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**The impact of harvesting practices on the biodiversity
of *Picea glauca* ectomycorrhizae.**

by



Lance W. Lazaruk

**A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment
of the requirements for the degree of Master of Science**

in

**Forest Biology and Management
Department of Renewable Resources**

Edmonton, Alberta

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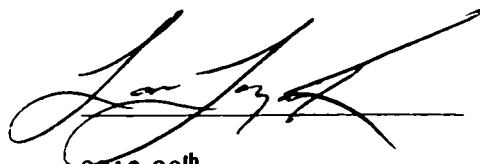
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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled The impact of harvesting practices on the biodiversity of *Picea glauca* ectomycorrhizae submitted by Lance W. Lazaruk in partial fulfillment of the requirements for the degree of Master of Science in Forest Biology and Management.



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Abstract

I assessed the impact of various harvesting practices (including those designed to emulate natural disturbances) on ectomycorrhizae associated with white spruce in northwestern Alberta, Canada. Treatments sampled included clear-cut, partial cut (with 20,50 and 75% live residual remaining), unharvested control and a burned stand. The percentage of active root tips, ectomycorrhizal richness and ectomycorrhizal diversity, observed in soil cores collected throughout the study site, all decreased with increasing harvesting intensity. Reductions in ECM biodiversity could be attributed to the sensitivity of late-stage ectomycorrhizae (e.g. *Cortinarius* spp., *Lactarius* spp., and *Russula* spp.). Harvesting practices that retained a percentage of permanent residual trees, either dispersed throughout the cutting unit (e.g. partial cutting) or in aggregated patches, were an effective means of maintaining ectomycorrhizal biodiversity. Mechanical site preparation, which is commonly used to improve the early performance of outplanted seedlings, significantly reduced the ectomycorrhizal status of planted white spruce seedlings.

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Chapter 1

General Introduction

Ectomycorrhizae

The symbiotic relationship that exists between fungi and the roots of many species of woody plants, termed ectomycorrhizae, can be observed on approximately 3% of vascular plant species worldwide (Smith and Read 1997). While this is a relatively small percentage globally, temperate regions are dominated by plants capable of forming this type of mycorrhizal symbiosis. This includes economically important tree species, such as those within the Pinaceae.

The antiquity of the ectomycorrhizal (ECM) association illustrates the significance of this relationship. Due to the microscopic size, soft tissues and the ephemeral nature of ectomycorrhizae fossil records are extremely rare, however, some specimens have been found by researchers. Using a cellulose acetate peel technique Lepage et al. (1997) examined well-preserved plant material obtained from the middle Eocene Princeton chert of British Columbia, Canada. The morphology of the root they observed, which they determined as *Pinus*, was strikingly similar to the ectomycorrhizae formed by present day basidiomycetes from the genera *Rhizopogon* and *Suillus*. This observation led the researchers to postulate that ectomycorrhizae were present in forest ecosystems at least 50 million years ago. While no fossil evidence exists prior to this Berbee and Taylor (1992) suggest, based on their molecular clock model, that ectomycorrhizae first appeared during the early Cretaceous, approximately 130 million years ago.

Ecological function of ectomycorrhizae

Many studies have shown that ectomycorrhizal plants are capable of outperforming their non-ectomycorrhizal counterparts. This has often been attributed to the fungal hyphae which, i) increase the surface area available for nutrient absorption, and ii) extend past the nutrient depletion zone, which forms adjacent to plant roots (Smith and Read 1997). However, until radioisotopic techniques became readily available and utilized by mycologists interested in mycorrhizal physiology, researchers were confined to performing descriptive studies (Tinker et al. 1991). This development finally allowed researchers to examine which mineral nutrients were involved, and at what rates these nutrients were being translocated to the host plant.

Current research suggests that ECM plants benefit mostly by acquiring nitrogen, which is a limiting nutrient characteristic to ecosystems dominated by ECM plants, and phosphorus (Finlay 1982; Kronzucker et al. 1997). Other important mineral nutrients include K, Cl and S. In addition, some ECM fungi also have the ability to produce extracellular enzymes, which allows them to extract organic nitrogen, such as protein and amino acids, directly from plant litter (Abuzinadah and Read 1986). According to Read (1991) the ability of ECM fungi to scavenge organic nitrogen is one of the most significant ecosystem roles of ectomycorrhizae. Rhizomorphs (linearly aggregated hyphae) have also been shown to play a key role in the absorption and long distances transportation of water, in addition to mineral nutrients (Lamhamedi et al. 1992; Smith and Read 1997).

ECM fungi benefit from the symbiotic association by obtaining photosynthetically derived carbon compounds from their host plant. Because ECM fungi lack certain enzymes, that their saprophytic cousins possess, they are for the most part obligate symbionts. Thus, the carbon received from their host plant is essential to their survival. Studies have shown that ECM fungi may receive up to 10-20% of the host plant's net primary productivity (Smith and Read 1997; Vogt et al. 1982). While this is a relatively low percentage, in certain situations (e.g. on high fertility sites where nutrients are not limiting and, thus, ectomycorrhizae don't provide a benefit to the host plant) this may be enough to restrict a host plants growth (Kropp and Langlois 1990).

In addition to the transfer of nutrients between fungus and plant Simard et al. (1997a) have shown that a significant translocation of carbon can exist between two plants (either the same or different species) via interconnected mycelium. In their experiment the net transfer of carbon from *Betula papyrifera* canopy trees to understory *Pseudotsuga menziesii* seedlings was over twice as great for seedlings growing in deep shade compared to those growing in partial shade. This finding implies that the translocation of nutrients, at least between host plants, is regulated by a source-sink relationship. While there is no evidence that a similar source-sink mechanism exists for phosphorus (Finlay and Read 1986) there is sufficient evidence that such a mechanism may exist for nitrogen (Simard et al. 1997a). Thus, in effect ectomycorrhizae may have the ability to mediate competition between ECM tree species (Perry et al. 1989) which, according to Read (1997), suggests that ectomycorrhizae play a key role in maintaining the productivity, stability and sustainability of forest ecosystems.

Other important ecological aspects of ectomycorrhizae include the production of polysaccharides which cement soil particles into macroaggregates (Jastrow 1996), enhancing the growth of otherwise subordinate plant species by mediating competition (Grime et al. 1987), the production of plant hormones and other secondary metabolites that influence plant growth (Kropp and Langlois 1990; Smith and Read 1997) and the production of antifungal compounds or phenols which provide protection from plant pathogens (Kropp and Langlois 1990). They are also important food source for microarthropods (Hiol et al. 1994), small mammals (Gehring and Whitham 1994; Johnson 1996) and humans (Pilz and Molina 1996).

Impact of disturbances on ectomycorrhizae

Mycorrhizal fungi are closely linked to their host plant, thus, any disturbance to the host plant will in turn affect the mycorrhizal fungi. For example, past research has shown that the biodiversity of ectomycorrhizae is significantly lower in both clear-cut sites (Bradbury et al. 1998; Durall et al. 1999; Hagerman et al. 1999) and burned sites (Stendell et al. 1999; Visser 1995) compared to undisturbed forest sites. In each of these studies the researchers reported an increase in the abundance of 'early stage' ECM fungi (such as *Inocybe*, *Laccaria*, *Thelephora* and E- strain) and a subsequent decrease of 'late stage' ECM fungi (such as *Lactarius*, *Cortinarius* and *Russula*). In effect, the disturbance of the above ground community resulted in a shift in the below ground ECM community.

The 'early' and 'late' stage distinction of ECM fungi was first proposed based on the spatial and temporal patterns of basidiocarp (mushroom) fruiting following tree establishment (Mason et al. 1983, Last et al. 1987). In these experiments the first

basidiocarps to be observed were located in close proximity to individual saplings. The ECM fungi associated with these basidiocarps were thus labeled as early stage ECM fungi. In subsequent years the researchers noticed that the fruiting of basidiocarps radiated out from the base of the saplings. Furthermore, different types of basidiocarps became more abundant over time while the abundance of those belonging to early stage ECM fungi began to decline. The ECM fungi associated with these new basidiocarps were labeled as late stage ECM fungi. While this is a somewhat superficial distinction, research has confirmed that physiological differences do exist between early and late stage ECM fungi (Deacon et al. 1983; Fox 1983).

The main distinction between these two broad groups of ECM fungi is now largely determined by the manner in which they colonize new root tips (Newton 1992). Inoculum sources of ectomycorrhizae in forest soils include vegetative structures (such as mycelial strands and mycorrhizal root tips), spores from reproductive structures, and resistant structures (such as sclerotia and chlamydospores) (Parke et al. 1984). Early stage ECM fungi have the ability to colonize new root tips via spores (Fox 1983), which makes them well adapted to disturbance. Thus, early stage ECM fungi tend to be generalists with a broad host range. On the other hand, late stage ECM fungi are characterized by their inability to colonize new root tips via spores (Fox 1983). Instead, late stage ECM fungi appear to require a substantial carbon source in order to colonize new root tips, thus, the primary source of inoculum appears to be an intact hyphal network that derives carbon from a host tree (Simard et al. 1997b). Furthermore, late stage ECM fungi tend to be specialists with a narrow host range. As a result, late stage ectomycorrhizae are slow to re-colonize disturbed sites as their reestablishment is

dependent upon the extension of fungal mycelium from an adjacent non-disturbed habitat (e.g. forest edge). However, there are drawbacks associated with using such a general distinction to classify such a diverse group of fungi.

Some ECM morphotypes (e.g. *Amphinema*, *Cenococcum*, *Hebeloma*, *Inocybe*, *Lactarius*, *Mycelium radialis atrovirens* (MRA) and *Thelephora*) may be observed on either young or mature trees (or stands) given that a source of inoculum is present. These species have been coined 'multistage' ectomycorrhizae. In terms of succession Visser (1995) suggests that these species would be more abundant in young forest stands (e.g. 2^o succession), as opposed to previously treeless sites (e.g. 1^o succession), because a source of inoculum may already be present in the soil. Therefore, the presence of these ECM fungi in harvested or burned stands would be determined by their ability to persist in the soil following disturbance. Mechanisms that would allow multistage fungi to remain viable in the absence of a host include the production of dormant structures, such as sclerotia (e.g. *Cenococcum* and *Hebeloma*) and chlamydoconidia (e.g. E-strain), and by obtaining nutrients saprophytically (Erland and Soderstrom 1991). It is also possible that these ectomycorrhizae persist by colonizing alternate hosts found on the site following disturbance. Woody shrubs and trees capable of providing refuge include species of *Alnus*, *Arctostaphylos*, *Betula*, *Populus*, *Salix*, *Shepherdia* and *Vaccinium* (Hagerman et al. 2001; Horton 1999; Kranabetter 1999). While this may explain the presence of multistage ectomycorrhizae with late stage (or K-selected) characteristics on disturbed sites it does not explain the presence of multistage ectomycorrhizae with early stage (or r-selected characteristics) characteristics (e.g. E-strain, MRA and *Thelephora*) in mature forest stands. The relative abundance of early stage ectomycorrhizae would be expected

to decrease over time as late stage ectomycorrhizae occupy the stand, however, research suggests that the relative abundance of multistage ectomycorrhizae remains constant throughout the stand's succession (Bradbury et al 1998; Visser 1995). Thus, one possible explanation is that multistage ectomycorrhizae (with r-selected characteristics) may have a competitive advantage, compared to early stage ectomycorrhizae, that enables them to persist in mature stands dominated by late stage ectomycorrhizae.

Ectomycorrhizal Diversity

The number of fungal species that form ectomycorrhizae is estimated to be between 5000 - 6000 worldwide (Molina et al. 1992). The identification of these species has been accomplished using a number of different techniques. This includes *in vitro* re-synthesis (Fortin et al. 1980), comparing cultures obtained from mycorrhizae and sporocarps (Hutchinson 1991) and linking mycorrhizae to fruiting bodies via hyphae (Agerer 1991; Ingelby et al. 1990). Another commonly used technique, termed morphotyping, involves comparing morphological and anatomical characters of a mycorrhiza to published descriptions (Agerer 1987-1998; Ingelby et al. 1990; Goodman et al. 1996). Compared to the previously mentioned techniques, morphotyping is a relatively quick process that doesn't require specialized laboratory equipment. However, the identification of individual species within a genus, which are often very similar morphologically, is usually quite difficult. Furthermore, macroscopic features (e.g. color and form) characteristic to certain ectomycorrhizae may be influenced by changing environmental conditions (Egger 1995; Egli et al. 1993). A more objective approach to identification involves molecular techniques, such as polymerase chain reaction (PCR)

and analysis of restriction fragment length polymorphisms (RFLP). The advantage of using molecular techniques, as opposed to morphotyping, is that the process is easy to learn, requires little time to process root tips, and the results are easily compared among researchers. The main disadvantage, however, is that specialized laboratory equipment is required and the processing of a large number of samples, which is generally required in order to make an accurate assessment of an ECM community, can be costly. Thus, given the advantages and disadvantages associated with the various identification techniques, the most suitable approach will depend on the nature of the study. If the objective is to identify new or unknown species of ectomycorrhizae then molecular techniques would be the most appropriate. If the objective is to compare various treatment effects on the composition and diversity of ectomycorrhizae then morphotyping will suffice, as long as the necessary precautions are taken in order to decrease the likelihood of identification errors (e.g. identifying to the genus level for genera with multiple, anatomically similar species).

Given the vast array of ecological interactions that ectomycorrhizae facilitate, maintaining ECM diversity is essential for preserving critical ecosystem processes. This is because a significant degree of physiological variation exists between species of ectomycorrhizal fungi. For example, ECM species differ in their ability to sequester and translocate various forms and types of nutrients; the rate at which these nutrient are sequestered and translocated; the production of various hormones and enzymes; their sensitivity to allelochemicals leaching from litter; and their tolerance to temperature and water stresses (reviewed by Bruns 1995; Cairney 1999; Kropp and Langlois 1990; Zak and Visser 1996). Different species of ECM fungi also respond differently to temperature

fluctuations which implies that the seasonal activity of different species also varies (Smith and Read 1997). Based on this intraspecific variation it has been hypothesized that ECM diversity enhances the ability of host plants to respond to spatial and temporal environmental variability (Perry et al. 1989). Thus, given the degree of functional diversity associated with ectomycorrhizae, the preservation of ECM species diversity is desirable as preserving species diversity will, in turn, preserve critical ecological interactions.

Ecosystem Management

In the past couple of decades the general public has become increasingly concerned about the state of our forests. As this concern has mounted forestry companies have responded by adopting more sustainable, ecosystem friendly policies. One of the main objectives associated with these policies pertains to the conservation of forest biodiversity. By conserving forest biodiversity it is argued that the ecological processes that these organisms govern will also remain intact and, thus, the health of the forest will not be compromised (Galindo-Leal and Brunell 1995). The natural disturbance paradigm of forest management (silvicultural practices that emulate natural disturbances such as fire) is being adopted by forest managers as a means of maintaining forest biodiversity (Bergeron et al. 1999). However, our current knowledge in regards to the structure and function of forest ecosystems is inadequate for forest managers to formulate sound practices and policies for natural disturbance management (Maini 1998). A number of large, multidisciplinary research projects have been designed throughout the world's

forests in order to address this problem. One such project is the Ecosystem Management by Emulating Natural Disturbances (EMEND) research program.

Study Site

The research reported in this thesis is one of the numerous studies undertaken at the EMEND research site, which is located in the mixed-wood boreal region of northwestern Alberta, Canada ($56^{\circ} 44' N$, $118^{\circ} 20' W$). One of the main objectives of the EMEND research site is “to determine which forest harvest and regenerative practices best maintain biotic communities, spatial patterns of forest structure and functional ecosystem integrity in comparison with mixed-wood landscapes that have originated through wildfire and other inherent natural disturbances” (Volney et al. 1999, p 244). One of the main questions that researchers will be addressing is “how much residual matter is required to preserve and protect the critical aspects of ecosystem function?” (Spence et al. 1999, p 584).

The EMEND research site encompasses four forest stand types: deciduous dominated (>70% deciduous), deciduous dominated with a conifer understory, mixedwood (with both deciduous and conifer sharing the canopy) and conifer dominated (>70% coniferous) (Volney et al. 1999). A variety of harvesting treatments was prescribed for each of the forest stand types. Originally, a series of controlled burns was planned for the experiment as well, however, only one burned stand (in the conifer dominated forest type) was available at the time of this study. Due to the logistical problems associated with sampling all of the stands (n=100) only those belonging to the

conifer dominated stand type were included in the present study (Appendix I). The white spruce trees in these stands ranged from 114 to 131 years old.

The dominant tree species at the EMEND research site are white spruce (*Picea glauca* [Moench] Voss), trembling aspen (*Populus tremuloides* Michx.), and balsam poplar (*Populus balsamifera* L.). Black spruce (*Picea mariana*), balsam fir (*Abies balsamea*), lodgepole pine (*Pinus contorta*) and paper birch (*Betula papyrifera*) may also be found scattered throughout the site. The understory is dominated by *Alnus spp.*, *Calamagrostis canadensis*, *Cornus canadensis*, *Epilobium angustifolium*, *Ribes triste*, *Rosa acicularis*, *Rubus pubescens*, *Sherpherdia canadensis* and *Viburnum edule* (Fenniak 2001). Forests in this region receive an average precipitation of 464mm a year, nearly two-thirds during the summer months, and have an average summer temperature of 12.8⁰C (Strong and Leggat 1992). Soils are fine-textured lacustrine deposits (Kishchuk, pers. comm.)

The study site was harvested during the winter of 1998-1999 in a randomized block design. My work focused on unharvested control, clear-cut and three partial cut treatments (with 20%, 50% and 75% live residual remaining). Three replicate stands for each treatment were positioned throughout the EMEND site. For comparison purposes a burned stand was also sampled. The partial cut stands were strip harvested using 5m wide machine corridors spaced 20m apart (ie. 20m wide residual strips). The machine corridors were oriented north-south (perpendicular to the prevailing winds) in order to protect the residual trees from wind-throw damage following harvesting (Volney et al. 1999). Residual trees were also left in all of the sites, including the clear-cut stands, in the form of two elliptical shaped patches (approximately 0.25 and 0.75 ha, respectively).

These ellipses were designed to emulate 'fire skips', which are common features inherent to wildfire dominated forest landscapes, and are believed to be an effective source of colonists for weak dispersing species at risk (Volney et al. 1999). All of the harvesting treatments were applied to areas approximately 10 ha in size.

A total of 150 sampling locations, randomly located on 30m long transects, were established within the EMEND research site (Figure 1-1). Three parallel transects (spaced 20m apart) were positioned within the un-harvested control and clear-cut stands. Six transects (oriented parallel to the machine corridors and spaced 10m apart) were positioned within each of the partial cut stands. This spacing allowed the center of 3 adjacent residual strips and machine corridors to be sampled. Within the burned stand three transects were established in both burned and unburned portions of the stand (at least 30m from a burn-unburned edge). Portions of the stand characterized as burned were void of an organic soil horizon and standing live trees. Unburned portions of the stand were untouched by fire and, thus, were visually similar to the un-harvested control stands.

Objectives

As discussed, ectomycorrhizae facilitate a host of ecological processes, all of which have implications for the productivity, stability and sustainability of forest ecosystems. However, little is known about the ectomycorrhizal community in the boreal forest of northwestern Alberta, as well as the impact timber harvesting is having on these communities. Therefore, the overall objectives of this thesis were to: 1) quantify the impact of various harvesting practices on the biodiversity of white spruce

ectomycorrhizae in northwestern Alberta; 2) determine whether mechanical site preparation influences the ectomycorrhizal status of planted white spruce seedlings; 3) provide guidelines which will assist forest managers in determining the best silvicultural practices for ensuring successful white spruce regeneration based on observed relationships between white spruce regeneration and the biodiversity of ECM fungi.

In all biodiversity studies the major challenges are to accurately and comprehensively document diversity and to understand at which taxonomic level, or by which ecological criteria, diversity is important. For example, many studies focus on species-level diversity while others examine diversity of functional types. Furthermore, studies in which species identification is difficult, or impractical, may examine diversity at higher taxonomic levels such as genus or family.

In this study, I examined the diversity of ECM morphotypes recognizing that some of these morphotypes are comprised of many different genera or species. Thus, in this thesis all references to ectomycorrhizal diversity refer to the diversity of ectomycorrhizal morphotypes.

This thesis is composed of three studies. The first study (Chapter 2) addresses the first objective. Specifically, this study was designed to assess the effectiveness of partial cutting in maintaining the biodiversity of white spruce ectomycorrhizae and to compare the ectomycorrhizal community of burned, partial cut and clear-cut stands. Two separate sets of soil cores were collected throughout the conifer dominated stands located within the EMEND research site. The first set was used to determine the density of fine root tips throughout the various stands while the second set was used in order to assess the ectomycorrhizal community associated with the active white spruce fine root tips.

The second study (Chapter 3) also addresses the first objective, however, the sampling for this study was limited to the clear-cut treatments. The aim was to determine if small patches of residual trees left within clear-cuts are an effective means of maintaining the biodiversity of ectomycorrhizae and, if the size of the residual patch influences the ectomycorrhizal community adjacent to the residual patches. Field sampling for this study involved collecting soil cores along transects, which were positioned perpendicular to the edge of the residual patches, in order to investigate how intact forest edges influence ectomycorrhizal communities.

