The impacts of broadcast burning after clearcutting on the diversity of ectomycorrhizal fungi associated with hybrid white spruce seedlings in central British Columbia using morphological and molecular characterization techniques

by

Karen Mah

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ABSTRACT

In British Columbia, broadcast burning following clearcutting has been used to meet forest management objectives such as site preparation for restocking timber species. However, effects of broadcast burning on ectomycorrhizae (ECM), which facilitate nutrient and energy cycling within forests, is poorly understood. Difficulties include a complex soil environment and uncertainties in ECM identification. To determine effects of broadcast burning following clearcutting on the diversity and abundance (percent colonization) of ectomycorrhizal fungi, morphological and molecular (PCR-RFLP) methods were used to assess naturally regenerating and outplanted hybrid white spruce seedlings growing in clearcut, clearcut plus burned, and adjacent mature sites in the Interior of British Columbia. Morphological characterization resulted in 24 fungal morphotypes. Significant treatment effects and seedling differences occurred between naturally regenerating seedlings in clearcut and mature sites and between naturally regenerating and planted seedlings in clearcut sites. The abundance of some morphotypes differed on planted seedlings in clearcut compared with clearcut plus burned sites and for planted seedlings in treated (clearcut and clearcut plus burned) compared with regenerating seedlings in untreated (mature) sites. A Russulaceae type and Thelephora were the most abundant morphotypes on regenerating seedlings in the mature and clearcut sites, respectively. Molecular characterization showed no significant differences for treatment effects or seedling type. Amplification of the ITS region for eight commonly occurring morphotypes revealed 12 genotypes (having a shared band pattern for one or none of three restriction endonucleases) with 18 variants (having similar band patterns for two restriction endonucleases). Cenococcum, Tuber, Hebeloma and Thelephora had only one genotype, however, Amphinema, E-strain, MRA, and a Russulaceae type each exhibited two or three genotypes. Morphology showed differences in occurrence and abundance of some ECM fungi following clearcutting, and clearcutting plus burning, suggesting that disturbance may be altering the fungal composition of hybrid white spruce seedlings on these sites towards ECM best able to adapt to changing environmental conditions. Using both characterization techniques provided a comprehensive estimate of diversity, specifically for total species richness when using morphology, and for increased understanding of inter- and intra-specific variation with respect to molecular characterization of ectomycorrhizal associations.

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Introduction

In British Columbia, broadcast burning (a form of prescribed burning) has been commonly used as a method of site preparation following clearcutting to help create a favourable environment for seedling establishment (Hawkes *et al.* 1990). To determine the efficacy of this treatment, it is necessary to examine the fire effects on the soil environment because the soil is a major determinant of site productivity (Agee 1993, Wells *et al.* 1979). For example, plant growth and productivity potential are affected by soil moisture-holding capacity, nutrient status and porosity (Hungerford *et al.* 1991). Numerous studies have been conducted on soil physical and chemical properties following clearcutting and burning. Burning effects are often confounded with those of clearcutting but in general, as the fire severity increases, negative impacts on the soil also occur, such as decreased soil porosity, increased soil erosion, and increased nutrient volatilization (Agee 1993, Wells *et al.* 1979).

The effects of broadcast burning on soil organisms have not been well documented. Of particular interest are fire effects on the overall diversity and abundance (percent colonization) of ectomycorrhizal fungi that live in symbiotic association with conifers, particularly commercial forest tree species. In forming ectomycorrhizae (ECM), these fungi contribute to nutrient and water uptake by roots and to protection against root pathogens (Harley and Smith 1983), providing a key link in nutrient and energy cycling within forest ecosystems (Dighton and Mason 1985). Some previous studies have reported a decrease in ECM abundance following fire and harvesting (Wright and Tarrant 1958; Harvey *et al.* 1980; Perry *et al.* 1982; Schoenberger and Perry 1982; Parke *et al.* 1984). However, other studies reported an increase (Pilz and Perry 1984; Brainerd and Perry 1987; Richter and Bruhn 1993) or no decrease (Visser 1995) in ECM abundance following these disturbances. Obstacles in determining responses of ECM formation to fire include the complexity of the soil environment, differences in response to fire intensity and severity as well as difficulty in identifying fungal symbionts.

Few studies have been conducted concerning the effects of fire on ECM diversity and these had limited or no descriptions of fungal types, making comparisons with current studies difficult. Recent efforts have been made to describe ECM using more detailed morphological characterization (Simard *et al.* 1997a; Horton *et al.* 1998; Visser *et al.* 1998) as well as molecular (the polymerase chain reaction-restriction

fragment length polymorphism, PCR-RFLP) methods (Kernaghan *et al.* 1997; Horton and Bruns 1998, Jonsson *et al.* 1999). Some weaknesses can be attributed to these two methods, including the inability of some morphotypes to be identified to the species level and the fact that some tips fail to amplify for molecular analysis. Traditional diversity indices (such as Shannon or Simpson) have been used in morphological analysis of ECM communities, however species uncertainty can be problematic because all species in a sample must be known (Magurran 1988). To assess diversity using molecular data, the Phi index has been derived by Egger (Baldwin 1999, M.Sc. Thesis). Using the Phi index, PCR-RFLP band patterns from ECM root tips are matched with every other tip in the sample and their distances (representative of their relatedness) are used instead of species richness and abundance data, in calculating molecular mycorrhizal diversity (Egger, pers. comm. 1999). By using a combination of morphological and molecular approaches, a more detailed assessment of mycorrhizal diversity and a better understanding of responses to burning effects can hopefully be obtained.

The main objective of the present study was to determine, using both morphological and molecular characterization (PCR-RFLP) methods, the effect of broadcast burning following clearcutting on the diversity of ECM on planted and naturally regenerating hybrid white spruce (*Picea engelmannii* (Parry ex Engelm.) x *glauca* (Moench) Voss). Mature, clearcut, and clearcut plus burned (cut plus burned) sites in the sub-boreal spruce (SBS) biogeoclimatic zone of central British Columbia were examined. In addition, the study was to explore differences in ECM diversity between planted and regenerating seedlings and to compare molecular results with previous morphological assessments. One of the main species usecl in reforestation in the Central Interior of British Columbia is hybrid white spruce, however, few studies have examined the ECM diversity of these seedlings planted on broadcast burned sites following harvesting.

Previous studies have reported decreased abundance of ECM tips with an increase in disturbance (from undisturbed to clearcut, to cut plus burned sites) (Wright and Tarrant 1958; Harvey *et al.* 1980; Perry *et al.* 1982; Schoenberger and Perry 1982; Parke *et al.* 1984). Due to the more extreme environments created by site disturbance and possible decreases in available fungal inoculum, this trend might be expected. As well, the fungal community composition between treatments may differ as those species best able to adapt to particular conditions of clearcut and cut plus burned sites may be favoured. Initial site changes

caused by clearcutting can include increased temperature extremes due to loss of shading from vegetation, destruction of the organic layer as well as disturbance of the mineral layer, and decreased soil porosity and water infiltration due to soil compaction. Following the removal of boles, crowns and forest floor, Amaranthus *et al.* (1996) reported that at moderate to severe soil compaction levels, decreased ECM abundance on outplanted Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco) and western white pine (*Pinus monticola* Dougl. ex D. Don) resulted. Disturbance of the organic and mineral layers is important, especially for fine roots. In a white spruce (*Picea glauca* [Moench] Voss) -subalpine fir (*Abies lasiocarpa* [Hook.] Nutt.) stand, Kimmins and Hawkes (1978) found that approximately 70% of overstory and understory fine-root biomass was in the LFH and Ae horizons, at an average depth of 8 cm. The initial flush of nutrients from the finer slash (Kimmins 1997) may be quickly lost when no roots or organisms are present to use them.

Depending on the fire severity and intensity, subsequent burning following clearcutting could destroy additional sources of fungal inoculum such as roots, fungal spores and sclerotia or might discourage the presence of remaining animal vectors. However, broadcast burns of light to moderate severity may in fact create an environment of increased nutrient availability and reduced competition, for those organisms remaining on, or colonizing the site. Furthermore, a few years after disturbance, soil conditions should improve, due to the regrowth of vegetation, increased shading, the formation of an organic soil layer and the return of animal vectors. Although perhaps not as great as might be seen immediately after disturbance, differences in ECM abundance and diversity might still be expected to persist.

ECM abundance and diversity might also be expected to be higher where there are many niches that specialized mycorrhizal fungi can colonize, such as in the mature forest. A steady supply of nutrients and water, regulation of temperature extremes and abundance of fungal inoculum sources and animal vectors should maintain a diverse and competitive community of ECM. Thus, there might be notable differences in fungal community composition between regenerating seedlings in the treated and untreated sites. Naturally regenerating seedlings in mature sites could provide a reference for the possible inoculum (native fungi) existing on adjacent sites following disturbance.

Differences may exist between regenerating and planted seedlings in the same site. Seedlings originating from greenhouse and nursery stock may initially possess fungal inoculum when outplanted two years later. However, new roots are gradually replaced or colonized by *in situ* fungi. Bledsoe and Tennyson (1982) reported that previously inoculated Douglas-fir seedlings, outplanted on dry, burned over sites in eastern Washington were colonized by native fungi within five months, although some fungi from the original inoculum were still present. Inoculated ectomycorrhizal fungi on outplanted black spruce (*Picea mariana* [Mill.] B.S.P.) and jack pine (*Pinus banksiana* Lamb.) nursery seedlings, declined sharply after two growing seasons and indigenous fungi were noted 11 weeks after outplanting (Browning and Whitney 1992). Another difference between planted and regenerating seedlings, grown under optimal conditions in the greenhouse and nursery for the first two years, might confer a greater chance of colonization by different and more ectomycorrhizal fungi when outplanted onto treated sites.

1. Literature Review

1.1 PRESCRIBED BURNING: OBJECTIVES AND CURRENT USES

Prescribed burning is the knowledgeable application of fire to a specific land area to accomplish predetermined forest management or other land use objectives (Merrill and Alexander 1987). In contrast, a wildfire is an unplanned or unwanted natural or human-caused fire (Merrill and Alexander 1987). Historically, wildfires have been the most important regenerative agent in coniferous forests (Ahlgren and Ahlgren 1960; DeByle 1976) but only recently have forest managers acknowledged this. After decades of fire suppression, forest managers are now attempting to utilize methods that mimic natural disturbance regimes, to meet goals of sustaining biological diversity and forest productivity (DeLong and Tanner 1996). In theory, fire should be reintroduced in the form of prescribed burns to those forest ecosystems that require periodic fire to maintain the character, diversity and vigour of the intrinsic plant and animal communities (Poulin *et al.* 1994). In British Columbia, such ecosystems include interior and northern forests in the sub-boreal spruce (SBS) biogeoclimatic zone (Vasbinder *et al.* 1996).

From a forest management standpoint, prescribed burning has been used to meet several objectives. These include reduction of the fire hazard, site preparation for replanting and seedling establishment, reduction of brush competition, facilitation of stand tending, site sanitization of disease organisms or insect pests, and natural ecosystem management as mentioned above (Hawkes *et al.* 1990; Weber and Taylor 1992; Mutch 1994; Feller 1996). Ninety percent of the prescribed burning in Canada has occurred in British Columbia, from the period of 1984 to 1992 (Feller 1996), and fire has been used as an economical and efficient tool to meet some of the above objectives (DeByle 1976; Weber and Taylor 1992). Since 1992, however, a decline in the amount of land burned in Canada for silvicultural purposes has occurred (Feller 1996). The main reasons for this decline are the logistical difficulties and economic costs in controlling fires that involve liability issues, shortage of qualified personnel and smoke concerns (Arno 1996). Meeting criteria of biodiversity hinders the use of fire in preference for other silvicultural treatments such as partial or selective cutting, combined with the increased availability of machines for mechanical site preparation (Feller 1996; Dow, pers. comm. 1997). Despite this downward trend, prescribed fire still remains an important tool in vegetation management (Feller 1996).

Normally, a burn prescription uses guides such as the Canadian Forest Fire Weather Index and Canadian Forest Fire Behavior Prediction system (Merrill and Alexander 1987) and is laid out once a site has been assessed. Three options are available to burn slash, or logging debris, namely broadcast, windrow (piling of slash into long rows) or pile burning. Broadcast burning is lower in intensity and its effects are homogeneous throughout the site as slash is distributed evenly on the ground, compared to windrow and pile burning where soil effects are more localized and severe (DeByle 1976; Hawkes *et al.* 1990). Broadcast burning imitates a natural fire better that the other two methods because there is less mineral soil disturbance, however, these fires are more difficult to control than slash pile burns (DeByle 1976).

1.2 TERMINOLOGY AND IMPORTANT DEFINITIONS RELATED TO FIRE

Critical concepts describing prescribed burns are the fire type, fire intensity, and fire severity. There are three basic types of fire in forests, categorized by the vertical strata where the burn occurs: the ground, surface and crown. In slash burns, only ground and surface fires occur. Ground fires, though of low intensity, are the most destructive as they smoulder slowly through packed organic matter and can kill roots in the forest floor. Surface fires exhibit flaming and burn rapidly, scorching bark and needles, killing seedlings and saplings, and opening serotinous cones. Each fire type releases different heat intensities and spreads at different rates, resulting in variable amounts of fuel consumed and levels of heating above-and belowground (Barbour *et al.* 1987; Hungerford *et al.* 1991; Kimmins 1997).

Fire intensity is defined as the rate of heat release per unit of ground surface area (kW/m) and is proportional to flame height and rate of spread (Wells *et al.* 1979). The rate of spread is the speed at which the leading edge of the fire travels downwind; duration refers to the time over which energy release occurs at any particular location (Kimmins 1997). The combined effects of fire intensity and duration are expressed by the term fire severity. This can be a qualitative assessment of litter, duff and soil appearance (or disappearance) after burning (Wells *et al.* 1979) or a quantitative measurement of the reduction in forest floor thickness (Merrill and Alexander 1987; Haeussler 1991; Feller 1996). Most fires in the SBS zone have been characterized as medium to high intensity surface and crown fires (Parminter 1992).

Fire severity is primarily influenced by four factors: fuel properties (compaction, composition and moisture content); weather conditions before and during fire (temperature, wind and precipitation); site conditions (topography and soil texture); and finally type of prescribed burn method used (Haeussler 1991). The timing of a fire is important in many respects. In particular, one must consider changes in plant phenology occurring throughout the year. For example, a fire in the early spring may not be as detrimental as one occurring in the summer because plants can resprout resulting in very little impact on the vegetation in the following year (Haeussler 1991; Kimmins 1997). Dormant plants (from late summer to early spring) are better protected against fire due to the presence of high levels of belowground carbohydrates and protected buds (Haeussler 1991).

1.3 FIRE AND THE SOIL ENVIRONMENT

Soil organisms play a major role in soil formation and nutrient cycling (Borchers and Perry 1990), yet soil biological responses to fire are some of the least studied aspects of the soil environment (Agee 1993). This may be due in part to the complexity of the soil environment, especially in the rhizosphere, the root surface and surrounding area where intense soil biological activity occurs (Borchers and Perry 1990). Here, nitrogen fixers, mycorrhizal fungi and root pathogens exist, interact, and use and/or produce carbon-rich root exudates, secretions of enzymes, chelators, growth hormones and antibiotics (Harley and Smith 1983). Extraction, identification and the study of small soil organisms such as bacteria and fungi often involves methods that are time-consuming, have a high degree of uncertainty, and that require constant revisions of taxonomy.

Perhaps the most important soil organisms affecting the survival of seedlings are the fungi that live in symbiotic association with living plant roots, forming mycorrhizae (Harley and Smith 1983). These symbiotic fungi represent about 10% of all recognized soil fungal species (Molina *et al.* 1992). Furthermore, an estimated 90% of all terrestrial plant species belong to families that are commonly mycorrhizal (Trappe 1987; Molina *et al.* 1992).

1.4 ECTOMYCORRHIZAL FUNGI DIVERSITY AND SPECIFICITY

Mycorrhizal fungi involved in mutualistic symbioses belong in the phyla Asco-, Basidio- and Zygomycotina and are grouped into seven currently recognized groups: vesicular-arbuscular, ecto- (ECM), ectendo-, arbutoid, monotropoid, ericoid, and orchid mycorrhizae (Harley and Smith 1983). ECM are symbiotic associations between fungi and angiosperm or gymnosperm hosts, many of which are important timber species worldwide and include species such as pine (*Pinus*), spruce (*Picea*), Douglas-fir (*Pseudotsuga*) and *Eucalyptus*. An excess of 5000 ectomycorrhizal fungi associate with likely more than 2000 plant host species (Kendrick 1992), providing for a multitude of combinations. Different hosts may possess a few or many mycorrhizal fungal partners and fungi may be host specific, to intermediate, to broad host ranging, capable of forming functionally compatible mycorrhizae on few to several members of diverse families. Douglas-fir and pines for example, have possibly 2000 associated fungal species worldwide, based on sporocarp-host associations (Trappe 1977). Currently, the data suggest that most ectomycorrhizal fungi are intermediate to broad host ranging (Molina *et al.* 1992). An example of a broad host ranging fungus is *Thelephora*, sometimes found on greenhouse-grown seedlings and capable of forming on members of many plant genera (Ingleby *et al.* 1990). However, several hundred species of fungi can be genus-specific such as *Suillus granulatus*, which only associates with *Pinus* species (Molina *et al.* 1992).

1.5 FUNCTIONS OF ECTOMYCORRHIZAL FUNGI

Increased absorption and access to minerals, increased nutrient and water uptake by plant roots, protection against root pathogens and the ability to act as large reservoirs of plant derived carbon are several documented functions of ectomycorrhizal fungi (Harley and Smith 1983). Different fungi vary in the degree to which they can perform these functions (Perry and Rose 1983) but the details of their specific functional contributions largely remain unknown.

The persistence and distribution of ectomycorrhizae in the absence of living hosts is not well documented (Harvey *et al.* 1980; Amaranthus 1991). There is potential for woody shrubs colonizing a planted site to provide a source of inoculum for host tree species. For example, *Arctostaphylos uva-ursi*, may provide ectomycorrhizal fungal inoculum for pine and spruce in clearcuts in B.C. (Molina *et al.* 1992). The suggestion of fungal linkages between plants of the same or different species implies a greater role by

mycorrhizae in seedling regeneration than is currently acknowledged. Newman (1988) proposed some important possibilities due to fungal linkages such as benefits to seedlings or to nutrient deficient plants that can link into a "hyphal network" and receive photosynthates from other hosts or from direct nutrient transfers from dying roots to living roots without going through the soil (where nutrients may be lost). Simard *et al.* (1997b), using reciprocal isotope labeling, showed bidirectional carbon transfer between birch (*Betula papyrifera* Marsh.) and Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) seedlings growing in the cedar-hemlock biogeoclimatic zone, and reported a net carbon gain by Douglas-fir. Transfer between hosts was facilitated by hyphal linkages but net carbon transfer must be demonstrated.

1.6 CHARACTERIZATION OF ECTOMYCORRHIZAE

1.6.1 Morphological techniques

Morphologically, ECM are different from other mycorrhizae in that the fungus forms a sheath or mantle that surrounds the plant root and a Hartig net develops between the root epidermal and cortical cells (Harley and Smith 1983). In angiosperms, the Hartig net intercellular penetration is barred by the exodermis, whereas in gymnosperms (which do not possess an exodermis) penetration occurs up to the endodermis (Kendrick 1992). ECM morphotypes are characterized by using light microscopy to distinguish features such as colour, mantle structure, external hyphae, and presence and structure of rhizomorphs (Agerer 1987-98; Ingleby *et al.* 1990; Agerer 1991; Goodman *et al.* 1996). Only a few ECM have been unambiguously identified to genus and species level using this characterization method (Mehmann *et al.* 1995) and the uncertainty may be partly due to the fact that morphology may vary according to changes in the root or soil environment, or to changes in host partner (Egger 1995).

Another approach to ECM identification is by linking reproductive structures or sporocarps (epigeous or hypogeous) to mycelia connected to underground root systems. ECM morphotypes in different areas of the world have been described and identified by this method (Agerer 1987-98; Ingleby *et al.* 1990; Agerer 1991; Agerer *et al.* 1996-98). ECM can also be identified by pure culture synthesis from sporocarps and re-inoculating seedlings to describe ECM. However a minority of fungal species have been successfully grown in culture (Danielson 1984).

1.6.2 Molecular techniques

Recent advances in molecular biology should help to provide a measure of ECM identification that is independent of environmental variation (Egger 1995). Restriction fragment length polymorphism (RFLP) analysis is used in combination with the polymerase chain reaction (PCR, Mullis and Faloona 1987), a process that amplifies target DNA sequences (from root tips, sporocarps or cultures) by the use of fungal-specific primers (Egger 1995; Mehmann *et al.* 1995; Gardes and Bruns 1996a). The internal transcribed spacer (ITS) region of the ribosomal unit of DNA (rDNA) has been chosen for amplification because it is variable enough to identify most fungi to the species or species group level, although it may not be variable enough to distinguish between closely related species (Gardes and Bruns 1996a). The amplified DNA is then digested by restriction enzymes and the fragments (target sequences) are run on a gel, where they migrate at different speeds and separate during electrophoresis, due to their varying lengths (Egger 1992, Gardes and Bruns 1996a). The resulting bands, visible by staining the gel, can be compared to known DNA patterns for identification or stored for reference if no match is found. The success in identifying (or matching) a given gel will depend on the database used for comparison (Gardes and Bruns 1996a).

