EFFECTS OF SEED SOURCES AND FUNGI ON ECTOMYCORRHIZAL FORMATION AND GROWTH OF CONTAINERIZED TAMARACK SEEDLINGS

by

© Hong Zhu

B. Sc., Northeastern For. Inst.

A thesis submitted in partial fulfillment

of the requirements for the degree of

Master of Science in Forestry

Lakehead University

Thunder Bay, Ontario

August, 1985

ABSTRACT

Zhu, H. 1985. Effects of seed sources and fungi on ectomycorrhizal formation and growth of containerized tamarack seedlings. Major Advisor: Dr. S. Navratil.

Additional Key Words: Larix laricina, mycorrhizal inoculation, root morphology, provenance, open-pollinated family, host specificity, pure culture synthesis.

The objectives of this study were to investigate the host specificity of mycorrhizal fungi to tamarack (Larix laricina (Du Roi) K. Koch.) in pure culture synthesis and to examine the effects of seed sources and fungal species on the ectomycorrhizal formation and growth of containerized tamarack seedlings in a greenhouse. Of nine mycorrhizal fungi tested in pure culture synthesis, Cenococcum geophilum, Laccaria laccata, Laccaria proxima, Hebeloma crustuliniforme and Pisolithus tinctorius demonstrated their ability to develop ectomycorrhizae with tamarack. Amanita porphyria, Rhizopogon vinicolor, Suillus granulatus and S. tomentosus failed to form ectomycorrhizae. The number of confirmed mycorrhizal symbionts of tamarack has been increased from three, previously known, to seven from the results of the pure culture synthesis.

Containerized seedlings of tamarack, representing four provenances and 17 open-pollinated families in Ontario, were inoculated with vegetative inocula of four fungal species in the greenhouse. During an 18-week period, L. laccata, P. tinctorius, and C. geophilum formed ectomycorrhizae with 60, 12 and 7% of the total feeder roots, respectively. Suillus granulatus failed to produce any mycorrhizae. The mycorrhizal formation was strongly governed by the seed sources and seed source x fungus interactions. The greatest difference in mycorrhizal formation by

L. laccata was 20% between provenances and 32% between families. Seedlings inoculated with L. laccata exhibited the best growth in diameter, shoot volume and dry weight. The development of root systems was differentially affected by different fungal inoculations. Feeder root proliferation was stimulated by C. geophilum, and lateral root growth was inhibited by L. laccata. Significant provenance effects were also found in shoot height and root descriptive variables. Although the effects of family-within-provenance were significant in most of the seedling traits, family variation was generally not constant, varying with fungal inoculations. Genetic correlations were positive between lateral root and shoot traits and between feeder root frequency and mycorrhizal formation, but negative between lateral root and mycorrhizal formation.

It is recommended that L. laccata is a suitable fungal species and C. geophilum, H. crustuliniforme, L. proxima and P. tinctorius are potential fungi for mycorrhizal inoculation of containerized seedlings of tamarack. This study reinforces the concept that seed sources should be tested with target fungi before a wide scale mycorrhizal inoculation is conducted.

ACKNOWLEDGEMENTS

I wish to express sincere thanks to my major advisor, Dr. S. Navratil, without whose guidance and encouragement, this thesis could not have been started and accomplished.

Dr. W.H. Parker and Dr. P. Knowles, also members of the Advisory Committee, provided much-needed advice, especially in genetic aspects of the research. For their help, I am very grateful.

I am also indebted to:

Dr. K.O. Higginbotham, Department of Forest Science, University of Alberta, for his constructive criticism and review of the manuscript.

Dr. A.D. Macdonald and Dr. T. Dickinson for their advice in the microtechnique of mycorrhizae.

Mr. L. Sevean and Mr. S. Elliott for their technical assistance in the Laboratory.

My classmate and good friend D. Lenthall for his assistance in proof-reading of the manuscript.

Petawawa National Forestry Institute, Chalk River, Ontario, and Sylvan Spawn Laboratory, Inc., Kittanning, Pennsylvania for providing tamarack seeds and mycorrhizal inocula for this research, respectively.

The Canadian Forestry Service and the School of Forestry, Lakehead University for the provision of research assistantships.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Larix laricina (Du Roi) K. Koch, commonly known as tamarack or eastern larch, is the most widely distributed conifer in Northern America (Roe, 1957). The tree occurs from Alaska, along the northern limit of coniferous tree distribution, through Canada to Newfoundland, and extends southward into the north-central and northeastern United States (Roe, 1957). Recently, tamarack has received increasing attention as an alternative conifer species for reforestation in Canada and the northern United States because of its relatively rapid growth rate, tolerance to poorly drained sites, and resistance to scleroderris canker and spruce budworm (Einspahr et al, 1984). In addition to its silvical characteristics, the genetics and tree improvement potential of tamarack are also of great interest. An intensive study of the population structure and genetic variation of the tree is now being carried out in northern Ontario (Parker[1], 1985, pers. commu.). Breeding and selection programs on tamarack are also underway which are designed to improve the growth performance of the species (Coles, 1979; Fowler, 1979; Fowler, et al, 1982).

As forest regeneration and mycorrhizal programs have intensified, the concept of inoculating seedlings with specific mycorrhizal fungi to improve their growth and survival in outplanting sites has been applied to the production of containerized seedlings (Marx and Barnett, 1975). Results show that future production of containerized seedlings and nursery stock for reforestation are not only possible with favorable shoot and root sizes but also with well-developed mycorrhizae (Marx et al, 1982). To meet the requirements of future forest practices, recent mycorrhizal research has focused on the selection of mycorrhizal

---

[1] School of Forestry, Lakehead University, Thunder Bay, Ontario, Canada.

symbionts and inoculation techniques. Evidence indicates that successful mycorrhizal inoculation which could result in abundant mycorrhizal formation must be based on a good understanding of the relationships between host and symbiont. Although thousands of fungi and numerous host trees have been studied for their mycorrhizal relationships (Trappe, 1962), little is known about mycorrhizal symbionts on tamarack, and there has been no report of mycorrhizal inoculation on tamarack. Research on mycorrhizal associations with tamarack and selection of suitable fungi for inoculation of the tree seedlings is greatly needed.

Among many criteria for the selection of mycorrhizal fungi, the compatibility between host and fungus genotypes has been emphasized recently (Marx, 1980; Cline and Reid 1982; Molina and Trappe, 1982; Navratil, 1985). Because of the wide distribution and suspected genetic diversity of tamarack, fundamental studies on tamarack mycorrhizae should consider the influence of host genotypes. In addition, genetic control and variation in root system development, root response to mycorrhizal inoculation, and interrelationships among root, shoot, and mycorrhizae could also be of interest to both tree improvement and reforestation programs.

The primary goal of this study was to investigate, from a genetic viewpoint, mycorrhizal relationships between tamarack and selected fungi. To accomplish this, the study was designed with the following objectives:

1. to determine the host specificity of ectomycorrhizal fungi to tamarack,
2. to evaluate the response of tamarack containerized seedlings to the inoculation of ectomycorrhizal fungi,
3. to examine the effects of seed sources on mycorrhizal formation of containerized seedlings, and
4. to examine the effects of mycorrhizal fungi on root development of containerized seedlings.

LITERATURE REVIEW

MYCORRHIZAL SYMBIONTS OF TAMARACK

Tamarack has been long known as a host of ectomycorrhizal symbionts, but this knowledge arises from only a few observations. Cooley (1904) and Beeftink (1951) reported that ectomycorrhizae were formed on tamarack seedlings in natural forests, but there were not detailed descriptions of the mycorrhizae, and the fungal species were not identified. Trappe (1962) listed three fungi, Cenococcum geophilum Fr., Gomphidius maculatus (Scop. ex Fr.) Fr., and Hygrophorus pseudolucorum A. H. Smith & Hesler, which formed mycorrhizae with tamarack. In a recent study Malloch and Malloch (1981) reported that C. geophilum and a number of unidentified fungi formed ectomycorrhizae or ectendomycorrhizae with tamarack in boreal forests of northeastern Ontario.

Relatively more mycorrhizal studies have been made on other larch species. Dominik (1950) reported that the fungi, Suillus grevillei (Klotzsch) Sing. and Boletus erythropus (Fr.) Pers., formed ectomycorrhizae with European larch (Larix decidua Mill.) in a natural stand in Poland. A successful inoculation using S. grevillei and Boletus caripes (Opat.) Kalchb. on paper-pot seedlings of European larch was made by Gobl (1974). Molina (1980) tested 15 ectomycorrhizal fungi and found that two of them, Laccaria laccata (Scope. ex Fr.) Bk. & Br. and C. geophilum, formed abundant mycorrhizae on containerized seedlings of western larch (Larix occidentalis Nutt.). In a pure culture synthesis study (Molina and Trappe, 1982), the fungi, L. laccata, Pisolithus tinctorius (Pers.) Coker & Couch, and S. grevillei, showed a great ability to form ectomycorrhizae with western larch seedlings. The

success of ectomycorrhizal formation by P. tinctorius on western larch supported the hypothesis by Trappe (1962) that although sporocarps may only form with a particular host or species within a genus, mycorrhizal formation with other hosts should not be ruled out.

Based on this review and the present knowledge of mycorrhizal fungus selection (Gobl 1975; Marx and Kenney, 1982; Navratil, 1981; Trappe, 1977), the fungal species, C. geophilum, L. laccata, and P. tinctorius, appear to have potential of forming ectomycorrhizae with tamarack through artificial inoculation. In addition to their mycorrhizal relationships with larches, their broad host range, adaptation to artificial inoculation, and rapid vegetative growth (except C. geophilum) also indicate that these fungi could be suitable for mycorrhizal inoculation of containerized seedlings of tamarack.

EFFECTS OF MYCORRHIZAL FUNGI ON HOST TREES

Several thousand papers have been published on mycorrhizae (Hacskaylo and Tomkins, 1973). Most of these papers relate to forest tree species and define the beneficial aspects of mycorrhizae to trees. Many forest trees, such as pine species, cannot grow without ectomycorrhizae in forest soil ecosystems. Trees with abundant ectomycorrhizae have a much larger, physiologically active, root-fungus area for nutrient and water absorption than the trees with few or no ectomycorrhizae. This increase in root surface area comes both from the multi-branching habit of ectomycorrhizae and from the extensive vegetative growth of fungal hyphae from the ectomycorrhizae to the soil. Ectomycorrhizae are able to absorb and accumulate nitrogen, phosphorus, potassium, and calcium in the fungus mantles more rapidly, and for longer periods of time. In the soil, ectomycorrhizae

are also able to break down certain complex minerals and organic substances into simple elements and transmit them to the roots. The tolerances of trees to drought, high soil temperatures, soil toxins, and extremes of soil pH appear to be increased by ectomycorrhizal formation (Marx, 1977a; and many others). The protective role of ectomycorrhizae against root diseases is an additional important aspect. This has been documented for feeder root pathogens, such as Phytophthora (Marx, 1969).

Because of their numerous benefits to trees, many mycorrhizal fungi have been intensively studied for inoculation of containerized or bare root seedlings in greenhouses, nurseries, and even in outplanting sites (Mikola, 1973). Seedling responses to mycorrhizal infection vary and are dependent on the fungi, the hosts, and growing conditions. In outplanting sites and nursery beds, mycorrhizal infections usually result in a positive response of seedlings, including increases in biomass, height and diameter growth, as well as survival (Marx, 1977a). In greenhouses, however, containerized seedlings often exhibit negative or a no-growth response to mycorrhizal formation. This is because the containers limit extension of fungal mycelia and root growth to obtain additional nutrient supplies. However, abundant mycorrhizal formation in greenhouses does help containerized seedlings in surviving, growing, and establishing mycorrhizal relationships in outplanting sites (Marx et al, 1982).

The area surrounding the roots is characterized by specific microbes and microenvironments. In the rhizosphere, symbiotic fungi strongly influence the activity and development of the root system. Fungal effects on root morphology including growth hormones and regulators produced by ectomycorrhizal fungi have been studied in detail (Slankis, 1973). From the data accumulated on growth

hormones related to ectomycorrhizae, it is evident that growth hormones and other exudates produced by symbiotic fungi affect root devlopment even without the establishment of a mycorrhizal relationship. Results have shown that mycorrhizal fungi stimulate feeder root proliferation and inhibit lateral root elongation (Slankis, 1958; Wilcox, 1968; Sohn, 1981).

GENETIC VARIATION IN MYCORRHIZAL FORMATION AND ROOT DEVELOPMENT

Although ectomycorrhizae have been the subject of scientific interest for many decades, the effects of host genetic composition on mycorrhizal formation have not been well defined. Few investigations have been made on genotypic effects of host trees on mycorrhizal formation. Linnemann (1960) found that the frequency of ectomycorrhizal roots on 1-2 year-old seedlings of Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) was different among seed sources. Wright (1963) and Wright and Ching (1962) found that mycorrhizal frequency on one-year-old Douglas-fir seelings varied significantly among provenances, but there were no significant differences on two-year-old seedlings. In the same study, Wright (1963) also found that seedlings which formed mycorrhizae earliest exhibited the best growth. Lundeberg (1968) reported higher frequencies of mycorrhizal formation on seedlings of Pinus sylvestris L. planted nearest the seed collection locality.

Although these findings were based on seedlings with unknown mycorrhizal fungi in field observations, the indications of genetic effects on mycorrhizal formation have brought forward a new interest in mycorrhizal studies. Recent studies have been carried out on seedlings inoculated with identified mycorrhizal symbionts in controlled environments. Marx and Bryan (1971) demonstrated that

the genotype of slash pine (Pinus elliotii Engelm. var. elliottii) influenced the degree of mycorrhizal formation with P. tinctorius in a pure culture synthesis. Long (1973) studied pot-grown seedlings of loblolly pine (Pinus taeda L.) from 15 full-sib families in growth chambers. Following inoculation with the mycorrhizal fungus P. tinctorius, the frequency of mycorrhizal feeder roots was significantly different among the families. In the same study, genotype x fungus interaction also showed a significant influence on seedling development indicated by the genetic variability of seedling characteristics in different fungal treatments. Mason (1975) carried out a pure culture synthesis with two seed sources of Betula pendula Roth and five fungal isolates of Amanita muscaria (L. ex Fr.) Pers. ex Hooker. The results indicated that both host and fungal genotypes affected the formation of ectomycorrhizae; the largest difference between seedlots within a single fungal isolate was 10% and between fungal isolates within a seedlot was 40%. Also, the degree of fungal stimulation on root production varied between the two seed sources as shown by a 30% difference in root numbers. Cline and Reid (1982) reported that the seed sources of Pinus contorta and P. ponderosa influenced the degree of ectomycorrhizal formation with Pisolithus tinctorius and Suillus granulatus. From their results, Cline and Reid concluded that no single mycorrhizal fungus was universally superior in growth improvement or in mycorrhizal formation among all seed sources within a tree species.

Genetic variation in root development has been studied on a few tree species, but most studies concentrated on gross root traits such as volume and weight (Bilan, 1971; Brown, 1969; Kriebel 1963). Additive gene effects on the number of lateral roots and total root weight have been found on loblolly pine seedlings (Stonecypher et al, 1965). The existence of genetic control of lateral roots and

root dry weight was also demonstrated by Long (1973) on four-month-old loblolly pine seedlings. In addition, he found that feeder root proliferation and feeder root dry weight were strongly affected by additive genes, and family means of the incidence of ectomycorrhizal feeder roots were significantly different. Genetic control of feeder root proliferation was also found on the seedlings from six half-sib progenies of slash pine by Marx and Bryan (1971).

Little is known about genetic variation in provenances and in families of tamarack. Considering its continuous and trans-continental distribution, genetic variation across the ranse of the species is expected to be clinal for some characteristics with gradual changes occurring along environmental gradients (Rauter and Graham, 1983). Significant differences between provenances of the tree have been found for height, diameter, and survival rate by Jeffers, (1975), Cech et al, (1977), and Park and Fowler (1983). Rehfeldt (1970) reported that root pattern and total height of two-year-old seedlings of tamarack were significantly different among families within a provenance. Based on that finding, he suggested that tamarack is highly variable at the intrapopulation level. The difference among families was also reported by Park and Fowler (1981) for germination and survival of tamarack seedlings.

MYCORRHIZAL SYNTHESIS APPROACHES

When a new mycorrhizal fungus is selected for artificial inoculation of a potential host, the first step is to test the fungus-host specificity. This test is usually done by using the technique of pure culture synthesis, which was first used by Melin (1922) and has been modified by various investigators (Marx and Zak, 1965; Trappe, 1967; Molina, 1979). Results from the technique not only enhance the

understanding of the complexity of mycorrhizal associations in nature but also provide the morphological and anatomical characterisics for distinguishing and classifying ectomycorrhizal fungi. Use of pure culture synthesis has also led to discovery of important physiological aspects of the symbiosis, including uptake of nutrients and water by the fungus and translocation to the host (Duddridge et al, 1980).

After the fungus-host specificity is confirmed by initial tests, large scale mycorrhizal inoculation can be conducted in greenhouses and nurseries. With control of growing conditions in greenhouses, a number of environmental factors has been studied for their influences on mycorrhizal development (Riffle and Maronek, 1982). Most mycorrhizal fungi are adapted to low fertility levels (Maronek, et al, 1981; Molina and Chamard, 1982). Temperature and soil moisture are also critical to mycorrhizal formation (Reid, 1978). As well, mycorrhizal development in inoculated soils and container growing media may be suppressed or prevented by indigenous microorganisms (Riffle and Maronek 1982). To avoid or reduce the effects of these environmental factors on mycorrhizal development, the maintenance of low fertility and water levels as well as sterilization of growing media are culture practices used in mycorrhizal inoculation programs in greenhouses and nurseries.

MATERIALS AND METHODS

SEED SOURCES AND FUNGAL SYMBIONTS

Seeds of tamarack were obtained from the nation-wide collection of the Petawawa National Forestry Institute, Canada. The seeds represented four provenances of tamarack in Ontario. Each provenance consisted of ten open-pollinated families. For each of the two provenances from Ignace Township(IT) and Manitouwadge(MA), seeds from ten open-pollinated trees were mixed into one seedlot, while the seeds from the remaining two provenances of Morley Township(MT) and Willison Township(WT) were kept separately for each of the families. Because of low germination of several families within provenances MT and WT, the numbers of families available for study were reduced to eight in the provenance MT and to nine in the provenance WT. Detailed descriptions of the seed sources are presented in Table 1.

The vermiculite-based inocula of four ectomycorrhizal fungus species, Pisolithus tinctorius (Pers.) Coker & Couch, Suillus granulatus (L. ex Fr.) O. Kuntze, Laccaria laccata (Scope. ex Fr.) Bk. & Br. and Cenococcum geophilum Fr. were provided as experimental samples by the Sylvan Spawn Laboratory, Kittanning, Pennsylvania, USA. The inocula were packed in sterilized plastic bags, air-shipped, and received on April 28, 1984. Cultures were made from each inoculum bag to check for contamination and to obtain pure cultures of these fungi. The inocula were then stored at 4C until used in the greenhouse experiment. Other fungal isolates, Hebeloma crustuliniforme (Bull. ex St. Am) Quel. and Rhizopogon vinicolor Smith, were also obtained from the Sylvan Spawn Laboratory, and Laccaria proxima Boudier, Amanita porphyria (A. & S. ex Fr.) Secor and Suillus tomentosus

Table 1. Provenances and open-pollinated families of tamarack from the Petawawa National Forestry Institute

Provenance	Lat.	Long.	Elev.	Seedlot	Family#	Germ.(%)
Morley Twp (MT)	48° 42′	94° 10′	350m	9554	MT1	55.4
"	"	"	"	9555	deleted	0
"	"	"	"	9556	MT2	60.4
"	"	"	"	9557	deleted	0
"	"	"	"	9558	MT3	76.2
"	"	"	"	9559	MT4	88.7
"	"	"	"	9560	MT5	77
"	"	"	"	9561	MT6	34.5
"	"	"	"	9562	MT7	78.3
"	"	"	"	9563	MT8	81.6
WillisonTwp (WT)	47° 52′	80° 28′	300m	9574	WT1	45.4
"	"	"	"	9575	WT2	87.9
"	"	"	"	9576	deleted	0
"	"	"	"	9577	WT3	88.7
"	"	"	"	9578	WT4	89.5
"	"	"	"	9579	WT5	89.5
"	"	"	"	9580	WT6	80.8
"	"	"	"	9581	WT7	75
"	"	"	"	9582	WT8	90
"	"	"	"	9583	WT9	85
Ignace Twp (IT)	49° 25′	91° 40′	450m	composite 9615-9624	-	60.4
Manitouwadge (MA)	49° 15′	86° 00′	335m	composite 20123-20132	-	69.1

(Kauf.) Sing., Snell & Dick were received from the Forest Pathology Laboratory, Lakehead University, Thunder Bay, Ontario, Canada.

PURE CULTURE SYNTHESIS

The seeds from two provenances MT and WT, mixed seeds from ten families within each of the provenances, were surface sterilized by soaking for 45 min in 30% hydrogen peroxide and were rinsed with 2 liters of sterilized distilled water. The sterilized seeds were then placed in Petri dishes containing 3% agar and were incubated in a germination chamber with the temperature at 23C, relative humidity at 70%, and light intensity of 6000 Lx. After germination, germinants 2-5mm in length were transplanted into glass test tubes (150 x 15mm). Prior to transplanting, the test tubes were partially filled with 25ml of peat moss and vermiculite substrate (1:10) and 18ml of modified Melin-Norkrans (MMN) nutrient solution (Marx 1969), and were autoclaved for 20min at 121C. Two discs (8mm in diameter) with mycelium from the edge of 2-4 week-old colony grown on MMN agar were aseptically transferred into each tube. Inoculation control tubes received MMN agar only. About 10ml of sterilized distilled water were added to each tube after transplanting, and the tube was covered by another 150x20mm test tube (Appendix Figure 1). All the synthesis tubes were randomly placed in a control growth chamber with temperatures 24/17C (day/night), humidity 70%, and light at approximately 11,000 Lx. This experiment included ten fungal treatments (9 fungi and 1 control), two seed provenances, and five replicates in each treatment combination.

After 16 weeks the seedlings were removed from the tubes and their roots were gently washed free of substrate. External ectomycorrhizal characteristics were

examined and described with the aid of a dissecting microscope. Mycorrhizal short roots were killed in formalin-acetic acid-alohol fixative (FAA), embedded in paraffin, sectioned at 10um thickness, and stained with safranin-fast green (Johansen 1940).

GREENHOUSE EXPERIMENT

Experimental Design

The greenhouse experiment involved four ectomycorrhizal inocula P. tinctorius, S. granulatus, C. geophilum and L. laccata and 19 seedlots from four provenances and 17 open-pollinated families. Based on these materials and the use of Ferdinand book containers (6 cells/book and 40cm^3/cell), a split-plot factorial design was made. This design was completed by using four replications as blocks, five fungal treatments (4 fungi and 1 control) as whole plots assigned at random in each block, and 19 seedlots as sub-plots assigned at random in each fungal treatment within each block. Each sub-plot had 6 seedlings to provide a mean of the treatment unit for statistical analysis. This design resulted in an experimental size of 2280 (4x5x19x6) seedlings in total.