The third study (Chapter 4) was also limited to the clear-cut and was designed to investigate the impact of mechanical site preparation on the ectomycorrhizal status of planted white spruce seedlings. This involved assessing the ectomycorrhizae associated with white spruce seedlings that were planted in scalped, mixed and mounded microsites. The ectomycorrhizal community of these seedlings was then compared to seedlings collected from the untreated control portions of the silviculture plots.

The final chapter of this thesis (Chapter 5) summarizes the results obtained from each of these studies. Within this chapter some limitations associated with these studies and suggestions for future research are also presented in order to address the third objective of this thesis.

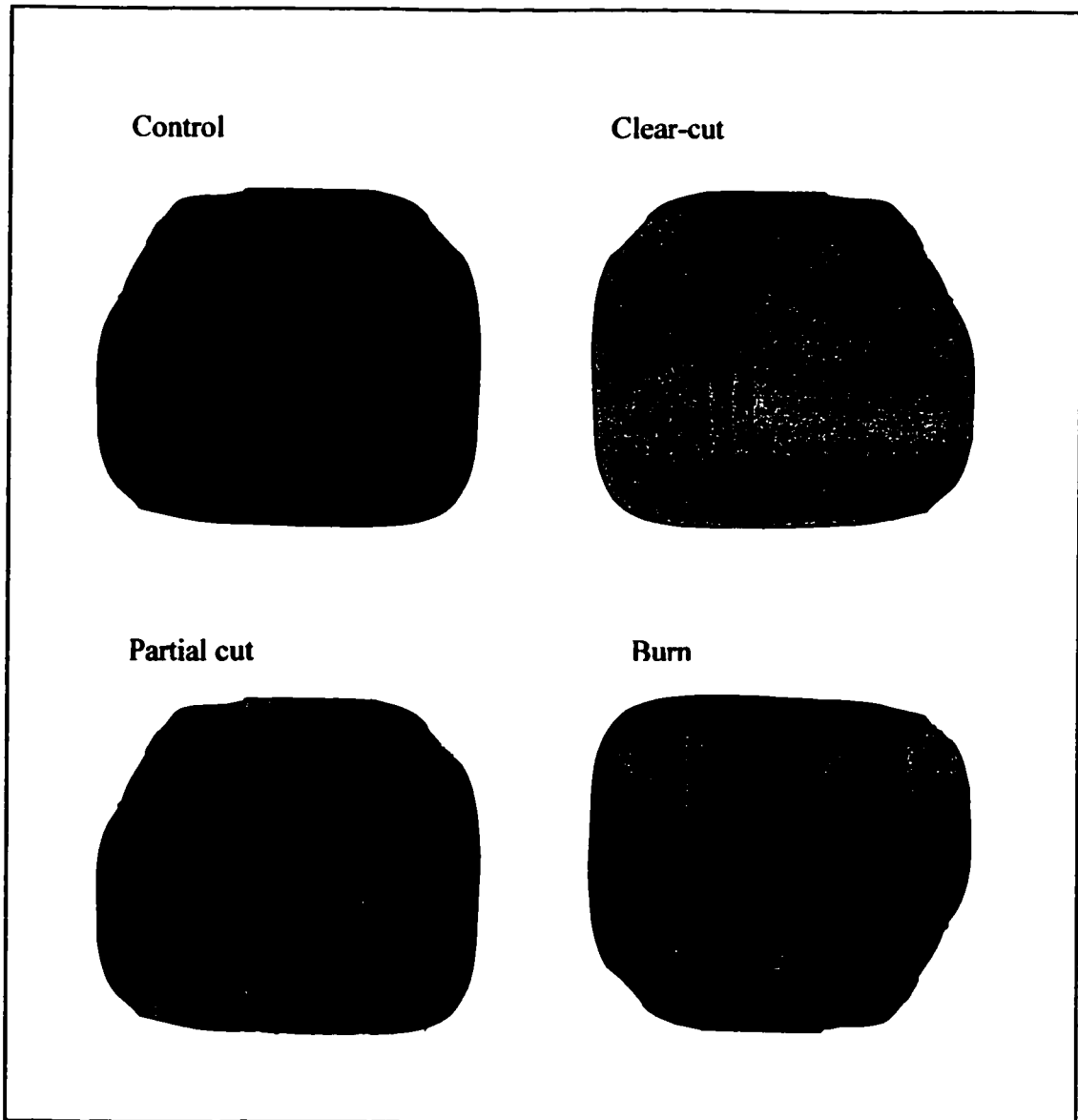


Figure 1-1. Diagram illustrating the positioning of sampling locations along 30m transects located within the burn, clear-cut, partial-cut and unharvested control stands at the EMEND research site. One burned stand and three replicate clear-cut, partial-cut and control stands were surveyed. Transects were 20m apart in the clear-cut and control stands, 10m apart in the partial cut stands [in order to sample both the machine corridors (light shading) and residual strips (dark shading)] and randomly positioned in burned (light shading) and unburned portions (dark shading) of the burned stand. Figure is not to scale.

*Note: Sampling positions were randomly positioned along each transect.

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Chapter 2

The impact of clear-cutting, partial cutting and burning on the abundance and diversity of *Picea glauca* ectomycorrhizae in northwestern Alberta

Introduction

The impact of disturbances on ectomycorrhizae is well documented. Stand replacing wildfires have been shown to significantly reduce the biodiversity of ectomycorrhizae. Stendell et al. (1999) reported an eight-fold decrease in fungal biomass 1-year post fire in a Ponderosa pine forest in the Sierra Nevada while Visser (1995), who sampled a chronosequence of jack pine stands originating from wildfire, noted that the ectomycorrhizal (ECM) species richness progressively increased with increasing stand age. Traditional forest practices, such as clear-cutting, have also been shown to significantly reduce the biodiversity of ectomycorrhizae in a variety of different forest types (Bradbury et al. 1998; Byrd et al. 1999; Durall et al. 1999; Hagerman et al. 1999a; Harvey et al. 1980a).

There is also strong evidence that ECM communities are positively influenced by forest edges. For example, both Hagerman et al. (1999a) and Kranabetter and Wylie (1998) observed a decrease in ECM biodiversity with increasing distance from a forest edge into a clear-cut. In both of these studies 'late stage' ectomycorrhizae, which appear to require intact hyphal connections with mature host trees in order to colonize new roots (see Chapter 1), became less abundant with increasing distance away from the intact forest edge. Based on this observation Hagerman et al. (1999b) hypothesized that

residual material (e.g. mature live trees) left on site after harvesting would be an effective way of preserving ECM biodiversity. This hypothesis is further supported by Kranabetter (1999) who noted that seedlings growing near residual mature trees in clear-cuts possessed similar ECM communities to seedlings growing in an adjacent forest, and that morphotype richness was greater on seedlings growing in proximity to mature trees in both the clear-cut and adjacent forest. One harvesting treatment that would retain residual mature trees is partial cutting, however, the impact of such a harvesting prescription on ECM communities has not been tested.

Forestry companies across Canada are beginning to adopt harvesting techniques designed to emulate natural disturbances (see Chapter 1). In northwestern Alberta, wildfire has historically been responsible for shaping the forest landscape. As opposed to traditional forestry practices, which uniformly remove the majority of trees in a harvesting area, forest landscapes created by wildfire tend to be a heterogeneous mosaic of burned and unburned patches (Eberhart and Woodard 1987; Johnson 1992). The residual live trees associated with these undisturbed patches are generally believed to act as refugia for species not suited to the pioneer life (Rowe and Scotter 1973).

The objectives of this study were to (i) assess the effectiveness of partial cutting in maintaining white spruce ECM biodiversity and, (ii) to determine how different harvesting prescriptions, designed to emulate natural disturbances, compare to fire with respect to effects on the ECM community. Two approaches were used to achieve these objectives. The first involved quantifying the density of white spruce fine root tips throughout the Ecosystem Management by Emulating Natural Disturbances (EMEND) research site to determine the total ECM abundance in the stands. The second approach

involved assessing the ECM community in each stand by identifying ectomycorrhizae, based on morphological and anatomical characteristics, associated with the active fine roots.

Materials and Methods

Study site

Field sampling was completed at the EMEND research site. A detailed description of the research site can be found elsewhere in this thesis (see Chapter 1). The treatments sampled included unharvested control stands, both machine corridors and residual strips located in partial cut stands (with 20, 50 and 75% live residual remaining) and both burned and unburned portions of a burned stand (Figure 2-1).

Field sampling

Fine root tip density. The density of white spruce fine roots was determined by obtaining a soil core (2cm diameter by 15cm deep) at each of the sampling locations (5 samples x 9 treatments x 3 replicates), with the exception of the unburned section of the burned stand, during June 2000 (two field seasons post disturbance). The soil cores were placed in a plastic 'zip-lock' bag, transported to the University of Alberta, and stored at 4°C for up to 3 months until processing was completed. This involved placing the soil cores within two nested soil sieves (32 mesh/inch and 115 mesh/inch) and isolating the root tips by gently rinsing off the adhering soil and organic debris. The fine roots from both sieves were subsequently characterized as inactive or active, using a stereomicroscope, based on morphological criteria outlined by Harvey et al. (1976).

Inactive root tips were characterized as having a wrinkled texture and a dark apex. In contrast, the majority of active root tips were smooth, turgid and had a pale or creamy white apex. Ectomycorrhizae formed by *Cenococcum geophilum* and *Tomentella* spp., however, were exceptions. The mantles formed by these species are hard and darkly pigmented, thus, root tips associated with these fungi are uniformly smooth and dark. For this reason root tips associated with these two morphotypes were carefully examined during the characterization process. Only white spruce fine root tips were examined and all root tips lacking a distinguishable apex were disregarded.

Ectomycorrhizal community. The white spruce ectomycorrhizal community at each of the sampling locations was characterized by obtaining a 3.5cm diameter by 15cm deep soil core at each of the sampling locations (n=150; 5 samples x 10 treatments x 3 replicates) during August 2000. The soil cores were stored in PVC pipe (to maintain the integrity of the core during transport), transported to the University of Alberta and stored for up to 4 months at 4°C during processing. The isolation of the fine roots was identical to that mentioned above. Once isolated, all active root tips were further classified as ectomycorrhizal or non-ectomycorrhizal. Using both stereo (Zeiss Stemi 2000-C, 7-40X) and compound (Leitz Labrolux K, 500-1000X) microscopes the ectomycorrhizal root tips were subsequently grouped based on their morphological and anatomical characteristics according to the protocol outlined by Goodman et al. (1996). Morphological features included the shape, color and texture of the ECM system and emanating elements (hyphae and mycelial strands). Some examples of anatomical features include mantle patterning; the size, color, ornamentation, and contents of hyphal cells; the type and

frequency of septa; and reactions to specific chemical compounds (e.g. KOH, sulphovanillin). Active root tips lacking distinguishable mantle features were designated “undetermined” according to Kranabetter and Wylie (1998). This included root tips with an immature or poorly developed mantle. When possible, 50 ectomycorrhizal root tips per core were grouped using this method, however, due to the limited number of active root tips observed in some of the cores (see Figure 2-3) this was not always possible. In this instance all of the active root tips encountered in the soil core were characterized. The distinct groups, or ‘morphotypes’, were then identified to the genus level by comparing them to published ectomycorrhizal descriptions (Agerer 1987-1998; Ingelby et al. 1990; Goodman et al. 1996; Kernaghan 2001). No attempt was made to identify the morphotypes past the genus level, except for those genera with only one known species, due to the similarities among species within genera. For the purpose of this study, which was to detect treatment effects on the ECM community, this level of identification was deemed sufficient.

Data analysis

Fine root tip density. The percentage of active fine root tips was calculated, for each soil core, as a proportion based on the total number of white spruce fine root tips observed.

Ectomycorrhizal community. The frequency of occurrence for each morphotype was calculated according to the number of soil cores and stands that the morphotype was observed in. The relative abundance of each morphotype was calculated, for each soil

core, as a proportion based on the total number of active root tips characterized. Ectomycorrhizal richness was then calculated for each soil core (simply the number of morphotypes present in each soil core) and for each stand (e.g. clear-cut and unharvested control) or portion of the stand (e.g. partial cut, burn) based on the total number of morphotypes observed in the 5 soil cores collected per treatment. Lastly, ECM diversity was quantified for each soil core and stand (according the average relative abundance of morphotypes from the 5 soil cores sampled per stand) using Shannon-Wiener diversity index (see Magurran 1988). Active fine root tips classified as non-mycorrhizal or undetermined were excluded from the calculation of both morphotype richness and diversity.

An analysis of variance (ANOVA) was used to test the treatment effects on the total number of fine root tips and the percentage of active fine root tips [main effects: Treatment (fixed), Block (random) and Treatment * Block (random)]. The blocks were considered the true replicates of treatment type while the soil cores collected within the various treatments were treated as subsamples. The data were log transformed prior to the analysis in order to meet the assumption of normality. The ECM richness and diversity data, at the soil core level, also had a non-normal distribution. Due to the frequency of zero abundance in many of the soil cores these distributions were highly skewed, thus, transformation of these data failed to improve normality. Therefore, a non-parametric test (Kruskal-Wallis H) was used to test the treatment effects on ECM diversity. The ECM richness, which is a count variable, was analyzed with the GLIMMIX macro (SAS Version 8.01, 1999-2000) using the Poisson error and the ANOVA model mentioned above. To test stand level ECM richness and diversity a

simplified ANOVA design, with treatment as the main effect and the three stands as replicates, was used. Significant ANOVA results ($p < 0.05$) were followed by Tukey-Kramer (honestly significant difference) test in order to detect differences among treatments. All data analysis, with the exception of ECM richness at the soil core level, was performed using SPSS Version 10.0 (SPSS 1999).

A detrended correspondence analysis (DCA) was used to further examine the treatment effects on the ECM community. Only common morphotypes, those observed in more than 5% of the soil cores, were included in the analysis which was performed using CANOCO version 4.0 (ter Braak and Smilauer 1998).

Results:

White spruce fine root tip density. Nearly 107,000 white spruce fine root tips were characterized as inactive or active throughout the study site. The total number of fine root tips per soil core increased from approximately 490 in the burned and clear-cut stands to just over 1300 in the control stands (Figure 2-2). Furthermore, the density of fine root tips in the thinned stands, an average of nearly 575, was intermediate to the burned/clear-cut and control stands. However, due to the high degree of variability within the treatments these differences were not statistically significant (Table 2-1; $p = 0.220$).

The percentage of active fine root tips differed significantly between the treatments (Table 2-1; $p < 0.001$) and appeared to be correlated with the level of disturbance (Figure 2-3). Highly disturbed stands, such as the burned and clear-cut stands, had a significantly lower percentage of active fine root tips (1.6 ± 1.1 and $4.5 \pm$

2.7 percent, respectively) compared to the undisturbed control stands (23.2 +/- 13.7 percent). Within the residual strip portions of the partial cut stands the percentage of active fine root tips increased from the 20 to the 50 and 75% partial cut stands, respectively, while the machine corridor portions were not significantly different from one another or from the burn and clear-cut stands. Furthermore, the percentage of active fine root tips was significantly greater in the residual strip portions of the 20 and 75 percent thinned stands compared to the machine corridor portions of the same stands.

Ectomycorrhizal community. A total of 4431 white spruce fine root tips were classified as active and, of these tips, an overwhelming majority was also characterized as mycorrhizal (99.4%). Throughout the entire study site a total of 19 distinct morphotypes were described (Table 2-2; refer to appendix II for a thorough description of each morphotype). The most common morphotypes was *Tomentella* spp. followed by *Cortinarius* spp., *Amphinema byssoides*, *Lactarius* spp., E-strain, *Mycelium radialis atrovirens* (MRA) and *Hebeloma* spp., respectively. These seven morphotypes formed the majority of the ectomycorrhizae (nearly 72% of the root tips), while none of the remaining 12 morphotypes were observed on more than 5% of the root tips. The most ubiquitous morphotype was *Tomentella* spp., which was observed in nearly 57% of the soil cores and was the only morphotype found in every stand.

Ectomycorrhizal richness was significantly different between the treatments at both the soil core ($X^2=109.53$; $p<0.001$) and stand levels (Table 2-3; $p<0.001$). At both levels, the number of morphotypes decreased with increasing harvesting intensity (Figure 2-4a,b). The richness of the residual strip portions of the partial cut stands decreased

somewhat according to the percentage of trees remaining on site, however, this decrease was not statistically significant compared to the control stands. Within the partial cut stands morphotype richness was lower in the machine corridors compared to the residual strip portions and, lastly, the number of morphotypes was lowest in the burn and clear-cut stands.

Ectomycorrhizal diversity was also significantly different between treatments, at both the soil core ($X^2=56.48$; $p<0.001$) and stand levels (Table 2-3; $p<0.001$), according to the level of disturbance (Figure 2-5a,b). The values were significantly greater in the control stands compared to the burn and clear-cut stands. Within the partial cut stands the diversity of residual strip portions increased slightly from the 20 to the 50 and 75% stands, respectively, while the 50% stands had the greatest value in the machine corridor portions. The diversity of ectomycorrhizae was greater in the residual strip portions compared to the machine corridor portions, however, this difference was not statistically significant. Furthermore, the ECM diversity was similar between control and the residual strip portions of partial cut stands. The lowest ECM diversity was observed in the burned stand.

The results from the ordination analysis provided more insight into the treatment effects on the ECM community (Figure 2-6). The clustering of the stands along the first ordination axis ($\lambda=0.4787$) imply that it was correlated with harvesting intensity. The site scores for the partial cut stands were intermediate to the heavily disturbed burn and clear-cut stands, which were positioned at the left end of the 1st DCA axis, and the undisturbed control stands, which were positioned on the right end of 1st DCA axis. Morphotypes on the left side of the diagram (e.g. MRA and *Tomentella* spp.) were

associated with the burn and clear-cut stands while morphotypes on the right side of the diagram (e.g. *Amphinema byssoides*, *Cortinarius* spp., E-strain, *Piloderma* spp. and *Lactarius* spp.) were associated with the unharvested control stands. Furthermore, *Cenococcum geophilum*, *Hebeloma* spp., *Russula* spp. and *Thelephora terrestris* were positioned among the partial cut stands.

Discussion

Root tip density

In this study harvesting significantly reduced the percentage of active fine root tips. In the clear-cut stands only 4.5% of the fine root tips were classified as active compared to 23.2% in the control stands. This is similar to the findings of Hagerman et al. (1999a) who studied the persistence of ECM fine roots in subalpine clear-cuts in the southern interior of British Columbia. In their study only 4% of the fine root tips observed in soil cores obtained from 2-year old clear-cuts were characterized as active compared to 30% in the adjacent forest. In addition, both Harvey et al. (1980a) and Parsons et al. (1994) reported the complete elimination of active fine roots two years after fall clear-cutting of Douglas-fir - larch stands and summer harvesting of lodgepole pine sites. There are a few explanations why in the present study a small proportion of root tips were able to persist in the clear-cut stands following the removal of the host trees. The root tips may have remained viable by utilizing the carbon reserves present in excised roots (Ferrier and Alexander 1985). It is also possible that the root tips were supplied with carbon, via hyphal linkages, by ectomycorrhizae associated with refuge plants. This has been suggested for paper birch (Kranabetter 1999) and a number of

woody understory plants (Hagerman et al. 2001) which, in clear-cut sites, have been shown to have a number ectomycorrhizae in common with the adjacent canopy trees. Lastly, the fungal mantle itself may have provided physical protection against desiccation as well as decay (Smith and Read 1997), which would enable certain root tips to persist, at least temporarily, without a host. This may have been the case for *Cenococcum geophilum* and *Tomentella* spp. which form thick, hard mantles. In fact, the majority of active root tips encountered in the burn and clear-cut soil cores in this study were morphologically similar to these ectomycorrhizae (per. obs.). This may also explain the increased abundance of both *Cenococcum* (Byrd et al.2000; Hagerman et al. 1999a; Schoenberger and Perry 1982) and *Tomentella* (Bradbury et al. 1998; Purdy et al. *in press*) morphotypes in disturbed sites.

The percentage of active fine root tips was significantly decreased in the residual strip portion of the partial cut stands that were thinned (e.g. 20 and 50% treatments) compared to the control stands. In contrast, Harvey et al. (1980b) failed to observe a difference in the density of active root tips in a partial cut (approximately 50% of the overstory removed) Douglas-fir – larch stand compared to an unharvested control stand. However, the results obtained by these authors should be treated with caution as only one partial cut and one control stand were available for comparison. The percentage of active fine roots in the residual strip portions of the 75% stands (which were not thinned) did not differ from the unharvested control stands. This is not surprising, as it is unlikely that the number of host root tips available for ectomycorrhizae to colonize would have been affected in this portion of the partial cut stands.

It was interesting that, despite being located within the rooting zone of adjacent trees, the fine root tip density in the machine corridors was drastically reduced. This finding is consistent with Parsons et al. (1994) who observed a significant reduction in active root tip density within 5-6m from the forest edge (into a clear-cut) and Hagerman (1997) who also reported a significant decrease in active root tip density in soil cores obtained only 2m from the forest edge. Based on these results it is logical to conclude that some factor, other than the removal of host trees, contributed to the observed reduction in root tip density in this study. Compaction, caused by harvesting equipment, is one likely candidate as it has been shown to increase the bulk density of soil, which in turn reduces the hydraulic conductance and infiltration rate of the surface soil (Startsev and McNabb 2000; Williamson and Neilson 2000). Thus, compaction caused by machine traffic can restrict root growth directly, due to physically impeding root growth, and indirectly, due to the anaerobic processes associated with these anoxic soil environments (Startsev and McNabb 2001). This hypothesis is further supported by Page-Dumroese et al. (1998) who observed a significant reduction in morphotype richness on outplanted seedlings collected from compacted microsites compared to seedlings excavated from non-compacted control portions of the sampling plot.