1.6.3 Comparison of characterization techniques

Recent studies indicate that the methods outlined above vary in the precision of fungal identification. Mehmann *et al.* (1995) conducted a study on ectomycorrhizal diversity in a 40-year-old pure spruce (*Picea abies*) stand in Switzerland, and reported 23, 18 and seven types respectively, using molecular (ITS, PCR-RFLP method), morphological (using a dissecting microscope) and sporocarp techniques for identification. Sporocarp identification was the least reliable for assessing belowground diversity as fruiting varied with environmental conditions such as temperature, humidity and precipitation (Mehmann *et al.* 1995). Morphotyping of soil cores was more reliable, however, molecular characterization revealed that one morphotype could represent one species, or that one morphotype could represent more than one species, or that several morphotypes could represent one species (Mehmann *et al.* 1995). Gardes and Bruns (1996b) examined ECM diversity of a 40-year-old bishop pine (*Pinus muricata*) stand in California and reported 10 sporocarps and 20 RFLP types over a four-year period. Due to the inherent challenges of characterization approaches, a combination of morphological and molecular techniques may provide

more information about mycorrhizae than if using only one method. Environmental differences in morphology may reflect differences in functional diversity whereas molecular techniques may provide understanding of inter- and intra-specific diversity.

1.7 CALCULATING ECTOMYCORRHIZAE DIVERSITY

Simple measures of diversity are richness (the number of species), and evenness (the distribution of species abundance). Richness measures can simply be counts of species or an average number of species per sampling unit to take unequal sample size into account, such as the Margalef index: D_{Mg} = (S-1) / In n, where S is the number of species and n is the number of individuals (Magurran 1988). Evenness measures the distribution of species abundance (Magurran 1988). Heterogeneity indices take both richness and evenness into account and two types are the information statistics indices and dominance measures, represented by the Shannon and Simpson index respectively (Magurran 1988). In some studies, morphological data has been analysed using the Shannon and Simpson composite diversity indices to measure species diversity for each seedling (Brainerd and Perry 1987; Simard 1997a). The Shannon index is calculated as H = $-\sum p_i \ln (p_i)$, where p_i = the proportional abundance of the ith species; Simpson index is computed as C = $1 - \sum p_i^2$ (Magurran 1988; Brewer 1994). H is sensitive to rare species (affected more by species richness), while C is heavily weighted to the most abundant (dominant) species (Magurran 1988). As the value of the indices increase, so does diversity. These indices are nonparametric, making no assumptions of normal data distribution however all species in the sample must be accounted for or known (Magurran 1988), which is rarely the case in soil microbial studies. A measure of evenness for the Shannon index can be calculated using the ratio of observed diversity to the maximum diversity: $E = H / \ln S$.

The Sorenson coefficient of similarity measures beta diversity, the variation in species composition between areas of alpha diversity, and indicates how closely sites are related (Magurran 1988; Mehmann 1995). It is calculated as: S = 2c / (a+b), where **a** is the number of morphotypes in one plot, **b** is the number of the other and **c** is the number of morphotypes in common (Magurran 1988). A value of one

indicates total similarity. This qualitative measurement does not take into account species abundance (Magurran 1988).

To calculate molecular diversity, which presents yet another level of complexity, the Phi index may be used, which was recently developed by Egger (Baldwin 1999, M.Sc. Thesis). The index is based on phylogenetic distances and attempts to resolve the problem of intraspecific variation more adequately than traditional diversity indices (Baldwin 1999, M.Sc. Thesis). Following the analysis of RFLP band patterns, distance values for each root tip, compared with every other root tip, are calculated using the reciprocal of Dice's index based on shared and unique band patterns. The Phi index value is calculated based upon the distance matrix (Egger, pers. comm. 1999). The more distantly related species are, the greater the phylogenetic distances. The larger the value of the Phi index, the more genetically diverse the site is (Egger, pers. comm. 1999).

1.8 FIRE EFFECTS ON MYCORRHIZAE

1.8.1 Fire effects on soil organisms, soil properties and fungi

Depending on the type of fire, there may be tremendous variation in the soil disturbance and consequently, in effects on mycorrhizae. Changes vary according to the severity of the fire; a single severe fire will likely have greater impacts on mycorrhizae than several light or moderately severe ones (Agee 1993; Wells *et al.* 1979). While examining the impact of fire on soils, it must also be kept in mind that prescribed burning is often used in conjunction with harvesting and that effects of both disturbances are often confounded (Hawkes *et al.* 1990; Agee 1993).

Fire affects mycorrhizae directly by consuming roots or other sources of fungal inocula in the soil. On clearcut and prescribed burn sites, fire intensity was positively correlated with the percent colonization of ECM roots on outplanted white pine (*Pinus strobus* L.), however this was not noted for red pine (*Pinus resinosa* Ait. (Herr *et al.* 1994). Fungi in chaparral soils were reported to tolerate temperatures of 155°C in dry soil and 100°C in wet soil (Dunn and DeBano 1977). In a low severity cool-burning prescribed fire in a mixed conifer forest in California, maximum surface temperatures only reached 100°C and at 5 cm

belowground, temperatures were only 50°C (DeBano *et al.* 1998). The effect of fire on soil temperature depends on how deep the soil was heated, the maximum temperature that was reached and how long this temperature was maintained (Agee 1993).

Indirectly, ECM can be affected by changes to the soil or aboveground environment. In a Douglas-fir/larch forest soil in western Montana, Harvey et al. (1976) reported that in the top 38 cm of soil, 95% of the active ECM were associated with organic material, mainly humus and decayed wood. Consumption of organic material and woody debris in severe fires should theoretically decrease fungal inocula for regenerating seedlings and decrease habitats for small mammals (Amaranthus 1991) which disperse spores of some ectomycorrhizal fungi (Maser et al. 1978). Phoenicoid fungi (those preferring post-fire environments) may have an advantage due to their ability to produce hydrolase enzymes and to use substrates in the postfire environment (Egger 1986). Fire can also alter the dynamics of competition between ECM and other soil organisms. Bacteria are generally less susceptible to heat than fungi (Ahlgren 1974). In the increased pH environment, six years after moderately severe fires in subalpine forests in B.C. and Alberta, bacteria were more abundant than microbial fungi (Bissett and Parkinson 1980). Rhizina undulata Fr., a parasitic fungus found on conifer roots such as those of Douglas-fir seedlings and growing in acid soil in the Pacific Northwest, increased after hot slash burns (Agee 1993; Wells et al. 1979). Although not a major problem in B.C., Rhizina root rot can be destructive on postburn plantations (Baranyay 1972 in Silversides et al. 1986). Actinomycete bacteria (Streptomyces) were reported to have produced antibiotics that inhibited mycorrhizal (Laccaria laccata [Scopp. ex Fr.] Bk. & Br.) and pathogenic ((Phellinus weirii (Murr.) Gilbertson) fungal growth in soils from clearcut and burned sites (Johnson and Curl 1972 in Perry and Rose 1983). Fire also resulted in a decrease in the activity of soil invertebrates, from three months to several years afterwards (Metz and Dindal 1980 in Borchers and Perry 1990). Heat, however, did not seem to be the cause of this decline but rather it was the postfire changes (drier environment, decreased food supply and greater temperature fluctuations) in the soil environment that was responsible (Ahlgren 1974).

Removal of the aboveground vegetation and the litter layer can lead to increased soil erosion and root strength loss, increased soil temperature extremes, decreased transpiration and decreased soil moisture due to the loss of shade (Wells *et al.* 1979; Agee 1993; Kimmins 1997). Less visible effects of fire include the loss of some nutrients, a decrease in soil acidity, decreased bulk density and porosity of the soil, and changes in water repellency of the soil. In the quick combustion of organic matter, there is an immediate loss of some nutrients, largely nitrogen and to a lesser extent other elements (Agee 1993; Wells *et al.* 1979). Nitrogen is the nutrient most limited in many forest ecosystems, volatilizing at 175°C to 200°C (White *et al.* 1973). In less severe fires, non-volatilized nitrogen can be leached from the system in the form of nitrate by nitrifying bacteria, that are sensitive to high temperatures (Agee 1993; Wells *et al.* 1979). Total losses in nitrogen caused by fire cannot be immediately replaced by natural sources (from precipitation and free living nitrogen fixation), however, over time, levels should return to normal (DeBell and Ralston 1970; Binkley 1991).

Sulfur, potassium and other nutrients are converted to a more available form in residual organic material if the fire is less severe (Agee 1993). Calcium, magnesium and sodium are transformed to soluble mineral forms (Wells *et al.* 1979) that are major components of ash (DeByle 1976; Agee 1993). These excess basic cations increase the pH of the soil, further affecting the availability of nutrients (Ahlgren and Ahlgren 1960; Wells *et al.* 1979). For example, chelated iron (Fe³⁺), the form available to plants was less soluble at a higher pH (6.16) in a broadcast burned soil (Perry *et al.* 1984). In addition, leaching of nutrients becomes greater in soils with low cation exchange capacity than in fertile soils where nutrients tend to adhere to clay and organic matter particles (Borchers and Perry 1990; Agee 1993).

1.8.2 Effects of fire on mycorrhizal abundance and formation

Although comparison between studies can be difficult, a majority of those reviewed reported decreases in the number of active mycorrhizal tips following fire disturbance (Table 1). Most studies were conducted in the Pacific Northwest using Douglas-fir as the preferred host. Different sources of mycorrhizae were examined, including naturally regenerating seedlings, soil cores, seedlings grown in the greenhouse from transferred soils and seedlings outplanted in disturbed and transferred soils. Total ectomycorrhizal

formation was usually measured by the number of active (live) root tips, and quantitative methods of assessment varied among investigators (Table 1). Differences found in these studies could be attributed to variation in the fire regimes, site conditions, host species and experimental protocol. Some studies reported an increase (Pilz and Perry 1984; Brainerd and Perry 1987), however many studies reported a decrease in ECM, from undisturbed to clearcut to burned sites (Harvey et al. 1980; Perry et al. 1982; Schoenberger and Perry 1982; Parke et al. 1984). Some interesting conclusions were also presented. Harvey et al. (1980) suggested that in difficult-to-regenerate sites, partial cutting may be less detrimental than burning in terms of ECM formation. Inoculum potential was examined in greenhouse studies conducted by Perry et al. (1982) and Parke et al. (1984), who found that seedlings grown in disturbed (clearcut plus burned) soils had decreased numbers of mycorrhizal tips than those in undisturbed (mature forest) sites. Brainerd and Perry (1987) examined mycorrhizae growing in soils in three sites along an elevation and moisture gradient and concluded that seedlings growing in cold, dry environments appeared to be more detrimentally affected by disturbance (clearcutting plus burning) than wetter and mesic sites. Greenhouse studies conducted by Schoenberger and Perry (1987) reported an increase of ECM in plantation soils previously clearcut plus burned (in Douglas-fir but not in western hemlock). Soil transfers from previously clearcut plus burned plantation sites were shown by Amaranthus and Perry (1987) to increase mycorrhizae formation in cold, dry sites. The authors speculated that mycorrhizae from the plantation soils were more compatible with seedlings in the clearcut environment than those in soil transferred from the mature forest.

	Mycorrhizae source/ treatment	Host* (age)/ location	Results: relative mycorrhizal abundance	Reference
field	seedlings/ undisturbed (Fd), light, and severe clearcut plus burn	Fd (1, 2 yr)/ Oregon, south- central Washington	 % mycorrhizal seedlings (1 < 2 yr-olds) in undisturbed> lightly burned≥ severely burned mycorrhizae at greater depths (1 yr-old) with increase in burn severity. 	Wright and Tarrant (1958)
field	soil cores/ undisturbed (Fd/larch, 250 yr), intensive cut (3 yr), partial cut plus burn (2 yr)	/western Montana	 number of mycorrhizal tips/volume of soil: undisturbed, intensive cut > partial cut plus burn. 	Harvey et al. (1980)
green- house	soil samples/ undisturbed 1 (Fd, 250+ yr; Hw, 100+ yr), undisturbed 2 (Fd, 200 yr; Hw, 100 yr), clearcut (1 yr), clearcut plus burn (<1 yr), natural burn (36-40 yr), plantation previously clearcut plus burned (18 yr)	Fd (4.5 mo.), Hw (6 mo.)/ west-central Oregon, Cascades	 average total mycorrhizal root tips/ seedling: Fd- clearcut > undisturbed 1, clearcut plus burn, natural burn > undisturbed 2, plantation; Hw-undisturbed 1 and 2, clearcut, natural burn > plantation, clearcut plus burn % mycorrhizal root tips: Fd- undisturbed 1, clearcut> clearcut plus burn, natural burn, undisturbed 2> plantation; Hw- natural burn, plantation> undisturbed 1 and 2, clearcut, clearcut plus burn, natural burn, undisturbed 1 and 2. 	Schoenberger and Perry (1982)
green- house	soil samples / undisturbed (Pl, 100+ yr), clearcut plus windrow, windrow burned (13 yr)	Fd, Se, Pl (4-6 mo.)/ south-west Montana	 total and mycorrhizal root tips/ seedling: undisturbed > clearcut plus windrow burned, clearcut, clearcut plus windrow small or no difference in % mycorrhizal tips. 	Perry et al. (1982)
green- house	soil samples / undisturbed, clearcut, clearcut plus burn (1-22 yr)	Pp, Fd (14-16 wks)/ south-west Oregon, northern California	 visual estimation of % mycorrhizal colonization of total root tips: undisturbed > clearcut > clearcut plus burn. 	Parke et al. (1984)
field	soil samples / undisturbed (Fd/Hw 80-250 yr), clearcut (2-3 yr), clearcut plus burn (1 yr) soils transferred to each undisturbed, clearcut, clearcut plus burn site	Fd (1yr 6.5 mo.)/ west-central Oregon, Cascades	 mean mycorrhizal tips/ seedling: by aboveground environment clearcut > clearcut plus burn > undisturbed; no difference looking at soil origin but greater number of non-mycorrhizal tips in clearcut and clearcut plus burned soils. 	Pilz and Perry (1984)
green- house	soil samples / undisturbed, clearcut, clearcut plus burn (3-5 yr)/ mesic coastal, moist and dry montane forest types	Fd, Pp (6 mo.)/ Oregon	 mycorrhizal % colonization in undisturbed soils: dry montane > moist montane > coastal. In disturbance soils, mycorrhizal colonization significantly increased in the coastal site, and no significant change occurred in other sites. 	Brainerd and Perry (1987)
field	soil samples / undisturbed (Fw/Fd/ Ps) and plantation (previously clearcut and, clearcut plus burn (10-15 yr)) soils were transferred	Fd, Ps (1yr 7 mo.)/ south-west Oregon, northern California	 increased mycorrhizal formation in Fd (doubling) from plantation soil transfers (previously clearcut plus burned) and in Ps from plantation soils (previously clearcut); no improvement in soil transfers from undisturbed sites. 	Amaranthus and Perry (1987)

1.8.3 Studies on mycorrhizal diversity and fire

Some studies reviewed above discussed mycorrhizal diversity based on general groupings such as white and brown types and the black *Cenococcum geophilum* Fr. Using such a broad classification scheme, an accurate estimate of the mycorrhizal diversity cannot be obtained and the analysis is not very insightful. In a study describing 12 ectomycorrhizal morphotypes, Pilz and Perry (1984) reported that there were fewer ECM types found in disturbed compared with the undisturbed sites and that the aboveground changes caused by disturbance probably influenced mycorrhizal formation more than the soil environment. Schoenberger and Perry (1982) identified five major ECM groups, and reported that those associated with Douglas-fir were greater in abundance in unburned, clearcut soil.

Diversity studies on mycorrhizae following disturbance have recently been conducted using traditional measures of diversity. Visser (1995) studied the effect of time in a successional study on ectomycorrhizal fungi in 6, 41, 65 and 122-yr-old jack pine stands following wildfire and reported a significant increase in mycorrhizal species richness between the 6 and 41-yr-old stands. Simard *et al.* (1997) reported a doubling in mean richness, diversity, and evenness of ECM on outplanted one-year-old Douglas-fir seedlings in untrenched compared to trenched sites in 90 to 120 year-old Douglas-fir and paper birch (*Betula papyrifera* Marsh.) dominated forests. Hagerman *et al.* (1999) reported reduced ECM richness and diversity from soil cores collected in clearcut compared to mature forest sites, in a subalpine forest dominated by 95 to 325 year-old subalpine fir and Engelmann spruce.

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2. Morphological Characterization of Ectomycorrhizae Associated with Hybrid White Spruce Seedlings in the Aleza Lake Research Forest in the Central Interior of British Columbia

ABSTRACT

Broadcast burning is a forest management practice used for site preparation in British Columbia but its effects on ectomycorrhizae (ECM), which provide a key link in nutrient and energy cycling within forest ecosystems, is not well understood. To assess the effects of broadcast burning on ECM abundance and diversity, 88 outplanted and naturally regenerating hybrid white spruce seedlings growing in two replicate sites, each of mature, clearcut, and clearcut plus broadcast burned sites in the sub-boreal spruce (SBS) biogeoclimatic zone were examined. Fungal symbionts were characterized by morphological assessment and the ECM abundance and diversity were determined for each seedling. A total of 24 distinct ECM morphotypes were described, 14 of which had known fungal affinities at the genus level. The dominant types, matching descriptions of E-strain, Cenococcum, MRA, Amphinema, Hebeloma, Thelephora, and a Russulaceae type showed variable treatment and seedling type differences. The morphotypes E-strain, MRA, and Amphinema and the lightly colonized or non-mycorrhizal group were significantly more abundant on planted seedlings in the treated (clearcut and clearcut plus burned) than untreated (mature) sites. With respect to regenerating seedlings, Russulaceae type 1 was the most abundant morphotype in the mature forest and Thelephora was the most abundant morphotype in the clearcut sites. The diversity of ECM on planted seedlings between clearcut and clearcut plus burned sites was not significantly different, however, Cenococcum was significantly less, and Hebeloma and Russulaceae type 1 were significantly more abundant in clearcut plus burned sites compared with sites which were only clearcut. ECM diversity of regenerating seedlings was significantly lower in clearcut sites, compared to those in adjacent mature sites and compared to planted seedlings in the clearcut sites. Results show that changes in the occurrence and abundance of some ECM fungi is occurring following clearcutting and clearcutting plus burning. This suggests that disturbance may be altering the fungal composition on hybrid white spruce seedlings on these sites towards those types best able to adapt to the changing environmental conditions.

2.1 INTRODUCTION

In British Columbia, broadcast burning has been commonly used following clearcutting to meet forest management objectives, one of which is site preparation for seedling establishment after harvesting. This practice impacts the soil environment, thus affecting site productivity (Wells *et al.* 1979; Agee 1993). Studies conducted on the impacts of fire on soil physical and chemical properties have shown that effects vary according to fire intensity and severity. Furthermore, burning effects are often confounded with clearcutting, which usually precedes it (Agee 1993). Most broadcast burns, planned to be of low to medium severity, should have less drastic impacts than severe burns. Burning impacts may include charring and blackening of the soil surface, removal of competing vegetation, partial consumption of slash and partial reduction of the organic layer (Silversides *et al.* 1996). Less obvious effects include increased soil pH, increased availability of soil nutrients and increased soil temperature extremes (Wells *et al.* 1979; Agee 1993). Severe burns may cause more deleterious impacts than less severe burns such as soil erosion, volatilization of soil nutrients, decreased soil porosity and decreased water infiltration (Wells *et al.* 1979; Agee 1993).

The effects of broadcast burning on soil organisms have not been extensively studied. One important group in the soil is mycorrhizal fungi that live in a symbiotic relationship with plant roots, and provide essential functions such as increased water and nutrient uptake and protection against root pathogens (Harley and Smith 1983). Mycorrhizal fungi thus are a key link in nutrient and energy cycling within forest ecosystems (Dighton and Mason 1985). Of the seven currently recognized mycorrhizal classes, ectomycorrhizae (ECM) are represented by an excess of 5000 fungi which associate with likely more than 2000 plant host species (Kendrick 1992), including commercially important conifer hosts such as spruce (*Picea*) and pine (*Pinus*) in the Central Interior of British Columbia. Ectomycorrhizal fungi have different physiological and ecological requirements such as optimum pH and temperature for growth (Cline *et al.* 1987), tolerance to drought (Nilsen *et al.* 1998) and resistance to root pathogens (Perry and Rose 1983). It has been proposed that seedlings growing in disturbed soil environments could benefit from having access to a diversity of fungi; those symbionts best able to function would be favoured and could provide a buffering capacity for seedlings to adapt to changes in the environment (Perry *et al.* 1987, Simard *et al.* 1997). Furthermore, the loss of fungal species in a functional group might result in a diminished capacity of the group to work (Staddon *et al.* 1996) and hence diminished ecosystem function or a reduction in

ability of seedlings to successfully grow in disturbed environments. Thus the effect of site treatment on seedling ECM abundance and diversity should be an important consideration in forest management practices. One of the main species used in reforestation in the Central Interior of British Columbia is hybrid white spruce, however, few studies have examined the ECM diversity of these seedlings planted on broadcast burned sites following harvesting.