Inoculation and Growing Conditions

A growing substrate containing equal volumes of sphagnum peat moss and vermiculite was autoclaved at 121C for 20min to kill resident mycorrhizal fungi. One part of inoculum was mixed thoroughly with six parts of the growing substrate. The mixed substrate was then filled into the containers. An equivalent amount of sterilized vermiculite was added for the inoculation control. Seed was sterilized by soaking in a 1% solution of sodium hypochlorite for 10min and was then rinsed in running tapwater for 30min. Two or three seeds were sown in each

cell, and the seeded tube was covered with a 5-10mm layer of sterilized vermiculite. The containers were placed in a mist chamber for a week and then moved to greenhouse benches. After germination the seedlings were thinned to one per cell and transplanting was done between replications within a treatment combination to fill empty cells. All the seedlings were grown at the temperatures at 24/17C (day/night), humidity at 50-65%, and light of approximately 12,000Lx over 17h provided by sunlight and sodium-vapor lamps. Seedlings were watered with tapwater as needed. Two weeks after germination, fertilization was started with a soluble 20-20-20 NPK fertilizer at a concentration of 100ppm. Fertilization was weekly until mid-July, then biweekly until mid-September. From July, 5g of 0.9% iron chelate were added to the soluble fertilizer at each time of fertilization to prevent chlorosis. The soluble fertilizer was dissolved in tapwater and evenly distributed over all seedlings. During the 18-week period, each seedling received approximately 34.32mg of N, 34.32mg of P, 34.32mg of K, 0.17mg of Fe, 0.09mg of Mn, 0.09mg of Zn, 0.09mg of Cu, 0.03mg of B, and 0.001mg of Mo. To reduce the effects of variable air flow and light on seedling growth in the greenhouse, the positions of blocks and whole plots on the benches were rearranged biweekly.

Assessment of Seedling Growth and Mycorrhizal Formation

After eighteen weeks of growth, all of the seedlings were harvested and their roots were washed free of the substrate with running tapwater. Attached pieces of vermiculite or peat moss were removed with a pair of forceps. Washed seedlings were wrapped in wet paper towel and stored at 4C for laboratory examination. In the laboratory, three seedlings were selected randomly from each treatment unit. Shoot height and diameter at root collar were measured, and the number of first order lateral roots longer than 5cm was counted for each sampled

seedling. Three first order lateral roots 9-10cm in length were selected from each seedling, and the number of second order lateral roots longer than 2cm was counted. Then the three first order lateral roots were cut into 2cm segments, mixed, and randomly chosen for mycorrhizal assessment under a dissecting microscope. Up to 100 feeder roots were counted from the selected segments, and the numbers of introduced ectomycorrhizae and indigenous ectomycorrhizae within the 100 feeder roots were recorded. Root and shoot dry weights were determined to 0.001g after drying at 75C for 24hr in a forced-air oven. In total fifteen variables were measured and generated for the seedlings:

1. shoot height cm (Height)
2. diameter at root collar mm (Diameter)
3. shoot volume cm^3 (Volume)
4. shoot dry weight g (Shoot W)
5. root dry weight g (Root W)
6. shoot:root ratio g/g (S/R ratio)
7. total dry weight g (Total W)
8. number of the 1st order lateral roots/seedling (1st LR)
9. frequency of the 2nd order lateral roots/cm (2nd LR)
10. frequency of introduced mycorrhizal feeder roots/cm (Myc FR)
11. frequency of introduced and indigenous mycorrhizal feeder roots/cm (Myc+Ind)
12. frequency of total feeder roots/cm (Feeder R)
13. percent of introduced mycorrhizal feeder roots (Myc %)
14. percent of indigenous mycorrhizal feeder roots (Ind %)
15. percent of introduced and indigenous mycorrhizae (Myc+Ind %).

The indicator of shoot volume was calculated using the equation introduced by Sinclair and Marx (1982):

$$\text{Shoot Volume} = \text{Shoot Height} \times \text{Diameter}^2 .$$

Qualitative observations were made on the shape, color, and mantle surface features of the ectomycorrhizae to compare with the results of the pure culture synthesis.

STATISTICAL ANALYSIS

Analysis of Variance

As a split-plot experimental design, the descriptive model for the effects of seed sources and mycorrhizal fungi on a single observation was developed as introduced by Anderson and McLean (1974). The linear model for analysis of variance (ANOVA) with provenances as a random factor is:

$$Y_{ijk} = u + B_i + a(i) + F_j + BF_{ij} + b(ij) + P_k + BP_{ik} + FP_{jk} + BFP_{ijk} \qquad \text{(Model 1)}$$

where, $i = 1...4$; $j = 1...5$; $k = 1...4$

Y_{ijk} = observation from the ith block, the jth fungal treatment, and the kth provenance
u = overall mean
B_i = effect of the ith block
$a(i)$ = restriction error due to the ith block
F_j = main effect of the jth fungal treatment (Fixed effect)
BF_{ij} = interaction effect of the ith block and the jth fungal treatment
$b(ij)$ = restriction error due to the jth fungal treatment within the ith block
P_k = main effect of the kth provenance (Random effect)
BP_{ik} = interaction effect of the ith block and the kth provenance
FP_{jk} = interaction effect of the jth fungal treatment and the kth provenance

BFPijk= residual, interaction effect of the ith block and the jth fungal treatment and the kth provenance.

Since the families were nested in the provenances, the model used to derive effects of family and family x fungus interaction is:

$$Yijkl = u + Bi + a(i) + Fj + BFij + b(ij) + Pk + BPik + FPjk + BFPijk + G(k)l + BG(k)il + FJG(k)l + BFG(k)ijl \qquad \text{(Nested model)}$$

where, i = 1...4; j = 1...3; k = 1,2; and l = 1...8

G(k)l = effect of the lth family within the kth provenance (Random effect)
BiG(k)l= interaction effect of the ith block and the lth family within the kth provenance
FjG(k)l= interaction effect of the jth fungal treatment and the lth family within the kth provenance
BFijG(k)l= interaction effect of the ith block and the jth fungal treatment and the lth family within the kth provenance

other terms are defined as in Model 1.

ANOVA tables for these two linear models are illustrated in Tables 2 and 3. To keep equal sample size, seedlings were selected randomly over all the families within each of the two provenances MT and WT for the analysis of variance with the model 1, and eight families were selected randomly from provenance WT for the analysis of variance with the nested model. In both ANOVA tables, the interaction effects of BFP and BFG were treated as estimate errors to test the effects of seed sources and seed source x fungus interactions. Since there was no appropriate F-ratio that could be provided by direct application of expected mean squares, the Quasi F-ratio method (Winer, 1971) was used to construct proper denominators for the test of fungal treatment. According to the variance components of the mean squares, the Quasi F-ratio in the ANOVA of model 1 was constructed as

Table 2. Table of analysis of variance for the model 1

Sources	df	Expected Mean Squares
B	3	$\sigma^2 + 5\sigma^2BP + 4\sigma^2b + 20\sigma^2a + 20\phi B$
a	0	$\sigma^2 + 4\sigma^2b + 20\sigma^2a$
F	4	$\sigma^2 + 4\sigma^2FP + 4\sigma^2b + 16\phi F$
BxF	12	$\sigma^2 + 4\sigma^2b + 4\phi BF$
b	0	$\sigma^2 + 4\sigma^2b$
P	3	$\sigma^2 + 20\sigma^2P$
BxP	9	$\sigma^2 + 5\sigma^2BP$
FxP	12	$\sigma^2 + 4\sigma^2FP$
Residual	36	σ^2

Table 3. Table of analysis of variance for the nested model

Sources	df	Expected Mean Squares
B	3	$\sigma^2 + 3\sigma^2BG + 16\sigma^2b + 48\sigma^2a + 48\phi B$
a	0	$\sigma^2 + 16\sigma^2b + 48\sigma^2a$
F	2	$\sigma^2 + 4\sigma^2FG + 16\sigma^2b + 64\phi F$
BxF	6	$\sigma^2 + 16\sigma^2b + 16\phi BF$
b	0	$\sigma^2 + 16\sigma^2b$
P	1	$\sigma^2 + 12\sigma^2G + 96\phi P$
BxP	3	$\sigma^2 + 3\sigma^2BG + 24\phi BP$
FxP	2	$\sigma^2 + 4\sigma^2FG + 32\phi FP$
BxFxP	6	$\sigma^2 + 8\phi BFP$
G	14	$\sigma^2 + 12\sigma^2G$
BxG	42	$\sigma^2 + 3\sigma^2BG$
FxG	28	$\sigma^2 + 4\sigma^2FG$
Residual	84	σ^2

$$F' = MS(Fi) / [MS(BFij)+MS(FPjk)-MS(BFPijk)]$$

and the degrees of freedom for the denominators were determined by :

$$\frac{[MS(BFij)+MS(FPjk)-MS(BFPijk)]^2}{MS(BFij)^2/df1 + MS(FPjk)^2/df2 + MS(BFPijk)^2/df3}.$$

For the nested ANOVA, the terms MS(FPjk), MS(BFPijk), df2, and df3 were replaced by MS(FG), MS(BFG), and their degrees of freedom.

When the variances of fungal treatments and seed sources were indicated significantly different at $P \leq 0.05$, the Tukey-HSD multiple range test was performed to assess the differences among treatment means.

Variance Component and Genetic Correlation

Variance components were derived only for random effects in the ANOVA tables by equating mean squares to their expectations and solving the resulting equations. For example, the equations in the nested ANOVA could be expressed in matrix form:

$$\begin{bmatrix} MS\ (G) \\ MS\ (FG) \\ MS\ (Residual) \end{bmatrix} = \begin{bmatrix} 1 & 0 & ij \\ 1 & i & 0 \\ 1 & 0 & 0 \end{bmatrix} * \begin{bmatrix} \sigma^2_r \\ \sigma^2_{FG} \\ \sigma^2_G \end{bmatrix}.$$

Negative variance components were interpreted as indicating a component of zero, or very small values. Positive variance components were expressed as percentages of the total variance to compare the relative importance of the source of variance between seedling traits.

Genetic correlations were computed for the variables between roots and shoots, within roots, and between roots and mycorrhizal traits by using the equation which was introduced by Falconer (1981) for half-sib families:

$$r_g = \overline{\sigma_{g(xy)}} \Big/ \sqrt{\sigma^2_{g(x)} * \sigma^2_{g(y)}}$$

where r_g is the genetic correlation of seedling variables x and y, and $\overline{\sigma_{g(xy)}}$, $\sigma^2_g(x)$, and $\sigma^2_g(y)$ are family components of covariance and variance on the x and y variables. The covariance components of x and y were calculated exactly the same as variance components from analysis of covariance (Table 4).

Canonical Discriminant Analysis

Canonical discriminant analysis is a multivariate approach that determines interrelationships among defined groups by classifying individuals. This technique has been widely used to solve taxonomic and systematic problems in biological studies (Pimentel, 1979).

The application of canonical discriminant analysis in this study was to answer the following questions:

1. Were there significant differences of overall seedling response among the fungal treatments?

2. If the fungal treatments exhibited statistical differences, to what extent did the fungal treatments differ?

3. What was the relative importance of each variable to the discrimination of fungal treatments?

From this multivariate analysis the parameters which strongly contributed to the difference between fungal treatments were derived by ordering the correlation

Table 4. Table of analysis of covariance with the nested model

Sources	df	Expected Cross Products
B	3	$\sigma\overline{} + 3\sigma\overline{BG} + 16\sigma\overline{b} + 48\sigma\overline{a} + 48\sigma\overline{B}$
a	0	$\sigma\overline{} + 16\sigma\overline{b} + 48\sigma\overline{a}$
F	2	$\sigma\overline{} + 4\sigma\overline{FG} + 16\sigma\overline{b} + 64\sigma\overline{F}$
BxF	6	$\sigma\overline{} + 16\sigma\overline{b} + 16\sigma\overline{BF}$
b	0	$\sigma\overline{} + 16\sigma\overline{b}$
P	1	$\sigma\overline{} + 12\sigma\overline{G} + 96\sigma\overline{P}$
BxP	3	$\sigma\overline{} + 3\sigma\overline{BG} + 24\sigma\overline{BP}$
FxP	2	$\sigma\overline{} + 4\sigma\overline{FG} + 32\sigma\overline{FP}$
BxFxP	6	$\sigma\overline{} + 8\sigma\overline{BFP}$
G	14	$\sigma\overline{} + 12\sigma\overline{G}$
BxG	42	$\sigma\overline{} + 3\sigma\overline{BG}$
FxG	28	$\sigma\overline{} + 4\sigma\overline{FG}$
Residual	84	$\sigma\overline{}$

$\sigma\overline{}$ Covariance component of seedling variable x and y.

coefficients and the magnitudes of these correlations in the canonical discriminant functions.

Two other statistical methods, Spearman's rank correlation and linear regressions, were also used to interpret the relationships between seedling variables.

All computations for above statistical procedures were done using the SPSS statistical package on the VAX11/780 computer at the Lakehead University Computer Centre.

Assumptions of Data Analysis

The validity of derived results from statistical methods such as analysis of variance depends upon assumptions of independence, normality, homogeneity of variances, and additivity of data. It was accepted without verification that the measurements of the designated traits were random variables whose error variances were additive in nature, and that the randomization procedure in the split-plot design produced independent observations. Homogeneity of variances among treatment groups was examined with Bartletts' test (Sokal and Rohlf, 1981) and the assumption was accepted at $P>0.05$. The relatively small number of observations prohibited normality of data for some variables, but the normal probability plot indicated close to a normal distribution pattern for most of the variables. Logarithmic transformation of data was made only for the variable of shoot/root ratio.

The assumptions for discriminant analysis are those of variance analysis extended to the multivariate situation. Multivariate homogeneity was examined by using Bartletts' Box test, and multivariate significances were tested by using Wilks' and Roys' procedures at $P\leq 0.05$ level.

RESULTS

ECTOMYCORRHIZAL FORMATION IN PURE CULTURE SYNTHESIS

Seedling shoots developed normally in most of the tubes and reached an average height of 4.9cm. No obvious differences in seedling growth and mycorrhizal formation existed between the two provenances. A very few seedlings inoculated with H. crustuliniforme and P. tinctorius were stunted in growth and had reddish needles. All attempts to reisolate the introduced mycobionts from culture substrate were successful.

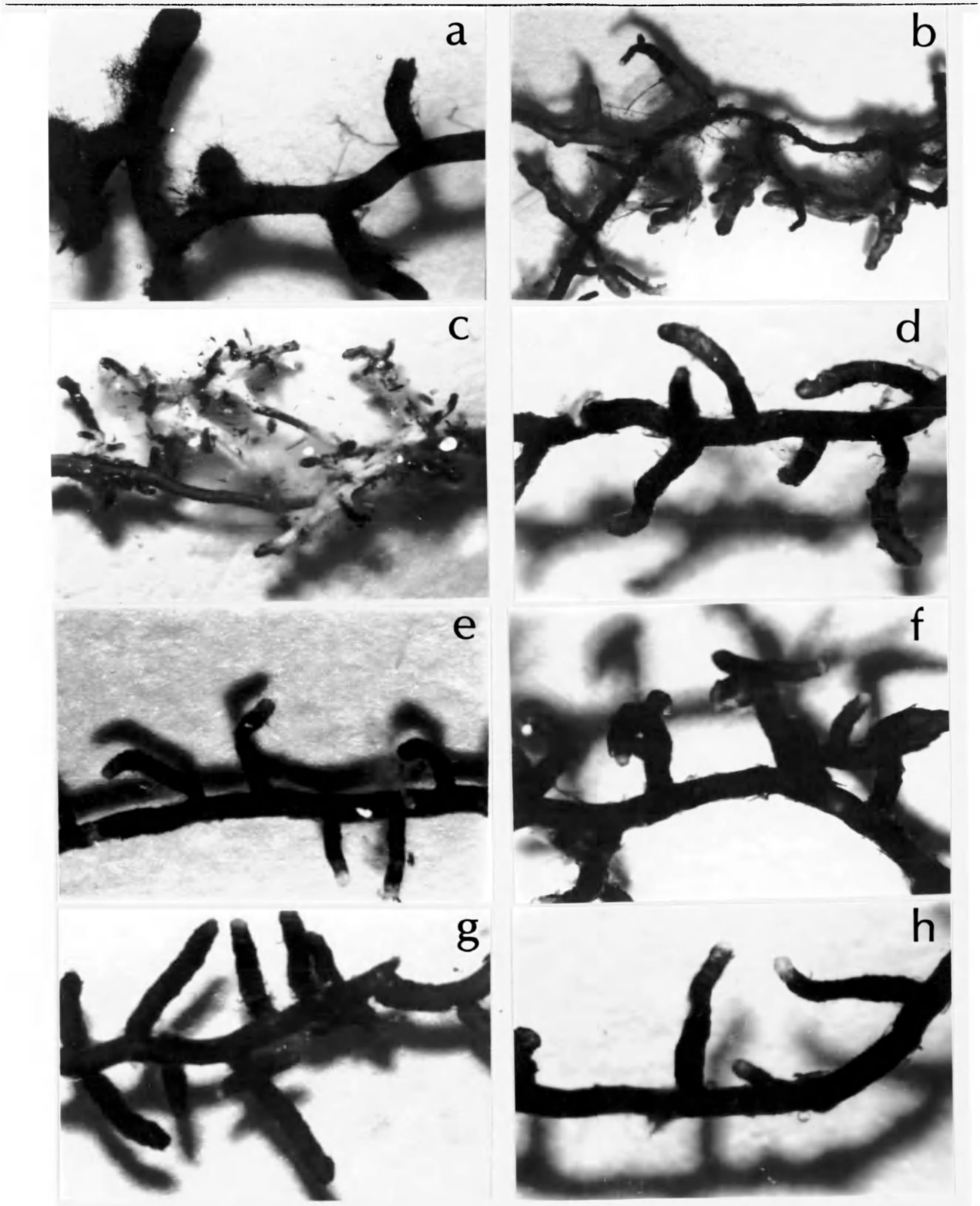
Degrees of ectomycorrhizal formation varied among fungus species: H. crustuliniforme, P. tinctorius, and C. geophilum formed more than 75 percent of mycorrhizae over the total feeder roots; L. laccata and L. proxima formed about 30 to 60 percent; A. porphyria, R. vinicolor, and S. granulatus failed to form any mycorrhizae, although the three fungi colonized most short roots. S. tomentosus grew very slowly in the test tubes and did not colonize root systems. The formation of feeder roots was stimulated in the seedlings inoculated with fungi. In one instance, a few seedlings inoculated with R. vinicolor exhibited a dichotomously branching habit.

Ectomycorrhizal morphology was fungus dependent and varied in macroscopic color, mantle hyphae, mantle texture, and Hartig net development. Details of the morphological and anatomical features are described below by individual fungus, and necessary references are made to previous studies on these fungi.

Cenococcum geophilum (Fig.1.a and Fig.2.a)

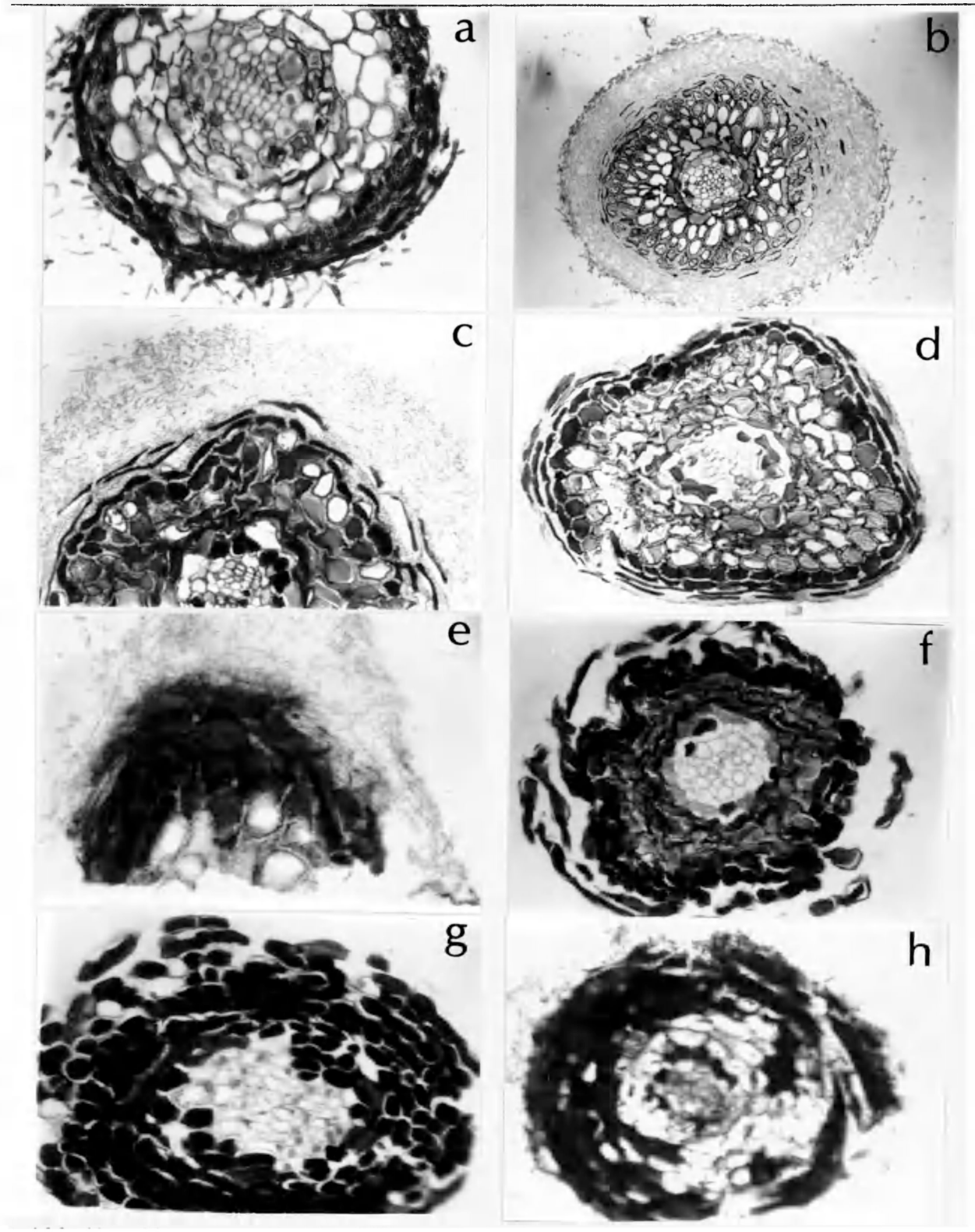
Mycorrhizae were well developed, jet black, heavily extensive, monopodial, and 1-5mm long. Mycelia were black, rhizomorph-like, extending from feeder roots to

Figure 1. Gross morphology of ectomycorrhizae and colonized feeder roots of tamarack seedlings in pure culture synthesis



* Figure b and c are at x1.6; and Figure a, d, e, f, g, and i are at x4.

Figure 2. Cross sections of ectomycorrhizae and colonized feeder roots of tamarack seedlings in pure culture synthesis



* All the figures are at x800.

lateral roots, and colonizing the whole root system. Cross sections revealed that mantles were well developed, 20-30 µm thick, and tightly interwoven. Mantle surface hyphae were black, thick-walled, stiff, coarse, and 2-5µm in diameter; simple septae and clamp connections were rarely present. Hartig net penetrated only between the outer two layers of unturgid cortical cells. These cortical cells were separated by 1-2 layers of hyphae.

C. geophilum formed about 80% of the total mycorrhizae with the feeder roots in pure culture synthesis. This fungus is the most ubiquitous and most easily recognizable ectomycorrhizal symbiont. More than 200 tree species including tamarack have been found to form mycorrhizae with this fungus (Trappe, 1962; Molina, 1980; Molina and Trappe, 1982).

