots by indigenous fungi was much weaker (< 25%) compared with that of pine roots (> 50%). In addition, the growth of spruce seedlings was positively correlated with the degree of root colonization.

Previous chapters addressed the relationship of soil temperature to four aspects of mycorrhizal efficacy: the ability to colonize roots of nursery seedlings (Chapter II); survival and colonization of new roots in forest soil (Chapter III); the ability to accelerate the acclimation of seedlings to cool soils during the transplant stress period (Chapter IV); and the ability to increase growth and nutrition of seedlings during a simulated field growing season (Chapter V). The purpose of this chapter is to discuss the implications of the main findings of the dissertation for applied forestry and research.

## Implications for Applied Forestry

The main findings of the dissertation research support the perspective that soil temperature has an important influence on all aspects of ectomycorrhiza efficacy. Although the results should be interpreted with caution because the experiments were conducted in controlled environment, they have some relevance to the production of inoculated seedlings in container nurseries and to the reforestation of cool forest sites.

1. Establishment of inoculant fungi in container nurseries

Growing mix temperature strongly influenced the extent of infection established on containerized seedlings. Overall, percent root infection was best for most fungal isolates, regardless of origin, at 16°C, with 6°C more detrimental to infection than 26°C. Primary infection (i.e., from "free" mycelial inocula) was inhibited less than secondary infection (i.e., from established mycorrhizae) in 6°C soil, indicating that nursery establishment of inoculant fungi would be inhibited by cool root-zone temperatures in the first three months after sowing. To determine the potential limitation of temperature to primary root colonization, it will be necessary to quantify infection success in more detail between 5 and 20°C root-zone temperatures. It is important to know the lower limits of the optimal temperature range for specific fungi. In culture, fungal growth decreases rapidly once this threshold temperature is reached (Dennis 1985, Samson and Fortin 1986).

The origin (field or nursery) of an isolate was not a predictor of response to temperature. Therefore, individual isolates must be screened for their response to low or high temperature in peat:vermiculite growing medium. This conclusion must be considered with caution because of (1) the limited number of isolates for each species of fungus, and (2) the different ages and cultural history of the various isolates used in the study. Nevertheless, the results are consistent with the results of *in vitro* studies of temperature response variation in ectomycorrhizal fungi (Dennis 1985, Samson and Fortin 1986, Cline *et al.* 1987), i.e., the temperature response of isolates is not related to their geographic origin.

Knowledge of the temperature tolerance of specific fungi will be useful in manipulating nursery infection. Rapid colonization by inoculant

fungi is important to prevent contamination by fungi indigenous (e.g., T. terrestris) to container nurseries. Fertilizer regimes have been shown to affect the composition of mycobionts on roots of container seedlings (Hunt 1989). Growing mix temperature could also be an important factor influencing the relative competitiveness of inoculant and indigenous nursery fungi. The two isolates of T. terrestris had a higher rate of mycorrhiza formation at 26°C than did most other inocula, indicating that this fungus would be more competitive at high growing mix temperatures. In the first three months after sowing, growing mix temperatures in southern B.C. container nurseries can exceed 30°C (Husted and Barnes 1987).

2. Importance of container stock with favorable root egress patterns

In general, inoculant fungi colonized a high proportion of the new roots formed outside the original container root mass during the three months after transplanting. This was attributed to several factors including (1) the successful establishment of inoculant fungi in the nursery and (2) the pattern of lateral root egress from the container plugs. New roots emerged from the entire root plug surface facilitating the spread of the inoculant fungi from old to new roots. Nursery practices which encourage this pattern of root growth should be an integral component of artificial inoculation programs, and probably container seedling production in general. Lateral root egress from the entire root plug would also speed the colonization of non-mycorrhizal container-grown seedlings by native soil ECM fungi.

3. Effect of inocula-induced changes in nutrition or size during the nursery production phase

Under the conditions of this study (i.e., slight nitrogen deficiency, cool soils), inoculation with specific mycorrhizae had more effect on seedlings physiology during the transplant stress period than did the soil temperature treatments (6 versus 12°C). Inoculation treatments influenced seedling acclimation in the 6°C soil as determined by net photosynthetic rate and resistance to water flow. These effects were related to differences in root size, nutrient content and distribution of nutrients between root and shoot tissues at the time of transplanting. Root biomass and shoot N and P were inversely related, respectively, to resistance to water flow and net photosynthetic rate.

The study results emphasize the importance of root size and morphology at the time of transplanting. In a review of seedling characteristics which correlate with field performance, Lavender (1988) noted that the growth of conifer seedlings after outplanting was often more closely related to root mass or volume at the time of planting than to root growth capacity, especially when new root growth was limited by droughty or cool soils.

Mycorrhizal inoculation has variable effects on the growth of container-grown seedlings, often resulting in smaller seedlings compared to non-inoculated seedlings (Castellano and Molina 1989). Cultural regimes may have to be modified to produce naturally or artificially inoculated mycorrhizal seedlings which meet the cull standards for nutrition and size. In particular, fungi which produce abundant sporocarps in the nursery may have detrimental effects on seedling nutrition, requiring compensatory fertilization.

