QUANTIFYING THE EFFECTS OF DIFFERENT CLEAR-CUT SIZES ON ECTOMYCORRHIZAL FUNGI AT A SUBALPINE FOREST: PERSISTENCE AND DIVERSITY

by

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Abstract

The overall objective of this thesis was to assess the influence of different clear-cut sizes on the survival of ectomycorrhizal fungi one and two growing seasons after logging. A comparative study involving a greenhouse bioassay and a survey of ectomycorrhizal roots from soil cores detected very different assemblages of the ectomycorrhizal fungal community one growing season after host removal. These differences are thought to be the result of variation in fungal activity and differences in epidemiology. Two growing seasons after host removal, a survey of ectomycorrhizal roots from soil cores revealed that both the numbers of active ectomycorrhizae and the diversity of ectomycorrhizae were significantly reduced at clear-cut plots relative to the undisturbed forest. Specifically, there was a significant decrease in diversity with increasing distance from the block edge. There were no differences in diversity at the same distance from the edge of different sized clear-cuts. A field bioassay showed that the diversity of ectomycorrhizal fungi capable of colonizing young spruce seedlings two growing seasons after host removal was significantly reduced with distance from the block edge. The critical distance beyond which diversity decreased was 16 - 25m. As with the results of the survey of ectomycorrhizae from soil cores, there were no differences in diversity at the same distance from the edge of different sized clear-cuts. These results suggest that, in clear-cuts ranging in size from 0.1 to 10 ha, distance from the edge, or proximity to overstory trees, is more important to patterns of ectomycorrhizal persistence and diversity than opening size. The use of vital stains revealed that fungal tissue associated with 'active' mycorrhizae is viable yet not metabolically active.

Table of Contents

List of Tables

List of Tables (Continued)

List of Figures

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Dedication

This thesis is dedicated to my mom, Patty Shane, who from the beginning of my life instilled in me the confidence and provided me with the support to pursue and realize my dreams. I could not have wished for a better role model in life.

Chapter 1

Effects of various silvicultural systems on the persistence and diversity of ectomycorrhizal fungi at a subalpine forest

General Introduction

Ectomycorrhizae, the symbiotic associations between the roots of many species of woody plants and dikaryomycotan fungi, are an important component of temperate forest ecosystems. Ectomycorrhizal fungi take up and transport to the host plant essential nutrients including nitrogen and phosphorus (Read 1991). As such, mycorrhization is said to be essential for adequate growth of conifers at both early and late stages of development (Christy *et al.* 1982, Perry *et al.* 1987, Villeneuve *et al.* 1991). In addition to enhancing the uptake of nutrients (Harley and Smith 1983) and water (Parke *et al.* 1983), ectomycorrhizae may also provide resistance to pathogens (Barham *et al.* 1974) and herbivory (Gehring and Whitham 1991). Mycorrhizae may also confer tolerance in plants to toxic heavy metals such as zinc, copper and nickel (Wilkins 1991). The formation of ectomycorrhizae may be particularly important for young seedlings as vigorous growth will help to ensure the early occupation of space and subsequent access to resources such as light and water (Perry *et al.* 1987).

The presence of mycorrhizal fungi in forest soils has ecological consequences beyond promoting plant growth. Mycorrhizal fungi contribute to structural properties of soil by forming soil aggregates (Tisdall and Oades 1979, Jastrow 1996). They provide a food source for microarthropods such as members of the Collembola (Hiol Hiol *et al.* 1994) as well as for small mammals (Fogel and Trappe 1978). Mycorrhizal fungi may also play a role in mediating competitive interactions amongst plants by enhancing the growth of otherwise subordinate species (Grime *et al.* 1987).

It is generally thought that ectomycorrhizal fungi are dependent on a host plant for carbon and to complete their life cycle (Trappe and Fogel 1977, Harley and Smith 1983). Support for the view that ectomycorrhizal fungi are obligate symbionts comes

from trenching experiments such as those described by Romell (1938): ectomycorrhizal sporocarps were absent on the far side of a trench that separated the fungal mycelium from tree seedlings, but were abundant on the near side of the trench where the mycelium had access to a mature tree. More recently, D. Durall (unpublished results) carried out a number of surveys of hypogeous sporocarps at Sicamous Creek: one and two growing seasons after host removal no fruiting structures were encountered in the clear-cuts whereas fruiting structures were present in the adjacent forest.

Persistence of ectomycorrhizal fungi following disturbance

If ectomycorrhizal fungi are obligate symbionts, then their ability to persist in the soil after removal of their hosts would be expected to be poor. Indeed, Hacskaylo (1973) hypothesized that ectomycorrhizal fungi are not persistent in the absence of a host and this has been confirmed in several studies. For example, Harvey *et al.* (1980) reported the complete elimination of active ectomycorrhizal roots at a high elevation Douglas-fir (*Pseudotsuga menziessii* (Mirb.)Franco) and western larch (*Larix occidentalis* Nutt.) forest in northwestern Montana one year after clear-cutting. Similarly, a progressive decrease in the number of active ectomycorrhizal root tips with increasing gap size was observed at a lodgepole pine (*Pinus contorta* ssp. *latifolia* (Engelm. ex Wats.)) forest in southeastern Wyoming (Parsons *et al.* 1994). Other studies using greenhouse bioassays have shown a decrease in mycorrhizal formation by seedlings following harvesting and burning (Perry *et al.* 1982, Parke *et al.* 1984).

There is evidence, however, that some ectomycorrhizal fungi can persist for some time in the absence of a live host. Ferrier and Alexander (1985) determined that excised mycorrhizal root tips of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) remained metabolically active, as determined by morphological criteria, for at least nine months after being disconnected from the host. Persson (1982), found that fine roots of Scots pine (*Pinus sylvestris* L.) can persist for up to 18 months after host removal by clear-

cutting. Similarly, Bauhas (1994) found that 50% of the original fine root biomass of a European beech (*Fagus sylvatica* L.) stand in Germany persisted in an active state two growing seasons following the formation of gaps 30 m in diameter.

The survival of excised roots suggests the potential for the persistence of associated ectomycorrhizal fungi but some ectomycorrhizal fungi may be viable and capable of colonization regardless of the viability of the associated root. Mechanisms that would enable the fungus to survive in the absence of a live host include obtaining nutrients saprophytically or the production of dormant structures. Although conclusive evidence of saprophytic growth by ectomycorrhizal fungi in the field is lacking, some ectomycorrhizal fungi are capable of restricted saprophytic growth under laboratory conditions. A number of studies have shown that certain ectomycorrhizal fungi have the ability to produce enzymes capable of degrading complex carbon sources (Todd 1979, Giltrap 1982, Trojanowski *et al.* 1984, Haselwandter *et al.* 1990, Durall *et al.* 1994). Furthermore, saprophytic growth of ectomycorrhizal fungi under controlled conditions has been observed. For example, *Paxillus involutus* has been observed to grow on unsterile humus (Laiho 1970, Erland and Söderström 1991), and several fungi have been observed to grow on sterile peat (Erland *et al.* 1990) without added carbon sources.

Little is known about the longevity of resistant structures such as sclerotia and chlamydospores, or the persistence of the vegetative mycelium and basidiospores (Miller *et al.* 1994). Sclerotia of *Cenococcum* persist in the soil several years following logging (Coley-Smith and Cooke 1971, Shaw and Sidle 1983) and basidiospores of species such as *Suillus brevipes, S. tomentosa, Lactarius scrobiculatus, Rhizopogon subcaerulescens* and *R. rubescens* persist in the soil for at least two years (Miller *et al.* 1994). The viability of these spores was not tested; however, it would be expected to decrease over time (Mosse *et al.* 1981). Mycelial strands of *Thelephora terrestris* associated with Sitka spruce seedlings have been

observed to persist throughout the fall and winter into the next growing season (Coutts and Nicoll 1990), however, the persistence of isolated strands was not investigated.

An additional factor that may affect the retention of fungal inocula at disturbed sites is the presence of alternate hosts. The association of ectomycorrhizal fungi with 'refuge species' has been documented (Smith *et al.* 1995, Harniman *et al.* 1996) and may provide a means for certain ectomycorrhizal fungi to survive following clear-cutting.

Diversity of ectomycorrhizal fungi

The concept of diversity can be regarded at a number of different scales: genetic, species, ecosystem, and functional (Whittaker 1977). Genetic diversity is the finest scale and refers to the variation in genotypes among individuals. Species diversity refers to the array of different species occurring in an area and can be quantified either as species richness, usually expressed in terms of numbers of species, or by indices that combine attributes of richness, evenness and dominance (Magurran 1988). Ecosystem diversity describes the differences between habitats or communities. Diversity can also be regarded in terms of functional differences among groups of organisms. In terms of ectomycorrhizal fungi and seedling performance, functional diversity may be the most applicable scale to consider.

Among the estimated 5000 to 6000 species of fungi capable of forming mycorrhizae (Molina *et al.* 1992), considerable differences exist with respect to their physiology and ecology (reviewed by Bruns 1995). For example, ectomycorrhizal fungi differ in their ability to take up various forms and types of nutrients (Abunzinadah and Read 1986, Dighton 1991), in their rates of nutrient uptake (Langlois and Fortin 1984), in their tolerance to water stress in pure culture (Mexal and Reid 1973, Dieblot and Mudge 1985) and in the field (Parke *et al.* 1983), as well as in their tolerance to temperature extremes (Slankis 1974). The functional diversity of ectomycorrhizal fungi

is poorly understood. There is a growing collection of laboratory evidence demonstrating differences among fungi, but the importance of these differences in the field remains unknown. The fact that different strains of fungi may perform differently under various circumstances further complicates this issue.

At all scales, investigation of ectomycorrhizal diversity is gaining momentum. In the past, research of this sort has been limited primarily by difficulties in identification. The fungal tissue associated with roots does not display sexual structures to aid in identification, and reliance on sporocarp production is often used to describe ectomycorrhizal fungal communities. Unfortunately, sporocarps are produced sporadically and may only represent a fraction of the root colonizing fungal community (Vogt *et al.* 1992). Many of the most common ectomycorrhizal fungi produce small, obscure, or below-ground fruiting structures and, thus, are underrepresented in surveys of epigeous sporocarps. Furthermore, some ectomycorrhizal fungi such as *Cenococcum* do not produce sexual stages that have been identified.

Recently, the use of molecular DNA techniques such as the polymerase chain reaction (PCR) and analysis of restriction fragment length polymorphisms (RFLP) have made it possible to quantify genetic diversity and to more accurately identify the ectomycorrhizal fungi associated with the roots. In addition, comprehensive morphological keys and descriptions of ectomycorrhizae continue to be updated and refined. Both molecular techniques as well as 'morphotyping' have proven to be useful tools in the study of ectomycorrhizal diversity.

There are advantages and disadvantages to both identification techniques. One of the benefits of using molecular tools for fungal identification is that the analysis is independent of variation induced by environmental conditions (Egger 1995). In addition, the results of molecular analysis are objective and can be compared amongst researchers providing the same primers and enzymes were used. Unfortunately, not all fungi generate a PCR amplification product (S. Sakakibara pers. comm.), and for

arbuscular mycorrhizae, more than one sequence has been observed in a single spore (Sanders 1996). Furthermore, processing large numbers of samples required for accurate description of the community can be costly. One of the primary disadvantages of morphological identification of ectomycorrhizae is that environmental conditions can greatly influence morphology, especially macroscopic features such as colour and form (Egli *et al.* 1993). In addition, the subjective nature of the technique can make comparison among researchers difficult (Egli *et al.* 1993). It is generally thought that the combined use of molecular and morphological characteristics will produce the most reliable results.

Many mycorrhizologists argue that ectomycorrhizal diversity is important for tree growth and ecosystem health (Perry *et al.* 1987. Perry *et al.* 1989a, Allen *et al.* 1995, Simard *et al.* 1997). At present, however, it has not been shown experimentally that associating with a diverse group of ectomycorrhizal fungi leads to improved seedling growth. It has been suggested that seedlings having access to a range of ectomycorrhizal fungi will be colonized by those mycobionts best adapted to the soil conditions present (Perry *et al.* 1987). Furthermore, seedlings associated with a variety of ectomycorrhizal fungi may be better able to adapt to changes in the environment (Simard *et al.* 1997a). At a larger scale, it has been hypothesized that "diversity in the microbial community plays a seminal role in buffering against disturbance and maintaining healthy links between plants and soils" (Perry *et al.* 1989a). The rationale is that if soils have a diverse community of microbes, the likelihood is increased that some species will persist following major disturbances. If this is true, some species of microbes, including mycorrhizal fungi, will remain viable in the soil and capable of associating with plants. In this way, mycorrhizal diversity would promote both seedling growth and ecosystem sustainability.

Sicamous Creek alternative silvicultural systems trial

Over the past few years there has been increased pressure on the subalpine forests of British Columbia to provide a timber supply (Feller 1997). Unfortunately, efforts to regenerate these sites following clear-cut logging have often resulted in poor plantations (Mather 1987, Farnden 1995). In an attempt to gain a better understanding of the processes occurring at high elevation forests, as well as in response to public pressure for alternatives to clear-cut logging, the B. C. Ministry of Forests Research Branch, Kamloops Region, initiated the Sicamous Creek Silvicultural Systems Trial. The study reported in this thesis is one of numerous investigations involving the alternative silvicultural systems currently being implemented throughout British Columbia.

The Sicamous Creek Silvicultural Systems Trial is situated on the Cariboo Plateau in the southern interior of British Columbia (51° N, 119° W). The study site, located in the Engelmann Spruce - Subalpine fir (ESSF) wc2 biogeoclimatic variant has a north-facing aspect and a slope gradient of 20-40%. The elevation ranges from 1500 - 1850 m (Lloyd and Inselberg 1997). The forest is comprised of mature Engelmann spruce (*Picea engelmannii* Parry Ex. Engelm.) and subalpine fir *(Abies lasiocarpa* (Hook.) Nutt.) ranging in age from 95-325 years. Engelmann spruce constitutes 10% of the canopy species (Parish 1997).

Forests located in the ESSF receive from 500 mm to 2200 mm of precipitation annually (Coupé *et al.* 1991). Much of this precipitation (50-70%) falls as snow resulting in a snow pack attaining depths of 1 to 4 meters. The mean annual temperature is 1 °C and the continuous frost-free period is less than 40 days occurring between mid July and mid August. The parent material is granitic gneiss (Coupé *et al.* 1991, Lloyd and Inselberg 1997). At Sicamous Creek, the understory at zonal sites is dominated by *Rhododendron albiforum, Vaccinium membranaceum, Vaccinium*

ovalifolium, Valeriana sitchensis, Arnica latifolia, Gymnocarpium dryopteris, Tiarella unifoliata and *Streptopus roseus* (Lloyd and Inselberg 1997). Soils on zonal sites are classified as Humo-Feric Podzols with a Hemimor humus form (Lloyd and Inselberg 1997). Overall, the predominating site series throughout the research area are 01 (mesic and submesic), 04 (subxeric and submesic) and 06 (subhygric and mesic) (Lloyd and Inselberg 1997). The specific areas studied for this thesis were located in the 01 or mesic site series.

During the winter 1994 - 1995 the study site was harvested in 30 ha treatment units in a randomized block design. There were five treatments for each of the three blocks; single tree selection, 0.1 ha, 1 ha and 10 ha clear-cuts. Within each treatment unit, approximately 33% of the total timber volume was removed according to one of the four treatments. No trees were removed in the fifth treatment. The three blocks ranged in elevation from approximately 1500 metres to 1850 metres.

Objectives

The objective of the Sicamous Creek study is to further what is known about the effects of alternative silvicultural practices at high elevation forests in B.C. (Vyse 1997). At high elevation clear-cuts, the establishment of a diverse array of ectomycorrhizal fungi with outplanted seedlings may be increasingly important as growth is limited by cool soil temperatures and a short growing season (Perry *et al.* 1987). The overall objective of this thesis was to assess the influence of different clear-cut sizes on the survival of ectomycorrhizal fungi one and two growing seasons after logging.

The specific hypotheses tested in this study were:

1) the diversity of ectomycorrhizal types will decrease following logging

2) the diversity of ectomycorrhizal types will decrease with increasing distance from the block edge

3) decreases in the diversity of ectomycorrhizal types will be greatest in the 10 ha clear-cut

4) the persistence of ectomycorrhizal types will differ

5) morphological assessment of root activity will vary with the results of physiological criteria

This thesis consists of four studies, each taking a different approach to achieving the objective. To investigate the persistence of ectomycorrhizal fungi one growing season after host removal, spruce seedlings were grown in soils obtained from the various plot locations (Chapter 2). The mycorrhizal types that formed with the greenhouse-grown seedlings indicated the fungi that had persisted through the first growing season after host removal. Chapter 2 compares the results of the 'greenhouse bioassay' with a survey of ectomycorrhizae occurring in soil cores i.e., roots once associated with the mature trees sampled from Sicamous Creek the same year. The latter survey tested how persistence and diversity was affected with clear-cut size and distance from the edge at the end of the first growing season after harvesting.

The second study (Chapter 3) investigated the persistence of the ectomycorrhizal roots remaining in the clear-cuts two growing seasons following logging. Morphological criteria were used to distinguish between active and inactive roots. Other studies have shown that following clear-cutting, ectomycorrhizal roots remain active from one to two years following host removal. The technique of morphotyping enabled the persistence of various types of mycorrhizae to be observed. This study also investigated the effect of clear-cut size and distance from the edge on the diversity of ectomycorrhizal types.

Chapter 4 describes a 'field bioassay' approach to assessing the persistence of ectomycorrhizal fungi two growing seasons after logging as the active ectomycorrhizal community identified in Chapter 3 may not neccesarily be capable of colonizing young seedlings. Eight-week-old non-mycorrhizal spruce seedlings were outplanted along transects of the different sized clear-cuts and used to 'bait' ectomycorrhizal fungi. The

advantage of this study was that the fungi colonizing the seedlings under field conditions could be observed without concern of prior contamination by common 'greenhouse fungi' such as *Thelephora terrestris*. The impact of clear-cut size and distance from the edge on the diversity of the ectomycorrhizal community was also tested in this investigation.

The study discussed in Chapter 5 investigated the use of vital stains as indicators of metabolic activity of ectomycorrhizal fungi. This study was undertaken to address the difficulty in assessing the activity of a root and its associated fungus. The samples for this study were also used for morphotyping in Chapter 3.

Chapter 6 summarizes the results from the four studies, discusses some of the limitations of this work and suggests areas for future research.

Chapter 2

Ectomycorrhizae at Sicamous Creek one growing season after host removal: a comparison of a greenhouse bioassay and a survey of roots obtained from soil cores

Introduction

The technique of growing seedlings in a greenhouse in soils obtained from the field (greenhouse bioassay) has been used by a number of researchers to assess the ectomycorrhizal inoculum potential of different soils (Perry *et al.* 1982, Schoenberger and Perry 1982, Parke *et al.* 1984, Pilz and Perry 1984, Jasper *et al.* 1991, Simard *et al.* 1997c). As detected by greenhouse bioassays, the effect of clear-cutting on ectomycorrhizal inocula is variable. In clear-cuts ranging in age and size, Parke *et al.* (1984) found that the inoculum potential of the clear-cut soils was reduced relative to adjacent undisturbed soils. Conversely, in a Douglas-fir forest in central Oregon, clear-cutting had no effect on the mycorrhizae that were able to colonize greenhouse-grown seedlings regardless of whether the soil originated from the clear-cuts or the forest (Pilz and Perry 1984).

Different types of ectomycorrhizal inocula include mycelia associated with fine roots, spores, isolated fragments of mycelia and hyphal strands, and sclerotia. As discussed in Chapter 1, little is known about the ability of these different types of inocula to persist in the soil following disturbance (Miller *et al.* 1994). Different types of inocula and their relative importance will differ in the field compared to the greenhouse. Similarly, various fungi and different types of inocula will vary in their ability to persist following logging. This variability will likely influence patterns of ectomycorrhizal colonization of both naturally regenerating and outplanted seedlings.

The objectives of this study were to: i) characterize the persistence of ectomycorrhizae associated with roots of harvested trees one growing season after

logging, and ii) to test the activity of these fungi by assessing their ability to colonize seedlings in a greenhouse.

Materials and Methods

Study Site

Field sampling of fine roots from mature trees harvested approximately 7 months previously was conducted during the first week in August, 1995. Plots were located along a north-south transect across each of the three 10 ha clear-cuts. Along the transect, plots were located at 2 and 25 metres from each of the north (south facing) and south (north facing) block edge as well as at the centre of the clear-cut (165 m). Along the same transect, plots were also located 40 m into the adjacent forest from the north and south edges of the 10 ha clear-cuts. One plot was located in the centre of the 0.1 ha clear-cut (16 m from the block edge), the centre of the 1.0 ha cut block (50 m from the block edge) and in the forest at the uncut treatment unit. The selection cut treatment was not studied. Therefore, there were 10 sampling locations per replicate block resulting in a total of 30 sampling locations (subset of points shown in Fig. 1).

Sampling

At each of the sampling locations, ten, 5 cm diameter x 30 cm deep soil cores (Leach Machine Works, Victoria) were removed at a distance of approximately 1 metre apart from each other (n=300). Soils were stored at 3° C for six months during processing. Two hundred randomly-selected live mycorrhizal roots were characterized. Active and inactive fine roots were separated based on morphological criteria outlined by Harvey (1976). Inactive roots were characterized as having a dark apex and wrinkled texture. Active tips had a pale apex and were turgid. Roots assessed as being active were further classified as mycorrhizal or non-mycorrhizal under the stereo-

microscope. The majority of roots were subsequently observed under 400X or 1000X either as whole mounts (entire root tip) or as a mantle peel (only the fungus). Mantle peels were made by separating the fungal tissue from the root with fine forceps. As the mycorrhizal roots were being counted, the ectomycorrhizal specimens were grouped into separate categories based on their morphological attributes. These attributes included both macroscopic and microscopic features of the ectomycorrhizae such as colour, texture and proportions of the whole root tip as well as ornamentation, colour and dimensions of the emanating elements and the pattern of the mantle (Appendix 2). In this way, each unique ectomycorrhizal morphotype was described according to the terminology used by Goodman *et al.* (1996). Mycorrhizae representative of each morphological grouping were photographed and stored in sterile water in Eppendorf tubes at -20 ° C. Grouping the ectomycorrhizae by morphological characteristics enabled fungal associations to be tentatively assigned for most of the ectomycorrhizae described. This was done by comparing the morphological descriptions from this study with published ectomycorrhizal descriptions (Agerer 1987-1995, Ingelby *et al.* 1990, Goodman *et al.* 1996). This is one of the methods for identification of ectomycorrhizae accepted by the Manual of Concise Descriptions of North American Ectomycorrhizae (Goodman *et al.* 1996).

Soils for the greenhouse bioassay were sampled during September 5-7, 1995. At each of the ten sampling locations, five soil cores were removed at a distance of approximately 1 m apart from each other. Care was taken to minimize the disturbance to the cores when transferring the soil into the sample bags. The cores were transferred to the lab at Okanagan University College (OUC) and stored at 3° C for two days. The soils were then cut lengthwise and each half was transferred to a 4 cm diameter X 21 cm deep Leach tube (Ray Leach 'Cone-tainer' Single Cell System) with the horizons intact. Three sterile *P. engelmannii* x *glauca* (seedlot # 6025) seeds were sown per conetainer. The seeds were sterilized by placing them in a flask containing 25 ml of

35% H_2O_2. After fifteen minutes 100 ml of sterile water was added and the seeds left to soak for 24 hours. This is a high elevation seed orchard seedlot originating from the Shuswap Adams zone. Seedlings were watered daily and grown with natural daylengths extended to twelve hours with sodium vapor lamps. Three half-cores per plot location were autoclaved for 1 hour at 250 °F and subsequently planted to test for the presence of greenhouse contaminants. After 35 weeks, seedlings and their surrounding soil were harvested and stored at 3° C during processing which lasted approximately two months. The seedlings were not thinned.

All fine roots of each seedling were assessed for mycorrhizal colonization. Each seedling was placed on a 1 mm^2 sieve and the adhering soil particles were gently washed free of the root system. Descriptions, criteria and storage of the ectomycorrhizae followed the same protocol as previously mentioned.

Analysis

Approximately 50% of seedlings failed to germinate or died before the root analysis. As a result of the extensive seedling mortality, there was insufficient replication and therefore no statistical analyses could be carried out regarding the distribution of ectomycorrhizae at the various plot locations. Nevertheless, the data were considered useful to characterize the active ectomycorrhizal fungal community existing in the soil one growing season following host removal. One growing season following host removal, there were no differences detected in the ectomycorrhizal fungal community when the soil cores were examined (M. Jones and D. Durall, unpublished results). For both studies, the relative abundance (RA) of each ectomycorrhizae type was calculated as a percentage of all the ectomycorrhizae classified. The community of ectomycorrhizae detected by the two methods was compared visually, using bar graphs.

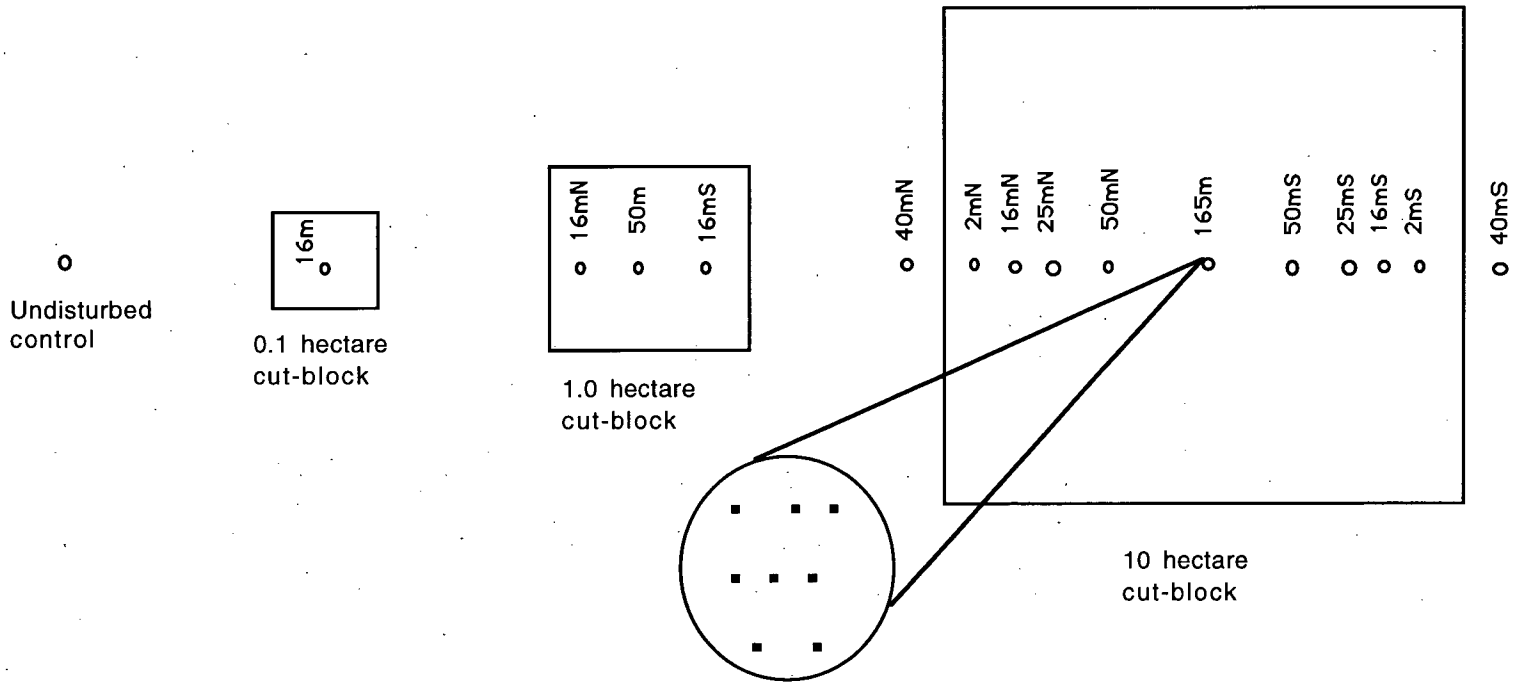

Figure 1. Diagram illustrating the 16 plot locations in and throughout the four types of treatment units at Sicamous Creek. The two markers outside of the 10 ha clear-cut border represent the 'local' undisturbed controls. For each study, three replicate treatment units were sampled. Actual numbers of cores or seedlings varies depending on the study.

Results

Assessment of the roots once associated with the mature trees revealed that 77% of the active fine roots from harvested trees were mycorrhizal. A total of 24 ectomycorrhizal types were observed on the roots from mature trees (Table 1) almost twice as many types as observed associated with the seedlings. Of these 24 types, eight types occurring with an abundance greater than 4% comprised 89.3% of all active mycorrhizae (Fig. 2). *Cenococcum* - like mycorrhizae were the most abundant (RA = 42%). Other dominant types include, *Hebeloma*-like (12.2%), *Piloderma*-like (9.0%), *Lactarius*-like (7.0%), *Amphinema*-like (6.5%), *Cortinarius*-like (5.4%), *Mycelium radicis atrovirens* Melin (MRA) (5.3%) and *Tuber*-like (4.7%) ectomycorrhizae.