Pisolithus tinctorius (Fig.1.b and Fig.2.b)

Mycorrhizae were bright yellow or bright golden color, swollen, simply branched or coralloid, 2-7mm long with a tomentose surface. Pale yellow to dark brown, thread-like rhizomorphs grew around the lateral roots and penetrated into the substrate. The mantle was uniformly developed, 90-110µm wide, tightly interwoven, and become loose at the mantle surface. Mantle surface hyphae were 2-4µm in diameter, yellowish, weft-like and thick-walled; septae and clamp connections were widely present. Hartig net extended into the endodermis for 4 to 5 layers of cells. Cortical cells and an exterior layer of endodermis cells were incorporated into the mantle; the inner layers of endodermis cells became radially elongated.

P. tinctorius formed abundant ectomycorrhizae with tamarack over about 85 % of the total feeder roots. Marx (1977b) reported that this fungus had proven experimentally to form mycorrhizae with 47 tree species and had been observed in

association with 25 additional host species. In a study of pure culture synthesis, Molina and Trappe (1982) found that this fungus formed more than 75% mycorrhizae with western larch seedlings.

Hebeloma crustuliniforme (Fig.1.c and Fig.2.c)

Individual mycorrhizae were often pale white at the root tips and became brown or dark brown near the bases; they were typically club-like, extensive and 2-5mm long. Attached mycelia and rhizomorphs appeared loose, weft-like, connected directly to the mantle surface, and grew along lateral roots. The mantle was well-developed, about 100μm thick, wooly or velvety in surface texture and was formed by white, thin-walled mantle hyphae with diameter at 2-3μm. Mantle hyphae were loosely interwoven but became compact near the root surface. The Hartig net penetrated the whole endodermis causing cells that were irregular in form and were separated by 1-4 layers of hyphae. Cortical cells became flaccid and were included in the mantle structure.

H. crustuliniforme formed about 85% ectomycorrhizae to total feeder roots in this pure culture condition. This fungus is one of the fastest growing mycorrhizal symbionts and has been successfully used for inoculation of containerized seedlings of white spruce (Picea glauca (Moench) Voss) (Navratil, 1985) and Douglas-fir in a pure culture synthesis (Trappe, 1967). Trappe (1962) listed more than 12 tree species associated with this fungus, but tamarack was not among them.

Laccaria laccata (Fig.1.d and Fig.2.d)

Mycorrhizae were pale yellowish or brown with white to pinkish tips; the surface was smooth or slightly tomentose, swollen near root tips, monopodial, and 1-4mm long. Thread-like mycelia grew along lateral roots and into the substrate

from the mycorrhizal root surface. Attached hyphae were pale white, thin-walled, and 2.6µm in diameter. The mantle was relatively thin, 10-30µm wide, and compact on the root surface. Mantle hyphae were parallel or interwoven, pale white, thin-walled, 1-3µm in diameter, and with septae and clamp connections. Hartig net was well developed, and extended into the endodermis 4-5 cells in depth; the cells were separated by 2-4 layers of hyphae. Two tiers of cortical cells at the periphery of the root became unturgid and were incorporated into the mantle.

L. laccata formed relatively fewer ectomycorrhizae, about 50% ectomycorrhizal feeder roots out of the total feeder roots. Trappe (1962) listed eight genera of tree hosts forming mycorrhizae with L. laccata. This fungus has been successfully used for inoculations of many coniferous species in greenhouses, nurseries, and outplanting sites (Molina, 1980; Shaw and Molina, 1980; Navratil, 1985). Studies of pure culture syntheses with this fungus also resulted in abundant mycorrhizal formation of several tree hosts (Molina and Trappe, 1982).

Laccaria proxima (Fig.1.e and Fig.2.e)

Mycorrhizae ranged from brown to dark brown in color; they were unswollen, monopodial, 2-5mm long and had a tomentose surface. Cross sections showed the thin mantle to range from 0 to 15µm thick, but became 50-100µm near root tips. Mantle hyphae were white, thin-walled, 1-3µm in diameter, multi-branched with septae and clamp connections present. The Hartig net penetrated into the endodermis for 3-4 cells separating cells by 1-3 layers of hyphae. Development of cortical cells was unaltered.

Little is known about the mycorrhizal assoication of this fungus with trees. Danielson and others (1984) reported that L. proxima formed abundant ectomycorrhizae with jack pine containerized seedlings in low fertilizer regimes. In

this pure culture synthesis this fungus formed ectomycorrhizae with about 45% of the total feeder roots.

Suillus granulatus (Fig.1.f and Fig.2.f)

S. granulatus failed to form ectomycorrhizae with tamarack seedlings in this study. Short roots were dark brown, simply branched, colonized by grayish hyphae, and showed a mantle-like, slightly tomentose root surface. Cross sections revealed that no mantle and Hartig net were formed although some cells in the endodermis had intracellular infections. Cortical cells appeared normal and often were deeply stained with safranin.

This fungus has been reported to form ectomycorrhizae with more than 30 tree species and is frequently associated with pines (Trappe, 1962). Experimental studies have shown that this fungus formed ectomycorrhizae in pure culture synthesis with numerous pines (Mary and Stewart, 1984; Cline and Reid, 1982).

Rhizopogon vinicolor (Fig.1.g and Fig.2.g)

Abundant hyphae grew to the substrate and colonized about 80 percent of the root system, but no ectomycorrhizae were formed. Colonized short roots were brown or dark brown, simply or dichotomously branched with pale white, loose hyphae on the root surface. Cross sections showed no regularly developed mantle or Hartig net. Cortical cells and most of the endodermis cells collapsed in various stages and were deeply stained with safranin.

R. vinicolor formed ectomycorrhizae with Douglas-fir and western larch in pure culture synthesis (Molina and Trappe, 1982). Mexal et al (1979) found that this fungus formed no true mantle and Hartig net, but caused abnormal cortical cell development in short roots of lodgepole pine (Pinus contorta Dougl.).

Amanita porphyria (Fig.1.h and Fig.2.h)

This fungus failed to form ectomycorrhizae with tamarack in this study. Mycelia penetrated the entire growth substrate and colonized about 60 percent of the root system. Short roots were dark brown, extremely narrow, simply branched and covered with a mantle-like mycelial mat. Cross sections showed cortical cells and endodermis cells that were normally developed and stained deeply with safranin.

Trappe (1962) indicated that some species of Picea and Abies as well as Pinus form ectomycorrhizae with this fungus.

ECTOMYCORRHIZAL FORMATION IN CONTAINERIZED SEEDLINGS

Qualitative Observations

Ectomycorrhizal formation with containerized seedlings was similar to the results of the pure culture synthesis. The ectomycorrhizae formed by L. laccata, C. geophilum and P. tinctorius were successfully identified and were easily distinguished from indigenous ectomycorrhizae. S. granulatus again failed to form ectomycorrhizae, though this fungus colonized about 20-30% of the feeder roots.

All the seedlings formed ectomycorrhizae with unidentified indigenous fungi, but the degree of the indigenous mycorrhizal formation varied with fungal treatments. The greatest degree was with the control and C. geophilum, and the lowest was with L. laccata. Mycorrhizal formation between indigenous and inoculated fungi appeared to be negatively correlated.

Macroscopic characteristics of the indigenous ectomycorrhizae were similar in all experimental units. In the top layer of the root system the indigenous mycorrhizae were reddish brown or dark brown, strikingly narrow cylindric, 1-3mm

long, and with no well-developed mantles; in the middle or bottom layer of the root system the indigenous mycorrhizae were pale white to yellowish in color, slightly swollen, simple-club-shaped, 2-5mm long, and with a smooth hyphal mantle.

Effects of Fungus Species and Seed Sources on Mycorrhizal Formation

Analysis of variance (Table 5 and 6) showed that the frequency and the percent of introduced mycorrhizae were significantly affected by fungal treatments, seed sources, and seed source x fungus interactions. The difference in mycorrhizal formation between fungal treatments was mainly attributable to the L. laccata treatment which accounted for 60% of mycorrhizae to the total feeder roots. In contrast the other two fungi formed mycorrhizae at much lower level, 6.6% and 12% of the total feeder roots for C. geophilum and P. tinctorius, respectively (Figure 3).

Provenances showed significantly different compatibilities to mycorrhizal formation with the fungi tested (Figure 4). Compared to the others, the provenance WT was superior in forming mycorrhizae with L. laccata, but not with the other two fungi. The provenance MA showed highest mycorrhizal formation with C. geophilum and P. tinctorius and secondly with L. laccata. The greatest overall mycorrhizal formation for all the provenances was associated with L. laccata, followed by P. tinctorius and C. geophilum.

The significant difference in mycorrhizal formation among families is shown in Table 6, and the differences between the means are illustrated in Figure 5. Since families were nested within provenances, it was necessary to estimate the family variance in mycorrhizal formation within a single provenance. Results of the partition ANOVA (Appendix Table 1) showed that family variability in mycorrhizal formation was different between provenances. The families in the provenance WT

Table 5. Mean squares in the ANOVA of the model 1 for tamarack seedling characteristics [a]

Source	df	Height	Diameter	Volume	ShootW	Root W
Fungus (F)	4	6.513	0.181	363.9	0.018	0.006
Block X F	12	1.805	0.030	61.99	0.005	0.004
Provenance(P)	3	21.59**	0.045	254.4	0.013	0.004*
Block X P	9	2.425	0.066	159.3	0.011	0.001
F X P	12	2.355	0.040	74.19	0.003	0.002
Residual	36	2.815	0.065	126.4	0.006	0.001

Source	df	S/R	Total W	1st LR	2nd LR	FeederR
Fungus (F)	4	0.332	0.076	8.535	0.143*	0.796
Block X F	12	0.391	0.022	2.419	0.039	0.227
Provenance(P)	3	0.209	0.023	12.94*	0.062	0.679*
Block X P	9	0.127	0.019	3.351	0.048	0.103
F X P	12	0.562**	0.012	1.309	0.034	0.337*
Residual	36	0.194	0.015	3.323	0.044	0.164

Source	df	MycInd /cm	Myc % [b]	Ind % [b]	MycInd%
Fungus (F)	4	10.958*	11277**	694.7*	5081.9**
Block X F	12	0.139	43.10	16.62	51.576
Provenance(P)	3	0.791**	241.8**	6.006	209.91**
Block X P	9	0.099	16.91	21.23	40.693
F X P	12	0.277*	83.62**	51.36	113.16*
Residual	36	0.109	20.13	32.91	43.84

a Fungus effect were estimated using Quasi F-ratio.
b Excluding the fungal treatment of S. granulatus, degrees of freedom of fungal treatment is 3; block x fungus is 9; fungus x provenance is 9; and residual is 27.
* Significance at $P \leq 0.05$ level; ** significance at $P \leq 0.01$ level.

Table 6. Mean squares in the ANOVA of nested model for tamarack seedling characteristics a

Source	df	Height	Diameter	Volume	ShootW	Root W
Fungus (F)	2	4.153	0.642**	1263.9**	0.037*	0.0108*
Block x F	6	2.030	0.033	62.360	0.005	0.0014
Provenance(P)	1	1.769	0.079	252.17	0.00004	0.0009
Block x P	3	0.252	0.002	7.2900	0.0003	0.0019
F X P	2	12.16	0.035	249.35	0.0088	0.0013
Family (G w P)	14	6.486**	0.158**	354.05**	0.017**	0.0050**
Block x G w P	42	1.834	0.034	67.310	0.005	0.0016
F X G w P	28	6.126**	0.050	144.23*	0.010*	0.0027
Residual	84	1.379	0.052	88.413	0.006	0.0020

Source	df	S/R	Total W	1st LR	2nd LR	FeederR
Fungus (F)	2	4.469*	0.031	29.342**	0.433**	0.797
Block x F	6	0.434	0.006	1.589	0.015	0.062
Provenance (P)	1	0.689	0.008	40.948*	0.019	0.026
Block x P	3	0.379	0.008	3.651	0.020	0.131
F X P	2	0.575	0.004	26.119**	0.085	0.162
Family (G w P)	14	0.964**	0.037**	6.561**	0.095**	0.397**
Block x G w P	42	0.284	0.010	1.988	0.025	0.084
F X G w P	28	0.423	0.168	3.965*	0.060	0.476**
Residual	84	0.308	0.016	2.189	0.040	0.112

Source	df	MycInd /cm	Myc %	Ind %	MycInd%
Fungus (F)	2	72.241**	66154.**	2982.3**	4188.0**
Block x F	6	0.0784	13.656	9.194	21.784
Provenance (P)	1	0.0093	97.759	103.06*	0.070
Block x P	3	0.0073	17.546	10.462	31.997
F X P	2	0.538*	492.97**	21.216	473.63**
Family (G w P)	14	0.218**	66.363**	21.443**	69.692**
Block x G w P	42	0.0578	18.645	6.078	21.537
F X G w P	28	0.185**	84.788**	36.501**	91.119**
Residual	84	0.737	18.301	7.138	20.732

a Fungus effect was estimated using Quasi F-ratio; * significant difference at $P \leq 0.05$ level; ** significant difference at $P \leq 0.01$ level.

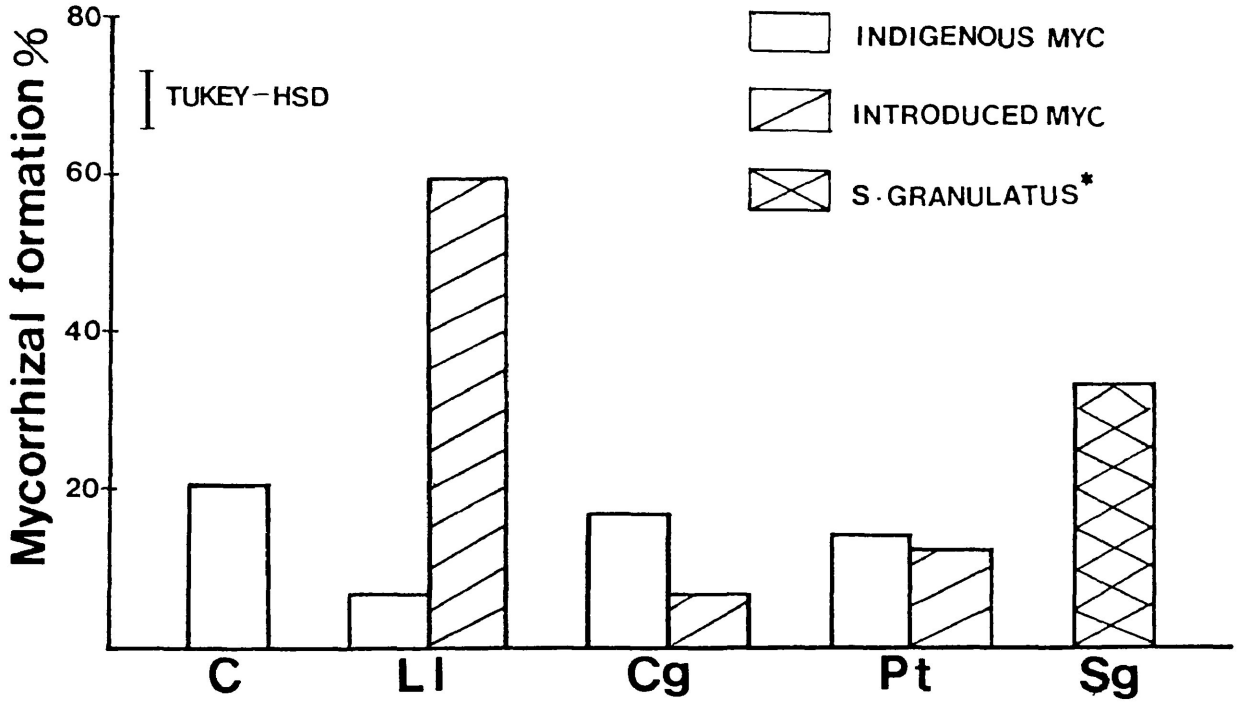


Figure 3. Mean percent of ectomycorrhizal feeder roots in five fungal treatment. * S. granulatus colonized and indigenous mycorrhizal feeder roots.

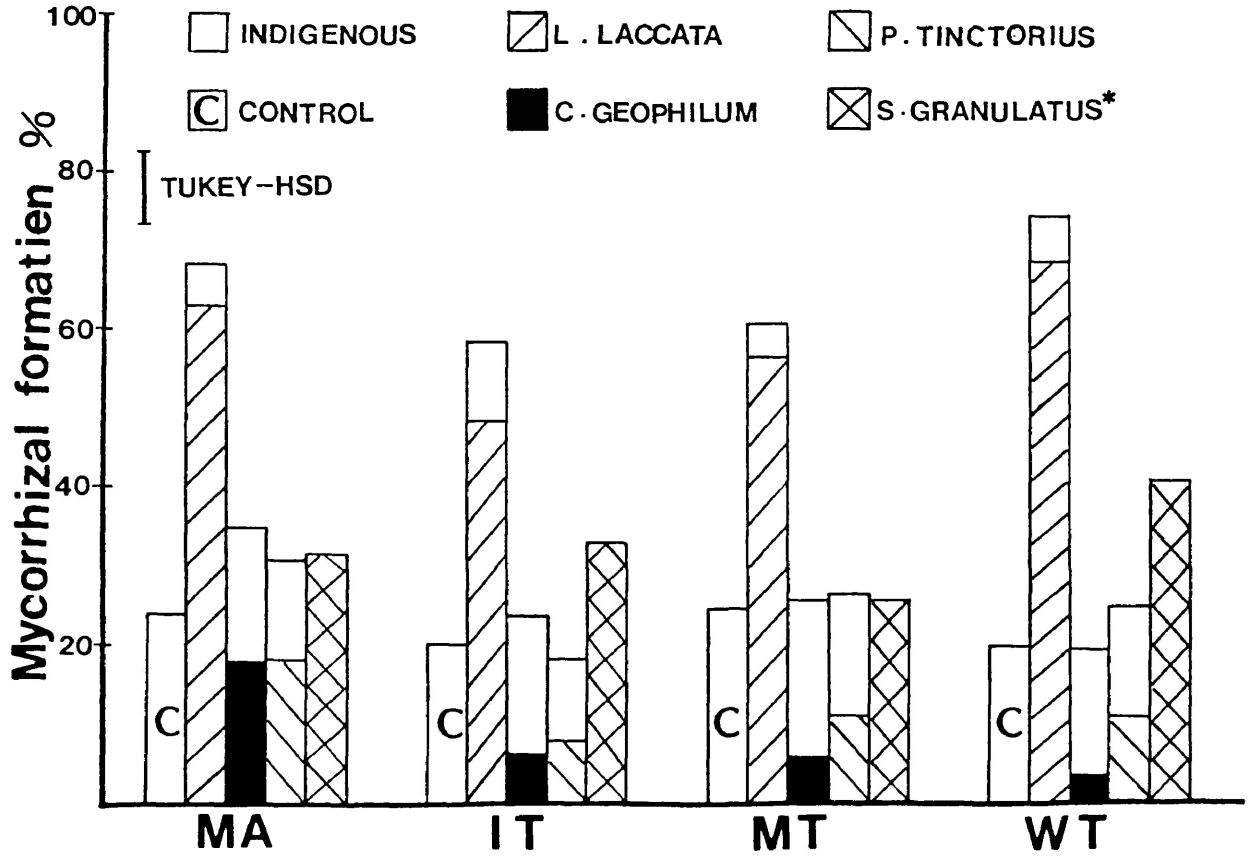


Figure 4. Mean percent of ectomycorrhizal feeder roots in four provenances with five fungal treatments. * S. granulatus colonized and indigenous mycorrhizal feeder roots.

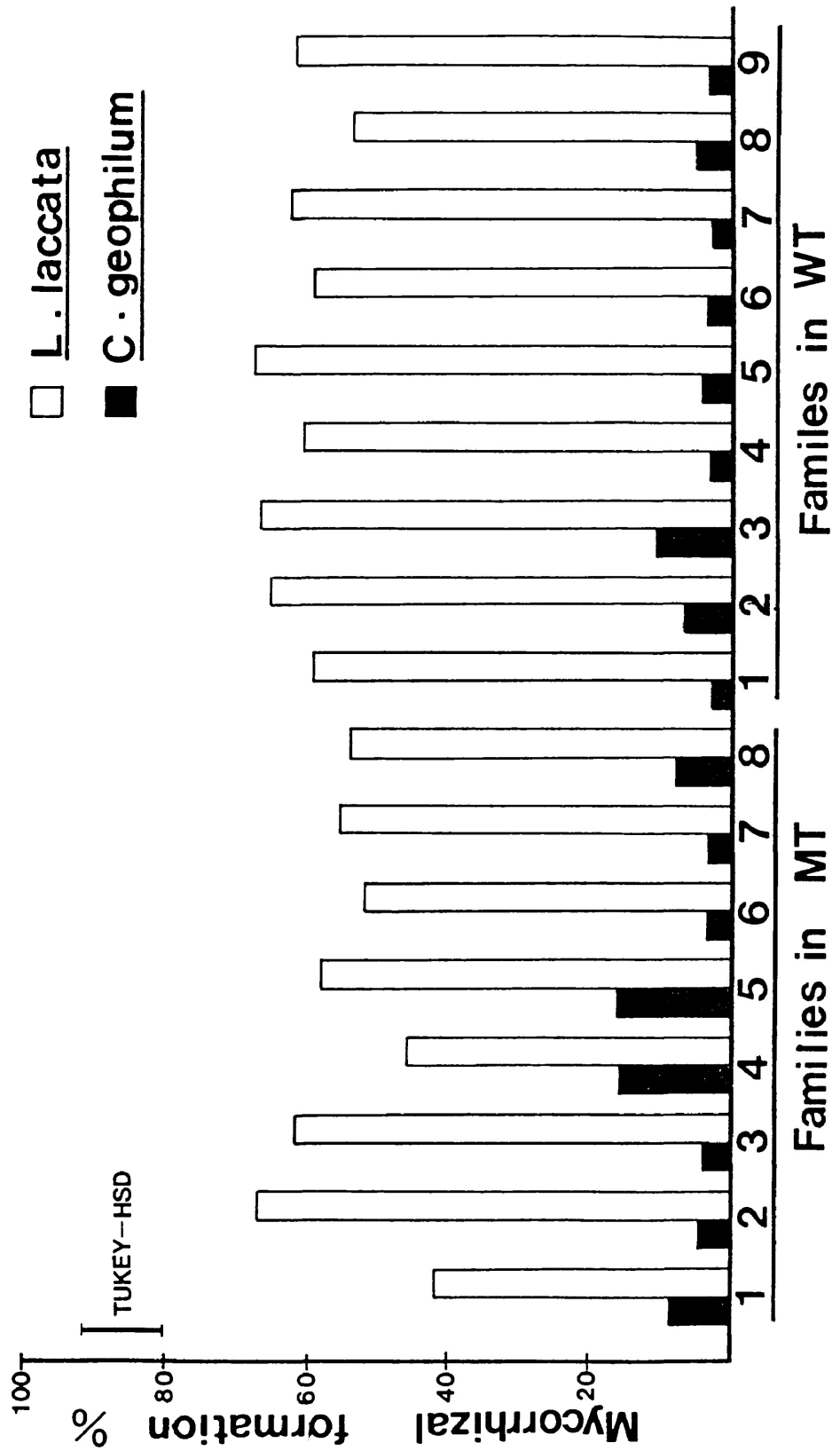


Figure 5. Mean percent of ectomycorrhizal feeder roots in 17 families from the provenance MT and WT

were generally constant in mycorrhizal formation with L. laccata and had no significant difference between their means. In contrast, the families in the provenance MT were significantly different in mycorrhizal formation with both L. laccata and C. geophilum.

Significant effects of family x fungus interaction (Table 6) indicated that the compatibility of families in mycorrhizal formation was not uniform. No family was superior in mycorrhizal formation with either L. laccata or C. geophilum. For instance, the family MT4 and MT5 had a greater mycorrhizal formation with C. geophilum than others, but not with L. laccata. The provenance x fungus interaction (Table 5) affected mycorrhizal formation in the same manner as the family x fungus interaction.