Differences among inoculation treatments (including the control, non-inoculated) in resistance to water flow might have been greater if storage conditions had been more severe or if the seedlings had been deficient in phosphorus. It is important to recognize (1) that the seedlings were cold-stored in relatively mild conditions compared to standard practices in B.C., (2) that shoot nitrogen concentrations were low and (3) root and shoot phosphorus concentrations were adequate. Beneficial effects of VA infection on root hydraulic conductance have been attributed to improvements in phosphorus nutrition by the symbiotic association (Nelsen and Safir 1982). The magnitude of inoculation effects on net photosynthetic rate would likely decrease for seedlings with higher levels of shoot nitrogen (> 1.8%).

4. Cold-storage of white spruce seedlings

Binder et al. (1987) noted that "hot planting" (i.e., with no cold storage) of white spruce seedlings in late summer is very successful, in part because this practice avoids fall-lifting and lengthy storage. Regardless of mycorrhizal infection, the results provide evidence that long periods of cold storage at below-freezing temperatures reduce the ability of white spruce seedlings to acclimate to cool soils. Acclimation of all test seedlings was faster and levels of seedling resistance to water flow lower when compared to other studies (Grossnickle and Blake 1985, Grossnickle 1987, 1988) in which spruce seedlings were cold-stored for long periods at below-freezing temperatures. Comparisons of the water relations of pine and spruce species made by these and other researchers may be seriously confounded by differences in storage treatments between the two species.

Binder et al. (1987) also suggested that the high shoot/root ratios of white spruce nursery stock are not appropriate for cool forest sites. Furthermore, they hypothesized that the shoot growth check observed in spruce plantations may be a period during which seedlings allocate more carbon to root growth rather than shoot growth. The relatively high shoot to root growth in 6°C soil versus 12°C soil suggests that white spruce seedlings may have a limited ability to adjust their growth patterns to maximize nutrient and water uptake. Carbon allocation in spruce seedlings needs further investigation.

5. Competitiveness of inoculant fungi at low soil temperatures

In the absence of indigenous inoculum, the spread of inoculant fungi to new roots formed outside the container root was independent of the two test soil temperatures (6, 12°C); whereas, in the presence of indigenous inoculum, the percentage of new roots colonized by inoculant fungi was less in 12°C soil than in 6°C soil. In the warmer soil, accelerated root elongation increased the likelihood that short roots at the tips of elongating laterals were colonized by native soil fungi.

The results suggest that the competitiveness (based on percent root infection) of inoculant fungi may be inversely proportional to soil temperature at routine reforestation sites. The generality of this result should be tested with replication over a wider range of soil temperatures and in a variety of soil environments including those in which indigenous fungal inoculum is attached to living plant roots. The indigenous inoculum in this study was "free" (i.e., not attached to living plant roots) as would likely be the case on backlog reforestation sites. Root colonization by "free" inoculum is probably more sensitive to soil temperature than

established inoculum; the latter inoculum has a supply of carbon and does not have to compete with rhizosphere organisms for carbohydrates during the initial stages of root colonization.

It is often assumed that inoculant fungi will be replaced by indigenous fungi after planting. The results of this study suggest that the replacement process will be slow in cool soils, and mycorrhizae established in the nursery will continue to dominate the root systems at least for the first growing season. This finding has two implications for artificial inoculation programs. First, artificial inoculation with specific fungi has greater potential to influence seedling performance, either positively or negatively, on cool northern or high altitude reforestation sites compared with warmer southern sites. There is greater likelihood that selected fungi will dominate the root system after planting, especially on backlog reforestation sites which may have lower and less predictable amount of indigenous inoculum than recently logged sites or more southern reforestation sites (Danielson 1985).

Second, selection of fungi efficient in promoting conifer growth will be more important for outplanting on cool sites than in warmer ones. Mycorrhizae established in the nursery may continue to dominate the root system even though they may not benefit, or even negatively affect, seedlings planted in cool soils. A high priority should be given to examining the efficacy in cool soils of fungi which naturally infect container-grown spruce seedlings (e.g., Thelephora terrestris, E-strain and Amphinema byssoides) in cool, wet soils. Although the isolates of E-strain and H. crustuliniforme used in this study met the first two criteria of effective fungi (aggressive in nursery, persistence in field) they did not promote the growth of spruce seedlings, at least in the first three months

after outplanting, in cool soils.

6. Superiority of specific inocula

Averaged across all inocula, the presence of mycorrhizae at the time of transplanting, did not significantly improve the growth of spruce seedlings transplanted into cool soils. This result does not support the premise that the presence of "any" fungus is better than "no" fungus for the particular study conditions, i.e., non-saturating light intensity, cool moist acidic forest soil with good potential for root colonization by indigenous ectomycorrhizal fungi. On average, mycorrhizal infection increased seedling nutrition and thus the water-use efficiency of mature white spruce needles. This suggests that "any" fungus might be better than "none" in cool, dry soils or on sites with a high evapotranspiration potential. The controlled environment seedlings were subject to less moisture stress compared to outplanted spruce seedlings in northern B.C. Mid-day xylem pressure potentials of approximately -1.6 MPa have been recorded for outplanted white spruce seedlings in late spring and early summer (Binder et al. 1987), whereas mid-day xylem pressure potentials in this study were above -1.0 MPa.