Ectomycorrhizal community

It is difficult to compare the number of morphotypes observed at the EMEND site directly with other studies because the morphotyping procedure used in each study varies somewhat. For example, in this study no attempt was made to distinguish between species within a genus while other studies often report multiple morphotypes for some

genera. However, my results are comparable to other studies if I group morphotypes into genera. For example, in this study 19 morphotypes were characterized two field seasons after harvesting while Hagerman et al. (1999a) reported 24 morphotypes associated with hybrid spruce in a subalpine site and Mah et al. (2001) reported 21 morphotypes associated with hybrid spruce in central BC.

At the EMEND site both ECM richness and diversity were negatively correlated with harvesting intensity. The clear-cutting treatment, which removed the greatest proportion of trees, had the greatest effect on ECM biodiversity. Significant reductions in ECM biodiversity for clear-cut sites have also been reported in lodgepole pine forests (Bradbury et al. 1998; Byrd et al. 1999), a high elevation Douglas fir - larch forest (Harvey et al. 1980a), a subalpine fir – Engelmann spruce forest (Hagerman et al. 1999a) and an interior cedar – hemlock forest (Durall et al. 1999). To my knowledge the impact of partial cutting on ectomycorrhizae has not been previously studied. However, a few studies have investigated the impact on thinning on the production of hypogeous sporocarps (Kranabetter and Kroger 2001; Waters et al. 1994). The main rationale of these studies is that because ECM fungi rely on photosynthetically derived carbon as an energy source they will be influenced by changes in host density. However, Kranabetter and Kroger (2001) failed to observe a relationship between basal area and mushroom productivity in partial cut western hemlock - redcedar stands located in northwestern British Columbia. Waters et al. (1994), who examined the effect of thinning on sporocarp production at two sites in northeastern California, also concluded that the total frequency and biomass of ECM sporocarps was not correlated with the level of thinning at their sites, however, they did observe a significant association between thinning and

the relative frequency of the most common genera of ECM fungi. In their study the relative frequency of *Gautieria* and *Hysterangium* was significantly greater in the control stands, compared to the thinned stands, while the relative frequency of *Gymnomyces* was greater in the thinned stands compared to the control stands. They concluded, based on this result, that while commercial thinning may not necessarily affect the productivity of ECM fungi, it may significantly alter the composition of ECM fungi.

The burn treatment also significantly reduced morphotype richness and diversity. This is supported by Visser (1995) who sampled a chronosequence of fire origin jack pine stands in northeastern Alberta. In her study the total number of fruiting bodies, the number of ECM fruitbodies and the number of morphotypes observed on root tips was significantly lower in the youngest stand (6 years old) compared to 41, 65 and 122 year old stands. Stand replacing wildfires differ from harvesting in that, in addition to the removal of trees, the organic layer of soil is often consumed (Johnson 1992). This is very significant with respect to ECM communities as the majority, up to 95%, of ectomycorrhizae are found in this portion of the soil (Goodman and Trofymow 1998; Harvey et al. 1976). To illustrate, Stendell et al. (1999) reported an 8-fold reduction in total ECM biomass in the litter/organic layer of a ponderosa pine stand two years after a prescribed ground fire.

The community assemblage associated with the various treatments at the EMEND site was comparable to past research involving harvesting (Hagerman et al. 1999a; Bradbury et al. 1998; Byrd et al. 1999) and wildfire (Visser 1995). While none of the common morphotypes were exclusive to the disturbed sites, the relative abundance of multistage ectomycorrhizae (e.g. *Hebeloma* spp., *Thelephora terrestris* and *Cenococcum*

geophilum) was greater on these sites. Furthermore, as illustrated by the ordination diagram, late stage ectomycorrhizae (e.g. *Cortinarius* spp., *Piloderma fallax* and *Lactarius* spp. and “cf *Tricholoma*”) were exclusive to the undisturbed sites. Thus, the reduction in ECM richness and diversity in the disturbed sites was, in large part, due to the exclusion of late stage ectomycorrhizae.

The positioning of *Tomentella* spp. among the disturbed stands in the ordination diagram suggests that it could be characterized as an early stage ectomycorrhizae, however, it was the only morphotype observed in every stand at the EMEND site. Thus, its positioning may be explained by the absence of other morphotypes in the disturbed habitats. E-strain possesses early stage characteristics (Deacon et al. 1983; Mason et al. 1983) and has previously been reported on seedlings grown in clear-cuts (Bradbury 1998; Hagerman et al. 1999b). However, in this study E-strain was observed in only 20 soil cores, and only 3 of these were from disturbed (e.g. machine corridors) habitats. This result is supported by Hagerman et al. (1999a) who also noted that E-strain was rarely observed in soil cores collected from subalpine clear-cuts in British Columbia, despite its presence on seedlings collected from the same sampling locations (Hagerman et al. 1999b). This suggests that root tips (colonized by E-strain) persisting in the soil following harvesting are not an important source of inoculum for this ectomycorrhiza. Instead, E-strain must rely on inoculum sources, such as spores or chlamydospores, which cannot be visibly detected in soil cores.

In summary, the results of this study suggest that partial cutting is an effective means of maintaining ECM biodiversity. At the stand level partial cutting also appeared to emulate the heterogeneity inherent to burned stands. However, both burning and clear-

cutting significantly decreased both ECM richness and diversity suggesting that emulating natural disturbance may not be synonymous with maintaining biodiversity (see Chapter 5). Furthermore, while this study was capable of addressing the immediate impacts of these harvesting methods, further research is required to assess the long-term impacts of these practices on ECM communities.

Table 2-1. Nested ANOVA model and results for tests of the total number of fine root tips and the percentage of active fine root tips. Tests performed using log transformed data.

Source	DF	Mean squares	<i>F</i> value	<i>p</i> > <i>F</i>
Total number of root tips				
Treatment	8	0.378	1.638	0.191
Block	2	0.167	0.722	0.501
Treatment * Block	16	0.231	2.566	0.020
Error	109	0.090		
Percentage of active root tips				
Treatment	8	2.524	13.630	< 0.001
Block	2	0.150	0.813	0.461
Treatment * Block	16	0.185	1.659	0.066
Error	109	0.112		

Table 2-2. The total number of active root tips colonized by a morphotype, relative abundance (% of root tips) and frequency of occurrence (% of soil cores or stands in which the morphotype occurred) of morphotypes encountered throughout the EMEND research site (n=150).

Morphotype	Tips	Abundance (%) ¹	Frequency (%) ²	
			Cores	Sites
<i>Tomentella</i> spp.	822	18.6	56.7	100.0
<i>Cortinarius</i> spp.	820	18.5	25.3	56.7
<i>Amphinema byssoides</i>	508	11.5	26.7	70.0
<i>Lactarius</i> spp.	368	8.3	13.3	40.0
E-strain	233	5.3	13.3	46.7
<i>Mycelium radicans atrovirens</i> (MRA)	224	5.1	24.7	66.7
<i>Hebeloma</i> spp.	221	5.0	14.0	53.3
<i>Cenococcum geophilum</i>	199	4.5	28.0	76.7
<i>Piloderma</i> spp.	170	3.8	10.7	33.3
<i>Russula</i> spp.	163	3.7	7.3	36.7
"cf <i>Tricholoma</i> "	156	3.5	8.7	26.7
<i>Tuber</i> spp.	118	2.7	4.0	20.0
"Unknown A"	87	2.0	4.0	20.0
"Basidiomycete 1"	81	1.8	2.0	10.0
<i>Thelephora</i> spp.	67	1.5	5.3	23.3
"Unknown D"	58	1.3	1.3	6.7
"Unknown B"	50	1.1	0.7	3.3
<i>Dermocybe</i> spp.	31	0.7	2.7	13.3
"non-mycorrhizal"	29	0.7	4.0	20.0
"cf <i>Paxillus involutus</i> "	17	0.4	0.7	3.3
"undetermined"	9	0.2	1.3	6.7
Total	4431			

¹ Relative abundance for each morphotype calculated as:
(number of root tips on which morphotype was observed) / (total number of root tips sampled) X 100.

² Frequency of occurrence for each morphotype calculated as:
(number of soil cores or sites in which morphotype was observed) / (total number of soil cores or sites) x 100.

Table 2-3. ANOVA model and results for tests of the per site values for ectomycorrhizal richness and diversity.

Source	DF	Mean squares	<i>F</i> value	<i>p</i> > <i>F</i>
Richness				
Treatment	9	33.052	8.309	< 0.001
Block	2	1.033	0.245	0.785
Error	18	4.219		
Diversity				
Treatment	9	1.039	9.614	< 0.001
Block	2	0.147	1.357	0.283
Error	18	0.108		

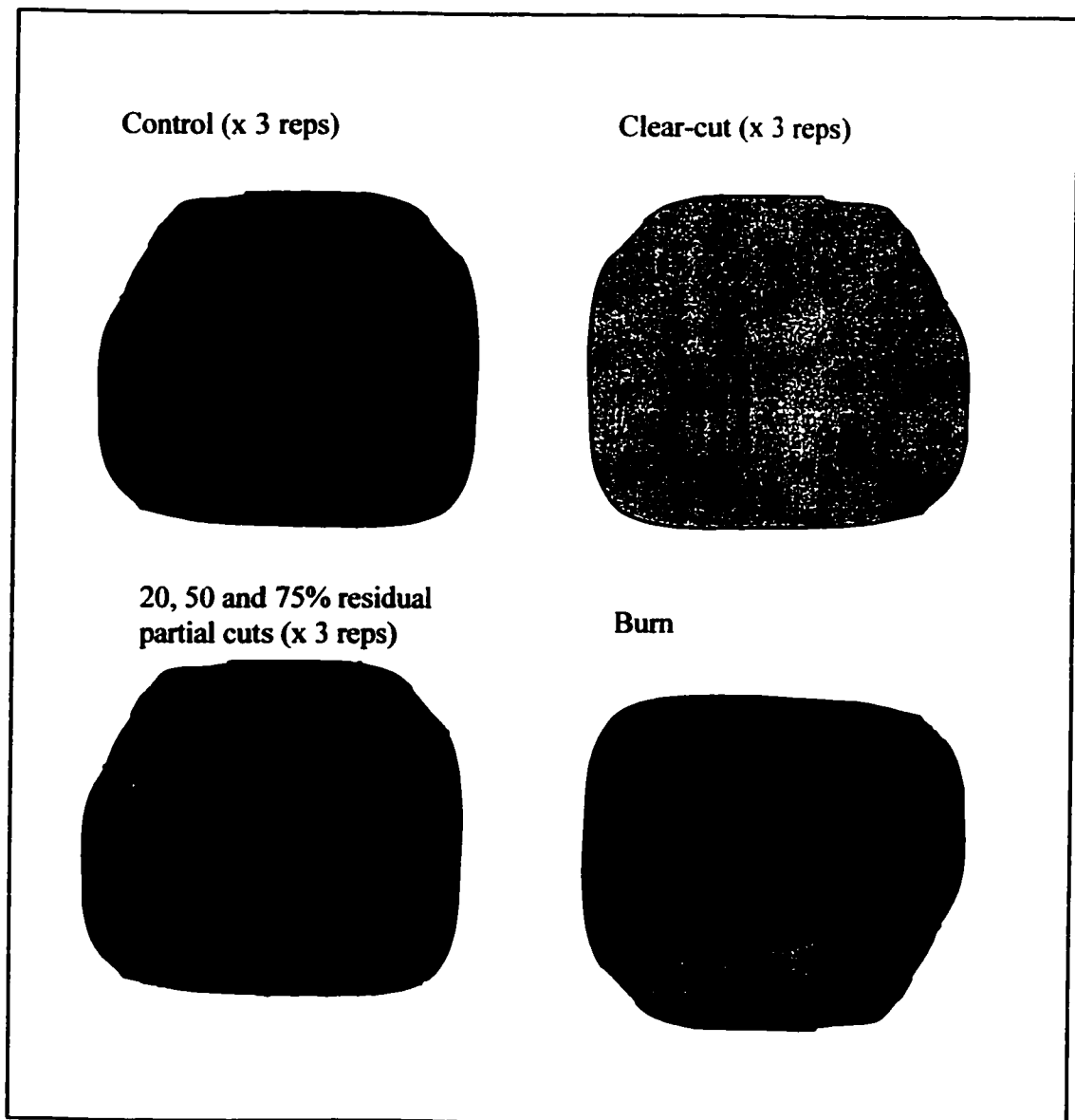


Figure 2-1. Diagram illustrating the positioning of transects (30m long) within the burn, clear-cut, partial-cut and unharvested control stands at the EMEND research site. One burned stand and three replicate clear-cut, partial cut and control stands were surveyed. Transects are 20m apart in the clear-cut and control stands, 10m apart in the partial cut stands [in order to sample both the machine corridors (light shading) and residual strips (dark shading)] and randomly positioned in burned (light shading) and unburned portions (dark shading) of the burned stand. Figure is not to scale.

***Note:** Sampling positions were randomly positioned along each transect.

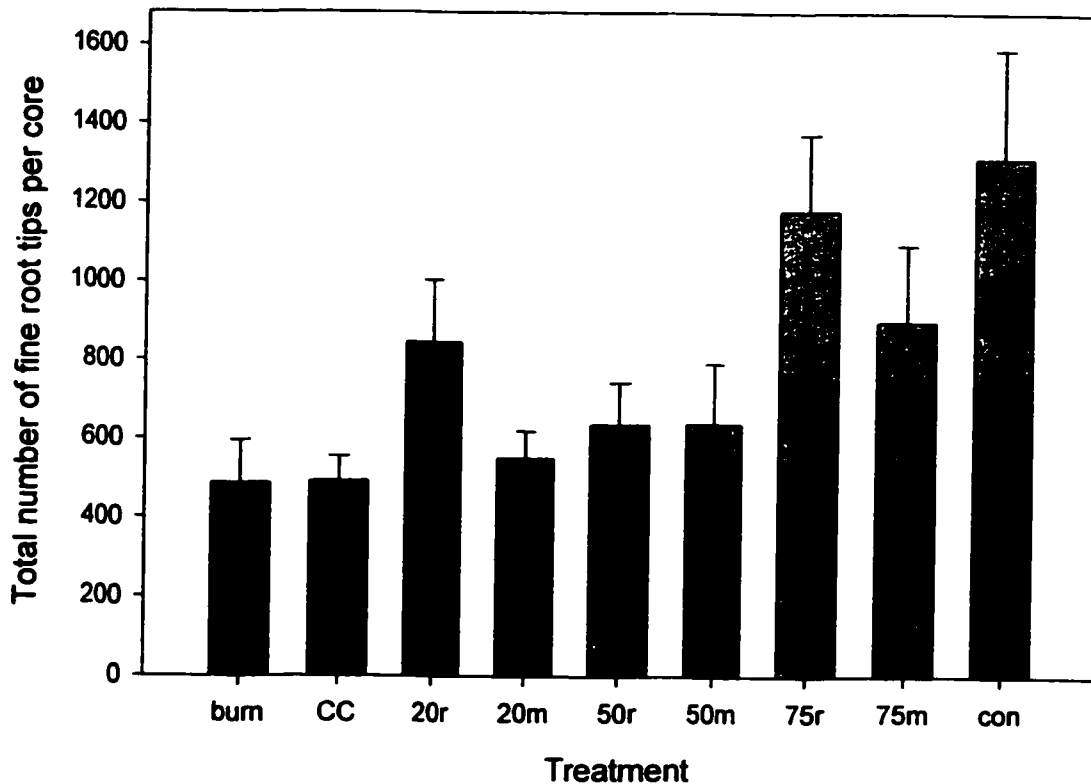


Figure 2-2. The mean number of white spruce fine root tips (active and inactive) observed in soil cores collected from the burn (burn), clear-cut (CC), partial cut [with 20, 50 and 75% residual remaining and sampled in both machine corridors (m) and residual strips (r)] and unharvested control stands (con) at the EMEND research site. There was no significant difference among treatments ($P=0.220$). Analysis performed using log transformed data. Error bars represent 1SE (based on the 15 soil cores per treatment).

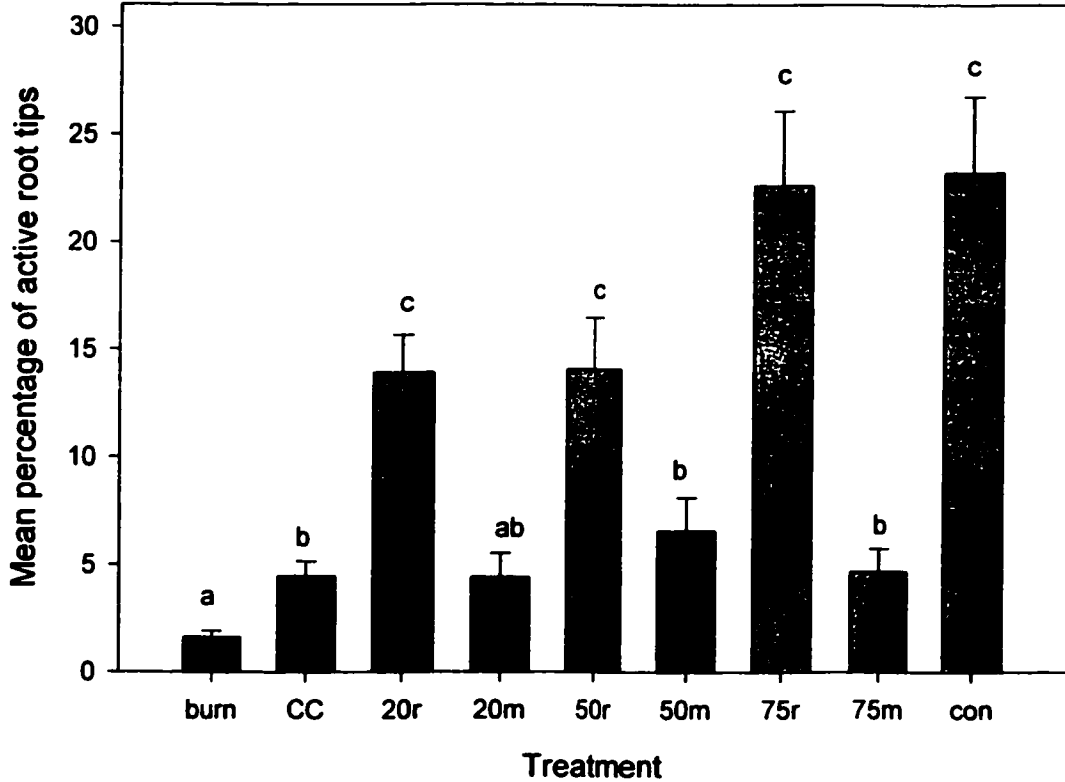


Figure 2-3. The mean percentage of active white spruce fine root tips in soil cores collected from burn (burn), clear-cut (CC), partial cut [with 20, 50 and 75% residual remaining and sampled in both machine corridors (m) and residual strips (r)] and unharvested control stands (con) at the EMEND research site. Treatment means with different letters are significantly different [$\alpha=0.05$; Tukey-Kramer (honestly significant difference) pairwise comparison test] (ANOVA: $P<0.001$). Analysis performed using log transformed data. Error bars represent 1SE (based on the 15 soil cores per treatment).