EFFECTS OF FUNGAL TREATMENT ON SEEDLING DEVELOPMENT

Relationships of Seedling Growth and Ectomycorrhizae

The relationships of seedling growth with the various ectomycorrhizae were examined using Spearman's rank correlations based on 204 seedlings in each of the three fungal treatments of L. laccata , C. geophilum, and control (Table 7). Percentages of mycorrhizal formation by L. laccata and C. geophilum were all positively correlated with shoot height, diameter, shoot and root dry weights. Consequently, total dry weight and shoot volume were similarly correlated with mycorrhizal formation. The relationships of root development and mycorrhizae varied with root variables. Lateral root development including both 1st and 2nd order lateral roots was negatively correlated with mycorrhizal formation for the L. laccata treatment. The frequency of total feeder roots was significantly and positively correlated with mycorrhizal frequency for all of the fungal treatments.

Since it had the highest correlation coefficient associated with mycorrhizal formation, the frequency of feeder roots could be considered as the most important factor for the development of mycorrhizae. The reverse relationship could also be deduced; mycorrhizal formation stimulated feeder root proliferation. The contribution of indigenous mycorrhizae to seedling growth was basically the same as that of introduced mycorrhizae in all of the treatment combinations. Therefore, the evaluation of seedling response to ectomycorrhizae could be expressed by combining inoculated and indigenous mycorrhizae in this experiment.

Regression analysis was used to derive the trend of mycorrhizal effects on seedling growth. Considering the higher degree of mycorrhizal formation, the regression analysis was applied to the L. laccata treatment only. Most of the seedling variables showed a positive linear relationship with mycorrhizal formation, but only a few were statistically significant. Based on the best fit to data, the effect of mycorrhizal formation on shoot volume and total dry weight appeared to be asymptotic (Figure 6 and 7). This result indicated that mycorrhizal formation by L. laccata enhanced the biomass accumulation of tamarack seedlings under the conditions of this study.

Seedling Response to Fungal Treatments

Throughout the analysis of variance, fungus effect was estimated by using denominators computed using the Quasi F-ratio method. The degrees of freedom varied with different seedling variables. Mean squares for all seedling traits are summarized in Tables 5 and 6. Results indicated that fungus effects were significant for seedling variables involving diameter, shoot volume, shoot dry weight, root dry weight, shoot/root ratio, and lateral roots based on the nested design model (Table 6). Only one trait, the frequency of 2nd order lateral roots,

Table 7. Spearman's correlation coefficients for ectomycorrhizae and tamarack seedling relationships a

Seedling Traits	L. laccata			C.geophilum			Control	
	Myc %	MycInd%	Myc FR	Myc %	MycInd%	Myc FR	Ind %	Ind FR
Height	0.23**	0.22**	0.16*	0.18**	0.10	0.19**	0.17**	0.17*
Diameter	0.13*	0.17**	0.16*	0.17**	0.24**	0.19**	0.25**	0.27**
1st LR	-0.05	-0.02	0.02	0.02	0.03	0.04	-0.05	-0.07
2nd LR	-0.06	-0.02	-0.04	0.01	-0.05	0.03	0.03	0.02
TotalFR	0.09	0.09	0.61**	0.36**	0.31**	0.62**	0.22**	0.48**
RootDW	0.04	0.08	0.13*	0.12*	0.08	0.14*	0.19**	0.18**
ShootDW	0.10	0.12*	0.13*	0.13*	0.14*	0.15*	0.24**	0.22**
S/R Ratio	0.03	-0.01	-0.08	-0.01	0.02	-0.02	-0.05	-0.05
ShootV	0.19**	0.23**	0.19**	0.19**	0.23**	0.21**	0.29**	0.30**
TotalDW	0.09	0.11*	0.13*	0.14*	0.13*	0.17**	0.24**	0.23**
Ind %	-0.46**	-0.20**		-0.16**	0.60**			
Ind FR/cm			-0.19**			-0.01		

a Correlation coefficients were based on 204 seedlings; * singnificant at P≤0.05 level; and ** significant at P≤0.01 level.

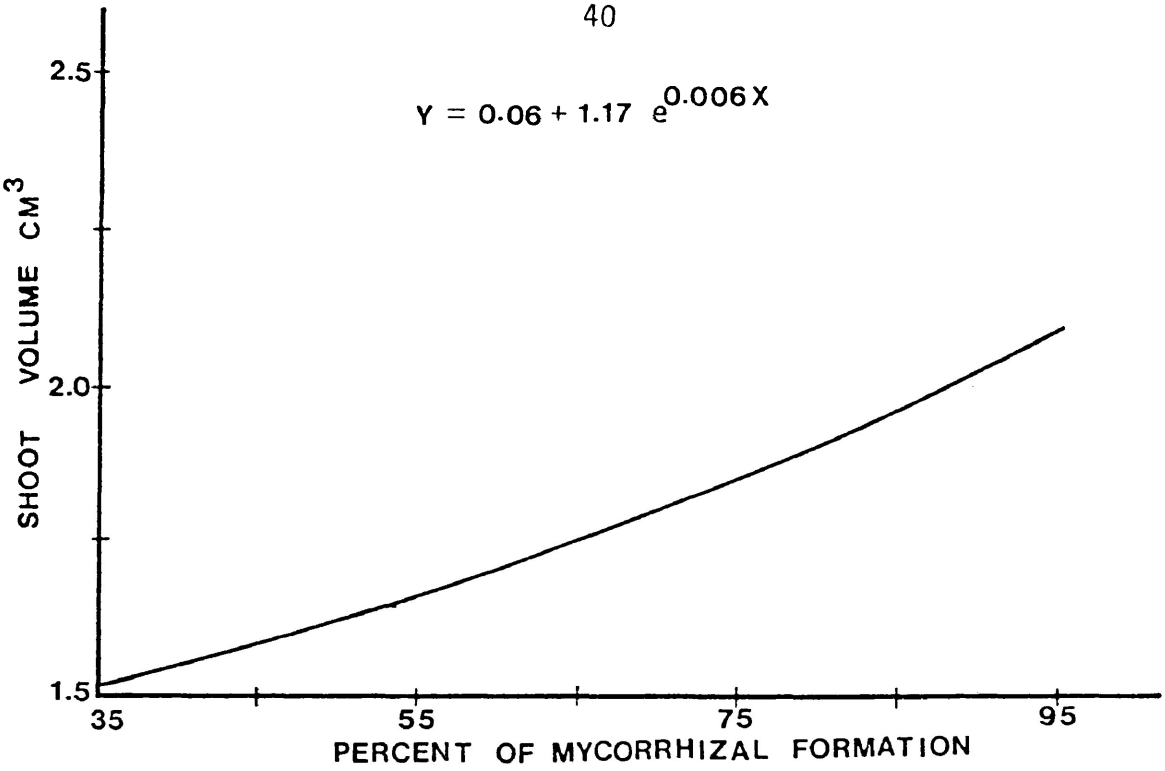


Figure 6. Relationship of L. laccata mycorrhizal formation and shoot volume of tamarack seedlings

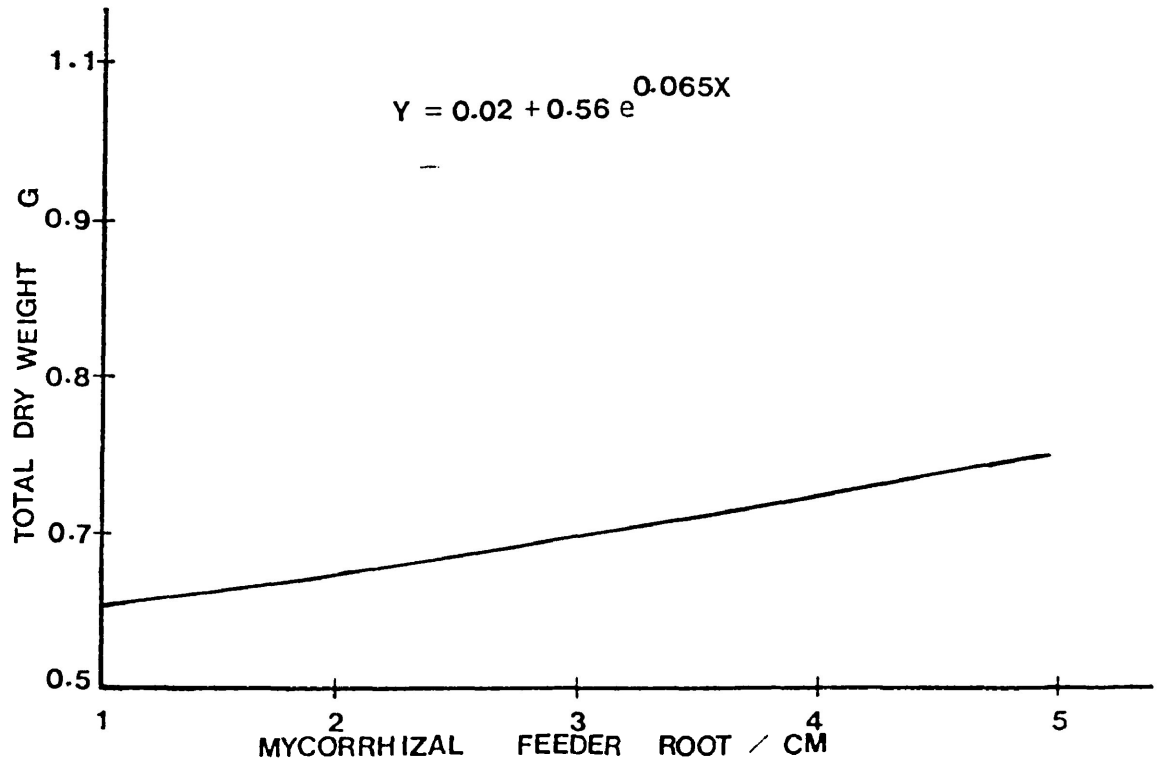


Figure 7. Relationship of ectomycorrhizal frequency and total dry weight in L. laccata treatment

was significantly affected by fungal treatments with the model 1 ANOVA (Table 5). Tukey-HSD mean comparison showed these significant differences among fungal treatments in Table 8 and Appendix Table 2. Both dimensional and biomass variables of the seedling shoot growth showed similar responses within a fungal treatment. The greatest mean values of diameter, shoot volume, and shoot dry weight were all in the L. laccata treatment. According to the relationship between shoot development and mycorrhizal formation, the increase of shoot growth resulted from the abundant mycorrhizal formation by L. laccata. In contrast, the effect of C. geophilum, which had much lower mycorrhizal formation, was not significantly different from the control treatment.

Root response to fungal treatment, unlike the shoot traits, varied with root variables within a fungal treatment. The frequency of 2nd order lateral roots and the number of 1st order lateral roots were reduced in the L. laccata treatment. The frequency of total feeder roots was significantly increased in the C. geophilum treatment, and L. laccata appeared to have a similar effect on feeder root frequency though it was not significant. Root dry weight was significantly increased in the L. laccata treatment only. This increase in root dry weight could have been caused by the accumulation of root biomass resulting from the beneficial effect of mycorrhizal formation. Fungal tissue could be a factor in the increase of root dry weight, but this contribution was very small and was ignored as indicated by Sinclair and Marx (1982).

The significantly lower shoot/root ratio in the C. geophilum treatment (Table 8) could be explained by the differential effects of this fungus on shoot and root biomass. Although seedlings in the L. laccata treatment exhibited a 19% increase in root dry weight and a 6% increase in shoot dry weight compared with the control,

Table 8. Mean values of tamarack seedling characteristics in three fungal treatments *

Trait	Fungal Treatment		
	Control	L.laccata	C.geophilum
Height cm	23.00**	23.68	23.21
Diameter mm	2.55b	2.74a	2.55b
Shoot Volume cm^3	1.52b	1.79a	1.52b
Shoot Dry Weight g	0.48b	0.51a	0.46b
Root Dry Weight g	0.16b	0.19a	0.18ab
Shoot:Root g/g	3.24a	3.01a	2.75b
Total Dry Weight g	0.64	0.69	0.65
1st order LR #	12.57a	11.35b	12.58a
2nd order LR/cm	1.09a	0.93b	1.01ab
Feeder root/cm	4.08b	4.22ab	4.32a
Myc + Ind FR/cm	0.79c	2.79a	0.98b
Mycorrhizal FR %		58.78a	6.12b
Indigenous FR %	19.32a	6.50c	16.29b
Myc + Ind FR %	19.32b	65.28a	22.42b

* Mean values are based on 204 seedlings.
** Means followed by no or same letter are not significant at P=0.05 according to Tukey-HSD test.

this differential increase between shoot and root dry weights did not result in a significant difference of shoot/root ratio in the L. laccata treatment.

In summary, the L. laccata treatment affected both the shoot and root growth of tamarack containerized seedlings; C. geophilum affected root development only; and P. tinctorius and S. granulatus inoculations did not produce any significant effect on seedling growth except on the frequencies of 2nd order lateral roots and feeder roots which were significantly affected by S. granulatus.

Estimates of Fungal Parameters and Effect Models

To provide an unbiased overall evaluation of fungal effects on seedling growth, seedling response was examined by multigroup discriminant analysis. This multivariate approach was applied to all seedling morphological variables except those mycorrhizal traits. The results of the three-group discriminant analysis showed that fungal treatment groups, control, L.laccata, and C. geophilum, were significantly different from each other (Figure 8). The 194 experimental units (each unit had 3 seedlings) in the three fungal treatments were correctly classified at 64.7% by the two canonical discriminant functions. The seedlings in the L. laccata treatment were distinguished by the first canonical discriminant axis (which summarized 65.4% of the variance) from the control and C. geophilum treatments. The seedlings in the control treatment were differentiated by the second canonical discriminant axis (which summarized 34.6% of the variance) from the C. geophilum and L. laccata treatments.

The same multivariate approach was also applied to the five-fungus-group case (Appendix Figure 2). Seedlings receiving the five fungal treatments were clustered into two groups; C. geophilum, P. tinctorius and control treatments were discriminated from S. granulatus and L. laccata.

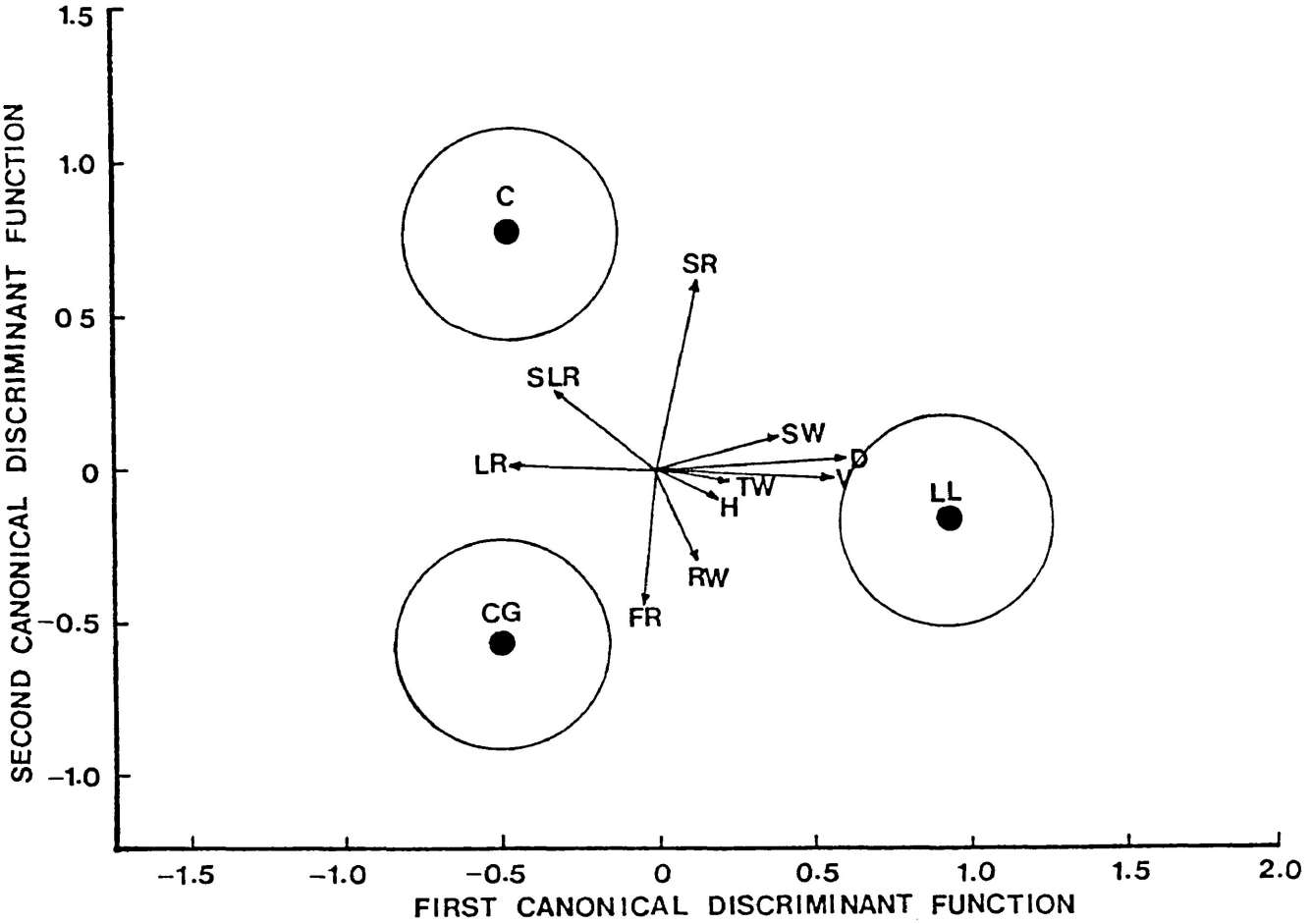


Figure 8. Graph of the first and second canonical axes of the centroids for three fungal treatments. Large dots are group centroids and circles are confidence circles (95%) for the centroides. Fungal treatments are labelled as C=control; LL=L. laccata; and CG=C.geophilum. Seedling variables are labelled as: LR=number of 1st order lateral roots; SLR=frequency of 2nd order lateral roots; SR=shoot/root ratio; SW=shoot dry weight; RW=root dry weight; TW=total dry weight; H=shoot height; D= diameter; V=shoot volume; and FR=frequency of feeder roots.

Another advantage provided by discriminant analysis was the comparison and the selection of indicator variables of fungal treatments. By ranking discriminant variables with the largest canonical correlation and magnitude of the correlation in discriminant functions, the sensitivities of seedling variables to a certain fungal treatment could be determined (Appendix Table 3). Diameter at root collar was the most indicative parameter of the L. laccata treatment, followed by shoot volume, lateral root traits, and root and shoot dry weights. Shoot/root ratio, frequency of feeder roots, and root dry weight were most sensitive to the C. geophilum treatment. The lesser importance of shoot/root ratio in the first discriminant function which separated the control from the L. laccata treatment indicated that shoot and root dry weights were affected to the same degree by the L. laccata treatment. Figure 8 shows the importance and behavior of each seedling variable in response to fungal effects as indicated by the length and direction of the variable in the discriminant space. For example, diameter and 1st order lateral roots could both be the best indicators for the L. laccata treatment, but the behaviors of the two variables were different; the growth of diameter was increased and the growth of 1st order lateral roots was inhibited by this fungus, as indicated by the different directions of the vectors.

Fungal Effect on the Relationships of Shoot and Root

The relationships between shoot and root variables were examined using Spearman's rank correlation within each of the fungal treatments (Appendix Table 4). These correlations were generally consistent among fungal treatments. However, significant differences of the correlations among fungal treatments occurred between the frequency of feeder roots and the shoot variables; highly significant ($P \leq 0.01$) correlation coefficients in the C. geophilum treatment were

obtained that ranged from 0.17 to 0.26. No significant correlation existed between feeder root frequency and any shoot variable in the L. laccata treatment. The development of both the 1st order and the 2nd order lateral roots was positively correlated with diameter growth, shoot volume, and shoot dry weight in all the fungal treatments. Shoot/root ratio was negatively correlated with shoot dry weight, shoot volume, and diameter.

Root dry weight was positively and significantly correlated with all the shoot variables in all the fungal treatments. Comparison of the linear regressions among fungus treatments showed that the relationships of root and shoot dry weights in both the C. geophilum and L. laccata treatments showed the same trend yielding regression lines with a common slope. The different intercepts indicated that shoot dry weight was greater in the L. laccata treatment than in the C. geophilum treatment while they both had the same root dry weights (Figure 9). This relationship was independent in the control from that in the C. geophilum or L. laccata treatments. The relationship between root dry weight and diameter was different among fungal treatments, although the regression lines had similar slopes (Figure 10). Larger gains in diameter growth with increasing root dry weight were evident for the L. laccata treatment than for the C. geophilum treatment.