Study results do support the second premise of artificial inoculation programs, i.e., some species of ectomycorrhizal fungi on certain sites are more beneficial than other fungi. The findings suggest mycorrhizal infections established in the nursery will influence, both negatively and positively, the field performance of spruce seedlings planted in cool soils (6-12$^{o}$C). It is important to recognize that establishment of forest soil fungi in the nursery benefited seedling growth after transplanting into cool soils even though non-inoculated seedlings were rapidly infected by forest soil fungi after transplanting. Inoculation treatments (e.g.,

forest floor, T. terrestris and L. bicolor) which most benefited total seedling biomass also increased the proportion of assimilated carbon invested in new photosynthetic capacity, suggesting that the growth benefits would continue into a second growing season.

Forest soil inoculum should be considered as an alternative to pure culture inoculum, if funding and technology is not available to investigate the efficacy of specific native ECM fungi. Seedlings inoculated with forest soil may benefit from the greater diversity of ectomycorrhizal fungi and rhizosphere organisms contained in soil inoculum compared to pure culture inoculum (Perry et al. 1987). However, there are several disadvantages to forest soil inoculum: (a) the need to handle large volumes of forest soil, (b) the risk of introducing soil-borne pathogens into container nurseries (c) the lack of control over the specific fungi forming mycorrhizae and (d) the ability of forest soil inoculum from cool sites to aggressively colonize roots in a warm container environment.

The operational problems associated with handling forest soil may be its major limitation as an inoculum source. I am not aware of any published reference documenting the introduction of pathogens with forest soil inoculum. In fact, forest soil may inhibit pathogens in nurseries, especially when the growing medium is relatively sterile[1]. Smith (1967) observed that Fusarium infection of pine seedlings declined after the seedlings were transplanted into forest soil. If a diverse population of ectomycorrhizal fungi is advantageous, the operational problems of applying forest soil need to be weighed against the difficulty of achieving mixed-species root infection from pure culture or spore inocula.

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Forest soil inoculum colonized roots well in 16 and 26°C peat:vermiculite mixes. However, it is important to note that it was applied to 8-week-old germinants. In an operational nursery, forest soil would likely be added to the growing mixture prior to sowing, and the inoculum would have to remain viable until germinants developed short roots. Further study is needed to determine the degree of root infection that could be expected if forest soil was applied under these conditions.

Of the fungal isolates studied, L. bicolor and T. terrestris appear to be most promising for further testing in cool, acidic moist forest soils. Although not too much should be inferred from the use of one isolate of each species, nevertheless these fungi were easy to establish in culture and in the nursery, strongly colonized new roots in cool soils and promoted seedling growth in cool soils. L. bicolor is a particularly promising ectomycorrhizal fungus for artificial inoculation and its basic physiology and genetics are being researched at the Université Laval (Kropp and Fortin 1988). This information will lead to the breeding of superior strains of L. bicolor.

In North America, T. terrestris is often considered a "weed fungus" which contaminates inoculation experiments and naturally infects conifer seedlings in both bare-root and container nurseries. T. terrestris had proven less effective than other fungi, especially Pisolithus tinctorius, on planting sites with a soil moisture deficit (Marx and Cordell 1987). However, T. terrestris may be better adapted to acidic poorly-drained forest soils than are other ectomycorrhizal fungi including P. tinctorius (Cruz 1974, Thomas and Jackson 1983, Wilson et al. 1987). In this study, T. terrestris infection significantly increased nitrogen uptake even in the 6°C soil. T. terrestris occurs over a wide range of tree ages and has good

potential to persist on outplanted seedlings (Thomas et al. 1983, Wilson et al. 1987). Thomas et al. (1983) observed T. terrestris mycorrhizae on nursery, 4- and 50-year-old outplanted Sitka spruce.

6. Selecting efficient mycorrhizal fungi

The efficacy of a particular ectomycorrhizal fungus will depend on three factors: (1) its aggressiveness in conifer nurseries (degree of root colonization), (2) its survival and colonization of new roots after planting and (3) its inherent ability to benefit the host plant (Trappe 1977). The main findings of this study emphasize the importance of gathering site-specific information on mycorrhiza efficacy. The well-established principle that forestry practices must be based on site-specific information must be extended to the mycorrhizal symbiosis. The effect of slow-release fertilizer on persistence of the E-strain isolate also points to the need to select specific fungi that interact favorably with silvicultural regimes.

In both the 6 and 12°C soils, inoculant fungi continued to be the dominant fungi colonizing new roots for the three months after transplanting. Persistence on new roots was positively correlated with the percentage of the container root plug colonized by the inoculant fungi before transplanting, emphasizing the importance of Trappe's first criterion of efficacy, i.e., fungi selected for artificial inoculation programs must be able to colonize roots aggressively in conifer nurseries.

The ability of established mycorrhizae to infect new roots formed after nursery-grown seedlings are planted into cool soils should be used as a screening variable when selecting potential fungal isolates for artificial inoculation of high altitude or latitude reforestation sites.