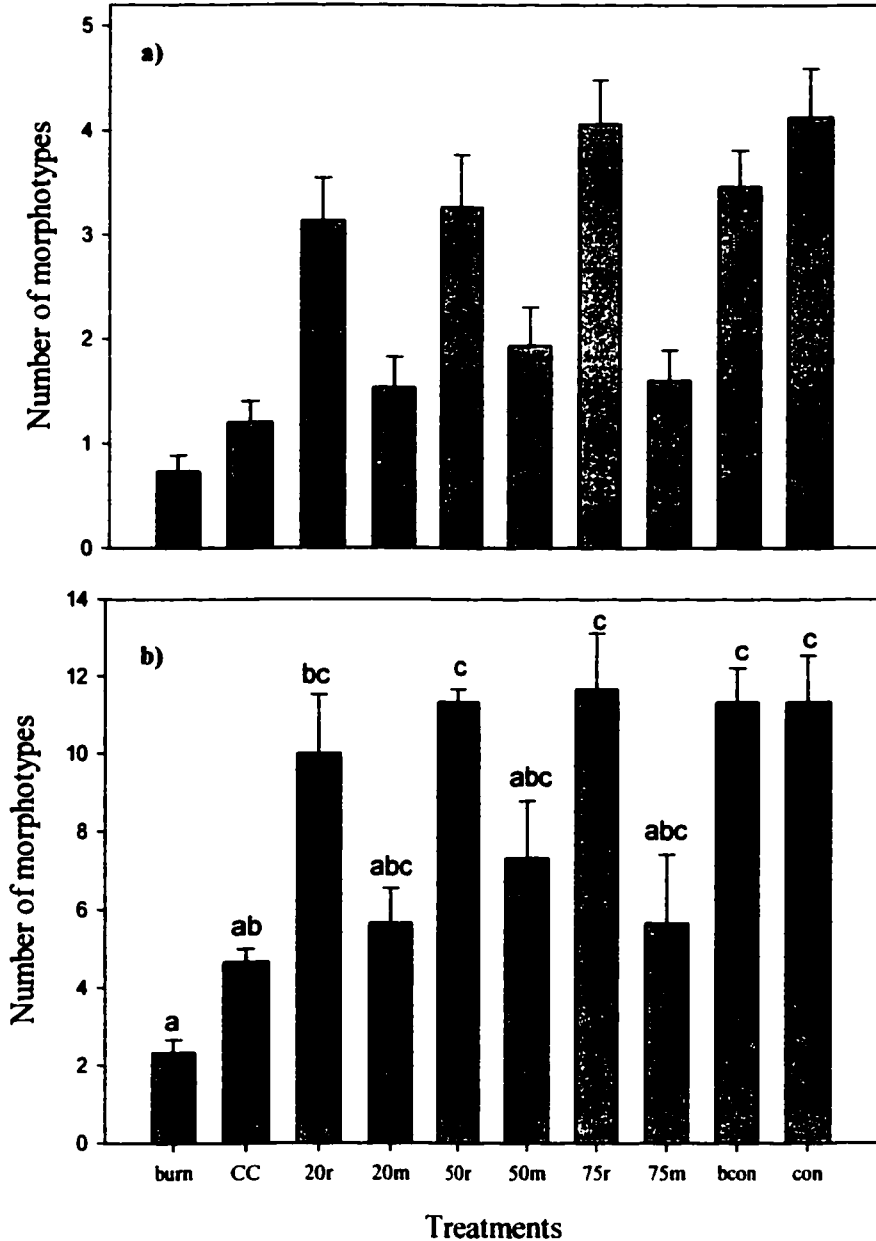


Figure 2-4a,b. Ectomycorrhizal richness calculated at the soil core (a) and stand (b) levels in burn (burn), control portion of the burn (bcon), clear-cut (CC), partial cut [with 20, 50 and 75% residual remaining and sampled in both machine corridors (m) and residual strips (r)] and unharvested control stands (con) two growing seasons after harvesting. Treatment means are significantly different ($P < 0.001$; $\alpha = 0.05$) at both the soil core (GLIMMIX; no post hoc treatment comparisons were made) and stand levels (ANOVA). Treatment means with different letters are significantly different according to Tukey-Kramer (honestly significant difference) pairwise comparison test. Error bars represent 1SE [based on 15 soil cores (a) and the 3 stand replicates (b), respectively].

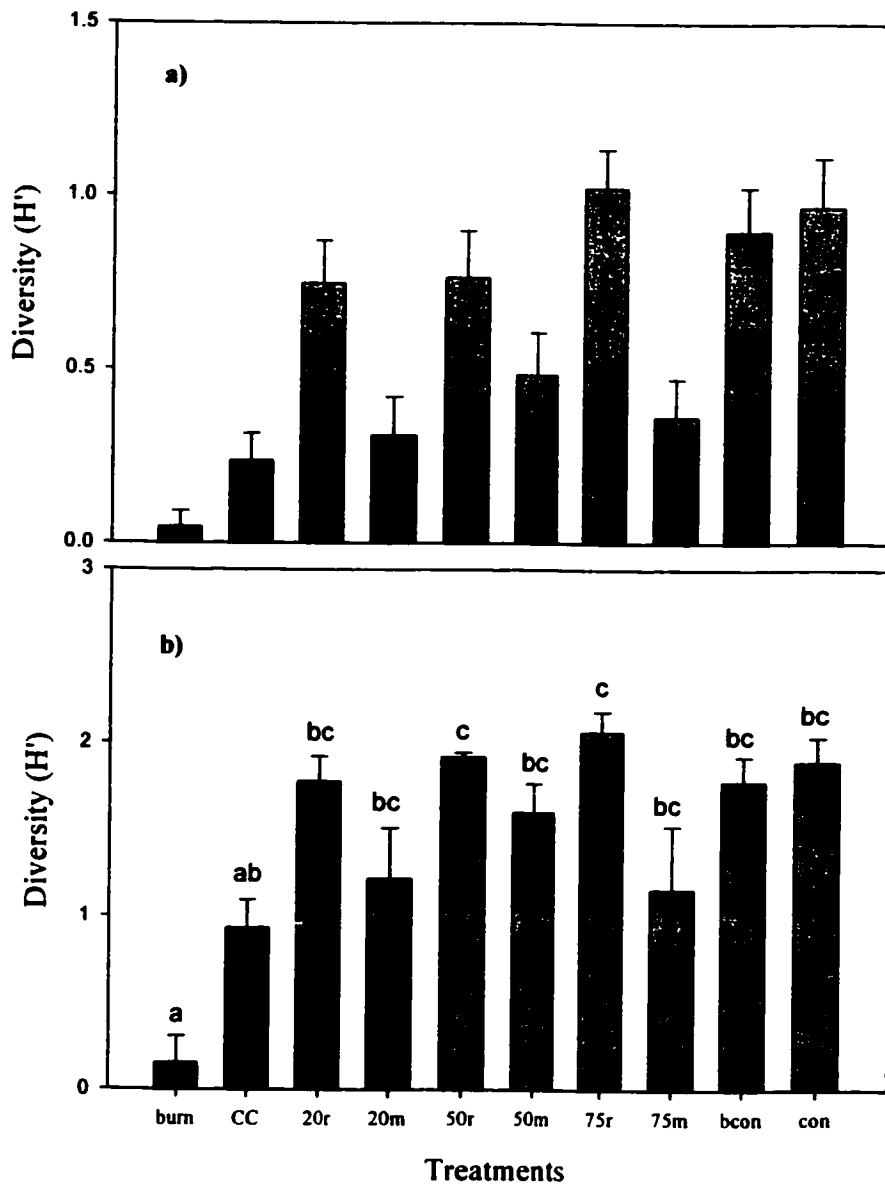


Figure 2-5a,b. Ectomycorrhizal diversity (H') calculated at the soil core (a) and stand (b) levels in burn (burn), control portion of the burn (bcon), clear-cut (CC), partial cut [with 20, 50 and 75% residual remaining and sampled in both machine corridors (m) and residual strips (r)] and unharvested control stands (con) two growing seasons after harvesting. Treatment means are significantly different ($P < 0.001$; $\alpha = 0.05$) at both the soil core (Kruskal – Wallis H; no post hoc treatment comparisons were made) and stand levels (ANOVA). Treatment means with different letters are significantly different according to Tukey-Kramer (honestly significant difference) pairwise comparison test. Error bars represent 1 SE [based on 15 soil cores (a) and the 3 stand replicates (b), respectively].

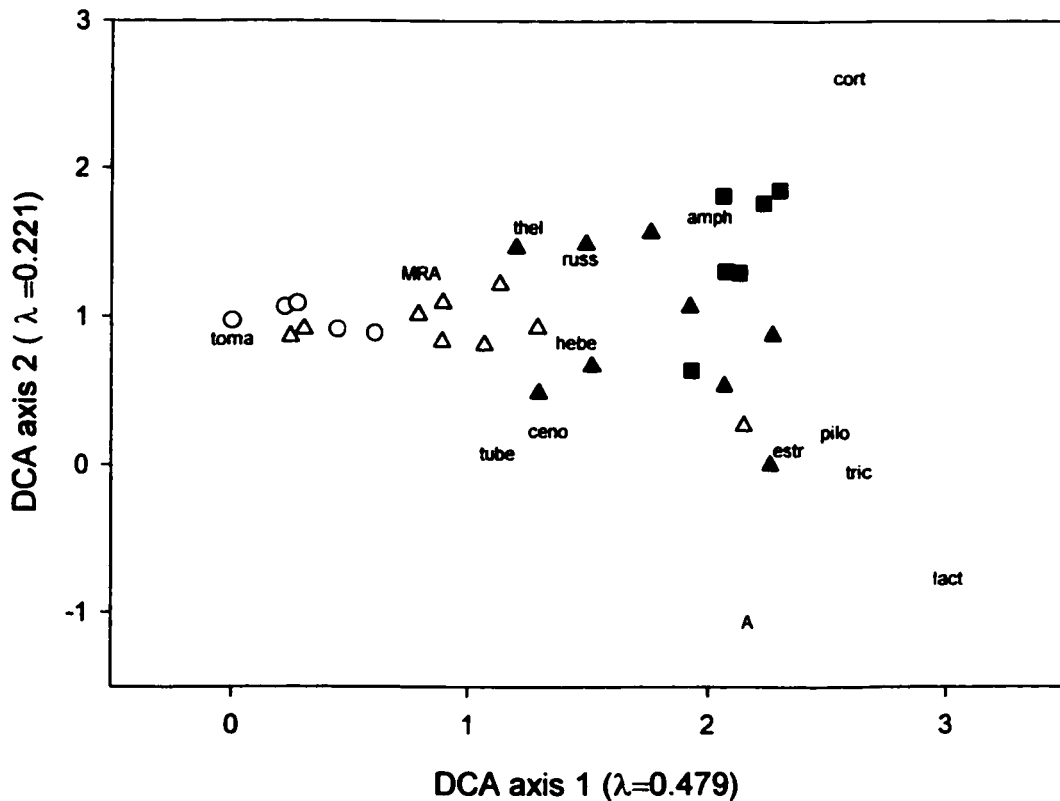


Figure 2-6. A detrended correspondence analysis (DCA) based on the relative abundance of common morphotypes encountered throughout the EMEND research site. Abbreviations are as follows: A = “Unknown A”, amph = *Amphinema byssoides*, cenoc = *Cenococcum geophilum*, cort = *Cortinarius* spp., estr = E-strain, hebe = *Hebeloma* spp., lact = *Lactarius* spp., MRA = *Mycelium radialis atrovirens*, pilo = *Piloderma* spp., russ = *Russula* spp., thel = *Thelephora terrestris*, toma = *Tomentella* spp., tric = “cf *Tricholoma*” and tube = *Tuber* spp. Symbols are as follows: ■ = control and burn control, ▲ = residual strips, △ = machine corridors and ○ = clear-cut and burn.

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Chapter 3

The ability of residual islands within clear-cuts to maintain ectomycorrhizal biodiversity

Introduction

Forestry companies are beginning to adopt harvesting techniques designed to emulate natural disturbances in an attempt to maintain forest biodiversity (see Chapter 1). The impact of some of these alternative harvesting practices on ectomycorrhizal (ECM) communities has been previously investigated in northwestern British Columbia (Durall et al. 1999; Kranabetter and Wylie 1998). However, the pattern of natural disturbance in these forests (small gap formations caused by single tree mortality) contrasts significantly to the natural disturbance processes inherent to the boreal region of Alberta. In the western boreal forest the prime natural disturbance is wildfire, which produces an uneven aged, multi-species forest landscape (Johnson 1992). Wildfires also introduce heterogeneity at a stand scale due to variations in fire severity and intensity caused by local factors (e.g. vegetation type and structure), soil moisture, topography and climate (Rowe and Scotter 1973). Thus, even within stand replacing wildfires patches of live trees, of various shapes and sizes, will be left untouched (Eberhart and Woodard 1987). These unburned patches, or fire residuals, are believed to be important ecosystem structures as they can act as refugia for wildlife and serve as a source of colonization for weakly dispersing species (Rowe and Scotter 1973).

Clear-cutting has been shown to negatively impact ECM communities (Bradbury et al 1998; Byrd et al. 1999; Hagerman et al. 1999a, 1999b; Harvey et al. 1980). In general, the ECM community in clear-cut sites is dominated by a few early stage (or r-

selected) ectomycorrhizae, especially as the distance from the undisturbed forest edge increases (Hagerman et al. 1999a, 1999b; Harvey et al. 1980; Kranabetter and Wylie 1998). In contrast, while many morphotypes may be observed in undisturbed sites, including early stage ectomycorrhizae, late stage (or K-selected) ectomycorrhizae are the most prevalent. As opposed to early stage ECM fungi, which can colonize new root tips via spores, late stage ECM fungi appear to require intact hyphal linkages in order to colonize new root tips (refer to Chapter 1). Thus, the shift in community composition immediately following harvesting can be attributed to the inability of certain ECM fungi (e.g. late stage ectomycorrhizae) to disperse from the edge of the forest into the clear-cut site.

Given the connection between intact forest and ectomycorrhizae, the primary objective of this study is to determine if residual patches, left within clear-cuts, are an effective means of maintaining ECM biodiversity. The second objective of this study was to determine if the ECM community associated with residual patches is influenced by the size of the residual patch. This objective was addressed by comparing the ECM community associated with $\frac{1}{4}$ and $\frac{3}{4}$ ha residual patches.

Materials and Methods

Study site

Field sampling for this study was completed at the Ecosystem Management by Emulating Natural Disturbances (EMEND) research site (refer to Chapter 1 for a detailed description of the EMEND site).

Field sampling

Two different sizes of residual ellipses (approximately $\frac{1}{4}$ and $\frac{3}{4}$ of a hectare) were sampled in three conifer dominated clear-cuts in May 2001 (three field seasons post disturbance). The sampling layout involved four transects, which were positioned around each residual ellipse perpendicular to the edge, with four sampling positions, located at the center of the ellipse, at the edge of the ellipse and within the clear-cut (10 and 20m from the edge) (n= 96; Figure 3-1). In order to assess the white spruce ectomycorrhizal community at each sampling point a 3.5 cm diameter by 15 cm deep soil core was collected. The soil cores were stored in plastic 'zip-lock' bags, transported to the University of Alberta and stored for up to 3 months at 4°C during processing. Both the isolation of the fine root tips and the morphotyping procedure used to characterize and identify the white spruce fine root tips were identical to that described elsewhere in this thesis (see Chapter 2).

Data analysis

The relative abundance of each morphotype (calculated as a proportion of the total number of ECM root tips characterized in each soil core), ECM richness (the total number of morphotypes observed in each soil core) and ECM diversity (calculated using Shannon's diversity index) were determined for each sampling position. A mean value for each of these calculations, based on the 4 transects per residual ellipse, was then determined for each sampling position. The frequency of occurrence of each morphotype was calculated based on the number of soil cores in which the morphotype was observed.

Root tips characterized as “undetermined” (see Kranabetter and Wylie 1998) were not included in the morphotype richness and diversity calculations.

The effect of increasing distance from the edge and residual ellipse size on the ECM community was tested with an analysis of variance (ANOVA) using a split-plot design with ellipse size as the main plot factor and sampling position as the sub-plot factor (Table 3-1). The test was performed using the mean ECM richness and diversity values (average of 4 transects) calculated for each ellipse. The data were tested for normality using a Kolmogorov – Smirnov test prior to the analysis. Significant ANOVA results ($P < 0.05$) were followed by a Tukey-Kramer (honest significant difference) test in order to detect differences among sampling positions. Further analysis was required to determine if differences in the ECM community, between the clear-cut and residual ellipse sampling positions, were due to differences in the community assemblages or, simply, due to the limited number of root tips encountered in clear-cut soil cores (see Table 3-3). This involved constructing a species abundance curve by randomly selecting 20 root tips (from soil cores collected from either the residual ellipse or clear-cut sampling positions) and plotting them against the total number of morphotypes encountered. This procedure was repeated 10 times (for both the clear-cut and residual ellipse) and the average values were graphed for comparison purposes. All data analysis was performed using SPSS Version 10.0 (SPSS 1999).

Results

During the course of this study a total of 2083 active white spruce root tips were characterized and 16 distinct morphotypes were described (Table 3-2; refer to Appendix

II for a thorough description of each morphotype). The most common morphotype was *Cortinarius* spp. followed by *Amphinema byssoides*, *Tomentella* spp., *Cenococcum geophilum*, E-strain, *Mycelium radialis atrovirens* (MRA), *Lactarius* spp. and *Piloderma* spp., respectively. These eight morphotypes formed the majority of the ectomycorrhizae (nearly 85%) while the other 8 morphotypes were observed in less than 5 percent of the soil cores.

The relative abundance of individual morphotypes varied according to the sampling position (Table 3-3). *Lactarius* spp., *Piloderma* spp., *Tuber* spp. and “cf *Tricholoma*” were exclusive to the center and the edge of the residual ellipse. While no morphotypes were exclusive to the sampling positions located within the clear-cut, the relative abundance of *A. byssoides*, E-strain and *Tomentella* spp. was greater in soil cores collected from the clear-cut compared to the soil cores collected from the center and at the edge of the residual ellipses. *Cenococcum geophilum* and *Tomentella* spp. were the only morphotypes observed at each sampling position, although *Amphinema byssoides* was absent from only the 10m sampling position of the $\frac{3}{4}$ ha ellipse. In addition, the average number of root tips observed, and thus characterized, in the soil cores was drastically reduced in the clear-cut sampling positions.

The relative abundance of morphotypes comprising the ECM community also differed according to the sampling position. The average ECM richness was nearly four times greater ($p=0.003$) in the soil cores collected from the center and at the edge of the residual ellipse compared to the soil cores collected in the clear-cut (Figure 3-2). Similarly, the average ECM diversity was significantly greater ($p=0.005$) in the center and at the edge of the residual ellipse compared to the clear-cut sampling positions

(Figure 3-3). No relationship was observed between the size of the residual ellipse and ECM richness ($p=0.878$) or diversity ($p=0.507$).

The species abundance curve constructed from the clear-cut soil cores differed from the curve constructed from the residual ellipse soil cores (Figure 3-4). In the clear-cut soil cores the maximum number of morphotypes was encountered after approximately 14 root tips had been selected while, in contrast, the number of morphotypes encountered in the residual ellipse soil cores continued to increase even after 20 root tips had been selected. This result suggests that the ECM biodiversity in the clear-cut soil cores would not have increased even if more root tips had been encountered. Thus, differences between the residual ellipse and clear-cut sampling positions may be attributed to difference in the community composition as opposed to the limited number of root tips encountered in the clear-cut soil cores.

Discussion

The results suggest that residual ellipses, as small as $\frac{1}{4}$ ha in size, are an effective means of maintaining ECM biodiversity in clear-cut sites as both ECM richness and diversity were significantly greater in and at the edge of the residual ellipse compared to the surrounding clear-cut. This result was expected given the findings of past research. For example, in a study designed to determine the effects of clear-cut logging on ECM biodiversity Hagerman et al. (1999a) reported a significant reduction in both morphotype richness and diversity in soil cores collected more than 16m from the edge of the intact forest compared to soil cores collected within the adjacent forest stand. The researchers also noted that this trend was similar in clear-cuts ranging in size from 0.1 to 10ha. Thus

ECM communities appear to be strongly correlated with, or dependent upon, intact forest edges. The change in community composition between the forest and clear-cut sampling positions in this study provide further evidence for this relationship.

In this study, *Lactarius* spp., *Piloderma* spp., *Russula* spp., “cf *Tricholoma*” and the 3 unknown morphotypes (“Unknown A”, “Unknown B” and “Basidiomycete 1”) were more abundant in soil cores collected from the center and at the edge of the residual ellipse. Ectomycorrhizae formed by species of *Lactarius*, *Piloderma* and *Russula* possess late stage characteristics (Last et. al 1987; Newton 1992). In particular, both *Lactarius* and *Russula* ectomycorrhizae are smooth and possess few emanating elements. Combined with their ability to produce extracellular phenoloxidases (Agerer 2000) and thus, their affinity for leaf litter, it is not surprising that these morphotypes were associated with the residual ellipses in this study. *Piloderma* is a resupinate basidiomycete that fruits on decomposing plant litters, thus, it has often been observed in association with decaying wood and fragmented litter in mature forest stands (Goodman and Trofymow 1998; Smith et al. 2000). Furthermore, according to Smith et al (2000) the correlation between well decomposed coarse woody debris (CWD) and the occurrence of *Piloderma fallax* suggests that it may be considered an indicator of old-growth forests. Therefore, it is not surprising that *Piloderma* was observed in association with the residual ellipses in this study, as this is where the majority of CWD in the advanced stages of decay was observed (pers. obs.).

Multistage ectomycorrhizae with early stage characteristics (see Chapter 1), such as *Hebeloma* and *Thelephora*, were only observed at the edge of the residual ellipses while MRA was only slightly more abundant in the clear-cut sampling positions.

Furthermore, E-strain, which is an early stage ectomycorrhizae, was only slightly more abundant in the clear-cut positions. Given that these ECM fungi are capable of colonizing root tips via spores, excised root tips and hyphal fragments it is unlikely that inoculum sources were absent from the clear-cut soil. However, given the distance to the residual ellipses and the absence of naturally regenerating white spruce seedlings (pers. obs.) few non-mycorrhizal root tips would have been available for these morphotypes to colonize. Therefore, in this study, the ECM community in the clear-cut positions consisted of root tips that had persisted in the soil since harvesting. This is further supported by Ferrier and Alexander (1985) who noted that excised ECM root tips were capable of remaining metabolically active for up to 9 months after being disconnected from their host and Hagerman et al. (1999a) who suggested that some root tips can survive up to three growing seasons in a clear-cut in the absence of a host. Furthermore, while Hagerman et al. (1999b) observed *Hebeloma* and *Thelephora* on seedlings collected from subalpine clear-cuts in British Columbia, they were absent from soil cores collected from the same sampling locations (Hagerman et al. 1999a).