EFFECTS OF SEED SOURCES ON SEEDLING DEVELOPMENT

The provenance effects were significant for five morphological variables, including shoot height, root dry weight, 1st order lateral root, and frequency of feeder roots (Table 5). The differences among provenance means were compared using the Tukey-HSD test (Table 9). Provenance MA exhibited the highest

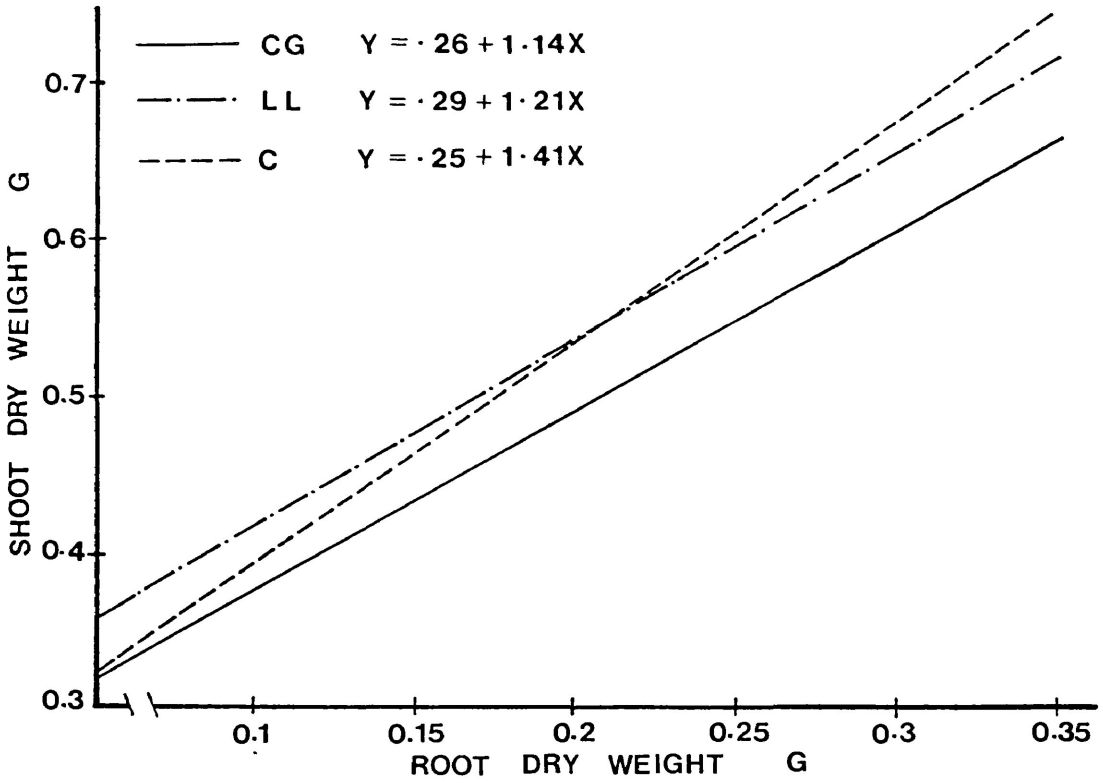


Figure 9. Relationships of shoot dry weight and root dry weight of fungal treatments of L. laccata, C. geophilum and control

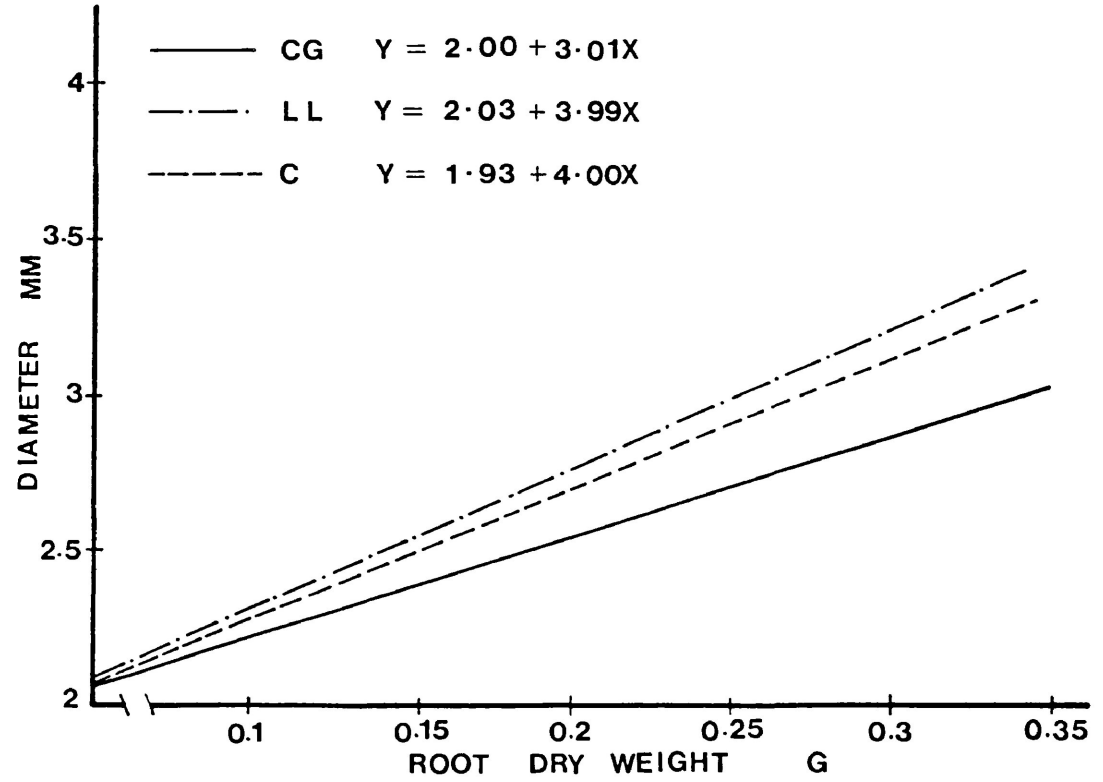


Figure 10. Relationships of diameter and root dry weight of fungal treatments of L. laccata, C. geophilum and control

frequency of feeder roots and the second largest number of 1st order lateral roots. In contrast, provenance IT showed the lowest frequency of feeder roots while the number of 1st order lateral roots was not different from the provenance MA. Shoot height was the only shoot variable that showed a significant difference among provenances. The largest mean value of shoot height was in the provenance MA.

The analysis of variance with the nested linear model (Table 6) showed that family effects were significant for all the seedling variables. In order to examine the family effects within a single provenance, the model 1 for analysis of variance was used as the partition ANOVA in which the provenance effects were replaced by the family effects. Results (Appendix Table 1) showed that the family effects were significant for five morphological variables in the provenance WT and for eight in the provenance MT. Diameter, total dry weight, shoot dry weight, and frequency of feeder roots showed significant differences in families within the provenance MT only. The number of 1st order lateral roots was significantly different among the families within the provenance WT. The ranges of family means within each of the two provenances are listed in Table 10. Generally, the family means in the provenance MT showed greater variation for most of the seedling variables than did the family means within the provenance WT. Therefore, the higher significance of family effects upon seedling variables in the nested ANOVA possibly could have resulted from the variance contribution of the families in the provenance MT.

Estimates of Variance Components

Variance components (Table 11) were calculated only for the random factors; the provenance and provenance x fungus interactions in the model 1 ANOVA; and the family and family x fungus interactions in the nested ANOVA. Being experimental

Table 9. Means values of tamarack seedling characteristics in four seed provenances

Trait	Overall mean	Provenance			
		MA	MT	IT	WT
Volume cm^3	1.694*	1.804	1.767	1.557	1.649
Height cm	23.454	24.882a	23.567ab	22.692b	22.675b
Diameter mm	2.693	2.671	2.724	2.611	2.693
1st LR #	12.167	12.417ab	13.100a	11.967ab	11.183b
2nd LR /cm	1.047	1.125	1.006	1.049	1.008
Feeder R/cm	4.245	4.515a	4.212ab	4.120b	4.134ab
Root W g	0.186	0.178	0.196	0.170	0.202
Shoot W g	0.503	0.507	0.528	0.468	0.507
S/R ratio g/g	2.862	2.934	2.813	2.960	2.742
Total W g	0.705	0.722	0.724	0.653	0.719
Myc FR % **	58.958	63.000ab	48.333b	56.500ab	68.000a
Myc FR /cm **	2.685	3.013ab	2.069b	2.505ab	3.042a

* Mean values were based on 60 seedlings and means followed by no or same letter are not significant at P=0.05 level according to Tukey-HSD test.

** For seedlings in the L. laccata treatment only.

Table 10. Range of family means and Tukey-HSD tests for tamarack seedlings from the provenances of MT and WT a

Trait	Provenance MT		Provenance WT		Overall mean
	Range of family means	Tukey-HSD	Range of family means	Tukey-HSD	
Height cm	22.15 - 24.54	1.92	22.40 - 24.31	1.91	24.11
Diameter mm	2.473 - 2.880	0.24	2.463 - 2.697	0.23	2.619
1st LR #	11.47 - 14.22	2.03	10.67 - 12.39	1.72	12.19
2nd LR #/cm	0.907 - 1.167	0.26	0.890 - 1.190	0.25	1.009
Volume cm^3	1.400 - 2.050	0.38	1.458 - 1.757	0.29	1.616
Total FR #/cm	3.924 - 4.582	0.36	4.019 - 4.388	0.37	4.205
RootDW g	0.155 - 0.214	0.05	0.140 - 0.213	0.05	0.177
ShootDW g	0.424 - 0.547	0.10	0.449 - 0.533	0.06	0.469
S/R ratio g/g	2.605 - 3.367	0.55	2.680 - 3.490	0.56	2.994
TotalDW g	0.552 - 0.747	0.14	0.594 - 0.746	0.13	0.639
Myc /cm *	1.730 - 2.719	0.61	2.333 - 2.965	0.66	2.473
Myc % *	42.58 - 67.17	11.3	60.66 - 74.50	13.8	61.51
Ind % **	18.17 - 23.50	5.33	14.08 - 23.67	5.00	19.39

* Seedlings in L. laccata treatment only; ** seedlings in control only.

a Mean values for each of the families are presented in Appendix Table 7-10.

errors, the components of residuals were the results of environmental and genetic differences, and therefore, were usually associated with the largest variance component. The interactions of seed source x fungus, if they were significant, were often associated with the second largest variance components. The variance components associated with family effect were generally the smallest for most of the variables. By comparing the sizes of the variance components between variables, the relative importance of genetic control on the development of 1st order lateral roots, frequency of feeder roots, and mycorrhizal formation were confirmed at both provenance and family levels. Relatively larger variance components associated with the seed source x fungus interactions indicated that the influence of genetic control on feeder root development and mycorrhizal formation probably varied with fungal treatments.

In order to compare the manifestation of the fungal effect on family variance, the variance components of families were also estimated within each of the fungal treatments. Results (Table 12) showed that the variation attributed to family effects was greater in the L. laccata treatment for diameter, root dry weight, and shoot/root ratio, but was less than for other fungal treatments for shoot dry weight and shoot height. Except for shoot/root ratio and frequency of 2nd order lateral roots, the variance components associated with family effects for all the variables were similar for the control and C. geophilum treatments. Significant family control of feeder root frequency and of mycorrhizal variables existed in all three treatments.

Genetic Correlation

Genetic correlations were estimated for all possible pairs of measurements based on 16 families from the provenance MT and WT (Table 13). However, the

Table 11. Estimates of variance components for the effects of provenance, provenance x fungus interaction, family, and family x fungus interaction

Trait	σ^2_P		σ^2_{FP}		σ^2_r *		σ^2_G		σ^2_{FG}		σ^2_r **	
	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%
Height	0.894	24.0			2.815	76.0	0.426	14.2	1.187	39.7	1.379	46.1
Diameter					0.095	100.0	0.009	14.8			0.052	85.4
Volume	0.006	4.5			0.126	95.5	22.13	17.8	13.95	11.2	88.41	68.2
ShootDW	0.350	5.5			6.000	94.5	0.942	11.9	0.918	11.7	6.001	76.3
RootDW	0.150	10.7	0.250	17.8	1.001	71.4	0.250	10.3	0.180	7.4	2.000	82.7
S/R Ratio	0.001	0.3	0.092	32.0	0.194	67.6	0.055	15.2	0.029	8.0	0.308	76.8
TotalDW	0.400	2.6			15.00	97.4	0.002	11.1			0.016	89.9
2nd LR	0.001	2.2			0.044	97.8	0.005	10.0	0.005	10.0	0.040	80.0
1st LR	0.481	12.6			3.323	87.4	0.364	12.2	0.444	14.8	2.189	73.0
TotalFR	0.026	11.2	0.043	18.5	0.164	70.3	0.024	10.6	0.091	40.0	0.112	49.3
MycInd%	8.304	12.3	17.33	25.7	43.84	62.0	4.080	9.6	17.59	41.5	20.73	48.9
Myc %	13.85	27.8	15.87	31.8	20.13	40.4	4.005	10.3	16.62	42.7	18.30	47.0
MycInd/cm	0.034	18.4	0.042	22.7	0.109	59.9	0.012	11.3	0.021	19.8	0.073	68.9

* Residual in the model 1 ANOVA; ** residual in the nested model ANOVA.

Table 12. Estimates of variance components of families for tamarack seedlings within individual fungal treatment, derived from fungal treatment partition nested ANOVA

Trait	σ^2_G Control		σ^2_G L. laccata		σ^2_G C.geophilum	
	Value	%	Value	%	Value	%
Height	1.9725**	63	0.4571*	19	1.1066**	42
Diameter	0.0097*	19	0.0128**	30	0.0069	12
Volume	0.0334**	31	0.0402*	20	0.0260*	25
Shoot W	0.0027**	29	0.0006	10	0.0017*	26
Root W	0.0001	5	0.0011**	44	0.0001	4
S/R ratio	0.0121	3	0.1843**	33	0.0311	15
Total W	0.0032*	20	0.0014	7	0.0025	17
1st LR #	0.4955*	23	0.4250	15	1.1101**	33
2nd LR/cm	0.0079	16	0.0085*	24	0.0107*	21
Feeder R/cm	0.0738**	59	0.1327**	48	0.0537**	33
Myc FR %			32.2785**	44	12.8918**	46
Myc+Ind FR %	7.6891**	53	28.3520**	47	11.1911**	30
My+In FR/cm	0.0249**	66	0.0431*	25	0.0279**	31

* and ** Significant variance components at $P \leq 0.05$ and $P \leq 0.01$ level; % magnitude of family variance in total variance.

relationships between root and shoot growth, root development and mycorrhizal formation, shoot development and mycorrhizal formation were the main interests of this study. In order to derive genetic correlations, the family variance components in Table 11 and the covariance components in Appendix Table 6 were used. The cross products of covariance, from which the covariance components were derived, are summarized in Appendix Table 5.

Lateral root traits and root dry weight were strongly correlated with diameter growth and shoot dry weight. However, no genetic relationship was expressed between feeder root frequency and shoot development. All of the root traits except feeder root frequency, were strongly and negatively correlated with mycorrhizal variables. Positive genetic correlation existed between feeder root frequency and mycorrhizal traits. These relationships between mycorrhizal and root traits verified again the hypothesis that mycorrhizal formation might inhibit lateral root development and stimulate feeder root proliferation. The correlations between mycorrhizal traits and shoot development varied from negative to positive; diameter and shoot dry weight were negatively correlated with mycorrhizal formation while height was positively correlated. These negative correlations are inconsistent with Spearman's correlation results. This disagreement may be caused by two factors; first, the negative correlations between mycorrhizal and lateral root traits may lead to a negative correlation between shoot and mycorrhizal traits since relatively stronger genetic correlation existed between lateral root traits and diameter and shoot dry weight; and secondly, estimates of genetic correlation are usually subject to rather large sampling errors when dealing with a small sample size, and therefore, the genetic correlations may not have been detected well in this study.

Table 13. Genetic correlations for tamarack seedling traits from 16 families in the provenances of MT and WT

Trait	Height	Diameter	ShootDW	# of 1st LR	#/cm 2nd LR	Total FR/cm	RootDW	S/R Ratio
Diameter	0.5234							
Volume	0.0237	0.0298						
ShootDW	0.0104	0.0228						
1st LR	0.0193	0.7098	0.0918					
2nd LR		0.7313	0.7845	0.0023				
Total FR	0.0019		-0.0421	0.2160				
RootDW	-0.0021	0.7930	1.0309	1.1018	0.8929	0.2040		0.1252
S/R Ratio	0.1444	-1.8288	-0.2364	-0.7187	-0.1239	-1.6216		0.1260
MycInd%	0.0189	-0.1414	-0.4740	-0.3678	-0.6470	0.4752	-0.4342	
Myc %	0.0317	-0.2739	-0.4136	-0.3325	-0.7173	0.4209	-0.3255	
MycInd/cm		0.1558	-0.2678	0.0194	-0.5675	0.9193	-0.1418	

EFFECT OF SEED SOURCE X FUNGUS INTERACTION

Analysis of variance (Table 5 and 6) indicated that the provenance x fungus and family x fungus interactions significantly influenced root system development and mycorrhizal formation. This effect was confirmed by the greater sizes of their variance components in Table 11, which were as large as or even larger than the components of the main factors. Variables showing this effect included feeder root frequency, shoot/root ratio, mycorrhizal formation to the level of provenance x fungus interaction, and shoot height, shoot volume, shoot dry weight, lateral roots, feeder root frequency and mycorrhizal formation to the level of family x fungus interaction. Because of the research interest in this study, the estimates of the seed source x fungus interaction were analyzed in detail only for root and mycorrhizal variables rather than for all traits.

Provenance x Fungus Interaction

Linear regression analysis was used to interpret the interaction of provenance x fungus. The general effect of each of five fungal treatments was first evaluated as the mean of all four provenances. Then the value of each provenance was plotted against the appropriate fungal treatment mean. Simple regressions were calculated using the joint mean points for each provenance with all the fungal treatments. The regression coefficient, expressed as slope (b), measured the responding sensitivity of each provenance to fungal treatments. A regression coefficient of 1.0 represented the average stability of all provenances. Provenances with $b < 1.0$ were considered more stable than the provenances with $b > 1.0$.

Results of joint regressions for feeder root frequency are presented in Figure 11. Two groups of provenance performances as affected by fungal treatments could be delineated based on their departure from the average sensitivity of 1.0 (fungal treatment mean slope). Provenance WT, with a slope of 0.04, showed a comparatively stable response to all the fungal treatments. Provenances MA and MT were similar in their feeder root frequency, and their relatively greater slopes (1.43 and 1.51) indicated that these provenances were very sensitive to the fungal treatments. For mycorrhizal traits, the percentage of mycorrhizae of total feeder roots was used as an example to interpret the interaction effects of provenances and fungal treatments on mycorrhizal formation (Figure 12). Provenances of IT, MT and MA showed a close performance to the average stability with slopes which ranged from 0.87 to 0.96. Provenance WT, which had a relatively greater slope (b=1.30), was more sensitive to the fungal treatments. The frequency of mycorrhizal feeder roots showed the same pattern as the percentage of mycorrhizae interpreted above.

Family x Fungus Interaction

Spearman's correlation coefficient was applied to family means in all the possible pairs of the fungal treatments. A strong, positive correlation coefficient indicated that the family rank remained generally constant and families were relatively stable in relation to the fungal treatments. A strong negative coefficient meant that family performance had a greater contribution to the variance of family x fungus interaction. The interaction of family x fungus could also occur, even with stable ranks shown in the environmental correlations, induced by changes in the difference between certain sets of the mean values. In addition, family ranks were based on a group of family means rather than a certain family,

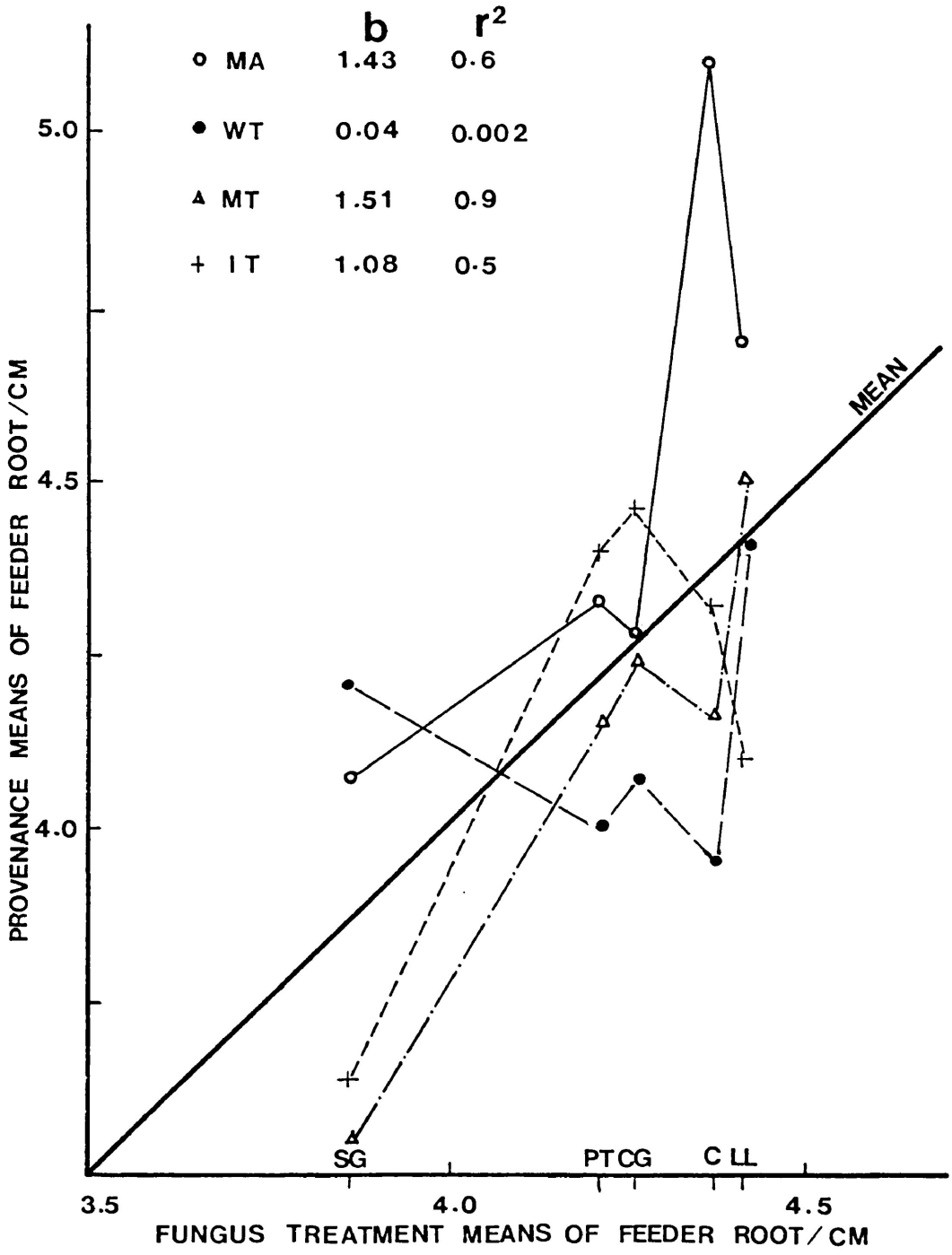
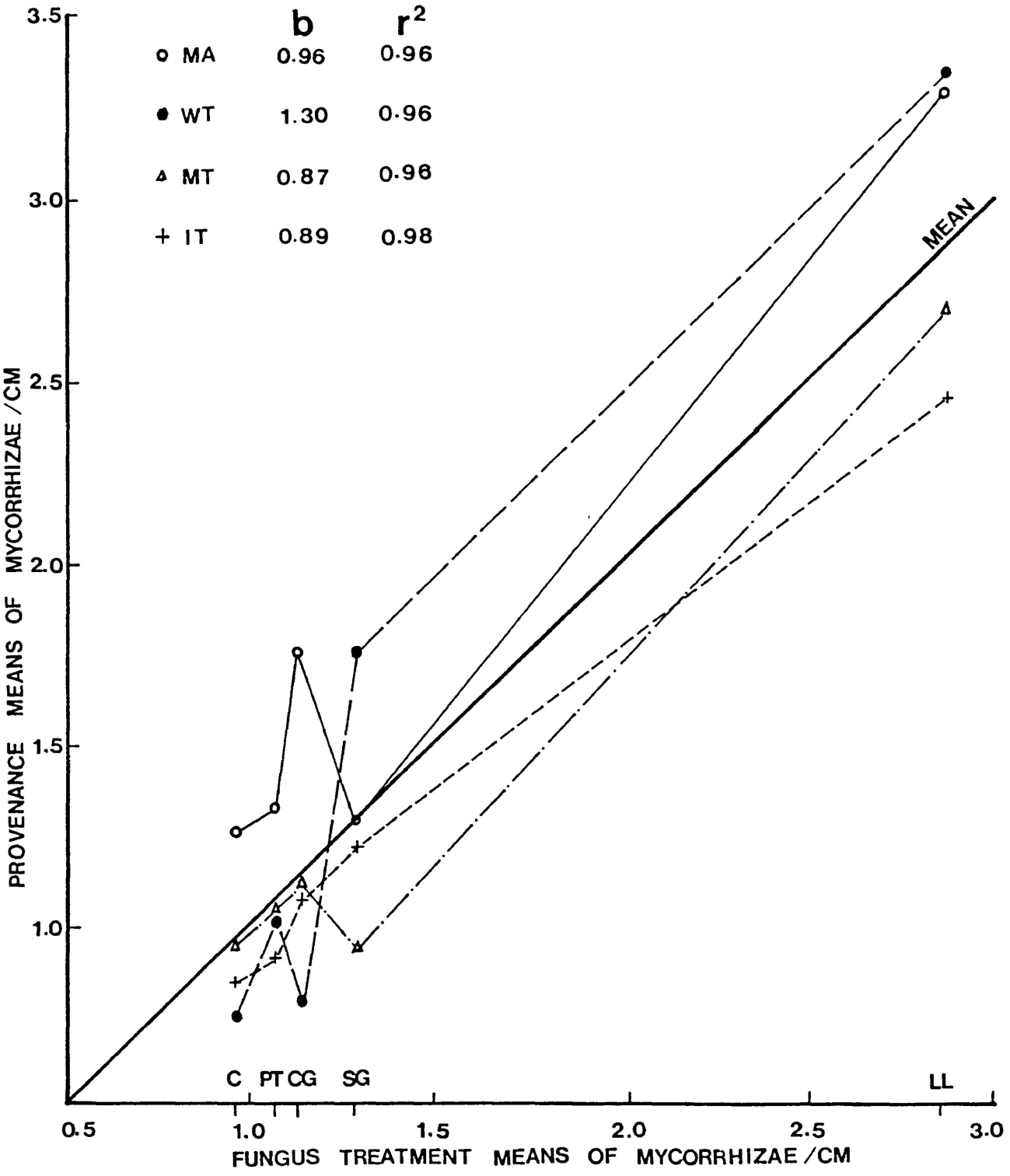


Figure 11. Relationship of the provenance and fungal treatment means of feeder root frequency. b=regression coefficient.