Reforestation of European sub-alpine forests has been conducted using "low temperature" strains of ectomycorrhizal fungi (Moser 1958). Studies of the temperature relations of naturally occurring ectomycorrhiza of Betula spp. growing on mine reclamation sites (Ingleby et al. 1985), suggest that spring and fall temperatures are more important to root colonization than are summer temperatures. These data should be collected in forest soils with indigenous microflora since the temperature response of fungi varies with the biological and chemical properties of the growing medium (Theodorou and Bowen 1971, Marx 1981). Differences between mycorrhizal fungi cannot be predicted from their growth in culture (Ingleby et al. 1985).

It is important to distinguish between the effects of soil temperature on primary and secondary infection processes. Primary infection of roots from "free" inoculum was inhibited more by cool soil temperatures than was secondary infection of the root system from mycorrhizal fungi already established on the root system. For example, neither T. terrestris isolate formed mycorrhizae in the 6°C peat:vermiculite mix, suggesting that this fungus may not colonize roots well in cool forest soil. However, established infections of T. terrestris successfully colonized new root growth of cold-stored seedlings transplanted into 6°C soil.

The main findings of the study showed that the first two criteria are appropriate only when the third criterion is satisfied. In this study, established H. crustuliniforme and E-strain fungi survived and colonized new roots in 6 and 12°C soils but did not promote the growth or nutrition of white spruce seedlings at these temperatures, supporting the conclusion of Marx et al. (1970) that ectomycorrhizal fungi do not benefit host plant

growth over the entire range of soil temperatures at which they form mycorrhizae. Marx and coworkers hypothesized that the physiological response of the host plant to particular soil temperatures determined whether a mycorrhizal symbiosis would be beneficial. White spruce seedlings did benefit from infection by other fungi (e.g., L. bicolor) in cool soils, indicating that interactions between the host plant and specific fungi are equally, or more important, than host plant physiology alone, in determining the benefits derived from the symbiosis in cool soils.

If E-strain and H. crustuliniforme had been less aggressive, in the nursery and after transplanting, allowing greater colonization by the indigenous fungal population, their depressive effect on seedling growth may have been less pronounced. Aggressive strains of fungi established in the nursery may impose an excessive carbon drain on outplanted seedlings (Stenström et al. 1990), particularly when carbon assimilation is restricted by low nutrient availability, droughty or cool soils. In addition, they can inhibit the colonization of new roots by a diversity of indigenous fungi adapted to the planting environment (Stenström et al. 1990).

## Implications for Forestry Research

These will be disussed under three broad areas. The first focuses on the need to broaden the prevailing concept of the mycorhizal symbiosis to include a wider range of host response. The second discusses the kinds of information reported in mycorrhizal studies, the methods used to assess mycorrhizae and the implications of the dissertation results to other areas of physiological research. The third area of concern is the current

emphasis on physiological and biochemical investigations of mycorrhizal efficacy.

1. Need for more detailed investigation of the full range of host response to mycorrhizal infection

The mycorrhizal symbiosis is commonly viewed as a mutualistic relationship between a fungus and host plant in which both partners benefit. It is commonly accepted that the mycorrhizal symbiosis is a good one in nature. However, the detrimental effects of specific fungi observed in this study are not without precedent. In their review of the mycorrhizal literature, Harley and Smith (1983) found published and unpublished reports documenting cases where the host plant did not benefit and even those where the growth of the host plant was depressed.

Harley and Smith (1983) and Wilcox (1983) emphasized the need to examine the fungus-host plant relationship in a broader context using the original definition of a symbiotic relationship. This definition includes (1) relationships in which one partner benefits but the other neither benefits nor loses (commensalism), and (2) those in which one partner benefits at the expense of the other (parasitism).

Harley (1969) hypothesized that the degree of benefits to the host plant depended on the balance between two factors (1) the benefit of improved nutrition and (2) the cost of carbon use by the fungal partner. Environmental conditions appear to influence the benefits accruing to each partner in the symbiosis. Growth depressions are most likely to occur in low light conditions and in very low or high nutrient regimes (Hatch 1937, Trappe 1977, Harley and Smith 1983 and references therein) where the benefits of improved nutrition may note compensate for the carbon cost of

the symbiosis. In low light environments (e.g., conifer plantation with brush competition), the rate of net photosynthesis may be too low to compensate for the carbon demand of mycobionts. This also may be the case when net photosynthesis and nutrient availability are reduced by cool soil temperatures. On average, mycorrhizal infection did not improve nutrient uptake in the 6°C soil but did in the 12°C soil.

Researchers have tended to ignore reports of negative or no host plant response to mycorrhizal infection. Harley and Smith (1983, p. 186) noted:

> "Results of this kind which show no effect of mycorrhizal infection or even a decrease in growth rate may be found scattered through the literature but are not often stressed."

More rigorous investigation of the effects of environment on carbon and nutrient exchanges between symbionts, and on the degree of host plant response to infection, are essential in order to estimate the potential benefits of mycorrhizal infection and to apply mycorrhizal technology to particular reforestation problems. Identification of environments or conditions which are likely to reduce host plant response to infection provides focus for artificial inoculation programs. Selection of specific fungi for these conditions is potentially rewarding. The results show that although some fungi were detrimental in the experimental conditions (cool, acidic, moist soils); others were very beneficial, increasing spruce seedling growth by 20 to 30%.