Although some studies have been conducted on mycorrhizal formation following fire, less is known concerning mycorrhizal diversity (Staddon et al. 1996). Burning might be expected to directly reduce the number of soil fungal species and sources of inoculum. Obstacles hindering fungal diversity studies following cutting and burning include the complexity of the soil environment, the difficulty in identifying fungal symbionts and differences in effects of fire intensity on ECM. The majority of the studies which examined mycorrhizal formation following harvesting and fire have reported decreased ECM abundance but comparisons between studies is difficult because of different methods used for assessment (Wright and Tarrant 1958; Harvey et al. 1980; Perry et al. 1982; Schoenberger and Perry 1982; Parke et al. 1984). For example, Wright and Tarrant (1958) assessed seedlings as being mycorrhizal or nonmycorrhizal, Schoenberger and Perry (1982) examined the percent of mycorrhizal root tips per seedling and Parke et al. (1984) used visual estimates on a scale of 20% increments. Descriptions of fungal types in these earlier studies were rudimentary, such as categorizing types into white, brown or black groups (Wright and Tarrant 1958), or using only macroscopic characteristics to describe types (Schoenberger and Perry 1982), compared to more recent studies on ECM diversity. The improved resolution and amount of information available has increased the scope of and confidence in ectomycorrhizae identification (Agerer 1987-98; Ingleby et al. 1990; Agerer 1991; Agerer et al. 1996-98; Goodman et al. 1996). For example, Hagerman et al. (1999) reported 39 mycorrhizal types from soil cores collected in a three year study of ECM on clearcuts in a subalpine forest in southern British Columbia.

The main objective of the present study was to determine, using morphological characterization, the effect of broadcast burning following clearcutting on the abundance and diversity of ECM on outplanted and naturally regenerating hybrid white spruce seedlings growing in the sub-boreal spruce (SBS) biogeoclimatic zone of central British Columbia. Three different site types were compared: mature (undisturbed), clearcut only, and clearcut plus broadcast burning (cut plus burned). A second objective

was to compare the ECM abundance and diversity between planted and regenerating seedlings on these sites to assess seedling difference.

2.2 MATERIALS AND METHODS

2.2.1 Site descriptions

The study area included four treated sites (two clearcut and two cut plus burned) and two adjacent mature forest sites, located approximately 36 km east of Prince George, British Columbia, near the Bowron River in the south-western portion of the Aleza Lake Research Forest (Figure 1). This area is in the SBS biogeoclimatic zone, willow wet, cool (wk1) variant. The SBS zone is situated in the Central Interior of British Columbia with a latitudinal range of 51° 30' to 59° N (Meidinger et al. 1991) and a variant elevation range of 660 to 1140 m (DeLong et al. 1996). The climate in the SBS zone is continental, with severe snowy winters, and warm, moist, short summers and is characterized by seasonal temperature extremes with averages below 0°C for four to five months of the year and above 10°C for two to five months (Meidinger et al. 1991). Mean annual precipitation is 440 to 900 mm, of which 25 to 50 percent is snow. Uneven aged and multi-storied canopies have resulted from fire suppression; stand destroying fires ranging from 20 to 1000 ha in size occur approximately every 200 years (DeLong et al. 1996). Hybrid white spruce (Picea engelmannii (Parry ex Engelm.) x glauca (Moench) Voss) and subalpine fir (Abies lasiocarpa (Hook.) Nutt.) are the climax species and hybrid spruce-oak fern is the zonal association (Meidinger et al. 1991). Dominant understory species at the zonal oak fern site include Lonicera involucrata (Richards.) Banks ex Spreng., Ribes lacustre (Pers.) Poir in Lamarck, Vaccinium membranaceum Dougl., Rubus parviflorus Nutt., Viburnum edule (Michx.) Raf., Oplopanax horridus (Smith) Miq., Gymnocarpium dryopteris (L.) Newm., Cornus canadensis L., Orthilia secunda (L.) House and Rubus pedatus J.E. Sm. (DeLong et al. 1996). Common soils in the Bowron River valley are Brunisolic Gray Luvisols and Gray Luvisols, formed on loam to clay glaciolacustrine deposits (DeLong et al. 1996). The humus forms are mor and moder types (Meidinger et al. 1991). The mature forest study sites were approximately 100 and 200 years old; vegetation and dominant canopy tree species were similar (Figure 2). The forest floor in the study area was dominated by both mor and moder types of humus and the mineral soils were silt loam to clay loam in texture.



Figure 1. Location of sites in the Aleza Lake Research Forest, Prince George Forest District (insert from Prince George Forest District recreation map, BC MOF, FRBC, Feb. 1997. Scale approximately 1:400 000).



Figure 2. A portion of the forest floor in the mature site located in the Aleza Lake Research Forest, Central Interior of British Columbia (June 1997)



Figure 3. a) cut plus broadcast burned site and b) clearcut site located in the Aleza Lake Research Forest, Central Interior of British Columbia (June 1997).
Northwood Pulp and Timber Limited held the license for both the clearcut and cut plus broadcast burned sites (pre-harvest silviculture data is shown in Appendix A). Table 2 summarizes site treatment and includes winter harvesting, fall burning and planting data. Planted hybrid white spruce seedlings consisted of two-year-old (one year greenhouse plus one year nursery) stock. Following harvesting, estimates of 30 to 46 cm of moderate to heavy compact slash were typical of the clearcut sites which were to be broadcast burned (Ron Jansen, pers. comm. 1997). Using a prescribed fire predictor/planner, the desired reduction of the slash was targeted at four to six cm, which is defined as a moderate fire severity (Feller 1996). However, no measurements were taken to determine fire severity such as depth of burn of the litter layer or fuel characterization. Therefore it cannot be certain that these prescribed burn objectives were met. Furthermore, fire intensity was not measured at the time of burning (Ron Jansen, pers. comm. 1997).

Site	Elevation (m)	Harvesting date/activity	Date/subsequent treatment	Hybrid white spruce planting
C 1	675	1/92-3/92 (96.9 ha)	9/92 windrowing (86.6 ha)	6/93 (82.9 ha)
			6/93 mounding (1.9 ha)	6/94 (49.7 ha)
				6/95 (90.3 ha)
C 2	675	1/92-3/92 (38.1 ha)	9/92 windrowing, windrow burned (36.8 ha)	6/93 (36.8 ha)
CB 1	686	11/93-3/94 (84.1 ha)	6/94 burn (56.3 ha), mounding (24.5 ha)	6/95 (66.72 ha)
			10/94 burn for hazard reduction (80.9 ha)	
CB 2	670	1/94-3/94 (38.2 ha)	10/94 burn (36.6ha), mounding (6.1 ha)	6/95 (18.47 ha)

Table 2. Site descriptions and dates for clearcut (C), and cut plus broadcast burned (CB) treatments in the Aleza Lake Research Forest.

2.2.2 Seedling sampling

On June 23 and 24, 1997, a 50 m x 50 m block was established in each study site for sampling; each block was situated at least 10 m inside site boundaries to minimize edge effects. Blocks in the clearcut sites were situated at least 10 m away from windrows. Within each clearcut or cut plus burned site (Figure 3a, b), 28 planted hybrid white spruce seedlings were tagged, of which fourteen were randomly selected (using a computer-generated random number table) and double tagged. In each of the nearby mature sites, 16 naturally regenerating hybrid white spruce seedlings were tagged and eight were randomly selected. Half of these double-tagged seedlings were harvested in June and the remaining seedlings were harvested on August 27 and 28, 1997. At this time, eight naturally regenerating hybrid white spruce seedlings were harvested in June and the remaining hybrid white spruce seedlings were harvested on August 27 and 28, 1997. At this time, eight naturally regenerating hybrid white spruce seedlings were harvested in June and the remaining hybrid white spruce seedlings were harvested on August 27 and 28, 1997. At this time, eight naturally regenerating hybrid white spruce seedlings were harvested in June and the remaining hybrid white spruce seedlings were harvested on August 27 and 28, 1997. At this time, eight naturally regenerating hybrid white spruce seedlings were harvested in June and the remaining hybrid white spruce seedlings were harvested in June and the remaining hybrid white spruce seedlings were harvested on August 27 and 28, 1997.

regeneration of hybrid white spruce appeared to be occurring on either of the cut plus burned sites, though seedlings were found growing on the landings and roads leading into these sites. Unfortunately, this precluded sampling of regenerating seedlings on cut plus burned sites. A summary of the sampling design is presented in Table 3.

Table 3. Sampling design for hybrid white	spruce seedlings harvested	from clearcut, cut plu	s burned and mature,
sites in the Aleza Lake Research Forest.			

Seedling type	Date	Site type			
		Mature	Clearcut	Cut plus burned	
Planted	Spring	-	14 (7 x 2 sites)	14 (7 x 2 sites)	
	Fall		14 (7 x 2 sites)	14 (7 x 2 sites)	
Naturally regenerating	Spring	8 (4 x 2 sites)	-	-	
	Fall	8 (4 x 2 sites)	16 (8 x 2 sites)		
Total		16	44	28	

Seedlings were harvested with the surrounding soil to a depth and radius of approximately 20 cm, then bagged and transported in 7 L plant pots to avoid disturbing the root systems. Adjacent to each harvested seedling (with the exception of regenerating seedlings), soil samples and slash measurements were taken in both clearcut and cut plus burned sites. In the mature sites, where most seedlings were growing on a woody substrate, representative soil samples were collected nearby. Local site conditions including soil horizon thickness, soil moisture, seedling substrate and microtopography as well as seedling height and leader growth were recorded. The seedlings (88 in total) were stored at 5°C until ECM characterization.

2.2.3 Soil and seedling analysis

In the laboratory, soil pH, total carbon and nitrogen of the mineral and organic horizons, and seedling age and basal diameter were measured. Soil pH was measured by the CaCl₂ method described in Hendershot *et al.* (1993). Air dried, sieved (2 mm mesh) mineral and organic soil samples were mixed with 0.01 M CaCl₂ in 50 ml conical tubes at 1:2 and 1:10 (soil:solution (g/ml)) ratios, respectively. They were then shaken mechanically (Eberbach shaker) for half an hour at high speed, allowed to settle for an hour and measured with a two point calibration pH meter (benchtop pH/ISE meter, model 420A). Soil analysis of total carbon and nitrogen were conducted on a Carlo Erba NA1500 Elemental Analyser using the standard Atropine with a detection limit of 0.01%. Approximately 5 to 10 mg of organic and 30 to 60

mg of mineral sieved (0.15 mm mesh) soil were used for analysis. Carbon/nitrogen (C/N) ratios were then calculated.

Using a dissecting microscope (Olympus SZ-30), seedling age was estimated by counting bud scars on the stem as well as growth rings on sanded cookies that had been cut just above the root collar. The 16 regenerating seedlings collected in the clearcut were aged by counting bud scars only and were estimated to be four years old. Basal diameter was averaged over the longest and shortest diameters of the cookies. To estimate the stand age of the mature sites, each of the five largest diameter hybrid spruce and subalpine fir trees in each site were cored and the growth rings were counted.

2.2.4 Ectomycorrhizae characterization

For each seedling, root systems were soaked in cold water for several hours, then carefully washed to eliminate soil particles and organic debris. Occasionally, fine forceps were used to remove remaining soil particles, under the magnification of the dissecting microscope. The entire root system of naturally regenerating seedlings was sampled (Figure 4a), however, for planted seedlings, only lateral and egressed roots growing from the soil plug were selected (Figure 4b). Root systems were floated in water over a grid consisting of 2 cm² cells. Root samples, 2 cm long, were randomly sampled until approximately 200 tips were selected. If a cell contained greater than 20 root tips, a sub-cell (1 cm² in size) was randomly sampled. Only healthy root tips (i.e. turgid root and intact meristem) with a length greater than three times the root width were selected. To avoid confusion with branching forms, an unbranched tip was considered as one mycorrhiza. If there were fewer than 200 root tips, all healthy tips were sampled. Initial macroscopic observations of ECM characteristics were made using the dissecting microscope. Subsequently, root squashes were prepared and viewed using a compound microscope (Olympus CH-2, 100-1000x). ECM features such as fungal mantle, presence of rhizomorph, emanating hyphae and other distinguishing characteristics were documented. Permanent slide mounts of squashes were made, fixed with high viscosity mountant (CMCP-10, Polysciences, Inc.). Macroscopic and microscopic features of root tips were photographed (Appendix B) using an automatic exposure (PM-10AK) photomicrographic system either attached to a dissecting microscope (Olympus BX-50) or a compound microscope (Olympus SZ-40).



Figure 4. Root systems of a) naturally regenerating and b) planted hybrid white spruce seedlings harvested from mature forest and cut plus broadcast burned sites, respectively, in the Aleza Lake Research Forest, Central Interior of British Columbia.

All root tips were categorized as mycorrhizal, non-mycorrhizal or mycorrhizal but lightly colonized (unidentifiable because of poorly developed features). Morphological descriptions of ECM were made with reference to a checklist (Appendix C) adapted from manuals by Agerer (1987-98), Ingleby *et al.* (1990), and Goodman *et al.* (1996). If an ECM could not be readily identified to genus or species, the morphotype was given a type name, based on conspicuous features (Appendix D). For each seedling, the number of ectomycorrhizal morphotypes and the proportional abundance (p) of each were calculated. If the morphotype was not found on a seedling, a value of 0 was assigned. As well, the site where morphotypes occurred and the frequency of occurrence of each morphotype (number of seedlings on which they occurred) were recorded. Non-mycorrhizal and lightly colonized tips were grouped together to calculate overall ECM abundance or formation (percent colonization) on hybrid white spruce for each type of study site.

2.2.5 Statistical analysis of morphotype abundance and diversity indices

General seedling, environmental and site characteristics were compared using Students t-test. In addition, correlations (Pearsons product-moment) of seedling measurements (leader growth, height and basal diameter) with abundance data were calculated. Differences in ECM abundance for seven of the most commonly occurring morphotypes (*Cenococcum*, E-strain, *MRA*, *Amphinema*, *Hebeloma*, *Thelephora* and Russulaceae type 1), as well as for the lightly colonized, unknown category, were determined using a one-way ANOVA (STATISTICA for Windows Release 5.1 G 1997 edition, Statsoft, Inc.) for a completely randomized design. Data for replicate sites and for season were pooled as determined by Students t-test using a Bonferroni correction of $\alpha = 0.004$ (α / number of comparisons = 0.05 / 13). To compensate for a skewed distribution, the data were transformed by the arcsine \sqrt{p} function (Sokal and Rohlf 1987), where p is the proportional abundance of a morphotype on a seedling.

Due to an incomplete experimental design (see Table 3 for sampling design), a Bonferroni correction of α = 0.01 (0.05 / 5) was used for five planned comparisons: 1) between planted seedlings in clearcut and cut plus burned sites to test for burning effects; 2) between regenerating seedlings in mature and planted seedlings in clearcut sites to test for cutting and seedlings effects; 3) between regenerating seedlings in mature and planted seedlings in cut plus burned sites to test for treatment and seedling effects; 4) between regenerating seedlings in mature and clearcut sites to test for cutting effects; and 5) between planted and regenerating seedlings in clearcut sites to test for seedling differences.

The ectomycorrhizal diversity for each seedling was measured using the Shannon and Simpson composite indices, Shannon evenness and Margalef richness measures (Magurran 1988) (see Appendix E for sample calculations). Data for lightly colonized and uncolonized tips were excluded when calculating diversity measures and morphotype data were not transformed. Preliminary analysis (Student t-test, Bonferroni correction of α = 0.004) indicated that replicate sites and seasonal data could be pooled. One-way ANOVA was used in a completely randomized design to determine treatment or seedling effects on diversity as stated above.

2.3 RESULTS

2.3.1 Site and seedling characteristics

Results comparing general site and seedling characteristics are presented in Table 4. The slash height in clearcut sites was significantly less than that on cut plus broadcast burned sites. The LFH layer thickness, ranging from 1.6 to 3.3 cm, only differed significantly between clearcut site 1 and cut plus burned site 1. The pH and C/N values were higher in the LFH layer than in the mineral layer. Cut plus burned site 2 had a higher pH value than other treated sites. No differences were found for the C/N values between any of the sites. Seedling ages ranged from four to seven years (at the time of sampling) with the youngest seedlings occurring in the cut plus burned site 2 and clearcut sites (regenerating seedlings, data not shown) and the oldest seedlings in mature site 1. Planted seedlings were significantly taller and had significantly greater leader growth and basal diameter than naturally regenerating seedlings harvested from the mature sites.

Table 4. General site, seedling^{\dagger} and soil characteristics (means ±SE)^{\ddagger} for mature, clearcut and cut plus broadcast burned sites sampled in the Aleza Lake Research Forest.

	Mature 1	Mature 2	Clearcut 1‡	Clearcut 2	Cut plus burned 1	Cut plus burned 2
Slash (cm)		-	5.9(0.5)a	7.1(0.8)a	10.4(0.9)b	11.0(1.1)b
Soil data						
LFH layer						
thickness (cm)	3.3(1.3) ab	2.8(0.3)ab	1.6(0.2)a	2.6(0.4)ab	3.0(0.3) b	2.2(0.3)ab
pH						
LFH layer	4.70(0.08)ab	4.57(0.08)ab	4.57(0.07)a	4.45(0.06)a	4.57(0.15)a	5.09(0.14)b
mineral layer	4.30(0.26)a	4.28(0.12)a	4.27(0.03)a	4.14(0.03)a	4.11(0.09)a	4.16(0.07)a
C/N						
LFH layer	35.48a	32.50a	34.25(8.90)a	25.99(2.47)a	26.00(2.44)a	22.96(0.79)a
mineral layer	11.48(3.31)a	16.8(2.6) a	16.56(2.30)a	14.9(2.2) a	16.62(3.00)a	13.7(1.1) a
Seedling data						
age(yr±)	6.5(0.7)a	5.5(0.7)abc	5.8(0.1)ab	6.0(0.2)ab	4.9(0.1)bc	4.3(0.2)c
leader growth						
(cm)§	3.2(0.3)a	3.4(0.2)a	16.6(1.2)b	16.0(1.4) b	20.1(1.6) b	18.5(1.3)b
height (cm)	17.0(2.1)a	13.3(1.1)a	67.1(4.3)b	64.9(2.3)b	69.3(3.0)b	66.5(2.2) b
basal diameter						
(cm)	0.3(0.1)a	0.3(0.1)a	1.7(0.1)b	1.6(0.1)b	1.5(0.1) b	1.5(0.1) b

†data does not include measurements for regenerating seedlings harvested from clearcut sites. ‡within rows, means followed by the same letters are not significantly different ($p \le 0.05$) as determined by one-way

twittin rows, means followed by the same letters are not significantly different ($p \ge 0.05$) as determined by one-way ANOVA.

§leader growth was log transformed. Values presented here are non-transformed.

2.3.2 ECM morphotype occurrence, frequency of occurrence and abundance

Morphotype occurrence for the different treatments as well as other categories is summarized in Table 5.

Overall, a total of 24 ECM morphotypes were described, four of which occurred on fewer than 5% (4) of

the seedlings. More basidiomycete (19) than ascomycete (5) fungal symbionts were described. Fourteen

types had morphological features that could be readily matched to descriptions in the published literature;

the remaining were more difficult to confirm (Appendix F).

Naturally regenerating seedlings from mature sites were associated with the most ECM morphotypes (20), whereas regenerating seedlings in clearcut sites had the fewest (12). Planted seedlings from the clearcut and cut plus burned treatments had a similar number of morphotypes (17 and 18). Regenerating seedlings were associated with more morphotypes than planted seedlings (22 versus 20) even though fewer regenerating seedlings were examined in this study. Lightly colonized tips represented 18% of approximately 17000 tips analyzed.

Table 5. Morphotype occurrence on naturally regenerating and planted hybrid white spruce seedlings in treated
(clearcut, and cut plus burned) and untreated (mature forest) sites in the Aleza Lake Research Forest, Central
Interior of British Columbia.

Site/category	n	Number of morphotypes	Occurrence*(%)	Mean [†] (±SE)
Mature (regenerating seedlings)	16	20	83	18 (2)
Clearcut (regenerating seedlings)	16	12	50	10(1)
Clearcut (planted seedlings)	28	17	71	14 (2)
Cut plus burned (planted seedlings)	28	18	75	15(1)
Shared in all sites	-	7	29	-
Over all sites	88	24	100	-
Ascomycetes	-	5	21	-
Basidiomycetes	-	19	79	-
On less than 4 seedlings	-	4	17	-
Regenerating seedlings	32	22	92	-
Planted seedlings	56	20	83	-

*number of occurrences (%) out of total number of ECM morphotypes (24).