For the greenhouse bioassay, 62% of all roots were mycorrhizal, 25% were non-mycorrhizal and 13% were assessed as being inactive (n=145 seedlings). None of the control seedlings grown in the sterilized soil formed mycorrhizae. The community of ectomycorrhizal fungi that formed with the greenhouse bioassay seedlings differed from the ectomycorrhizae persisting on the roots of mature trees. A total of 14 ectomycorrhizal morphotypes were detected (Table 1). Six dominant morphotypes accounted for 96.3% of all mycorrhizae with the remaining types occurring infrequently and with low abundance (Fig. 2). *Hebeloma*-like (31.4% of active mycorrhizae), E-strain (23.9%) and *Thelephora*-like (23.2%) ectomycorrhizae predominated on the roots of seedlings. The remaining dominant types were represented by *Amphinema*-like (9.1%), *Cenococcum* -like (5.9%) and ITE -1 - like (4.7%) ectomycorrhizae.

Interestingly, E-strain and *Thelephora*-like ectomycorrhizae dominated the roots of seedlings and yet both types were absent from roots of harvested trees. Conversely, *Lactarius* - like, *Cortinarius* - like and *Tuber* - like ectomycorrhizae were absent from seedling roots. *Cenococcum* - like mycorrhizae were nearly ten times more abundant on the roots of mature trees than when on greenhouse grown seedlings. *Amphinema* -

like and *Hebeloma* - like ectomycorrhizae were present in both surveys, with *Hebeloma* occupying a greater proportion of the community on seedlings as compared with the roots of harvested trees (25% versus 12.2%).

Discussion

Different types and proportions of ectomycorrhizae were found on seedlings grown in a greenhouse in soil collected from Sicamous Creek when compared to the mycorrhizal types already present in that soil. This suggests that some types of ectomycorrhizae encountered in the soil cores could not act as inoculum, either due to low activity or because the fungi were not adapted to colonize young spruce seedlings under disturbed conditions. In addition, the results show that important sources of inoculum other than ectomycorrhizal roots exist in these soils.

The terms 'early' and 'late-stage' fungi were first used to describe temporal patterns of ectomycorrhizal sporocarp occurrence in association with an early successional mixed birch (*Betula pendula* Roth and *B. pubescens* Ehrh.) stand planted in agricultural soil in Scotland (Mason *et al.* 1982, 1983, Deacon *et al.* 1983). The researchers observed that certain groups of fungi were characteristically associated with young trees whereas other groups were associated with older trees. Subsequent studies in other ecosystems have supported the concept of succession as it relates to mycorrhizal fungi (Chu-Chou 1979, Richter and Bruhn 1993). However, as with the initial study in Scotland, most of these studies have been carried out in areas influenced by man-made disturbance events such as sites that are logged and subsequently planted. Recently, Visser (1995) investigated the concept of ectomycorrhizal succession in naturally-regenerated jack pine (*Pinus banksiana* Lamb.) stands of various stages of development following wildfires. Her findings also support the view that ectomycorrhizal fungi follow a successional sequence over time. Furthermore, Visser

(1995) refers to 'multi-stage' fungi as those capable of persisting (not necessarily fruiting) between disturbance events.

The use of the terms 'early' and 'late-stage' fungi has been criticized or at least met with reservation, by many researchers (reviewed by Deacon and Fleming, 1992). As Newton (1992) points out, one of the unfortunate connotations of the terms, is the implication that late-stage fungi are restricted to older hosts. In fact, Fleming (1984) has demonstrated that seedlings planted amongst forest trees are capable of developing ectomycorrhizae with late-stage fungi. Chapter 4 of this thesis also reports the occurrence of late-stage fungi colonizing young seedlings. The observation that late-stage fungi are not commonly associated with young hosts is more likely the result of the conditions that exist where young hosts are found. For example, mycelial connections with mature trees are severed in clear-cuts where outplanted seedlings are growing.

Despite the debate over terminology, there are real biological differences that exist between groups of fungi with respect to growth habit and epidemiology over a wide range of ecosystems. Used in this context, distinguishing early from late-stage fungi can be informative. In general, early-stage fungi can be characterized by their ability to colonize from spores or fragments of mycelia (Deacon and Fleming 1992). It is also suspected that many early-stage fungi have low carbon requirements (Deacon and Fleming 1992) although this may not always be the case. Conversely, late-stage fungi are generally unable to colonize from spores or fragments of mycelia and it is speculated that high amounts of carbon are required for colonization (Deacon 1983, Fleming 1983, 1985, Deacon and Fleming 1992).

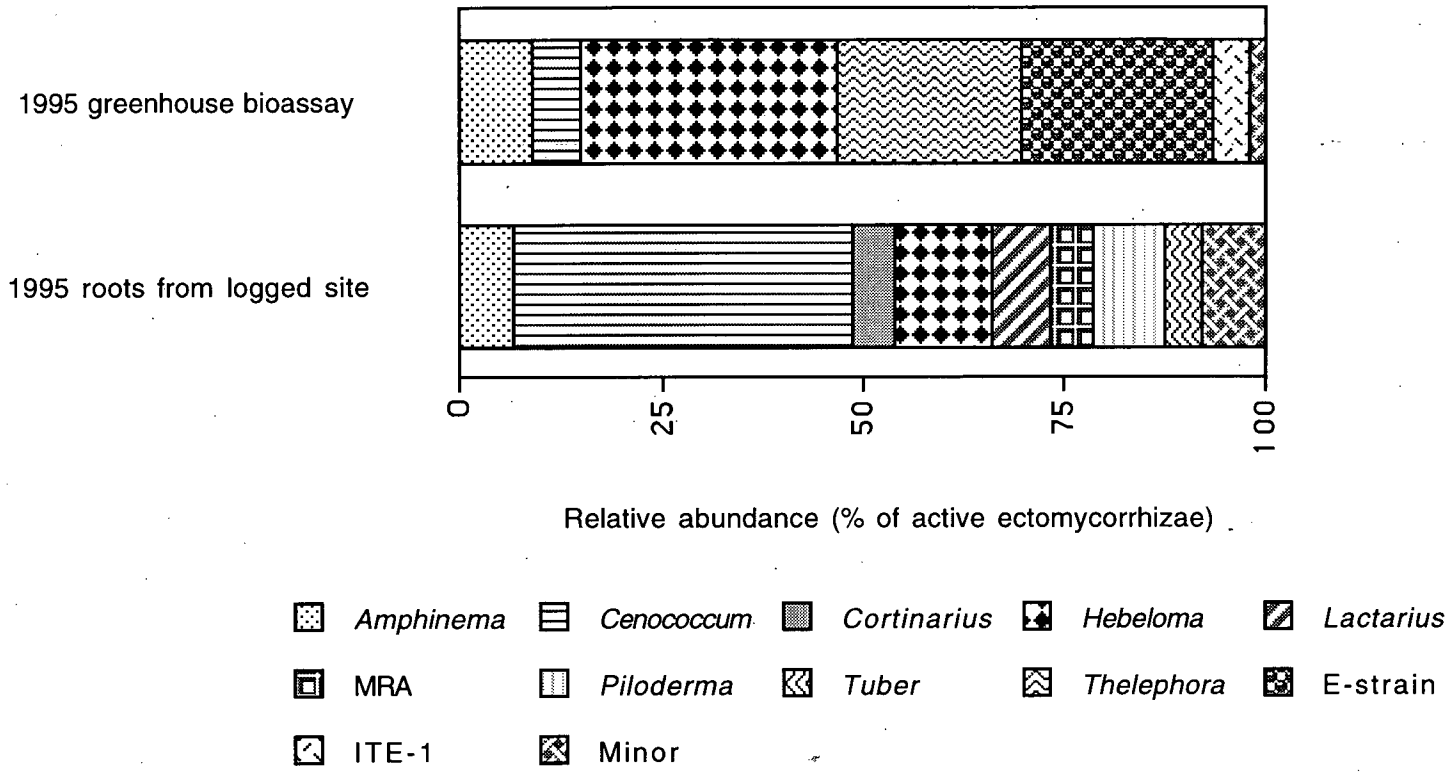

Figure 2. Relative abundances of the twelve major ectomycorrhizal types and the combined minor types (RA<4%) described directly from soil cores or from*Picea engelmannii* x *glauca* seedlings grown for 35 weeks in a greenhouse in soil cores collected at Sicamous Creek approximately 8 months after winter logging.

Table 1. Mean relative abundance (RA) of the ectomycorrhizae encountered on the roots from soil cores (SC) as well as ectomycorrhizae colonizing greenhouse-grown spruce seedlings (GB) one growing season after winter logging at Sicamous Creek. See Appendix 2. for complete morphological descriptions of the mycorrhizae encountered.

Thesis reference	Possible fungal associate	RA (%) SC	RA (%) GB
SC#150 / GB#10	*Amphinema* - like I	6.4	9.1
SC#10	*Amphinema* - like unknown II	0.1	-
SC#50 / GB#40	*Cenococcum* - like	42.0	5.9
SC#120	*Cortinarius* - like I	5.4	-
GB#63	E-strain type I	-	22.0
GB#60	E-strain type II	-	1.8
SC#70 / GB#200	*Hebeloma* - like	12.2	31.38
GB#62	E-strain - like IV	-	0.16
SC#170 / GB#	ITE-1 - like	0.1	4.7
SC#80	*Laccaria* -like	2.2	-
SC#200	*Lactarius* - like IV	0.2	-
SC#190	*Lactarius* - like II	4.3	-
SC#310	*Lactarius* - like III	0.3	-
SC#160	*Lactarius* - like I	2.2	-
SC#90 / GB#120	*Mycelium radicis atrovirens* (MRA)	5.3	0.33
SC#130 / GB#110	*Piloderma* - like	9.0	0.16
SC#240	*Rhizopogon* - like	0.5	-
SC#210	*Russula* - like I	0.6	-
SC#60	*Russula* - like II	0.7	-
SC#20	*Thelephora* - like II	0.3	-
GB#80	*Thelephora* - like I	-	23.17
SC#30 / GB#61	*Tomentella* - like II	0.6	0.25
SC#181	*Tomentella* - like I	0.03	-
SC#140	*Tuber* -like	4.7	-
SC#40	Unidentified	2.2	-
SC#100	Unidentified	0.6	-
SC#220	Unidentified	0.006	-
SC#250	Unidentified	0.2	-
GB#45	Unidentified	-	0.08
GB#140	Unidentified	-	0.25
GB#130	Unidentified	-	0.60

Lactarius spp. and *Cortinarius* spp., have been described as late-stage fungi (Deacon *et al.* 1983, Fleming 1984, Visser 1995). As such, these fungi are thought to require an intact hyphal connection with a mature host in order to colonize young seedlings (Fleming 1984, Simard *et al.* 1997a) because the seedlings cannot support the entire carbon demands of the fungi (Simard *et al.* 1997a). It has recently been demonstrated that carbon transfer can occur between trees and is likely facilitated by the mycorrhizal mycelium (Simard *et al.* 1997b). As seedlings in the present study were grown in Leach tubes and thus were isolated from other trees it is not surprising that *Lactarius* - like, *Cortinarius* - like and *Piloderma* - like mycorrhizae were not found associated with the seedling roots even though these types of mycorrhizae were abundant in the soil.

Fungi belonging to the E-strain group and *Thelephora*-like mycorrhizae are common contaminants of commercial greenhouses (Hunt 1991). These fungi have early-stage characteristics in that they aggressively colonize from spores and fragments of mycelia (Danielson and Visser 1989, Danielson 1991). Both of these types of mycorrhizae were abundant on the bioassay seedlings but absent from the roots of mature trees. The non-mycorrhizal status of the control seedlings allows us to conclude that these fungi originated in the forest soils. Furthermore, the absence of these mycorrhizal types on the roots of the mature trees suggests that the type of inocula that colonized the seedlings was spores or fragments of mycelia resident in the soil. Apparently, these types of inocula are capable of persisting in the soil in the absence of an association with a host and are capable of colonizing seedlings. Thus E-strain fungi and *Thelephora* may also be described as multi-stage fungi (Visser 1995). As previously mentioned, the ability of these fungi to efficiently colonize from spores is characteristic of early-stage fungi, however, their additional ability to persist in the absence of a host makes them well characterized as multi-stage fungi. Many types of E-strain fungi produce thick walled chlamydospores (Danielson 1982) and these

structures may remain viable in soil for extended periods of time. The mechanism by which *Thelephora* is able to persist is unknown although rhizomorphs of *T. terrestris* have been observed to persist in association with Sitka spruce seedlings through the fall and winter into the following growing season (Coutts and Nicholl 1990).

An alternate explanation for the presence of *Thelephora* - like and E-strain mycorrhizae on the roots of seedlings may be that dispersal of these types of fungi is efficient, (within 8 months) and thus inoculum levels are rapidly re-established even on large (10 ha) disturbed areas. The ability of spores to constitute a biologically significant input to the inoculum potential of disturbed soils is of considerable interest for landscape management decisions. At present, spores are not thought to be a significant source of inocula for largely disturbed areas (Fries 1987, Perry *et al.* 1989b), although Danielson (pers. comm.) has observed that only two or three spores of *T. terrestris* are able to establish mycorrhizae on a seedling.

Hebeloma has early-stage characteristics such as the ability to colonize from spores (Fox 1983, 1986a) and it is often associated with young stands (Last *et al.* 1983). Although less commonly associated with roots from the harvested trees, *Hebeloma* - like ectomycorrhizae were found on the roots of seedlings as well on mature trees. Visser (1995) observed *Hebeloma* fruiting bodies exclusively in young (less than 41 years) naturally regenerating jack pine stands following fire disturbance in northeastern Alberta. From this, it was concluded that the association of *Hebeloma* with young stands implies the persistence of the fungus in the soil over the lifetime of the stand and therefore, the designation multi-stage was assigned. The observation that *Hebeloma* - like ectomycorrhizae were associated with both bioassay seedlings as well as with excised roots supports this designation.

Alternatively or in addition to the above explanation, the production of sclerotia by *Hebeloma* may have played a role as a source of inocula for seedlings (Fox 1986b). Sclerotia resident in the soil may have been the primary or an additional source of

inoculum for the greenhouse seedlings. This could explain the differences in the proportion of the ectomycorrhizal community that *Hebeloma* occupied as sclerotia were not counted in the assessment of the ectomycorrhizal roots.

Cenococcum - like mycorrhizae comprised only 5.9% of the ectomycorrhizal community associated with the greenhouse-grown seedlings in comparison with having an abundance of 42.0% when associated with the roots of the mature trees. Perhaps, the mycelium associated with the severed roots present in the soil were not a viable source of inoculum for seedlings under these conditions. Due to its dark colouring, determining whether or not this type of mycorrhizae is active can be very difficult. It is possible that the vitality of *Cenococcum* - like mycorrhizae was overestimated in the survey of mycorrhizae classified from cores. In laboratory studies, *Cenococcum* has been shown to be tolerant of low water potential (Coleman *et al.* 1989). The regular irrigation of the greenhouse seedlings may have favored the growth of other fungi to the detriment of *Cenococcum*. *Cenococcum* has been characterized as a multi-stage fungus (Danielson and Visser 1989). Interestingly, Danielson and Visser (1989) observed that *Cenococcum* preferentially colonized the older roots of 1-3 year-old jack pine seedlings outplanted at an oil-sands containment dyke in Alberta. Perhaps the colonization potential of *Cenococcum* was reduced in association with the young root systems of the greenhouse-grown seedlings. Similarily, Christy *et al.* (1982) found that older seedlings of western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) were colonized to a greater extent than younger seedlings.

The observation that certain types of mycorrhizae were absent or reduced on the greenhouse-grown seedlings may be the result of differences in metabolic activity. It may be that one growing season following logging, ectomycorrhizae such as *Lactarius* - like, *Cortinarius* - like, *Tuber* - like, *Piloderma* - like and *Cenococcum* - like are declining in activity and are either incapable or limited in their ability to colonize greenhouse-grown seedlings.

Competition amongst ectomycorrhizal fungi may have also played a role in the types of mycorrhizae that formed on the greenhouse-grown seedlings. McAfee and Fortin (1988), observed competitive effects when more than one type of inoculum was added to controlled pot experiments involving jack pine and American larch (*Larix laricina* (DuRoi) Koch). In combination, *Laccaria bicolor* formed mycorrhizae to the exclusion of *Pisolithus tinctorius*. However, *P. tinctorius* was capable of forming mycorrhizae when inoculated as the sole isolate. The researchers attribute this observation to *P. tinctorius* having a slower growth rate than *L. bicolor* under the controlled conditions. When *Hebeloma cylindosporum* and *L. bicolor* were inoculated in combination, the former was the more competitive fungus. Interestingly, when a suspension of pine soil was added *L. bicolor* became more competitive. This finding illustrates the complexity of the mycorrhizosphere and the likelihood that other soil microorganisms such as mycorrhization helper bacteria (MHBs) play and important role in determining mycorrhizal colonization (Garbaye 1994). In this study, the reduced abundance of *Cenococcum* - like mycorrhizae, for example, may have been the result of its inability to compete under the imposed conditions and in the presence of other fungi that perhaps have faster growth rates. Under field conditions, *Cenococcum* has been observed to colonize seedlings slowly relative to other fungi (Dahlberg and Sternström 1991).

The tree species predominating at Sicamous Creek are subalpine fir and Engelmann spruce, with the latter constituting an average of only 10% of the canopy (Parish 1997). Therefore, the majority of the roots from the cores are likely those of subalpine fir. As spruce seedlings were used for the bioassay, host specificity may have contributed to some of the differences in the types of ectomycorrhizae observed between the two methods. It is possible that some fungi associated with subalpine fir roots are incapable of associating with spruce roots. Most research, however, has shown that spruce and fir associate with a broad range of mycobionts and do not

exhibit limitations due to host specificity (Molina *et al.* 1992). The genera of fungi encountered in this study that have been observed to be restricted to a specific plant genus are associated with *Picea* spp. and not *Abies* spp. For example *Cortinarius citrinofulvescens*, *C. claricolor* and *C. lundelli* are restricted to members of *Picea* spp. Similar restrictions apply to *Hebeloma candipes* as well as *Lactarius bresadolianus* and *L. deterrimus* (Molina *et al.* 1992).

Despite the insights that a greenhouse bioassays such as this may offer into the inoculum potential of soils, there are a number of limitations. First, the mycelium present in the Leach tubes is fragmented and therefore, this method likely selects against those fungi which are incapable of forming ectomycorrhizae from spores, fragments of mycelial and / or sclerotia. Thus, in this type of experiment, only a portion of the active ectomycorrhizal fungal community is being surveyed. However, with the exception of refuge plants, excised mycorrhizal roots are the most common type of inocula on logged sites. Secondly, ectomycorrhizal formation is influenced by differences in light, temperature and moisture (Slankis 1974). It may be that certain types of fungal inocula present in soil were not adapted to colonizing seedlings under greenhouse conditions and, therefore, were not detected. Thirdly, seedlings have commonly been observed to associate with a lesser number of ectomycorrhizal species than do mature trees (Last *et al.* 1987, Danielson and Pruden 1989).

Contrary to similar studies, we elected not to mix the soil samples with sterile media such as vermiculite. This was done for two reasons: firstly, soil disturbance has been shown to reduce the infectivity of propagules of vesicular arbuscular mycorrhizae (Jasper *et al.* 1991), and secondly, the forest floor at Sicamous Creek is thin with humus layers at mesic sites ranging from 1.5 - 9 cm (Hope 1997). Inclusion of this portion of the soil profile was deemed important as studies have shown that ectomycorrhizal fungi are often concentrated in organic fractions of the soil profile (Harvey *et al.* 1976, Harvey *et al.* 1979). The admixture of sterile media is done

primarily to create air spaces in the soil which helps to prevent compaction. It is well known that fine roots are often positioned in air spaces within the soil (Babel 1987, cited in Read, 1992). In fact, the soil in the Leach tubes was quite compacted and this may have been a factor contributing to the poor seedling growth as well as limiting the growth of certain fungi.

In summary, the results of this comparative study illustrate that not all the types of ectomycorrhizae found in the soil were effective inoculum for greenhouse-grown seedlings. These differences may be the result of the requirement of certain fungi such as *Lactarius* spp., *Cortinarius* spp. and *Piloderma* spp. for an intact mycelial connection with a live tree to effectively colonize another host. Differences in the activity of the excised mycorrhizae may have also influenced the results. Finally, effective sources of inoculum other than ectomycorrhizal roots were present in the soil for some fungi. Due to differences in fungal epidemiology, specifically the ability of certain fungi to initiate colonization from spores, the results of greenhouse bioassays are thought to be most useful in predicting the inoculum potential of disturbed sites.

Chapter 3

Effects of alternative silvicultural systems on ectomycorrhizal fungi two years post harvest: quantifying persistence and diversity of ectomycorrhizae remaining in the soil

Introduction

The fungal mycelium extending from ectomycorrhizal roots is thought to be the primary source of inocula initiating new colonization events (Read 1984). Over time, other types of inocula such as spores, will disperse into disturbed areas but their relative contribution to the inoculum potential of the soil is thought to be low (Fries 1987, Perry *et al.* 1987, Perry *et al.* 1989b). This may be particularly true in areas of large scale disturbance.

Estimates of the persistence of metabolically active fine roots following removal of the host shoot have varied. For example, Harvey *et al.* (1980) reported the complete elimination of active ectomycorrhizal fine roots beyond 4.6 m from the edge of 1.7 ha clear-cuts located in a high elevation Douglas-fir and larch forest in northwestern Montana, two growing seasons after fall logging. In a similar study, two growing seasons after summer logging, a progressive decrease in active ectomycorrhizal fine roots was reported with distance from the edge into the center of gaps in a Wyoming lodgepole pine stand (Parsons *et al.* 1994). The number of active fine roots declined to zero beyond 3-5 m from the edge of 30-trees gaps. Ferrier and Alexander (1985), determined that excised mycorrhizal fine roots of Sitka spruce remained metabolically active for at least nine months after being disconnected from the host. In contrast, Persson (1982), found that fine roots of Scots pine can persist for a least 18 months after host removal. Similarly, Bauhus (1994, cited in Bauhus and Bartsch1996) found that 50% of the original fine root biomass of a European beech stand in Germany persisted in an active state two growing seasons following the

formation of gaps 30 metres in diameter. The studies mentioned above used morphological criteria to determine whether or not roots were active or inactive. The terms 'active' and 'inactive' are used because it is extremely difficult to determine whether or not a root is alive or dead (Atkinson 1992).

Most studies investigating the persistence of fine roots following disturbance describe the mycorrhizae in very broad terms. In many cases, ectomycorrhizal roots are grouped based on colouring (e.g. white, brown or yellow) (Kropp 1982, Dahlberg and Stenström 1991, Andersson and Söderström 1995, Harvey *et al.* 1996). For some studies, no distinction is made and ectomycorrhizal roots are simply separated based on whether or not they are active (Harvey *et al.* 1976, Harvey *et al.* 1980, Parsons *et al.* 1994). This is one of a few studies investigating fine root persistence that has attempted a thorough morphological description of the ectomycorrhizal types involved.

This chapter and Chapter 4 describe two approaches to address the question of mycorrhizal persistence and activity. The objectives of this study were (i) to investigate the persistence of ectomycorrhizal fine roots throughout clear-cuts ranging in size from 0.1 - 10 hectares two growing seasons after winter logging, (ii) to describe patterns of ectomycorrhizal distribution (i.e. persistence of individual mycorrhizal types) and (iii) to quantify diversity throughout and between the different sized clear-cuts. Chapter 4 describes the correlation between mycorrhizal persistence and colonization of outplanted seedlings. This second approach was taken as some ectomycorrhizal roots assessed as being active may be incapable of colonizing young seedlings.

Materials and Methods

Sampling

Field sampling was conducted on July 8 and 9, 1996. Cores were sampled at the same plot locations described in Chapter 2 for the greenhouse bioassay. Six

additional locations were added for this study: 16 m from the north and south edge of the 1.0 ha and 10 ha clear-cuts and 50 m from the north and south edge of the 10 ha clear-cut. At each of the 48 plot locations (Fig. 1), eight, 5 cm diameter x 30 cm deep soil cores were removed at a distance of approximately 1 m apart from each other (n=384). Samples were placed in plastic bags and transferred to the lab at OUC and stored at 3° C during processing for approximately six months.

The processing involved placing the entire core in a tray, gently mixing the soil and removing approximately one-quarter of the total volume of the core (200 ml), taking care to include a portion of the humus layer if present. This is an adaptation of the sampling procedure used previously for the mycorrhizal study at Sicamous Creek (Durall and Jones 1996, unpublished results, Chapter 1): in 1995, up to 200 active mycorrhizal roots were examined per core. This technique was modified in 1996 due to the large numbers of inactive roots encountered two growing seasons after logging. The former method would have been extremely time consuming as active roots would be encountered very infrequently. Furthermore, the current technique allowed for comparisons to be made between the ratios of active to inactive roots as the same volume of soil was analyzed for each sample.

For each subsample of soil, counts of active mycorrhizal, active non-mycorrhizal and inactive roots were recorded. Enumeration and classification of mycorrhizal roots follow the procedure described in Chapter 2. No distinction could be made between non-mycorrhizal and mycorrhizal inactive roots. Distinction between active and inactive mycorrhizal fine roots follow the criteria described by (Harvey *et al.* 1976; see Chapter 2). No attempt was made to distinguish the roots of Engelmann spruce from subalpine fir.

Statistical analysis

The relative abundance of each ectomycorrhizal type was calculated as a proportion of the total number of active mycorrhizal roots averaged over the eight cores sampled at each plot location. Mycorrhizal colonization was calculated as the percentage of the total number of active fine roots that were mycorrhizal. Diversity was measured by Shannon's diversity index, richness and evenness using PC-ORD (MjM Software Design 1995) where richness is expressed as the number of unique ectomycorrhizal morphotypes. Shannon's diversity index was chosen as it is sensitive to the presence of rare types; other indices such as Simpson's index are primarily affected by changes in the common types and hence give less weight to rare types (Peet 1974).

One-factor planned contrasts were performed to test for the effects of three broad micro-environments: plots located in the uncut forest (control treatment units and local controls located 40 metres into the forest) were classified as the undisturbed treatment, plots located at 2 m from the block edge were classified as the root-zone treatment, and the remainder of the samples located at distances ≥ 16 m from the block edge formed the third treatment. The response of the diversity indices were analyzed according to these groupings. The data were grouped in this way to account for the observation that roots of *Abies lasiocarpa* can extend up to 13 m (Stone and Kalisz 1991) thus creating the possibility that cores sampled along the interior edge of the clear-cut may contain roots associated with trees located in the adjacent forest. Furthermore, the three locations may represent unique micro-environments due to canopy closure, shading effects, and different moisture and temperature regimes.

The effect of clear-cut size on the diversity of mycorrhizae was tested by comparing samples collected at the same distance from the block edge but from clear-cuts of different sizes, i.e. samples collected at 16 m from the edge of the 0. 1 ha clear-cuts were compared with those collected at 16 m from the edge of the 1. 0 ha or 10 ha

clear-cuts. The effect of planting at increasing distance from the edge was tested by one-factor planned contrast by grouping all samples collected at the same distances from the edge regardless of clear-cut size. Significant ANOVA results ($p < 0.05$) were evaluated using the Tukey-Kramer (honestly significant difference) pairwise comparison test to identify differences among means. This particular test was chosen as it can accommodate different sample sizes and is more conservative than Fisher's least significant difference or Duncan's new multiple range test (Dowdy and Wearden 1991).

Using the Shapiro-Wilk W test, it was determined that the distribution of the counts of individual mycorrhizal types at each plot location was not normally distributed. Therefore, the count data was log transformed [log (x + 1)] prior to analyses to account for the log-normal distribution which was recognized by analyzing histogram plots of the distribution. However, despite transformation, the data did not strictly conform to assumptions of normality and homogeneity of variances and therefore non-parametric Kruskal -Wallis tests were employed to test for treatment effects on the counts and relative abundances of individual types of mycorrhizae.

The effect of north and south facing edges on diversity and the proportion of inactive roots was tested by one factor planned contrasts. Plots located at 2, 16 and 25 metres from the south block boundaries were classified as the south edge treatment (north-facing), plots located at 50 and 165 metres were classified as the centre treatment, and plots located at 2, 16 and 25 metres from the north boundary were classified as the north edge treatment (south-facing). These groupings were based on observations that trees in the adjacent forest are approximately 30 m in height (Novak *et al.* 1997) thereby potentially affecting light, temperature, and moisture regimes along the clear-cut boundaries. Analysis of variance and non-parametric tests were performed using JMP (Version 3.1 SAS Inc.).

Detrended correspondence analysis

To detect plots having similar assemblages of ectomycorrhizae, the transformed data matrix of dominant ectomycorrhizal types was subjected to detrended correspondence analysis (DCA) ordination (Hill 1979b, Hill and Gauch 1980) by PC-ORD. Spearman's rank correlation coefficients were calculated by SYSTAT (Version 5 SYSTAT Inc.) to determine the underlying gradients contributing to the variation expressed by the first DCA axis.