2. Experimental technique

When I began comparing my results to other studies, I quickly realized that published results of field and controlled environment studies

(including my own) would be more valuable to other workers if they included descriptions of the initial nutritional status of seedlings, the relative growth rates of the various inoculation treatments, and the environmental conditions of the experiment. It is misleading to generalize about the benefits of a specific mycorrhizal fungus to a particular host plant without describing the nutritional status of the seedlings and the environmental conditions (e.g., acidity, temperature, fertility, moisture status of soil) of the study. Initial size differences are usually reported. However, the results of this study do not support the contention of Mexal (1980) that the effects of various inoculation treatments on the survival and growth of outplanted seedlings are primarily due to differences in the initial size of seedlings. Equally important to growth in this study were initial nutrient contents and distribution of nutrients, and relative growth rates after transplanting.

Reliable assessments of mycorrhiza formation are an integral part of mycorrhizal studies. At least for small spruce seedlings, visual or low power magnification assessments may not accurately classify infected and uninfected short roots. At low power magnification, short roots of spruce seedlings with a Hartig net but lacking a well-developed mantle may be classified as non-mycorrhizal. Formation of the Hartig net, which is the diagnostic criteria for mycorrhiza formation, preceded mantle formation. The early stages of infection, have been shown to influence host plant physiology (Nylund and Unestam 1982) and their detection is important to understanding the mycorrhizal symbiosis. Gross characteristics (e.g., lack of root hairs, distinctive colours, branching and swelling) commonly used to identify infected roots visually or at low power magnification can be found on non-mycorrhizal root systems (Nylund and Unestam 1982) and are not

reliable indicators of mycorrhiza formation.

Finally, the results of this study have implications for any study of seedling physiology. Inoculation treatments accounted for a significant proportion of the variability in physiogical parameters (e.g., net photosynthetic rate, xylem pressure potential) especially in the first five weeks of the experiment. It is important for physiologists to be aware of this source of variability and its influence on physiological parameters when they select "uniform" populations of seedlings for experiments and when they interpret physiogical responses.

3. Direction for future research

The inconsistent results of artificial inoculation experiments reflects many factors including differences in (1) the physiology of ectomycorrhizal fungi (Harley and Smith 1983), (2) the adaptation of fungal symbionts to the environment of the planting site (Trappe 1977, Parke 1985), and (3) the inability of inoculant fungi to persist in the field in the presence of indigenous inoculum (Trappe 1977) or fungal grazers (Fitter 1985).

Research on mycorrhizal efficacy has focused on the physiology of mycorrhizae in culture or in symbiosis (usually excised mycorrhizae) with the aim of discovering the characteristics which cause one fungal species or strain to be more effective than others in promoting the growth or nutrient uptake of host plants. One long-term goal of artificial inoculation research is to select and breed fungi which have superior abilities to promote host plant growth. Knowledge of the biochemical and physiological attributes which endow fungi with superior efficacy is considered essential to achieve this goal (Harley 1985).

So far, physiological research has not revealed the underpinnings of efficiency. In a review of past mycorrhizal research, Harley (1985, p. 28) concludes:

> "But regardless of more than 50 years of research on their cultural behavior and growth physiology we do not know, at all, what properties an efficient mycorrhizal fungus should possess. Although we know that they vary in effectiveness both between species and between variants of a single species, it is clear that we have not yet asked the right questions."

Harley (1985) recommends asking more questions in the areas of biochemistry and intermediate physiology of mycorrhizal fungi. I wonder, however, if these questions are the most "profitable" ones to meet the stated applied objectives of selecting superior strains of fungi.

First, physiological and biochemical characteristics (e.g., high rates of hormone, acid phosphatase synthesis) may not be useful criteria for selecting fungi. The rates of physiological processes, such at photosynthetic rate, are often poorly correlated with plant productivity; plant productivity is more dependent on the amount of photosynthetic capacity (leaf area) and the length of time it is functional (Hunt 1982). Second, breeding for specific physiological characteristic may not be a desirable goal unless the host plant lives in a physical and biological environment which is uniform in time and space. Jones (1983) cautioned that breeding for specific attributes, such as drought resistance, is risky for plants grown in fluctuating environments. Breeding for lower hydraulic conductivity, for example, will improve water conservation in droughty years but will not be beneficial to plant growth in years when water is adequate.

Third, differences in efficacy between ectomycorrhizal fungi are just as likely to result from difference in adaptation to different environments

as from differences in fungal physiology. We know that there is considerable ecological variation within and between fungal species and we have the opportunity to select fungi which are adapted to a broad range or particular soil environments. Although it is recognized that it is essential to select fungi that are adapted to particular environments (Trappe 1977, Harley and Smith 1983, Perry et al. 1987), there has been little research directed to studying the mycorrhizal symbiosis in different soil environments or the efficacy of specific ectomycorrhizal fungi in different soil environments. As a result:

> "we are still in the "Dark Ages" with regard to applying these features of ecological specialization to agriculture, forestry or in restoring native vegetation" (Parke 1985, p. 107).