†mean number of morphotypes are pooled over replicate sites (2) and seasons (fall and spring). Regenerating seedlings in clearcut sites were sampled only in the fall.

The abundance and frequency of occurrence for all ECM morphotypes as well as treatment and seedling differences for the seven most commonly occurring morphotypes and for lightly colonized tips are shown in Table 6. Detailed statistical analyses for treatment and seedling differences are provided in Appendix G. The most common types of ECM included the ascomycetes *Cenococcum*, E-strain and *MRA* and the basidiomycetes *Amphinema*, *Hebeloma*, *Thelephora* and a Russulaceae type. Preliminary data analysis using the Students t-test indicated that replicate sites as well as seasons could be pooled (using a Bonferroni correction of $\alpha = 0.004$), with one exception, E-strain, which showed significant differences for the cut plus burned replicate sites in the spring. This variation may have been due to the burning of site 1 in the summer as well as in the fall.

Analysis showed that the abundance of *Hebeloma* (14% versus 6%) and Russulaceae type 1 (5% versus 1%) was significantly greater in the cut plus burned sites compared to the clearcut sites. In contrast, the abundance of *Cenococcum* (1% versus 4%) was significantly less.

Comparing treated sites (planted seedlings) to mature (regenerating seedlings), the abundance of Estrain (6 to 13% versus 0.1%), *MRA* (23 to 26% versus 4%), and *Amphinema* (14 to 18% versus 2%) as well as the lightly colonized tips category (19 to 26% versus 11%) was significantly greater in both clearcut and cut plus burned sites. In contrast, *Cenococcum* (6 versus 1%) was more abundant in mature than cut plus burned sites but was similar in abundance in mature and clearcut sites. Russulaceae type 1 (35% versus 1 to 5%) was significantly more abundant in mature sites than on any of the other sites, whether seedlings were planted or regenerating. *Thelephora* (35% versus 3 to 8%) was significantly more abundant on regenerating seedlings in the clearcut site than in the mature, cut plus burned, or on planted seedlings in the clearcut sites. *Piloderma* was only found on regenerating seedlings in both mature and clearcut sites and did not occur on planted seedlings (Table 6).

Morphotype	Mature-r		Clearcut-r		Clearcut-pl		Cut plus bu	rn-pl	
	n=16		n=16		n=28	n=28		n=28	
	mean†	freq	mean	freq	mean	freq	mean	freq	
Cenococcum	5.5 (1.6)a	56	1.6 (0.6)ab	44	4.1 (0.9)a	75	1.1 (0.5)b	36	
E-strain	0.1 (0.1)a	13	14.1 (7.0)ab	63	6.3 (1.3)b	75	12.7 (3.4)b	61	
MRA	4.3 (2.5)a	38	11.2 (3.6)a	69	26.2 (2.8)b	100	23.3 (4.1)b	89	
Tuber	0.1 (0.1)	6	5.7 (2.9)	38	1.3 (1.3)	4	-		
ascomycete unknown	0.3 (0.3)	6	-		-				
Amphinema	2.0 (0.9)a	38	14.7 (6.4)ab	44	17.7 (4.7)b	79	13.9 (2.8)b	64	
Hebeloma	7.4 (2.4)ab	69	4.7 (2.6)a	19	6.2 (1.5)a	61	14.3 (2.3)b	82	
Inocybe	-		-		-		0.4 (0.3)	18	
Laccaria	0.5 (0.5)	6	-		2.1 (1.2)	14	0.5 (0.3)	21	
Piloderma	5.8 (2.7)	38	0.5 (0.4)	13	-		-		
Russulaceae 1	35.4 (6.4)a	94	3.8 (3.3)bc	19	1.0 (0.6)b	21	5.1 (1.7)c	57	
Russulaceae 2	1.4 (1.0)	13	-		1.6 (1.6)	7	0.2 (0.1)	11	
Thelephora	8.3 (6.0)a	44	34.9 (6.8)b	75	2.7 (1.4)a	29	4.0 (1.7)a	43	
Thelephoraceae-like			0.1 (0.1)	6			-		
Tomentella 1	3.1 (1.4)	50	0.8 (0.7)	13	0.1 (0.1)	4	0.1 (0.1)	4	
Tomentella 2	1.0 (0.9)	13	-		1.0 (1.0)	7	0.3 (0.3)	4	
Tomentella 3	-		0.1 (0.1)	6	-		-		
non-rhizomorphic olive- green	0.8 (0.3)	25			-		0.1 (0.1)	4	
non-rhizomorphic thin mantled	4.3 (1.4)	50	-		2.7 (1.3)	39	2.7 (1.4)	43	
non-rhizomorphic unclamped	-		-		0.7 (0.5)	7	0.1 (0.1)	4	
non-rhizomorphic white	0.4 (0.3)	19	-		0.1 (0.1)	4	1.5 (1.4)	7	
rhizomorphic brown	0.9 (0.9)	13	-		0.6 (0.3)	14	0.4 (0.3)	7	
rhizomorphic orange	4.3 (3.9)	13	-		-		0.1 (0.1)	4	
rhizomorphic white	3.0 (1.3)	44	-		0.1 (0.1)	4	-		
lightly colonized	11.0 (3.1)a	94	7.9 (1.8)a	94	25.8 (2.9)b	100	19.4 (2.1)b	100	

Table 6. Mycorrhizae morphotype abundance* (mean percent $(\pm SE)$) and frequency of occurrence (%) for planted (pl) and naturally regenerating (r) hybrid white spruce seedlings in treated (clearcut, and cut plus burned) and mature sites in the Aleza Lake Research Forest. Central Interior of British Columbia.

*percent abundance= number of root tips for each fungal type / total number of root tips sampled per seedling x 100. †within rows, means followed by the same letter are not significantly different (Bonferroni correction of α =0.01) as determined by separate one-way ANOVA comparisons for treatments and two seedling types. Transformed data (arcsin \sqrt{p} , where p is morphotype abundance) were used. Means (±SE) presented are non-transformed values.

In addition to abundance and frequency differences, ten of the 22 assessed morphotypes were

significantly correlated (p≤0.05, data not shown) to several seedling variables: leader growth, basal

diameter and height.

2.3.3 ECM diversity

For the diversity indices and evenness and richness measures, data for planted and regenerating seedlings in the mature, clearcut, and cut plus burned sites were not significantly different with respect to replicate site and season (spring and fall) (Students t-test, $p \le 0.004$). Subsequently, replicate site and season data were pooled for further analysis. Graphical analysis (boxplots) of the pooled data indicated a few outliers, however these were not removed due to the small sample size. Analysis (one-way ANOVA) did not indicate violation of the assumptions. The seedling measurements such as leader growth, basal diameter and height were weakly correlated or non-significant for the diversity indices ($p \le 0.05$) and were not included in statistical analysis.

No significant differences were found between clearcut, and cut plus burned sites for planted seedlings with respect to richness, evenness or the Shannon and Simpson diversity indices (Table 7). However, ECM diversity of regenerating seedlings in clearcut sites was significantly lower than regenerating seedlings in mature sites and planted seedlings in the clearcut sites for both the Shannon (p=0.008) and Margalef (p=0.001) measures (Bonferroni correction of $\alpha = 0.01$). Simpson index values were also low (p=0.059 and p=0.022) for these seedlings but were not significant.

Similarity coefficients (Sorenson) supported these results; the least similar values resulted from comparisons between regenerating seedlings in clearcut sites to those in mature sites (0.63), and to planted seedlings in clearcut (0.62), and cut plus burned sites (0.53)(Table 8). The similarity coefficient for planted seedlings in the clearcut treatment versus the cut plus burned treatment was fairly high (0.86).

Table 7. Ectomycorrhizae richness, evenness and diversity measures (Shannon¹ and Simpson², Shannon Evenness³ and Margalef⁴) showing mean values (\pm SE). Indices were assessed using one-way ANOVA to test for treatment effect (clearcut, cut plus burned, and unburned, mature) and to test for seedling differences (naturally regenerating, (n=16) versus planted (n=28) of hybrid white spruce seedlings growing in the Aleza Lake Research Forest, Central Interior of British Columbia.

Treatment	/ seedling type	F-statistic	p-value†	
Clearcut /planted	Cut plus burned/ planted	df (1, 54)		
$1.19(0.08)^{1}$	1.16 (0.06)	0.067	0.797	
$0.61 (0.03)^2$	0.61 (0.03)	0.003	0.954	
$0.71(0.03)^3$	0.71 (0.03)	0.000	0.988	
$0.90(0.06)^4$	0.90 (0.06)	0.000	0.978	
Mature/ regenerating	Clearcut/ regenerating	df (1, 30)		
1.30 (0.52)	0.87 (0.08)	8.174	0.008	
0.61 (0.22)	0.48 (0.04)	3.844	0.059	
0.69 (0.05)	0.62 (0.04)	1.355	0.254	
1.07 (0.12)	0.59 (0.05)	14.567	0.001	
Mature/ regenerating	Clearcut/ planted	df (1, 42)		
1.30 (0.52)	1.19 (0.08)	0.579	0.451	
0.61 (0.22)	0.61 (0.03)	0.000	0.991	
0.69 (0.05)	0.71 (0.03)	0.058	0.810	
1.07 (0.12)	0.90 (0.06)	2.244	0.142	
Mature/ regenerating	Cut plus burned/ planted	df (1, 42)		
1.30 (0.52)	1.16 (0.06)	1.054	0.311	
0.61 (0.22)	0.61 (0.03)	0.004	0.952	
0.69 (0.05)	0.71 (0.03)	0.082	0.776	
1.07 (0.12)	0.90 (0.06)	1.954	0.169	
Clearcut/ planted	Clearcut/ regenerating	df (1, 42)		
1.19 (0.08)	0.87 (0.08)	7.849	0.008	
0.61 (0.03)	0.48 (0.04)	5.618	0.022	
0.71 (0.03)	0.62 (0.04)	2.591	0.115	
0.90 (0.06)	0.59 (0.05)	13.453	0.001	

means are pooled for both replicate sites and season.

 \pm significant differences indicated in bold (p \leq 0.01, Bonferroni correction for planned comparisons). P-values < 0.0015 have been designated as 0.001.

Table 8. Sorenson similarity coefficients calculated for ectomycorrhizae of naturally regenerating (r) and planted
(pl) hybrid white spruce seedlings from unburned mature, clearcut, and cut plus burned sites in the Aleza Lake
Research Forest, Central Interior of British Columbia.

Treatment/ seedling comparisons*	Similarity coefficient	Visualization		
Mature-r versus clearcut-r	0.63	Mat	ure-r	
Mature-r versus clearcut-pl	0.87	0.87	0.63	
Mature-r versus cut plus burned-pl	0.84	Clearcut-pl 0.62	Clearcut-r	
Clearcut-r versus clearcut-pl	0.62	0.86	0.53	
Clearcut-r versus cut plus burned-pl	0.53	0.84		
Clearcut-pl versus cut plus burned-pl	0.86	Cut plus burned-pl		

*Site data were pooled for treatment/seedling comparisons.

2.4 DISCUSSION

2.4.1 ECM morphotype abundance

The 24 ECM types found on hybrid white spruce in the present study, of which 14 were of recognizable

taxonomic affinities, is comparable to the numbers reported in recent studies characterizing ECM.

Twenty-two morphotypes have been described on regenerating hybrid spruce seedlings growing in the Stone wildfire site, a Lodgepole pine (*Pinus contorta* var. *latifolia* Engelm.) dominated stand that was burned in the summer of 1992 (Egger and Massicotte 1999). On Sitka spruce (*Picea sitchensis* (Bong.) Carr.), 25 distinct mycorrhizal types (of which 14 were known) were reported on seedlings and trees growing in four forest types and four nurseries in the British Isles (Thomas *et al.* 1983). However, 13 ECM morphotypes were reported for naturally regenerating Sitka spruce growing in an uneven-aged plantation forest in southern Scotland that was selectively logged (Flynn *et al.* 1998). For young to mature urban white spruce (*Picea glauca* (Moench) Voss) and blue spruce (*Picea pungens* Engelm.) growing in Calgary, Alberta, 25 mycorrhizal types were reported (Danielson and Pruden 1989). Bruns (1995) recently reviewed seven studies of small monoculture forests that examined fungal fruitbodies and mycorrhizae, and reported an average of 20 to 35 species typically found on those sites. In other studies, 20, 22 and 19 ECM types, of which 14, 19 and 14 were identified to genus level or group, were described by Simard *et al.* (1997), Visser *et al.* (1998) and Hagerman *et al.* (1999), respectively.

The 20% ascomycetes and 80% basidiomycetes reported in the present study were also found to be similar to the percentages for six-year-old jack pine stands (Visser 1995). Danielson and Pruden (1989) reported different but highly variable values of 47(±27)% ascomycetes and 31 (±21)% basidiomycetes for urban blue and white spruce.

Mycorrhizal formation, as measured indirectly by lightly colonized, unidentified tips and non-mycorrhizal tips was increased on planted seedlings in treated sites compared to regenerating seedlings in clearcut and mature forest sites (Table 6). This was most likely due to seedling differences rather than to treatment effect: planted seedlings were larger and had correspondingly larger root systems, with more root tips that could potentially be colonized. Several studies have examined burning effects on ECM formation, mainly conducted in the Pacific Northwest with Douglas-fir. Sources of mycorrhizae varied and included naturally regenerating seedlings, soil cores, seedlings grown in the greenhouse on soils transferred from disturbed sites and seedlings planted in the field growing in disturbed and transferred soils. Some studies reported an increase (Pilz and Perry 1984; Brainerd and Perry 1987; Richter and Bruhn 1993) or no decrease (Visser 1995) in ECM abundance following disturbance. However, many

studies reported a decrease in ECM, from undisturbed to clearcut to burned sites (Harvey *et al.* 1980; Perry *et al.* 1982; Schoenberger and Perry 1982; Parke *et al.* 1984).

In a greenhouse bioassay of Douglas-fir and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), Schoenberger and Perry (1982) grew seedlings in soils from central Oregon that had been clearcut, cut plus burned, naturally burned, and undisturbed (old growth and young growth). Douglas-fir had more roots and ECM root tips in the unburned clearcut soils, followed by intermediate ECM colonization in the cut plus burned soils compared to the other sites. Western hemlock had the fewest roots and ECM root tips in the cut plus burned soils.

In another greenhouse study, Perry *et al.* (1982) examined mycorrhizal formation (number of active root tips per seedling) on Lodgepole pine, Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) and Douglas-fir seedlings grown in soils from 16-year-old clearcut, clearcut plus windrowed, windrow burned and adjacent mature forest sites in Montana. A significant decrease in the number of total and ECM root tips occurred in clearcut, clearcut plus windrowed and windrow burned sites compared to undisturbed forest sites.

Parke *et al.* (1984) grew Ponderosa pine and Douglas-fir seedlings in soil retrieved from clearcut, cut plus burned, and undisturbed sites in southwest Oregon and northern California in a greenhouse study to determine total ECM inoculation potential. Mycorrhizal colonization was quantified by visual estimates using a scale of zero to five that represented 20% increments. After growing seedlings 14 to 16 weeks, ECM colonization was greatest in undisturbed forest soils (80 to 100%), followed by clearcut soils (20% less) and cut plus burned soils (40% less). All treatments were significantly different.

Seasonal differences in ECM abundance were not observed in the present study (no significant differences in seasonal abundance for the seven common types). Soil moisture was not measured but the soil was observed to be drier in August compared to June. Regenerating seedlings in the clearcut sites, which were only sampled in early fall when soil moisture was low, had fewer lightly colonized tips compared to planted seedlings in the disturbed sites. Reports in the literature, with respect to season and soil moisture are variable. An increase in numbers of ECM have been reported at the beginning of the

growing season for mature (Harvey *et al.* 1978) and clearcut sites (Richter and Bruhn 1993). However, ECM colonization was higher for soils in dry montane compared to moist montane and mesic coastal sites in Oregon (Brainerd and Perry 1987). Brainerd and Perry (1987) suggested that in dry montane environments with limited moisture and shorter growing season, ECM may be more important in maintaining tree moisture and nutrient status. In contrast, under drought conditions, ECM was decreased in studies examining soil cores only (Nilsen *et al.* 1998) and studies examining both soil cores and Norway spruce (*Picea abies* L. (Karst.)) seedlings (Feil *et al.* 1988) compared to control sites and seedlings that were not subjected to drought.

Many of the morphotypes found in the present study, such as the seven commonly occurring types discussed here, are considered to be broad host ranging, and match descriptions of those previously reported for spruce seedlings as well as other conifers, such as Pinus, Pseudotsuga, and Abies. Danielson and Pruden (1989) reported E-strain as the most common morphotype (38% found on urban white and blue spruce), followed by Amphinema byssoides, Hebeloma, Tuber and Tomentella (approximately 30%). Thelephora and E-strain have also been reported on greenhouse or nursery grown spruce seedlings (Thomas et al. 1983; Ingleby et al. 1990). Some of the dominant ECM that have been reported in clearcut subalpine forest sites include E-strain, Lactarius, Cenococcum, Piloderma, Hebeloma, Amphinema and Cortinarius (Hagerman et al. 1999). Most of these ECM with the exception of Lactarius were also found in clearcut sites in the present study. In the Eagle wildfire site, located adjacent to the Aleza Lake Research Forest, the most common ECM morphotypes reported on regenerating and planted spruce seedlings in burned salvaged-logged, burned unsalvaged and unburned sites, were similar to the seven most commonly occurring types reported in the present study (Egger and Massicotte 1998). Morphotypes found on jack pine stands, six years following wildfire, also included Cenococcum, E-strain, MRA, and Russula spp. and in 122-year-old jack pine stands, Cenococcum, Hebeloma, MRA, Piloderma, Russula spp. and Tomentella spp. were found (Visser 1995).

In the present study, some differences in ECM abundance occurred between clearcut and cut plus burned sites, and between treated (regardless of treatment type) and untreated (mature) sites. For example, *Hebeloma* and Russulaceae type 1 were more abundant in cut plus burned sites than clearcut sites but *Cenococcum* was less abundant. Similarly, *Amphinema*, E-strain, and *MRA* were more

abundant in all treatment sites (planted seedlings) compared to untreated (mature) sites whereas Russulaceae type 1 was less abundant.

Cenococcum was less abundant on planted seedlings in cut plus burned sites compared to planted seedlings in clearcut sites as well as seedlings in mature, undisturbed sites. ECM that successfully colonize seedlings in burned sites may need to possess structures or propagules capable of surviving burns and tolerating adverse conditions in post-fire environments such as moisture stress. Cenococcum, a common but not abundantly occurring ECM (Wright and Tarrant 1958; Ingleby et al. 1990), is thought to be drought tolerant (Trappe 1969). It forms sclerotia, vegetative structures that may confer an advantage by increasing survival in disturbed sites. Cenococcum geophilum sclerotia, collected from soil samples in Wyoming, were found up to two years after fire in burned sites (Miller et al. 1994). Visser et al. (1998) also found Cenococcum sclerotia up to two years following clearcutting in a mixedwood site in Alberta. Egger and Massicotte (1998) found Cenococcum ECM to be significantly less abundant on regenerating hybrid spruce in burned salvaged-logged sites than in mature, unburned sites. Schoenberger and Perry (1982) noted a decrease in the abundance of *Cenococcum geophilum* Fr. on western hemlock greenhouse seedlings growing in soils from cut plus burned plantations. Contrary to these reports and the present study, Pilz and Perry (1984) reported increased abundance of Cenococcum ECM on Douglas-fir grown in clearcut plus burned areas than in clearcut areas or in the undisturbed forest. Results by Pilz and Perry (1984) were based on macroscopic ECM characteristics; no Cenococcum mycorrhizae descriptions were provided in the study by Schoenberger and Perry. Furthermore, it is possible to confuse Cenococcum with other black mycorrhizae such as some Tomentella spp. and MRA (Danielson 1991).