Results

There was no block (elevation) effect detected for any of the variables measured.

The ectomycorrhizal community

Twenty-nine different morphotypes of ectomycorrhizae were detected on the severed roots of mature trees (Table 2). *Cenococcum* - like, *Lactarius*- like type I, *Lactarius* - like type II -, *Piloderma* - like, *Hebeloma* - like, *Amphinema* - like and *Paxillus* - like mycorrhizae accounted for 80.6% of all ectomycorrhizae. The remaining types occurred with a relative abundance of less than 4%.

Effects of location on root persistence

Over all plot locations, including the undisturbed controls, the percentage of ectomycorrhizal roots assessed as being inactive was 77.5%. The proportion of inactive roots was significantly different between the three treatments ($p = 0.006$). Although Tukey's pairwise comparison tests did not detect differences among the means, the trend was for an increase in the proportion of inactive roots from the forest (68.8% assessed as inactive) to the root-zone (77.6%) to the remainder of the plots located $\geq$ 16 m from the block edge (80.2%). There was no difference between the

three treatments for the actual numbers of inactive roots encountered ($p = 0.653$), however, the number of live ectomycorrhizal roots was significantly greater at plots located in the forest as compared with plots located in the root-zone and the remaining clear-cuts (Fig. 3). There were no differences detected in the number of inactive or active roots at the same distances within clear-cuts of different sizes. There were differences detected in the number of active roots encountered with increasing distance from the block edge ($p = 0.038$, Fig. 4) but Tukey's pairwise comparisons did not distinguish among means.

Effect of location on the abundance of specific ectomycorrhizal types

Counts of mycorrhizal types

Of the dominant ectomycorrhizal types encountered, there were significantly fewer *Cenococcum* - like and *Hebeloma*-like mycorrhizae at plots located ≥ 16 m into the clear-cuts than in the undisturbed forest and root-zone locations (Table 3). This trend also applies to *Cortinarius* - like mycorrhizae. Although not a dominant mycorrhizae encountered on the excised roots, *Cortinarius* did comprise a substantial portion of the community associated with the field bioassay seedlings (Chapter 4) and, therefore, it is considered here. *Lactarius* type II - like and *Amphinema* - like ectomycorrhizae were seemingly unaffected by the treatments as no differences were detected in their numbers two years post harvest. The effects of location on the numbers of mycorrhizal roots of *Piloderma*-like, *Lactarius* type I - like, and *Paxillus*-like are ambiguous. Analysis of the counts of the dominant ectomycorrhizal types throughout Sicamous Creek using non-parametric Kruskal -Wallis tests revealed that the counts of all ectomycorrhizal types were equally distributed throughout the plot locations one year before and after logging.

Relative abundance of mycorrhizal types

The relative abundance of *Hebeloma* - like and *Cortinarius* - like mycorrhizae were both significantly affected by the three treatments (Table 4). Although Tukey's pairwise comparisons did not distinguish among means for any of the mycorrhizae tested, for *Hebeloma*, the trend was for a decrease in relative abundance from the plots located in the forest to plots ≥ 16 m into the clear-cuts. For *Cortinarius*, the relative abundance was greatest at root-zone. The relative abundance of *Lactarius* type I - like mycorrhizae, was greatest at plots located at all locations within the clear-cuts. For *Cenococcum* - like, *Amphinema* - like and *Lactarius* type II - like mycorrhizae, there were no differences in relative abundance with treatment. The difference in the relative abundance for *Piloderma* - like mycorrhizae was ambiguous with a borderline significance value ($p = 0.131$).

Diversity

Both diversity and richness were significantly greater at the forest plot locations relative to the plots in the root-zone and those located ≥ than 16 m into the clear-cuts (Fig. 5). Evenness did not vary with treatment. There was no effect of clear-cut size on diversity or richness of ectomycorrhizal morphotypes at plots located at 16 or 50 metres from the block edge. There was no difference in richness with distance into the clear-cuts from the adjacent forest ($p = 0.386$, Fig. 6). The effect of distance on evenness ($p = 0.068$) and diversity ($p = 0.158$) was less clear. Mean diversity did decline with increasing distance from the block edge. Interestingly, simple linear regressions performed on richness and diversity indices against distance from the edge resulted in a highly significant relationship between variables (Fig. 7). The relationship between evenness and distance was comparable to the results of ANOVA.

Table 2. Mean relative abundance (%) ± SE over all plot locations (n = 48) for the twenty-nine ectomycorrhizal types encountered on fine roots obtained from cores in 1996 at Sicamous Creek two growing seasons after winter logging. See Appendix 2. for complete morphological descriptions of the mycorrhizae encountered.

Thesis reference	Possible fungal associate	RA (%)
SC #50	*Cenococcum* - like	25.1 ± 2.5
SC #160	*Lactarius* - like I	15.3 ± 2.3
SC #190	*Lactarius* - like II	12.3 ± 2.3
SC #130	*Piloderma* - like	10.1 ± 2.5
SC #70	*Hebeloma* - like	8.2 ± 1.6
SC #150	*Amphinema* - like	5.5 ± 2.1
SC #270	*Paxillus* - like	4.1 ± 1.0
SC #260	E-strain III - like	2.5 ± 1.1
SC #170	ITE-1 - like	2.4 ± 1.1
SC #120	*Cortinarius* - like I	2.2 ± 1.2
SC #80	*Laccaria* - like	1.1 ± 0.4
SC #90	*Mycelium radicis atrovirens* - like	1.1 ± 0.6
SC #20	*Thelephora*- like II	0.1 ± 0.1
SC #60	*Russula*- like II	0.1 ± 0.1
SC #181	*Tomentella* I - like	0.7 ± 0.4
SC #100	*Lactarius* - like	0.6 ± 0.4
SC #40	Unidentified	0.5 ± 0.2
SC #30	*Tomentella* II - like	0.3 ± 0.2
SC #110	*Lactarius* - like III	0.3 ± 0.3
SC #210	*Russula* - like I	0.2 ± 0.1
SC #220	Unidentified	0.1 ± 0.1
SC #250	Unidentified	0.2 ± 0.1
SC #290	Unidentified	2.0 ± 0.9
SC #295	Unidentified	1.5 ± 0.5
SC #300	*Leccinum* - like	1.3 ± 0.8
SC #310	ITE-6 - like	0.2 ± 0.2
SC #330	*Dermocybe* - like	1.1 ± 0.8
SC #340	Unidentified	0.3 ± 0.3
SC #350	*Suillus* - like	0.4 ± 0.3

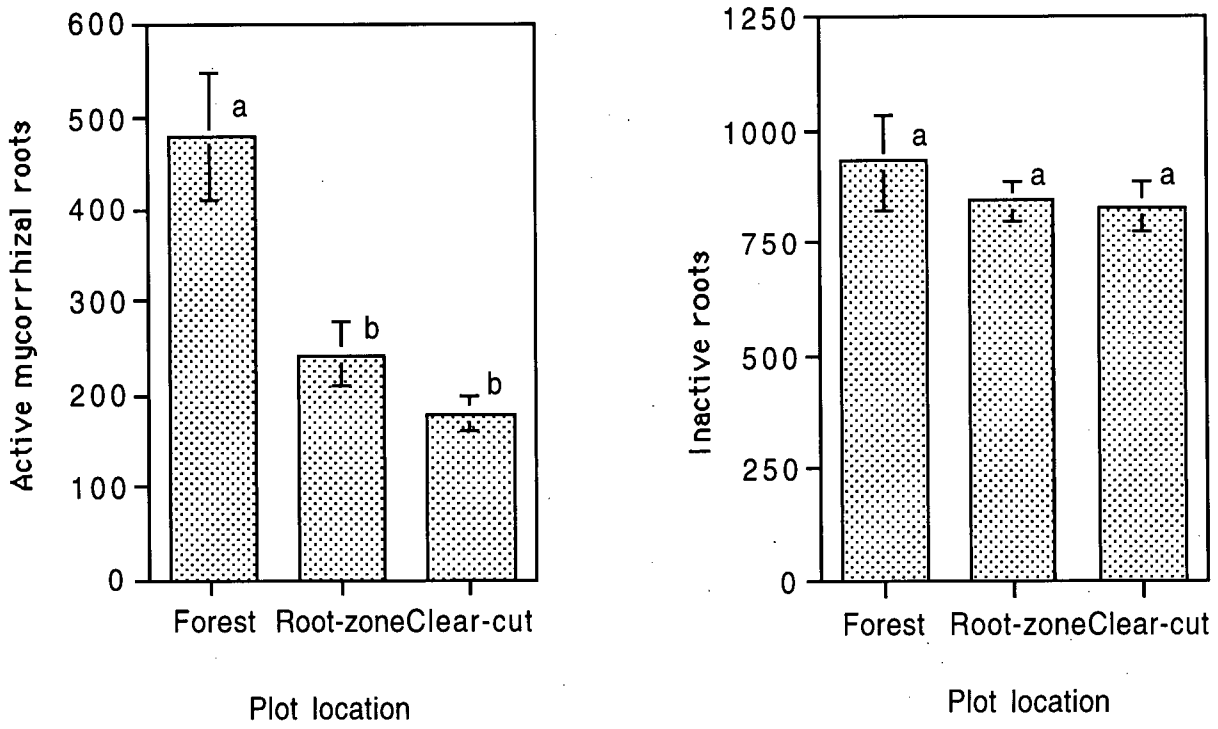

Figure 3. Numbers of active mycorrhizal and inactive fine roots encountered in 1/4 of a soil core at three different plot locations at Sicamous Creek two growing seaons after logging. Active mycorrhizal roots (ANOVA: p = 0.001), inactive roots (ANOVA: p = 0.653). Means denoted by different letters differ significantly (alpha = 0.05). Forest plots (n=9) include samples taken at local controls (40 m into the forest) as well as uncut treatment unit, root-zone plots (n=6) inlcude samples taken at 2m from the north and south edges of the 10 ha cut-block, clear-cut plots (n = 33) include samples taken at all plots greater than or equal to 16 m from the edge of 0.1, 1.0 and 10 ha cut-blocks. Error bars represent standard error of the mean.

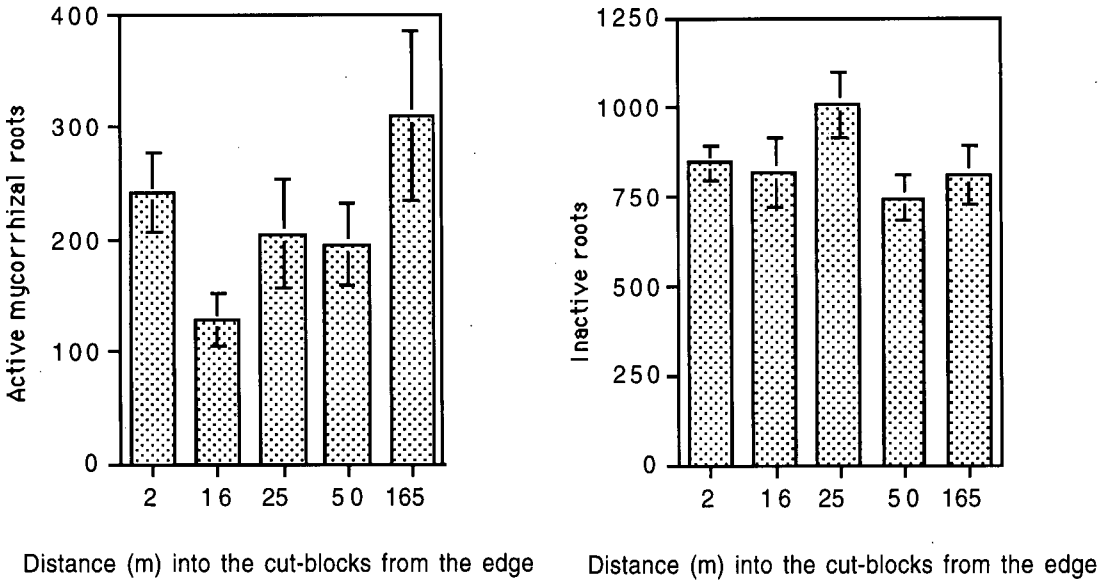

Figure 4. Numbers of active mycorrhizal and inactive fine roots encountered in soil cores with increasing distance from the block edge two growing seasons after logging at Sicamous Creek. Active mycorrhizae (ANOVA: $p = 0.038$), inactive (ANOVA: $p = 0.515$). Sample sizes at respective distances: 2m (n=6), 16m (n=15), 25m (n=6), 50m (n=9), 165m (n=3).See Fig.1 for sampling details.

Table 3. Numbers and standard errors of roots per 1/4 core of the most common ectomycorrhizae encountered at each plot location grouped as forest, root-zone and clear-cut plot locations at Sicamous Creek 1996.

Ectomycorrhizal type	**Forest** [1]	**Root-zone** [2]	**Clear-cut** [3]	**p-value**
Cenococcum - like	106. 2 a (19.5)	60.5 a (21.8)	44.2 b (7.4)	0.012
Hebeloma - like	76.2 a (17.5)	24.8 a (14.0)	8.09 b (2.9)	0.0002
Lactarius type I - like	25.0 (8.9)	47.0 (6.6)	26.4 (6.9)	0.041
Piloderma - like	53.1 (19.1)	3.3 (1.9)	30.3 (10.9)	0.12
Lactarius type II- like	34.2 (13.2)	17.3 (10.5)	19.9 (6.3)	0.53
Paxillus - like	35.1 (15.6)	4.5 (2.5)	6.5 (2.1)	0.079
Amphinema - like	9.67 (6.5)	42.0 (38.1)	12.7 (6.1)	0.63
Cortinarius - like	11.7 a (6.6)	9.3 a (4.3)	4.2 b (3.8)	0.011

Within rows, means with the same letter are not significantly different as tested by Kruskal-Wallis non-parametric tests.

[1] Includes cores sampled from 40 m into the forest from the north and south edges of the 10 ha clear-cuts and cores sampled from uncut treatment units; n = 9.

[2] Includes cores sampled 2 m from the north and south edges of the 10 ha clear-cuts; n = 6.

[3] Includes cores sampled from the centre of the 0.1 ha clear-cuts (16 m from the edge), 16 m from the north and south edges and in the centre (50 m) of the 1.0 ha clear-cuts, and 16, 25 and 50 m from the north and south edges and in the centre (165 m) of the 10 ha clear-cuts; n = 33.

Table 4. Relative abundance and standard errors of ectomycorrhizae (expressed as a proportion of the total mycorrhizal community detected) encountered at each plot location grouped as forest, root-zone and clear-cut plot locations at Sicamous Creek 1996.

Ectomycorrhizal type	**Forest** [1]	**Root-zone** [2]	**Clear-cut** [3]	**p-value**
Cenococcum - like	0.239 (0.03)	0.25 (0.08)	0.25 (0.03)	1.00
Hebeloma - like	0.136 (0.02)	0.094 (0.05)	0.06 (0.02)	0.009
Lactarius type I - like	0.062 (0.02)	0.217 (0.04)	0.17 (0.03)	0.041
Piloderma - like	0.15 (0.05)	0.01 (0.01)	0.10 (0.03)	0.131
Lactarius type II - like	0.083 (0.03)	0.074 (0.04)	0.145 (0.03)	0.842
Paxillus - like	0.056 (0.03)	0.026 (0.016)	0.04 (0.012)	0.540
Amphinema - like	0.017 (0.01)	0.134 (0.10)	0.052 (0.02)	0.585
Cortinarius - like	0.02 (0.01)	0.04 (0.02)	0.02 (0.02)	0.006

Within rows, means with the same letter are not significantly different as tested by Kruskal-Wallis non-parametric tests.

[1] Includes cores sampled from 40 m into the forest from the north and south edges of the 10 ha clear-cuts and cores sampled from uncut treatment units; n = 9.

[2] Includes cores sampled 2 m from the north and south edges of the 10 ha clear-cuts; n = 6.

[3] Includes cores sampled from the centre of the 0.1 ha clear-cuts (16 m from the edge), 16 m from the north and south edges and in the centre (50 m) of the 1.0 ha clear-cuts, and 16, 25 and 50 m from the north and south edges and in the centre (165 m) of the 10 ha clear-cuts; n = 33.

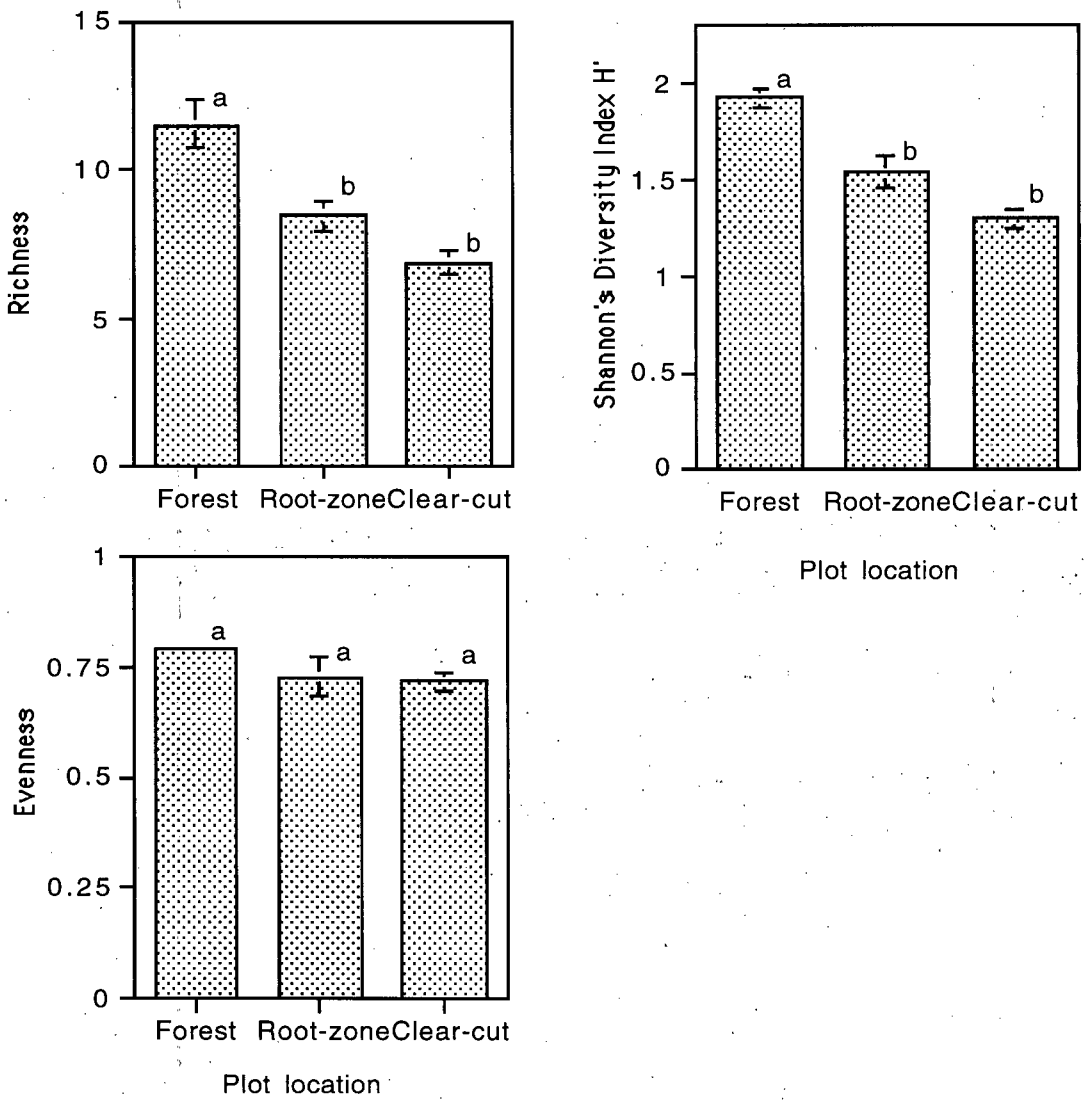

Figure 5. Richness, Shannon diversity index and evenness of ectomycorrhizae encountered in soil cores taken from Sicamous Creek two growing seasons after logging. Richness (ANOVA: $p < 0.0001$), diversity (ANOVA: $p < 0.0001$), evenness (ANOVA: $p = 0.244$). Means denoted by different letters differ significantly (alpha = 0.05). Forest plots (n=9) include samples taken at local controls (40 m into the forest) as well as uncut treatment units, root-zone plots (n = 6) inlcude samples taken at 2m from the north and south edges of the 10 ha cut-block, clear-cut plots (n=33)include samples taken from all plots greater than or equal to 16 m from the edge of 0.1, 1.0 and 10 ha cut-blocks. Error bars represent standard error of the mean. The absence of an error bar indicates that the error was too small to be depicted in the graph.

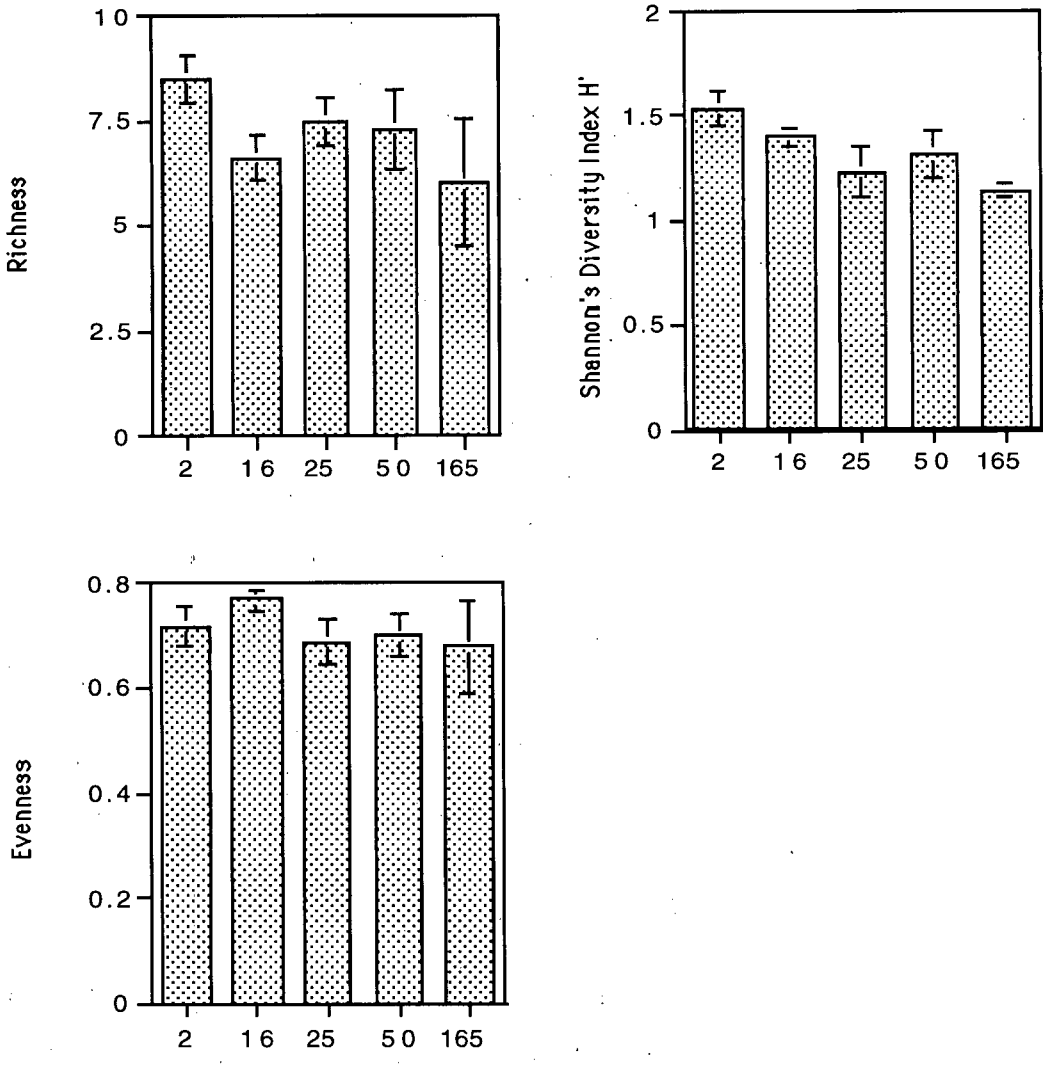

Figure 6. Richness, Shannon diversity index and evenness of ectomycorrhizae encountered in soil cores taken from Sicamous Creek two growing seasons after logging with increasing distance from the block edge. Richness (ANOVA: $p = 0.386$), diversity (ANOVA: $p = 0.158$), evenness (ANOVA: $p = 0.068$). Sample sizes at respective distances: 2m (n=6), 16m (n=15), 25m (n=6), 50m (n=9), 165m (n=3). See Fig. 1 for sampling details.

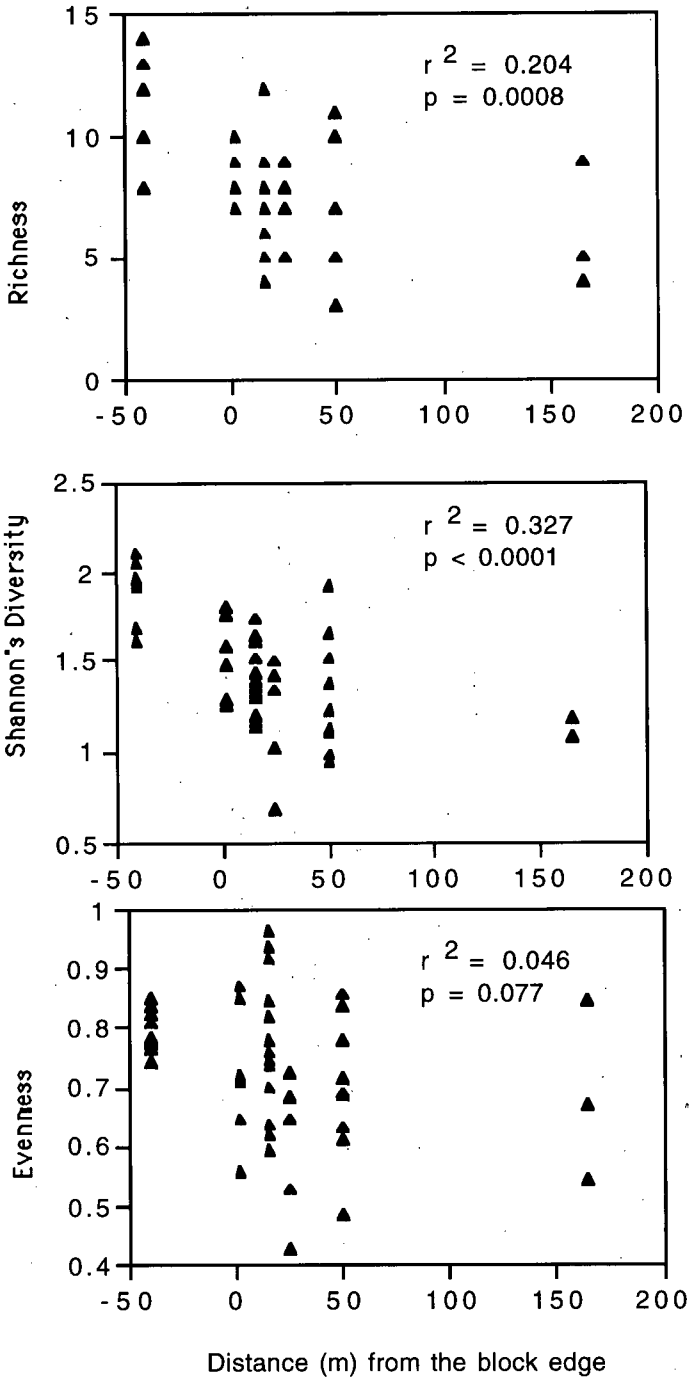

Figure 7. Simple linear regressions for richness, diversity and evenness of ectomycorrhizae encountered in soil cores against increasing distance from the block edge two growing seasons after winter logging at Sicamous Creek. Triangular symbols represent the mean for each plot location where 8 cores were sampled.

Edge effects of north and south block boundaries

There were no differences in richness ($p = 0.848$), evenness ($p = 0.408$) or diversity ($p = 0.207$) at north, south or centre treatments. Similarly, the numbers of inactive roots did not differ significantly between treatments ($p = 0.155$).

Detrended correspondence analysis (DCA)

Detrended correspondence analysis separated the undisturbed and root-zone plots from the majority of the clear-cuts along the first DCA axis (Fig. 8). The clear-cut plots closest to the root-zone and forest plots were all from the south side of the opening. Although Spearman's rank correlations revealed a relationship between treatment (undisturbed and root-zone versus open clear-cut; DCA1 $r = - 0.717$, DCA2 $r = - 0.015$)) and the first axis, it is difficult to state for certain the underlying factors contributing to the variation.