Given the relatively small amount of research directed to the ecology of mycorrhizal symbiosis, it can be argued that questions concerning ecology may have more potential to improve our understanding of efficacy and the selection of superior fungi than those concerned with the biochemistry or physiology of mycorrhizal fungi. Field studies of the seasonal periodicity of mycorrhiza development, root and shoot growth of conifer seedlings would improve our understanding (1) of the processes affecting persistence of inoculant fungi after planting and (2) the interactions between fungal and host plant development and mycorrhizal symbioses.

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# APPENDIX A

## INDEX OF COMMON AND SCIENTIFIC NAMES

| | |
|---|---|
| arbutus | Arbutus menziesii Pursh. |
| ash | |
| green ash | Fraxinus pennsylvanica Marsh. |
| birch | |
| silver birch | Betula pendula Roth |
| Douglas-fir | Pseudotsuga menziesii (Mirb.) Franco |
| fir | |
| amabilis fir | Abies amabilis Dougl. ex Forbes |
| hemlock | |
| western hemlock | Tsuga heterophylla (Raf.) Sarg. |
| pine | |
| eastern white pine | Pinus strobus L. |
| Italian stone pine | P. pinea L. |
| jack pine | P. banksiana Lamb. |
| loblolly pine | P. taeda L. |
| lodgepole pine | P. contorta Dougl. ex. Loud. |
| radiata pine | P. radiata D. Don |
| red pine | P. resinosa Ait. |
| Scots pine | P. sylvestris L. |
| slash pine | P. elliotii Engelm. |
| spruce | |
| black spruce | Picea mariana (Mill.) B.S.P. |
| Engelmann spruce | P. engelmannii Parry |
| Sitka spruce | P. sitchensis (Bong.) Carr. |
| white spruce | P. glauca (Moench) Voss |
| soybean | Glycine max L. Merr. |

# APPENDIX B

## KEY CHARACTERISTICS OF MYCORRHIZA FORMED BY INOCULANT AND INDIGENOUS FUNGI

### 1. Inoculant Fungi

**E-strain or** Complexipes moniliformis Walker (Danielson 1982, Danielson and Pruden 1989)

| | |
|---|---|
| Mycorrhizae: | simple, medium to dark brown, glabrous with sparse hyphae. |
| Mantle: | thin, discontinuous and textura intricata. |
| Extramatrical (EM) hyphae: | 4-5 μm diam., simple septate with Woronin bodies visible in young hyphae. Hyphae stiff, pale and smooth when young to tawny coloured with blister-like ornaments on mature hyphae. |

Amphinema byssoides (Danielson and Pruden 1989)

| | |
|---|---|
| Mycorrhizae: | simple, covered with abundant to dense whitish to yellow mycelium. |
| EM hyphae: | 2-3 (-4) μm in diam. with keyhole clamps, finely ornamented, color varying from pale cream to yellow. |
| Mycelial strands | common, whitish to yellow. |
| Other: | hyphae, mycelial strands and ectomycorrhizae turning bright yellow in 3% KOH. |

Hebeloma crustuliniforme 5249

| | |
|---|---|
| Mycorrhiza: | simple, woolly, covered with abundant to dense mycelium, white. |
| Mantle: | loose, textura intricata. |
| EM hyphae: | abundant, binding roots and soil together, 3-4 μm diam., most septa clamped, hyaline, smooth to verrucose with fine, deciduous ornaments; no colour change in 3% KOH. |

Laccaria bicolor

Mycorrhizae: smooth to subfloccose, plump, pale violet-brown to deep brown.

Mantle: outer layer-textura intricata, densely interwoven, frequently forked, septate hyphae 3-5 μm diam.

EM hyphae: smooth, hyaline, clamped, 2.5-4 μm diam, frequent branches, frequent elbow-like protrusions.

inner layer-textura epidermoidea, hyphae 4-10 μm in diam.

Mycelial strands: uncommon, not organized.

Thelephora terrestris

Mycorrhizae: simple, light to grey-brown, cystidia frequent.

EM hyphae: 3-4 μm diam.

Mantle: outer layer-loose textura intricata, hyphae 2-7 μm diam., septate with few clamp connections.
inner layer-textura epidermoidea, hyphae 5-7 μm in diam., no clamps.

Cystidia: 2-3 μm diam., up to 100 μm, septate with a clamp connection at the mantle surface, some retraction septa as described by Schramm (1966).

Mycelial strands: frequent, undifferentiated; cystidia present.

2. ECM Indigenous to Forest Soil Mixture

Cenococcum geophilum (Chilvers 1968, Danielson and Pruden 1989)

Mycorrhizae: black, simple, club-shaped with stiff,dark hyphae radiating from the mantle.

Mantle: stellate pattern of cells in plan view.

EM hyphae: dark brown (500x), 4-6 μm diam., smooth, simple-septate with Woronin bodies.

**Tuber-like** (Danielson and Pruden 1989)

Mycorrhizae: plump, pallid, becoming darker reddish-brown with age; surface smooth or bearing hyaline cystidia.

Mantle: textura "jigsaw" to textura epidermoidea on young tips with cells up to 30 x 4 μm.

Cystidia: thin-walled, simple-septate at base, 4 μm at base, up to 110 μm long and pointed at the tip.

**Tomentella-like with cystidia** (Danielson and Pruden 1989)

Mycorrhizae: simple, dark brown; many hyphoid cystidia radiating from tip.