The presence of *Amphinema byssoides* and *Cenococcum geophilum* in all of the sampling positions supports the belief that these species of ectomycorrhizae are best characterized as multi-stage (Visser 1995). As such, they are capable of colonizing root tips, given that a source of inoculum exists, irrespective of the successional status of the site. In addition, the increase in relative abundance of *Cenococcum geophilum* and *Tomentella* spp. in the clear-cut sampling positions concurs with the findings of Bradbury (1998), Byrd et al. (2000), Hagerman et al. (1999a) and those previously reported in this thesis (see Chapter 2).

While, to my knowledge, no other study has investigated the ability of residual ellipses to act as refuge for ectomycorrhizae, comparable studies have been conducted in northwestern British Columbia (Durall et al. 1999; Kranabetter and Wylie 1998). These studies were designed to examine the effects of different stand openings on the ECM community. Furthermore, the stand openings were designed such that they would emulate the naturally occurring small-scale disturbances inherent to this region. Durall et al. (1999) found that the number of ECM morphotypes observed on both outplanted lodgepole pine and western hemlock seedlings decreased slightly with increasing distance from the intact forest edge. Similarly, Kranabetter and Wylie (1998) observed an average of 13.1 morphotypes on naturally regenerating western hemlock seedlings growing under the forest canopy, 9.6 morphotypes on seedlings growing at the forest edge and 7.8 morphotypes on seedlings growing in the forest openings. While both of these studies reported a reduction in ectomycorrhizae, the decrease was more pronounced in this study. This is most likely due to differences in sampling techniques. For example, the study by Durall's group involved outplanted lodgepole pine and hemlock seedlings, while Kranabetter and Wylie sampled naturally regenerating seedlings. In contrast, in this study the ECM community was determined using soil cores. As previously mentioned, the significance of this discrepancy is that very few, if any, intact root systems were present in the soil cores. This is obviously not the case with seedlings. Thus, any fungal inoculum that was present in the soil would have the opportunity to colonize the seedlings and, subsequently, researchers would note their presence. Furthermore, the seedlings used in the Durall et al. (1999) study were likely colonized by common nursery ectomycorrhizae, such as *Thelephora* and E-strain, prior to outplanting.

The ECM community in this study was not influenced by the size of the residual ellipse. This is supported by Kranabetter (1999) who examined the effect of refuge trees on ECM communities. He found that, within clear-cuts, seedlings growing within 5m of a single mature birch tree were associated with significantly more morphotypes than seedlings collected 25-50m from mature birch trees. This suggests that even single trees left within clear-cuts are capable of providing refuge for ECM communities.

In summary, the results of this study suggest that residual patches of trees are capable of providing refuge for ECM communities. Furthermore, ECM richness and diversity was similar among sampling positions associated with both $\frac{1}{4}$ and $\frac{3}{4}$ ha residual patches suggesting that ECM communities are more dependent upon the proximity to host trees (e.g. a carbon source) as opposed to the size of the residual patches. Future research will be required to determine the dispersal ability of the ectomycorrhizae associated with these residual structures which, in turn, will provide additional information regarding how these structures influence reforestation efforts in the surrounding clear-cuts.

Table 3-1. Split plot ANOVA model and results for tests of the ellipse size (main plot) and sampling position (sub-plot).

Source	DF	Mean squares	F value	p > F
Richness				
Block	2	1.17	1.00	0.500
Ellipse	1	0.04	0.04	0.868
Ellipse x block	2	1.17	0.42	0.666
Position	3	55.26	19.90	< 0.001
Ellipse x position	3	2.38	0.86	0.491
Error	12	2.77		
Diversity				
Block	2	1.18	14.33	0.065
Ellipse	1	0.04	3.10	0.220
Ellipse x block	2	0.01	0.08	0.928
Position	3	3.02	17.97	< 0.001
Ellipse x position	3	0.09	0.58	0.665
Error	12	1.68		

Table 3-2. The total number of root tips sampled, relative abundance (%) and frequency of occurrence (%) of morphotypes encountered in the soil cores (n=96) collected in clear-cut sites (in the center, at the edge, and at 10m and 20m from the edge of residual ellipses) three growing seasons after logging.

Morphotype	Tips	Abundance ¹	Frequency ²
<i>Cortinarius</i> spp.	376	18.1	21.9
<i>Amphinema byssoides</i>	275	13.2	26.0
<i>Cenococcum geophilum</i>	256	12.3	31.3
<i>Tomentella</i> spp.	269	12.9	25.0
E-strain	177	8.5	11.5
<i>Mycelium radialis atrovirens</i> (MRA)	166	8.0	11.5
<i>Lactarius</i> spp.	127	6.1	6.3
<i>Russula</i> spp.	126	6.1	12.5
<i>Piloderma</i> spp.	113	5.4	5.2
"cf <i>Tricholoma</i> "	64	3.1	5.2
"Unknown A"	40	1.9	1.0
<i>Tuber</i> spp.	35	1.7	4.2
<i>Thelephora terrestris</i>	15	0.7	2.1
"Undetermined"	15	0.7	2.1
<i>Hebeloma</i> spp.	12	0.6	1.0
"Unknown B"	13	0.6	1.0
"Basidiomycete 1"	4	0.2	1.0
Total	2083		

¹ Relative abundance for each morphotype calculated as:
(number of root tips on which morphotype was observed) / (total number of root tips sampled) X 100.

² Frequency of occurrence for each morphotype calculated as:
(number of soil cores in which morphotype was observed) / (total number of soil cores) x 100.

Table 3-3. The average relative abundance¹ (%) of morphotypes and number of root tips characterized at both residual ellipses and at each sampling position.

Morphotype	0.25 ha				0.75 ha			
	middle	edge	10m	20m	middle	edge	10m	20m
<i>Cortinarius</i> spp.	13.3	15.1	16.7	-	33.8	12.5	-	3.0
<i>Amphinema byssoides</i>	10.8	7.1	28.6	6.0	10.4	19.0	-	30.3
<i>Cenococcum geophilum</i>	15.1	13.9	50.0	16.7	14.4	1.9	9.1	5.4
<i>Tomentella</i> spp.	6.0	12.6	4.8	22.7	12.8	12.6	90.9	5.4
E-strain	4.7	5.6	-	-	4.9	13.9	-	21.5
<i>Mycelium radicans atrovirens</i>	14.9	6.5	-	21.4	6.7	-	-	-
<i>Lactarius</i> spp.	9.0	-	-	-	5.2	14.1	-	-
<i>Russula</i> spp.	7.4	26.2	-	-	3.2	2.4	-	1.1
<i>Piloderma</i> spp.	7.9	1.8	-	-	8.5	10.0	-	-
"cf <i>Tricholoma</i> "	2.3	4.5	-	-	-	4.6	-	-
"Unknown A"	6.3	-	-	-	-	-	-	-
<i>Tuber</i> spp.	-	6.7	-	-	0.2	1.6	-	-
<i>Thelephora</i> spp.	-	-	-	-	-	2.4	-	-
<i>Hebeloma</i> spp.	-	-	-	-	-	4.0	-	-
"Unknown B"	2.4	-	-	-	-	-	-	-
"Basidiomycete 1"	-	-	-	-	-	1.1	-	-
Number of root tips	552	409	50	59	496	432	43	42

¹ Relative abundance for each morphotype calculated as:
(number of root tips on which morphotype was observed) / (total number of root tips sampled) X 100.

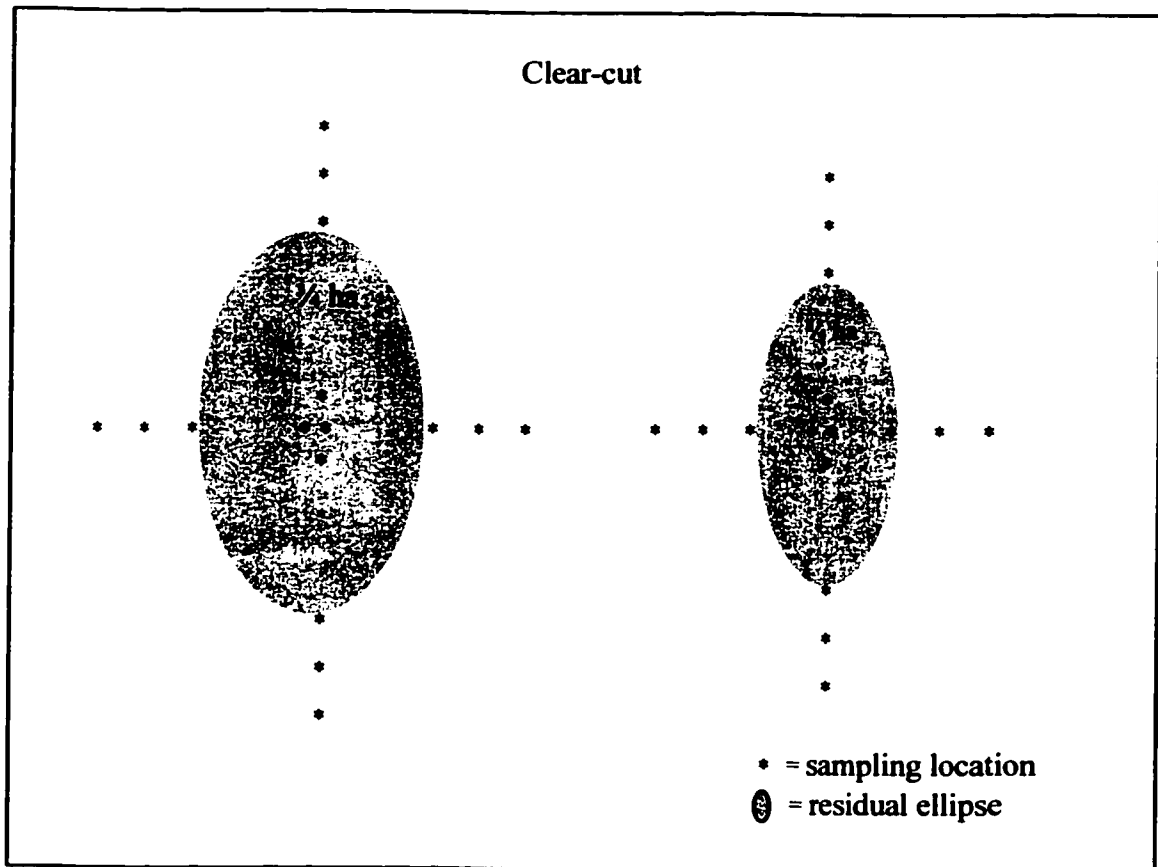


Figure 3-1. Diagram illustrating the sampling locations in relation to the residual ellipses. Sampling locations positioned within the clear-cut are 10 and 20m from the edge of the residual ellipses. Figure is not to scale.

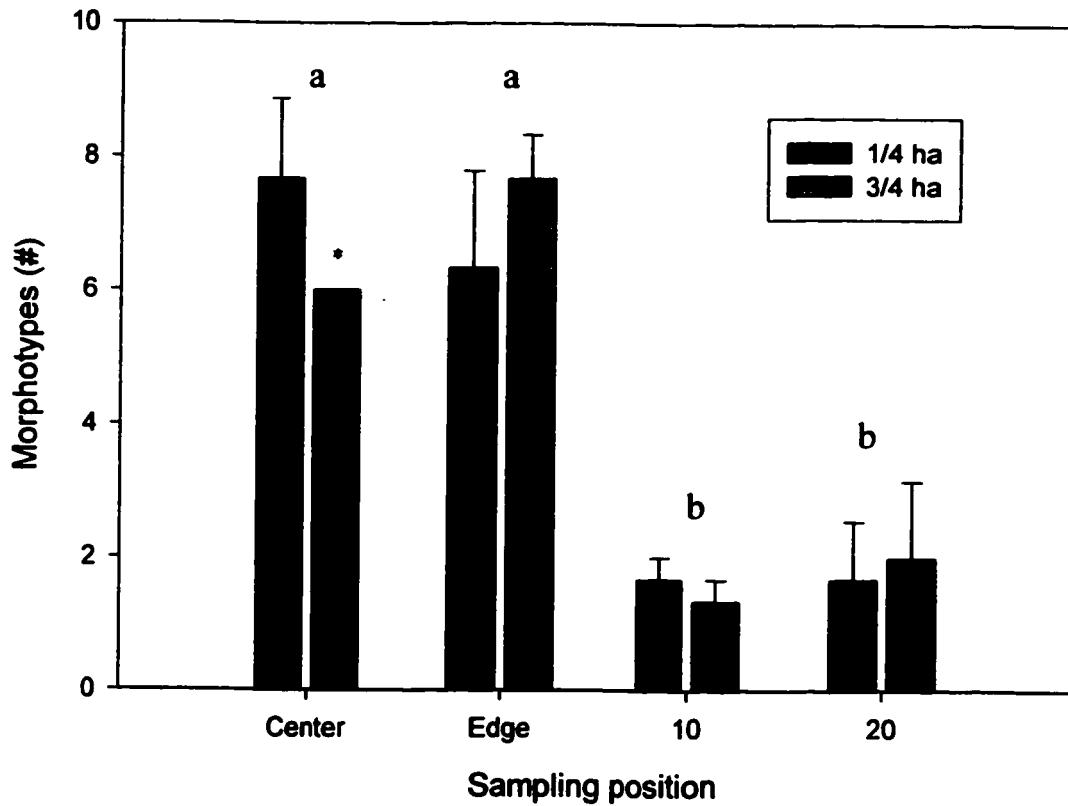


Figure 3-2. The mean ectomycorrhizal richness based on the relative abundance of morphotypes in soil cores collected from the center, at the edge and 10 and 20m from the edge of residual ellipses located in 3 year old clear-cuts. Treatment means with different letters are significantly different [$\alpha=0.05$; Tukey-Kramer (honestly significant difference test)]. Error bars represent 1 SE. * denotes SE = 0.

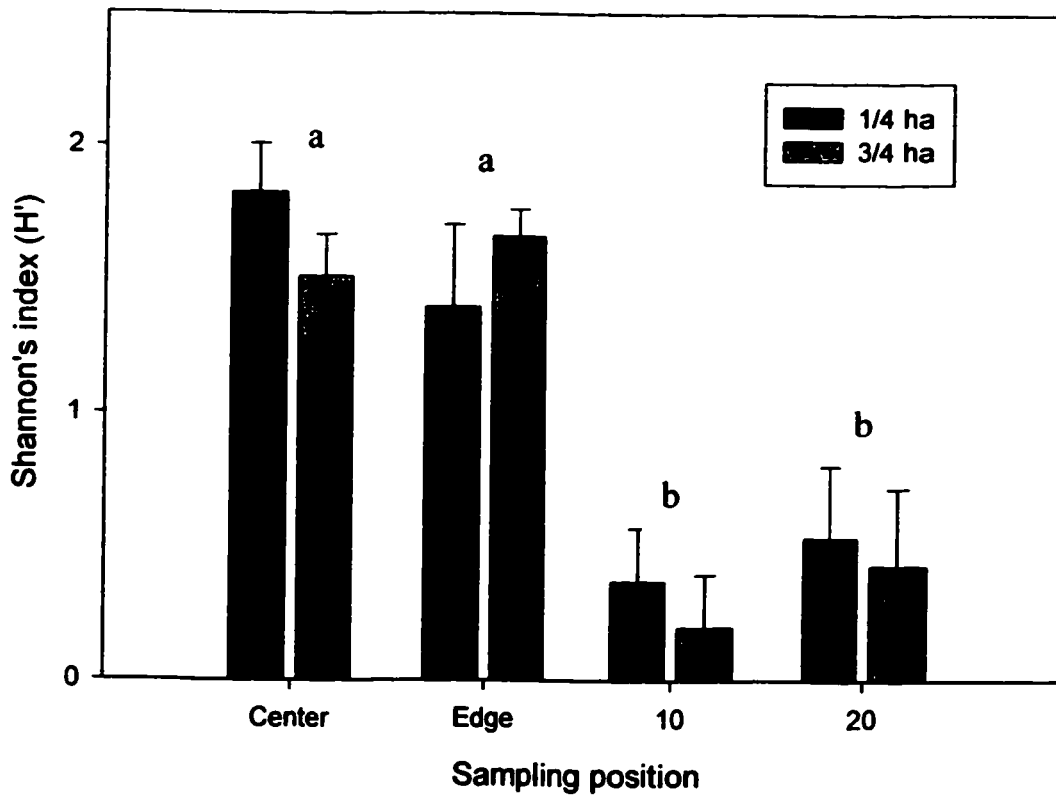


Figure 3-3. The mean ectomycorrhizal diversity (H') based on the relative abundance of morphotypes in soil cores collected from the center, at the edge and 10 and 20m from the edge of residual ellipses located in 3 year old clear-cuts. Treatment means with different letters are significantly different [$\alpha=0.05$; Tukey-Kramer (honestly significant difference test)]. Error bars represent 1 SE.

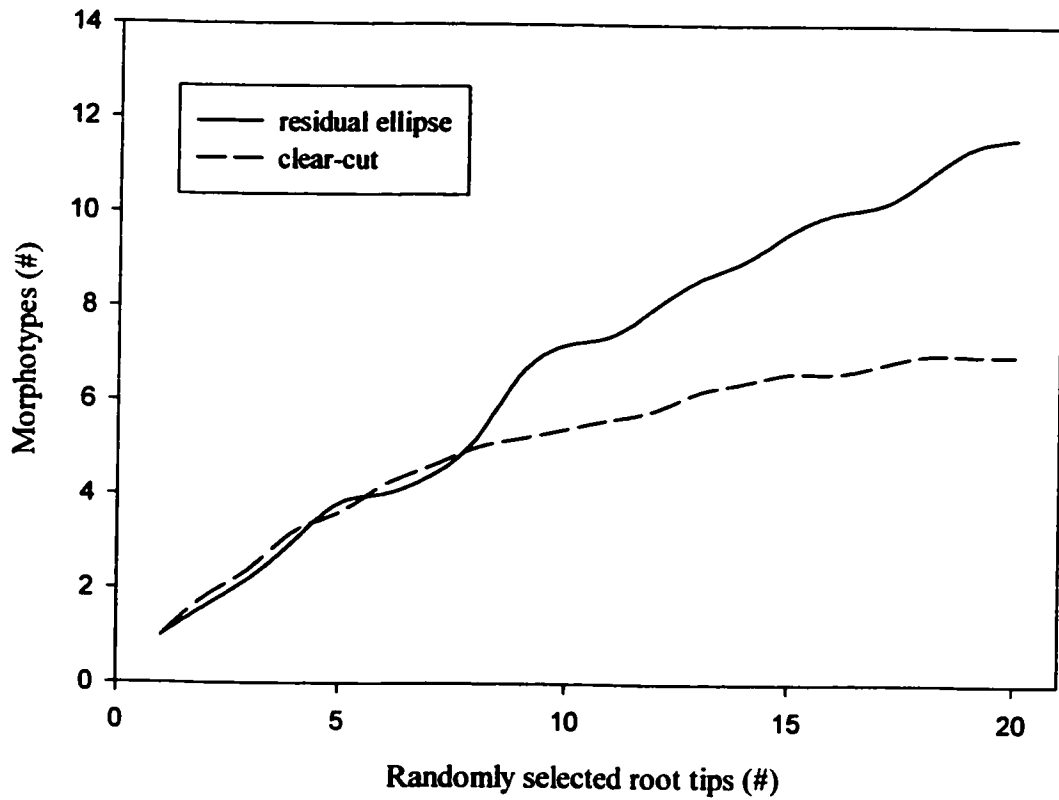


Figure 3-4. The relationship between the number of randomly selected root tips and the total number morphotypes encountered in soil cores collected from residual ellipse and clear-cut sampling positions. Values represents the average of 10 replicates.

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Chapter 4

The effect of mechanical site preparation on the ectomycorrhizal status of planted white spruce seedlings

Introduction

In the North American boreal forest, the regeneration of white spruce (*Picea glauca*) following clear-cutting has often proved difficult (Navratil et al. 1991). White spruce seedlings have slow growth rates (Nienstadt and Zasada 1990), and as a consequence they are often overtopped by faster growing broadleaf trees, grasses and a variety of early successional shrubs (Drew 1988; Lieffers et al. 1993). In addition to competition for light, white spruce seedlings must also compete with these species for other essential resources such as water and mineral nutrients (Kimmins 1997).

Mechanical site preparation (MSP) is commonly used to improve the early performance of white spruce seedlings, and ultimately ensure the successful regeneration of harvested sites. Common treatments include scalping (removing the overlying organic layer and exposing the mineral soil), mixing (incorporating the underlying mineral soil with the overlying organic layer) and mounding (inverting the soil horizons such that the organic layer is sandwiched between the organic layer and a mineral 'cap') (McMinn and Hedin 1998). The objectives of MSP include increasing the availability of nutrients, increasing the soil temperature, reducing competing vegetation and, in the case of mounding, elevating the seedling above competing vegetation (Orlander et al. 1990).