Figrue 12. Relationship of the provenance and fungal treatment means of ectomycorrhizal feeder root frequency. b= regression coefficient.

Table 14. Spearman's family rank correlation coefficients for all possible comparisons of fungal treatments based on the means of 17 families of tamarack seedlings

Trait	Control: L.laccata	Control: C.geophilum	L.laccata: C.geophilum
Height	-0.3516	-0.3102	0.2961
Diameter	0.0836	0.2038	0.0818
Volume	0.1444	-0.0125	-0.0125
Shoot W	-0.6697**	-0.6697**	-0.5780**
Root W	-0.3607	0.1775	-0.2429
S/R Ratio	0.1284	0.5432*	-0.0758
Total W	-0.4325*	0.0400	0.0234
1st LR	0.0547	0.1872	0.3847
2nd LR	0.2590	0.1394	0.1759
Feeder R/cm	-0.4364*	0.2824	0.1025
Myc+Ind %	0.2073	0.4772*	0.0426

* Significant at P≤0.05 level; ** significant at P≤0.01 level.

and therefore, the locations of interactions could not be revealed to a certain family by this method. Family mean comparison between fungal treatments could be helpful, but was beyond the scope of this study.

Results of the family rank correlations (Table 14) showed that control and L. laccata were more independent than any of other two pairs. Family proformance of feeder root frequency, lateral roots, and shoot height in control and L. laccata was significantly different and could be major sources responsible for the interaction of family x fungus. For the two pairs of C. geophilum with the control and with L. laccata, the family ranks for lateral roots and feeder roots were similar. For shoot dry weight, family ranks were independent in all pairs of fungal treatments as indicated by significant negative coefficients ranging from -0.57 to -0.68. Mycorrhizal traits were not significantly independent between fungal treatments. This result agrees well with the results of family variance components for mycorrhizal traits presented in Table 12.

DISCUSSION

HOST SPECIFICITY TO MYCORRHIZAL FUNGI

Mycorrhizal host specificity to tamarack varied widely among the tested fungi in pure culture synthesis. Of nine fungal species, C. geophilum, P. tinctorius, L. proxima, H. crustuliniforme and L. laccata formed ectomycorrhizae with tamarack seedlings. R. vinicolor, S. granulatus, S. tomentosus and A. porphyria failed to form mycorrhizae with the host seedlings. Thus, the results of this study increase the number of confirmed mycorrhizal symbionts on tamarack from three (Trappe, 1962) to seven.

Mycorrhizal specificity has been studied for many combinations of symbiotic fungi and host trees. Evidence indicates that a certain degree of specificity exists (Harley and Smith, 1983). However, many mycorrhizal symbionts exhibit wide host ranges as well as ecological and physiological adaptability. From a study of mycorrhizal specificity, Molina and Trappe (1982) suggest that fungi with broad host ranges may share a common compatability with many or all ectomycorrhizal hosts. This suggestion is supported by the results of this study. All of the five fungi that formed mycorrhizae with tamarack, except L. proxima, are well-known for their wide host ranges, and have been intensively studied on various tree species (Trappe, 1962; Marx, 1977a).

Since the reasons explaining why four fungi failed to form mycorrhizae under the conditions of this study are not clear, conclusions on the host specificities of the four fungi to tamarack must be drawn with caution. The lack of mycorrhizal formation in pure cultures does not necessarily mean that these fungi are not mycorrhizal symbionts of tamarack. As pointed out by Molina and Palmer (1982),

positive synthesis results confirm the ability of that particular host-fungus association, and negative results are not conclusive in themselves but do suggest that the mycorrhizal union of the fungus and host is unlikely.

One of the important applications of pure culture synthesis is to evaluate the morphological and anatomical features of ectomycorrhizae. These characteristics are not only used to confirm mycorrhizal associations, but also are useful for classification of mycorrhizae in nature (Trappe, 1967; Riffle, 1973). For each successful synthesis of fungus-tamarack combination in this study, ectomycorrhizae were well-developed, easily discernible in external appearance, and without questionable characteristics. The color, branching habit, mantle surface structure, Hartig net development, and the morphology of mantle hyphae varied obviously with the different fungal species. These mycorrhizal features could be used for reference for mycorrhizal classification in field studies.

A great difference in mycorrhizal formation was noted between the pure culture synthesis and the greenhouse experiment. C. geophilum and P. tinctorius, which formed mycorrhizae with more than 80% of the total feeder roots in the pure culture synthesis, formed mycorrhizae with only 6-12% of feeder roots on the containerized seedlings. The reasons for this difference between culture approaches are not clear. Possible explanations could include the following aspects: 1. low efficiency and poor quality of the vegetative inoculum; 2. improper inoculation technique; 3. inhibition or competition effects by indigenous mycorrhizae or other microbes; and 4. sensitivity of inoculum to fertilization. The efficiency of vegetative inoculum is usually determined by the age of the inoculum and the ability of the inoculum to develop in a growth medium. The time from inoculum production to greenhouse inoculation was about one and a half months.

This period could have been a factor reducing the inoculum efficiency of C. geophilum and P. tinctorius. Indigenous mycorrhizal fungi and other soil microorganisms may prevent the mycorrhizal formation of introducted fungi, as indicated by the negative correlations between introduced and indigenous mycorrhizal formation (Table 7). For the inoculation technique, Molina (1980) points out that certain mycorrhizal fungal species or isolates cannot withstand the disturbance involved in inoculation preparation or cannot survive within the vermiculite particles until the young germinants produce feeder roots for mycorrhizal colonization. However, the inoculation technique used in this experiment has been successfully used in many other mycorrhizal programs (Marx, et al 1982; Navratil, 1985). In addition, abundant mycorrhizal formation by L. laccata in this study indicated that the inoculation technique did not significantly affect mycorrhizal development, at least of L. laccata. However, the efficiency of inoculum could be dramatically affected by the inoculation time which was about three weeks after seed germination had occurred. Fertilization may be critical in controlling mycorrhizal formation, particularly to the fungus P. tinctorius, since a number of studies have proven that high fertilization inhibits ectomycorrhizal formation by this fungus (Maronek, et al 1981).

GENETIC ASPECT OF MYCORRHIZAL FORMATION

The influence of host genotype on ectomycorrhizal development has been studied previously on various tree species other than tamarack (Linnemann,1960; Wright and Ching, 1962; Lundeberg, 1968; Marx and Bryan 1971; Long, 1973; Mason, 1975; Cline and Reid, 1982, Navratil, 1985). Host genotype effects on mycorrhizal formation have been demonstrated at the levels of provenance, half-sib family, and

full-sib family. In this study, mycorrhizal formation was significantly influenced by the seed sources of tamarack, and the effects of host genotype within a seed source were also pronounced. All seedlings from the provenances and families formed some ectomycorrhizae with compatible fungi. The greatest difference in mycorrhizal formation in the L. laccata treatment was 20% between provenances and 32% between families. In general, no seed source of tamarack was identified that was superior in mycorrhizal formation over all the mycorrhizal fungi tested. The effects of family on mycorrhizal formation were different between provenances. The analysis of variance for family effect within provenance indicated that this variaton was relatively greater among the families in the provenance MT than in WT. A similar result has been reported by Marx and Bryan (1971), i.e. that the mycorrhizal formation of Thelephora terrestris was not influenced by the host genotype of Pinus elliottii. From the negative results, it could be suggested that genotype effects on mycorrhizal formation may not be significant for all the combinations of tree host and fungus.

The mechanisms of genotypic effects on mycorrhizal formation are nuclear. It has been suggested that the genotypic effects on mycorrhizal formation might be governed by the host susceptibility to a partcular fungus (Marx and Bryan, 1971). The susceptibility appears to be superficially similar to that found in pathogenic associations but the susceptible genes may not be dominant. There has been no evidence documenting the gene-for-gene phenomenon for mycorrhizal association (Harley and Smith, 1983). A strong and positive association between feeder root and mycorrhizal frequencies was found in this study. This relationship may indicate that the feeder root development may enhance mycorrhizal formation at least in some portions of lateral roots. Therefore, the genetic control of feeder

root development may be also responsible for the difference in mycorrhizal formation between genotypes, even though no difference in physiological susceptibility to mycorrhizal fungi existed among host genotypes.

In summary, successful ectomycorrhizal formation with five fungal species in the pure culture synthesis indicates that tamarack could be a compatible host for many mycorrhizal fungi. More ectomycorrhizal fungi of tamarack may be found through synthesis experiments and field examinations. Genetic influences of tamarack on mycorrhizal development could be direct and/or indirect. Higher levels of mycorrhizal formation could be achieved by selecting the most compatible combinations of fungus species and seed sources. Vegetative inocula of L. laccata, C. geophilum and P. tinctorius can be successfully used to inoculate containerized seedlings of tamarack. However, the inoculation time, fertilization levels and other environmental factors need to be studied further.

SEEDLING RESPONSE TO MYCORRHIZAL FUNGI AND SEED SOURCES

Seedling response to mycorrhizal treatments varied among seedling variables, and this variation was dependent on fungal treatments. As the results showed, L. laccata stimulated both shoot and root growth except the development of lateral roots. C. geophilum significantly enhanced root biomass and feeder root proliferation but not shoot growth. Both the stimulation and inhibition of seedling response could result directly from the ectomycorrhizal formation and/or from growth regulators produced by mycorrhizal fungi. Strong, positive correlations of shoot dry weight, volume, and diameter with mycorrhizal formation indicated that the effect of mycorrhizal formation on these traits was important. This effect was particularly obvious in the L. laccata treament. However, significant effects of

mycorrhizal fungi on seedling growth may not necessarily result only from the higher degree of mycorrhizal formation. For example, in this study the development of feeder roots was stimulated by C. geophilum but which formed only 6% mycorrhizae of total feeder roots. Perhaps this result can be explained by the action of growth regulators produced by the fungus on feeder root proliferation.

L. laccata is an ectomycorrhizal fungal species that has been intensively studied for inoculation of many other tree species (Molina, 1982; Molina and Chamard, 1982; Shaw, et al, 1982; Molina, 1980; Navratil, 1985). In contrast to its effect on tamarack seedlings found in this study, these other studies showed that L. laccata did not increase seedling shoot growth, and sometimes, it even reduced shoot and root development. By comparing seedling response of white spruce to L. laccata and to Amanita muscaria, Shaw and others (1982) stated that different requirements for host photosynthate between fungi may result in different host responses. The reduction of seedling growth by L. laccata was assumed to result from great demand of this fungus on host photosynthate. They also suspected that prolific mycelial colonization of potting medium and production of sporophores by L. laccata reduced the host photosynthate availability for seedling growth. Assuming this hypothesis is true, the positive response of tamarack seedlings to the mycorrhizal formation with L. laccata could be in part explained by the lack of sporophore production in this study. In addition, mycelial colonization of the potting medium by this fungus was not so prolific as described in other studies.

As discussed above, different species of mycorrhizal fungi may affect host seedling growth differently. A mycorrhizal fungus may markedly increase or decrease seedling growth by affecting only certain seedling parameters; each parameter may vary in usefulness as an indicator of mycorrhizal influence (Sinclair

and Marx, 1982). Thus, comparison of the sensitivities of seedling parameters to fungal treatments can lead to a more meaningful evaluation of the effects of the mycorrhizal fungi. Diameter growth and 1st order lateral root development were the most important indicators of the effect of L. laccata on tamarack seedlings, and feeder root frequency, root dry weight, and shoot/root ratio were the most sensitive to C. geophilum. Considering all three fungal treatments, their magnitude of effect on shoot parameters is: L. laccata > C. geophilum > control, and on root parameters is: C. geophilum > L. laccata > control.

In addition to fungal effects, seedling growth was also strongly influenced by seed sources and the seed source x fungus interaction. Provenance variation in seedling growth was confirmed by variance analysis for shoot height and three root variables. Mean comparison among four provenances indicated that the provenance MA was generally different from others on most of the seedling variables. Seedlings from the provenance MA also had a relatively higher degree of mycorrhizal formation.

Although family effects were significant for all seedling variables, their effects on shoot growth appear to be stronger than on root growth. The family x fungus interaction had a similar effect for most seedling variables. Strong genotype influence on seedling growth in mycorrhizal experiments was also found by Long (1973) on Pinus taeda and by Cline and Reid (1982) on Pinus contorta.

Relationships between shoot and root variables were generally constant among the fungal treatments. For most traits the relationships were under genetic control. The stable relationships may be due to the lack of, or the same degree of fungal effects on shoot and root growth.

In sum, it can be concluded that the performance of containerized seedlings of tamarack was influenced by both mycorrhizal fungi and seed sources. Certain mycorrhizal fungi affect shoot and root growth differently, but not necessarily in direct relation to the degree of mycorrhizal formation. L. laccata is a suitable mycorrhizal fungus for the inoculation of tamarack containerized seedlings. It should be pointed out that these observations may be limited to this particular study condition, since this experiment was done with limited number of seed sources. It may be possible that seedling responses might not be the same under other study conditions.

EFFECTS OF FUNGAL TREATMENT AND SEED SOURCE ON ROOT DEVELOPMENT

Analysis of variance revealed that lateral and feeder root growth was markedly altered by mycorrhizal inoculations and strongly affected by seed source. Thus, the results on root development must be discussed in detail from the considerations on the fungal treatments, seedling genetic variation, and fungus x genotype interaction.

Role of Mycorrhizal Fungi in Root Development

The influences of mycorrhizal fungi on roots of host plants has been studied in field experiments, pot and pure cultures. Inhibition of lateral root elongation by mycorrhizal fungi has been found by Wilcox (1968) on red pine and by Sylvia and Sinclair (1982) on Douglas-fir. Recently, Sohn (1981) reported that second and third order lateral roots of red pine seedlings were inhibited by P. tinctorius. In contrast to lateral roots, feeder root development is usually stimulated by mycorrhizal fungi (Marx and Bryan, 1971; Long, 1973). It was also reported that

mycorrhizal fungi enhanced rooting of woody plant cuttings by increasing both percentage rooting and root ball size (Linderman and Call, 1977; Navratil and Rochon; 1981). In addition to the effect of mycorrhizal infection, it has been shown that the inhibition of lateral root growth and the stimulation of feeder root proliferation can be caused by fungus exudates alone (Slankis, 1973). Similar phenomena were also found in this study. Lateral root development was inhibited by L. laccata, and feeder root proliferation was stimulated by both C. geophilum and L. laccata.

The mechanisms and pathways of fungal effects on root development are little known. Acceptable explanations probably relate to hormonal relationships in mycorrhizal development. According to Harley and Smith (1983), the activity of ectomycorrhizal fungi in setting up and maintaining a close association with the root tissue of trees must involve synthesis of metabolite activities in mycorrhizal formation. The fungal symbiont provides the host with growth hormones, including auxins, cytokinins, gibberellins, and growth-regulating B vitamins. These substances have been intensively studied for many mycorrhizal fungi. The effects of these compounds on lateral and feeder roots are different and often vary with concentrations applied (Slankis, 1973). The ability to produce growth regulators differs among fungal species and among strains within a fungal species (Harley and Smith, 1983). This sort of difference may explain the differential effects on lateral roots produced by L. laccata and C. geophilum.

The increases in the frequency of feeder roots observed on sampled lateral roots may not indicate an increase of feeder roots when the total root system is considered. The observed increase could be accompanied by inhibition of lateral

root development, consequently increasing the number of feeder roots per unit length of lateral roots (Slankis,1958).

Genetic Variation in Root Development

Genetic variance is usually used for estimating potential advances in tree improvement programs. In such cases, genotype x environment interaction can present substantial bias for estimating genetic gain. When the main objective is to identify the pattern of genetic control in root development and mycorrhizal relationships, the estimation can be made whether additive variance is estimated alone or confounded with the interaction term. In this study, family variance component was used as an estimate for comparing the relative magnitude of the additive variance among different traits. These family effects on root growth were significant for all root variables. No pattern was evident from the analyses, showing that some root characteristics were under a particular genetic control. Assuming that these traits are geneticaly comparable based on the variance components, the differences in their heritabilities would show up in environmental fitness. The significantly higher variance component of family x fungus interaction observed for feeder root frequency may imply that this trait has a lower heritability than other root traits. Low heritabilities may closely relate to environmental fitness. The lack of significance of the provenance x fungus interaction coupled with the significant family effect confirmed the strong genetic control of first order lateral roots. The other two traits, root dry weight and feeder root frequency, were strongly affected by the provenance x fungus interaction.

Genetic variation in root development has been reported in other mycorrhizal programs (Marx and Bryan, 1971; Long, 1973; Mason, 1975). However, the

mechanism of genetic control of root development is not clear. The processes, involved in root development, cell division, differentiation, longitudinal expansion, and radial elongation, appear to be independently controlled by different metabolic elements (Scott, 1972). Such independence may indicate that the various growth processes are subject to different controlling elements. On the basis of their activity in other growth and development phenomena, auxins are the earliest compounds suspected of regulatory capabilities in roots (Long 1973). Generally, auxin effects are related to their concentration; root elongation is enhanced at low concentrations in the root tip; higher concentrations inhibit elongation and promote the initiation of lateral root primordia (Leopold, 1955). Other compounds are also involved in the regulatory processes of root formation. They include thiamine, nicotinic acid, pyridoxin, Kinetin, adenine, and several micronutrients. In theory, all these growth promoters and inhibitors could be under genetic control. Thus, genetic inheritance of root proliferation may imply a genetic control over at least some of these regulators.

If, as suggested, genetic diversity in root development is physiologically oriented, a model for seedling root development could be developed. First, genetic control provides a primary influence on seedling root morphology. Then, superimposed on this basic genetic control are the effects of environmental factors, of which an important component is mycorrhizal fungus.

EVALUATION OF TECHNIQUES

The use of small test tubes as a synthesis apparatus meant a size reduction compared to standard synthesis devices such as large glass jars and tubes. Successful synthesis with five mycorrhizal fungus species on tamarack

demonstrated that the size of the culture apparatus was not critical for mycorrhizal formation in pure cultures. The small size of the device allowed the arrangement of a large synthesis experiment in a limited space, and the tube covers also provided an aseptic condition for both shoot and root interaction with the inoculum. The limitation on dimensional growth of seedling roots and nutrient supply is a drawback of the small size apparatus. Therefore, this method may not be suitable for the evaluation of seedling response to mycorrhizal formation. When the purpose of a synthesis study is merely to demonstrate the specificity between a given fungal isolate and a potential mycorrhizal host, this method could probably be much simplified.

In an attempt to increase applicability of the study results, the greenhouse experiment was designed to duplicate as closely as possible the practices of containerized seedling production. Deviations from realistic practices were reduction in fertilization and sterilization of growth substrate. Sterilization of growth medium did not maintain aseptic conditions. Containers were exposed to a variety of air-borne microorganisms, and contamination was not eliminated. Assuming that potential inoculum of contaminant fungi was randomly distributed, effects of indigenous mycorrhizae likely occurred but were homogeneous. Consequently, the results of this experiment were still meaningful concerning the introduced fungi.

The use of pot or container cultures in genetic and mycorrhizal experiments generally entails an attempt to control environmental variation. Besides the uniform condition of soil or substrates, sufficient replications are needed to reduce such environmental variation. With a relatively larger greenhouse experiment, blocking seedlings into groups, random arrangement and frequent

changes of block positions are recommended. A more formal procedure would be the use of split-plot design, such as the experimental design of this study. The resulting factorial design would provide data on fungus and host genotype effects, and on their interactions. Removal of these interaction effects from the genetic variation would enhance estimation of family variance components. If a factorial design is to be used in a mycorrhizal study, the design should be based on sufficient samples. Otherwise, large variation within experimental units will affect reliable estimates.

When a mycorrhizal experiment deals with a large number of seedlings, the time needed to examine the mycorrhizal root systems can be a problem. For this reason, examination of mycorrhizal and root variables is often based on randomly selected roots rather than on the whole root system. There has been no standard procedure for selecting the root subsamples, and the number of roots selected from each seedling varies. Subsampling procedures may include: random selection of a predetermined number of roots; random selection of roots until a predetermined total length is reached; selection of the major lateral root; and mycorrhizae counted on a predetermined length of root (Grand and Harvey, 1982). Variation could be encountered due to these subsampling techniques. However, if all the seedlings are studied in the same manner, the comparison between and among treatments remains valid.

The application of canonical discriminant analysis enhanced the interpretation of the seedling response from the multivariate viewpoint. Unlike the common use of this multivariate approach to classify a single observation into proposed groups, the main purpose in this study was to examine the weight of each seedling variable in the discriminant functions. By comparing the weights of these

variables, the importance of seedling responses was derived. Multivariate statistics have been widely used in biological studies. It is known in general that organisms are integrated units where characters are intercorrelated to varying degrees. By not taking character interdependency into account, numerous univariate analyses may overestimate divergence. Based on the results of this study, multivariate analysis is recommended when an experiment deals with a number of fungal treatments and a number of measurements which are used to predict the seedling response.

CONCLUSIONS

Five out of nine fungal species formed ectomycorrhizae with tamarack seedlings the pure culture synthesis. It was the first time that H. crustuliniforme, P. tinctorius, L. laccata, and L. proxima were proven as ectomycorrhizal symbionts of tamarack, and C. geophilum was confirmed forming ectomycorrhizae with tamarack experimentally. The successful mycorrhizal formation by the five fungi indicates that they have great potential for artificial inoculation of tamarack seedlings at a large scale.

In the greenhouse experiment, L. laccata, P. tinctorius, and C. geophilum formed ectomycorrhizae with containerized seedlings of tamarack, but the degree of mycorrhizal formation was different among the three fungi. Abundant mycorrhizal formation occurred only in the L. laccata treatment. It remains unknown why C. geophilum and P. tincorius produced much lower incidence of ectomycorrhizae on containerized seedlings than in pure culture synthesis. However, the effects of inoculation time, growing condition, and inoculum quality could have been critical for these two fungi.

A beneficial effect of ectomycorrhizae on shoot growth occurred only on seedlings inoculated with L. laccata, as shown by significant increases in shoot dry weight, volume, and diameter.

Root development of containerized seedlings of tamarack was significantly affected by L. laccata and C. geophilum. Seedlings in the L. laccata treatment showed an increase in root dry weight and a decrease in lateral root growth. Feeder root proliferation, measured as the number of feeder roots per unit length of lateral roots, was stimulated by both C. geophilum and L. laccata. Correlation analysis indicated that lateral root development was negatively associated with mycorrhizal formation while feeder root frequency was strongly and positively correlated with mycorrhizal variables.

Overall evaluation of seedling response showed that the best growth performance of tamarack containerized seedlings was in the L. laccata treatment. In view of this and its abundant mycorrhizal formation, L. laccata is suggested as a suitable fungal species for mycorrhizal inoculation of containerized seedlings of tamarack. The performance of tamarack seedlings inoculated with P. tinctorius and C. geophilum could likely be improved by using modified growth conditions and inoculation techniques.