Hebeloma and Russulaceae type 1 were more abundant in the present study, on planted seedlings in cut plus burned sites than in clearcut sites. One possible reason may be due to the differences in slash heights and LFH layers in these treated sites. The depth of slash was greater in cut plus burned than in clearcut sites where windrowing most likely removed much of the larger woody debris. Windrowing may also have disrupted the LFH layer on the clearcut sites, and removed the nutrient rich forest floor. In contrast, burning of finer slash would add organic matter to the LFH layer on the cut plus burned sites. These activities may partially explain the significant differences between clearcut and cut plus burned

sites. More slash or a thicker LFH horizon could have beneficial moisture and nutrient effects and the higher abundance of *Hebeloma* and Russulaceae type 1 in cut plus burned sites may reflect this. Visser *et al.* (1998) reported the presence of *Hebeloma* only on clearcut sites that received 10 cm of wood chips and its absence on clearcut sites with no added wood chips or with 5 cm of added wood chips, in a mixedwood site in Alberta. The increased abundance of *Hebeloma* and Russulaceae type 1 in cut plus burned sites may also be due to an association of these morphotypes with other woody shrubs (willow) or trees (birch, poplar) present on these sites that perhaps provided a source of inoculum. However, the relative abundance of *Hebeloma* fruitbodies under tall willows (*Salix*) and Visser *et al.* (1998) reported *Hebeloma* and Russulaceae ECM on aspen (*Populus tremuloides*) roots. Kernaghan *et al.* (1997) speculated that the Russulaceous ECM they studied were capable of colonizing woody shrubs such as *Betula* and *Salix.*

Although the abundance of *Amphinema*, E-strain and *MRA* (as well as lightly colonized tips) was similar between planted seedlings in clearcut and cut plus burned sites, significant decreases were seen when comparing regenerating seedlings in the undisturbed mature sites to both types of treatment. Results in the present study suggest that both seedling type and treatment effect may be influencing colonization by these fungi for *Amphinema* and E-strain because intermediate abundance of these types occurred on regenerating seedlings in clearcut sites. However, for *MRA* and for lightly colonized tips, seedling type was likely influencing colonization as no significant differences were found between regenerating seedlings in mature and clearcut sites. In the Eagle fire study, E-strain, *MRA* and the lightly colonized group were more abundant and *Amphinema* was less abundant on regenerating spruce seedlings in burned salvaged-logged sites compared to mature sites (Egger and Massicotte 1998). However, on planted seedlings in that study, *Amphinema* was more abundant and the lightly colonized group was less abundant in burned salvaged-logged, than in burned unsalvaged sites. ECM abundance differences between the Eagle fire and the present study may be due to different fire types (wildfire versus broadcast burning).

Rhizomorphs are an adaptive feature conferring advantages in disturbed sites and ECM that possess such mycelial networks may increase access to or storage of soil "nutrients" (Harley and Smith 1983). As

well, mycelia emanating from active mycorrhizal roots are thought to be an important source of inoculum for outplanted seedlings (Hagerman *et al.* 1999). In the present study, *Amphinema* was the dominant rhizomorphic fungi found on planted seedlings in disturbed sites and this may be due to its ability to increase access to and storage of soil nutrients as well as the ability to spread to and colonize other seedlings via rhizomorphs. The number of years after disturbance could also be a factor in the abundance of *Amphinema*; Danielson (1991) reported a large increase in abundance of *Amphinema* on outplanted white spruce growing on coal mine spoils, four and seven years after treatment with peat, fertilizer and sewage sludge.

E-strain is believed to consist of a complex group of species (Danielson 1982; Ingleby *et al.* 1990); possibly including post-fire ascomycetes belonging to the order Pezizales that are commonly found following burning of forest habitats (Petersen 1970 in Egger and Paden 1986). E-strain has been reported as a dominant ECM on disturbed sites such as coal spoils (Danielson 1991) and clearcuts (Hagerman *et al.* 1999). Additionally, E-strain fungi possess thick walled chlamydospores that may enhance survival in the soil after disturbance (Thomas *et al.* 1983). Perhaps due to its limited mantle development, it does not compete as well in mature sites with other fungi that have thicker mantles, rhizomorphs or numerous emanating hyphae, but is able to thrive in extreme environmental conditions provided by disturbed sites.

MRA occurs globally and is broad host ranging. It has been reported as both ectomycorrhizal and pathogenic though it is poorly understood in terms of its ecological function ($J_{\mu}m_{E}$)ponen and Trappe 1998). Little is known about the species that comprise *MRA* and possible candidates include post-fire ascomycetes. In the present study, *MRA* varied morphologically: mantles were thin to well developed, with few to abundant emanating hyphae. While the well developed *MRA* may be able to persist in disturbed as well as undisturbed sites, *MRA* with poorly developed (patchy) mantles might be restricted to disturbed areas. *MRA* has been reported as dominant in disturbed sites such as trenched soils (Simard *et al.* 1997) and amended oil sands (Danielson 1991).

In the present study, Russulaceae type 1 had the greatest abundance of all ECM in the mature forest. It has been suggested that Russulaceae species may have a preference for fruiting in decaying wood in

North American coniferous forests (Schaffer 1975 in Kernaghan *et al.* 1997). *Piloderma* was also abundant in mature sites in the present study and absent from all other sites except for a minor component of regenerating seedlings in clearcut sites. This fungus may prefer organic matter, a condition found in mature sites and is known to possess proteolytic enzymes to enable it to extract nitrogen from organic compounds (Dahlberg *et al.* 1997). It also prefers fruiting in decayed wood and litter (Visser *et al.* 1998). In a 100-year-old Norway spruce stand in southern Sweden, *Piloderma croceum* Erikss. & Hjortst. accounted for 19% of the total mycorrhizal tips examined (Dahlberg *et al.* 1997). In mixedwood stands of similar age in Alberta, *P. byssinum* (Karst.) Jül. was equally abundant (Visser *et al.* 1998). In the Eagle fire study, both Russulaceae type 1 and *Piloderma* ECM of regenerating spruce were more abundant in mature than in burned salvaged-logged sites (Egger and Massicotte 1998).

A possible difference in ECM colonization between regenerating and planted seedlings is that fungi commonly found in greenhouses (such as *Thelephora*) might be an additional source of inoculum for outplanted seedlings. Richter and Bruhn (1993) reported that *Thelephora terrestris* colonized *Pinus* roots for all three years after outplanting. In the present study, seedlings were not assessed for ECM before planting. However, *Thelephora* was found on all sites, and was most abundant on regenerating seedlings in the clearcuts. It may be likely that some of the *Thelephora* tips reported on regenerating seedlings in clearcuts were a *Laccaria* species, as these types have similar mantle and hyphal characteristics. Both *Thelephora* and *Laccaria* are reported to occur in a variety of habitats (Ingleby *et al.* 1990). In addition, *Thelephora* possesses rhizomorphs, and therefore may be better able to colonize roots of regenerating seedlings than non-rhizomorphic types.

2.4.2 Treatment effects or seedling type differences on ectomycorrhizal diversity

In the present study, broadcast burning did not appear to affect the ECM diversity of planted seedlings when clearcut sites were compared to cut plus burned sites. This is in contrast to the few burn studies that have examined ECM diversity. Pilz and Perry (1984) examined ECM on Douglas-fir seedlings grown in three western Cascade Mountain sites in undisturbed, mature (80 to 250 year old Douglas-fir/western hemlock), clearcut, and cut plus burned soils that had been transferred to each of the undisturbed, clearcut, and clearcut plus burned sites. They found more types of ECM in undisturbed than disturbed ones. Similarly, in a study of ectomycorrhizal fungal succession following wildfire in northeastern Alberta,

Visser (1995) reported a significant increase in mycorrhizal species richness between the six- and 122year old stands, using soil cores for ECM assessment. In a greenhouse bioassay, Brainerd and Perry (1987) examined the diversity of six-month-old Douglas-fir and ponderosa pine seedlings grown in soil from disturbed (three- to five-year-old clearcut plus burned) and undisturbed forest sites in Oregon. These sites represented a moisture/elevation gradient. Diversity (Shannon index) was highest in the dry montane site and lowest in the mesic coastal sites for undisturbed soils. Diversity decreased in disturbed soils in all site types. ECM morphotype information was not provided in this study.

In the present study, a significant decrease in ECM diversity for the naturally regenerating seedlings in clearcut compared to mature sites was noted. Other studies have reported decreased diversity following disturbance. Simard *et al.* (1997) conducted a trenching study in 90 to 120 year-old Douglas-fir and paper birch (*Betula papyrifera* Marsh.) dominated forests in the southern interior of British Columbia to determine the effect of ECM occurrence on one-year-old Douglas-fir seedlings outplanted for six to 16 months. They reported a doubling in mean richness, diversity, and evenness of ECM per seedling in the untrenched versus trenched treatment. Hagerman *et al.* (1999) examined clearcut size effects on ECM diversity and persistence in a subalpine forest dominated by 95 to 325 year-old subalpine fir and Engelmann spruce in southern British Columbia. They reported reduced ECM richness and diversity as well as reduced numbers of active fine roots in soil cores from clearcut compared to mature forest sites, sampled two and three growing seasons after logging.

One reason for the difference in ECM diversity noted in the present study could be that the majority of hybrid spruce seedlings found in mature sites were rooted in woody substrates. Similarly, in approximately 100 to 200 year-old sub-boreal spruce stands in central British Columbia, 48% of hybrid spruce seedlings were found on rotting wood substrates (Kneeshaw and Burton 1997). Woody substrates provide a source of moisture (Harvey *et al.* 1978), a haven for possible animal vectors that help to disperse mycorrhizal inocula (Maser *et al.* 1978) and possibly better access to sunlight than seedlings on the forest floor. In a 250 year-old Douglas-fir/larch forest in western Montana, Harvey *et al.* (1976) found that in the top 38 cm of soil, 95% of the active ECM were associated with organic material, mainly humus and decayed wood. In a later study (Harvey *et al.* 1981), they reported increased ECM numbers with increases in organic matter (up to 45% by volume) in the top 30 cm of the soil, with more

tips in decayed wood than in humus. This could partly account for differences seen in the present study: the regenerating seedlings in the mature sites could benefit from the nutrient rich, moist, and shaded environment and from less stress associated with drought and temperature extremes. Another possible reason for differences in ECM diversity may be related to seedling age. Regenerating seedlings in mature sites were approximately two years older than those on clearcut sites.

Regenerating seedlings in clearcut sites in the present study were also significantly less diverse than planted seedlings in the same site. Seedling measurements showed that planted seedlings were larger than regenerating seedlings and were therefore able to support the formation of a larger root system which could exploit larger soil volumes and reach more fungal propagules than regenerating seedlings. As well, planted seedlings had two years initial growth in the greenhouse and nursery soils and may have been colonized by fungi such as *Thelephora* and E-strain, respectively. Fewer regenerating seedlings were sampled on clearcut sites compared to planted seedlings. This, in combination with smaller root systems, may account for some of the lower species richness: rare, less abundant species comprised most of the ECM missing on regenerating seedlings.

The fact that very few regenerating seedlings were found on cut plus burned sites was unexpected. It is unlikely that the absence of seedlings was related to the availability of mycorrhizal inoculum (planted seedlings in the same sites were colonized) but rather was related to seed source. High seedling regeneration may have occurred after 1993, a time when the seed crop was rated as good (John Revel pers. comm. 1999). At this time, clearcut sites were one year old and were potentially ideal for seedling regeneration. However, on the cut plus burned sites, cones and seedlings surviving the clearcutting (in the winter of 1993) would have been burned in 1994. The optimum conditions for Engelmann spruce (*Picea engelmannii*) regeneration in the Engelmann Spruce-Subalpine Fir biogeoclimatic subzone are seedbeds created by clearcutting with exposure of mineral soil compared to a seedbed of undisturbed or burned forest floor (Feller 1998). Seedbeds created by low severity burns supported the largest number of living spruce seedlings after three growing seasons (Feller 1998). If the seed source is insufficient, white spruce does not readily regenerate after logging, requiring the outplanting of one- or two-year-old nursery (or greenhouse) grown seedlings (Silversides *et al.* 1986).

Some differences in ECM diversity and abundance may be attributed to the general site, seedlings and soil characteristics. Those discussed previously include slash height and LFH layer thickness. Soil characteristics such as pH and C/N ratios did not appear to differ greatly and cannot be correlated to ECM abundance and diversity in this study as these measurements may not be representative of rhizosphere conditions. However, they are useful for general site descriptions and determining variability within and between sites. Carbon/nitrogen ratios were within the range of reported values in the literature; the minimum C/N ratios for organic and mineral soils are 20, and 10 to 12, respectively (Brady 1974). A higher ratio represents a lower rate of decomposition and less readily available nitrogen and this would be expected for the LFH layer, where nitrogen would be bound and less available than in the mineral layer (Brady 1974). Seedling age varied among sites (four to seven years), generally being lower in cut plus burned sites due to a later planting date than those in clearcut sites and lower in regenerating seedlings in clearcut sites. Seedlings were older in the mature sites compared to all other sites. Finally, although the diversity indices in this study were not strongly correlated with the measured seedling parameters, the abundance values of nearly half of the assessed morphotypes were significantly correlated with leader growth, seedling height and basal diameter. For example, MRA abundance positively increased with increase in leader growth. More rigorous examination of these measurements (soil and site characteristics and seedling parameters) followed by other types of analysis such as canonical correspondence analysis may explain differences in ECM abundance.

Difference in diversity may not have been detected due to the uncertainties in resolving and identifying some morphotypes, in particular some of the lightly colonized types. In the present study, the most abundant morphotypes appeared to be fairly easy to distinguish. The Shannon and Simpson indices assume that all species are known in the sample (Magurran 1988). Some ECM types were identifiable to the species level but others could only be resolved to the genus or family level. Walker (1987) discussed classification of Sitka spruce ECM claiming it was possible but not easy to determine mycorrhizae to the genus level. Use of traditional diversity indices (Shannon and Simpson) may not reflect real differences in diversity if a morphotype does not represent a species. The absence of some of the rare ECM on regenerating seedlings in the clearcut sites is probably the cause of significant differences seen in the richness measure and Shannon index, both which are sensitive to changes in the number of rare species.

The Shannon and Simpson indices may also be sensitive to sample size (Magurran 1988), which varied in the present study between the naturally regenerating and planted seedlings.

In conclusion, results in the present study suggest that broadcast burning following clearcutting does not appear to be affecting ECM diversity. However, some changes in ECM abundance occurred as a result of both types of disturbance. The impact of these changes in abundance to seedling establishment and growth are unknown and require further studies. Although broadcast burning did not appear to have more adverse effects on mycorrhizal diversity than clearcutting, fungal diversity was reduced in regenerating seedlings in clearcut sites. Applications of results in the present study to forest management is limited to hybrid white spruce seedlings in the SBS biogeoclimatic zone willow wet, cool (wk1) variant. ECM abundance and diversity could not be correlated to a specific burn severity without depth of burn measurements, although a general estimate of a moderate severity was made. Future studies should endeavor to accurately measure fire severity and intensity. It should be noted that very few hybrid spruce seedlings regenerated on cut plus burned sites, reaffirming the current practice to replant these sites.

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3. Molecular Characterization of Mycorrhizae Associated with Hybrid White Spruce Seedlings in the Aleza Lake Research Forest, Central Interior of British Columbia

ABSTRACT

Broadcast burning is a common form of site preparation following clearcutting in coniferous forests of British Columbia. Associated with many conifer and deciduous species, ectomycorrhizae (ECM) provide a key link in nutrient and energy cycling within forest ecosystems. To assess the impacts of broadcast burning practices on ectomycorrhizae (ECM) diversity, planted and naturally regenerating hybrid white spruce seedlings, growing in the sub-boreal spruce (SBS) biogeoclimatic zone in the Central Interior of British Columbia, were sampled from two treated (clearcut, and clearcut plus burned) as well as adjacent mature forest sites (uncut and unburned). During an initial study on ECM morphology, seedling root systems were characterized for mycorrhizae and approximately 1800 tips were subsampled for molecular analysis (PCR-RFLP), RFLP analysis of eight commonly occurring ECM morphotypes as well as lightly colonized tips revealed 12 genotypes (those that shared one or no band patterns of three restriction endonucleases) with 18 variants (those sharing similar band patterns for two of the three restriction endonucleases). Analysis of the commonly occurring morphotypes revealed that four (Cenococcum, Tuber, Hebeloma and Thelephora) exhibited only one molecular genotype. However, Hebeloma and Thelephora had several variants. The other four morphotypes (Amphinema, E-strain, MRA, and Russulaceae type 1) possessed two or three genotypes, some with several variants. The number of genotypes and variants appeared to increase with increasing disturbance from regenerating seedlings in mature sites (6 genotypes, 9 variants) to planted seedlings in clearcut plus burned sites (11 genotypes, 17 variants). However, ECM molecular diversity, assessed using the Phi index, was not significantly different between treatments or for planted versus naturally regenerating seedlings. This suggests that the molecular diversity of ECM on hybrid white spruce seedlings was not affected by clearcutting or clearcutting plus broadcast burning. Molecular characterization provided a more comprehensive estimate of diversity, specifically for total species richness, in combination with morphological methods and increased understanding of inter- and intra-specific variation with respect to ectomycorrhizal associations.

3.1 INTRODUCTION

Broadcast burning on clearcut sites has been used as a common method of site preparation prior to outplanting of seedlings (Hawkes *et al.* 1990). The impacts of this practice have been summarized in reviews of numerous studies examining changes in soil chemical, physical and biological characteristics (Wells *et al.* 1979; Agee 1993). Results vary, but in general more severe fires cause the most deleterious effects (e.g. increased soil erosion, decreased soil porosity, increased volatilization of plant nutrients and a decreased number of soil organisms). A growing number of studies have focussed on burning effects on important soil components such as mycorrhizae. Mycorrhizae are symbiotic fungal-plant root associations in which the fungal partner enhances moisture and nutrient uptake in exchange for plant carbohydrates (Harley and Smith 1983). They are believed to be essential to seedling growth and survival. Ectomycorrhizae (ECM), one of several types of symbioses, are associated with angiosperm and gymnosperm hosts; many of these are important commercial forest species (e.g. *Picea, Pinus, Abies*) in British Columbia.

In general, previous studies of burning effects have reported decreases in ECM abundance, however, comparisons and interpretations are sometimes difficult due to different methods of assessment. For example, on clearcut plus burned sites in the field, Wright and Tarrant (1958) only assessed seedlings as being mycorrhizal or non-mycorrhizal, Pilz and Perry (1984) examined the mean number of mycorrhizal root tips per seedling and Parke *et al.* (1984) used visual estimates on a scale of 20% increments. Descriptions of fungal types in these studies were rudimentary, such as categorizing types into white, brown or black groups (Wright and Tarrant 1958). In the last decade, the resolution of morphological identification has constantly improved, due to the publication of description standards (Ingleby *et al.* 1990; Agerer 1987-98; Agerer *et al.* 1996-98; Goodman *et al.* 1996). However, morphological identification requires considerable skill and this may be hampered by problems such as the phenotypic variation of ECM on different hosts and under varying environmental conditions (Egger 1995). In contrast, molecular characterization of ECM is in theory easier to learn, is less time consuming in the processing of tips, and examines ECM genotypes that are independent of environmental variation (Egger 1995; Gardes and Bruns 1996). However, in order to make inferences from molecular data, molecular analysis still relies on comparisons with identified mycorrhizal tips, based on morphological data (Horton and Bruns 1998;

Varga 1998, M.Sc. Thesis; Egger and Massicotte 1999); on sporocarp data (Kårén *et al.* 1997; Kernaghan *et al.* 1997), or on cultures from identified root tips (Mehmann *et al.* 1995).

Molecular assessment includes DNA amplification by the polymerase chain reaction (PCR, Mullis and Faloona 1987), digestion of selected targeted sequences by restriction endonucleases and analysis of the restriction fragment length polymorphism (RFLP) band patterns (Gardes and Bruns 1996). The internal transcribed spacer (ITS) region of the ribosomal RNA gene unit of DNA (rDNA) has been widely used for amplification. This region lies between the 18S and 25S rRNA coding genes and contains the 5.8S rRNA gene flanked by two non-coding spacers called ITS1 and ITS2 (Gardes and Bruns 1996). Due to its relatively rapid rate of evolution, the ITS region is suitable for identification to the species or species group for most fungi (White et al. 1990; Gardes and Bruns 1996). Several studies on ECM fungi have examined this region using the PCR-RFLP method. A study by Kraigher et al. (1995) attempted to distinguish two species of Lactarius that are difficult to separate by morphological typing. Another study by Kernaghan et al. (1997) compared morphological characterization of Russulaceae mycorrhizae to sporocarp tissue to confirm identification. Recently, Horton and Bruns (1998) examined fungal associates for possible linkages between Douglas-fir (Pseudotsuga menziesii D. Don) and bishop pine (Pinus muricata D. Don) and Horton et al. (1998) examined ECM and dark septate fungal colonization on bishop pine after wildfire. Jonsson et al. (1999) examined mycorrhizae and sporocarps in a chronosequence study of ECM community and composition following wildfire in Scots pine (Pinus sylvestris) stands. Thus, PCR-RFLP analysis of the ITS region is currently being used in a variety of applications to address questions concerning ECM.