Discussion

A total of twenty-nine ectomycorrhizal morphotypes were encountered, some of which occurred with unequal distribution throughout the different plot locations. Diversity and richness were both significantly greater at undisturbed plot locations relative to root-zone and clear-cut plots. There was a significant decline in diversity and richness with increasing distance from the undisturbed forest. There was no difference in diversity or richness at corresponding distances from different opening sizes.

Effect of location on root persistence

Two years following host removal the percentage of inactive roots at plots located greater than 16 m into the clear-cuts was 80.2%. This is substantially greater

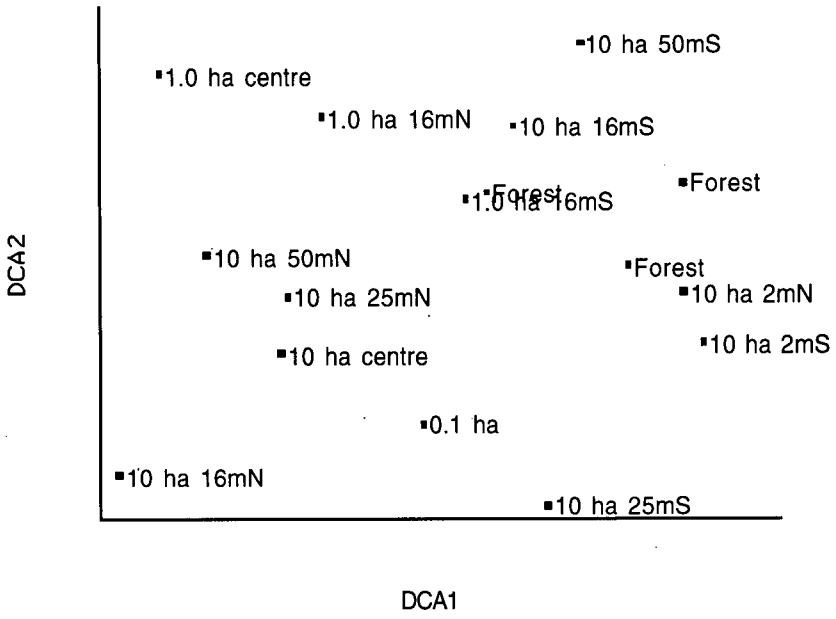

Figure 8. Detrended correspondence analysis ordination of plots based on assemblages of ectomycorrhizae. The first axis represents the change in the ectomycorrhizal community from mainly open clear-cuts at left, to mainly root zone and forest locations at right.

than the results from the 1995 core sampling (one growing season following logging) where 39.2% of roots in the clear-cuts were classified as inactive. However, the increase in the percentage of inactive roots two growing seasons following logging was also observed in the control plots (72.5% at control and root-zone plots in 1996, 36.9% in 1995).

The most likely explanation for the dramatic increase in proportion of inactive to active fine roots between 1995 and 1996 is due to changes in the sampling procedure between years. In 1995, the roots were cut into 2 cm sections prior to assessment. Up to 200 active mycorrhizal roots were counted per core. In addition to the 200 mycorrhizal roots, dead and non-mycorrhizal roots were recorded on randomly-selected root segments. As previously mentioned, due to the decomposition of the roots, the sampling procedure for 1996 was altered and all roots in approximately 1/4 of the total core (200 ml), were enumerated (inactive, mycorrhizal and non-mycorrhizal). As the amount of sample was not consistent among cores for the 1995 sampling, the data cannot be used as an indication of the proportion of active to inactive roots. Therefore, no comparisons regarding root persistence can be made between years. This is particularly unfortunate because the time between the first and second growing season following host removal seems to be critical in terms of the duration of time fine roots persist in an active state. The results of the 1997 sampling (three years post harvest) will provide useful, consistent results.

The proportion of inactive fine roots detected in this study is high but within the range previously reported for root turnover of conifers (Fogel and Hunt 1979). At Sicamous Creek, the ground remains covered by snow well into June. Sampling for the 1996 cores took place the first week of July and therefore, water stress and increased soil temperatures thought to influence root turnover (Lyr and Hoffman 1967, Teskey and Hinckley 1981) are not likely to be factors. It is well documented that naturally

occurring fine root turnover in trees exhibits modal or bimodal peaks with maximum root growth usually taking place in the early spring and autumn, followed in each case, by a period of senescence (Fogel and Hunt 1979, Vogt *et al.* 1986). In addition to environmental factors such as temperature and moisture, increases in root senescence following spring root growth may be influenced by the onset of shoot growth (Vogt *et al.* 1982). However, at this high elevation site, shoot growth is not thought to have resumed at the time of sampling. The hypothesis that environmental conditions dictate the life span of a root is one of two models regarding root senescence. The other hypothesis states that the life span of a root is determinate and dependent on a finite supply of carbohydrate (reviewed by Vogt and Bloomfield 1992).

The finding that there were significantly more active mycorrhizal roots in forested plots illustrates that despite the high numbers of inactive roots detected, roots are turning over and regenerating. This exemplifies the pattern of root senescence and turnover previously mentioned. Conversely at the clear-cut plots, the roots are dying but are not being replaced.

Effect of location on the counts and relative abundance of specific ectomycorrhizal types

As discussed above, the counts of active mycorrhizal roots were significantly lower at plots located within the clear-cuts as compared with the forest plots. Specifically, *Cenococcum* - like, *Hebeloma* - like and *Cortinarius* - like mycorrhizae were reduced at plots located ≥ 16 m into the clear-cuts. This suggests that these types of ectomycorrhizae are less persistent in clear-cuts than *Lactarius* type II - like and *Amphinema* - like mycorrhizae which were not affected by treatment.

Cenococcum

The numbers of *Cenococcum* - like mycorrhizae encountered one growing season after logging did not vary with plot location ($p = 0.637$). Two growing seasons after logging, however, there was a decrease in the numbers of *Cenococcum* at plots located ≥ 16 m into the clear-cut. Nevertheless, the proportion of the community that this mycorrhizae occupied did not vary among the three treatments (Table 4). This discrepancy may be explained by a simultaneous decrease in another dominant type of mycorrhizae such as *Hebeloma*. It should be noted that the dark brown-black colour of *Cenococcum* makes morphological assessment difficult and may have contributed to the variable results.

Hebeloma

There were no significant differences in the numbers of *Hebeloma* - like mycorrhizae encountered among treatments one growing season after logging ($p = 0.104$). Two growing seasons after logging, both the numbers and the proportion of the community that *Hebeloma* - like mycorrhizae occupied was significantly reduced at plots located ≥ 16 m into the clear-cut. These results strongly suggest that mycorrhizae of this type begin to decline between one and two growing seasons following winter logging. The difference in the relative abundance of *Hebeloma* implies that the position it occupies in the community is being replaced by other types of ectomycorrhizae.

Cortinarius

There were no differences in the numbers of *Cortinarius* - like mycorrhizae encountered among treatments one growing season after logging ($p = 0.191$). Two growing seasons after logging, the numbers of *Cortinarius* - like mycorrhizae were

significantly reduced at plots located ≥ 16 m into the clear-cuts. This indicates that this fungus is poorly adapted to survive in the absence of a live host with loss of vitality occurring between one and two growing seasons. Late-stage ectomycorrhizal fungi such as *Cortinarius* are speculated to have high carbon requirements (Deacon and Fleming 1992). If this is so, this fungus may be expected to persist for a shorter period of time relative to fungi that use carbon reserves in the root tissue at a slower rate.

Persistence of ectomycorrhizae two growing seasons following logging

The numbers of *Lactarius* type II and *Amphinema* - like mycorrhizae encountered in soil cores did not vary significantly with plot location one and two growing seasons following logging. Based on morphological criteria, these particular ectomycorrhizal types remained active in the absence of a live host for over eighteen months. This result corroborates previous findings indicating that at disturbed clear-cuts, ectomycorrhizal fine roots can persist for up to eighteen months (Bauhus 1994, cited in Bauhus and Bartsch1996). Notably this is the upper limit reported for ectomycorrhizal persistence as other studies have shown that ectomycorrhizal tips persist in an active state for nine months to two years following logging (Harvey *et al.* 1976, Persson 1982, Ferrier and Alexander 1985, Parsons *et al.* 1994).

The mechanism by which these ectomycorrhizal fungi were able to persist was not examined in this study. In the past, the accepted view was that ectomycorrhizal fungi are exclusively dependent on the host to satisfy their carbon demands (Trappe and Fogel 1977, Harley and Smith 1983). More recently, various studies have shown that certain ectomycorrhizal fungi have the ability to produce carbohydrate degrading enzymes (Todd 1979, Giltrap 1982, Trojanowski *et al.* 1984, Haselwandter *et al.* 1990, Durall *et al.* 1994). For example, *Paxillus involutus* can grow saprophytically on unsterile humus (Laiho 1970, Erland & Söderström 1991), and other fungi, including *Piloderma croceum,* can grow on sterile peat (Erland *et al.* 1990). The

evidence for both obligate and facultative associations indicates that there is likely a range of host dependency among ectomycorrhizal fungi.

Lactarius

In the laboratory, *Lactarius* spp. have been shown to produce significant quantities of polyphenol oxidases; the group of enzymes implicated in the breakdown of lignin. Therefore, Giltrap (1982), speculated that members of this genus may possesses saprophytic capabilities and concluded that *Lactarius* spp. may be facultative symbionts. The results presented here reveal that two growing seasons after host removal, two types of *Lactarius* mycorrhizae were equally abundant in the clear-cuts and in the undisturbed forest. The trend for *Lactarius* type I was to occupy a greater proportion of the ectomycorrhizal community at plots located in the clear-cuts. Thus, the abundance of this mycorrhiza may, in part, have influenced the reduction in the relative abundance that *Hebeloma* - like occupied. The relative abundance of *Lactarius* type II did not vary across treatments. The persistence of this fungus at a length of time previously reported to be the upper limit of ectomycorrhizal fine root survival offers support for Giltrap's assertion.

Piloderma

There was a weakly significant treatment effect on the numbers of *Piloderma* - like mycorrhizae encountered in the soil cores (Table 3). The highest counts were from plots located ≥ 16 m into the clear-cuts as well as in the forest. This trend was similar for the relative abundance of *Piloderma. Piloderma* is commonly found in the humus layers of soils (Dahlberg 1990) and has been suggested as an indicator of old growth stands (Smith *et al.* 1996). However, isolates of *Piloderma* have been shown to grow saprophytically on sterile humus (Erland *et al.* 1990). Perhaps this fungus is able to persist in localized patches of organic matter throughout the clear-cuts by obtaining

nutrients saprophytically. At Sicamous Creek, this fungus has been observed to persist nearby stumps into the third growing season following logging (pers. observation). *Piloderma* has also been observed nearby stumps persisting into the third growing season following logging of a lodgepole pine forest located approximately 50 km south-east of Kelowna, British Columbia (pers. observation).

Paxillus

Paxillus involutus has been referred to as a facultative ectomycorrhizal fungus (Laiho 1970, Haselwandter *et al.* 1990). This designation has been formulated based on the results of laboratory studies were *Paxillus involutus* has been shown to grow on unsterile humus (Laiho 1970, Erland and Söderström 1991) and to degrade lignin (Haselwandter *et al.* 1990). If this ability to degrade complex polymers is also true in natural ecosystems, *Paxillus* may be less dependent on hosts for carbon than other fungi and might be able to persist in soils following logging and to provide a viable inoculum source for outplanted seedlings. The observed persistence of *Paxillus* at the upper limit previously observed for fine root persistence offers support for the designation of *Paxillus* as a facultative fungus in nature.

Implications for colonization of outplanted seedlings

The results presented here indicate that planting eighteen months after logging may result in a decrease in the formation of *Cenococcum* - like, *Hebeloma - like* and *Cortinarius* - like mycorrhizae on outplanted seedlings if fine roots are, in fact, the primary inoculum source. One factor that was not quantified and may impact on the levels of inoculum present in the soil is the production of sclerotia by *Hebeloma* and *Cenococcum* fungi. Sclerotia are resistant structures known to persist in the soil for extended periods of time (Shaw and Sidle, 1983) and are capable of colonization (Fox

1986b, Trappe 1969). Finally, the results discussed in Chapter 2 have shown that other types of inocula such as spores may be important for seedling colonization.

Diversity of mycorrhizae encountered in soil cores two growing seasons after winter logging

Diversity and richness were significantly greater at the forested plots as compared with root-zone and plots located ≥ 16 m into the clear-cut. This implies that at the scale of a plot (approximately $8m^2$ for this study), there is a greater number of more evenly-distributed types co-existing in the undisturbed forest. The clear-cuts are characterized by a lesser number of co-existing types at this scale. The implications of this finding is that at Sicamous Creek, seedlings planted in clear-cuts will encounter fewer types of ectomycorrhizae than seedlings naturally regenerating in the adjacent forest. This could be regarded as a detriment to plantation success as the presence of a diverse array of ectomycorrhizal fungi in the soil is thought to be important for both seedling growth and the sustainability of ecosystems (Perry *et al.* 1987, 1989a).

Analysis of variance detected only slightly significant effects of distance into the clear-cuts on diversity, and no effect on richness. When values from the forest were included, regression analysis revealed a negative relationship between both richness and diversity with distance from the edge (Fig. 7). The latter analysis indicates that two growing seasons after winter logging, diversity and richness of mycorrhizae were significantly reduced with distance from the block edge. Presumably, the significantly lower numbers of *Hebeloma* - like and *Cortinarius* - like mycorrhizae at plots located ≥ 16 m into the clear-cut is contributing to the reduction in diversity. Differential rates of persistence for the minor types are also likely to have contributed to this result although their rarity precluded statistical testing for location effects. The lack of an effect due to clear-cut size suggests that for clear-cuts ranging in size from 0.1 ha and 10 ha,

distance from the edge is more important to patterns of ectomycorrhizal persistence than clear-cut size.

Processes influencing diversity

A number of hypotheses have been proposed to explain patterns of local species diversity (Connell 1978, Shmida and Wilson 1985). Local species diversity refers to processes occurring at the scale of the community and does not address larger scale influences such as latitude or altitude which, are also known to impact on patterns of ectomycorrhizal fungal diversity (Allen *et al.* 1995). Bruns (1995) recently argued that two general models, those based on resource partitioning and disturbance or density dependent processes such as competition, predation, disease and herbivory, may be particularly applicable to maintaining patterns of local diversity of ectomycorrhizal fungi. These two general models as well as the influence of mass effect are chosen for this discussion.

Niche diversification hypothesis

The niche diversification hypothesis (Whittaker 1960, MacArthur 1965) falls under the general heading of equilibrium hypotheses which state that the composition of species in communities is in a state of equilibrium (Connell 1978). Specifically, the niche diversification hypothesis states that diversity can be explained by the specialization of organisms to occupy specific niches in a community (Connell 1978) i.e. resource partitioning. Therefore, spatial heterogeneity can be seen to provide the opportunity for species coexistence (Bengtsson *et al.* 1994). There are numerous causes of spatial heterogeneity including patchy disturbance regimes, herbivory, soil factors and localized climatic influences such as frost effects in depressions. Spatial heterogeneity may also arise from biological components of the community itself. For

example, the production of secondary chemicals by organisms and the accumulation of organic matter during succession may be influential.

Spatial heterogeneity and its role in contributing to species coexistence is directly linked to competitive interactions. The competitive exclusion principle (Gause 1934) states that niche differentiation facilitates the coexistence of two competing species in a stable environment. Therefore, a spatially heterogeneous environment would be conducive to niche differentiation as there are a variety of habitats to which organisms may differentially respond. Conversely, a homogenous environment lacks this variation and, as a result, niche differentiation is less likely to occur.

The division of niche space is the result of competition for resources. In plant communities, these resources include light, water and nutrients, all of which are essential to all plant growth. Obviously, plants cannot survive without light, for example, so partitioning occurs along gradients, with different plants adapted to different light conditions. A similar situation applies to fungi. The basic requirements for fungal growth are fixed carbon and mineral nutrients (Kendrick 1992). Therefore, these two resources can be defined as those that fungi compete for. For ectomycorrhizal fungi, carbon is obtained from the associated host and mineral nutrients are obtained from the soil and litter (Bruns 1995). Bruns (1995) describes four ways in which resources could be partitioned: in time and space, along roots, with soil depth, and within the heterogeneous litter.

Intermediate disturbance hypothesis

The intermediate disturbance hypothesis states that diversity is greatest at intermediate scales of disturbance. The intermediate disturbance hypothesis falls under the group of hypotheses that explain diversity in terms of non-equilibrium processes (Connell 1978). Within this group of hypotheses, competitive exclusion is avoided due to a community composition that is in constant flux. For ectomycorrhizal fungi,

disturbance may include grazing by mycophagous microbes or the production of chemicals released by neighboring plants or fungi. Density dependent processes such competitive replacement may also occur with one fungi replacing another on host roots.

Mass effect

Communities do not exist in isolation exempt from processes occurring in adjacent areas. The fact that natural communities are not closed systems is the basis of a mechanism described as mass effect (Shmida and Whittaker 1981). Shmida and Wilson (1985) proposed mass effect as an additional mechanism thought to influence diversity and defined it as the dispersal of individuals from areas where they are well adapted to exist to less favorable areas where the individuals are not likely to be self-maintaining. The influence of mass effect on determining the diversity of ectomycorrhizae in clear-cuts may be significant. Conceivably there will be dispersal of inocula from the adjacent forest into the clear-cut where individuals may not necessarily be self-maintaining. Conversely, individuals adapted to disturbed conditions are likely to be dispersed into the adjacent forest although their success may be limited. However, even a small number of individuals may contribute to the biological interactions between plants, microbes and fungi. Shmida and Wilson (1985) proposed that mass effect increases species diversity and suggested that the occurrence of species outside of their 'core' habitat is a substantial contributor to noise or patterns of diversity that are difficult to explain.

Diversity indices

There are numerous indices available to quantify diversity. Among these are species richness, Shannon's, Simpson's, the log series, Margalef, Brillouin and McIntosh indices (Magurran 1988). There is a lack of consensus regarding the value of

the various indices, however, the most commonly employed indices are species richness, Shannon's and Simpson's. For this study, Shannon's diversity index was chosen primarily because it gives equal weight to all species (Magurran 1988). Furthermore, Egger (1996) tested a number of diversity indices for their sensitivity to sample size and found that the Shannon index gave the most consistent results. Other indices tested, including Simpson's and log series gave highly variable results. Conversely, other researchers have found the Shannon index to be unreliable (May 1975) stating that the Simpson index is preferable (Routledge 1979). Still others cite the Brillouin index as the most reliable (Pielou 1975). Each index has its own inherent limitations and characteristics. For this study, it was deemed important that both dominant and rare types be equally considered in the analysis and, therefore, Shannon's index was chosen.

For ectomycorrhizal research, the use of evenness, one of the components of all diversity indices, has been criticized on the basis that individuals of ectomycorrhizal fungi are rarely identified (Bruns 1995). Thus, interpretation of indices such Shannon's or Simpson's can be considered unsuitable and Bruns (1995), concludes that richness is the most applicable index to consider in the context of ectomycorrhizal fungi. However, the use of evenness applied to ectomycorrhizae has been justified by Jones *et al.* (1997 in press) in the context that it is the individual mycorrhizal root that functions in physiological processes such as nutrient uptake that impacts on plant growth. For this study we are ultimately interested in the diversity of mycorrhizae capable of colonizing the fine roots of outplanted seedlings and so, as Jones *et al.* (1997 in press) argue, this index is felt to be informative.

DCA

One of the underlying factors contributing to the variation expressed by the first DCA axis may have been the treatment of clear-cutting. Visually, the first axis can be

seen to represent a change in the ectomycorrhizal community occurring at clear-cut plots and the undisturbed and root- zone plots. The discontinuous separation of the two groups along the first axis supports this interpretation.

It is interesting that the four clear-cut plots falling closely with the undisturbed and root-zone plots were from the south edge of the 10 ha clear-cut. Perhaps the additional shade offered at these locations created micro-climate differences more similar to the local environment beneath the canopy than at the centre or the north edge of the clear-cuts. Soil temperature, known to impact on ectomycorrhizal formation (Slankis 1974), may have been particularly influential regarding the distribution of certain mycorrhizal fungi. During the growing season, the soil temperature underneath the canopy at Sicamous Creek ranges from 7-10 °C and there is an edge effect that results in cool soil temperature extending 12 metres from the south edge into the 10 ha clear-cuts (R. Adams unpublished data). Beyond 12 m from the south edge, the temperatures are much warmer (up to 17 °C). At the north edge of the clear-cuts, warm soil temperatures persist up to and slightly into the adjacent forest.

Edge effects of north and south block boundaries

Although the DCA ordination detected similarity among plots located at the south edge and the forest, analysis of variance was unable to detect any differences with respect to diversity. This is not surprising as the ordination is capable of simultaneously analyzing plots and species whereas the univariate approach only considers one variable at a time and therefore, does not reveal the complex relationships between groups of organisms. As previously mentioned, the similarity between plots located at the south edge and the forest may have been influenced by the cooler soils which are present at these locations.

Chapter 4

Ectomycorrhizal colonization of *Picea engelmannii* x *glauca* seedlings planted across clear-cuts of different sizes

Introduction

Greenhouse bioassays such as the method described in Chapter 2, are commonly used to test the inoculum potential of soils (Perry *et al.* 1982, Schoenberger and Perry 1982, Parke *et al.* 1984, Pilz and Perry 1984, Jasper *et al.* 1991, Simard *et al.* 1997c). Although this technique can be informative, seedlings are grown under unnatural conditions. The technique of a 'field bioassay' using outplanted non-mycorrhizal seedlings improves upon the limitations of a greenhouse bioassay but has been employed relatively infrequently (McAfee and Fortin 1989, Andersson and Söderström 1995) because growing sterile seedlings can be very difficult. This difficulty is compounded when seedlings are grown at commercial greenhouses where contaminants such as *Thelephora terrestris* are prevalent. Despite colonization by greenhouse contaminants, some researchers have used commercially grown seedlings for a field bioassay (Pilz and Perry 1984, Danielson 1990, Richter and Bruhn 1993). The obvious drawback is that the results have to be treated cautiously, taking into account the influence that greenhouse fungi may have an affect on the ability of indigenous fungi to colonize the seedlings in the field.

In this study, eight-week-old, non-mycorrhizal seedlings were used to observe the ectomycorrhizal fungi capable of colonization at Sicamous Creek two growing seasons after logging. Specifically, this study was carried out to investigate if patterns of ectomycorrhizal persistence, as determined from soil cores (Chapter 3), correlated with patterns of colonization. The objectives were (i) to identify the active inocula in the soil that would be available for operationally planted seedlings by observing the types of ectomycorrhizae that were capable of colonizing sterile spruce seedlings two

growing seasons after logging, and (ii) to evaluate the effects of clear-cut size and distance from the edge on ectomycorrhizal diversity and community composition.

Materials and Methods

Seedling Growth and Outplanting

On April 30, 1996 *Picea engelmannii* x *glauca* seeds (seedlot #6025) were germinated and grown for 8 weeks in a greenhouse in a sterile mixture of peat and vermiculite (1:1 v/v). Prior to sowing, the mix was autoclaved for one hour at 250 °F, left to sit for 24 hours and subsequently autoclaved for an additional hour. Daylengths were extended to 18 hours with sodium vapor lamps. No fertilizer was added. In late June 1996, 12 non-mycorrhizal seedlings (determined by clearing and staining a subset of roots) were planted at each of the 48 plot locations shown in Fig. 1 (n=576). At each plot, seedlings were planted approximately 1 m apart from each other in soil that was 'undisturbed' relative to the actual mounded site preparation. Attempts were made to disturb the soil as little as possible while planting. A small conical wire mesh cage (1/4 inch openings) was placed around each seedling to prevent browse damage. Thirteen weeks later, the seedlings were harvested, placed in plastic bags with some surrounding soil and transported to the laboratory where they were stored at 3 ° C for up to 3 months during processing. Because of variation in the survival of the seedlings at the various plot locations, 7 seedlings from each plot location were randomly selected for analysis (n=336).

Seedling assessment

The entire root system of each seedlings was washed and the ectomycorrhizae characterized as described in Chapter 2. In addition, shoot dry weights were

determined after drying at 60 °C for 30 hours. Root length of the entire root system was estimated prior to mycorrhizal assessment by the line intercept method (Tennant 1975).

Statistical analysis

The relative abundance of each ectomycorrhizal type was calculated as a proportion of the total number of ectomycorrhizal roots averaged over the seven seedlings from each planting location. Ectomycorrhizal colonization was calculated as the percentage of the total number of roots that were mycorrhizal. Shoot dry weight, root length, percent colonization and the total number of roots are expressed as means of seven seedlings at each of the 48 plot locations. The diversity of ectomycorrhizae, based on the number of distinct ectomycorrhizal morphotypes encountered at each planting location, was expressed by Shannon's diversity index, richness and evenness. One-factor planned contrasts were performed to test for the effects of three broad micro-environments: plots located in the uncut forest (control treatment units and local controls located 40 metres into the forest) were classified as the undisturbed treatment, plots located at 2 metres from the block edge were classified as the root-zone treatment and the remainder of the samples located at distances ≥ 16 metres from the edge formed the third treatment. The response of all seedling variables and diversity indices were analyzed according to these planned comparisons. The effect of clear-cut size and edge influence on diversity was tested as described in Chapter 3.

Despite transformation, the distribution of individual ectomycorrhizal types did not meet assumptions of normality or homogeneity of variances between treatments. Therefore, non-parametric Kruskal - Wallis tests were used to test for treatment effects on the abundances of individual fungi.

Classification analysis

The polythetic divisive clustering technique, TWINSPAN (Two-way indicator species analysis; Hill 1979a) was used to detect differences in the community of ectomycorrhizal fungi at the various plot locations. This program first subjects the sample matrix to ordination by reciprocal averaging; groupings of samples are then constructed based on the presence and abundance of species (vanTongeren, 1987). The results are presented in a table of plots by species. Presence / absence data for the six ectomycorrhizal types occurring with an overall frequency greater than 3% were included in the analysis. The data were edited in a number of different ways prior to TWINSPAN analysis. Analysis was performed on abundance, frequency and presence / absence data. Additionally, separate analyses were performed using transformed (arcsin) and untransformed data using all ectomycorrhizal types and only the most frequently occurring (> 3%) types.

Other analyses

A number of other multivariate analyses were performed with the objective of detecting differences in the ectomycorrhizal community that may have occurred as a result of the of the various treatments. Cluster analysis as well as detrended correspondence analysis and non-metric multidimensional scaling were performed on the various data sets but no discernible trends could be identified.

Results

There was no block (elevation) effect for any of the variables measured. Similarly, no effect of clear-cut size was detected for any of the diversity indices at plots located at 16 or 50 metres from the block edge. Significant differences were observed among plots located in the forest, root-zone and greater than 16 metres into the clear-cut. More specifically, throughout the clear-cuts, diversity, richness and evenness were significantly reduced with increasing distance from the edge.

Shoot and root systems for the 21 week old seedlings

Seedling shoot and root systems were larger at the clear-cut locations as compared with forest locations (Table 5). Seedlings grown in the root-zone were intermediate with respect to dry weight, root length, and the number of fine roots, and not significantly different from either the clear-cut or forest plots. The mean mycorrhizal colonization for all seedlings after 13 weeks was 35.3%, and was slightly greater on seedlings planted in the root-zone as compared with seedlings planted in the forest or the clear-cuts ($p= 0.087$). A maximum of three ectomycorrhizal types were found on one seedling. The average number of ectomycorrhizal types per seedlings was 0.92. Thirty-one percent of seedlings were entirely non-mycorrhizal.

Summary of ectomycorrhizal types

Seventeen ectomycorrhizal types were identified on the roots of the outplanted seedlings (Table 6). The community of ectomycorrhizal fungi colonizing young seedlings was characterized by a small number of dominant types and numerous rare types. Six dominant ectomycorrhizal types occurred at an overall frequency greater than 3% and comprised 90.5% of all mycorrhizal roots. *Hebeloma* - like ectomycorrhizae were twice as abundant as any other ectomycorrhizal type.

Classification

Of all the data sets subjected to TWINSPAN, presence - absence data resulted in the clearest separation of plots (Table 7), although the classification generated from the abundance data was very similar (results not shown), the classification was the same regardless of whether all ectomycorrhizal types or only the most frequent types were included. TWINSPAN separated the forest and root-zone plots (cluster 2) from

Table 5. Root and shoot system size, mycorrhizal colonization and standard errors of *Picea engelmannii* x *glauca* seedlings grown in clear-cut or forest locations at Sicamous Creek for 13 weeks.

	Clear-cut [1]	**Root-zone** [2]	**Forest** [3]	**p-values**
Shoot dry weight (mg)	8.14 a (0.35)	7.02 ab (0.29)	6.19 b (0.31)	0.014
Root length (mm)	101.79 a (3.2)	84.46 ab (6.8)	75.28 b (4.4)	0.001
Total no. root tips per seedling	17.82 a (1.0)	14.98 a (2.3)	7.29 b (1.0)	0.0001
Proportion of mycorrhizal colonization	0.33 a (0.03)	0.5 a (0.05)	0.36 a (0.08)	0.087
Number of types seedling per seedling *	0.91 ab (0.05)	1.26 a (0.1)	0.78 b (0.2)	0.039

Within rows, means with the same letter are not significantly different as tested by Tukey's pairwise comparison tests p = 0.05.