Ectomycorrhizae are believed to be key components of healthy forest ecosystems as they help facilitate a host of interactions. This includes providing protection from plant pathogens (Kropp and Langlois 1990), providing the host plants with water and

mineral nutrients (Smith and Read 1997) and mediating interspecific competition by transferring carbon and mineral nutrient between interconnected host plants (Simard et al. 1997). These interactions are especially important for regenerating seedlings, as they are particularly susceptible to competitive pressures during this stage of their life cycle. Yet, despite the importance of ectomycorrhizae few studies have addressed the impact of MSP on the ectomycorrhizal (ECM) communities associated with regenerating seedlings. Given that MSP treatments are designed to displace the upper litter/organic layer, it is expected that these treatments will also disrupt ECM communities by severing the hyphal connections that link ectomycorrhizae, and their hosts, to one another. Thus, the objective of this study was to assess the impact of MSP on the ectomycorrhizal status of planted white spruce seedlings.

Methods and Materials

Study site

Field sampling for this study was completed at the Ecosystem Management by Emulating Natural Disturbances (EMEND) research site (refer to Chapter 1 for a detailed description of the EMEND site). The study was located within three 0.5 hectare silviculture plots that were established within separate clear-cut sites in the spring of 1999 (one field season post harvest). Prior to harvesting, the sites were dominated by 113-130 year old white spruce (Appendix 1). The silviculture plots were divided into four 50 x 25m quadrants and each quadrant was randomly assigned one of 4 mechanical site preparation treatments (mound, scalp, mix and untreated control) (Figure 4-1). The mineral cap of each mound was approximately 80cm wide x 100cm long and was

between 10-15cm deep at the center. Scalp microsites were approximately 100 cm² and were established such that the mineral soil was exposed throughout the microsite. Mixed microsites were 140cm wide by 100 cm long and were created using a Meri-Crusher high-speed horizontal drum mulcher. All of the MSP treatments were created using a 200 series excavator equipped with a mounding bucket (mound and scalp treatments) and the Meri-Crusher attachment. Ten one-year-old white spruce seedlings (stock type 415B 1+0) obtained from a local nursery were planted in each of the quadrants (one seedling per microsite in the MSP treatments and approximately 1m apart in the untreated control) in July 1999.

Field sampling

The seedlings were collected in May 2001 (two growing seasons after planting). This involved carefully excavating the entire seedling, clipping the seedlings at the root collar, storing the root system in a plastic 'zip-lock' bag and transporting them to the University of Alberta where they were stored for up to 3 months at 4°C until processing. The isolation of root tips was accomplished by gently rinsing the root system over a soil sieve (32 mesh/inch), in order to remove adhering soil and debris, and cutting the roots into 2 to 3 cm long sections. Sections of the root system were then randomly selected and, when possible, 200 fine root tips (also randomly selected) were examined for each of the 120 seedlings. Only roots extending from the soil plug were sampled, however, it was not always possible to find 200 suitable root tips for some of the seedlings. In this situation every suitable root tip encountered on the seedling was examined. The

morphotyping procedure used to characterize and identify the fine root tips was identical to that described elsewhere in this thesis (see Chapter 2).

Data analysis

The relative abundance of each morphotype (calculated as a proportion of the total number of ECM root tips characterized on each seedling), ECM richness (the total number of morphotypes observed on each seedling) and ECM diversity (calculated using Shannon's diversity index) was determined for each seedling. The frequency of occurrence for each morphotype was also calculated based on the number of seedlings on which the morphotype was observed. Root tips characterized as "undetermined" (see Kranabetter and Wylie 1998) were not included in the morphotype richness and diversity calculations.

The impact of mechanical site preparation, on both the average morphotype richness and diversity, was tested with an analysis of variance (ANOVA) using a completely randomized block design. The three silviculture plots were considered blocks (random effect), the four treatments (control, mix, mound and scalp) as main effects (fixed) and individual seedlings within a quadrant (treatment) were treated as subsamples. The ECM richness and diversity data were first tested for normality using a Kolmogorov – Smirnov test. Planned contrasts, between the untreated control and MSP treatments (mix, mound and scalp combined), were also performed using the ECM richness and diversity data in order to further test the impact of MSP on the ECM status of the white spruce seedlings. All data analysis was performed using SPSS Version 10.0 (SPSS 1999).

Results

During the course of this study more than 11,000 root tips were analyzed and 16 distinct morphotypes were described (Table 4-1; refer to Appendix II for a description of each morphotype). The most common morphotype was *Thelephora terrestris*, followed by E-strain, *Amphinema byssoides* and *Mycelium radialis atrovirens* (MRA), respectively. These four morphotypes were observed on the majority of the root tips (nearly 81%) while the other 12 morphotypes had an overall relative abundance less than 3 percent.

No treatment effect was detected for either ECM richness ($p=0.118$) or diversity ($p=0.173$) according to the ANOVA test (Table 4-2). However, the planned contrast showed that white spruce seedlings excavated from the mechanically site prepared microsites had significantly lower ECM richness ($F=4.578$; $df=3$; $p=0.005$) and diversity ($F=4.013$; $df=3$; $p=0.009$) compared to seedlings collected from the untreated control portion of the study plot. An average of approximately 2 morphotypes per seedling was observed on seedlings collected from the site prepared microsites compared to nearly 3 morphotypes per seedling in the untreated control quadrant (Figure 4-2). Furthermore, the average ECM diversity was nearly twice as great on the control seedlings compared to the seedlings obtained from the site prepared treatments (Figure 4-3). No difference was detected, for either ECM richness or diversity, between the MSP treatments (Table 4-2).

The relative abundance of individual morphotypes varied according to the treatment (Table 4-3). *Thelephora terrestris*, E-strain, *Amphinema byssoides* and MRA were observed on seedlings collected from all of the microsites, however, *Amphinema*

byssoides was relatively rare on seedlings collected from the scalp microsites. E-strain and MRA were most abundant on seedlings collected from the mixed microsites while *Thelephora terrestris* was most abundant in the mounded and scalped microsites. *Piloderma* spp. was observed exclusively in the site prepared microsites while, in contrast, *Tuber* spp. was only observed in the control portion of the sampling plots. Furthermore, the majority of the rare morphotypes (*Hebeloma* spp., *Russula* spp., *Lactarius* spp., *Dermocybe* spp. and the 2 unknown Basidiomycetes) were each observed in only one type of MSP treatment.

Discussion

The results of this study suggest that mechanical site preparation is capable of altering the ECM status of out-planted white spruce seedlings as both ECM richness and diversity were lower on seedlings collected from the site prepared microsites compared to the control portions of the sampling plots. This is comparable to Jones et al. (1996) who quantified the impact of mechanical site preparation (scalping) on ECM status of lodgepole pine seedlings outplanted in a dry grassy site located in south central British Columbia. Twenty-eight months after outplanting both ECM richness and diversity were significantly greater on the control seedlings compared to seedlings collected from the scalped portion of their study plot (Jones et al. 1996). Page-Dumroese et al. (1998) reported similar findings in their study, which was designed to determine the impact of tree stump removal (designed to decrease the incidence of root disease, especially *Armillaria*, on seedlings) and soil compaction on the development of ectomycorrhizae on

Douglas-fir seedlings. Three years after planting both the morphotype richness and the number of ectomycorrhizal root tips encountered on the seedlings were significantly lower for seedlings grown in the stump removal plots versus the control seedlings (Page-Dumroese et al. 1998). Furthermore, while compaction did not appear to affect the number of ECM root tips encountered on these seedlings, seedlings collected from the compacted plots had significantly lower morphotype richness compared to the control seedlings (Page-Dumroese et al. 1998). The adverse effect of soil compaction, caused by harvesting equipment, on ECM communities was also observed in a related study at the EMEND research site and is described elsewhere in this thesis (see Chapter 2).

Given that ectomycorrhizae are largely confined to the organic layer of forest soils the results obtained in this study, and those mentioned above, are not surprising. Harvey et al. (1976) determined that the majority of active root tips (nearly 95%) observed in soil cores collected from a mature Douglas-fir – larch forest were encountered in the upper organic soil layer. This has also been reported in lodgepole pine stands in Wyoming (Byrd et al. 1999) and both mature and old – growth Douglas-fir stands on southwestern Vancouver Island (Goodman and Trofymow 1998). Furthermore, the destruction of the litter/organic layer during a prescribed fire event in a Sierra Nevada ponderosa pine forest resulted in an 8-fold reduction in total ECM biomass (Stendell et al. 1999). In addition to the vertical stratification of ectomycorrhizae in forest soils, ectomycorrhizae also appear to have an affinity for woody debris in the soil. For example, Goodman and Trofymow (1998) observed significantly more ECM root tips in log and stump microsites compared to mineral soil. Similarly, 21% of the active root tips encountered by Harvey et al. (1976) were associated with decaying wood. Thus, the

removal or disruption of the forest floor during mechanical site preparation directly influences the ECM community, as the majority of ectomycorrhizae are found in the organic/litter layer of forest soils.

The pattern of root development observed on seedlings planted in mechanically site prepared microsites further illustrates the relationship between organic matter and ectomycorrhizal root tips. In response to site preparation Harvey et al. (1997) noted that the highest density of ECM root tips observed on western white pine and Douglas-fir seedlings was correlated with organic matter, particularly decayed wood or mixtures containing decaying wood. Similar patterns of root development were observed on the white spruce seedlings in this study (pers. obs.). For example, the majority of egressed roots on the seedlings collected from the mounded microsites originated from the bottom of the root plug while the majority of egressed roots on the seedlings collected from the control seedlings originated from the upper half of the root plug. Seedlings collected from the mixed microsites had uniform, as well as extensive, root development.

The removal of competing vegetation, which is also an objective of mechanical site preparation, may also have negative impacts on ectomycorrhizal communities. This is because a number of herbs and shrubs, found on clear-cut sites after harvesting, may be alternate hosts for ectomycorrhizae. This includes *Vaccinium* sp., *Chimaphila umbellata* and *Ledum glandulosum* (Largent et al. 1980); *Amelanchier alnifolia*, and *Sheperdia canadensis* (Hagerman et al. 2001); *Arctostaphylos* sp. (Hagerman et al. 2001; Horton 1998); and *Salix* sp. (Hagerman et al. 2001; Kernaghan and Currah 1998). Regenerating hardwood trees (e.g. alder, aspen and birch) may also provide refuge for ectomycorrhizae (Hagerman et al. 2001; Kranabetter 1999). All of the plants and trees mentioned, or

related species, were observed in the conifer dominated clear-cuts at the EMEND site (Fenniak 2001; Frey 2001). Therefore, the exclusion of competing vegetation on the site prepared microsites may also have contributed to the observed reduction in ECM richness and diversity in this study.

The community assemblage throughout the study site was somewhat as expected given the disturbed environment in which the seedlings were outplanted. For example, the majority of ectomycorrhizae were formed by early stage (e.g. E-strain) and multistage ectomycorrhizae (e.g. *Amphinema byssoides*, MRA and *Thelephora terrestris*) while late stage ectomycorrhizae, such as *Piloderma* spp., *Lactarius* spp. and *Russula* spp., were each observed on less than 1% of the root tips. However, it was rather difficult to determine any clear trends, with respect to specific morphotypes, between the different treatments. The exception was *Amphinema byssoides*, which was observed on only 2.9% of the root tips in the scalp treatment compared to an average of nearly 20% in the other three treatments. *Amphinema* is a basidiomycete that forms resupinate basidiocarps on decomposing plant litter, thus, the removal of the litter/organic layer from the scalp microsites may have contributed to its reduced abundance. The other 3 common morphotypes (E-strain, MRA and *Thelephora terrestris*) were rather ubiquitous. As all three of these morphotypes are common nursery ectomycorrhizae it is possible that they were present on the seedlings prior to outplanting. This would explain why these morphotypes were so abundant throughout this study and why their relative abundance remained somewhat constant among the treatments. The remaining 12 morphotypes were each observed on less than 3% of the root tips, therefore, suggesting a habitat preference for these morphotypes would be inappropriate.

In conclusion, mechanical site preparation is capable of significantly altering the ECM status of planted white spruce seedlings. Clear-cutting has been shown to significantly reduce ECM biodiversity in northwestern Alberta (see Chapter 2). As expected, MSP in these clear-cut sites resulted in further reduction to the ECM community. Initially, these reductions are likely offset by the favorable growing conditions created by the MSP treatments, however, it is unclear how the seedlings will be affected over time (see Chapter 5). Further research is also required to clarify how specific ECM fungi are influenced by the various MSP treatments.

Table 4-1. The total number of root tips sampled, relative abundance (% of root tips) and frequency of occurrence (% of seedlings) of morphotypes encountered on white spruce seedlings (n=120) collected from scalped, mixed, mounded and control microsites located within three clear-cut sites in northwestern Alberta.

Morphotype	Tips	Abundance ¹	Frequency ²
<i>Thelephora terrestris</i>	3239	29.31	46.67
E-strain	2294	20.76	40.00
<i>Amphinema byssoides</i>	1857	16.80	45.00
<i>Mycelium radialis atrovirens</i> (MRA)	1512	13.68	42.50
"cf <i>Laccaria</i> "	249	2.25	5.00
<i>Tomentella</i> spp.	227	2.05	8.33
<i>Cortinarius</i> spp.	213	1.93	5.83
<i>Tuber</i> spp.	190	1.72	3.33
<i>Cenococcum geophilum</i>	141	1.28	5.00
<i>Lactarius</i> spp.	100	0.90	0.83
<i>Piloderma</i> spp.	96	0.87	5.83
"Basidiomycete 3"	65	0.59	0.83
<i>Dermocybe</i> spp.	60	0.54	0.83
<i>Russula</i> spp.	24	0.22	1.67
<i>Hebeloma</i> spp.	19	0.17	2.50
"Basidiomycete 2"	5	0.05	0.83
"undetermined"	717	6.49	25.83
non-mycorrhizal	44	0.40	5.00
Total	11052		

¹ Relative abundance for each morphotype calculated as:
(number of root tips on which the morphotype was observed) / (total number of root tips sampled) X 100.

² Frequency of occurrence for each morphotype calculated as:
(number of seedlings on which the morphotype was observed) / (total number of seedlings) x 100.

Table 4-2. ANOVA model and results for test of the ectomycorrhizal richness and diversity on seedlings planted in control and mechanically site prepared microsites.

Source	DF	Mean squares	<i>F</i> value	<i>p</i> > <i>F</i>
Richness				
Treatment	3	4.900	2.985	0.118
Block	2	1.575	0.959	0.435
Treatment * Block	6	1.642	1.534	0.174
Error	108	1.070		
Diversity				
Treatment	3	0.625	2.341	0.173
Block	2	0.076	0.286	0.761
Treatment * Block	6	0.267	1.714	0.125
Error	108	0.156		

Table 4-3. The average relative abundance (%) of morphotypes characterized on white spruce seedlings collected from control, mixed, mounded and scalped microsites located within three clear-cuts in northwestern Alberta.

Morphotype	Control	Mix	Mound	Scalp
<i>Thelephora terrestris</i>	11.70	19.10	43.00	34.17
E-strain	23.97	27.20	14.30	11.00
<i>Amphinema byssoides</i>	18.07	20.80	20.13	2.90
<i>Mycelium radicis atrovirens</i> (MRA)	12.57	20.67	7.77	9.40
"cf <i>Laccaria</i> "	2.57	2.83	-	2.90
<i>Tomentella</i> spp.	5.90	-	1.67	-
<i>Cortinarius</i> spp.	1.90	-	3.27	1.93
<i>Tuber</i> spp.	6.33	-	-	-
<i>Cenococcum geophilum</i>	3.63	1.00	0.07	-
<i>Lactarius</i> spp.	-	-	-	3.33
<i>Piloderma</i> spp.	-	0.60	2.27	0.33
"Basidiomycete 3"	-	-	-	2.17
<i>Dermocybe</i> spp.	-	-	2.00	-
<i>Russula</i> spp.	-	-	0.80	-
<i>Hebeloma</i> spp.	-	-	-	0.63
"Basidiomycete 2"	-	0.17	-	-
"undetermined"	9.12	8.02	5.54	2.19
non-mycorrhizal	0.98	0.19	0.17	0.36

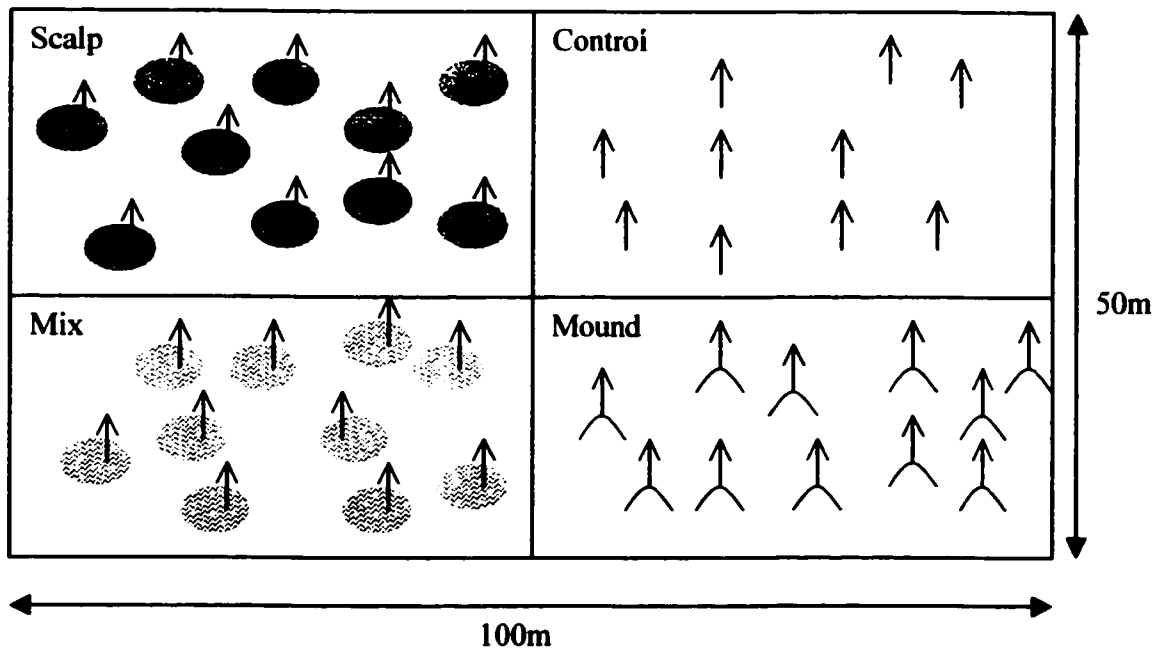


Figure 4-1. Diagram illustrating the positioning of the mechanical site preparation (mix, mound and scalp) and untreated control treatments within the silviculture plots. One seedling was planted per microsite in the mechanical site preparation treatments and approximately 1m apart in the untreated control quadrant (a total of 10 seedlings per treatment). Three replicate silviculture plots were sampled. Diagram is not to scale.

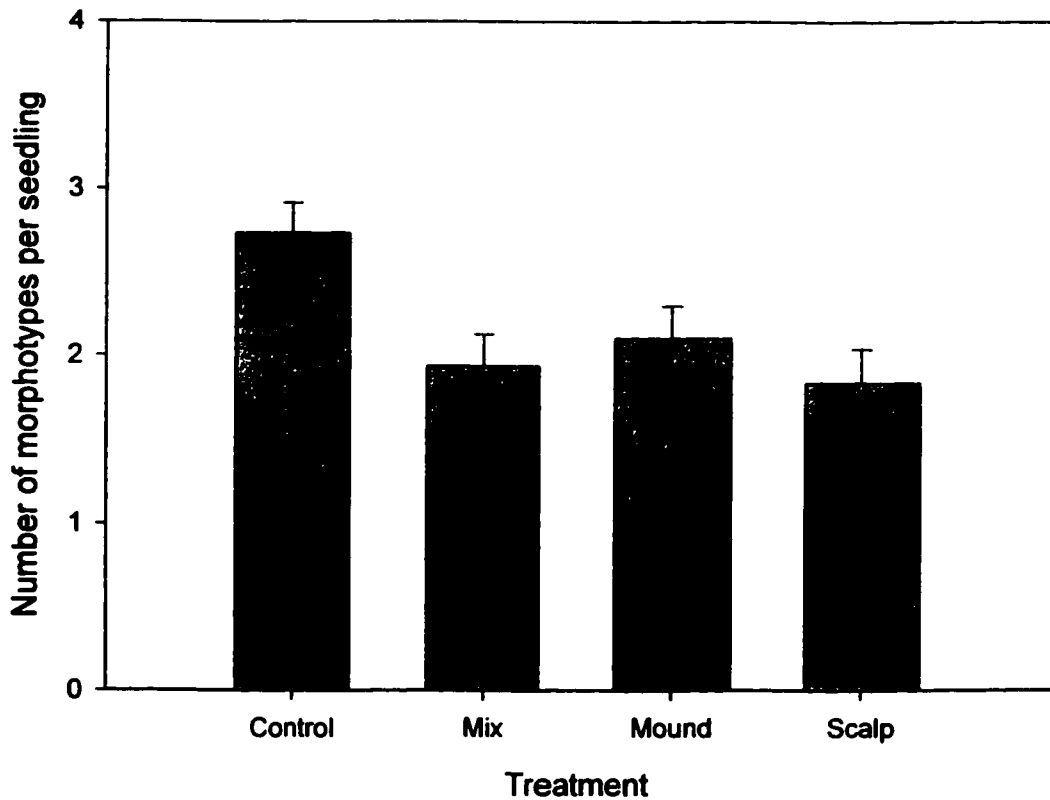


Figure 4-2. The mean ectomycorrhizal richness based on the relative abundance of morphotypes observed on seedlings obtained from mechanically site prepared microsites and control portions of sampling plots located within 3 year old clear-cut sites in northwestern Alberta. There were no significant differences among treatments ($P=0.118$; $n=3$). Error bars represent 1 SE based on the 10 seedlings sampled per treatment.