The Shannon and Simpson indices are commonly used diversity indices that have been applied to ECM morphological data (Brainerd and Perry 1987; Simard *et al.* 1997; Houston *et al.* 1998). However, using these indices for ECM may be problematic. For example, for some ECM, it is possible that one morphological type (morphotype) could represent more than one species; for other ECM, one species may simply have more than one assemblage of morphological characteristics depending on its growth stage or on environmental conditions (Mehmann *et al.* 1995). This problem of species uncertainty could violate the assumption of the diversity indices: that all species in a sample are known (Magurran 1988). However, these indices currently appear to be the best measure of diversity for morphological data. To

assess diversity using molecular data, the Phi index has been derived by Egger (Baldwin 1999, M.Sc. Thesis). Using the Phi index, ECM root tips are matched with every other tip in the sample and their distances (representative of their relatedness) are used instead of species richness and abundance data, in calculating mycorrhizal molecular diversity of the entire sample (Egger, pers. comm. 1999).

The main objective of this study was to determine, using molecular characterization (PCR-RFLP methods), the effect of broadcast burning following clearcutting on the diversity of ECM on planted and regenerating hybrid white spruce growing in mature, clearcut, and cut plus burned sites in the SBS biogeoclimatic zone of central British Columbia. In addition, the study was to explore differences in ECM diversity between planted and regenerating seedlings and to compare molecular results with previous morphological assessments. The study further examined the Phi index as a useful measure of diversity for molecular analysis in place of traditional methods.

3.2 MATERIALS AND METHODS

3.2.1 Ectomycorrhizae sampling

The study area included four treated (two clearcut and two cut plus burned) and two adjacent mature forest sites, located approximately 36 km east of Prince George, British Columbia, near the Bowron River in the south-western portion of the Aleza Lake Research Forest. The study area is part of the SBS biogeoclimatic zone, willow wet, cool (wk1) variant; climax tree species are hybrid white spruce (*Picea engelmannii* (Parry ex Engelm.) x *glauca* (Moench) Voss) and subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) and the hybrid spruce-oak fern is the zonal association (Meidinger *et al.* 1991). A total of 88 hybrid white spruce seedlings were harvested, half in late June and half in late August 1997, from 50 m x 50 m blocks. Sampling included 16 regenerating seedlings from mature sites, 28 planted and 16 regenerating seedlings (only sampled in the fall) from clearcut sites and 28 planted seedlings from cut plus burned sites. Roots systems were washed and floated over a grid in water and 200 healthy (i.e. turgid roots with intact meristems) tips were randomly sampled for each seedling. Ten percent of the tips representing each ECM morphotype for each seedling were selected for molecular analysis (approximately 1800 root tips) and stored in 1 ml microcentrifuge tubes at -20°C until processed. Pre-harvest silviculture prescriptions are presented in Appendices B, C and D.

3.2.2 DNA extraction

Isolation of fungal DNA was conducted using a modified CTAB protocol (Zolan and Pukkila 1986). A thin section of the apical end of each root tip (approximately 2 mm) was excised, using a sterile dissecting blade for each morphotype, and was placed in a separate glass micromortar (Mandel Scientific) on ice (-20°C) for at least 15 minutes. The frozen tips were then quickly ground and suspended in 175 µl of 2X CTAB buffer (8.6 ml autoclaved, millipore water, 3.54 ml 5M NaCl, 1.41 ml 1M Tris-HCl (12.1 g Tris (hydroxymethylamino-methane, Trizma® Base, Sigma Chemical Co.) and approximately 4.2 ml HCl to pH 8.0), 578 µl of 0.5M EDTA (Ethylenediaminetetraacetic acid) at pH 8.0, 2.89 ml of 10% CTAB (Hexadecyltrimethly-ammonium bromide, Sigma Chemical Co.), and 28.9 µl of 2-mercapto-ethanol solution. Tips were then reground and then transferred to sterile 1 ml microcentrifuge tubes. A final 175 µl rinse with 2X CTAB was added to the micromortars and transferred to the microcentrifuge tubes. The contents of the tubes were incubated at 60°C in a heatblock (VWR Scientific) for 45 to 60 minutes. Following incubation, an equal amount (approximately 350 µl) of 24:1 chloroform:isoamyl alcohol solution was added to the tubes, which were spin vortexed, then centrifuged for 10 minutes at 13000 rpm.

To precipitate the DNA, the top aqueous layer was pipetted into a new microcentrifuge tube and an equal amount (approximately 350μ l) of absolute isopropanol (stored at -5° C) was added. The tubes were mixed by inverting for 1 minute, then stored at -5° C for 10 minutes before being centrifuged at 13000 rpm. Contents of the tubes were removed by air suction, leaving the pellet and approximately 100 µl of liquid. To remove salts from the pellet, about 175 µl of 70% ethanol (stored at -5° C) was added and the tubes were finger vortexed a few times and then centrifuged for 3 to 5 minutes at 13000 rpm. Two more washes with ethanol followed, then the remaining liquid was removed by air suction, leaving approximately 50 µl of solution, which was removed by placing the tubes in a dessicator (VWR Scientific) overnight. The remaining pellet was used for DNA amplification.

3.2.3 DNA amplification

The DNA pellet was resuspended with 50 μl of 8 mM of NaOH and heated to 60°C for 10 minutes in a heatblock. Subsequently, 4 μl was added to 27 μl of master mix (17.2 μl autoclaved, millipore water, 3.0 μl 10X DNA Polymerase Buffer (BIO/CAN Scientific), 3.0 μl 2mM dNTP stock solution (2mM each of

dATP, dCTP, dGTP and dTTP, Pharmacia Biotech), 2.4 µl 25mM MgCl₂, 1.2 µl each of 10 µM oligonucleotide primers ITS 1 (TCC GTA GGT GAA CCT GCG G) (White *et al.* 1990) and NL6Bmun (CAA GCG TTT CCC TTT CAA CA) (Egger 1995), and 0.08 µl of 5units/µl pure or 1:1 UltraTherm[™] DNA Polymerase to Polymerase Buffer (BIO/CAN) and put into 0.6 ml microcentrifuge tubes kept on ice. A drop of mineral oil (Sigma Chemical Co.) was added to prevent evaporation and the tubes were briefly spun to 10000 rpm. The Perkin Elmer Cetus Thermocycler was used for DNA amplification at two settings: 1) for robust, well colonized roots, denaturation at 94°C for 45 sec., annealing temperature of 48°C for 45 sec., and an extension step at 72°C starting at 130 sec., increasing 1 second per cycle for 35 cycles and; 2) for lightly colonized roots or roots which were weakly amplified using the first protocol, similar settings as above except cycles were extended to 40, and annealing temperature was decreased to 46°C to increase possibility of amplification. As well, pure and 1:1 diluted DNA Polymerase were used for lightly colonized and robust tips, respectively.

To determine whether there was sufficient DNA and to visualize double amplifications (double bands indicating the presence of two types of fungal DNA, called doublets), about 4 μ l of loading buffer (0.003% bromophenol blue and 0.45% glycerol) was added to 4 μ l of PCR product, loaded and then run with a 1 Kb ladder standard (Life Technologies) on a 0.7% agarose (Sigma Chemical Co.) gel (0.7 g agarose in 100ml of 10X TBE (108 g TRIZMA® Base, Sigma Chemical Co.), 55 g Boric acid and 40 ml 0.5 M EDTA and deionized water to make 1 litre). To stain the resulting bands, 22 μ l of ethidium bromide was added to the gel when it was poured. Moderate to strong bands, ranging in size from 800-1200 base pairs, were selected for digestion. Doublets were stored for analysis in a future study.

3.2.4 Digestion, gel electrophoresis and photography

For digestion of PCR products, three restriction endonucleases were used. Approximately 7.4 μ l of amplified DNA was added to 0.2 ml microcentrifuge tubes, each containing an endonuclease (0.5, 0.3 and 0.4 μ l of *Alu* I, *Hinf* I and *Rsa* I (Pharmacia Biotech), respectively), 2 μ l of the corresponding buffer solution (Pharmacia Biotech) and 5 μ l autoclaved filtered (millipore) water. These tubes were placed in an incubator at 37°C for a minimum of 5 hrs and then centrifuged briefly to remove condensation from the caps (if products were refrigerated) and 4 μ l of loading buffer was added to the solution. The contents of

the tube were loaded and run on 2.5% high resolution gel (1.0% NuSieve agarose, 1.5% agarose, 10X TBE buffer) at approximately 90mV. Ethidium bromide (approximately 7 μl/100 ml) was added before pouring the gel, enabling band patterns to be viewed under the UV light. Digital images of gels were taken using the Gel Print 2000i photographic system (BioPhotonic Corp.) and were saved on disk as well as printed on Mitsubishi thermal paper (K65H Mitsubishi Electric Corp.).

3.2.5 Analysis of molecular data

Band patterns were assessed using the RFLP analysis application software RFLPscan Plus, Version 3.0, (©1990-1996 Scanalytics). Band size was calibrated using the Desmile calibration method with log piecewise linear curve fitting and bands in all lanes were matched simultaneously at a 2% tolerance level; banding patterns across different gels were compared at a 6% variation level to compensate for differences in gels. Fragments less than 75 base pairs were not counted to reduce the possibility of including primer dimer products. Using RFLPscan Database, Versions 2.1 and 3.0 (©1990-1996 Scanalytics), 14 databases were created, separated by replicate site and season for each treatment. In addition, databases for eight commonly occurring morphotypes (*Amphinema, Cenococcum*, E-strain, *Hebeloma, MRA*, Russulaceae type 1, *Thelephora*, and *Tuber*) and for the group of lightly colonized but unidentified tips were created.

Pairwise comparisons of all banding patterns were compiled for each database. Pairs of tips were matched using a modification of Dice's index; the modification was that Dice's index was converted to a distance value (i.e. 1-Dice's index). As calculated using the RFLPscan software, modified Dice's index = Σ (polymorphic bands) / (shared bands + total bands) / 3 (Egger unpublished). Once all the possible pairwise combinations were examined, a distance matrix was created. Cluster analysis using the unweighted pair-group method with arithmetic means (UPGMA) of the distance matrix was done using the Neighbor-Joining/UPGMA module in PHYLIP (Phylogeny Inference Package) Version 3.5c (©1986-1993 Joseph Felsenstein). The UPGMA phenograms produced were viewed using TreeView, Version Win 3.2 (©1998 Roderic DM Page).

From each phenogram, clusters of tips were selected to determine intra- or inter-specific variation by comparing the band patterns for all three enzymes. If the band size differed by more than 6% of the total molecular weight, the variation was considered a polymorphism. Tips with similar band patterns for two enzymes but differing for the third enzyme were classified as intraspecific variants (Gardes and Bruns 1996). If only one or no enzyme band pattern was shared, then tips were considered to be different interspecific genotypes. Due to the large sample size, not all band patterns for all the tips were considered when reporting the major genotypes and variants; excluded tips were those that occurred infrequently (less than 10 in a cluster) or tips that were separated by a large distance (greater than 20%) in the phenogram.

The newly derived Phi index (see Appendix H for calculations) was used to assess genetic diversity between treatments and for each of the eight commonly occurring morphotypes using band patterns from all successfully amplified and digested tips. One-way ANOVA (STATISTICA for Windows Release 5.1 G 1997 edition, Statsoft, Inc.) was used to determine significant differences between treatments and between seedling types using a Bonferroni correction of alpha = 0.01 (α / number of comparisons =0.05 / 5) for five planned comparisons due to an incomplete experimental design. Comparisons included: 1) between planted seedlings in clearcut and cut plus burned sites to test for burning effects; 2) between regenerating seedlings in mature and planted seedlings in cut plus burned sites to test for cutting and seedlings effects; 3) between regenerating seedling effects; 4) between regenerating seedlings in mature and cut planted and regenerating seedlings in clearcut sites to test for sites to test for seedling in clearcut sites to test for seedlings in mature and clearcut sites to test for seedlings in mature and seedling effects; 4) between regenerating seedlings in clearcut sites to test for seedlings in mature and clearcut sites to test for seedlings in mature and planted and regenerating seedlings in clearcut sites to test for seedling differences.

3.3 RESULTS

3.3.1 Amplification and digestion success rates

Of all tips selected for morphological characterization, 69% (1276) were successfully amplified and digested for RFLP analysis (Table 9). The eight commonly occurring morphotypes (*Cenococcum*, E-strain, *MRA*, *Tuber*, *Amphinema*, *Hebeloma*, Russulaceae type 1 and *Thelephora*) plus the lightly colonized category, were further examined for band patterns. These comprised 91% of all successfully amplified tips. The level of amplification varied among types but in general was high among some of the commonly occurring ECM (e.g. *Cenococcum*, E-strain, *Amphinema*, *Hebeloma* and Russulaceae type 1). For the well colonized ECM *Tomentella* type 1 and *Piloderma*, amplification rates were expected to be higher but a number of tips were lost in early extractions.

For most well colonized tips favourable settings for amplification included denaturation at 94°C for 45 sec., annealing temperature of 48°C for 45 sec., and an extension step at 72°C starting at 130 sec., increasing 1 second per cycle for 35 cycles. The amplification success rate for E-strain, *MRA*, and non-rhizomorphic thin mantled and unclamped types as well as for lightly colonized tips was improved by using a lower annealing temperature (48°C) and increasing the number of cycles from 35 to 40 cycles.

Unsuccessful amplification included weak bands (showing insufficient DNA for further processing) or doublets. The morphotypes *MRA*, *Thelephora* and the category of lightly colonized tips had the highest percentage of doublets (8, 9 and 7%, respectively) followed by E-strain (5%).

Table 9. Summary of DNA amplification (PCR*) of mycorrhizal root tips from naturally regenerating and planted hybrid white spruce seedlings growing in the Aleza Lake Research Forest, Central Interior of British Columbia.

Morphotype	Туре	Total	Tips	Amplification	Doublets	Doublets
	code	tips	amplified [†]	rate (%)		% total
Cenococcum	2	68	53	77.9	1	1.5
E-strain*	1	149	124	83.2	8	5.4
MRA*	3	326	204	62.6	27	8.3
Tuber	4	29	17	58.6	-	-
Ascomycete unknown	5	1	-	-	-	-
Amphinema	9	258	221	85.7	3	1.2
Hebeloma	Ι	145	115	79.3	5	3.4
Inocybe	H	4	4	100.0	-	-
Laccaria	E	17	14	82.4	-	-
Piloderma	A	20	10	50.0	-	-
Russulaceae 1	F	164	139	84.8	1	0.6
Russulaceae 2	G	23	15	65.2	-	-
Thelephora	8	176	113	64.2	15	8.5
Thelephoraceae-like	Р	1	1	100.0	-	-
Tomentella 1	6	17	3	17.6	-	-
Tomentella 2	7	9	7	77.8	-	-
Tomentella 3	0	1	1	100.0	-	-
Non-rhizomorphic olive-green	J	3	2	66.7	-	-
Non-rhizomorphic thin mantled*	L	44	32	72.7	2	4.5
Non-rhizomorphic unclamped*	М	3	2	66.7	-	-
Non- rhizomorphic white	K	9	8	88.9	-	-
Rhizomorphic brown unclamped	D	10	6	60.0	-	-
Rhizomorphic orange unclamped	С	15	8	53.3	-	-
Rhizomorphic white	В	12	8	66.7	-	-
lightly colonized*	N/X	339	169	49.9	25	7.4
Totals / meanst		1843	1276	69.2‡	87	4.7±

*settings include denaturation at 94°C for 45 sec., annealing temperature of 46°C for 45 sec., and an extension step at 72°C starting at 130 sec., increasing 1 second per cycle for 40 cycles. Otherwise, different annealing temperature (48°C) and number of cycles (35) were used.

tincludes tips which were further digested and analysed for RFLP patterns and excludes those tips showing weak or double bands.
3.3.2 Band patterns of selected ECM morphotypes

Molecular band patterns for eight commonly occurring ECM morphotypes and the lightly colonized, unknown group are presented in Tables 10 to 12. Table 10 shows the genotype (band pattern difference in more than one endonuclease) and variant (band pattern difference in only one enzyme) patterns for the four ascomycetes: *Cenococcum*, E-strain, *MRA* and *Tuber*. Band patterns of *Cenococcum* were represented by one major genotype. E-strain showed two genotypes; for genotype 2, band patterns of variants differed by the addition of a band in *Hinf* I, as well as a restriction site for *Rsa* I. For *MRA*, although genotype 2 only differed in the *Rsa* I endonuclease, it had more and different restriction sites and was distinctly larger in size than genotype 1. The distance on the phenogram was also sufficient (approximately 20%) to justify designating the two groups as separate genotypes. Like *Cenococcum*, *Tuber* showed one genotype. For ascomycetes, the total band size was highest for *Tuber* and lowest for *Cenococcum*. Most of the variants occurred on both regenerating and planted seedlings and in more than one treatment. *MRA* was an exception where genotype 2, occurred only on planted seedlings.

Band patterns for the four basidiomycetes (*Amphinema*, *Hebeloma*, Russulaceae type 1 and *Thelephora*) are presented in Table 11. Three different sets of band patterns for *Amphinema*, varying for *Alu* I and *Rsa* I, resulted in three genotypes. Of these, restriction sites varied from none (genotype 2, variant 1) to three (genotype 3) for *Rsa* I and from two to three sites for Alu I, producing a total of six variants. Interestingly, all band patterns for *Hebeloma* were similar to those of genotype 1 for *Amphinema*, including its three variants. Two genotypes were defined for Russulaceae type 1: genotype 2 differed from genotype 1 for all endonucleases in band pattern (having fewer restriction sites) but not for total band size for *Alu* I and *Rsa* I. Variants of genotype 1 differed in total band size for *Hinf* I. For *Thelephora*, only one genotype was resolved with two variants, differing in band patterns for *Alu* I.

Most band patterns were seen in more than one treatment or type of seedling (Table 11). Exceptions included all three genotypes for *Amphinema*. Genotype 1, variant 3 for *Amphinema*, only occurred on regenerating seedlings in clearcut sites, genotype 2, variant 1, occurred on cut plus burned sites only, and genotype 3 only occurred on planted seedlings.

Туре	Genotype [†] (no. tips)	Band p	atterns	using	Genotype (no. tips)	Band	pattern	susing
(no. amplified	Variant [‡] (no. tips)	three e	endonuc	leases	Variant (no. tips)	three	endonu	cleases
tips)	Occurrence§	Alu I	Hinf I	Rsa 1	Occurrence	Alu I	Hinf I	Rsa I
Cenococcum	Gen. 1 (45)	438	270	261				
(53)	Var. 1 (45)	149	159	182				
	M-r/ C-pl/ CB-pl	110	127	137				
		697	94	99				
			650	679				
E-strain	Gen. 1 (65)	693	493	923	Gen. 1 (65)	686	507	983
(124)	Var. 1 (36)	186	160		Var. 2 (29)	185	161	
	C-pl/C-r/CB-pl	113	146		C-pl/ C-r/ CB-pl	118	144	
		992	799			989	812	
	Gen. 2 (30)	672	490	886				
	Var. 1 (30)	183	177	92				
	C-pl/ C-r/ CB-pl	116	166	978				
		971	148					
			981					
MRA	Gen. 1 (108)	620	428	558				
(204)	Var. 1 (108)	147	248	175				
	M-r/ C-pl/ C-r/ CB-pl	113	168	733				
		880	844					
	Gen. 2 (32)	635	437	442				
	Var. 1 (32)	147	248	155				
	C-pl/CB-pl	113	164	142				
		895	849	124				
				863				
Tuber	Gen. 1 (10)	587	327	357				
(17)	Var. 1 (10)	184	307	303				
	M-r/C-pl/C-r	144	176	255				
	1	114	125	91				
		1029	935	1006				

Table 10. RFLP band patterns of four ascomycete morphotypes amplified (PCR*) from naturally regenerating and planted hybrid white spruce seedlings in the Aleza Lake Research Forest, Central Interior of British Columbia.

* primers used for the ITS region of rDNA were ITS1 and NL6Bmun.

† genotypes were defined as tips having band pattern differences in more than one enzyme (Alu I, Hinf I, or Rsa I).
† variants were defined as tips having band pattern differences in only one enzyme. Genotypes and variants

reported here include clusters on phenograms with ≥10 tips.

§ M- mature, C- clearcut, CB- cut plus burned, r-regenerating seedling, pl-planted seedling.

¶ band patterns presented were taken from a representative tip within each variant cluster in the phenogram.