Genetic control of mycorrhizal compatibility was demonstrated at both the levels of provenances and open-pollinated families of tamarack. Variation among provenances and the significant effect of provenance x fungus interaction indicated that no single fungus was universally superior with all the provenances. Both fungal species and seed sources governed mycorrhizal formation.

Significant provenance effects were found in feeder root frequency, number of 1st order lateral roots, root dry weight, and shoot height. Feeder root frequency

was strongly affected by the provenance x fungus interaction. Family effects within provenance were significant in most of the seedling traits, but only shoot/root ratio, and shoot volume showed relatively higher variability.

The examination of family variance components within individual fungal treatments indicated that family variation was altered by the mycorrhizal fungus. L. laccata inoculation enhanced diameter growth, root dry weight, and shoot/root ratio, and reduced shoot height and dry weight. The family response to C. geophilum inoculation was generally similar to that in the control treatment. L. laccata not only affected family variability, but also reduced the positive correlations of family ranks between the control and fungal treatments. This indicated that L. laccata tended to equalize the family genetic expression.

Strong and positive genetic correlations between root dry weight, lateral roots, shoot dry weight and diameter indicate that selection for shoot characteristics is associated with desirable changes in root systems. Feeder root frequency did not directly correlate with shoot development, but its positive correlation with root dry weight could indirectly affect certain shoot traits. Additionally, the positive correlation of feeder root frequency with mycorrhizal formation may indicate that the proliferation of feeder roots is an important characteristic in the selection of seed sources for mycorrhizal inoculation.

Based on the conclusions presented above, basic implications of this study for mycorrhizal and reforestation programs with tamarack are as follows:

1. tamarack appears to be a compatible host tree for several ectomycorrhizal fungi, and its mycorrhizal relationships could be important to seedling survival and growth in plantations;

2. the fungi C. geophilum, H. crustuliniforme, P. tinctorius, L. laccata, and L. proxima showed compatibility with tamarack seedlings, and L. laccata demonstrated an ability to develop ectomycorrhizae in containerized tamarack seedlings;

3. tamarack seed sources influence mycorrhizal formation, and the degree of this influence is dependent on both species of fungus and seed sources. Therefore, selection of fungi having higher compatibility to a broad range of host genotypes may be possible;

4. selection of seed sources for mycorrhizal formation should consider the genetic control of root characteristics, since the lateral and feeder roots are affected by mycorrhizal fungi.

LITERATURE CITED

Anderson, V.L. and R.A. McLean. 1974. Design of experiments: a realistic approach. Marcel Dekker Press. 418p.

Beeftink, H.H. 1951. Some observations on tamarack or eastern larch Larix laricina (Du Roi) K.Koch in Alberta. For. Chron. 27:38-39.

Bilan, M.V. 1971. Some aspects of tree root distribution. In Mycorrhizae Proc. First North American Conf. on mycorrhizae. E. Hacskaylo ed. USDA Misc. Publ. 1189. pp 69-80.

Brown, J.H. 1969. Variation in roots of greenhouse grown seedlings of different Scotch pine provenances. Silvae Genetica. 18:111-117.

Cech, F.C. et al. 1977. Seventh-year results of a tamarack provenance study. In Proc. 24th Northeast. For. Tree Impr. Conf. pp55-59.

Cline, M.L. and C.P.P. Reid. 1982. Seed source and mycorrhizal fungus effects on growth of containerized Pinus contorta and Pinus ponderosa seedlings. Forest Sci. 28:237-250.

Coles, J.F. 1979. New Brunswick tree improvement council makes impressive strides. For. Chron. 55:32-33.

Cooley, G.E. 1904. Silvicultural features of tamarack. Forestry Quart. 2:148-160.

Danielson, R. M. 1984. Effects of fertilization on the growth and mycorrhizal development of container-grown jack pine seedlings. Forest Sci. 30:828-835.

Dominik, T. 1950. The larch in the Czerniejewo forests near Gniezno and the developmental dynamics of its mycorrhizae. Acta Societatis Bot. Polonia. 20:305-330.

Duddridge, J.A. et al. 1980. Structure and function of mycorrhizal rhizomorphs with special reference to their role in water transport. Nature 287:834-836.

Einsphar, D.W. et al. 1984. Larch-a fast growing fiber source for the Lake States and Northeast. J. For. 82:104-106.

Falconer, D.S. 1981. Introduction to quantitative genetics. 2nd edition. Longman Press. 339p.

Fowler, D.P. 1979. Provenance research in Canada. In Tree seed production and tree improvement in Canada - research and development needs in

1977-1987. Canada Forestry Service. Petawawa Forest Experi. Station Information Report PS-X-74. pp49-61.

Fowler, D.P. et al. 1982. Tree breeding at the Maritimes Forest Research Centre, 1979 and 1980. Proc. 18th Meeting. Can. Tree. Imp. Assoc. Part 1. pp193-198.

Fortin, J.A. 1980. Technique for the observation of early morphological changes during mycorrhizal formation. Can. J. Bot. 58:361-365.

Gobl, V.F. 1974. Mycorrhiza-versuche bei paperpot-samlingen: I. impfungen von larch. Cbl. ges. Forestwesen. 91:74-87.

Gobl, V.F. 1975. Erahrungen bei der enzucht von mykorrhiza impfmaterial. Cbl. ges. Forestwesen. 92:227-237.

Grand, L.F. 1968. Conifer association and mycorrhizal syntheses of some Pacific Northwest Suillus species. Forest Sci. 14:304-312.

Grand, L.F. and A.E. Harvey. 1982. Quantitative measurement of ectomycorrhizae on plant roots. In Methods and principles of mycorrhizal research. (N.C.Schenck ed) Amer.Phytopathol.Society. 244p.

Hacskaylo, E. and C.M. Tomkins. 1973. World literature on mycorrhizae. Cont. of Reed Herbarium. No. XXII. The Paul M. Harrod Co. Baltimore. 14:304-312.

Harley, Frs. J.L. and S.E. Smith. 1983. Mycorrhizal symbiosis. Academic Press. 461p.

Jeffers, R.M. 1975. Survival and height growth of tamarack planted in northern Wisconsin. Res. Note NC-190 USDA For.Ser.

Johanson, D.A. 1940. Plant microtechnique. McGraw-Hill, New York, 523p.

Kriebel, H.B. 1963. Selection for drought resistance in sugar maple. FAO World Con. on forest genetics and tree improv. Stockholm, Sweden. 5pp.

Leopold, A.C. 1955. Auxins and plant growth. Univ.of Californ. Press. 354p.

Linderman, R.G. and C.A. Call. 1977. Enhanced rooting of woody plant cuttings by mycorrhizal fungi. J. Amer. Soc. Hort. Sci. 102:629-632.

Linnemann, G. 1960. Rassenunterschiede bei Pseudotsuga taxifolia hinsichtlich der mycorrhiza. Alg Forest Jagdztg. 131:41-47.

Linnemann, G. 1971. Erfahrungen bei synthese-versuchen, insbesondere mit Pseudotsuga menziesii II. Zentbl Bakt, Parasitk, infektionskrankheit, Hygiene. 126:230-241.

Long, A.J. 1973. Genotype and ectomycorrhizal fungi as factors in feeder root development and patterns of growth in four and one-half-month-old lablolly pine(Pinus taeda) seedlings. Ph. D. diss, N C State Univ. Raleigh. 127p.

Lundeberg, G. 1968. The formation of mycorrhizae in different provenances of pine (Pinus sylvestris) Svensk Bot. Tidskr. 62:249-255.

Malloch, D. and B. Malloch. 1981. The mycorhizal status of boreal plants: species from northern Ontario. Can. J. Bot. 59:2167-2172.

Maronek, D.M. and T.W. Hendrix. 1979. Slow release fertilizer for optimizing mycorrhizal production in pine seedlings of Pisolithus tinctorius. In Program and abstracts 4th No. Amer. conf. myc. Colorado State Univ. Callins Colo.

Maronek, D.M. et al. 1981. Fertility-mycorrhizal interactions in production of containerized pine oak seedlings. Scientia Hortic 15:283-289.

Marx, D.H. 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I, II, III, and IV. Phytopathol. Vol: 59.

Marx, D.H. 1976. Synthesis of ectomycorrhizae on loblolly pine seedlings with basidiospores of Pisolithus tinctorius. Forest Sci. 22:13-20.

Marx, D.H. 1977a. The role of mycorrhizae in forest production. MPPI Conf. Pap. pp151-161.

Marx, D.H. 1977b. Tree host range and world distribution of the ectomycorrhizal fungus Pisolithus tinctorius. Can. J. Microbiol. 23:217-223.

Marx, D.H. 1980. Variability in ectomycorrhizal development and growth among isolations of Pisolithus tinctorius as affected by source,age, and reisolation. Can.J.For.11:168-174

Marx, D.H. and J.P. Barnett. 1975. Mycorrhizae and cpntainerized forest tree seedlings. In Proc North. Am. Containerized Forest Tree Seedling Symp. (R.W. Tinus et al eds) pp85-92.

Marx, D.H. and W.C. Bryan. 1971. Formation of ectomycorrhizae on half-sib progenies of slash pine in aseptic culture. Forest Sci. 17:488-492.

Marx, D.H. and D.E. Kenney. 1982. Production of ectomycorrhizal fungus inoculum. In Methods and principles of mycorrhizal research. (N.C. Schenck ed) Amer. Phytopathol. Society. 244p.

Marx, D.H. and B. Zak. 1965. Effect of pH on mycorrhizal formation of slash pine in aseptic culture. Forest Sci. 11:66-75.

Marx, D.H. et al. 1982. Commercial vegetative inoculum of Pisolithus tinctorius and inoculation techniques for development of ectomycorrhi[illegible]n container grown tree seedlings. Forest Sci. 28:373-400.

Mary, E.P. and E.L. Stewart. 1984. In vitro synthesis of mycorrhizae between presumed specific and nonspecific Pinus + Suillus combinations. Mycologia. 76:579-600.

Mason, P. 1975. The genetics of mycorrhizal association between Amanita muscaria and Betula verrucosa. In The development and function of roots. (J.G. Torrey & D.T. Clarkson eds) Academic Press. pp567-564.

Melin, E. 1922. Untersuchungen uber die Larix mycorrhiza. I. synthese der mykorrhiza in reinkulture. Svensk Bot. Tidskr. 15:192-203.

Mexal, J.G.,C.P.P. Reid. and E.J. Burke. 1979. Scanning electron microscopy of lodgepole pine roots. Bot. Gaz. 140:318-323.

Mikola, P. 1973. Application of mycorrhizal symbiosis in forestry practice. In ectomycorrhizae: their ecology and physiology (G.C.Marks and T.T. Kozlowski, eds) pp383-411. Academic press. New York.

Molina, R. 1980. Ectomycorrhizal inoculation of containerized western conifer seedlings. USDA For.Serv. Res. Note PNW-375.

Molina, R. 1979. Pure culture synthesis and host specificity of red alder mycorrhizae. Can. J. Bot. 57:1223-1228.

Molina, R. 1982. Use of the ectomycorrhizal fungus laccaria laccata in forestry. I. Consistency between isolates in effective colonization of containerized conifer seedlings. Can. J. For. Res. 12:469-473.

Molina, R. and J. Chamard. 1982. Use of the ectomycorrhizal fungus Laccaria laccata in forestry. II. Effects of fertilization forms and levels on ectomycorrhizal development and growth of container-grown Douglas-fir and ponderosa pine seedlings. Can. J. For. Res. 13:89-95.

Molina, R. and J.G. Palmer. 1982. Isolation, maintenance and pure culture manipulation of ectomycorrhizal fungi. In Methods and principles of mycorrhizal research. (N.C. Schenck ed) Amer. Phytopathol. Society. 244p.

Molina, R. and J.M. Trappe. 1982. Pattern of ectomycorrhizal host specificity and potential among Pacific Northwest conifers. Forest Sci. 28:423-458.

Navratil, S. 1981. Manipulation of mycorrhizae in nursery production: an emerging technology. In Nurserymen's meeting. St. Williams. Ontario

Ministry of Natural Resources. pp44-83.

Navratil, S. 1985. Mycorrhizal inoculation of white spruce containerized seedlings. In the Poster Session of the Interior Spruce Seedling Performance Seminer. Prince George, B.C.

Navratil, S. and G.C. Rochon. 1981. Enhanced root and shoot development poplar cutting induced by Pisolithus inoculum. Can. J. For. Res. 11:844-848.

Park, Y.S. and D.P. Fowler. 1981. Effects of inbreeding and genetic variances in a natural population of tamarack (Larix laricina (Du Roi) K.Koch) in Eastern Canada. Silvae Genetica. 31:21-26.

Park, Y.S. and D.P. Fowler. 1983. A provenance test of Japanese larch in eastern Canada including comparative data on European larch and tamarack. Silvae Genetica 32:96-101.

Pimentel, R.A. 1979. Morphometrics. Kendall and Hunt Press. 276p.

Rauter, R.M. and B.J. Graham. 1983. The genetics and tree improvement potential of larches in Ontario. In Larch symposium-potential for the future. Proc. Symposium by the Ontario Ministry of Natural Resources and the Univer. of Toronto. pp11-26.

Reid, C.C.P. 1978. Mycorrhizae and water stress. In Root physiology and symbiosis (A. Riedaker et al eds). pp392-408.

Rehfeldt, G.E. 1970. Genecology of Larix laricina (Du Roi) K. Koch. in Wisconsin. I. Patterns of natural variation. Silvae Genetica 19:9-16.

Riffle, J.W. 1973. Pure culture synthesis of ectomycorrhizae on Pinus ponderosa with species of Amanita, Suillus and Lactarius. Forest Sci. 19:242-250.

Riffle, J.W. and D.M. Maronek. 1982. Ectomycorrhizal inoculation procedures for greenhouse and nursery studies. In Methods and principles of mycorrhizal research. (N.C.Schenck ed) 243p.

Roe, E.I. 1957. Silvical characteristics of tamarack (Larix laricina) USDA For. Ser. Lake States For. Exp. Station. Pap. No 52. p22.

Scott, T.K. 1972. Auxins and roots. Ann. Rev. Plant Physiol. 23:235-258.

Shaw, C.G. and R. Molina. 1980. Formation of ectomycorrhizae following inoculation of containerized sitka spruce seedlings. USDA Res. Note PNM-351. 8p.

Shaw, C.G. et al. 1982. Development of ectomycorrhizae following inoculation of containerized sitka and white spruce seedlings. Can. J.

For. Res. 12:191-195.

Sinclair, W.A. and D.H. Marx. 1982. Evaluation of plant response to inoculation. A. Host variables. In Methods and principles of mycorrhizal research.(N.C. Schenck, ed) Amer. Phytopathol.Society. 244p.

Slankis, V. 1958. The role of auxin and other exudates in mycorrhizal symbiosis of forest trees. In Physiology of forest trees. (K.V. Thimann ed) pp427-437. Ronald Press.

Slankis, V. 1973. Hormonal relationships in mycorrhizal development. In Ectomycorrhizae -their ecology and physiology. (G.C. Marks and T.T. Kozlowski. eds) Acad. Press. New York. 444p.

Sohn, R.F. 1981. Pisolithus tinctorius forms long ectomycorrhizae and affects root development in seedlings of Pinus resinosa. Can. J. Bot. 59:2129-2134.

Sokal, R.R. and F.J. Rohlf. 1981. Biometry. 2nd edtion. Freeman and Company Press. 859p.

Stonecypher, R.W. et al. 1965. Estimates of components of variance and covariance in root and shoot characteristics of loblolly pine after one growing season. In Proc. 8th Southern Conf. on forest tree improv. pp86-95.

Sylvia, D.M. and W.A. Sinclair. 1982. Suppressive influence of Laccaria laccata on Fusarium oxysporum and on Douglas-fir seedlings. Phytopathol. 73:384-389.

Trappe, J.M. 1962. Fungus associates of ectotrophic mycorrhizae. Bot. Review. 28:538-606.

Trappe, J.M. 1967. Pure culture synthesis of Douglas-fir mycorrhizae with species of Hebeloma, Suillus, Rhizopogon and Astraeus. Forest. Sci. 13:121-130.

Trappe, J.M. 1977. Selection of fungi for ectomycorrhizal inoculation in nurseries. Ann. Rev. Phytopathol. 15:203-222.

Winer, B.J. 1971. Statistics principles in experimental design. 2nd ed. McGraw-Hill. New York.

Wright, E. 1963. Effects of seed source on formation of ectomycorrhizae on Douglas-fir seedlings. In Mykorrhiza(J.G. Fischer). 203p.

Wright, E. and K.K. Ching. 1962. Effect of seed source on mycorrhizal formation on Douglas-fir seedlings. Northwest Sci. 36:1-6.

Wilcox, H.E. 1968. Morphological studies of the roots of red pine Pinus ponderosa II. Fungal colonization of roots and the development of mycorrhizae. Amer. J. Bot. 55:686-700.

Wyckoff, G.W. 1982. Larch and tamarack-alternative conifers for the Lake States and northeast. In Proc. artificial regeneration of conifers in upper great lake region. (D.M. Glerr ed). pp168-172.

APPENDICES

App. Table 1.1 Mean squares in analysis of variance for seedling characteristics within the provenance WT a

Source	df	Height	Diameter	Volume	ShootW	Root W
Fungus (F)	2	13.676	0.4896**	1.0861**	0.0116	0.0037
Block x F	6	1.9971	0.0161	0.0423	0.0031	0.0018
Family (G)	8	4.0728**	0.0882	0.1438	0.0128	0.0057**
Block x G	24	1.5987	0.0401	0.0650	0.0046	0.0012
F x G	16	7.2457**	0.0611	0.1785**	0.0063	0.0036*
Residual	48	1.1294	0.0513	0.0716	0.0061	0.0017

Source	df	S/R	Total W	1st LR	2nd LR	Feeder R
Fungus (F)	2	2.0432*	0.0214	58.587**	0.3145*	0.6816
Block x F	6	0.5931	0.0057	2.5379	0.0354	0.0342
Family (G)	8	1.2354**	0.0263	4.5118*	0.0985*	0.2065
Block x G	24	0.2399	0.0109	1.6918	0.0221	0.0609
F x G	16	0.5740	0.0157	5.0165**	0.0473	0.3109**
Residual	48	0.3919	0.0128	1.8243	0.0356	0.1090

Source	df	Myc %	MycInd%	MycInd/cm
Fungus (F)	2	43481.6**	28964.7**	49.937**
Block x F	6	12.139	16.0951	0.0631
Family (G)	8	40.358	106.984**	0.2037*
Block x G	24	21.899	27.0236	0.0762
F x G	16	25.999	61.9536**	0.1785*
Residual	48	21.215	21.2137	0.0784

a Fungus effect was estimated using Quasi F-ratio method.
* Significant at P≤0.05 level; ** significant at P≤0.01 level.

App. Table 1.2 Mean squares in analysis of variance for seedling characteristics within the provenance MT a

Source	df	Height	Diameter	Volume	Shoot W	Root W
Fungus (F)	2	7.2677	0.3654*	0.0857	0.0355	0.0081
Block x F	6	2.2691	0.0411	0.0855	0.0088	0.0009
Family (G)	7	8.8869**	0.2454*	0.5632**	0.0215**	0.0062*
Block x G	21	2.0458	0.0293	0.0690	0.0046	0.0019
F x G	14	4.8239**	0.0434	0.1268	0.0124*	0.0015
Residual	42	1.5173	0.0518	0.1020	0.0051	0.0021

Source	df	S/R	Total W	1st LR	2nd LR	FeederR
Fungus (F)	2	3.0763*	0.0189	2.9031	0.1722	0.4096
Block x F	6	0.1076	0.0162	2.0088	0.0276	0.0693
Family (G)	7	0.8474**	0.0537*	9.2749*	0.0818	0.5588**
Block x G	21	0.3037	0.0094	2.4610	0.0333	0.1209
F x G	14	0.2707	0.0157	2.2096	0.0763	0.6278**
Residual	42	0.2373	0.0184	3.0250	0.0456	0.1234

Source	df	Myc %	MycInd %	MycInd/cm
Fungus (F)	2	28058.**	16471.**	30.332**
Block x F	6	15.488	14.554	0.0574
Family (G)	7	79.367**	37.397*	0.2277*
Block x G	21	14.675	14.835	0.0472
F x G	14	140.30**	124.64**	0.2118**
Residual	42	14.485	19.949	0.0747

a Fungus effect was estimated using Quasi F-ratio method.
* Significant at P≤0.05 level; ** significant at P≤0.01 level.

App. Table 2. Mean values of tamarack seedling characteristics in five fungal treatments *

	Fungus treatment				
Trait	L1	Cg	Pt	Sg	Control
Height cm	23.53**	23.19	24.35	22.35	23.60
Diameter mm	2.85a	2.57b	2.66ab	2.68ab	2.62ab
Volume cm^3	1.94a	1.54b	1.73ab	1.64ab	1.63ab
Shoot W g	0.55a	0.46b	0.51ab	0.51ab	0.49ab
Root W g	0.22a	0.17b	0.19ab	0.19ab	0.17b
Shoot/Root Ratio w/w	2.68	2.87	2.85	2.83	3.08
Total W g	0.82a	0.63b	0.70ab	0.70ab	0.68b
1st LR #	11.52	12.69	13.10	11.42	12.10
2nd LR/cm	0.97ab	1.06ab	1.09ab	0.94b	1.17a
Feeder R/cm	4.42a	4.27ab	4.22ab	3.87b	4.38a
Myc+Ind FR/cm	2.88a	1.12b	1.07b	_____	0.96b
Myc FR %	58.96a	8.71b	11.67b	_____	_____
Ind FR %	6.46c	17.19b	13.65b	_____	22.12a
Myc+Ind %	65.42a	25.91bc	25.31c	32.69b	22.12c

* Mean values were based on 48 seedlings.

** Means followed by no or the same letter are not significant at P=0.05 level according to Tukey-HSD test.

App. Table 3. Discriminant analysis for estimates of fungal treatments and seedling parameters based on means of experiment units

Actual Group		No Of Cases	Predicted Group Membership 1	2	3
1	Control	68	49	8	11
			72.1%	11.8%	16.2%
2	L. laccata	68	9	44	15
			13.2%	64.7%	22.1%
3	C. geophilum	68	14	15	39
			20.6%	22.1%	57.4%

Ranks of discrimination variables by the functions with largest correlation and the magnitude of the correlation

Trait	Function 1	Function 2
Diameter	0.571*	0.012
Volume	0.562*	-0.019
1st LR	-0.459*	0.001
Shoot W	0.376*	0.105
2nd LR	-0.337*	0.292
Total W	0.217*	-0.011
Height	0.205*	-0.097
S/R	0.137	0.589*
FeederR	-0.051	-0.428*
Root W	-0.134	-0.298*

App. Table 4. Spearman's rank correlation coefficients for the relationships between seedling traits under fungal treatments [a]

Trait		Height	Diameter	Volume	ShootW	1st LR	2nd LR	Tot FR	Root W	S/R Ratio
Diameter	Cg	0.27								
	Ll	0.25								
	C	0.28								
Volume	Cg	0.61	0.91							
	Ll	0.56	0.93							
	C	0.60	0.93							
ShootW	Cg	0.38	0.68	0.68						
	Ll	0.39	0.77	0.81						
	C	0.44	0.77	0.80						
1st LR	Cg	-0.02	0.26	0.19	0.23					
	Ll	0.09	0.26	0.24	0.18					
	C	-0.17	0.16	0.08	0.13					
2nd LR	Cg	0.01	0.30	0.25	0.27	0.28				
	Ll	0.09	0.28	0.28	0.20	0.27				
	C	-0.01	0.19	0.18	0.19	0.10				
Tot FT	Cg	0.17	0.25	0.27	0.26	0.12	0.20			
	Ll	-0.01	0.11	0.01	0.09	0.03	0.07			
	C	0.08	0.15	0.16	0.07	0.00	-0.02			
Root W	Cg	0.07	0.66	0.54	0.71	0.34	0.35	0.21		
	Ll	0.06	0.75	0.66	0.68	0.27	0.39	0.17		
	C	0.06	0.72	0.61	0.69	0.27	0.29	0.08		
S/R Ratio	Cg	0.28	-0.27	-0.12	-0.12	-0.27	-0.23	-0.09	-0.73	
	Ll	0.27	-0.37	-0.22	-0.12	-0.26	-0.36	-0.16	-0.77	
	C	0.30	-0.25	-0.10	-0.03	-0.21	-0.26	-0.05	-0.68	
Total Weight	Cg	0.28	0.72	0.67	0.95	0.32	0.33	0.30	0.88	-0.37
	Ll	0.29	0.82	0.81	0.96	0.21	0.28	0.13	0.85	-0.36
	C	0.33	0.81	0.78	0.97	0.20	0.23	0.07	0.85	-0.26

[a] Correlations were based on 204 seedlings; significant coefficients at $P=0.05 \geq |0.12|$; at $P=0.01 \geq |0.17|$.