* Includes seedlings that were entirely non-mycorrhizal

[1] Includes seedlings planted in the centre of the 0.1 ha clear-cuts (16 m from the edge), 16 m from the north and south edges and in the centre (50 m) of the 1.0 ha clear-cuts and 16, 25 and 50 m from the north and south edges and in the centre (165 m) of the 10 ha clear-cuts; n = 33.

[2] Includes seedlings planted 2 m from the north and south edges of the 10 ha clear-cuts; n = 6.

[3] Includes seedlings planted 40 m into the forest from the north and south edges of the 10 ha clear-cuts and seedlings planted in uncut treatment units; n = 9.

Table 6. Mean relative abundance (%) ± SE over all plot locations (n = 48) for the seventeen ectomycorrhizal types encountered on spruce seedlings outplanted at Sicamous Creek for 13 weeks, two growing seasons after winter logging. See Appendix 2. for complete morphological descriptions of the mycorrhizae encountered.

Thesis reference	**Possible fungal associate**	**RA (%)**
FB #200	*Hebeloma*- like	43.3 ± 4.1
FB #40	*Cenococcum* - like	23.3 ± 3.5
FB #63	E-strain	9.9 ± 2.2
FB #10	*Amphinema* - like	6.3 ± 1.5
FB #325	*Cortinarius* - like II	5.7 ± 1.7
FB #310	*Lactarius* - like	2.8 ± 1.3
FB #400	Unidentified	2.2 ± 1.1
FB #120	*Mycelium radicis atrovirens*	2.0 ± 1.0
FB #12	Unidentified	1.4 ± 0.8
FB #140	Unidentified	0.8 ± 0.7
FB #300	Unidentified	0.8 ± 0.5
FB #420	*Russula* - like I	0.4 ± 0.4
FB #520	Unidentified	0.4 ± 0.2
FB #500	E-strain IV	0.1 ± 0.1
FB #440	*Inocybe* - like	0.2 ± 0.2
FB #110	*Piloderma* - like	0.2 ± 0.2
FB #510	Unidentified	0.1 ± 0.1

Table 7. TWINSPAN classification showing the six dominant ectomycorrhizal types and their occurrence at different plot locations. Numbers in the matrix refer to presence (1) absence (-) of ectomycorrhizae.

LOCATION [1]	**2**	**4**	**6**	**7**	**8**	**9**	**10**	**1**	**5**	**12**	**11**	**13**	**14**	**16**	**3**	**15**
CLUSTER [2]	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Mycelium radicis atrovirens	-	1	1	1	1	1	1	-	1	1	-	-	-	-	-	-
Amphinema	-	1	1	1	1	1	1	1	1	-	-	1	1	1	1	1
Cenococcum	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
E-strain	1	1	1	1	1	1	1	1	1	-	1	1	1	1	-	-
Hebeloma	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Cortinarius	-	-	-	-	-	-	-	-	1	1	1	1	1	1	1	1

[1] Numbers represent plot location; 1 = 0.1 ha, 2 = 1.0 ha 16 mS, 3 = 1.0 ha centre, 4 = 1.0 ha 16 mN, 5 = 10 ha 2-3 mS, 6 = 10 ha 16 mS, 7 = 10 ha 25 m S, 8 = 10 ha 50 mS, 9 = 10 ha centre, 10 = 10 ha 50 mN, 11 = 10 ha 25 mN, 12 = 10 ha 16 mN, 13 = 10 ha 2-3 mN, 14 = 40 mN, 15 = 40 mS, 16 = control.

[2] Showing the first division of the TWINSPAN classification.

the majority of the clear-cut plots (cluster 1). The division is defined by the presence of *Cortinarius* - like ectomycorrhizae in forest and root-zone plots whereas *Mycelium radicis atrovirens* ectomycorrhizae were mainly restricted to seedlings planted at distances greater than 16 m into the clear-cut.

Effect of treatment on the relative abundance of individual ectomycorrhizal types

The distributions of the six dominant ectomycorrhizal types were tested by Kruskal-Wallis non-parametric statistics to detect differences amongst the three groups of planting locations. *Cenococcum* - like mycorrhizae were significantly more abundant at plots located ≥ 16 m from the block edge as compared with forest (Table 8). The distribution of *Hebeloma* - like ectomycorrhizae also varied among treatments, occurring with greater abundance in the forest than at the block edge. The distribution of *Cortinarius* - like mycorrhizae differed significantly among treatments (Kruskal-Wallis $p = 0.019$) however, Tukey's pairwise comparison tests did not reveal significant differences among the means at alpha = 0.05. No differences were detected in the distribution of E-strain, *Amphinema* - like or MRA ectomycorrhizae.

Ectomycorrhizal diversity

At each of the 48 plot locations, richness varied from 2 - 7 ectomycorrhizal types. Both richness and diversity of ectomycorrhizae observed on seedlings planted at the root-zone were significantly greater than in clear-cut or forest plots. Evenness did not vary between the three plot groupings ($p = 0.119$). (Fig. 9). There was a significant decline in ectomycorrhizal richness, diversity and evenness with increasing distance from the edge (Fig. 10). The critical distance at which the indices declined was 16-25 m from the block edge; however, due to the large degree of error associated with plots

Table 8. Relative abundance and standard error of ectomycorrhizae (expressed as a proportion of the total mycorrhizal community detected) encountered at each plot location grouped as forest, root-zone and clear-cut plot locations at Sicamous Creek 1996.

Ectomycorrhizae	**Forest** [1]	**Root-zone** [2]	**Clear-cut** [3]	**p-values**
Hebeloma - like	0.61 b (0.10)	0.23 a (.023)	0.421 ab (.04)	0.031
Cenococcum - like	0.044 b (.02)	0.246 ab (.06)	0.282 a (.04)	0.008
E-strain	0.03 (.02)	0.186 (.07)	0.103 (.02)	0.091
Amphinema - like	0.13 (.05)	0.067 (.04)	0.045 (.02)	0.171
Cortinarius - like	0.08 (.0004)	0.096 (0.03)	0.041 (.02)	0.019
Mycelium radicis atrovirens	-	0.01 (.01)	0.028 (.01)	0.265

Within rows, means with the same letter are not significantly different as tested by Kruskal-Wallis non-parametric tests and Tukey's pairwise comparison tests p = 0.05.

[1] Includes seedlings planted 40 m into the forest from the north and south edges of the 10 ha clear-cuts and seedlings planted in uncut treatment units; n = 9.

[2] Includes seedlings planted 2 m from the north and south edges of the 10 ha clear-cuts; n = 6.

[3] Includes seedlings planted in the centre of the 0.1 ha clear-cuts (16 m from the edge), 16 m from the north and south edges and in the centre (50 m) of the 1.0 ha clear-cuts and 16, 25 and 50 m from the north and south edges and in the centre (165 m) of the 10 ha clear-cuts; n = 33.

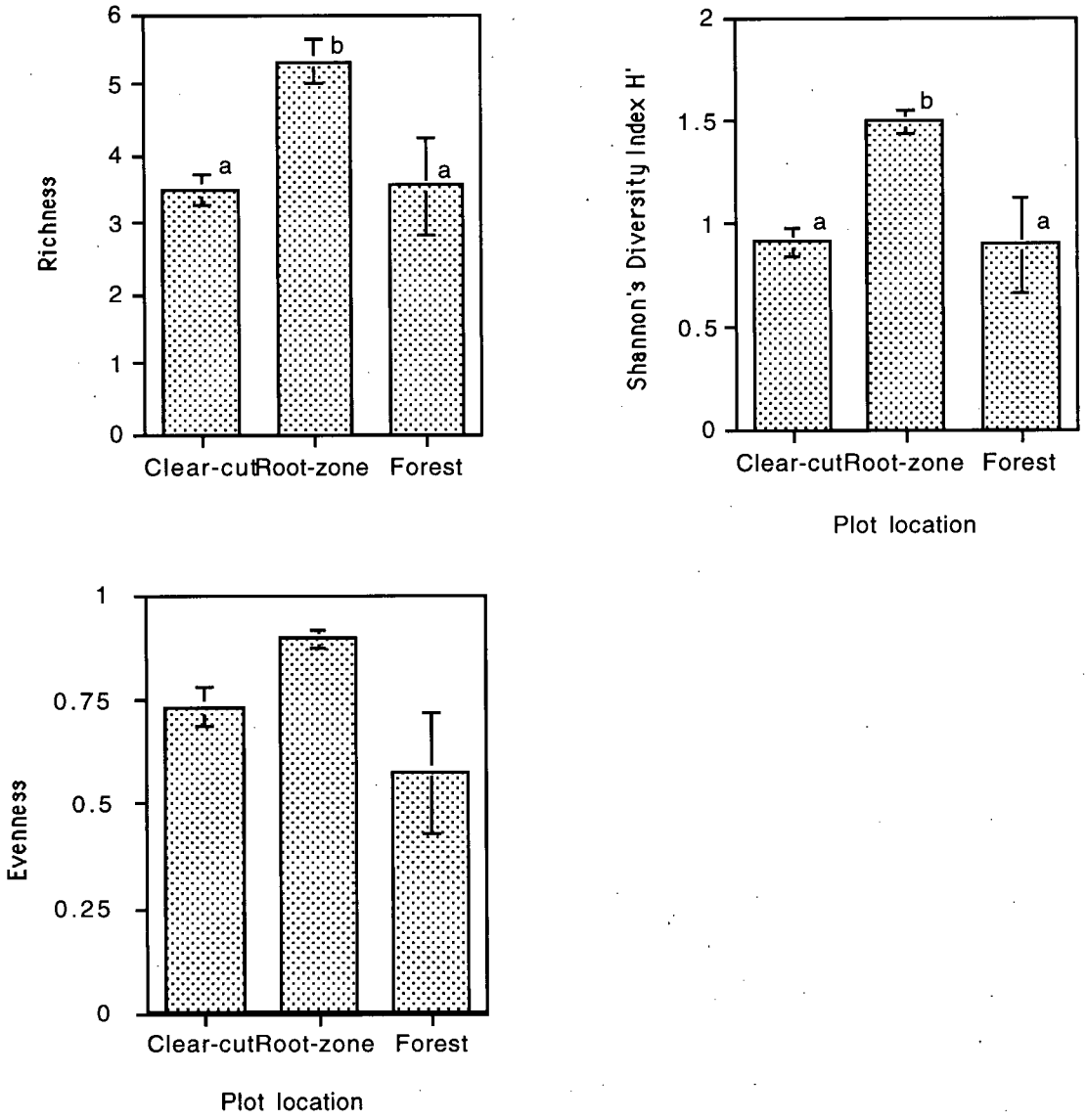

Figure 9. Richness, Shannon diversity index and evenness of ectomycorrhizae on 8-week-old spruce seedlings. Richness (ANOVA: p = 0.022), diversity (ANOVA: p = 0.022), evenness (ANOVA: p = 0.119). Means denoted by different letters differ significantly (alpha = 0.05). Forest plots (n=9), include samples taken at local controls (40m into the forest) as well as uncut treatment units (n=9), root-zone plots (n=6),include samplestaken at 2m from the north and south edges of the 10 ha cut-block. Error bars represent standard error of means.

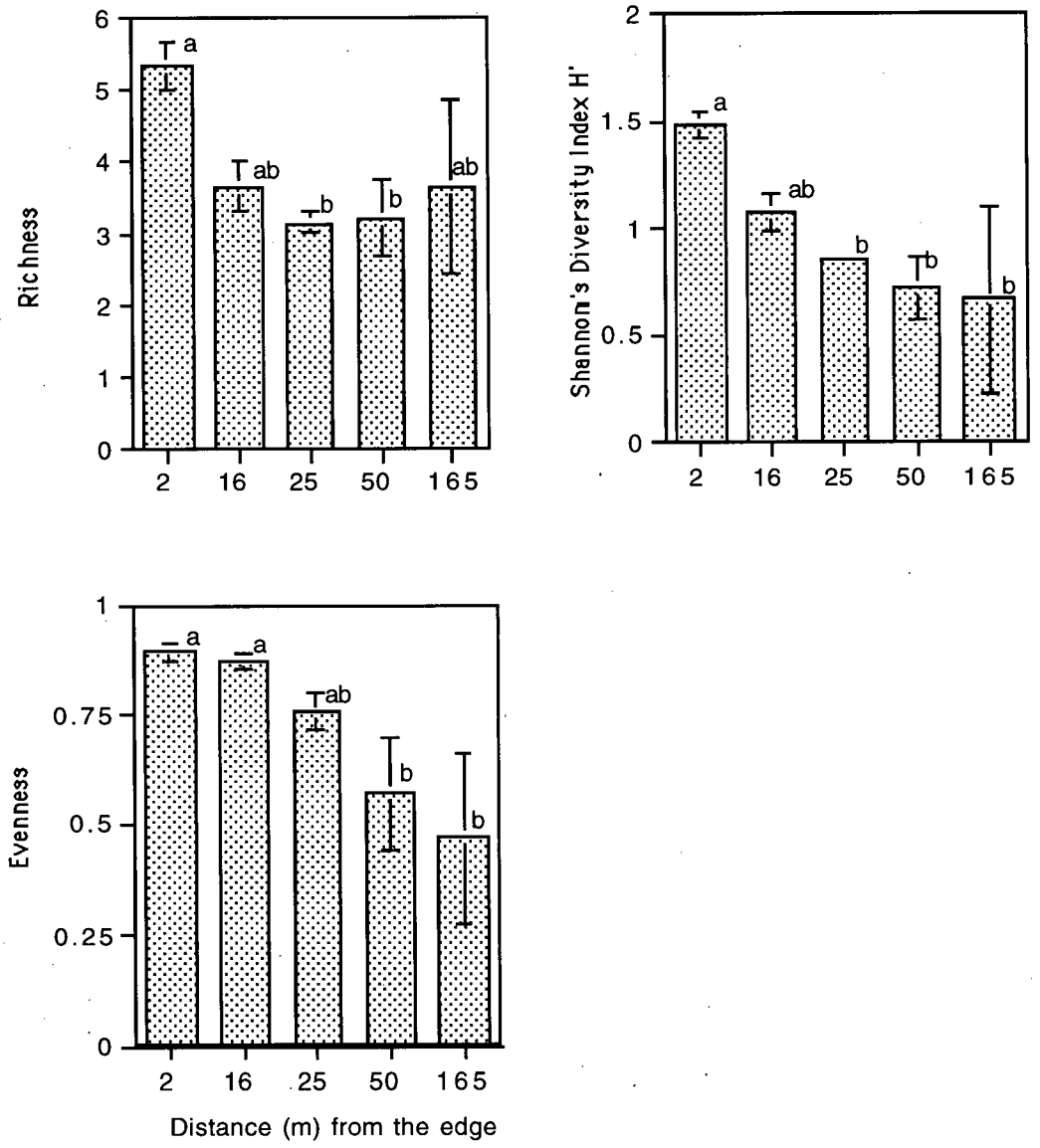

Figure 10. Richness, Shannon diversity index and evenness of ectomycorrhizae on 8-week-old spruce seedlings planted at increasing distances into cut-blocks. Richness (ANOVA: $p = 0.0409$), diversity (ANOVA: $p = 0.0041$), evenness (ANOVA: $p = 0.0035$). Means denoted by different letters differ significantly (alpha = 0.05). Sample sizes at respective distances: 2m (n=6), 16m (n=15), 25m (n=6), 50m (n=9), 165m (n=3). See Fig.1 for sampling details. Error bars represent standard error of means.

located at 165 m from the edge, there were no significant differences in richness between 2m and 165 m. There were also no significant differences in any of the diversity indices detected between the three clear-cut sizes at 16 or 50 m from the block edge.

Edge effects of north and south block boundaries

Evenness ($p = 0.002$) and diversity ($p = 0.028$) differed significantly between the north, south and centre plots. However, these differences are the result of higher values at the edges relative to the centre. Pairwise comparisons indicated that the two edges did not differ with respect to diversity.

Discussion

Seedling growth

The superior growth of seedlings in all parts of the clear-cuts as compared with the forest can be explained by the physiology of *P. engelmannii*. At high elevations, *P. engelmannii* is relatively shade intolerant because it utilizes the low levels of solar radiation present under the canopy inefficiently (Krajina 1965, Knapp and Smith 1982). *A. lasiocarpa,* a more shade-tolerant species, comprises the majority of seedlings regenerating under the canopy of subalpine forests (Miller 1970, Whipple and Dix 1979). Furthermore, at high elevations, net photosynthesis of conifers has been shown to be limited by low soil temperatures (Tranquillini 1979). Throughout the growing season, the soils under the canopy at Sicamous Creek range from 7-10 °C whereas soil temperatures in the clear-cuts range from 13-17 °C (R. Adams unpublished data). The cool soils in the forests very likely contributed to the reduced growth of *P. engelmannii.*

Colonization levels

Mycorrhizal colonization per seedling tended to be greater at the root-zone than at the forest or clear-cut plot locations. This result corroborates the findings of Pilz and Perry (1984) who found that levels of ectomycorrhizal colonization on outplanted Douglas-fir were similar in clear-cuts relative to the undisturbed forest. The clear-cuts, located in the Oregon Cascade mountains, were 2-3 years old and the authors suggest that replanting within a certain time after logging may avoid a biologically significant decrease in inoculum potential. The clear-cuts in this study were two years old and this may have been within an unknown critical time frame that enables adequate ectomycorrhizal formation. One of the long term objectives of the mycorrhizal research at Sicamous Creek (beyond the scope of this thesis) is to answer this question.

In contrast, Parke *et al.* (1984) found a significant reduction in mycorrhizal colonization on greenhouse bioassay seedlings grown in soils from clear-cut and clear-cut and burn sites, relative to soils from forested sites. The clear-cuts in their study, also located in Oregon, were identified as difficult to regenerate and ranged in age from 1-22 years and varied in size from 40 - 160 acres. Interestingly, the authors reported that ectomycorrhizal formation was not correlated with the age of the clear-cut. This result contradicts the hypothesis that prompt regeneration avoids a decrease in levels of mycorrhizal inoculum.

As previously discussed, the ability of different types of inocula to persist in soil is relatively unknown. Although the types of inocula colonizing outplanted seedlings could not be determined in this study, two growing seasons after logging there was sufficient inoculum present in clear-cut soils to result in 71.7% of seedlings becoming colonized within 13 weeks. The predominance of *Cenococcum* - like ectomycorrhizae associated with seedlings outplanted in the clear-cuts suggests that this fungus can persist for up to eighteen months following host removal. Similarly, *Hebeloma* - like ectomycorrhizae were abundant on seedlings planted in clear-cuts as

well as forest plots. Therefore, this fungus also seems to have also persisted for eighteen months. Although constituting less than 10% of the ectomycorrhizal community detected, there was active inocula of E-strain - like, *Amphinema* -like, and MRA - like present in clear-cut soils. The near absence of *Cortinarius* - like ectomycorrhizae on seedlings grown in the clear-cuts illustrates that certain types of inocula of this fungus such as the mycelia associated with excised mycorrhizae is not capable of colonization under these conditions. These findings support the assertion that certain fungi and certain types of inocula are better able to persist in the absence of a host than others.

Effect of treatment on the distribution of ectomycorrhizal fungi

The distribution of some ectomycorrhizal types varied with treatment. Specifically, *Cenococcum* - like ectomycorrhizae were more abundant at plots located ≥ 16 m into the clear-cut than at plots in the forest. This finding is consistent with observations made by Dahlberg and Stenström (1991), who investigated the formation of mycorrhizae on outplanted Scots pine seedlings in a boreal Scots pine and Norway spruce stand in Sweden. They found that *Cenococcum* colonized seedlings outplanted in clear-cuts more frequently than those outplanted in forests. Similarly, in Wyoming and Oregon, sclerotia of *Cenococcum* have been observed to be more abundant in some clear-cuts relative to undisturbed areas (Miller *et al.* 1994). In that study, the researchers suggested that as the photosynthate reserves of the root systems of harvested trees decrease, sclerotia production may be stimulated. If sclerotia are a viable source of inocula, this may explain the increased abundance of *Cenococcum* mycorrhizae associated with seedlings in clear-cut soils relative to undisturbed soils.

The epidemiology of *Cortinarius* colonization may offer an explanation for its abundance in the forest and root-zone. Research involving mycofloristic surveys commonly reveal that this fungus is associated with the roots of mature trees and,

therefore, it is often described as a late-stage fungus (Deacon *et al.* 1983; see discussion in Chapter 2). Furthermore, *Cortinarius* has been shown to be unable to colonize from spores (Fox 1986a). In this study, the presence of *Cortinarius* fungi associated with spruce seedlings at the forest and the root-zone plots, but only rarely in the clear-cuts, is further evidence that colonization is facilitated by mycelial links with mature trees.

Mycelium radicis atrovirens mycorrhizae have been found in both mineral soils (Danielson and Visser 1989) as well as in humus rich soils (Fernando and Currah 1995). Due to the mechanical disturbance caused by logging and site preparation, mineral soil was extensive throughout the clear-cuts. Despite our efforts to plant seedlings in organic fractions of soil, this was not always possible due to the extent of the site preparation at some locations. If these fungi exhibit a preference for mineral soils as has been previously reported (Danielson and Visser 1989), this could have been a factor contributing to the complete absence of this mycorrhizae at undisturbed plots. However, as the humus layers are fairly thin throughout the study site (1.5 - 9 cm); (Hope 1997) it is possible that the roots of seedlings grown at undisturbed plots were at least in part, growing in mineral fractions. An alternate explanation for the occurrence of MRA mycorrhizae in the clear-cuts may be the result of its ability to readily colonize from spores in a newly disturbed environment. Perhaps this early-stage fungus is poorly adapted to compete with the late-stage fungi present in the undisturbed soils.

Relationships between proximity to the edge, clear-cut size and ectomycorrhizal diversity

The simplest explanation for the patterns of diversity observed can be explained by the fact that certain types of fungi are incapable of colonizing seedlings from spores, sclerotia and fragments of mycelia attached to non-living roots. It is hypothesized that some fungi require an intact mycelial connection with a live host to colonize seedlings.

This phenomenon may have contributed to the ectomycorrhizal fungal community of seedlings planted in the root-zone and the forest relative to seedlings planted further into the clear-cut by providing the opportunity for late-stage fungi such as *Cortinarius* to colonize seedlings. Also present at root-zone plots were E-strain and MRA mycorrhizae which may be ill-suited to compete in the forest due to characteristics of the forest soils or possibility as a result of certain fungi being stronger sinks for fixed carbon than others (Deacon and Fleming 1992). If, for example, *Cortinarius* is a strong sink for carbon, it could be seen to have a competitive advantage over other fungi. Although having slower growth rates, late-stage fungi are highly competitive in symbiosis and it is commonly observed that early-stage fungi are quickly replaced. The finding that there is an increase in richness and diversity at the root-zone may have important consequences for plantation success as a diverse community of ectomycorrhizal fungi is thought to be important for seedling growth (Perry *et al.* 1987).

The upper limit of radial extension of *Abies lasiocarpa* roots is reported as being 13 metres (Stone and Kalisz 1991). Presumably, the fungal mycelium associated with the roots will extend even further. Therefore, beyond this approximate distance into clear-cuts, seedlings will not be in contact with inocula associated with overstory trees. The reduction in the diversity of fungi colonizing outplanted seedlings at distances greater than 16 - 25 m supports this hypothesis. The results presented here are similar to the findings of Simard *et al.* (1997a), who investigated ectomycorrhizal colonization of outplanted Douglas-fir seedlings at trenched and untrenched plots in the southern Interior of British Columbia. Seedlings grown at untrenched plots, where the roots had access to adjacent overstory trees, were colonized by a significantly richer and more diverse array of fungi. Observed patterns of colonization were attributed to untrenched seedlings having access to a greater diversity of fungi facilitated by links with overstory

trees. Furthermore, Simard *et al.* (1997a), conclude that the higher levels of diversity contributed to seedling success thus supporting the hypothesis of Perry *et al.* (1987).

Effects of north and south block boundaries

Univariate analysis detected no differences between the north and south block edges. This indicates that the variation in above ground conditions that may have existed between the two locations did not affect mycorrhizal colonization.

Summary

The results of this study suggest that proximity to overstory trees may be more important than clear-cut size for patterns of ectomycorrhizal diversity and colonization. The effects of partial cutting on these dynamics should be investigated as this silvicultural method would leave networks of mature trees scattered throughout the block that could act as reservoirs for mycorrhizal fungi.

Chapter 5

The use of vital stains to determine the physiological status of ectomycorrhizal fungi

Introduction

Among other harvesting practices, clear-cut logging removes the plant hosts that ectomycorrhizal fungi depend on for carbon (Trappe and Fogel 1977, Harley and Smith 1983). It is, therefore, not surprising that ectomycorrhizal roots decline in metabolic activity and eventually die one to two growing seasons following logging (Harvey *et al.* 1980, Parsons *et al.* 1994). The criteria used to identify active roots in the aforementioned studies as well as in Chapter 4 of this thesis, involved morphological characterization of the whole ectomycorrhizae (Harvey *et al.* 1976). As such, assessment of active and inactive ectomycorrhizae encompasses the vitality of both the root and the fungus. Since the overall objective of this study was to understand how host removal impacts on the inoculum potential of soils, we are primarily interested in the vitality of the fungus, not the root.

Numerous researchers have utilized vital stains to assess cellular activity and viability (Whittmann 1962, McGee and Smith 1990, Schaffer and Peterson 1993). Fluorescein diacetate (FDA) is one such vital stain that is commonly used in investigations of soil fungi (Söderström 1977, Ingham and Klein 1984, Ritter *et al.* 1989, Hamel *et al.* 1990, Miller *et al.* 1993, Torres and Honrubia 1994). Fluorescein diacetate is a non-fluorescent, non-polar compound which is taken up by cells by active transport (Rotman and Papermaster 1966). In the cytoplasm of active cells, FDA is hydrolyzed by esterases which converts FDA to the fluorescent, polar compound, fluorescein. Due to the differences in the polarity of the two compounds, fluorescein accumulates in the cell and the result can be observed under the microscope at 490 nm.

A positive FDA reaction indicates activity; FDA does not stain cells that are inactive yet still viable. Recently, an assay has been developed that simultaneously assesses active and dead cells (Molecular Probes, Live/Dead Cytotoxicity Kit). This assay uses calcein acetoxy methyl ester (calcein AM) in combination with ethidium homodimer (EthD-1) (Moore *et al.* 1990). Calcein AM, like FDA, is an indicator of metabolic activity. When cleaved by esterases, the fluorochrome emits a bright green fluorescence at 515-535 nm. The advantage of calcein AM over FDA is that it fluoresces with greater intensity, is not as prone to bleaching and there is less leaching from the cells as compared with FDA (Kaneshiro *et al.* 1993). Ethidium homodimer is a nucleic acid stain that is able to enter cells that have damaged cell membranes. The stain binds to nucleic acids and emits a red fluorescence at 617 nm. Thus the combination of calcein AM and ethidium homodimer allows active and dead cells to be characterized simultaneously.

Two other vital stains, aceto-iron haematoxilin and the nitro-blue tetrazolium (NBT) stain for succinate dehydrogenase, were considered for use at the outset of this study. Aceto-iron haematoxilin, a stain that binds to chromatin, has been used as an indicator of basidiospore viability (Torres and Honrubia 1994). However, it is unknown how long chromatin can persist following cell death (K. Egger pers. comm.) and, therefore, this method was rejected. The NBT assay was considered as an indicator of activity but was rejected in favor of FDA which has been shown to give more consistent results with mycorrhizal fungi (Hamel *et al.* 1990).

The objective of this study was to use vital stains to test the accuracy of the classification of ectomycorrhizae as 'active' or 'inactive' based on morphological criteria in Chapter 4. Fluorescein diacetate was used as a measure of fungal activity, and calcein AM combined with ethidium homodimer was used as a measure of fungal viability.

Materials and Methods

During the process of characterizing and describing mycorrhizal roots obtained from soil cores, samples of roots were set aside for approximately 3-4 hours for physiological assessment. Four out of the eight soil core replicates sampled at each plot location at Sicamous Creek were used for vital staining; two were used for FDA staining and two were used for calcein AM/EthD-1

Morphological criteria

The morphological criteria used to distinguish between active and inactive roots were described in Chapter 2. For each soil sample obtained from within the clear-cuts, up to 10 roots of each ectomycorrhizal type (Table 2) assessed as being active were placed in vials of sterile deionized water and stored in the refrigerator for up to 24 hours (usually for 3-4 hours) and subsequently tested with vital stains to detect false positive characterization of 'active' ectomycorrhizal fungi. Tests indicated that this storage period did not influence the efficacy of the stain. Roots assessed as being inactive were not tested with vital stains because it was impossible to determine if the fungi associated with the roots were mycorrhizal or some other saprophytic non-mycorrhizal fungi. Furthermore, the mantles of most inactive roots were decaying and could not be removed, intact from the root. The influence of location within the clear-cut on staining was not tested.