Mantle: textura angularis to textura "jigsaw" encrusted with discontinuous pigment; cells up to 4 x 25 μm.

Cystidia light gold, up to 60 μm x 2-3 μm.

**Tomentella-like without cystidia** (Danielson et al. 1984d, Danielson and Pruden 1989)

Mycorrhizae: simple, dark brown.

Mantle: textura angularis to textura "jigsaw" encrusted with discontinuous pigment; cells up to 4 x 25 μm.

EM hyphae: 3-5 μm, simple septate or clamped with thick yellow-brown walls; hyphae emerge from inflated cells (15-20 μm diam.) at the mantle surface; stiff and rarely branched.

Mycelial strands: rare, undifferentiated.

**Hebeloma-like** (Danielson and Pruden 1989)

Mycorrhiza: simple, covered with abundant to dense mycelium, white to tan.

Mantle: textura intricata.

EM hyphae: 3-4 μm diam., most septa clamped, hyaline; no colour change in 3% KOH.

Mycelium radicis atrovirens (Visser 1986, Thomas and Jackson 1979)

Mycorrhizae: dark brown to black, simple, often covered with a loose net of dark hyphae.

Mantle: irregular, discontinuous textura epidermoidea, hyphae associate with mantle are typically sinuous, smooth, and 3-4 μm diam.

EM hyphae: olive-brown, 2-2.5 μm, verrucose, simple septa.

Unknown type 1

Mycorrhizae: simple, light yellow brown.

Mantle: discontinuous, textura intricata, strands of hyphae (formed by parallel hyphae) form a loose interwoven net, hyphae 1.5 μm diam.

EM hyphae: 1-1.5 μm diam, hyaline, septate, no clamps.

Sphaerosporella-like (Danielson 1984a)

Mycorrhizae: simple, brown.

Mantle: discontinuous textura epidermoidea, hyphae smooth, 4-15 μm.

EM hyphae: coarse, cinnamon-brown, smooth or verrucose, 4-12 μm diam., indented at septa.

# APPENDIX C

## NUTRIENT CONCENTRATIONS AT 0, 5, AND 12 WEEKS

| Week | 6°C soil | | | | 12°C soil | | | |
|---|---|---|---|---|---|---|---|---|
| | N | P | Ca | Fe | N | P | Ca | Fe |
| Uninoculated seedlings | | | | | | | | |
| | | | mature shoot tissue | | | | | |
| 0 | 1.1 | 0.26 | 0.49 | 74 | 1.2 | 0.24 | 0.57 | 70 |
| 5 | 0.5 | 0.10 | 0.32 | 40 | 0.5 | 0.12 | 0.33 | 40 |
| 12 | 0.5 | 0.12 | 0.32 | 50 | 0.6 | 0.20 | 0.48 | 41 |
| | | | root tissue | | | | | |
| 0 | 1.6 | 0.28 | 0.43 | -- | 1.2 | 0.23 | 0.37 | -- |
| 5 | 1.3 | 0.18 | 0.32 | -- | 1.1 | 0.18 | 0.33 | -- |
| 12 | 1.4 | 0.20 | 0.34 | -- | 1.2 | 0.18 | 0.35 | -- |
| | | | new foliage | | | | | |
| 5 | 0.7 | 0.19 | 0.12 | 22 | 0.7 | 0.19 | 0.20 | 23 |
| 12 | 0.7 | 0.17 | 0.27 | 25 | 0.9 | 0.22 | 0.42 | 32 |
| Seedlings inoculated with forest soil | | | | | | | | |
| | | | mature shoot tissue | | | | | |
| 0 | 1.4 | 0.35 | 0.49 | 69 | 1.4 | 0.36 | 0.56 | 65 |
| 5 | 0.5 | 0.14 | 0.28 | 24 | 0.6 | 0.17 | 0.40 | 30 |
| 12 | 0.5 | 0.15 | 0.27 | 32 | 0.8 | 0.21 | 0.40 | 44 |
| | | | root tissue | | | | | |
| 0 | 1.6 | 0.35 | 0.42 | -- | 1.6 | 0.35 | 0.42 | -- |
| 5 | 1.3 | 0.18 | 0.32 | -- | 1.4 | 0.19 | 0.31 | -- |
| 12 | 1.3 | 0.21 | 0.32 | -- | 1.1 | 0.18 | 0.28 | -- |
| | | | new foliage tissue | | | | | |
| 5 | 0.8 | 0.20 | 0.07 | 23 | 0.8 | 0.17 | 0.16 | 21 |
| 12 | 0.6 | 0.15 | 0.20 | 25 | 1.0 | 0.21 | 0.44 | 40 |