App. Table 5. Cross products of family effect and residuals for the analysis of covariance on all the possible pairs of seedling variables *

Source	df	Height x Diameter	Volume	ShootDW	1st LR	2nd LR	FeederR	RootDW	S/R	TotalW	MycInd%
Family	14	0.3902	0.9775	0.0916	0.1989	-0.0015	0.0253	-0.0102	0.4341	0.0254	1:5235
Residual	84	0.0014	0.1043	0.0125	0.1076	-0.0429	0.0230	-0.0016	0.1689	0.0208	1.2268

Source	df	Height x Myc%	MycInd/cm	Diameter x Volume	ShootDW	1st LR	2nd LR	FeederR	RootDW	S/R	TotalW
Family	14	1.2664	-0.0329	0.2249	0.0394	0.5774	0.0744	0.0031	0.0205	-2.9322	0.0055
Residual	84	0.7637	0.0717	0.0648	0.0139	0.0905	0.0149	0.0238	0.0074	-0.0569	0.0225

Source	df	Diameter x MycInd%	Myc %	MycInd/cm	Volume x ShootDW	1st LR	2nd LR	FeederR	RootDW	S/R	TotalW
Family	14	-0.3389	-0.7438	0.0268	0.0565	0.7283	0.0956	0.0531	0.0253	-0.2309	0.0708
Residual	84	-0.0138	-0.1194	0.0074	0.0183	0.1168	0.0159	0.0303	0.0089	-0.0554	0.0291

Source	df	Volume x MycInd%	Myc %	MycInd/cm	ShootDW x 1st LR	2nd LR	FeederR	RootDW	S/R	TotalW	MycInd%
Family	14	-0.2960	-0.7662	-0.0375	0.1661	0.0254	-0.0075	0.0061	-0.0303	0.0227	-0.3161
Residual	84	-0.0923	-0.0688	-0.0153	0.0332	0.0047	-0.0049	0.0003	-0.0098	0.0082	-0.0091

Source	df	ShootDW x Myc %	MycInd/cm	1st LR x 2nd LR	FeederR	RootDW	S/R	TotalW	MycInd%	Myc %	MycInd/cm
Family	14	-0.3914	-0.0178	0.4364	0.3769	0.1414	-1.3667	0.3435	-5.6130	-4.9296	0.0393
Residual	84	-0.0421	0.0009	0.0424	0.1344	0.0153	-0.1462	0.0381	-0.2337	-0.1137	0.0240

App. Table 5 (Cont.)

Source	df	2nd LR x FeederR	RootDW	S/R	TotalW	MycInd%	Myc %	MycInd/cm	RootDW x S/R	TotalW
Family	14	-0.0032	0.0146	-0.1660	0.0379	-1.2638	-1.2982	-0.0604	-0.0888	0.0122
Residual	84	-0.0402	0.0023	-0.0355	0.0064	-0.1554	-0.0795	-0.0079	-0.0165	0.0045

Source	df	RootDW x MycInd%	Myc %	MycInd/cm	FeederR x RootDW	S/R	TotalW	MycInd%	Myc %	MycInd/cm
Family	14	-0.1816	-0.1405	-0.0085	0.0101	-0.0925	0.0084	2.1969	1.8828	0.2474
Residual	84	-0.0154	-0.0172	0.0047	0.0042	-0.0384	0.0100	0.4131	0.3166	0.0597

Source	df	S/R x Total	MycInd%	Myc %	MycInd/cm	Total x MycInd%	Myc %	MycInd/cm	MycInd% x Myc %	MycInd/cm
Family	14	-0.0888	1.1660	1.0325	0.0031	-0.6069	-0.6151	-0.0147	57.3071	2.9852
Residual	84	-0.0216	0.4542	0.3232	0.0070	-0.0220	-0.096	0.0022	15.9476	1.0532

Source	df	Myc % x MycInd/cm
Family	14	2.4185
Residual	84	0.8052

* Cross products of family effect and residuals were computed from the Table 4. analysis of covariance.

App. Table 6. Family components of covariance from analysis of covariance for seedling traits of 16 tamarack open-pollinated families

	Volume	Dia-meter	ShootW	1stLR	2ndLR	FeederR	RootW	S/R ratio	TotalW	MycInd%	Myc%	MycInd/cm
Height	.0727	.0324	.0066	.0076		.0002	-.0007	.0221	.0025	.0247	.0419	-.1046
Volume		.0133	.0032	.0510	.0066	.0019	.0014	-.0146	.0035	-.0169	-.0581	-.0019
Diameter			.0021	.0406	.0049		.0012	.2396		-.0271	-.0520	.0016
Shoot W				.0110	.0017	-.0002	.0005	-.0017	.0012	-.0256	-.0291	.0014
1st LR					.0001	.0202	.0105	-.1017	-.0255	-.4483	-.4013	.0013
2nd LR							.0011	-.0109	.0025	-.0924	-.1016	-.0044
Feeder R							.0005	-.0045		.1487	.1305	.0156
Root W								-.0060	.0006	-.0139	-.0103	.0007
S/R ratio									-.0006	.0593	.0591	
Total W										-.0487	-.0487	
MycInd%											3.4466	.1610
Myc%												.1344

App. Table 7. Mean values of open-pollinated families for seedling traits with three fungal treatment *L. laccata*, *C. geophilum*, and control pooled

Family	Height cm	Diameter mm	1stLR #	2ndLR /cm	FeederR /cm	RootDW g	ShootDW g	S/R w/w	TotalW g	Volume cm^3	MycInd %	MycInd /cm
MT1	22.74	2.51	12.25	1.06	4.03	0.16	0.49	3.37	0.65	1.44	32.69	1.36
MT2	23.67	2.59	11.47	0.91	3.92	0.17	0.47	2.99	0.61	1.62	37.06	1.44
MT3	22.63	2.47	12.50	1.01	4.18	0.18	0.46	2.82	0.64	1.40	36.03	1.53
MT4	24.54	2.72	14.22	1.05	4.44	0.21	0.51	2.65	0.71	1.84	35.08	1.66
MT5	23.11	2.55	12.19	0.92	4.58	0.17	0.45	3.21	0.62	1.52	38.72	1.78
MT6	22.15	2.79	13.67	1.04	4.15	0.21	0.53	2.61	0.74	1.73	35.39	1.55
MT7	23.59	2.64	12.42	1.03	4.16	0.16	0.42	2.82	0.55	1.66	35.39	1.46
MT8	24.44	2.88	12.97	1.17	4.09	0.20	0.55	2.81	0.72	2.05	36.92	1.43
WT1	23.07	2.50	11.44	0.90	4.02	0.17	0.45	3.24	0.65	1.46	33.64	1.42
WT2	23.12	2.51	11.94	0.97	4.19	0.16	0.47	3.41	0.61	1.46	37.81	1.56
WT3	23.67	2.62	11.67	0.89	4.31	0.16	0.49	3.27	0.65	1.64	40.75	1.68
WT4	22.94	2.66	12.28	1.06	4.05	0.19	0.49	2.74	0.69	1.63	33.97	1.37
WT5	22.40	2.60	12.25	1.02	4.30	0.19	0.45	2.68	0.66	1.53	36.75	1.59
WT6	22.73	2.69	12.38	1.19	4.35	0.21	0.53	2.69	0.75	1.68	33.25	1.43
WT7	23.08	2.56	10.67	0.97	4.39	0.17	0.47	2.92	0.63	1.52	38.17	1.68
WT8	24.31	2.68	11.67	1.01	4.14	0.18	0.53	3.27	0.71	1.76	32.64	1.38
WT9	23.78	2.46	10.86	0.93	4.19	0.14	0.45	3.49	0.59	1.47	32.22	1.39

App. Table 8. Mean values of open-pollinated families for seedling traits within fungal treatment control

Family	Height cm	Diameter mm	1stLR #	2ndLR /cm	FeederR /cm	RootDW g	ShootDW g	S/R w/w	TotalW g	Volume cm^3	MycInd %	MycInd /cm
MT1	22.08	2.55	11.92	1.02	3.71	0.14	0.48	3.61	0.62	1.45	23.50	0.91
MT2	24.95	2.61	11.42	0.99	4.16	0.17	0.52	3.41	0.69	1.75	18.17	0.75
MT3	21.33	2.29	13.58	1.18	3.84	0.15	0.39	2.95	0.55	1.13	18.33	0.71
MT4	24.92	2.66	13.49	1.20	3.94	0.18	0.54	3.19	0.71	1.79	22.91	0.90
MT5	24.41	2.59	11.58	0.86	4.63	0.17	0.48	3.85	0.64	1.64	22.17	1.01
MT6	23.58	2.78	13.67	1.32	3.74	0.20	0.58	3.13	0.78	1.83	20.67	0.77
MT7	23.17	2.51	11.50	0.99	4.16	0.12	0.35	3.03	0.47	1.47	19.17	0.79
MT8	23.87	2.88	12.67	1.29	4.32	0.20	0.55	2.94	0.75	1.99	21.59	0.93
WT1	21.25	2.42	13.83	0.94	3.93	0.21	0.39	2.96	0.59	1.24	14.42	0.56
WT2	22.75	2.44	12.75	1.14	3.82	0.15	0.45	3.16	0.59	1.35	15.33	0.58
WT3	21,83	2.49	12.25	0.98	4.61	0.14	0.48	3.57	0.63	1.36	23.33	1.10
WT4	23.91	2.57	13.08	0.99	4.03	0.16	0.47	3.04	0.63	1.58	14.08	0.57
WT5	20.54	2.60	13.92	1.19	4.23	0.17	0.44	2.82	0.61	1.39	18.33	0.77
WT6	20.95	2.58	12.83	1.15	4.13	0.18	0.48	3.13	0.66	1.39	19.67	0.81
WT7	24.13	2.47	11.08	1.08	4.35	0.15	0.50	3.54	0.64	1.47	23.67	1.04
WT8	25.12	2.67	12.25	1.14	3.86	0.17	0.56	3.55	0.72	1.80	16.25	0.64
WT9	22.21	2.30	11.92	1.02	3.84	0.14	0.41	3.27	0.55	1.18	16.92	0.66

App. Table 9. Mean values of open-pollinated families for seedling traits within fungal treatment

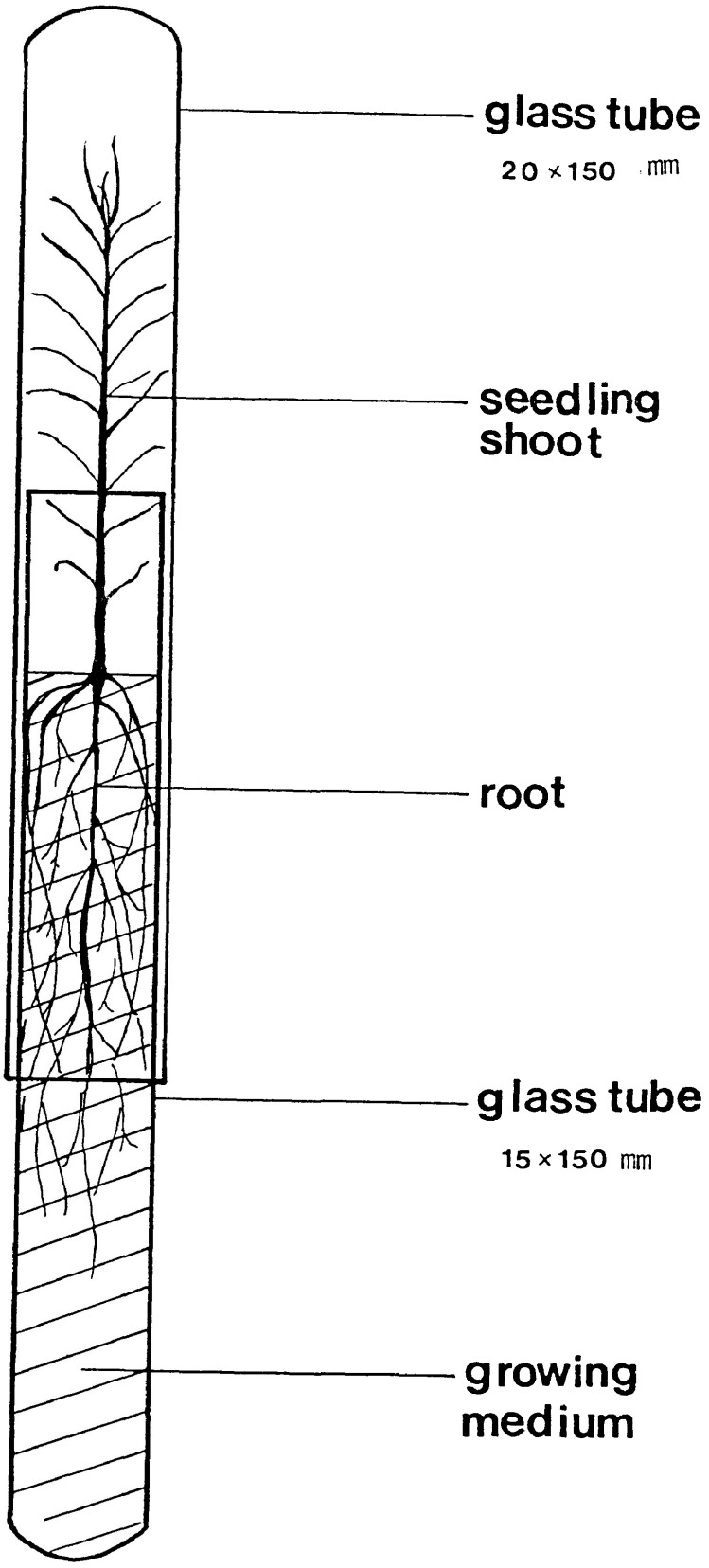
L. laccata

Family	Height cm	Diameter mm	1stLR #	2ndLR /cm	FeederR /cm	RootDW g	ShootDW g	S/R w/w	TotalW g	Volume cm^3	MycInd %	MycFR /cm
MT1	23.63	2.50	11.17	0.89	4.98	0.25	0.49	3.44	0.75	1.48	48.75	1.98
MT2	23.04	2.74	11.25	0.77	3.78	0.18	0.49	2.96	0.66	1.75	72.08	2.74
MT3	24.33	2.67	11.58	0.97	4.18	0.19	0.52	2.84	0.71	1.74	68.67	2.88
MT4	23.58	2.74	14.08	1.00	5.18	0.23	0.46	2.35	0.69	1.80	55.50	2.91
MT5	23.00	2.69	12.42	0.92	4.70	0.17	0.53	3.26	0.70	1.67	61.67	2.87
MT6	22.36	2.85	13.75	0.96	4.64	0.24	0.53	2.23	0.79	1.82	60.58	2.85
MT7	24.17	2.85	12.99	1.03	4.05	0.21	0.53	2.65	0.75	1.98	64.00	2.59
MT8	25.62	3.07	13.58	1.21	3.52	0.21	0.59	2.53	0.80	2.42	64.75	2.28
WT1	24.79	2.65	10.25	0.85	4.33	0.16	0.52	3.54	0.67	1.75	65.92	2.85
WT2	22.25	2.55	10.17	0.75	3.95	0.16	0.49	3.99	0.62	1.44	70.92	2.78
WT3	24.17	2.62	11.50	0.77	3.87	0.14	0.48	3.40	0.63	1.66	72.66	2.78
WT4	23.17	2.81	10.83	1.04	3.99	0.18	0.51	2.81	0.70	1.82	67.33	2.69
WT5	23.45	2.73	10.83	0.88	4.32	0.25	0.49	2.28	0.78	1.75	74.50	3.24
WT6	23.83	3.05	10.08	1.05	4.22	0.25	0.62	2.52	0.86	2.22	66.75	2.82
WT7	22.00	2.68	10.33	0.96	4.28	0.17	0.46	2.70	0.63	1.59	67.00	2.92
WT8	23.33	2.65	8.67	0.78	4.36	0.14	0.49	3.96	0.63	1.65	60.66	2.63
WT9	25.49	2.77	9.50	0.94	4.38	0.13	0.50	3.21	0.63	1.96	68.00	2.99

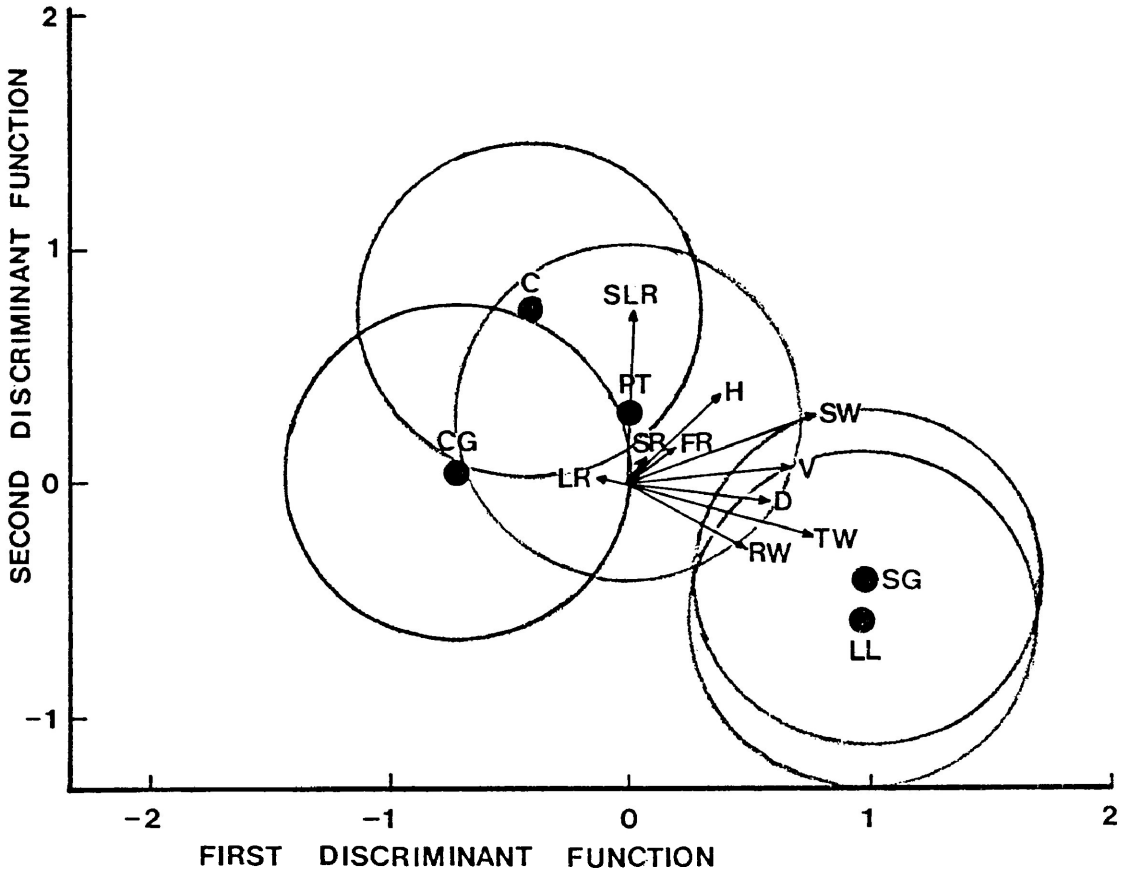
App. Table 10. Mean values of open-pollinated families for seedling traits within fungal treatmeat

C. geophilum

Family	Height cm	Diameter mm	1stLR #	2ndLR /cm	FeederR /cm	RootDW g	ShootDW g	S/R w/w	TotalW g	Volume cm^3	MycInd %	MycInd /cm
MT1	22.50	2.48	13.67	1.26	4.39	0.17	0.49	3.05	0.66	1.39	25.83	1.18
MT2	23.00	2.41	11.75	0.95	3.83	0.16	0.39	2.59	0.56	1.36	20.92	0.81
MT3	22.21	2.45	12.33	0.89	4.53	0.19	0.47	2.66	0.67	1.34	21.08	0.98
MT4	25.13	2.77	15.08	0.94	4.21	0.22	0.52	2.41	0.74	1.93	26.83	1.17
MT5	21.92	2.36	12.58	0.97	4.41	0.18	0.35	2.52	0.53	1.24	32.33	1.44
MT6	20.50	2.73	13.58	0.85	4.07	0.19	0.48	2.46	0.67	1.54	24.92	1.00
MT7	23.46	2.57	12.75	1.07	4.26	0.15	0.38	2.79	0.54	1.54	23.00	0.99
MT8	23.83	2.69	12.67	0.99	4.43	0.18	0.51	2.96	0.69	1.74	24.42	1.08
WT1	23.17	2.44	10.25	0.92	3.79	0.15	0.44	3.20	0.59	1.38	20.58	0.82
WT2	24.38	2.53	12.92	1.00	4.81	0.16	0.47	3.07	0.63	1.57	27.17	1.32
WT3	25.00	2.76	11.25	0.94	4.43	0.18	0.51	2.84	0.69	1.91	26.25	1.16
WT4	21.75	2.61	12.92	1.15	4.12	0.22	0.50	2.37	0.72	1.49	20.50	0.84
WT5	23.20	2.48	11.99	1.02	4.34	0.15	0.43	2.94	0.58	1.45	17.42	0.77
WT6	23.41	2.46	14.25	1.35	4.70	0.25	0.49	2.00	0.71	1.45	13.33	0.65
WT7	23.13	2.54	10.58	0.87	4.53	0.18	0.43	2.53	0.62	1.51	23.83	1.14
WT8	24.45	2.72	14.08	1.12	4.19	0.21	0.54	2.59	0.76	1.82	21.00	0.89
WT9	23.67	2.32	11.17	0.84	4.33	0.15	0.44	3.26	0.60	1.29	11.75	0.51



App. Figure 1. Design of tube pure culture synthesis for ectomycorrhizal formation between tamarack seedlings and selected fungi



App. Figure 2. Graph of the 1st and 2nd canonical axes of the centroids for three fungal treatments. Large dots are group centroids and circles are confidence circles (95%) for the centroides. Fungal treatments are labelled as C=control; LL=L. laccata; PT=P.tinctorius; SG=S. graunlatus; and CG=C.geophilum. Seedling variables are labelled as: LR=number of 1st order lateral roots; SLR=frequency of 2nd order lateral roots; SR= shoot/root ratio; SW=shoot dry weight; RW=root dry weight; TW=total dry weight; H=shoot height; D=diameter; V=shoot volume; and FR=frequency of feeder roots.