Physiological criteria: FDA

The roots were removed from the vials and the fungal mantle was peeled from the root surface and placed onto a microscope slide. This was done to facilitate penetration of the stain and to reduce interference from fluorescence of the root cells. Preliminary tests showed that when the stain was applied to a root squash, it was difficult to determine whether root or fungal tissue was fluorescing. A stock solution

of 5 mg FDA ml^{-1} acetone was kept at - 20 ° C. The staining solution was prepared by adding 1 ml of 0. 1 M phosphate buffer at pH 7.4 to 20 µg FDA stock solution (Bornman 1989). The samples were stained with a drop of the FDA solution and observed immediately using epifluorescence microscopy on an Olympus BHS microscope equipped with a 450-490 nm excitation filter. A green fluorescence of the fungal cytoplasm, distinguishable from yellowish autofluorescence, indicated a positive reaction. For each sample, five randomly selected fields were observed at 400X. The reaction of the fungus was recorded as a percentage of the mantle fluorescing; 0 = no reaction, 1 = 1 - 5%, 2 = 6 - 30 % , 3 = 31 - 60, 4 = > 60%. Photographs were taken using Kodak Royal Gold film (1000 ASA).

Physiological criteria: calcein AM and ethidium homodimer

Two separate stock solutions consisting of 4 mM solution of calcein AM dissolved in dimethylsulfoxide (DMSO) and 2 mM solution of ethidium homodimer - 1 (EthD-1) in 1: 4 DMSO / H_20 were stored at - 20 ° C. For staining, the reagents were first warmed to room temperature and briefly vortexed. The EthD-1 solution was prepared by adding 10 µl of the EthD-1 stock solution to 1.5 ml phosphate buffer, pH 7.4 subsequently 2.5 µl calcein AM was added to the EthD-1 solution.

A drop of stain was placed on the mantle peels. The slides were covered with a petri dish to limit evaporation and set to incubate at room temperature for 30 minutes. Samples were observed with epifluorescence microscopy on an Olympus BHS microscope equipped with a 450-490 nm excitation filter. A positive calcein AM reaction (activity) was indicated by bright green fluorescence of the cytoplasm. A positive EthD-1 reaction (non-viable cells) was indicated by deposits of bright red fluorescence. For each sample, five randomly chosen fields were observed at 400X. The reaction of the fungus was recorded as the presence of each stain fluorescing (all reactions occurred with less than 40% of the mantle fluorescing); 0 = no reaction, 1 =

dead (positive EthD-1, negative calcein AM), 2 = active (negative EthD-1, positive calcein AM), 3 = dead and active (positive EthD-1, positive calcein AM). Photographs were taken using Kodak Royal Gold film (1000 ASA). As a positive control, the reaction of FDA and EthD-1 / calcein AM was applied to live cultures of *Coprinus* sp. and *Armillaria ostoyae (*Romagnesi) Herink as well as to mantles of mycorrhizae associated with field bioassay seedlings. The stains were also applied to these fungi after the tissue had been boiled for 20 minutes to act as a negative control.

Results

Fluorescein diacetate

The FDA method gave a strongly positive reaction when applied to fungal cultures of *Coprinus* sp. and *Armillaria ostoyae* (Romagnesi) Herink and to mantles of *Cortinarius* - like mycorrhizae associated with the field bioassay seedlings. However, when the stain was applied to the mantles of excised mycorrhizae, fluorescence was rarely observed (Table 9). Positive FDA reactions were, almost exclusively, restricted to the emanating elements (individual hyphae, rhizomorphs or cystidia).

Calcein AM and ethidium homodimer

The cytotoxicity method indicated that, although the mantle tissue from the majority of the roots was not active with respect to esterase activity, the cells remained viable in that the plasma membrane remained intact (Table 10). As with the FDA staining procedure, positive staining of either component of the cytotoxicity stain was restricted to the emanating elements. Approximately 1% of mantles were characterized as having tissue that was both dead and active.

Response of individual fungi to FDA and cytotoxicity staining

Of the common ectomycorrhizal types tested with FDA, *Amphinema* - like, *Cortinarius* - like, *Paxillus* - like, and to a lesser extent *Piloderma* - like mycorrhizae exhibited positive staining more frequently than did *Lactarius* type I - like, *Hebeloma* - like, and *Lactarius* type II - like mycorrhizae (Table 11). This result may have been influenced by the fact that positive FDA fluorescence was most commonly observed with fungi that had emanating hyphae and rhizomorphs which are commonly formed by the former group.

Of the common mycorrhizae tested with calcein AM and ethidium homodimer, *Lactarius* type I - like, *Piloderma* - like, and *Amphinema* - like mycorrhizae did not exhibit positive calcein AM staining. Just under one-quarter of all mantles from *Hebeloma* - like mycorrhizae and approximately 30% of all *Paxillus* - like mycorrhizae tested positive for dead tissue (Table 12). *Cortinarius* also showed moderate levels of dead tissue; however, a similar proportion of the mantles tested were characterized as having both active and dead tissue.

Discussion

The use of EthD-1 and calcein AM confirmed that the majority of mycorrhizal fungi associated with roots characterized as active by morphological criteria were viable although metabolically inactive.

FDA and calcein AM

Over 87% of the fungal samples classed as active by morphological criteria did not exhibit positive staining when subjected to FDA. Less than 10% yielded weakly positive results. FDA has been shown to be correlated with O_2 utilization and CO_2 evolution (Ingham and Klein, 1984). As such it is said to be a good indicator of overall

Table 9. FDA staining of fungal mantles associated with excised roots of harvested trees two growing seasons after logging at Sicamous Creek.

% of mantle fluorescing	# of roots / 690	%
0	604	87.5
1-5	58	8.4
6-30	20	2.9
31-60	8	1.2
> 60	0	0

Table 10. Calcein AM and ethidium homodimer staining of fungal mantles associated with excised roots of harvested trees two growing seasons after logging at Sicamous Creek. Where a positive reaction was observed, the percent of the field of view fluorescing was always < 40%.

	# of roots / 679	%
no reaction	567	83.5
dead [1]	97	14.3
active [2]	5	0.7
active/dead [3]	10	1.5

[1] Positive EthD-1, negative calcein AM
[2] Negative EthD-1, positive calcein AM
[3] Positive EthD-1, positive calcein AM

Table 11. Responses (% of fungal mantles from roots tested) of different types of ectomycorrhizal fungi to FDA staining.

Ectomycorrhizal type	no reaction	1-5%	6-30%	31-60%	total # of root tips
Lactarius - like type I	98.6	1.4	-	-	149
Hebeloma - like	90.5	9.5	-	-	95
Piloderma - like	88.8	6.9	2.7	1.6	72
Lactarius deterrimus - like	93.75	6.25	-	-	48
Amphinema - like	61.53	15.4	23.1	-	39
Cortinarius - like	73.5	14.7	-	11.8	34
Paxillus - like	69.5	26.1	-	4.4	23

Table 12. Responses ((% of fungal mantles from roots tested) of different types of ectomycorrhizal fungi to cytotoxicity staining. Where a positive reaction was observed the percent of the field of view fluorescing was always < 40%.

Ectomycorrhizal type	no reaction	dead	active	dead/active	total # of root tips
Lactarius - like type I	88.8	11.2	-	-	125
Hebeloma - like	76.3	22.7	1.0	-	118
Piloderma - like	89.7	10.3	-	-	88
Lactarius deterrimus -like	90.0	7.15	-	2.85	70
Paxillus - like	59.3	30.0	4.0	6.7	54
Amphinema -like	83.0	17.0	-	-	41
Cortinarius - like	69.0	15.4	-	15.6	13

metabolic activity. The results presented here indicate that the fungi associated with roots of trees severed two years previously are not metabolically active with respect to esterase activity.

It has been suggested that mycorrhizal fungi are generally inactive or that they are assimilating carbon from stores present in the root and thus do not recognize the acetate groups on the FDA as a carbon source (E. Ingham pers. comm.). However, the observation that mantles of *Cortinarius* - like mycorrhizae associated with the field bioassay seedlings stained positively with FDA discounts both of these suggestions. It seems more likely that there is a reduction with time in the activity of mantle tissue associated with the excised roots. This finding broadly correlates with the results of Söderström (1977) who found that actively growing pure cultures of fungi stained with FDA yielded positive staining, whereas when the stain was applied to older cultures, no staining was observed. Furthermore, in one of the first studies using FDA as an indicator of activity, Rotman and Papermaster (1966) stated that one of the factors preventing the accumulation of fluorescein is aging.

In a study investigating aging of Sitka spruce mycorrhizae, Downes *et al.* (1992) also observed that positive FDA fluorescence was restricted to the emanating elements and almost never observed for the fungal mantle. The researchers observed FDA staining in longitudinal sections of *Tylospora fibrillosa* and *Paxillus involutus* mycorrhizae. Downes *et al.* (1992) suggest that the mantle hyphae may be less permeable to the stain. However, for the study presented here, the mantles were removed from the root to improve penetration of the stain and yet the results were similar.

The use of calcein AM as a measure of fungal activity was found to have properties making it preferable to FDA for testing on fungi. With FDA, there was considerable leaching of the fluorescein from the cells which resulted in high levels of background fluorescence. The loss of contrast between the sample and the background

made observation difficult. Conversely, calcein AM was not leached from the cell by any detectable amount within the observation time. Furthermore, calcein AM appeared to fluoresce more intensely. This supports observations of Kaneshiro *et al.* (1993) who found similar comparisons between FDA and calcein AM. For investigations of fungal activity, calcein AM was found to be a more effective stain.

Factors that may have influenced the accuracy of assessment include autofluorescence of the fungal tissue, as well as properties of the stain itself. Many of the fungi observed were strongly autofluorescent. In most cases the pale yellow to orange autofluorescence could be distinguished from the bright green FDA fluorescence, but mantles of *Hebeloma* - like, *Lactarius* type II - like, and *Cortinarius* - like mycorrhizae exhibited very strong autofluorescence and faint FDA reactions may not have been detected in some fungi. This would have resulted in an underestimation of activity. The observation that *Hebeloma* and *Cortinarius* fungi are highly autofluorescent corroborates the observation of Torres and Honrunbia (1994) who observed similar fluorescent properties of basidiospores of these fungi.

Ethidium homodimer

The results of the cytotoxicity assay indicate that although the majority of cells were inactive with respect to esterase activity (as measured by both FDA and calcein AM) the plasma membrane remained intact and, thus, the cells would be considered viable. It should be noted that cells devoid of cytoplasm may also yield negative reactions to both stains. Although 14% of the fungal samples assessed as being live by morphological criteria exhibited positive ethidium homodimer staining (i.e., damaged cell membranes), the degree of fluorescence was consistently below 40 % of the sample. Therefore, it seems reasonable to conclude that in this 14% of the samples there were portions of the tissue that were no longer viable, while other areas remained

viable. As such, we can conclude that morphological criteria did not substantially overestimate the proportion of live ectomycorrhizal roots.

The cytotoxicity assay has primarily been used on mammalian cells (Moore *et al.* 1990), although recently it has been used on protists, bacteria and fungi (Kaneshiro *et al.* 1993). In mammalian cells, ethidium homodimer binds exclusively to nucleic acids. In protists and fungi, however, additional binding sites have been identified. The cytoplasm of protists has been observed to stain positively with ethidium homodimer as have the cell walls of yeast (Kaneshiro *et al.* 1993). The positive staining of yeast cell walls was suggested to result from the binding of ethidium homodimer to polysaccharides such as chitin. As chitin is a component of fungal cell walls, a portion of the positive results perceived in this study may have been overestimated. In this study, a positive calcein AM reaction was occasionally observed where the adjacent cell walls were also stained positively with ethidium homodimer. A true positive reaction of ethidium homodimer is the staining of the nuclei, observed as red deposits within the hyphae. Unfortunately, due to inexperience with this technique, the distinction between the reactions was not made throughout the study. This error is not thought to account for the double positive reactions; rather the existence of active and dead tissue within a sample is likely due to the metabolic status of the fungi. The patchy distribution of active tissue has been observed in other mycorrhizal systems (Downes *et al.* 1992).

An additional difficulty encountered with both FDA and the cytotoxicity assay was that darkly pigmented fungi including *Cenococcum* did not respond to staining. This is thought to be due to either the inability of the stains to penetrate thick cell walls, or to the interference by pigments in the staining process. The darkly pigmented portion of live cultures of *Armillaria ostoyae* did not react with EthD-1. This finding does not agree with the results of Söderström (1977) who did observe positive staining with darkly pigmented hyphae.

Associated microbes

Although not quantified, rod-like, spherical and filamentous bacteria fluoresced abundantly in almost all samples. Bacteria were found imbedded in the mantles, along the emanating hyphae, as well as surrounding the preparation. The bacteria stained much more intensely than the fungi. Thus, both FDA as well as the calcein AM and ethidium homodimer may be useful as an indicator of bacterial activity, viability, and biomass estimation in soils. This finding supports the observation of Söderström (1977), who observed positive FDA staining of bacteria along with fungi in soil.

In itself, the abundance of bacteria associated with the ectomycorrhizal fungi is intriguing. Research has shown that the presence of certain types of bacteria termed 'mycorrhization helper bacteria' (MHB) can promote mycorrhizal development (as reviewed by Garbaye (1994). The mechanism by which this occurs is still unknown although currently, the favored hypothesis is that the bacteria produce substances that stimulate or enhance the growth of fungi in their free living stage prior to colonization (Garbaye 1994). In addition to influencing colonization, nitrogen-fixing bacteria have been detected within tubercules of Douglas-fir ectomycorrhizae and their presence is thought to contribute to nitrogen acquisition (Li *et al.* 1992). Furthermore, some strains of *Bacillus polymyxa* have been shown to increase the growth of lodgepole pine and hybrid spruce seedlings by mechanisms unrelated to mycorrhizal fungi (Shishido *et al.* 1996).

One of the interesting aspects of MHBs in association with ectomycorrhizal fungi, is the question of how closely are the bacteria associated with the fungi. The answer to this question is currently unknown. Given the complexity and diversity of organisms occurring in the rhizosphere, and more specifically in the mycorrhizosphere, equally complex interactions can be expected to be occurring. Alternatively, the bacteria observed associated with the fungi in this study may have been obtaining nutrients from the fungus and thus not 'helper' in nature at all.

Chapter 6

Synthesis

The broad objectives of this thesis were to assess the influence of different clear-cut sizes on the survival of ectomycorrhizal fungi one and two growing seasons after logging. Specifically the goal was to quantify the diversity and persistence of ectomycorrhizal fungi between and throughout the clear-cuts. Four approaches were taken to address these objectives: a greenhouse bioassay was carried out to determine which fungi were active and capable of colonizing greenhouse grown seedlings one growing season after logging; the persistence and diversity of mycorrhizae two growing seasons after logging was investigated using roots from soil cores; the activity of ectomycorrhizal fungi two growing seasons after logging was investigated by a field bioassay and the accuracy of morphological criteria used to determine the activity of severed tree roots was tested by vital stains.

Persistence

One growing season after winter logging (approximately 7-8 months) there were no differences in persistence of the various types of mycorrhizae at the various plot locations throughout the clear-cuts and the controls. This is not surprising as most studies have found that excised roots begin to die after approximately 9 months. It is thought that the mycorrhizal fungi are able to persist by continuing to assimilate carbon from reserves present in the root tissue (Ferrier and Alexander 1985).

The greenhouse bioassay confirmed that many of the major types of mycorrhizae present in the soil cores were, indeed, capable of colonizing greenhouse-grown seedlings. These types included *Amphinema* - like, *Cenococcum* - like, and *Hebeloma* - like mycorrhizae. It is thought that the mycelium extending from active mycorrhizae are the primary source of inoculum for initiating new colonization events

(Read 1984). However, there is always the possibility that these mycorrhizae originated from alternate inoculum sources such as sclerotia. The unique presence of *Thelephora* and E-strain mycorrhizae associated with the seedlings demonstrates that spores can be an important for colonization.

Not surprisingly, two growing seasons after winter logging (approximately 19 months), there was a decline in the numbers of active fine roots in the clear-cuts as compared with the forest. The technique of 'morphotyping' enabled us to identify the mycorrhizal types persisting and those in decline. Specifically, *Cenococcum* - like, *Hebeloma* - like, and *Cortinarius* - like mycorrhizae were less persistent than *Amphinema* - like, *Lactarius* - like I and II, and *Piloderma* - like mycorrhizae. The mechanism by which the latter group was able to persist may be the result of saprophytic capability. For *Lactarius* spp. and *Piloderma* spp., there is laboratory evidence which supports this hypothesis. I was unable to find any reference to *Amphinema* studied for saprophytic potential.

As demonstrated by the field bioassay, the persistence of a mycorrhiza is not necessarily correlated with its colonization potential. For instance, *Cenococcum* - like mycorrhizae, the second most abundant type found on the field bioassay seedlings was significantly more abundant at clear-cut plots than at forested or root-zone plots (Table 9). However, the numbers of persisting *Cenococcum* - like mycorrhizae were lower at plots located ≥ 16 metres into the clear-cut relative to forest and root-zone locations. Similarly, *Hebeloma* - like mycorrhizae (the most abundant mycorrhizae associated with field bioassay seedlings) were equally distributed throughout clear-cut and undisturbed plots and yet the numbers of *Hebeloma* - like mycorrhizae were also significantly reduced beyond the root-zone. Therefore, either the reduction detected by the method of soil cores does not translate into a real biological deficit for seedlings or the source of inoculum of *Cenococcum* and *Hebeloma* was not the mycelium extending from mycorrhizal roots. Conversely, *Lactarius* - like I and II mycorrhizae occupied

27.6% of the community assessed as persisting two growing seasons after logging and yet they were entirely absent on the roots of the seedlings.

The ectomycorrhizal fungi which were active in the clear-cuts and capable of colonizing young spruce seedlings two growing seasons after logging, were *Hebeloma* - like, *Cenococcum* - like, E-strain and *Amphinema* - like. The type of inocula that these mycorrhizae originated from remains uncertain.

The use of vital stains revealed that the mantle tissue of ectomycorrhizal fungi associated with excised roots was not very active two growing seasons after host removal. However, the dual stain of calcein AM and EthD-1 revealed that although the majority of samples were inactive, they were viable. Thus, we can conclude that morphological criteria used to characterize active roots is relatively accurate.

Epidemiology

The persistence of ectomycorrhizal fine roots is not necessarily correlated with colonization potential due, in part, to the variation in the epidemiology of individual fungi. As previously discussed, ectomycorrhizal fungi can be grouped as early or late-stage based on their reproductive and dispersal strategies and, notably, the ability to colonize from various types of inocula. Epidemiological differences are thought to have contributed to patterns of colonization observed in both the greenhouse and field bioassays.

Lactarius and *Cortinarius* are among the genera of fungi commonly referred to as late-stage. On the other hand, E-strain, *Hebeloma*, *Amphinema*, *Cenococcum* and *Thelephora* are described as early or multi-stage fungi. The results presented here support these designations. *Lactarius* - like, *Cortinarius* - like and *Piloderma* -like mycorrhizae were abundant on the excised roots one growing season after logging, and yet were absent in association with greenhouse-grown seedlings.

For the field bioassay, *Cortinarius* - like mycorrhizae were almost exclusively found within the root-zone or in the forest. This observation supports the hypothesis that late-stage fungi require an intact connection with a mature tree to support colonization. Alternatively, the reduction in the numbers of excised *Cortinarius* - like mycorrhizae beyond the root-zone in the clear-cuts may have contributed to the pattern of colonization observed for the seedlings. However, the observation that this type of mycorrhiza was equally persistent one growing season after logging and yet absent in association with the greenhouse-grown seedlings supports the previous explanation. Interestingly, *Lactarius* - like mycorrhizae I and II encountered in soil cores two growing seasons after logging were equally abundant at all plot locations, and yet were entirely absent in association with the field bioassay seedlings. It is possible that these fungi preferentially colonize older root systems.

Past studies have shown that the types of ectomycorrhizae forming with greenhouse bioassay seedlings broadly correlate with the main ectomycorrhizae forming with outplanted seedlings during the first two years after outplanting (Jones *et al.* in press). The results presented in this thesis corroborate this finding. For this study, the dominant types of ectomycorrhizae associated with field bioassay seedlings (see Chapter 3) were also found associated with the roots of the greenhouse bioassay seedlings. Interestingly, the only common ectomycorrhizal type that was associated with the field bioassay seedlings and absent from the greenhouse bioassay seedlings was *Cortinarius*-like, a fungus known to have late-stage characteristics such as the inability to colonize from spores (Fox 1986a), and the apparent requirement of an intact mycelium to initiate formation.

Diversity

Clear-cut blocks exhibited significant decreases in the richness and diversity of excised mycorrhizae two growing seasons after logging. Specifically, richness and

diversity were significantly reduced with increasing distance from the forest. The diversity of the mycorrhizae associated with the field bioassay seedlings also declined with increasing distance from the edge. Whether or not this reduction was due to epidemiological differences or from a reduction in the inoculum potential of soils due to the death of certain types of mycorrhizae is not known for certain.

For the mycorrhizae forming with field bioassay seedlings, diversity and richness were greatest at the root-zone. Perhaps the root-zone is a more heterogeneous micro-habitat due to its proximity to both the forest as well as the clear-cuts. For example, there may be temperature and moisture conditions that allow early-stage fungi such as MRA and E-strain to remain competitive with late-stage fungi. These observations may support the niche diversification hypothesis as maintaining local species diversity.

General patterns of diversity

In general, for the studies presented in this thesis, the community of mycorrhizae is characterized by the presence of a few dominant types and numerous rare types. This distribution coincides with patterns of ectomycorrhizal distribution observed in both tropical (Su See and Alexander 1996) and temperate ecosystems (Dahlberg 1990, Newton 1991). Differences in the distribution of mycorrhizae were observed, however, when rank / abundance graphs were examined. Rank / abundance plots can be used to describe the characteristics of a community based on the distribution and abundance of species (May 1975).

The community of excised mycorrhizae persisting one-growing season after logging conform visually to the model of a log-normal distribution (Fig. 11); (Whittaker 1975, Magurran 1988). This is a common pattern observed for many communities (Sugihara 1980). The concept of resource partitioning due to niche

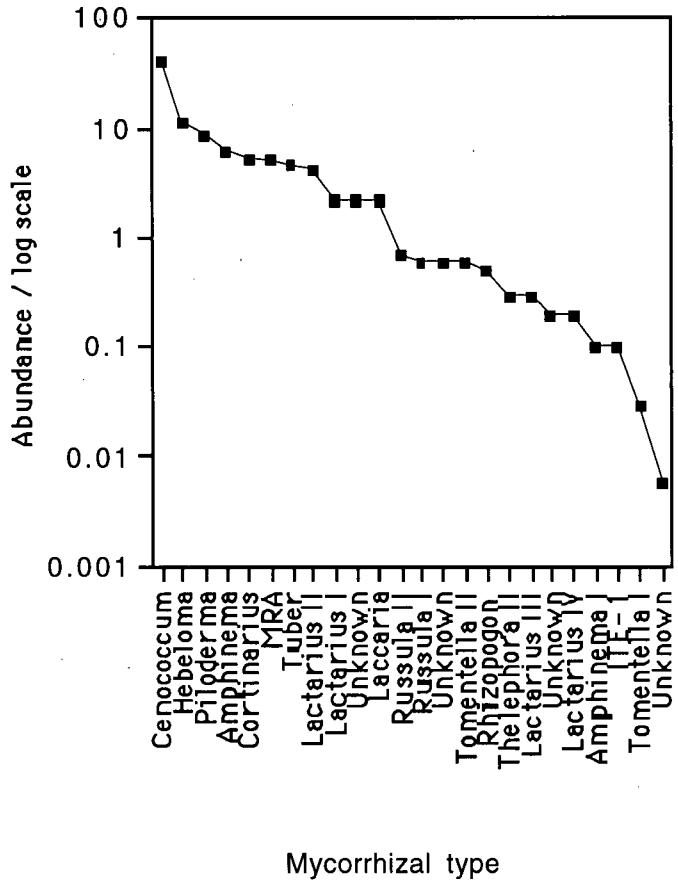

Figure 11. Rank abundance diagram showing the diversity of mycorrhizae in all samples from clear-cut, root-zone and forest plots one growing season after winter logging. The abundance of mycorrhizal types follows a log normal distribution (n=300 soil cores).

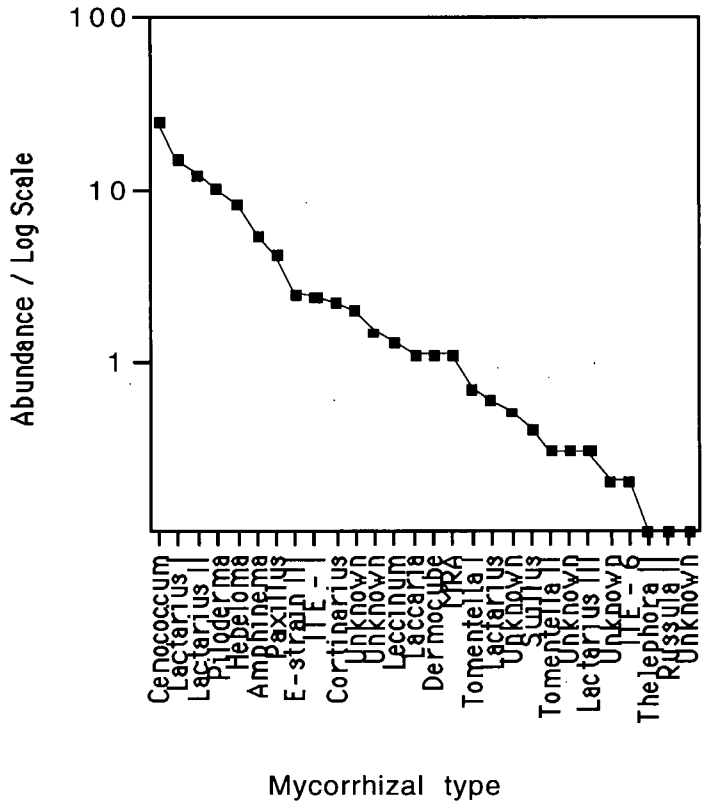

Figure 12. Rank abundance diagram showing the diversity of mycorrhizae in all samples from clear-cut, root-zone and forest plots two growing seasons after winter logging. The abundance of mycorrhizal types follows a geometric distribution (n=384 soil cores).

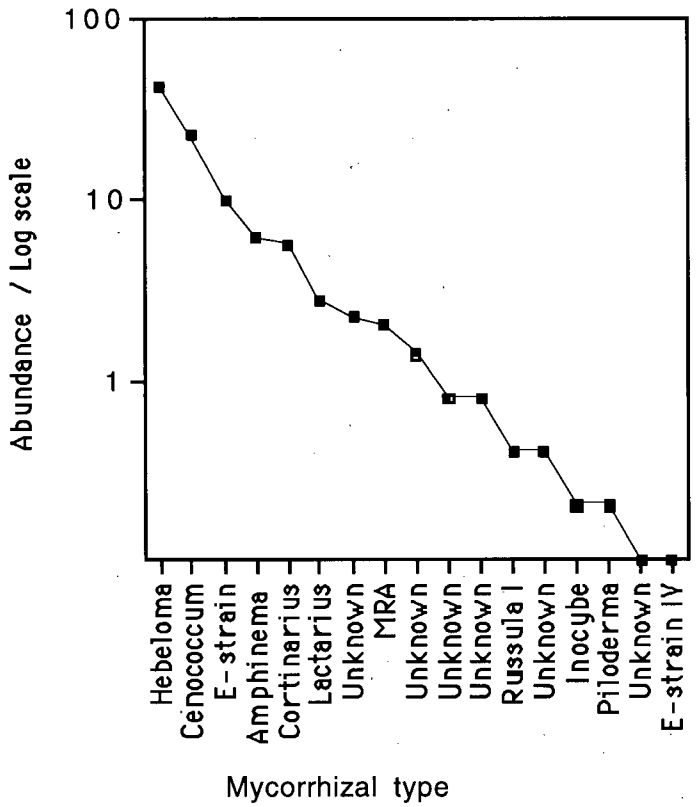

Figure 13. Rank abundance diagram showing the diversity of mycorrhizae forming with outplanted spruce seedlings two growing seasons after winter logging in all samples from clear-cut, root-zone and forest sites. The abundance of mycorrhizal types follows a geometric distribution (n=336 seedlings).

diversification is often invoked to explain this type of distribution (Sugihara 1980, Huston 1994). As previously discussed, this hypothesis states that the organisms in the community are utilizing resources in different ways. Although we are uncertain about the specific functional differences which exist between fungi in the field, this observation supports the suggestion that the fungi perform different roles and occupy various niches within the ecosystem.

In contrast, the rank / abundance diagram for the communities assessed by coring and the field bioassay two growing seasons after logging conforms to the geometric series. The distribution is much less equitable than the community observed one growing season after logging, and the existence of few dominant types is more pronounced (Fig. 12 and 13); (Whittaker 1975, Magurran 1988). The simplest explanation for the differences in patterns observed between years is due to differential rates of persistence: some types are better able to persist in the absence of a host (dominants), whereas others are lose dominance as they die. The greenhouse bioassay is not discussed in this context as the study took place under controlled conditions.