Seedlings inoculated with E-strain

mature shoot tissue

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 0 | 0.9 | 0.28 | 0.46 | 46 | 0.9 | 0.25 | 0.42 | 55 |
| 5 | 0.5 | 0.13 | 0.29 | 40 | 0.5 | 0.12 | 0.29 | 35 |
| 12 | 0.4 | 0.10 | 0.30 | 33 | 0.8 | 0.23 | 0.49 | 52 |

root tissue

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 0 | 1.9 | 0.48 | 0.42 | -- | 1.8 | 0.36 | 0.46 | -- |
| 5 | 1.5 | 0.24 | 0.34 | -- | 1.4 | 0.27 | 0.33 | -- |
| 12 | 1.4 | 0.21 | 0.32 | -- | 1.7 | 0.23 | 0.35 | -- |

new foliage tissue

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 5 | 0.8 | 0.20 | 0.17 | -- | 0.7 | 0.19 | 0.17 | -- |
| 12 | 0.5 | 0.13 | 0.23 | -- | 1.7 | 0.22 | 0.57 | -- |

Seedlings inoculated with L. bicolor

mature shoot tissue

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 0 | 1.2 | 0.39 | 0.48 | 39 | 1.1 | 0.34 | 0.51 | 58 |
| 5 | 0.5 | 0.17 | 0.33 | 59 | 0.7 | 0.22 | 0.40 | 34 |
| 12 | 0.6 | 0.19 | 0.30 | 30 | 0.8 | 0.28 | 0.45 | 38 |

root tissue

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 0 | 1.4 | 0.33 | 0.41 | -- | 1.2 | 0.25 | 0.37 | -- |
| 5 | 1.9 | 0.29 | | -- | 1.7 | 0.30 | | -- |
| 12 | 1.7 | 0.23 | 0.28 | -- | 1.5 | 0.22 | 0.32 | -- |

new foliage tissue

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 5 | 0.8 | 0.22 | 0.09 | 20 | 0.8 | 0.20 | 0.17 | 19 |
| 12 | 0.7 | 0.20 | 0.23 | 19 | 0.9 | 0.26 | 0.47 | 30 |

Seedlings inoculated with H. crustuliniforme

mature shoot tissue

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 0 | 0.8 | 0.20 | 0.41 | 62 | 0.9 | 0.27 | 0.48 | 68 |
| 5 | 0.5 | 0.14 | 0.28 | 35 | 0.5 | 0.16 | 0.37 | 35 |
| 12 | 0.4 | 0.13 | 0.35 | 30 | 0.7 | 0.20 | 0.47 | 90 |

root tissue

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 0 | 1.3 | 0.25 | 0.38 | -- | 1.4 | 0.25 | 0.43 | -- |
| 5 | 1.4 | 0.20 | 0.33 | -- | 1.3 | 0.20 | 0.34 | -- |
| 12 | 1.3 | 0.17 | 0.35 | -- | 1.3 | 0.16 | 0.36 | -- |

Seedlings inoculated with H. crustuliniforme (cont.)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | new foliage tissue | | | | | |
| 5 | 0.8 | 0.24 | 0.16 | -- | 0.7 | 0.17 | 0.16 | -- |
| 12 | 0.5 | 0.14 | 0.26 | -- | 0.9 | 0.20 | 0.46 | -- |

Seedlings inoculated with A. byssoides

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | mature shoot tissue | | | | | |
| 0 | 1.1 | 0.27 | 0.40 | 60 | 1.1 | 0.25 | 0.43 | 64 |
| 5 | 0.6 | 0.13 | 0.32 | 38 | 0.6 | 0.16 | 0.36 | 41 |
| 12 | 0.5 | 0.17 | 0.33 | 39 | 0.8 | 0.22 | 0.50 | 45 |
| | | | root tissue | | | | | |
| 0 | 1.4 | 0.30 | 0.41 | -- | 1.5 | 0.30 | 0.39 | -- |
| 5 | 1.5 | 0.19 | 0.33 | -- | 1.4 | 0.19 | 0.34 | -- |
| 12 | 1.5 | 0.19 | 0.35 | -- | 1.3 | 0.17 | 0.31 | -- |
| | | | new foliage tissue | | | | | |
| 5 | 0.8 | 0.21 | 0.09 | 24 | 0.8 | 0.19 | 0.16 | 23 |
| 12 | 0.8 | 0.19 | 0.22 | 21 | 1.0 | 0.23 | 0.41 | 33 |

Seedlings inoculated with T. terrestris

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | mature shoot tissue | | | | | |
| 0 | 0.8 | 0.21 | 0.42 | 77 | 0.8 | 0.24 | 0.42 | 71 |
| 5 | 0.5 | 0.14 | 0.26 | 30 | 0.6 | 0.21 | 0.34 | 39 |
| 12 | 0.6 | 0.18 | 0.28 | 51 | 1.0 | 0.29 | 0.47 | 56 |
| | | | root tissue | | | | | |
| 0 | 1.3 | 0.25 | 0.41 | -- | 1.1 | 0.23 | 0.39 | -- |
| 5 | 1.5 | 0.23 | 0.30 | -- | 1.4 | 0.24 | 0.34 | -- |
| 12 | 1.5 | 0.20 | 0.27 | -- | 1.8 | 0.29 | 0.34 | -- |
| | | | new foliage tissue | | | | | |
| 5 | 0.8 | 0.23 | 0.08 | 25 | 0.8 | 0.18 | 0.17 | 24 |
| 12 | 0.8 | 0.18 | 0.24 | 42 | 1.2 | 0.25 | 0.43 | 38 |

NOTE: Sufficient tissue was not available to analyze for active Fe in all root and new foliage samples.