Type (no. amplified	Genotype† (no. tips) Variant‡ (no. tips)	Band pa three e	atterns¶ ndonucl	using eases	Genotype (no. tips) Variant (no. tips)	Band three	patterns endonuc	using leases
tips)	Occurrence§	Alu I	Hinf 1	Rsa 1	Occurrence	Alu l	Hinf 1	Rsa 1
Amphinema	Gen. 1 (117)	562	316	763	Gen. 1 (124)	360	321	779
(221)	Var. 1 (42)	191	285	180	Var. 2 (60)	189	289	176
	M-r/ C-pl/ CB-pl	117	158	943	M-r/ C-pl/ C-r/	112	165	955
		97	146		CB-pl	661	152	
		967	905				927	
					Gen. 1 (124)	651	322	750
					Var. 3 (15)	189	282	176
					C-r	120	167	926
						960	<u>153</u> 924	
	Gen. 2 (24)	361	321	988				
	Var. 1 (24)	189	290					
	CB-pl	111	165					
		661	<u>150</u>					
			926					
	Gen. 3 (37)	579	320	323	Gen. 3 (37)	362	319	321
	Var. 1 (18)	189	286	292	Var. 2 (19)	190	292	286
	C-pl/ CB-pl	116	169	150	C-pl/ CB-pl	118	166	151
		97	157	136		670	153	90
		981	932	901			930	848
Hebeloma	Gen. 1 (77)	577	320	764	Gen. 1 (77)	357	312	770
(115)	Var. 1 (20)	193	293	178	Var. 2 (31)	189	285	174
	M-r/ C-pl/ CB-pl	121	158	942	M-r/ C-pl/ CB-pl	110	160	944
		99	149			656	146	
	<u></u>	990	920			610	903	= 10
					Gen. 1 (77)	648	319	749
					Var. 3 (26)	195	275	178
					M-r/ C-pl/ CB-pl	113	165	927
						956	150	
December 1	C 1 (100)	507	212	702	Con 1 (100)	506	909	701
Kussulaceae I	Gen. 1 (108)	327	313	/83	Gen. 1 (108)	320	238	/91
(139)	Var. $I(78)$	162	258	1/3	Var. 2(30)	100	217	179
	м-п С-рі СВ-рі	150	109	930	IVI-I/ C-I/ CB-PI	100	105	970
		070	154			109	151	
		970	91			982	92	
	Con 2 (22)	670	227	567			003	
	V_{2} (23)	197	282	100				
	$\frac{Val}{\Gamma(23)}$	107	160	199				
	M-1/ C-pi/ C-1/ CD-pi	068	154	047				
		900	943	747				
Thelephora	Gen. 1 (101)	576	310	783	Gen. 1 (101)	602	317	823
(113)	Var. 1 (45)	185	254	201	Var. 2 (56)	191	261	206
	C-pl/ C-r/ CB-pl	118	161	984	C-r/ CB-pl	159	167	1029
		113	147			123	154	
		992	104			1075	107	
			976				1006	

Table 11. RFLP band patterns of four basidiomycete morphotypes amplified (PCR*) from naturally regenerating and planted hybrid white spruce seedlings in the Aleza Lake Research Forest, Central Interior of British Columbia.

*primers used for the ITS region of rDNA were ITS1 and NL6Bmun.

†genotypes were defined as tips having band pattern differences in more than one enzyme (Alu I, Hinf I, or Rsa I). †variants were defined as tips having band pattern differences in only one enzyme. Genotypes and variants reported here include clusters on phenograms with ≥ 10 tips.

§M- mature, C- clearcut, CB- cut plus burned, r-regenerating seedling, pl-planted seedling.

¶band patterns presented were taken from a representative tip within each variant cluster in the phenogram.

Table 12 shows the analysis of band patterns for the lightly colonized category. A total of three genotypes were resolved (one variant each) and all patterns were similar to those of four previously described morphotypes (E-strain, *MRA*, *Amphinema* and *Hebeloma*) (Tables 10 and 11). The first band pattern closely resembled that of E-strain (genotype 1, variant 2). The second band pattern, representing the largest cluster of tips in the phenogram for the lightly colonized group, matched the most common band pattern for *MRA* (genotype 1, variant 1). The third band pattern matched the most common band pattern of *Amphinema* as well as that of *Hebeloma* (genotype 1, variant 2). All band patterns for the lightly colonized group were found in sites where the matched morphotype also occurred. No new band patterns were resolved for the lightly colonized groups using the criterion (a minimum of 10 tips per cluster in the phenogram) for reporting band patterns.

Table 12. Comparison of RFLP band patterns of lightly colonized but unknown ECM with known morphological types amplified (PCR*) from naturally regenerating and planted hybrid white spruce seedlings in the Aleza Lake Research Forest, Central Interior of British Columbia.

Lightly colonia	zed group			Matched refe	erence type		
Genotype [†] (no. tips)	Band	patterns	for	Genotype (no. tips)	Band	l pattern	s for
Variant [‡] (no. tips)	unknov	vn type u	using	Variant (no. tips)	referen	nce type	using
Occurrence§	three en	ndonucle	eases	Occurrence	three e	endonuc	leases
	Alu I	Hinf I	Rsa I		Alu I	Hinf I	Rsa I
Lightly colonized a	661	504	967	E-strain	686	507	983
Gen. a (11)	182	164		Gen. 1 (65)	185	161	
Var. a (11)	114	148		Var. 2 (29)	118	144	
C-pl/ CB-pl	957	816		C-pl/ C-r/ CB-pl	989	812	
Lightly colonized b	634	444	559	MRA	620	428	558
Gen. b (60)	149	246	173	Gen. 1 (108)	147	248	175
Var. b (60)	113	162	732	Var. 1 (108)	113	168	733
M-r/ C-pl/ C-r/ CB-pl	896	852		M-r/ C-pl/ C-r/ CB-pl	880	844	
Lightly colonized c	359	318	760	Amphinema	360	321	779
Gen. c (11)	190	288	177	Gen. 1 (124)	189	289	176
Var. c (11)	111	160	937	Var. 2 (60)	<u>112</u>	165	955
M-r/ C-pl/ CB-pl	660	148		M-r/ C-pl/ C-r/ CB-pl	661	152	
		914				927	
				Hebeloma	357	312	770
				Gen. 1 (71)	189	285	174
				Var. 2 (31)	110	160	944
				M-r/ C-pl/ CB-pl	656	146	
						903	

*primers used for the ITS region of rDNA were ITS1 and NL6Bmun.

†genotypes were defined as tips having band pattern differences in more than one enzyme (Alu I, Hinf I, or Rsa I). †variants were defined as tips having band pattern differences in only one enzyme. Genotypes and variants reported here include clusters on phenograms with ≥ 10 tips.

§M- mature, C- clearcut, CB- cut plus burned, r-regenerating seedling, pl-planted seedling.

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3.3.3 Molecular diversity for commonly occurring morphotypes

For the eight morphotypes examined plus the lightly colonized, unknown group (representing 74% of the 1155 tips amplified), 16 genotypes and 24 variants were identified, of which four genotypes and six variants appeared to be duplicates (Tables 10 to 13). These included the band patterns for *Hebeloma* and the lightly colonized group. Combining these similar band patterns left 12 distinct genotypes and 18 variants. *Cenococcum* and *Tuber* were the least diverse, each with one genotype and variant representing 85% and 59% of tips amplified, respectively, for these types. *Thelephora* and *Hebeloma* also had only one genotype each, but had two and three variants, respectively. E-strain, *MRA* and Russulaceae type 1 were moderately diverse, with two genotypes and two to three variants, while *Amphinema* and the lightly colonized group were the most diverse, with three genotypes and three to six variants. Morphotypes with the three lowest and three highest Phi index values were *Tuber, Thelephora*, *Cenococcum* and the lightly colonized group, *MRA* and E-strain, respectively.

Table 13. Molecular genotype and variant occurrence and diversity (Phi index)* for commonly occurring ECM morphotypes found on naturally regenerating and planted hybrid white spruce seedlings growing in treated (clearcut, and cut plus burned) and untreated (mature) sites in the Aleza Lake Research Forest, Central Interior of British Columbia.

Morphotype	n	Genotypes†	Variants [‡]	Variants (% tips)	Phi
Cenococcum	53	1	1	85	0.056
E-strain	124	2	3	77	0.135
MRA	204	2	2	69	0.158
Tuber	17	1	1	59	0.036
Amphinema	221	3	6	81	0.098
Hebeloma	115	1	3	67	0.063
Russulaceae 1	139	2	3	94	0.074
Thelephora	113	1	2	89	0.055
Lightly	169	3	3	49	0.267
colonized§					
Total/Mean¶	1155	16	24	74¶	-

*all amplified tips (n) are included in diversity analysis for each morphotype.

†genotypes were defined as tips having band pattern differences in more than one enzyme (Alu I, Hinf I, or Rsa I). ‡variants were defined as tips having band pattern differences in only one enzyme. Genotypes and variants reported here include clusters on phenograms with ≥ 10 tips.

§lightly colonized tips possessed Hartig nets but were not identifiable.

Table 14 shows an assessment of genotype and variant occurrence for treatment and for regenerating versus planted seedlings for the eight ECM morphotypes and lightly colonized group. Previous morphotyping showed that morphotypes were found on all sites and types of seedlings. Blank cells refer to tips that either were not similar to any of the reported band patterns (Tables 10 to 14) and did not meet the criterion or did not successfully amplify to determine band patterns. *Tuber*, an exception, did not

occur on cut plus burned sites. *MRA* and *Amphinema* had twice as many genotypes and variants on planted compared to regenerating seedlings (Table 14). A trend of increasing genotypes and variants occurred for commonly occurring morphotypes as disturbance increased. Naturally regenerating seedlings in mature sites had the lowest number of distinctive genotypes (six and nine) and planted seedlings in cut plus burned sites had the most (11 and 17). Regenerating seedlings in clearcut sites had intermediate numbers (8 genotypes and 11 variants). Planted seedlings in both treated sites had similar numbers of genotypes and variants (Table 14).

Table 14. Number of molecular genotypes and variants for ECM found on naturally regenerating and planted hybrid white spruce seedlings growing in treated (clearcut, and cut plus burned) and untreated (mature) sites in the Aleza Lake Research Forest, Central Interior of British Columbia.

Morphotype	Mature/regenerating	Clearcut/regenerating	Clearcut/planted	Cut plus burned/ planted
Cenococcum	1 genotype*	-	1 genotype	1 genotype
	1 variant†		1 variant	1 variant
E-strain	-	2 genotypes	2 genotypes	2 genotypes
		3 variants	3 variants	3 variants
MRA	1 genotype	1 genotype	2 genotypes	2 genotypes
	1 variant	1 variant	2 variants	2 variants
Tuber	1 genotype	1 genotype	l genotype	-
	1 variant	1 variant	1 variant	
Amphinema	1 genotype	1 genotype	2 genotypes	3 genotypes
	2 variants	2 variants	4 variants	5 variants
Hebeloma	l genotype	-	1 genotype	1 genotype
	3 variants		3 variants	3 variants
Russulaceae 1	2 genotypes	2 genotypes	2 genotypes	2 genotypes
	3 variants	2 variants	2 variants	3 variants
Thelephora		1 genotype	1 genotype	1 genotype
		2 variants	l variant	2 variants
Lightly colonized [‡]	2 genotypes	1 genotype	3 genotypes	3 genotypes
	2 variants	1 variant	3 variants	3 variants
Total distinct	6 genotypes	8 genotypes	11 genotypes	11 genotypes
types§	9 variants	11 variants	15 variants	17 variants

*genotypes were defined as tips having band pattern differences in more than one enzyme (Alu I, Hinf I, or Rsa I). †variants were defined as tips having band pattern differences in only one enzyme. Genotypes and variants reported here include clusters on trees with ≥ 10 tips.

‡lightly colonized tips possessed Hartig nets but were not identifiable.

§all Hebeloma band patterns matched those of Amphinema; lightly colonized tips matched band patterns of E-strain, MRA, Amphinema and Hebeloma.

3.3.4 Treatment effects on ECM molecular diversity using the Phi, Shannon and Simpson indices

Phenograms (a total of 14 (see Appendix I for example)) generated for each site database separated by

season (spring and fall) and replicate (1 and 2) generally displayed large, distinct groups both for putative

ascomycetes (Types 1 to 5, Table 9) and basidiomycetes (Types 6 to P, Table 9). These groups were

separated by large distances (greater than approximately 30%).

Preliminary analyses on Phi index values (Student's t-test, Bonferroni correction of α =0.01) showed that neither season nor replicate site values differed significantly and that those databases could be pooled. However, pooling both replicate sites and season would have reduced the sample size of Phi values (calculated for one database) to 1, precluding ANOVA. As a result, only season databases were pooled and analysis was done on both unpooled and pooled databases to examine the outcome of pooling on diversity assessment. No significant differences were found for treatment effect (clearcut, cut plus burned and undisturbed) or for seedling type (naturally regenerating versus planted) using the Phi index values as a measure of molecular diversity (one way ANOVA, α =0.01 using a Bonferroni correction) for either pooled or unpooled databases (Table 15). Pooling of the databases for season resulted in an increase in the Phi values for clearcut as well as cut plus burned sites and a decrease for the mature sites but this did not change ANOVA results.

One way ANOVA (α =0.01 using a Bonferroni correction) of Shannon and Simpson composite indices were not significant for either treatment or seedling effect. Databases were kept separate for replicate site and season but Shannon and Simpson values were pooled for these variables, after conducting preliminary Student t-tests (Bonferroni correction of α =0.01). Table 15. Statistical summation^{*} for treatment effect and seedling type on molecular diversity assessed using Phi, Shannon and Simpson index values (mean±SE)) for ECM associated with naturally regenerating and planted hybrid white spruce seedlings growing in treated (clearcut, and cut plus burned) and untreated (mature) sites in the Aleza Lake Research Forest, Central Interior of British Columbia.

Treatment/s	seedling type comparison	F-statistic (df), p-value	
Clearcut/planted	Cut plus burned/planted		
$0.268(0.006)^1$	0.240(0.013)	F(1,6)=3.664, p=0.104	
$0.270(0.006)^2$	0.253(0.008)	F(1,2)=2.667, p=0.244	
$3.221(0.061)^3$	3.245(0.107)	F(1,6)=0.036, p=0.856	
0.937(0.008)4	0.942(0.007)	F(1,6)=0.295, p=0.606	
Mature/regenerating	Clearcut/regenerating		
0.276(0.038)	0.220(0.027)	F(1,4)=0.877, p=0.402	
0.191(0.044)	0.220(0.027)	F(1,2)=0.322, p=0.628	
3.079(0.199)	3.135(0.139)	F(1,4)=0.032, p=0.866	
0.935(0.017)	0.936(0.014)	F(1,4)=0.005, p=0.948	
Mature/regenerating	Clearcut/planted		
0.276(0.038)	0.268(0.006)	F(1,6)=0.037, p=0.854	
0.191(0.044)	0.270(0.006)	F(1,2)=3.199, p=0.216	
3.079(0.199)	3.221(0.061)	F(1,6)=0.470, p=0.519	
0.935(0.017)	0.937(0.008)	F(1,6)=0.011, p=0.919	
Mature/regenerating	Cut plus burned/planted		
0.276(0.038)	0.240(0.013)	F(1,6)=0.783, p=0.410	
0.191(0.044)	0.253(0.008)	F(1,2)=1.961, p=0.296	
3.079(0.199)	3.245(0.107)	F(1,6)=0.538, p=0.491	
0.935(0.017)	0.942(0.007)	F(1,6)=0.173, p=0.692	
Clearcut/planted	Clearcut/regenerating		
0.268(0.006)	0.220(0.027)	F(1,4)=6.425, p=0.064	
0.270(0.006)	0.220(0.027)	F(1,2)=3.285, p=0.212	
3.221(0.061)	3.135(0.139)	F(1,4)=0.483, p=0.525	
0.937(0.008)	0.936(0.014)	F(1,4)=0.001, p=0.994	

^{*}One-way ANOVA, p≤0.01, Bonferroni correction for planned comparisons.

¹Phi values, databases with season and replicate site data kept separate (unpooled).

²Phi values, databases with season data (pooled).

³Shannon index, databases with season and replicate site data kept separate (unpooled).

⁴Simpson index, databases with season and replicate site data kept separate (unpooled).

3.4 DISCUSSION

3.4.1 Genetic diversity between treatments and between seedling type

In the present study, the genetic diversity of ECM associated with hybrid white spruce, as calculated by

the Phi, Shannon and Simpson indices, did not appear to be significantly affected by treatment or

seedling type. Similarly, Baldwin (1999, M.Sc. Thesis) also found no difference in ECM diversity (Phi,

Shannon and Simpson indices) for regenerating black spruce (Picea mariana) seedlings growing in

clearcut and cut plus burned sites (of low and high intensity burns) in a mixedwood paper birch (Betula

papyrifera)-black spruce forest. In a recent study examining wildfire and salvage-logging effects on

planted and naturally regenerating hybrid white spruce seedlings, the Phi, Shannon and Simpson indices

showed no significant effects of treatment on ECM diversity (Egger and Massicotte 1999).

Traditional diversity indices assume that all species in the sample are known and this may be difficult to determine with ECM. Molecular analysis differs from morphological assessment (which uses species counts based on descriptions) because the band patterns produced could represent interspecific or intraspecific variation of ECM. In this respect, the Phi index is a more appropriate measure than traditional diversity indices because it uses phylogenetic distance, which may be less variable than species descriptions, as a measurement of species relatedness. However, the accuracy of the Phi as a measure of molecular diversity depends on the number of tips and types that are successfully amplified.

3.4.2 Genetic variation of hybrid white spruce ectomycorrhizae

The 12 genotypes and 18 variants found on hybrid white spruce compares favourably to numbers reported in other studies on ECM diversity. Varga (1998, MSc. Thesis) found 14 and 31 distinct ECM RFLP topologies (or variants) for Sitka alder (*Alnus sinuata* [Regel] Rydb) and Lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.), respectively, in the Central Interior of British Columbia, SBS biogeoclimatic zone. Mehmann *et al.* (1995) reported 23 RFLP types (or variants) from fungal sporocarps and cultures originating from a 40-year-old Norway spruce stand in Switzerland and Horton *et al.* (1998) reported 14 ECM molecular types (or variants) on Bishop pine seedlings five months after wildfire. Phenograms generated in the present study were very large and one consequence of this size was that RFLP patterns for the less abundant types as well as clusters of less than 10 tips were not examined. Therefore the number of genotypes and variants reported in the present study is most likely a conservative estimate.

In the present study, the morphotypes *Cenococcum* and *Tuber* were each composed of one genotype and one variant. These ECM also had the lowest Phi values, suggesting a high degree of similarity among their isolates. Other studies on *Cenococcum* have reported variable levels of genetic variation. LoBuglio *et al.* (1991), examining numerous isolates of *Cenococcum* over a large geographic area, found high genetic variation. However, studies (more limited in geographic range) by Varga (1998, M.Sc. Thesis), Baldwin (1999, M.Sc. Thesis) and Egger and Massicotte (1998) reported two, four, and six types (or variants) respectively, for *Cenococcum* ECM.

The *MRA*, and E-strain types are believed to consist of complexes of fungal species (Ingleby *et al.* 1990). These morphotypes had intermediate numbers of genotypes and variants (represented by approximately 70 and 80% of the amplified tips for these types) but had the highest Phi values (which included all tips in the analysis). As well, *MRA* had more genotypes and variants on planted compared to regenerating seedlings; morphological results reported an increase in the abundance of this morphotype as well as E-strain on planted compared to regenerating seedlings (Chapter 2). Although individual morphotypes by treatment using the Phi values was not assessed, high index values suggest that these more abundant types may also be more diverse, having more genotypes or variants. Similar to the present study, Varga (1998, M.Sc. Thesis) reported two genotypes and three variants for *MRA* on Lodgepole pine. Egger and Massicotte (1998) reported five variants each for both E-strain and *MRA*.

The Russulaceae species are often difficult to separate morphologically due to similar features (Horton and Bruns 1998). Horton and Bruns (1998) found three different Russulaceae ECM RFLP variants and Egger and Massicotte (1998) reported four variants. In the present study, Russulaceae type 1 was abundant in mature sites (morphologically) and amplified well for molecular analyses. Although it possessed similar numbers of genotypes and variants (accounting for 94% of amplified tips for this type), it had a lower Phi value and may have been less variable genetically. Phi index values are partially determined by distance; a low value may indicate that although several genotypes exist, these may be genetically quite similar. If there were many tips of closely related species having small distances and only few diverse species having larger distances, Phi values might be expected to remain low, although diverse species contribute more to the Phi index on average than closely related species *(Fc)ger pers. comm.* 1999).

Amphinema and *Hebeloma* ECM are also difficult to distinguish morphologically (Ingleby *et al.* 1990). The molecular band patterns for *Hebeloma* were identical to those for one genotype and its variants for *Amphinema*. It is possible that *Hebeloma* was not found in the present study or that some *Hebeloma* tips were mistaken for *Amphinema*. If the second scenario were true, the number of genotypes and variants for *Amphinema* should be lower. Similar to the present study, Egger and Massicotte (1998) found that *Amphinema* and *Hebeloma* had a variant in common. On Sitka alder, 2 genotypes were reported for *Hebeloma* (Varga 1998, M.Sc. Thesis). Egger and Massicotte (1998) reported many variants for

Amphinema and eight variants for Hebeloma. Amphinema and Hebeloma had intermediate Phi values in the present study.

Thelephora had only one genotype and two variants and these were found on both planted and regenerating seedlings. This suggests that *Thelephora* occurred independently of greenhouse inoculum, though some may have been on planted seedlings initially. This was also true for all E-strain variants, suggesting its occurrence was not solely due to nursery inoculum. In contrast, eight variants were reported for *Thelephora* from mature forest, burned-salvaged-logged, and burned-unsalvaged sites (Egger and Massicotte 1998).