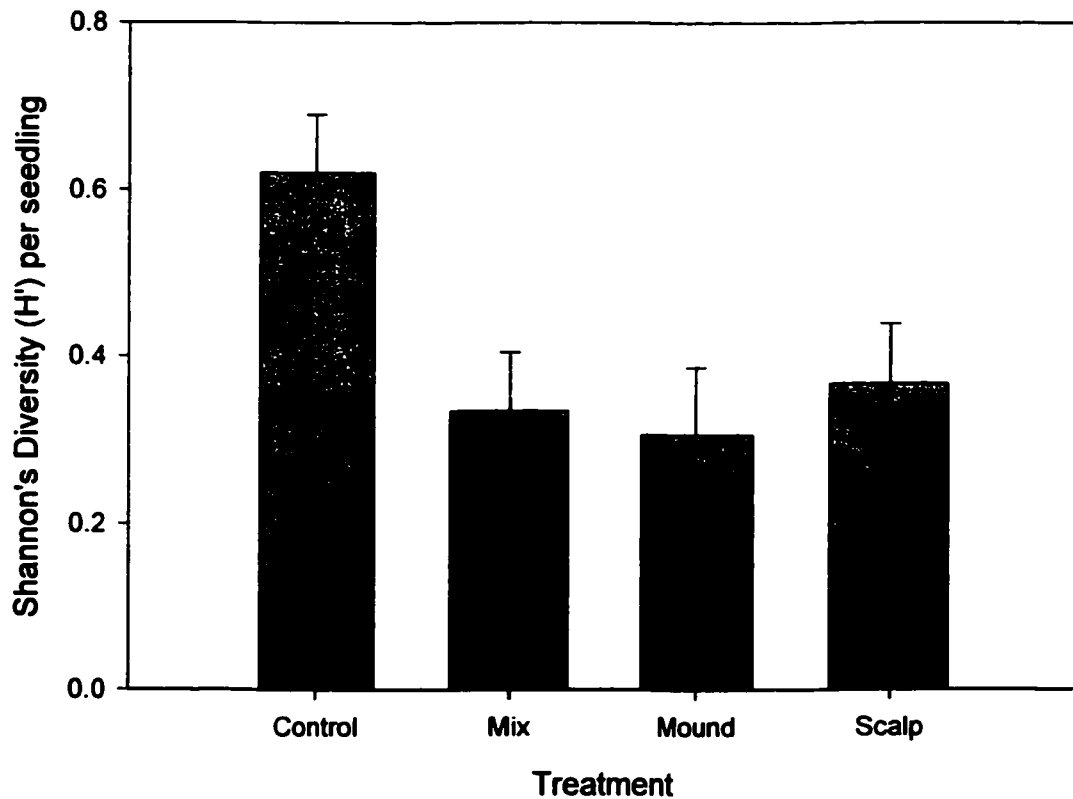


Figure 4-3. The mean ectomycorrhizal diversity (H') based on the relative abundance of morphotypes observed on seedlings obtained from mechanically site prepared microsites and control portions of sampling plots located within 3 year old clear-cut sites in northwestern Alberta. There were no significant differences among treatments ($P=0.173$; $n=3$). Error bars represent 1 SE based on the 10 seedlings sampled per treatment.

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Chapter 5

General discussion and conclusions

Summary

The objectives of this thesis were to: 1) assess the impact of various harvesting practices on the biodiversity of white spruce ectomycorrhizae in northwestern Alberta, 2) determine whether mechanical site preparation influences the ectomycorrhizal status of planted white spruce seedlings and, 3) discuss some of the management implications of this research.

The objective of the first study (Chapter 2) was to determine the impact of clear-cutting, partial cutting and a controlled burn on ectomycorrhizal (ECM) communities. The results suggested that as the harvesting intensity increases there is a corresponding decrease in the percentage of active fine root tips, ECM richness and ECM diversity. Within the partial cut stands the residual strip portions were comparable to the unharvested control stands suggesting that partial cutting is an effective method of maintaining ECM biodiversity. However, the percentage of active fine root tips, ECM richness and ECM diversity in the machine corridor portions of the partial cut stands were significantly lower than in both the both residual strips and the unharvested control stands. In fact, the ECM communities in the machine corridors were comparable to the clear-cut stands. Thus, on a stand level, partial cutting did influence the ECM community. Finally, the burn treatment, which removed the litter/organic layer all together, had the greatest impact on the ECM community. The percentage of active fine root tips, ECM richness and ECM diversity in the burned portion was even lower than in the clear-cut stands.

The second study (Chapter 3) also investigated the influence of residual trees on ECM communities, however, as opposed to partial cutting (which leaves an even, linear distribution of residual trees) it involved spatially clumped distributions. In particular, the objective of this study was to determine if residual patches of trees left within clear-cuts act as refuges for ECM communities. As expected, both the ECM richness and diversity were greater in the center and at the edge of residual ellipses compared to 10 and 20m into the adjacent clear-cut, regardless of the residual ellipse size (1/4 versus 3/4 ha). While the residual ellipses are capable of harboring a diverse ECM community, the results of this study do not suggest that the residual ellipses influenced the adjacent clear-cut ECM community (as evident by the sharp decline in ECM richness and diversity in the clear-cut sampling positions). However, this is likely to change over time as ectomycorrhizae extend out from the residual ellipses.

In the third study (Chapter 4) I investigated the impact of mechanical site preparation on the ECM status of outplanted white spruce seedlings. Seedlings excavated from the mechanically site prepared microsites (scalped, mixed and mounded) had lower ECM richness and diversity than seedlings obtained from the control portion of the sampling plots, however, no difference between the types of site preparation were detected.

Management Implications

The results of this study suggest that, from an ectomycorrhiza point of view, both partial and clear-cutting are capable of emulating natural disturbances (e.g. wildfire) depending upon the scale at which the comparison is made. Wildfires introduce heterogeneity at a stand scale due to variations in fire severity and intensity caused by

local factors (e.g. vegetation type and structure), soil moisture, topography and climate (Rowe and Scotter 1973). The unburned patches of residual vegetation commonly observed within a burned landscape (Eberhart and Woodard 1987; Turner et al.1994) best illustrates this point. Within the partial cut stands the impact on the ECM community was severe in the machine corridors and, for the most part, negligible in the residual strips. Similarly, in the burned stand the ECM community in the unburned portion of the stand was comparable to the unharvested control stands while in the burned portion ectomycorrhizae were almost entirely absent. In this sense the partial cut treatment was comparable to the burned stand. Partial cutting may also be of interest to forest managers because, by altering the thinning prescription, they would be able to further address the variability inherent to boreal forest ecosystems.

The results from chapter 3 indicate that residual ellipses are capable of harboring a diverse ECM community compared to the surrounding clear-cuts. Thus, an alternative method of introducing variability at the stand level would be to incorporate variable retention cutting with residual trees aggregated throughout the harvested site.

At the landscape scale wildfire dominated ecosystems may be regarded as a mixture of burned and unburned stands. With respect to ectomycorrhizae, wildfire may be considered similar to clear-cutting in that they both remove host trees and disturb the upper organic/litter soil layer. For example, both the burning and clear-cutting treatments in this study had similar ECM communities, and significantly reduced ECM richness and diversity compared to the unharvested control stands. This result also implies that emulating natural disturbances may not be synonymous with maintaining biodiversity. Ecological processes inherent to the boreal forest further support this idea. For instance,

the western boreal forest of Canada is characterized by frequent stand replacing wildfires. Given that ECM fungi are heavily dependent upon, and thus have co-evolved with, their hosts it seems logical to conclude that ECM fungi would have evolved mechanisms enabling them to adapt to these disturbance events. The different life strategies of ECM fungi (e.g. early stage ectomycorrhizae with r-selected attributes versus late stage ectomycorrhizae with K-selected attributes) imply that at least some species require disturbances in order for them to become established. Some researchers have suggested that dormant structures (e.g. sclerotia and chlamydospores) formed by some species of ECM fungi may be analogous to disturbance dependent structures associated with trees (e.g. serotinous cones) (Visser 1995). Furthermore, elevated soil temperature has been found to stimulate the germination of spores produced by ECM fungi (especially ascomycetes) (Wicklow 1988).

Mechanical site preparation (MSP) is commonly used in northwestern Alberta to increase the early performance of white spruce seedlings and to ensure the successful regeneration of harvested sites. In this study the white spruce seedlings collected from the site prepared microsites had lower ECM biodiversity compared to seedlings excavated from the untreated control portion of the study site. However, while the control seedlings may have had greater ECM biodiversity, past research suggests that they may also experience higher rates of mortality (Jones et al. 1996; Sutton and Weldon 1995; Weber et al. 1995), although this is not always the case (Macdonald et al. 1998). Furthermore, increased seedling performance on site prepared microsites should be expected given that one important objective of these treatments is to create favorable

growing conditions, however, this may (Heineman et al. 1999; Sutton and Weldon 1995; Weber et al. 1995) or may not (Macdonald et al. 1998; Munson et al. 1993) be the case.

The soil temperature in site prepared microsites is often elevated compared to untreated planting locations (Frey 2001; Stewart 2000), which may lead to an extended growing season for seedlings planted in those microsites (Weber et al. 1995). Disruption of the forest floor may also reduce the density of competing vegetation (e.g. *Calamagrostis canadensis*, *Epilobium angustifolium* and *Populus tremuloides*) immediately following the disturbance. However, while fewer plants may be competing for nutrients, past studies suggest that MSP may actually decrease the overall nutrient availability. For example, MSP has been shown to reduce the availability of N, P and K (Munson et al. 1993; Schmidt et al. 1996); decrease foliar P, K and Mg concentrations (Macdonald et al. 1998); and increase N mineralization and nitrification (Munson et al. 1993), which can lead to increased leaching of cations (Likens 1970). This reduction becomes increasingly significant over time as both seedlings and vegetation develop and their nutrient requirements become greater. As resources become more limiting seedlings will become increasingly dependent upon ectomycorrhizae. Thus, the detrimental effect these treatments have on ECM communities may become more significant over the long term.

Differences between the MSP treatments were not detected in this study, however, other researchers have noted that the more intensive methods generally have a greater impact on soil nutrient dynamics (Schmidt et al. 1996) and seedling performance (Haeussler et al. 1999). The more intensive treatments (those that mix or displace the organic/litter layer) would also be expected to have a greater impact on ectomycorrhizae

given the disruption to the mycelial network. One method that may help to reduce the impacts of MSP on ECM communities would be to leave residual trees scattered throughout the silviculture treatment. The diverse ECM community associated with these trees would allow developing seedlings to compete for limiting resources and, thus, improve the long-term productivity of the site.

Future Research

These studies were able to assess the immediate post-disturbance ECM community associated with the various harvesting treatments. However, additional research will be required in order to determine how the ECM communities will change over time. While the ECM community in all of the treatments is expected to eventually converge towards its pre-disturbance assemblage, the recovery rate for the different treatments should differ. For example, based on the greater proportion of active root tips observed in the partial cut stands, compared the clear-cut stands, the ECM community in the partial cut stands should converge to its pre-disturbance assemblage at a quicker rate than in the clear-cut stands. Verifying this hypothesis would provide additional insight into how the various harvesting prescriptions surveyed in this study impact the ECM community. Further research is required to determine the dispersal ability of the ECM community associated with the residual ellipses and, thus, the long-term effectiveness of these structures in maintaining ECM biodiversity in clear-cut sites.

One of the drawbacks associated with using soil cores to quantify ECM communities is that the method does not take all of the sources of ECM inoculum into consideration. While it may provide a portrayal of ectomycorrhizae associated with fine

root tips there is no way to measure other sources of ECM inoculum, such as fragments of mycelia, spores, chlamydospores and to a certain extent sclerotia. This is illustrated by two related studies performed by Hagerman and her colleagues (1999a,b) at the Sicamous Creek Silvicultural Systems Trial site in the southern interior of British Columbia. In Hagerman et al. (1999a) the ECM community was assessed using the soil core method while in Hagerman et al. (1999b) the ECM community was assessed using a field bioassay. Even though the seedlings and the soil cores were collected from the same sampling locations, the ECM community reported in these studies differed from one another. Therefore, in order to get an idea of what ECM species are capable of forming ectomycorrhizae on seedlings a field bioassay should be performed at the EMEND research site. In addition, given that white spruce seedlings are typically planted following harvesting in northwestern Alberta, a field bioassay would also provide forest managers with some insight into how the harvesting prescriptions influence commercial reforestation efforts.

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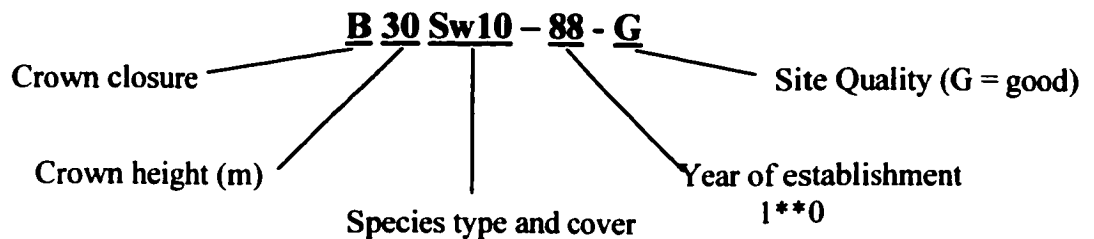
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Appendix I. Stand descriptions

Replicate	Compartment	Treatment	stand #	Tree Age	AVI Description	
					Overstory	Understory
1	889	control	31	127.4	B30Sw10-88-G	B19Sw8Aw2-90-G
1	890	75%				
1	892	clear-cut				
1	896	20%	43	129.2	C26Sw9Aw1-89-G	A21Sw9Aw1-90-G
1	898	50%				
2	918	control	314	113.9	C26Sw9Aw1-89-G	A21Sw10-90-G
2	919	20%				
2	920	50%				
2	921	75%				
2	922	clear-cut				
3	929	50%	445	131.3	B28Sw9Aw1-88-G	B22Sw9Aw1-90-G
3	930	control				
3	931	75%				
3	932	clear-cut				
3	933	20%				
-	926	burn	306	116.4	B28Sw8Aw2-88-G	B14Sw8Sb2-94-G

Key to AVI codes



Crown closure

A 6-30 %
B 31-50 %
C 51-70 %
C 71-100 %

Tree species

Aw *Populus tremuloides*
Sb *Picea mariana*
Sw *Picea glauca*

Appendix II. Morphotype descriptions

This section contains the morphological and anatomical features associated with the morphotypes presented in this thesis. The procedure used to characterize the morphotypes was that outlined by Goodman et al. (1996a). Thus, for a glossary of terms used in this section refer to Goodman et al. (1996b).

References:

- Goodman, D.M., Durall, D.M., and Trofymow, J.A. 1996. Describing ectomycorrhizae. *In* a manual of concise descriptions of North American ectomycorrhizae. *Edited by* Goodman, D.M., Durall, D.M., Trofymow, J.A., and Berch, S.M. Mycologue Publications, Sidney, B.C.
- Goodman, D.M., Durall, D.M., Trofymow, J.A., and Berch, S.M. 1996. A manual of concise descriptions of North American ectomycorrhizae. Mycologue Publications, Sidney, B.C.

***Amphinema byssoides* (Pers.: Fr.) Erikss.**

Encountered on: *Picea glauca* seedlings; excised roots of *Picea glauca*

DISTINGUISHING FEATURES: yellowish white, felty or stringy tips; mycelial strands common (undifferentiated) and emanating hyphae with large 'keyhole clamps', strongly yellow in KOH.

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:

Shape: unbranched systems; straight tips 400-600 μ m wide.

Color and texture: cream to yellow, finely grainy, woolly

Emanating elements:

Mycelial strands: common, smooth or hairy

Hyphae: common, cottony, white to yellow

ANATOMY (Compound Microscope)

Mantle in plan view: medium thickness, type B (Agerer 1991)

Outer layer: a felt prosenchyma of hyaline cells 100 μ m by 3-4 μ m, no matrix, smooth to finely verrucose, commonly septate, large 'keyhole' clamps, H-shaped anastomoses

Inner layer: a net prosenchyma of hyaline cells 6-15 μ m by 3-7 μ m, smooth, simple septate

Mycelial strands: loose undifferentiated, smooth to finely verrucose, large 'keyhole' clamps, H-shaped anastomoses, bright yellow in KOH

Emanating hyphae: common, 3-4 μ m wide, smooth, commonly septate, large 'keyhole' clamps, H-shaped anastomoses, bright yellow in KOH

Cystidia: not observed

OTHER FEATURES: none observed

References:

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

Encountered on: *Picea glauca* seedlings; excised roots of *Picea glauca*

DISTINGUISHING FEATURES: black; thick, hard mantle forming a stellate pattern, blackish red; emanating hyphae black, 'whisker'-like (straight and thick).

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:

Shape: unbranched system; straight tips 500µm wide.

Color and texture: black, coarsely grainy, reflective and shiny

Emanating elements:

Mycelial strands: none observed

Hyphae: common, straight, thick and wiry

ANATOMY (Compound Microscope)

Mantle in plan view: thick mantle, type G (Agerer 1991)

Outer layer: a net synenchyma of thick walled cells 20µm by 4-5µm wide, blackish red, forming a stellate pattern

Inner layer: not observed

Mycelial strands: none observed

Emanating hyphae: common; 20-30µm long by 4-5µm wide, straight and thick walled, rarely septate (simple)

Cystidia: not observed

OTHER FEATURES:

Sclerotia: dark brown to black, hard, spherical, up to 3mm in diameter.

References:

- Agerer, R. 1991. Characterization of ectomycorrhiza. *In* Techniques for the study of mycorrhiza. *Edited by* Norris, J.R., Read, D.J., and Varma, A.K. Academic Press, London. pp 25-73.
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- Ingleby, K., Mason, P.A., Last, F.T., and Fleming, L.V. 1990. Identification of Ectomycorrhizas. Institute of Terrestrial Ecology, Scotland. p. 71

Cortinarius spp.

Encountered on: *Picea glauca* seedlings; excised roots of *Picea glauca*

DISTINGUISHING FEATURES: bright white tips, cottony, commonly clamped.

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:

Shape: monopodial pinnate systems; straight tips 600µm wide.

Color and texture: bright white to pinkish, reflective, cottony to stringy

Emanating elements:

Mycelial strands: common, white, resembling dental floss

Hyphae: white, cottony

ANATOMY (Compound Microscope)

Mantle in plan view: medium thickness, no specialized cells

Outer layer: a felt prosenchyma of hyaline cells 3-5µm wide, cylindrical, smooth, clamped

Inner layer: a net synenchyma of hyaline cells 3-4µm wide, cylindrical, smooth, rarely clamped

Mycelial strands: common; smooth - undifferentiated, hyphal cells cylindrical, smooth, clamped

Emanating hyphae: common; 2-4µm wide, smooth, cylindrical, commonly septate, clamped

Cystidia: not observed

OTHER FEATURES: none observed

References:

Agerer, R. 1987. Studies on Ectomycorrhizae X. Mycorrhizae formed by *Cortinarius obtusus* and *C. venetus* on spruce. *Mycologia*, 79: 524-539.

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. p. 16.

Gronbach, E., and Agerer, R. 1988. *Cortinarius obtusus*. In Color Atlas of Ectomycorrhiza, plate 12. Edited by Agerer, R. Einhorn-Verlag, Schwabisch Gmund.

Dermocybe spp.

Encountered on: *Picea glauca* seedlings; excised roots of *Picea glauca*

DISTINGUISHING FEATURES: white, felty or stringy tips; undifferentiated mycelial strands common; large globular and crystalline ornamentation on emanating hyphae, clamps common.

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:

Shape: unbranched system; tips are straight or slightly bent

Color and texture: white, woolly, reflective

Emanating elements:

Mycelial strands: common, white, smooth

Hyphae: common, white

ANATOMY (Compound Microscope)

Mantle in plan view: medium thickness, no specialized cells observed

Outer layer: a felt or net prosenchyma of hyaline cells 4-5 μ m wide, smooth, rarely clamped

Inner layer: a net synenchyma of hyaline cells 6-10 μ m wide, smooth, simple septate

Mycelial strands: common; loose undifferentiated, cells 4-5 μ m wide; ornamentation crystalline, globular or lacking

Emanating hyphae: common; 4-5 μ m wide; ornamentation crystalline, globular or lacking; simple septate

Cystidia: not observed

OTHER FEATURES: none observed

References:

Agerer R., and Uhl, M. 1989. *Dermocybe semisanguinea*. In Color Atlas of Ectomycorrhiza, plate 25. Edited by Agerer, R. Einhorn-Verlag, Schwabisch Gmund.

E-strain

Encountered on: *Picea glauca* seedlings; excised roots of *Picea glauca*

DISTINGUISHING FEATURES: reddish brown tips, non-interlocking synenchyma mantle with restricted septa, emanating elements rare.

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:

Shape: unbranched system; tips are straight, 300 μ m wide.