Improvements, future research and management implications

There are numerous ways in which this research could have been improved. Firstly, the use of molecular techniques would have reduced the margin of error involved in describing the 'morphotypes'. It is likely that the ectomycorrhizal types identified throughout this study are an underestimate of the diversity encountered. That is to say, in a given morphotype group, there is the possibility that more than one species were included. Currently in the lab at OUC, preliminary comparisons of morphotype groupings with PCR and RFLP analysis have found that the morphological groupings are predominantly formed by fungi with one RFLP pattern and in some cases, more than one pattern (S. Sakakibara pers. comm.). Although not

as precise as molecular identification, identification of the various groups of fungi could have been accomplished by growing the fungi in culture and analyzing their physiological attributes and morphological characteristics (Hutchinson 1991). However, this is a very time consuming technique and not all ectomycorrhizal fungi are easily grown in culture.

As the stand at Sicamous Creek is comprised predominantly of subalpine fir, an additional improvement to this study would have been to use subalpine fir as well as spruce in the greenhouse bioassay and the field bioassay experiments. This was considered in the spring of 1996; however, seeds of subalpine fir require extended scarification (three months) and there was not enough time to fulfill its germination requirements prior to the scheduled field bioassay. Moreover, subalpine fir is not a preferred species for use in silviculture; foresters are primarily interested in patterns which relate to the growth of spruce at these high elevation forests.

It is speculated in this thesis that a connection between seedling roots and the mycelium extending from the ectomycorrhizae of mature trees facilitated certain mycorrhizal associations with seedlings. Identifying this connection by careful excavation and tracing of the mycelium would have confirmed the existence of mycelial connections in forested plots. Finally, as ectomycorrhizal formation is known to be influenced by a variety of factors including soil pH, moisture and temperature, some basic soil measurements would have helped to gain a better understanding regarding the distribution of the various fungi.

Future directions that this work could take include investigations into the functional diversity of the ectomycorrhizal fungi persisting in the clear-cuts. It would be interesting to ascertain the mechanisms by which the certain fungi are capable of persisting while others seem to decline. The ectomycorrhizae associated with naturally regenerating conifers have been investigated in the northern interior of British Columbia (M. Kranabetter pers. comm.). This would be another useful means to investigate the

naturally occurring inoculum in the soil without being limited to a certain seedlot, or species, or concern regarding prior contamination.

There are a number of additional studies that have been set up for continued research on ectomycorrhizal fungi at Sicamous Creek. As the vitality of inactive roots could not be determined by the vital stains, a bioassay was set up where seedlings were germinated in soils that contained roots that were assessed as being inactive by morphological criteria as well as live roots of the dominant ectomycorrhizal types. It will be interesting to determine the ability of various fungi to colonize seedlings from the excised roots as well as to see if any ectomycorrhizae are formed by fungi associated with inactive roots. In addition, in July of 1996, commercially grown spruce seedlings were outplanted throughout the entire harvested area of the trial as well as at the 48 plot locations investigated in this thesis. One year after planting, in 1997, the roots of the outplanted seedlings will be observed and the mycorrhizae associated with them characterized. The destructive sampling of outplanted seedlings is planned to continue at years 2, 3, 5, 10 and 20 after planting. Finally, another field bioassay and a survey of soil cores will be carried out in 1997, three growing seasons after logging. This will provide important information regarding the persistence of ectomycorrhizal fungi beyond the upper limit reported for most types of inocula including ectomycorrhizal roots.

The results presented in this thesis suggest that for clear-cuts ranging in size from 0.1 to 10 hectares, proximity to overstory trees is more important to patterns of colonization and diversity than clear-cut size. Therefore, further investigation appears warranted into silvicultural systems which would maximize the perimeter to area ratio of clear-cuts , or systems such as partial cutting which would leave networks of standing live trees throughout the harvested forests.

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Appendix 1. Formulae for the diversity indices.

Index	**Formula**
Richness	S = the number of ectomycorrhizal types
Shannon diversity index	$H' = - \sum p_i \ln p_i$ where p_i is the proportion of live ectomycorrhizae found in the *i*th type (see Magurran 1988 p. 35)
Shannon evenness index	$E = H'/ \ln S$ (Pielou 1969)

Appendix 2. Complete descriptions of the ectomycorrhizal morphotypes encountered throughout the various studies presented in this thesis. Classification follows the taxonomy outlined in Dictionary of the Fungi (Hawksworth *et al.* 1983).

Reference:

Hawksworth, D.L., Sutton, B.C., Ainsworth, G.C. 1983. Dictionary of the fungi. 7th ed. Commonwealth Mycological Inst., Kew. 445 pp.

Amphinema byssoides (Pers.: Fr.) Erikss. - like I

Class: Basidiomycotina
Order: Aphyllophorales
Family: Corticiaceae
Encountered on: excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm., and *Picea engelmanni* x *glauca* seedlings; greenhouse bioassay
DISTINGUISHING FEATURES: tawny brown to yellowish tips; mycelial strands loose undifferentiated, strands and emanating hyphae, clamped with 'keyhole' spaces and strongly yellow in KOH
MORPHOLOGY (Dissection Microscope)
Ectomycorrhizal system:
Shape and dimensions: unbranched systems; tips are straight, 3-5mm by 400µm
Colour and texture: tawny brown, white to yellow, finely grainy, woolly
Emanating elements:
Mycelial strands: common, smooth or hairy
Hyphae: common cottony white and thin
ANATOMY (Compound Microscope)
Mantle in plan view: mantle is of medium thickness, type B (Agerer 1991) no specialized cells
Outer layer: a felt prosenchyma of hyaline cells 3-4 µm by 100 µm, no matrix,
some finely verrucose hyphae, clear contents, bulbous, keyhole clamps, hyphal junctions common, H-shaped not clamped anastomoses abundant
Inner layer: a net synenchyma of hyaline cells 2-4 µm, no ornamentation, clear contents, no clamps
Mycelial strands in plan view: loose undifferentiated, 50-60 µm by 4-8 µm, smooth to finely verrucose, clear contents, commonly septate, 'keyhole' clamps, H-shaped anastomoses
Emanating hyphae: common, hyaline cells 3-4 µm by 100 µm, some smooth hyphae other are finely verrucose, clear contents, bulbous, keyhole clamps, hyphal junctions common, H-shaped not clamped anastomoses abundant
Cystidia: none observed
OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
References:
Agerer, R. 1991. Characterization of ectomycorrhizae. *In* Techniques for the study of mycorrhiza. Edited by Norris J.R., Read D.J and A.K. Varma. Academic Press. London. pp 25-73.
Harniman, S.M.K. and D.M. Durall. 1996. *Amphinema byssoides* - like, CDE6. *In* A manual of concise descriptions of North American ectomycorrhizae. Ed's. Goodman, D.M., Durall, D.M., Trofymow, J.A. and S.M.Berch. Mycologue Publications, Sidney, B.C.
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Weiss, M. 1988. *Amphinema byssoides* In Agerer, R. (ed.) Colour Atlas of Ectomycorrhizae, plate 23. Einhorn-Verlag, Schwäbisch Gmünd.
Thesis reference: SC#150 / GB#10 (OUC#20)

Cenococcum **Fr. - like**
Class: Deuteromycotina
Order:
Family:
Encountered on: excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm., *Picea engelmanni* x *glauca* seedlings; field bioassay, greenhouse bioassay
DISTINGUISHING FEATURES: dark brown to black thick, hard mantle forming a distinct stain glass pattern observable at 200X, emanating hyphae are straight, brittle, very linear, dark brown with a slightly purple tinge
MORPHOLOGY (Dissection Microscope)
Ectomycorrhizal system:
Shape and dimensions: unbranched system; tips are straight, 3-5 mm by 500 µm
Colour and texture: dark brown, black, coarsely grainy, reflective and shiny
Emanating elements:
Mycelial strands: none observed
Hyphae: common, straight, black and wiry
ANATOMY (Compound Microscope)
Mantle in plan view: thick mantle, type G (Agerer, 1991), fungus completely obscures host, no specialized cells
Outer layer: a net synenchyma comprised of dark brown cells 4-5 µm by 20 µm, no matrix, most hyphae are not ornamented, occasionally finely verrucose, commonly septate, no clamps, no hyphal junctions, no anastomoses
Inner layer: not observed
Mycelial strands in plan view: none observed
Emanating hyphae: common, cells 4-5 µm by 20-30 µm, very linear, sometimes finely verrucose, thick walled, brittle
Cystidia: none observed
OTHER FEATURES:
Sclerotia and Microsclerotia: dark brown, hard, spherical 2 (1.5-2.5 mm) diameter
Chlamydospores: none observed
Reference:
Agerer, R. 1991. Characterization of ectomycorrhizae. *In* Techniques for the study of mycorrhiza. Edited by Norris J.R., Read D.J and A.K. Varma. Academic Press. London. pp 25-73.
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Thesis reference: GB#40 / FB#40 /SC#50 (OUC#30)

Cortinarius-like I

Class: Basidiomycotina
Order: Agaricales
Family: Cortinareaceae
Encountered on: excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm.

DISTINGUISHING FEATURES: bright white tips, mycelial strands are smooth-undifferentiated with granular or oil-like bodies within the hyphae, commonly clamped

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:
Shape and dimensions: unbranched systems; tips are straight, 2 - 3 mm by 600 µm
Colour and texture: bright white, reflective due to trapped air within the mantle and the mycelial strands, stringy

Emanating elements:
Mycelial strands: common, white, resembles dental floss
Hyphae: white, cottony, curved

ANATOMY (Compound Microscope)

Mantle in plan view: mantle of medium thickness, no specialized cells
Outer layer: a felt prosenchyma of hyaline cells 4-5 µm, granular matrix, no ornamentation, clear to granular hyphal contents, commonly septate, clamps common
Inner layer: a patchy net synenchyma of hyaline cells 3-4 µm wide, no ornamentation, clear contents

Mycelial strands in plan view: smooth-undifferentiated, hyphal cells 6-15 µm by 30 µm, contents are clear, granular and oil-like bodies, commonly septate
Emanating hyphae: common, hyaline cells 4-6 µm wide, no ornamentation, granular contents, commonly septate
Cystidia: none observed

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

Reference:
Danielson, R.M. 1991. Known and putative genera of ectomycorrhizal fungi with ecological information, characteristics of the mycorrhizae, and selected references to descriptive material. Kananaskis Centre for Environmental Research. University of Calgary 16 pp.

Thesis reference: SC#120 (OUC#40)

Cortinarius - like II

Class: Basidiomycotina
Order: Agaricales
Family: Cortinareaceae
Encountered on: *Picea glauca x engelmanni* seedlings, field bioassay

DISTINGUISHING FEATURES: white - mauve tips and sclerotia bruising purple when touched, smooth undifferentiated mycelial strands, matrix in the inner mantle

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:

Shape and dimensions: unbranched systems; tips are straight, 2-3 mm by 500 µm
Colour and texture: whitish - mauve, smooth, reflective, bruising purple when touched, mycelial strands stringy

Emanating elements:

Mycelial strands: common, white, stringy
Hyphae: white cottony

ANATOMY (Compound Microscope)

Mantle in plan view: medium-thick mantle, no Hartig net observed, no specialized cells

Outer layer: a net prosenchyma - synenchyma of hyaline cells, 5-10 µm wide, no ornamentation, commonly septate, no clamps, no matrix materials
Inner layer: a distinct not-interlocking irregular synenchyma, resembling sausage links with large spaces in between(matrix), cells hyaline, 10-12 µm wide and 30 -50 µm long, no ornamentation, clear contents, no clamps

Mycelial strands in plan view: smooth undifferentiated

Emanating hyphae: none observed

Cystidia: none observed

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

Reference:

Godbout, C. and Fortin, J.A. 1985. Synthesized ectomycorrhizae of aspen: fungal genus level of structural characterization. Canadian Journal of Botany **63**: 252-262.
Thesis reference: FB #325

Dermocybe-like

Class: Basidiomycotina
Order: Agaricales
Family: Cortinariaceae
Encountered on: excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm.

DISTINGUISHING FEATURES: white tips with abundant bright white undifferentiated mycelial strands, ornamentation of emanating hyphae is distinct; large, globular and crystalline, clamps present

MORPHOLOGY (Dissection Microscope)
Ectomycorrhizal system:
Shape and dimensions: unbranched or monopodial pinnate; tips are straight or bent, 1-3 mm by 300 µm
Colour and texture: white, woolly, reflective
Emanating elements:
Mycelial strands: common, white and smooth
Hyphae: white, curved

ANATOMY (Compound Microscope)
Mantle in plan view: mantle of medium thickness, no specialized cells observed
Outer layer: a felt prosenchyma or net prosenchyma of pale yellow cells 4-5 µm wide, no matrix, no ornamentation, rarely clamped, long sweeping parallel sections
Inner layer: a net rounded net synenchyma, no matrix, cells pale yellow 5 µm wide, no ornamentation, clear contents, no clamps

Mycelial strands in plan view: loose or smooth-undifferentiated, pale yellow cells 4-5 µm wide, variable ornamentation; none, large crystalline or globular deposits (3 µm diameter
Emanating hyphae: pale yellow cells 4-5 µm wide, variable ornamentation; none, large crystalline or globular deposits (3 µm diameter), commonly septate, clamps rare or common
Cystidia: none observed

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

Reference:
Agerer, R. and M. Uhl 1989. *Dermocybe semisanguinea.* In: Agerer, R. (ed.) Colour Atlas of Ectomycorrhizae, plate 25. Einhorn-Verlag, Schwäbisch Gmünd.
Thesis reference: SC#330 (OUC#50)

E-strain I

Class: Ascomycotina
Order:
Family:
Encountered on: *Picea engelmanni* x *glauca* seedlings; field bioassay, greenhouse bioassay

DISTINGUISHING FEATURES: milky white to reddish brown tips with white apices, extensive Hartig net, no emanating elements

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:
Shape and dimensions: unbranched systems; tips are straight, bent or tortuous, 3 mm by 400 µm
Colour and texture: milky white to reddish brown tips with white apices, smooth, reflective
Emanating elements:
Mycelial strands: none observed
Hyphae: none observed

ANATOMY (Compound Microscope)
Mantle in plan view: a thin discontinuous mantle, Hartig net extensive, no specialized cells
Outer layer: a net synenchyma of hyaline cells 3-5 µm by 10-12 µm, no matrix, no ornamentation, constricted at the septa
Inner layer: none observed

Mycelial strands in plan view: none observed
Emanating hyphae: none observed
Cystidia: none observed

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

Reference:
R.M. Danielson. 1982. Taxonomic affinities and criteria for identification of the common ectendomycorrhizal symbionts of pines. Canadian Journal of Botany **60:** 7-18.
Thesis reference: GB#63 / FB#63

E-strain II

Class: Ascomycotina
Order:
Family:
Encountered on: *Picea engelmanni* x *glauca* seedlings; field bioassay

DISTINGUISHING FEATURES: smooth, glossy reddish brown tip, incomplete and thin regular synenchyma type K (Agerer 1991) mantle comprised of red hyphae constricted at the septa, no clamps.

MORPHOLOGY (Dissection Microscope)
Ectomycorrhizal system:
Shape and dimensions: not branched, tips straight, 2-5 mm by 300 µm
Colour and texture: brown to reddish brown, shiny and smooth
Emanating elements:
Mycelial strands: none observed
Hyphae: none observed

ANATOMY (Compound Microscope)
Mantle in plan view: mantle is discontinuous, in places resembling type K (Agerer, 1991)
Outer layer: a net prosenchyma, no matrix; cells 8 (6-9) µm by 13 (12-15) µm, yellowish red, no ornamentation, clear contents, commonly septate, restricted septa, no clamps
Inner layer: none observed

Mycelial strands in plan view: none observed
Emanating hyphae: none observed
Cystidia: none observed

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

Reference:
Agerer, R. 1991. Characterization of ectomycorrhizae. *In* Techniques for the study of mycorrhiza. Edited by Norris J.R., Read D.J and A.K. Varma. Academic Press. London. pp 25-73.

Thesis reference: GB#60

E-strain III

Class: Ascomycotina
Order:
Family:
Encountered on: excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm.

DISTINGUISHING FEATURES: brown to dark brown tips with light brown orange apices, incomplete mantle, Hartig net visible, hyaline, verrucose emanating hyphae rare

MORPHOLOGY (Dissection Microscope)
Ectomycorrhizal system:
Shape and dimensions: unbranched systems comprised of straight to tortuous tips; tips 2 mm by 500 µm
Colour and texture: brown to dark brown with orange brown apices, smooth and shiny
Emanating elements:
Mycelial strands: none observed
Hyphae: none observed

ANATOMY (Compound Microscope)
Mantle in plan view: mantle is discontinuous and thin, no specialized cells, Hartig net visible and extensive
Outer layer: an irregular synenchyma comprised of orange cells 9 µm by 12 µm, no ornamentation, no clamps
Inner layer: none observed

Mycelial strands in plan view: none observed
Emanating hyphae: rare, hyaline, 6 µm wide, verrucose ornamentation
Cystidia: none observed

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

Reference:
R.M. Danielson. 1982. Taxonomic affinities and criteria for identification of the common ectendomycorrhizal symbionts of pines. Canadian Journal of Botany **60:** 7-18.
Thesis reference: SC#260 (OUC#60)

E-strain IV

Class: Ascomycotina
Order:
Family:
Encountered on: *Picea engelmanni* x *glauca* seedlings; field bioassay, greenhouse bioassay

DISTINGUISHING FEATURES: reddish brown, smooth, glossy tips, patchy mantle, extensive Hartig net, brown, emanating hyphae 8-10 µm wide and heavily verrucose

MORPHOLOGY (Dissection Microscope)
Ectomycorrhizal system:
Shape and dimensions: unbranched systems; straight tips, 3 mm by 400 µm
Colour and texture: reddish brown with white apices, smooth, glossy
Emanating elements:
Mycelial strands: none observed
Hyphae: rare to common, curved

ANATOMY (Compound Microscope)

Mantle in plan view: a thin discontinuous mantle, Hartig net extensive, no specialized cells
Outer layer: a discontinuous net prosenchyma or net synenchyma of hyaline cells 10-25 µm by 20-40 µm, no ornamentation, clear contents, commonly septate
Inner layer: not observed

Mycelial strands in plan view: not observed
Emanating hyphae: rare to common, brown cells are 8-10 µm by 60-300 µm, medium to large verrucose or globular ornamentation, clear contents, no clamps, commonly septate
Cystidia: none observed

OTHER FEATURE:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

Reference:
Ingleby, K., Mason, P.A., Last, F.T and L.V. Fleming. 1990. *Identification of ectomycorrhizas*. Institute of Terrestrial Ecology (ITE) research publication no. 5. HMSO London. 112 pp.
Thesis reference: GB#62 / FB#500

***Hebeloma* - like**

Class: Basidiomycotina
Order: Agaricales
Family: Cortinariaceae
Encountered on: excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm. and *Picea engelmanni glauca* seedlings; field bioassay, greenhouse bioassay

DISTINGUISHING FEATURES: tawny brown reddish tips, abundant clamped, verrucose emanating hyphae

MORPHOLOGY (Dissection Microscope)
Ectomycorrhizal system:
Shape and dimensions: unbranched systems; tips are straight, 2-4 mm by 600 µm
Colour and texture: tawny brown reddish tips with white apices
Emanating elements:
Mycelial strands: none observed
Hyphae: common, straight, white

ANATOMY (Compound Microscope)
Mantle in plan view: mantle of medium thickness, no specialized cells
Outer layer: a felt prosenchyma of hyaline cells 3-4 µm, verrucose, clamped commonly septate, abundant H-shaped anastomoses
Inner layer: a net synenchyma of hyaline cells 3 µm, no ornamentation, clear contents

Mycelial strands in plan view: none observed
Emanating hyphae: common, hyaline cells 3-4 µm wide, both smooth and verrucose hyphae, abundant clamps and H-shaped anastomoses
Cystidia: none observed

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

Reference:
Ingleby, K., Mason, P.A., Last, F.T and L.V. Fleming. 1990. *Identification of ectomycorrhizas*. Institute of Terrestrial Ecology (ITE) research publication no. 5. HMSO London. 112 pp.
Thesis reference: GB#200 / FB#200 /SC#70 (OUC#80)

Inocybe-like

Class: Basidiomycotina
Order: Agaricales
Family: Cortinariaceae
Encountered on: *Picea engelmanni x glauca*, field bioassay seedlings

DISTINGUISHING FEATURES: white, smooth tip; surface mantle is a net prosenchyma comprised of grainy cells 4-5 µm wide, rare emanating hyphae 4-5 µm wide

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:
Shape and dimensions: monopodial pinnate, straight tips, 2-3 mm by 300 µm
Colour and texture: creamy white tip, smooth and matte
Emanating elements:
Mycelial strands: none observed
Hyphae: rare, cottony, white

ANATOMY (Compound Microscope)
Mantle in plan view: mantle is of medium thickness, no specialized cells

Outer layer: net prosenchyma; cells 4-5 µm, hyaline, granular contents, commonly septate, rarely clamped; septa stain very darkly in Toluidine blue
Inner layer: not observed

Mycelial strands in plan view: none observed
Emanating hyphae: rare; appear as extensions of the mantle, tortuous, hyaline, 4-5 µm, clamps are rare, keyhole
Cystidia: none observed

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

Reference:
Ingleby, K., Mason, P.A., Last, F.T and L.V. Fleming. 1990. *Identification of ectomycorrhizas*. Institute of Terrestrial Ecology (ITE) research publication no. 5. HMSO London. 112 pp.
Thesis reference: FB #440 (OUC#95)

ITE-1-like

Class: Ascomycotina
Order:
Family:
Encountered on: excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm. and *Picea engelmanni* x *glauca* seedlings; greenhouse bioassay

DISTINGUISHING FEATURES: milky grey sometimes silvery tips, surface mantle is a net prosenchyma of cells 5 µm wide surrounded by a granular matrix, no emanating elements

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:
Shape and dimensions: irregularly branched, bent tips, 2-8 µm long, 500 µm wide
Colour and texture: milky grey, sometimes silvery, finely grainy, matte
Emanating elements:
Mycelial strands: none observed
Hyphae: none observed

ANATOMY (Compound Microscope)

Mantle in plan view: a thin net prosenchyma, no specialized cells
Outer layer: net prosenchyma of cells 5 µm wide, grainy matrix between cells, no ornamentation, hyaline, hyphal contents granular, no clamps, stains in Toluidine blue, some parallel sections
Inner layer: similar to the outer layer net prosenchyma, more of a random distribution, no ornamentation, granular hyphal contents, no clamps

Mycelial strands in plan view: none observed
Emanating hyphae: none observed
Cystidia: none observed

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

References:
Ingleby, K., Mason, P.A., Last, F.T and L.V. Fleming. 1990. *Identification of ectomycorrhizas*. Institute of Terrestrial Ecology (ITE) research publication no. 5. HMSO London. 112 pp.
Thesis reference: SC#170 / GB #150 (OUC#100)

ITE-6-like

Encountered on: excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm.

DISTINGUISHING FEATURES: shiny brown tips, no emanating elements, surface mantle is a net synenchyma of clear cells 3-4 µm wide

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:
Shape and dimensions: straight tips, 2 mm long
Colour and texture: smooth, shiny brown

Emanating elements:
Mycelial strands: none observed
Hyphae: none observed

ANATOMY (Compound Microscope)

Mantle in plan view: mantle of medium thickness, Hartig net not observed, no specialized cells present
Outer layer: a net synenchyma of clear cells 3-4 µm wide, no ornamentation, no clamps
Inner layer: none observed

Mycelial strands in plan view: none observed
Emanating hyphae: none observed
Cystidia: none observed

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

Reference:
Ingleby, K., Mason, P.A., Last, F.T. and L.V. Fleming. 1990. *Identification of ectomycorrhizas*. Institute of Terrestrial Ecology (ITE) research publication no. 5. HMSO London. 112 pp.
Thesis Reference: SC#310 (OUC#125)

Laccaria-like

Class: Basidiomycotina
Order: Agaricales
Family: Tricholomataceae
Encountered on: excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm.

DISTINGUISHING FEATURES: whitish-yellow tip, surface mantle is a net prosenchyma of cells 8-10 µm, undifferentiated mycelial strands and emanating hyphae

MORPHOLOGY (Dissection Microscope)
Ectomycorrhizal system:
Shape and dimensions: irregularly branched, straight tips, 4 mm long by 500 µm
Colour and texture: whitish-yellow, finely grainy and matte
Emanating elements:
Mycelial strands: common whitish woolly
Hyphae: common, curved and tortuous, cottony

ANATOMY (Compound Microscope)
Mantle in plan view: surface mantle is a medium, thick net prosenchyma, no specialized cells
Outer layer: a net prosenchyma of cells 8-10 µm, clear in colour, no ornamentation, contents granular and with oil-like bodies, clamps present, H-shaped clamped, H-shaped not clamped anastomoses
Inner layer: a net synenchyma / interlocking irregular synenchyma of hyaline cells 10 µm, no ornamentation, no clamps, no anastomoses

Mycelial strands in plan view: rare, loose and smooth-undifferentiated, cells 4-5 µm wide, hyaline, no ornamentation, contents clear and granular, commonly septate
Emanating hyphae: common, tortuous, cells 6 µm wide, hyaline, no ornamentation, contents clear, granular and oily, no anastomoses, clamps present
Cystidia: none observed

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

Reference:
Ingleby, K., Mason, P.A., Last, F.T and L.V. Fleming. 1990. *Identification of ectomycorrhizas.* Institute of Terrestrial Ecology (ITE) research publication no. 5. HMSO London. 112 pp.
Thesis reference: SC# 80 (OUC#130)

Lactarius deterrimus **Gröger-like II**

Class: Basidiomycotina
Order: Russulales
Family: Russulaceae
Encountered: on excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm.

DISTINGUISHING FEATURES: bright green, bluish tip sometimes with milky tan areas, surface mantle is a net prosenchyma / net synenchyma of cells 3-4 µm wide, laticifers in the inner mantle, wiry mycelial strands

MORPHOLOGY (Dissection Microscope)
Ectomycorrhizal system:
Shape and dimensions: irregularly branched, straight tips, 3 mm long by 700 µm wide
Colour and texture: bright green, bluish with tan apices, smooth and shiny
Emanating elements:
Mycelial strands: rare, green and wiry
Hyphae: rare, straight, curved

ANATOMY (Compound Microscope)
Mantle in plan view: mantle is medium-thick type E (Agerer, 1991) laticifers found in the inner mantle
Outer layer: a net prosenchyma / net synenchyma of hyaline cells 3-4, hyphae have elbow-like protrusions, no ornamentation, contents, clear and granular, no clamps, contact not clamped and H-shaped not clamped anastomoses
Inner layer: net synenchyma of clear cells 4-6 µm wide, parallel sections, no ornamentation, clear contents, no clamps, contact not clamped and H-shaped not clamped anastomoses

Mycelial strands in plan view: undifferentiated 4-5 µm wide, no ornamentation
Emanating hyphae: rare, 3 µm wide, hyaline, no ornamentation, clear contents
Cystidia: none observed

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed
References:
Agerer, R. 1987. *Lactarius deterrimus*. In: Agerer, R. (ed.) Colour Atlas of Ectomycorrhizae, plate 3. Einhorn-Verlag, Schwäbisch Gmünd.
Agerer, R. 1991. Characterization of ectomycorrhizae. *In* Techniques for the study of mycorrhiza. Edited by Norris J.R., Read D.J and A.K. Varma. Academic Press. London. pp 25-73.
Thesis reference: SC#190 (OUC#142)

***Lactarius glyciosmus* (Fr.:Fr.) Fr. - like III**
Class: Basidiomycotina
Order: Russulales
Family: Russulaceae
Encountered on: excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm.

DISTINGUISHING FEATURES: creamy white, beige, brown tip, surface mantle is a net synenchyma of clear cells 3-5 µm no emanating elements

MORPHOLOGY (Dissection Microscope)
Ectomycorrhizal system:
Shape and dimensions: not branched, straight, 2 mm by 300 - 500 µm
Colour and texture: creamy white, beige to brown, smooth, shiny, silvery
Emanating elements:
Mycelial strands: none observed
Hyphae: none observed

ANATOMY (Compound Microscope)
Mantle in plan view: mantle is a medium thick net synenchyma, mantle type C (Agerer 1991) laticifers 10 µm wide
Outer layer: net synenchyma; cells 3-4 µm wide, 5-6 µm long, pale in colour, no ornamentation, clear contents, no clamps; matrix material stains strongly in Toluidine blue
Inner layer: net synenchyma, in places resembling an interlocking irregular synenchyma; similar to the outer mantle but cells more tightly arranged, 3-4 µm, pale in colour, no ornamentation, clear contents, no clamps

Mycelial strands in plan view: none observed
Emanating hyphae: none observed
Cystidia: none observed

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none
References:
Agerer, R. 1991. Characterization of ectomycorrhizae. *In* Techniques for the study of mycorrhiza. Edited by Norris J.R., Read D.J and A.K. Varma. Academic Press. London. pp 25-73.