Color and texture: brown to reddish brown, smooth and glossy

Emanating elements:

Mycelial strands: none observed

Hyphae: none observed

ANATOMY (Compound Microscope)

Mantle in plan view: mantle discontinuous, mostly type K (Agerer 1991)

Outer layer: a net synenchyma to non-interlocking synenchyma, hyphal cells 6-9 μ m wide (restricted at septum), smooth, simple septate

Inner layer: not observed

Mycelial strands: none observed

Emanating hyphae: rare; 6-10 μ m wide, verrucose ornamentation, simple septate

Cystidia: not observed

OTHER FEATURES: none observed

References:

- Agerer, R. 1991. Characterization of ectomycorrhiza. *In* Techniques for the study of mycorrhiza. Edited by Norris, J.R., Read, D.J., and Varma, A.K. Academic Press, London. pp 25-73.
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- Egger, K.N., Danielson, R.M., and Fortin, J.A. 1991. Taxonomy and population-structure of E-strain mycorrhizal fungi inferred from ribosomal and mitochondrial-DNA polymorphisms. *Mycol. Res.* 95:866-872.
- Ingleby, K., Mason, P.A., Last, F.T., and Fleming, L.V. 1990. Identification of Ectomycorrhizas. Institute of Terrestrial Ecology, Scotland. pp. 15-20.

Hebeloma spp.

Encountered on: *Picea glauca* seedlings; excised roots of *Picea glauca*

DISTINGUISHING FEATURES: straight, cottony white tips; emanating hyphae verrucose, abundantly clamped

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:

Shape: unbranched system, tips straight

Color and texture: cottony, white

Emanating elements:

Mycelial strands: none observed

Hyphae: common, straight, white

ANATOMY (Compound Microscope)

Mantle in plan view: medium thickness

Outer layer: a felt prosenchyma of hyaline cells 3-4 μ m wide, verrucose ornamentation, clamped

Inner layer: a net synenchyma of hyaline cells 3mm wide, smooth, no clamps

Mycelial strands: none observed

Emanating hyphae: common; hyaline cells 3-4 μ m wide, verrucose , clamped.

Cystidia: not observed

OTHER FEATURES: none observed

References:

Brunner, I., Amiet, R., Schneider, B. 1991. Characterization of naturally grown and in vitro synthesized ectomycorrhizas of *Hebeloma crustuliniforme* and *Picea abies*. Mycol. Res. 95:1407-1413.

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. p. 16.

Ingleby, K., Mason, P.A., Last, F.T., and Fleming, L.V. 1990. Identification of Ectomycorrhizas. Institute of Terrestrial Ecology, Scotland. p. 43

***Laccaria* spp.**

Encountered on: excised roots of *Picea glauca*

DISTINGUISHING FEATURES: whitish-brown tips; outer mantle a net prosenchyma, inner mantle a net synenchyma; tortuous, hyaline hyphae with clamps abundant.

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:

Shape: unbranched to monopodial pinnate systems, tips straight

Color and texture: creamy white to brownish, smooth to felty

Emanating elements:

Mycelial strands: none observed

Hyphae: rare to common; white to hyaline

ANATOMY (Compound Microscope)

Mantle in plan view:

Outer layer: a net prosenchyma of smooth, hyaline cells 2.5–4 µm wide; clamps rare to common

Inner layer: a net synenchyma of smooth hyaline cells, simple septate

Mycelial strands: none observed

Emanating hyphae: common; tortuous, hyaline, 2–5 µm wide, irregularly formed clamps common

Cystidia: not observed

OTHER FEATURES: none observed

References:

Ingleby, K., Mason, P.A., Last, F.T., and Fleming, L.V. 1990. Identification of Ectomycorrhizas. Institute of Terrestrial Ecology, Scotland. pp. 51-58.

***Lactarius* spp.**

Encountered on: excised roots of *Picea glauca*

DISTINGUISHING FEATURES: creamy white to brownish tips, mantle a net synenchyma to non-interlocking irregular synenchyma (often contrasting between outer and inner layer), lacticifers abundant, emanating elements rare.

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:

Shape: monopodial pinnate to pyramidal, tips straight

Color and texture: creamy white to brownish, smooth

Emanating elements:

Mycelial strands: rare

Hyphae: rare

ANATOMY (Compound Microscope)

Mantle in plan view: thick mantle; type C, E (depending on species) (Agerer 1991), lacticifers abundant

Outer layer: a net, regular or interlocking 'jig-saw' synenchyma, smooth

Inner layer: a net synenchyma, smooth; lacticifers abundant, turning blue in sulphovanillin

Mycelial strands: rare; undifferentiated

Emanating hyphae: rare; hyaline, 2-3.5µm wide, simple septate

Cystidia: not observed

OTHER FEATURES: none observed

References:

Agerer, R. 1987. *Lactarius deterrimus*. In Color Atlas of Ectomycorrhiza, plate 3. Edited by Agerer, R. Einhorn-Verlag, Schwabisch Gmund.

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

Kernaghan, G., and Berch, S.M. 1996. *Lactarius scrobiculatus* (Fr.) Fr., CDE12. In A manual of concise descriptions of North American ectomycorrhizae. Edited by Goodman, D.M., Durall, D.M., Trofymow, J.A., and Berch, S.M. Mycologue Publications, Sidney, B.C.

Kernaghan, G., Currah, R.S., and Bayer, R.J. 1997. Russulaceous ectomycorrhizae of *Abies lasiocarpa* and *Picea engelmannii*. Can. J. Bot. 75: 1843-1850

***Mycelium radialis atrovirens* Melin**

Encountered on: *Picea glauca* seedlings; excised roots of *Picea glauca*

DISTINGUISHING FEATURES: tips dark green to black; mantle a net prosenchyma, rarely septate, restricted; hyphae thick walled, 'whisker'-like, simple septate, finely verrucose.

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:

Shape: unbranched system; straight tips

Color and texture: black, coarsely grainy, reflective and shiny

Emanating elements:

Mycelial strands: none observed

Hyphae: common, straight, thick and wiry

ANATOMY (Compound Microscope)

Mantle in plan view: thin mantle, Hartig net present

Outer layer: a net prosenchyma of thick walled cells 2µm wide (restricted at septum), simple septate

Inner layer: not observed

Mycelial strands: none observed

Emanating hyphae: common; dark green to black, 2-3µm wide, thick walled, curved, rarely septate (simple), finely verrucose

Cystidia: not observed

OTHER FEATURES: none observed

References:

- Ingleby, K., Mason, P.A., Last, F.T., and Fleming, L.V. 1990. Identification of Ectomycorrhizas. Institute of Terrestrial Ecology, Scotland. p. 33.
- Currah, R.S., and Tsuneda, A. 1993. Vegetative and reproductive morphology of *Phialocephala fortinii* (Hyphomycetes, *Mycelium radialis atrovirens*) in culture. Trans. Mycol. Soc. Japan. 34: 345-356.

***Piloderma* spp.**

Encountered on: *Picea glauca* seedlings; excised roots of *Picea glauca*

DISTINGUISHING FEATURES: white, coarsely felty tips; mantle is a thick felt prosenchyma of crystalline hyphae; abundant undifferentiated mycelial strands; hyphae verrucose and/or crystalline, simple septate.

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:

Shape: irregular systems, tips curved.

Color and texture: white with some yellow patches, cottony

Emanating elements:

Mycelial strands: common, white

Hyphae: common, white

ANATOMY (Compound Microscope)

Mantle in plan view: medium thickness

Outer layer: a thick felt prosenchyma, cells 3-4 μ m wide, verrucose and crystalline ornamentation, simple septate

Inner layer: a net synenchyma, cells 3 μ m wide, smooth, simple septate

Mycelial strands: common; loose, undifferentiated

Emanating hyphae: common; 3-4 μ m wide, hyaline, verrucose and crystalline ornamentation, simple septate, H-shaped anastomosis common.

Cystidia: not observed

OTHER FEATURES: none observed

References:

- Arocena J.M., Glowa, K.R., and Massicotte, H.B. 2001. Calciumrich hyphal encrustations on *Piloderma*. *Mycorrhiza*, 10:209-215.
- Brand, K. 1991. *Piloderma croceum*. In *Color Atlas of Ectomycorrhiza*, plate 62. Edited by Agerer, R. Einhorn-Verlag, Schwabisch Gmund.
- Goodman, D.M., and Trofymow, J.A. 1996. *Piloderma fallax* (Libert) Stalpers, CDE1. In *A manual of concise descriptions of North American ectomycorrhizae*. Edited by Goodman, D.M., Durall, D.M., Trofymow, J.A., and Berch, S.M. Mycologue Publications, Sidney, B.C.

***Russula* spp.**

Encountered on: *Picea glauca* seedlings; excised roots of *Picea glauca*

DISTINGUISHING FEATURES: systems monopodial pinnate, tips smooth, creamy white to brown, lacticifers lacking, simple septate, emanating elements lacking, cystidia may be present.

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:

Shape: monopodial pinnate to pyramidal systems, tips straight

Color and texture: creamy white to brownish, smooth (may be fuzzy due to cystidia)

Emanating elements:

Mycelial strands: none observed

Hyphae: none observed

ANATOMY (Compound Microscope)

Mantle in plan view: thick mantle; no specialized cell observed

Outer layer: a net prosenchyma or non-interlocking irregular synenchyma (some forming rosettes that turn blue in sulphovanillin), smooth, hyaline

Inner layer: a net synenchyma (some with partially interlocking epidermoidal cells), smooth, simple septate, hyaline

Mycelial strands: none observed

Emanating hyphae: none observed

Cystidia: rare to abundant depending on species; ampule shaped, 3.5-6.5µm wide x 13-30µm long

OTHER FEATURES: none observed

References:

- Agerer, R. 1987. *Lactarius deterrimus*. In Color Atlas of Ectomycorrhiza, plate 3. Edited by Agerer, R. Einhorn-Verlag, Schwabisch Gmund.
- Agerer, R. 1991. Characterization of ectomycorrhiza. In Techniques for the study of mycorrhiza. Edited by Norris, J.R., Read, D.J., and Varma, A.K. Academic Press, London. pp 25-73.
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***Thelephora terrestris* (Ehrh.) Fr.**

Encountered on: *Picea glauca* seedlings; excised roots of *Picea glauca*

DISTINGUISHING FEATURES: creamy white to brown shiny tips, thin net synenchyma to non-interlocking synenchyma mantle, awl-shaped cystidia with basal clamps abundant.

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:

Shape: systems not branched

Color and texture: creamy white to brownish, shiny, smooth

Emanating elements:

Mycelial strands: none observed

Hyphae: none observed

ANATOMY (Compound Microscope)

Mantle in plan view: thin; no specialized cells observed

Outer layer: a thin net synenchyma to non-interlocking irregular synenchyma of hyaline cells 4 μ m wide, smooth, simple septate

Inner layer: a net synenchyma of hyaline cells 4 μ m wide, smooth, simple septate

Mycelial strands: none observed

Emanating hyphae: none observed

Cystidia: rare to common; awl-shaped with basal clamp, 4 μ m wide by 125 μ m long

OTHER FEATURES: none observed

References:

- Agerer, R. 1991. Characterization of ectomycorrhiza. *In* Techniques for the study of mycorrhiza. Edited by Norris, J.R., Read, D.J., and Varma, A.K. Academic Press, London. pp 25-73.
- Ingleby, K., Mason, P.A., Last, F.T., and Fleming, L.V. 1990. Identification of Ectomycorrhizas. Institute of Terrestrial Ecology, Scotland. pp. 39-41.
- Gillespie, S.M.K., Durall, D.M., and Hagerman, S.M. 1996. *Thelephora terrestris*, CDE20. *In* A manual of concise descriptions of North American ectomycorrhizae. Edited by Goodman, D.M., Durall, D.M., Trofymow, J.A., and Berch, S.M. Mycologue Publications, Sidney, B.C.

Tomentella spp.

Encountered on: *Picea glauca* seedlings; excised roots of *Picea glauca*

DISTINGUISHING FEATURES: dark brown to black tips, coarsely grainy and reflective; mantle is a regular to non-interlocking irregular synenchyma; hyphae rare to abundant, curly brown and clamped; short awl shaped cystidia common.

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:

Shape: systems not branched, tips straight

Color and texture: dark brown to black, coarsely grainy, matte to reflective

Emanating elements:

Mycelial strands: none observed

Hyphae: rare to abundant, brown, tortuous

ANATOMY (Compound Microscope)

Mantle in plan view: thick; no specialized cells observed

Outer layer: a regular to non-interlocking irregular synenchyma of brown to black hyphae 4-5 μ m wide, smooth

Inner layer: a net synenchyma 4 μ m wide, smooth

Mycelial strands: none observed

Emanating hyphae: rare to abundant; 4mm wide, brown, clamped, smooth, curly

Cystidia: rare to common; awl shaped, 50-70 μ m long

OTHER FEATURES: none observed

References:

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

Goodman, D.M. 1996. *Tomentella*, CDE2. *In* A manual of concise descriptions of North American ectomycorrhizae. *Edited by* Goodman, D.M., Durall, D.M., Trofymow, J.A., and Berch, S.M. Mycologue Publications, Sidney, B.C.

Raidl, S. 1998. *Tomentella ferruginea*. *In* Color Atlas of Ectomycorrhiza, plate 137. *Edited by* Agerer, R. Einhorn-Verlag, Schwabisch Gmund.

Tuber spp.

Encountered on: *Picea glauca* seedlings; excised roots of *Picea glauca*

DISTINGUISHING FEATURES: Cream to yellow tips with abundant long awl to bristle shaped cystidia.

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:

Shape: monopodial pyramidal systems; tips straight to bent

Color and texture: cream to yellow, smooth to felty

Emanating elements:

Mycelial strands: none observed

Hyphae: none observed

ANATOMY (Compound Microscope)

Mantle in plan view: often obscured by abundant cystidia

Outer layer: an irregular to interlocking or non-interlocking synenchyma of thick-walled hyphae

Inner layer: an irregular non-interlocking synenchyma

Mycelial strands: none observed

Emanating hyphae: none observed

Cystidia: abundant; long (often curved), hyaline, simple septate, bristle to awl shaped

OTHER FEATURES: none observed

References:

Ingleby, K., Mason, P.A., Last, F.T., and Fleming, L.V. 1990. Identification of Ectomycorrhizas. Institute of Terrestrial Ecology, Scotland. pp. 63-65.

cf *Tricholoma*

Encountered on: excised roots of *Picea glauca*

DISTINGUISHING FEATURES: White cottony tips, thin tortuous hyphae, needle-like crystals abundant.

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:

Shape: no systems observed; tips straight

Color and texture: white, reflective, cottony

Emanating elements:

Mycelial strands: none observed

Hyphae: common, cottony

ANATOMY (Compound Microscope)

Mantle in plan view: thin; no specialized cells observed

Outer layer: a felt prosenchyma of hyaline cells, needle-like crystal ornamentation abundant, simple septate

Inner layer: a net synenchyma of hyaline cells, smooth, simple septate

Mycelial strands: none observed

Emanating hyphae: common; thin, needle-like crystal ornamentation abundant, simple septate

Cystidia: none observed

OTHER FEATURES: none observed

References:

Agerer, R. 1987. *Tricholoma sulphureum*. In Color Atlas of Ectomycorrhiza, plate 9. Edited by Agerer, R. Einhorn-Verlag, Schwabisch Gmund.

Lefevre, C.K. and Muller, W.R. 1996. *Tricholoma magnivelare* (Peck) Redhead, CDE18. In A manual of concise descriptions of North American ectomycorrhizae. Edited by Goodman, D.M., Durall, D.M., Trofymow, J.A., and Berch, S.M. Mycologue Publications, Sidney, B.C.

cf Paxillus involutus

Encountered on: excised roots of *Picea glauca*

DISTINGUISHING FEATURES:

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:

Shape: monopodial pyramidal

Color and texture: dark brown to red, felty to woolly

Emanating elements:

Mycelial strands: none observed

Hyphae: common

ANATOMY (Compound Microscope)

Mantle in plan view: thin; no specialized cells observed

Outer layer: a felt prosenchyma of thick walled hyaline cells, smooth, simple septate

Inner layer: a net to interlocking irregular synenchyma of hyaline cells, smooth

Mycelial strands: none observed

Emanating hyphae: common; thick walled, smooth, simple septate

Cystidia: none observed

OTHER FEATURES: none observed

References:

Ingleby, K., Mason, P.A., Last, F.T., and Fleming, L.V. 1990. Identification of Ectomycorrhizas. Institute of Terrestrial Ecology, Scotland. pp. 83-85

Unknown A (C-25)

Encountered on: excised roots of *Picea glauca*

DISTINGUISHING FEATURES: Creamy white tips with thick mantle of simple septate hyphae.

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:

Shape: monopodial pinnate to pyramidal systems; tips straight

Color and texture: creamy white, smooth

Emanating elements:

Mycelial strands: none observed

Hyphae: rare

ANATOMY (Compound Microscope)

Mantle in plan view: thick, type E (Agerer 1991)

Outer layer: a net prosenchyma of hyaline cells, commonly septate (simple)

Inner layer: none observed

Mycelial strands: none observed

Emanating hyphae: rare; smooth, hyaline, simple septate

Cystidia: none observed

OTHER FEATURES: none observed

Unknown B (F-28)

Encountered on: excised roots of *Picea glauca*

DISTINGUISHING FEATURES: dark reddish brown tips with cream apices, thick mucigel-like mantle; undifferentiated mycelial strands and thin, simple septate hyphae common.

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:

Shape: monopodial pinnate to pyramidal systems, tips straight

Color and texture: young tips red turning dark red to brown with maturity, apices hyaline to cream; velvety

Emanating elements:

Mycelial strands: rare to common; restricted point to flat angle

Hyphae: common; curved to tortuous, white to hyaline

ANATOMY (Compound Microscope)

Mantle in plan view: thick, no specialized cells observed

Outer layer: a felt prosenchyma of hyaline cells, smooth, simple septate

Inner layer: none observed

Mycelial strands: common; smooth-undifferentiated, simple septate

Emanating hyphae: common; hyaline, smooth, simple septate

Cystidia: none observed

OTHER FEATURES: none observed

Unknown D (#26)

Encountered on: excised roots of *Picea glauca*

DISTINGUISHING FEATURES: Pink to orange tips; thick mantle, hyphal cells with abundant oil bodies.

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:

Shape: monopodial pinnate to pyramidal systems, tips straight to slightly bent

Color and texture: pink to orange, smooth

Emanating elements:

Mycelial strands: none observed

Hyphae: none observed

ANATOMY (Compound Microscope)

Mantle in plan view: thick, oil-like bodies prominent

Outer layer: a net prosenchyma of hyaline cells, smooth, simple septate

Inner layer: none observed

Mycelial strands: none observed

Emanating hyphae: none observed

Cystidia: none observed

OTHER FEATURES: none observed

Unknown Basidiomycete 1 (E-27)

Encountered on: excised roots of *Picea glauca*

DISTINGUISHING FEATURES: Bright yellow reflective tips; abundantly clamped, slightly verrucose hyphae.

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:

Shape: monopodial pinnate systems, tips bent

Color and texture: bright yellow, felty, reflective

Emanating elements:

Mycelial strands: none observed

Hyphae: common

ANATOMY (Compound Microscope)

Mantle in plan view:

Outer layer: a felt prosenchyma, commonly septate, clamped, granular or oil-like bodies present in matrix

Inner layer: none observed

Mycelial strands: none observed

Emanating hyphae: common; smooth to slightly verrucose, commonly septate, clamped, granular contents

Cystidia: none observed

OTHER FEATURES: none observed

Unknown Basidiomycete 2 (G-30)

Encountered on: *Picea glauca* seedlings

DISTINGUISHING FEATURES: mantle a felt prosenchyma of smooth cells, clamps common; pale yellow to hyaline, commonly clamped hyphae abundant.

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:

Shape: not branched, tips straight to beaded

Color and texture: cream to brown, cottony

Emanating elements:

Mycelial strands: none observed

Hyphae: common, white to hyaline

ANATOMY (Compound Microscope)

Mantle in plan view: thick, no specialized cells observed

Outer layer: a felt prosenchyma of hyaline cells, smooth, clamps common

Inner layer: none observed

Mycelial strands: none observed

Emanating hyphae: common; pale yellow to hyaline, smooth, clamps common

Cystidia: none observed

OTHER FEATURES: none observed

Unknown Basidiomycete 3 (H-31)

Encountered on: excised roots of *Picea glauca*

DISTINGUISHING FEATURES: Dark tips with creamy swollen apex; localized areas of mantle staining pink in KOH; thin, tortuous, clamped hyphae with globular ornamentation

MORPHOLOGY (Dissection Microscope)

Ectomycorrhizal system:

Shape: monopodial pinnate, tips straight

Color and texture: dark brown to black, swollen hyaline to cream apex

Emanating elements:

Mycelial strands: none observed

Hyphae: common, tortuous, white to hyaline

ANATOMY (Compound Microscope)

Mantle in plan view: localized area staining pink in KOH

Outer layer: a felt prosenchyma of hyaline cells

Inner layer: a net synenchyma of hyaline cells

Mycelial strands: none observed

Emanating hyphae: common; thin, tortuous, clamped, abundant globular ornamentation

Cystidia: none observed

OTHER FEATURES: none observed