Ingleby, K., Mason, P.A., Last, F.T and L.V. Fleming. 1990. *Identification of ectomycorrhizas*. Institute of Terrestrial Ecology (ITE) research publication no. 5. HMSO London. 112 pp.
Thesis reference: SC#310 / SC#110 (OUC#125)

***Lactarius pubescens* (Fr.: Krombh.) Fr. -like V**

Class: Basidiomycotina
Order: Russulales
Family: Russulaceae
Encountered: on excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm.

DISTINGUISHING FEATURES: milky orange tips, surface mantle is a net synenchyma / regular synenchyma of cells 6 µm wide, awl shaped cystidia, no clamps

MORPHOLOGY (Dissection Microscope)
Ectomycorrhizal system:
Shape and dimensions: irregularly branched, straight tips 4 mm long by 500 µm
Colour and texture: milky orange, smooth and matte
Emanating elements:
Mycelial strands: none observed
Hyphae: none observed

ANATOMY (Compound Microscope)
Mantle in plan view: surface mantle is a thick net synenchyma / regular synenchyma, no specialized cells observed
Outer layer: net synenchyma / regular synenchyma of cells 5-7 µm wide and 8-10 µm long, hyaline, clear contents, commonly septate, no clamps
Inner layer: a net synenchyma of cells 3-4 µm wide and 8-10 µm long, no ornamentation, commonly septate, no clamps

Mycelial strands in plan view: no observed
Emanating hyphae: none observed
Cystidia: rare, awl shaped 50-100 µm long medial width is 3 µm, no ornamentation, clear contents, no septa or clamps

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

References:
Ingleby, K., Mason, P.A., Last, F.T and L.V. Fleming. 1990. *Identification of ectomycorrhizas*. Institute of Terrestrial Ecology (ITE) research publication no. 5. HMSO London. 112 pp.
Thesis reference: SC#100 (OUC#650)

Lactarius rufus (Scop.: Fr.) Fr. - like I

Class: Basidiomycotina
Order: Russulales
Family: Russulaceae
Encountered on: excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm. and on *Picea engelmanni* x *glauca;* field bioassay

DISTINGUISHING FEATURES: creamy white/grey to orange/brown tip; surface mantle is a not-interlocking irregular synenchyma comprised of cells 15 µm in diameter, no emanating hyphal elements

MORPHOLOGY (Dissection Microscope)
Ectomycorrhizal system:
Shape and dimensions: irregular system; tips are 3-6 mm long and 300-400 µm wide.
Colour and texture: creamy white/grey becoming reddish brown with age; smooth and matte
Emanating elements:
Mycelial strands: none observed
Hyphae: none observed

ANATOMY (Compound Microscope)
Mantle in plan view: mantle is of medium thickness, no specialized cells
Outer layer: a not-interlocking irregular synenchyma of cells 8-15 µm in diameter; resembles a 'jigsaw puzzle' no ornamentation, no clamps
Inner layer: a net synenchyma of cells 4-5 µm in diameter, no ornamentation, clear contents, no clamps

Mycelial strands in plan view: none observed
Emanating hyphae: none observed
Cystidia: none observed

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

Reference:
Ingleby, K., Mason, P.A., Last, F.T and L.V. Fleming. 1990. *Identification of ectomycorrhizas*. Institute of Terrestrial Ecology (ITE) research publication no. 5. HMSO London. 112 pp.
Thesis reference: SC#160 / FB#400 (OUC#150)

Leccinum-like

Class: Basidiomycotina
Order: Boletales
Family: Boletaceae
Encountered on: excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm.

DISTINGUISHING FEATURES: tips are pale yellow sometimes with a pink tinge, hairy undifferentiated mycelial strands, hyphae in mantle and strands lacking clamps

MORPHOLOGY (Dissection Microscope)
Ectomycorrhizal system:
Shape and dimensions: unbranched systems; tips 2 mm by 400-600 µm
Colour and texture: pale yellow with a pink tinge, woolly or stringy, matte
Emanating elements:
Mycelial strands: common, hairy, pale yellow
Hyphae: none observed

ANATOMY (Compound Microscope)
Mantle in plan view: mantle is thick, no specialized cells observed
Outer layer: a net synenchyma, no matrix, yellow or hyaline cells 3 µm, ornamentation varies from none to verrucose, clear contents, no clamps
Inner layer: not observed

Mycelial strands in plan view: loose or smooth-undifferentiated, yellow or hyaline cells 3 µm wide, commonly verrucose, commonly septate, no clamps
Emanating hyphae: none observed
Cystidia: none observed

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

Reference:
Ingleby, K., Mason, P.A., Last, F.T and L.V. Fleming. 1990. *Identification of ectomycorrhizas*. Institute of Terrestrial Ecology (ITE) research publication no. 5. HMSO London. 112 pp.
Thesis reference: SC#300 (OUC#160)

Mycelium radicis atrovirens **Melin**

Class: Deuteromycotina
Order:
Family:
Encountered on: excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm. *Picea engelmanni* x *glauca* seedlings; field bioassay, greenhouse bioassay

DISTINGUISHING FEATURES: dark brown, grey colour, mantle surface is a felt prosenchyma; hyphae 2 µm wide

MORPHOLOGY (Dissection Microscope)
Ectomycorrhizal system:
Shape and dimensions: not branched; tips 4 (3-5) mm by 300 µm
Colour and texture: grey to dark brown with grey apices; finely grainy; host visible through mantle
Emanating elements:
Mycelial strands: none observed
Hyphae: rare, straight

ANATOMY (Compound Microscope)
Mantle in plan view: thin mantle, Hartig net present, no specialized cells present
Outer layer: a felt prosenchyma; cells 2 µm wide, dark brown, no ornamentation observed, clear contents, rarely septate, no clamps, straight, thin hyphae
Inner layer: a net synenchyma; cells 2-3 µm wide, yellowish brown, 'brain-like' pattern

Mycelial strands in plan view: none observed
Emanating hyphae: rare; 2 µm wide, dark brown, no ornamentation, commonly septate, clear contents, no clamps
Cystidia: none observed

OTHER FEATURE:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

Reference:
Ingleby, K., Mason, P.A., Last, F.T and L.V. Fleming. 1990. *Identification of ectomycorrhizas*. Institute of Terrestrial Ecology (ITE) research publication no. 5. HMSO London. 112 pp.

Thesis reference: GB#120 / FB#450 / SC#90 (OUC#170)

Paxillus-like

Class: Basidiomycotina
Order: Boletales
Family: Boletaceae
Encountered on: excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm.

DISTINGUISHING FEATURES: brownish white tips, mycelial strands common, undifferentiated, hyphae in mantle and emanating hyphae pale yellow and with clamps

MORPHOLOGY (Dissection Microscope)
Ectomycorrhizal system:
Shape and dimensions: irregularly branched systems; tips 4 mm by 600 µm
Colour and texture: brownish white with red, brown or white apices, white patches in mantle bruise when touched, stringy or felty, matte to reflective
Emanating elements:
Mycelial strands: common, hairy and thin
Hyphae: common, curved or tortuous, cottony

ANATOMY (Compound Microscope)
Mantle in plan view: mantle of medium thickness, no specialized cells observed
Outer layer: a felt prosenchyma or net prosenchyma, no matrix, cells hyaline to pale yellow, 4 (3-5) µm, no ornamentation, clear contents, commonly clamped, hyphal junctions common, contact not clamped anastomoses common
Inner layer: a net synenchyma, no matrix, hyaline cells 4 (3-5) µm wide, no ornamentation, clear contents, commonly clamped, some parallel sections

Mycelial strands in plan view: loose or smooth-undifferentiated, 4 (3-5) µm, pale yellow or hyaline, no ornamentation, frequently septate, contents clear
Emanating hyphae: common, cells hyaline or pale yellow, 4 (3-5) µm wide, no ornamentation, contents clear or granular rare anastomoses
Cystidia: none observed

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed
Reference:
Ingleby, K., Mason, P.A., Last, F.T and L.V. Fleming. 1990. *Identification of ectomycorrhizas*. Institute of Terrestrial Ecology (ITE) research publication no. 5. HMSO London. 112 pp.
Thesis reference: SC #270 (OUC#180)

Piloderma-like

Class: Basidiomycotina
Order: Aphyllophorales
Family: Corticiaceae
Encountered on: excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm. *Picea engelmanni* x *glauca* seedlings; greenhouse bioassay

DISTINGUISHING FEATURES: bright yellow, coarsely felty; mantle is a thick felt prosenchyma, mycelial strands are abundant undifferentiated, hyphae common and verrucose

MORPHOLOGY (Dissection Microscope)
Ectomycorrhizal system:
Shape and dimensions: irregular systems; difficult to discern the length of roots due to the wefts of hyphae extending from it, 450-500 µm wide
Colour and texture: bright yellow, coarsely felty, mantle completely obscures the root
Emanating elements:
Mycelial strands: abundant, bright yellow
Hyphae: common, curved to tortuous

ANATOMY (Compound Microscope)
Mantle in plan view: thick mantle, Hartig net not observed, no specialized cells observed
Outer layer: a thick felt prosenchyma; cells 3-4 µm wide, clear-yellow, commonly septate, verrucose ornamentation, clamps common, H-shaped anastomoses
Inner layer: not observed

Mycelial strands in plan view: Loose-undifferentiated, clear - yellow; 3-4 µm wide, verrucose and smooth elements, commonly clamped, anastomoses common
Emanating hyphae: common 3-4 µm wide, verrucose and smooth, yellow, commonly septate, H-shaped anastomoses common
Cystidia: none observed
OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed
Reference:
Brand, K. 1991. *Piloderma croceum* In: Agerer, R. (ed.) Colour Atlas of Ectomycorrhizae, plate 62. Einhorn-Verlag, Schwäbisch Gmünd.

Thesis reference: GB #110 / SC #130 (OUC#200)

***Russula illota* Romagn. - like I**

Class: Basidiomycotina
Order: Russulales
Family: Russulaceae
Encountered on: excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm., *Picea engelmanni* x *glauca* seedlings; field bioassay

DISTINGUISHING FEATURES: white tip with a reflective silvery sheen caused by abundant cystidia of two different morphologies; surface mantle is a net prosenchyma comprised of cells 3-4 µm in diameter

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:
Shape and dimensions: unbranched system, tips straight 2-4 mm long, 300 µm wide
Colour and texture: white to buff in colour, reflective and shiny
Emanating elements:
Mycelial strands: none observed
Hyphae: none observed

ANATOMY (Compound Microscope)

Mantle in plan view: mantle is of medium thickness; Hartig net not observed, no specialized elements
Outer layer: a net prosenchyma comprised of cells 3-4 µm wide; some parallel sections; clear with granular contents; commonly septate; no clamps
Inner layer: none observed

Mycelial strands in plan view: none observed
Emanating hyphae: none observed
Cystidia: Abundant; two types; bottle shaped, straight neck, thick walled, 60 µm in length, apex is 2 µm wide, base is 10 µm wide, no clamps; non capitate form, bulbous 30 µm in length, 6 µm wide

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

Reference:
Brand F. 1991. *Russula illota* In: Agerer, R. (ed.) Colour Atlas of Ectomycorrhizae, plate 64. Einhorn-Verlag, Schwäbisch Gmünd.
Thesis reference: FB #420 / SC #210 (OUC#220)

Russula-like II

Class: Basidiomycotina
Order: Russulales
Family: Russulaceae
Encountered: on excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm.

DISTINGUISHING FEATURES: yellow/gold tip, surface mantle is a regular synenchyma of cells 5 µm in diameter, no emanating elements

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:
Shape and dimensions: systems unbranched and monopodial pinnate, straight, 2 mm long by 500 µm
Colour and texture: yellow/gold, smooth and shiny

Emanating elements:
Mycelial strands: none observed
Hyphae: none observed

ANATOMY (Compound Microscope)
Mantle in plan view: medium-thin regular synenchyma mantle, no specialized cells observed
Outer layer: a regular synenchyma comprised of cells 5 µm in diameter, no ornamentation, clear contents, no clamps, no anastomoses
Inner layer: not observed

Mycelial strands in plan view: none observed
Emanating hyphae: none observed
Cystidia: none observed

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

Reference:
Danielson, R.M. 1991. Known and putative genera of ectomycorrhizal fungi with ecological information, characteristics of the mycorrhizae, and selected references to descriptive material. Kananaskis Centre for Environmental Research. University of Calgary 16 pp.

Thesis reference: SC#60 (OUC#223)

Suillus-like

Class: Basidiomycotina
Order: Boletales
Family: Boletaceae
Encountered on: excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm.

DISTINGUISHING FEATURES: very white tips with common white hairy undifferentiated mycelial strands, no clamps observed

MORPHOLOGY (Dissection Microscope)
Ectomycorrhizal system:
Shape and dimensions: irregularly branched systems; tips straight or bent, 3 mm by 500 µm
Colour and texture: very white, woolly or stringy
Emanating elements:
Mycelial strands: common, white and hairy
Hyphae: common, curved

ANATOMY (Compound Microscope)
Mantle in plan view: thick mantle, no specialized cells
Outer layer: a felt prosenchyma of hyaline cells 5 (4-6) µm wide, no matrix, crystalline ornamentation giving the hyphae a bumpy appearance, clear contents, rarely septate
Inner layer: not observed

Mycelial strands in plan view: loose or smooth-undifferentiated, 5 (4-6) µm wide, crystalline ornamentation, clear contents, no clamps
Emanating hyphae: common, hyaline cells 5 (4-6) µm wide, crystalline ornamentation, clear contents, no clamps
Cystidia: none observed

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

Reference: Agerer, R. 1987. *Suillus plorans*. In: Agerer, R. (ed.) Colour Atlas of Ectomycorrhizae, plate 46. Einhorn-Verlag, Schwäbisch Gmünd.
Thesis reference: SC #350 (OUC#230)

***Thelephora terrestris* (Ehrh.) Fr. - like I**

Class: Basidiomycotina
Order: Thelephorales
Family: Thelephoraceae
Encountered on: *Picea engelmanni* x *glauca* seedlings; greenhouse bioassay

DISTINGUISHING FEATURES: milky white to beige-brown, can be copperish looking due to air bubbles; smooth; thin mantle of felt prosenchyma, abundant cystidia with basal clamps

MORPHOLOGY (Dissection Microscope)
Ectomycorrhizal system:
Shape and dimensions: systems not branched; tips 7 (4-10) µm long
Colour and texture: milky white to beige and light- dark brown, smooth, coarsely grainy, reflective
Emanating elements:
Mycelial strands: none observed
Hyphae: none observed

ANATOMY (Compound Microscope)
Mantle in plan view: thin mantle, extensive felt prosenchyma
Outer layer: a felt prosenchyma with cells 2-4 µm wide and 8-10 µm long; hyphae are hyaline, not ornamented, commonly septate
Inner layer: patchy net synenchyma of brown hyphae, 5-8 µm wide. NO ornamentation, clear contents, no clamps.

Mycelial strands in plan view: none observed
Emanating hyphae: none observed
Cystidia: abundant awl shaped cystidia with basal clamps; 70-350 µm long and 2-4 µm wide; basal clamps

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

References:
Agerer, R. and M. Weiss. 1989 Studies on ectomycorrhiza XX. Mycorrhizae formed by *Thelephora terrestris* on Norway spruce. *Mycologia* **81:** 444-453
Ingleby, K., Mason, P.A., Last, F.T and L.V. Fleming. 1990. *Identification of ectomycorrhizas*. Institute of Terrestrial Ecology (ITE) research publication no. 5. HMSO London. 112 pp.

Thesis reference: GB #80 / FB #300

Thelephora-like II

Class: Basidiomycotina
Order: Thelephorales
Family: Thelephoraceae
Encountered on: excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm.

DISTINGUISHING FEATURES: tan, gold tip, surface mantle is a net prosenchyma / net synenchyma, no cystidia or clamps, no emanating elements

MORPHOLOGY (Dissection Microscope)
Ectomycorrhizal system:
Shape and dimensions: Monopodial pinnate, irregular systems, tips are 3-4 mm long by 500 µm
Colour and texture: tan/gold, sometimes grey, finely grainy, reflective

Emanating elements:
Mycelial strands: none observed
Hyphae: none observed

ANATOMY (Compound Microscope)

Mantle in plan view: surface mantle is a medium thick net prosenchyma / net synenchyma, resembling mantle type L and P (Agerer 1991), no specialized cells observed
Outer layer: a net prosenchyma / net synenchyma of hyaline cells 5-6 µm wide and 15-30 µm long, no ornamentation, clear contents, commonly septate, no clamps, no anastomoses

Inner layer: a not-interlocking irregular synenchyma of cells 10 µm in diameter, no ornamentation, clear contents, no anastomoses

Mycelial strands in plan view: none observed
Emanating hyphae: none observed
Cystidia: none observed

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed
Reference:
Agerer, R. 1991. Characterization of ectomycorrhizae. *In* Techniques for the study of mycorrhiza. Edited by Norris J.R., Read D.J and A.K. Varma. Academic Press. London. pp 25-73.
Thesis reference: SC #20 (OUC#240)

Tomentella-like I

Class: Basidiomycotina
Order: Thelephorales
Family: Thelephoraceae
Encountered on: excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm.

DISTINGUISHING FEATURES: light tan tip, regular synenchyma of clear cells, no emanating elements

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:
Shape and dimensions: unbranched to irregularly branched systems; tips 3 (2-4) mm by 450 (300-6000 µm
Colour and texture: light tan, milky tip, smooth to finely grainy, matte, host is obscured by the fungus

Emanating elements:
Mycelial strands: none observed
Hyphae: none observed

ANATOMY (Compound Microscope)

Mantle in plan view: thin mantle, no specialized cells observed
Outer layer: a regular synenchyma of hyaline cells 12 µm in diameter, clear contents, no clamps, no hyphal junctions, no anastomoses
Inner layer: not observed

Mycelial strands in plan view: none observed
Emanating hyphae: none observed
Cystidia: none observed

OTHER FEATURES:

Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

Reference:
Thesis reference: SC#181 (OUC#253)

Tomentella-like II

Class: Basidiomycotina
Order: Thelephorales
Family: Thelephoraceae
Encountered on: excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm., *Picea engelmanni* x *glauca* seedlings; greenhouse bioassay

DISTINGUISHING FEATURES: dark brown to black; coarsely grainy and reflective; mantle is not-interlocking irregular synenchyma with dark brown cells 10-15 µm diameter

MORPHOLOGY (Dissection Microscope)
Ectomycorrhizal system:
Shape and dimensions: system is not branched; tips straight to bent, 2-3 mm by 700 µm
Colour and texture: tips dark brown to black, coarsely grainy and reflective
Emanating elements:
Mycelial strands: none observed
Hyphae: rare to common, straight to curved and black

ANATOMY (Compound Microscope)
Mantle in plan view: mantle is a thick not-interlocking irregular synenchyma, resembles type N (Agerer 1991), fungus entirely obscures the root
Outer layer: a not-interlocking irregular synenchyma, rarely a net prosenchyma cells 10-15 µm by 15 µm, brown, no ornamentation, clear contents
Inner layer: not observed

Mycelial strands in plan view: none observed
Emanating hyphae: rare to common, 4-6 µm wide, dark reddish brown, no ornamentation, clear contents, septate, no clamps
Cystidia: none observed

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

References:
Agerer, R. 1991. Characterization of ectomycorrhizae. *In* Techniques for the study of mycorrhiza. Edited by Norris J.R., Read D.J and A.K. Varma. Academic Press. London. pp 25-73.

Thesis reference: GB #61 / SC #30 (OUC#251)

Unidentified

Encountered on: *Picea engelmanni* x *glauca* seedlings; field bioassay, greenhouse bioassay

DISTINGUISHING FEATURES: Tawny brown tips with cream colored apex; surface mantle is a felt prosenchyma of light brown to grey cells 3-4µm, emanating hyphae are 4-5 µm, grey - brown and common

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:
Shape and dimensions: monopodial pinnate system, straight, tips are 2-3 mm long and 300 µm wide
Colour and texture: Tawny brown with pale apices, finely grainy, cottony and reflective
Emanating elements:
Mycelial strands: none observed
Hyphae: cottony, pale brown to brown

ANATOMY (Compound Microscope)
Mantle in plan view: thin mantle, no specialized cells
Outer layer: a thin felt prosenchyma, cells 3-4 µm , brown to grey, no ornamentation, granular contents in places, commonly septate, anastomoses H-shaped not clamped, no clamps
Inner layer: a faint net synenchyma, cells 8-10 µm, granular contents, no ornamentation, commonly septate, no clamps

Mycelial strands in plan view: none observed
Emanating hyphae: common, 4-5 µm wide, brown, grey, no ornamentation, granular contents, commonly septate, H-shaped anastomoses
Cystidia: none observed

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

Reference:
Thesis reference: FB#520

Unidentified

Encountered on: *Picea engelmanni* x *glauca* seedlings; field bioassay

DISTINGUISHING FEATURES: pale brown, grey tip, surface mantle is a thin net synenchyma of cells 6-12 µm in diameter, no emanating elements

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:
Shape and dimensions: unbranched system, straight tip, 3 mm long by 300 µm
Colour and texture: buff to light brown, older tips darker brown, shiny and smooth
Emanating elements:
Mycelial strands: none observed
Hyphae: none observed

ANATOMY (Compound Microscope)
Mantle in plan view: Thin net synenchyma, no specialized cells
Outer layer: Thin net synenchyma of cells 6-12 µm wide, no ornamentation, no clamps, hyphal junctions common, constricted at the septa, some parallel sections other less elongated and more like a not-interlocking net synenchyma
Inner layer: not observed

Mycelial strands in plan view: none observed
Emanating hyphae: none observed
Cystidia: none observed

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

Reference:
Thesis reference: FB#510

Unidentified

Class:
Order:
Family:
Encountered on: excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm., *Picea engelmanni* x *glauca* seedlings; greenhouse bioassay

DISTINGUISHING FEATURES: white, grayish tip with abundant hyphae 4-5 µm wide, mantle surface is a felt prosenchyma of clear hyphae

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:
Shape and dimensions: unbranched, straight to bent tip, 4 mm long and 300µm wide
Colour and texture: white to grey, cottony to stringy
Emanating elements:
Mycelial strands: rare, grey, hairy
Hyphae: abundant, straight, grey wefts of hyphae

ANATOMY (Compound Microscope)
Mantle in plan view: thin felt prosenchyma, no specialized cells observed
Outer layer: a felt prosenchyma of hyaline cells 4-5 µm wide; both smooth and crystalline ornamentation, commonly septate, no clamps, abundant H-shaped and contact anastomoses
Inner layer: not observed

Mycelial strands in plan view: loose undifferentiated, 5 µm wide; no ornamentation, clear contents, common anastomoses
Emanating hyphae: abundant, 5 µm wide, clear, commonly septate, no clamps, abundant H-shaped and contact anastomoses
Cystidia: non observed

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

Reference:
Thesis reference: GB #140 / SC #40 (OUC#380)

Unidentified

Class:
Order:
Family:
Encountered on: excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm.

DISTINGUISHING FEATURES: pale beige, surface mantle an irregular synenchyma with grainy cell walls, no emanating elements

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:
Shape and dimensions: irregularly branched; tips bent to tortuous, 3 mm by 600-800 µm
Colour and texture: pale beige, finely grainy, coarsely grainy, matte
Emanating elements:
Mycelial strands: none observed
Hyphae: none observed

ANATOMY (Compound Microscope)
Mantle in plan view: mantle of medium thickness, type M (Agerer, 1991), no specialized cells observed
Outer layer: an irregular synenchyma, no matrix; cells hyaline, 5 (3-7) µm by 6 (4-8) µm, verrucose to globular ornamentation, clear contents, no clamps
Inner layer: net synenchyma, no matrix, hyaline cells 3 µm by 45 µm, no ornamentation, clear contents, rarely septate, no hyphal junctions, no clamps

Mycelial strands in plan view: not observed
Emanating hyphae: not observed
Cystidia: not observed

OTHER FEATURES:
Sclerotia and Microsclerotia: not observed
Chlamydospores: not observed

Reference:
Thesis reference: SC #250 (OUC#250)

Unidentified

Class:
Order:
Family:
Encountered on: excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm.

DISTINGUISHING FEATURES: grey-white translucent sheath covering natural root tip colour, irregularly ornamented hyphae without clamps

MORPHOLOGY (Dissection Microscope)
Ectomycorrhizal system:
Shape and dimensions: unbranched, straight to bent tips, 1-2 mm long by 500-700 µm wide
Colour and texture: white to grey-white, smooth, finely grainy and matte
Emanating elements:
Mycelial strands: none observed
Hyphae: rare or common, cottony

ANATOMY (Compound Microscope)
Mantle in plan view: mantle of medium thickness, no specialized cells observed
Outer layer: a felt prosenchyma, no matrix; cells 3 (2-4) µm wide, yellow to hyaline, ornamentation can be crystalline, verrucose or globular, clear contents, no clamps, hyphal junctions common, contact, not clamped, H-shaped not clamped anastomoses
Inner layer: not observed

Mycelial strands in plan view: none observed
Emanating hyphae: rare or common, 3 (2-4) µm wide, irregular ornamentation (see mantle description), clear contents, no clamps, contact not clamped and H-shaped not clamped anastomoses
Cystidia: none observed

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

Reference:
Thesis reference: SC #220 (OUC#660)

Unidentified

Class:
Order:
Family:
Encountered on: excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm.

DISTINGUISHING FEATURES: smooth, orange to yellow tips, no emanating elements, mantle surface is a felt prosenchyma or net prosenchyma of clear cells 1-3 µm wide

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:
Shape and dimensions: unbranched to monopodial pinnate and sometimes irregularly branched tip, 3-6 mm long, 500 µm wide
Colour and texture: smooth, orange to yellow tips

Emanating elements:
Mycelial strands: none observed
Hyphae: none observed

ANATOMY (Compound Microscope)

Mantle in plan view: mantle of medium thickness, no Hartig net observed, no specialized cells
Outer layer: a felt prosenchyma or net prosenchyma of hyaline cells 1-3 µm wide, no ornamentation, clear contents, commonly septate, no clamps, contact and H-shaped anastomoses, parallel sections
Inner layer: a net synenchyma of hyaline cells 3-4 µm wide no ornamentation, clear contents, commonly septate, no clamps, parallel sections

Mycelial strands in plan view: none observed
Emanating hyphae: none observed
Cystidia: none observed

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

Reference:
Thesis reference: SC #290

Unidentified

Class:
Order:
Family:
Encountered on: excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm.

DISTINGUISHING FEATURES: green - gold tips occasionally with mycelial strands, surface mantle is a net synenchyma of yellow cells 5 µm wide

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:
Shape and dimensions: unbranched , straight tips, 3 mm long and 500 µm wide
Colour and texture: green - gold, finely grainy, woolly and cottony
Emanating elements:
Mycelial strands: can be rare or common, green, commonly branched
Hyphae: can be rare or common, curved

ANATOMY (Compound Microscope)

Mantle in plan view: thick mantle, Hartig net not observed, no specialized cells
Outer layer: a net synenchyma of yellow cells 5 µm wide, clear contents, rarely septate, no clamps
Inner layer: a net synenchyma or interlocking irregular synenchyma of cells 6 µm wide, yellow, granular matrix materials present, no ornamentation, clear contents

Mycelial strands in plan view: loose-undifferentiated, 6 µm wide, clear yellow cells, no ornamentation, commonly septate, no clamps
Emanating hyphae: rare or common, yellowish grey cells are 6 µm wide, no ornamentation, clear contents, commonly septate
Cystidia: none observed

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

Reference:
Thesis reference: SC# 295

Unidentified

Class: Basidiomycotina
Order:
Family:
Encountered on: excised roots of *Abies lasiocarpa* [Hook.] Nutt. or *Picea engelmanni* Parry ex Engelm.

DISTINGUISHING FEATURES: White crusty mucilaginous thick tip that bruises blue and milky, surface mantle is a felt prosenchyma of clear cells 3 µm wide

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:
Shape and dimensions: unbranched, straight tips 5 mm long and 1.5 mm wide
Colour and texture: white to cream coloured tip with a bluish tinge

Emanating elements:
Mycelial strands: common, white and hairy
Hyphae: common, white, curved and tortuous

ANATOMY (Compound Microscope)

Mantle in plan view: very thick mantle, Hartig net not observed, no specialized elements
Outer layer: a felt prosenchyma of clear cells 3 µm wide, no ornamentation, clear contents, commonly septate, clamps common, contact clamped and H-shaped anastomoses, extension of emanating hyphae
Inner layer: a not-interlocking synenchyma of cells 10-15 µm wide, no ornamentation, clear contents, no clamps

Mycelial strands in plan view: loose-undifferentiated
Emanating hyphae: clear cells 3 µm wide, no ornamentation, clear contents, commonly septate, clamps common
Cystidia: none observed

OTHER FEATURES:
Sclerotia and Microsclerotia: none observed
Chlamydospores: none observed

Reference:
Thesis reference: SC #340