One of the most interesting groups was that of the lightly colonized, unknown group. The three genotypes resolved were all previously characterized as belonging to *MRA*, E-strain, and the similar *Amphinema* and *Hebeloma* band patterns. This suggests that lightly colonized tips may often belong to ECM that have been characterized. Scoring these as a group may be affecting abundance rather than richness measures as only three distinct morphotypes were found examining 50% of the tips.

With respect to the molecular variation for the commonly occurring morphotypes, the total number of genotypes and variants appeared to increase with increase in disturbance (from mature to clearcut to cut plus burned sites) as well as increase from regenerating to planted seedlings. Jonsson *et al.* (1999) did not find any changes in community composition following wildfire, but they did note an increased dominance in the commonly occurring types in fire-disturbed sites. In the present study (Chapter 2, Table 14), an increase in the number of some commonly occurring types (*MRA* and *Amphinema*) occurred in disturbed sites.

Caution should be used when interpreting intraspecific variation using the ITS region for amplification. In some instances, the ITS region may not be variable enough to determine differences in closely related species that other regions, such as the intergenic region (IGR) may detect (Gardes and Bruns 1996). In other situations, some fungi isolates may exhibit so much variation in the ITS region that they would be classified as different species (Gardes and Bruns 1996). Thus, the use of the ITS region may not reflect real differences in intraspecific variation of some morphotypes.

The percentage of tips available for RFLP analysis after amplification and digestion in the present study was 69%. This compares favourably with other studies (49% for alder and 63% for lodgepole pine (Varga 1998, M.Sc. Thesis); 65% for black spruce (Baldwin 1999, M.Sc. Thesis); 60% for hybrid white spruce (Egger and Massicotte 1999); 56% for soil cores collected in one to 62 year-old Scots pine stands (Jonsson *et al.* 1999). Amplification rates in the present study for commonly occurring morphotypes were more variable than those in the study by Varga (1998 M.Sc. Thesis), who reported 67, 75 and 75% for *Cenococcum*, *MRA* and *Amphinema*, respectively.

The percentage of doublets in the present study (approximately 5%) was similar to other studies (6% (Baldwin 1999 M.Sc. Thesis); 7% (Egger and Massicotte 1999) however, a higher rate of doublets (15%) was reported by Jonsson et al. (1999). One of the explanations for doublet formation could be due to heteroduplex DNA products formed in the PCR reaction (Jonsson et al. 1999). The authors further suggested that heteroduplexes could be attributed to heterogeneity in the amplified segment or from cross-hybridization between slightly different amplifications products in the PCR reaction (Jonsson et al. 1999). Another reason that doublets might occur is if ECM tips are additionally colonized with another ECM fungi or if fungal endophytes are present and are amplified. Endophytes grow within plant root cells (unlike ECM which grow between cells), are widely and abundantly distributed and easy to isolate. However, they are poorly understood in terms of their ecological function (Jumpponen and Trappe 1998). MRA has been reported to vary from being non-pathogenic (called dark septate endophytes) to pathogenic; little is known about which species or functional groups comprise this morphotype (Jumpponen and Trappe 1998). Nevertheless, endophytic types could have been inadvertently amplified during molecular analysis of MRA. In the present study, MRA had one of the highest rates (8%) of doublets. Thelephora also had a high rate of doublets (9%) as did the group of lightly colonized tips (7%). Interestingly, a recent study examining doublets from post-fire ECM also found a high percentage in Thelephora (34%) and MRA (10%) morphotypes as well as in the lightly colonized group (25%) (Rosling et al. unpublished).

3.4.3 Comparison between molecular and morphotyping characterization Both molecular and morphological characterization methods have advantages and disadvantages in the identification of ECM. With respect to morphological techniques, a problem often reported is the

tremendous environmental variation of ECM morphotypes. Types that look and are reported as different may actually be the same, resulting in over-representation of species. In some instances, less conspicuous or common looking (e.g. white rhizomorphic or Russulaceae) mycorrhizae are lumped as similar morphotypes, resulting in under-representation of species. Molecular analyses appears to partially address the concern of environmental variation. In the present study, three situations occurred: 1) one morphotype represented one RFLP genotype (e.g. *Cenococcum*, *Tuber*); 2) one morphotype had one RFLP genotype but several variants (e.g. *Hebeloma*, *Thelephora*); and 3) one morphotype had more than one RFLP genotype and variant (E-strain, *Amphinema*, Russulaceae type 1). In some instances, morphotyping methods appeared to agree with molecular assessments whereas in others it may have underestimated the ECM diversity.

Morphotyping is a process that requires considerable time to examine root systems, especially when large samples need to be analysed. Molecular analysis (e.g. amplification, digestion, database matching) does not require a lot of time beyond the initial preparation, however, considerable time can be spent on analysing phenograms and determining the number of genotypes and variants.

The task of identifying ECM using morphological techniques can be subjective. For example, differentiation between morphotypes is based on the observer's opinion of root colour, texture and mantle pattern. Differences in researcher interpretation may have contributed to some of the differences in diversity (Shannon and Margalef index) with respect to regenerating seedlings in the clearcut sites. Eight unknown, rare types were absent on these regenerating seedlings while two other types were unique to them (Chapter 2). Some errors attributed to morphotyping differences might be restricted to those rare types. However, many broad host-ranging types have been consistently reported in studies possibly because these types may be easier to identify (such as *Amphinema*). Molecular data in the present study confirm this: tips of commonly occurring morphotypes found on the same regenerating seedlings in clearcut sites. Similar to morphological techniques, analysis of band patterns is also subjective, both in determining band presence when examining gels, as well as determining whether a partial digestion or a double amplification has occurred. It was observed, however, that when tips of one morphotype were combined on one gel, differences were minimized and comparisons within a type were

easier to assess. Determining the number of genotypes and variants from the phenograms also requires individual interpretation. Using the criterion of ten tips per band pattern to determine whether genotypes or variants could be reported appeared to work well for large ECM databases, however, it may not have assessed smaller ECM databases (e.g. *Tuber*) as well.

Differences in scientific terminology and methods are common problems for both morphological and molecular techniques (Mehmann *et al.* 1995). This makes comparisons between studies challenging. Differences in morphological methods were discussed previously (see Introduction). With respect to molecular methods, different protocols, primers or endonucleases make it difficult to compare band patterns from different studies. ITS1-F (Gardes and Bruns 1991) and ITS4 (White *et al.* 1990) have been frequently used (Kårén *et al.* 1997; Kernaghan *et al.* 1997; Horton and Bruns 1998) but few studies have used NL6Bmun. The primer NL6Bmun was used in the present study as it is preferential for basidiomycetes but it also amplifies ascomycetes. The use of different endonucleases results in different fragment sizes and band patterns. In addition, bands may be matched at different levels of tolerance and different fragment size calculation algorithms may be used in other studies. This can result in different numbers of variants being reported.

A problem unique to molecular methods was the difficulty of adequately amplifying the lightly colonized tips, and some thin-mantled *MRA*, thereby possibly biasing diversity analysis towards the types that amplified well. Sometimes even well colonized tips did not amplify due to unforeseen difficulties with extractions. However, as discussed earlier, lightly colonized tips appeared to be well represented by previously identified species, suggesting that failure to amplify tips may not greatly affect species richness results (genotypes and variants). Nevertheless, Phi values and diversity results that are influenced in part by the numbers of tips, may still be affected by the inability of some tips to be amplified. Failure to amplify some tips also made it difficult to calculate species abundance. For diversity analysis, the variable success rate in PCR amplification meant that databases could not be created for individual seedlings as was done with the morphological data. As a result, only one number could be calculated for each site treatment database using the Phi index. Morphological comparisons (ANOVA) based on 16 to 28 diversity values for each site appeared to be a stronger statistical test than those based on two Phi values.

Despite the differences in number of diversity values, diversity measures obtained for molecular (Phi, Shannon and Simpson indices) and morphological (Shannon and Simpson values, evenness and richness measures) analytical techniques were mostly in agreement. Both studies showed no differences in ECM on planted seedlings growing in clearcut and cut plus burned sites. However, results for the morphology assessment between regenerating seedlings in mature and clearcut sites and between planted and naturally regenerating seedlings in the clearcut site (see Chapter 2) differed from molecular assessment, which showed no differences for these sites. This may partly be explained by the fact that rare types that were characterized morphologically, did not always successfully amplify. The Shannon composite index and Margalef richness measure, both sensitive to the number of morphotypes (Magurran 1988), were perhaps better able to detect differences whereas the Phi index may not have been as sensitive to either increases or decreases in the number of types. Rare types counted in morphological analysis also may not have been included in molecular analysis because they did not amplify. The Phi index may be likened to the molecular counterpart of the Simpson index, as it appeared to be more sensitive to the most abundant species (genotypes and variants) whose distances contribute to the total distance more than do the less abundant ones. In the morphology assessment, the Simpson index was not found to be significant for either treatment or seedling effects in terms of diversity.

Studies using species diversity and similarity indices to measure fungal community composition in the past, have not directly addressed community dynamics such as function and stability (Zak 1992). However, in a recent greenhouse study conducted by van der Heijden *et al.* (1998), it was shown that arbuscular mycorrrhizal diversity was a major factor in contributing to the maintenance of plant biodiversity. Shoot and root biomass, hyphal length, and plant phosphorus showed increasing trends with increase in the number of mycorrhizal fungal species. Similar studies conducted for ECM would lend support to the belief that an increase in ECM diversity is beneficial for ecosystem functioning. Zak (1992) also suggests that examining species-abundance distributions will help to determine changes in fungal communities over time and to predict return times as well as determine better the role of disturbance events. In the present study however, treated sites were only compared for one growing season. In the assessment of using two methods of ECM characterization, it was found that morphological characterization provided a portrait of the fungal community, including rare types which may be missed or

may not amplify well using molecular methods. An assessment of morphotyping accuracy as well as the variation within morphotypes (species or isolate difference) can be obtained from molecular analysis. Research methods that choose several characterization techniques that complement each other provide a more comprehensive view of ECM abundance and diversity.

3.4.4 Conclusions

In conclusion, both morphological and molecular techniques showed no differences in ECM diversity for planted hybrid white spruce seedlings in the clearcut plus broadcast burned treatment compared to the clearcut treatment. However, morphological results showed a significant treatment effect between regenerating seedlings in clearcut and mature forest sites as well as a seedling effect between naturally regenerating and planted seedlings in clearcut sites. Morphologically, a total of 24 distinct morphotypes were described, 14 which were of known fungal affinities. Molecular analysis produced 12 genotypes and 18 variants for eight common ECM types plus the lightly colonized, unknown group. Differences in the distribution and in the inter- and intra- specific variation of the commonly occurring morphotypes (Amphinema, Cenococcum, E-strain, Hebeloma, MRA, Russulaceae type 1, Thelephora, and Tuber) were also shown by morphological and molecular techniques. These results are limited to hybrid white spruce seedlings, growing in mature forest sites in the SBS biogeoclimatic zone willow wet, cool (wk1) variant as well as to windrowed clearcuts and to burns estimated to be of moderate severity. The limitations of morphological and molecular characterization techniques seem to be shared; they both have some subjective aspects and comparisons with other studies are difficult due to differences in materials and protocols. Using both methods together provides a more comprehensive view of ECM diversity as well as a verification or validation on the accuracy of each method. Improved standardization of both methods would facilitate the ability to assess ECM diversity in complex forest ecosystems.

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Site characteristic		Burn site 1		Bui	m site 2	Clearcut site 1	Clearcut site 2
Ecology: association size (ha) moisture nutrition	Sxw*-oak fern (21.90) mesic mesotrophic	Sxw-devil's club (43.28) subhygric mesotrophic - permesotrophic	Sxw - pink spirea - oak fern (10.60) subhygric submesotrophic - mesotrophic	Sxw-oak fem (3.88) mesic mesotrophic	Sxw-devil's club (29.06) subhygric mesotrophic - permesotrophic	Sxw-oak fern/ devil's club (86.7) subhygric mesotrophic	Sxw-oak fem/ devil's club (36.6) subhygric mesotrophic
Geography: elevation (m) slope % aspect	686 0 n/a	0 n/a	0 n/a	670 0-5 SE	0-5 n/a	~670 n/a n/a	675 19 S
Soils: humus form humus depth (cm) texture % coarse fragments total depth (cm)	mor 5 n/a 0 >50	moder 8-10 silty loam 0 >50	mor 8-14 silty loam 0 >50	moder 5 silty loam 0 >50	moder 5-8 silty loam to silty clay loam 0	n/a	n/a
Soil protection: compaction displacement erosion mass wasting forest floor sensitivity rating	high medium nedium medium medium			high low high high medium		n/a	n/a
Site degradation (%) = block road landings bladed structure	13.0 2.0 8.0			13.7 1.8 3.9 8.0		9	9

Appendix B. Macroscopic and microscopic characteristics of selected ectomycorrhizae found on naturally regenerating and planted hybrid white spruce seedlings in mature forest, clearcut, and cut plus burned sites in the Aleza Lake Research Forest, Central Interior of British Columbia.



hyphae and hyaline apex; c) Amphinema with patchy yellow mantle and dark yellow rhizomorphs; d) Hebeloma with patchy white mantle and abundant loosely attached Ascomycetes (a-b) and basidiomycetes (c-g) ectomycorrhizae (bar is equal to 1 mm): a) Cenococcum with abundant emanating hyphae; b) E-strain with few emanating emanating hyphae; e) Inocybe opaque coloured smooth mantle; f) Russulaceae type 1 smooth mantle and robust tip; and g) Tomentella type 1 with warty appearance.



Amphinema inner mantle showing net synenchyma pattern as well as a clamped hypha in the bottom left corner (arrow); k) E-strain Hartig net; l) Cenococcum h) outer mantle of Cenococcum showing stellate pattern; i) outer mantle of Tomentella type 1 showing regular synenchyma pattern and mounding of cells; j) unclamped, septate (arrows), emanating hypha; and m) Thelephora rhizomorph (bar is equal to 10 µm). Appendix C: Checklist for ectomycorrhizae morphological data (adapted from Goodman et al. 1996).

Fungus:	Date:
Host:	Location:
Dissecting Mi	croscope:
Colour:	
Texture:	smooth/ finely grainy/ felty/ velvety/ warty/ woolly/ cottony/ stringy/ short spiny/ long spiny/ other
Lustre:	matte/ shiny/ reflective
Branching:	monopodial pinnate/ monopodial pyramidal/ dichotomous/ irregular/ coralloid/ tuberculate/ not branched/ other
Tip shape:	straight/ beaded/ club-shaped/ tortuous/ bent
Dimensions:	length of system:mm tip length:mm tip width:mm
Rhizomorphs: Notes:	no yes (attachment and abundance)

Compound Microscope:

Mantle:

- Outer: felt prosenchyma/ net prosenchyma/ net synenchyma/ interlocking irregular synenchyma/ non-interlocking irregular synenchyma/ regular synenchyma
- Inner: felt prosenchyma/ net prosenchyma/ net synenchyma/ interlocking irregular synenchyma/ non-interlocking irregular synenchyma/ regular synenchyma

Thickness: µm Cell Width: µm

Hartig net: yes no

Emanating Hyphae:

Type:	cystidia/ i	ndeterminant			
Width:	μm	Length:µm		Colour:	
Septa:	yes no	Clamps (location): n	o yes_		
Ornam	entation:	none/ crystalline/ verruc	ose/ glol	bular/ other	
Notes:					

Mycelial Strands:

Type: loose, undifferentiated/ smooth, undifferentiated/ slightly undifferentiated/ differentiated, random hyphae/ differentiated, central core/ highly differentiated

Hyphae: as per emanating hyphae

Appendix D. Ectomycorrhizae descriptions of naturally regenerating and planted hybrid white spruce seedlings growing in treated (clearcut, and cut plus burned) and untreated (mature) sites in the Aleza Lake Research Forest, Central Interior of British Columbia.

Ectomycorrhizae (macroscopic features)	Mantle and Hartig net	Emanating hyphae (EH)	Rhizomorph	Site*
Ascomycetes Cenococum geophilum: mostly single (1-4 mm long, 0.25-0.4 mm wide), some pinnate, finely grainy, black, robust, sclerotia sometimes present	outer mantle (OM)/ inner mantle (IM): net synenchyma stellar pattern, both spherical and elongated cells (3.5-5 μm wide), mantle 20-40 μm thick, Hartig net (HN) labyrinthic	EH: rare to frequent, 3.5-6 μ m wide, thick- walled (~1 μ m wide), sometimes verrucose (with ornaments to 0.5 μ m), dark brown to black, sometimes branching but usually straight, septate, no clamps seen	none observed	M-r, C-r, CB-pl, CB-pl
E-strain: single (1-3 mm long, 0.2 mm wide), smooth to pinnately branched, gray, yellow, or rusty brown, apex sometimes appearing uncolonized	OM: net prosenchyma, hyphae can be constricted at septa ($3-15 \mu m \times 5-35 \mu m$); IM: net synenchyma, patchy, typically large cells (to 14 μm wide, to $34 \mu m$ long), walls $0.5 - 1 \mu m$ wide; mantle not always covering tip, thin, 2 to 3 cell layers (12-22 μm) thick, HN labyrinthic	EH: often not seen, 5-10 μm wide, smooth to strongly verrucose, occasionally branched, hyaline to light tan to reddish-brown, no clamps seen	none observed	M-r, C-r, C-pl, CB-pl
<i>Mycelium radicis atrovirens (MR4)</i> : mostly single (1.5-4 mm long, 0.2 mm wide), finely grainy, thin to well developed mantle, dark brown, reddish brown, brown-black, grey- black or black, sometimes with a hyaline apex	OM: net prosenchyma to net synenchyma (1.5-9 μm wide to 20 μm long); IM: net synenchyma, mantle 10-15 μm thick, often incomplete but may be well colonized, inflated cells and constricted septa, septa sometimes lens-shaped, HN labyrinthic	EH: rare to abundant, 1.5-4 μm wide, smooth to finely verrucose, hyaline, reddish-brown, or grey-black, no clamps seen	none observed	M-r, C-r, C-pl, CB-pl
<i>Tuber</i> : single to pyramidal (1-3 mm long, 0.4 mm wide), finely grainy or bristle-like to short spiny, robust, pale yellow, sandy or yellow-brown colour to reddish-gold or rusty-brown	OM: variable, net prosenchyma connecting with cystidia, to interlocking irregular synenchyma (3-8 μm x 10-15 μm) sometimes non-interlocking or regular synenchyma; IM: net synenchyma (3-5 μm x 10-25 μm) to irregular interlocking, mantle 12-40 μm thick, HN labyrinthic	EH: frequent to abundant, tapering bristle-like cystidia (45-130 μm long), 1 μm at tip, 3-5 μm wide at base, enlarged basal cell at mantle edge, often lower basal septa plus one or more above, thick-walled, hyaline, no clamps seen, occasionally very large hyaline hyphae seen near root tips. <u>Variation</u> : occasionally with fusiform cells (~15 μm wide at base x 25 μm long), or short hyphae (~4 x 30 μm)	none observed	M-r, C-pl
ascomycete unknown: single (1 mm long) to pinnate (2 mm long, 0.4mm wide), smooth, finely grainy, or warty, robust, pale green, or reddish- brown	OM: non-interlocking irregular synenchyma to regular synenchyma (4-15 x 7-30 μm); IM: net synenchyma, mantle 12-23 μm thick, HN labyrinthic	EH: rare, 8-13 μm wide, walls 1.5-2 μm thick, strongly verrucose, branched, yellow-brown, no clamps seen	none observed	M-r

Basidiomycetes				
<i>Amphinema</i> : single (0.25 mm wide) to pinnate (7 mm long), tips only slightly enlarged, cottony, patchy yellow or white, few to many loosely associated EH	OM: felt prosenchyma; IM: net synenchyma (hyphae 2-3 μm wide), mantle 20-23 μm thick, variable, HN labyrinthic	EH: often very abundant, 1.5-5 μm wide, sometimes slightly thick-walled, usually finely verrucose, branched, anastomosis 'H', hyaline to pale yellow, large clamps at most septa	strands few to abundant, loose, undifferentiated, 20- 30 μm wide, white, cream, straw or yellow colour, some possibly differentiated (5 μm wide)	M-r, C-r, C-pl, CB-pl
<i>Hebeloma</i> : single (3 mm long) to pinnate (7 mm long, 0.2 mm wide), cottony, dark yellow-brown, pale yellow or white, often patchy.	OM: felt to net prosenchyma, elongated cells; IM: net synenchyma (1.5-3 μm wide), mantle 17-19 μm thick, HN labyrinthic	EH: often very abundant, 2-3 µm wide, smooth to finely verrucose, branched, hyaline to occasionally pale straw colour, round clamps at most septa, anastomosis 'H' most with clamps	occasional rhizo- morphs observed, appear undifferen- tiated hyphae similar to mantle EH	M-r, C-r, C-pl, CB-pl
Inocybe: single (1.5 mm long) to pinnate (6 mm long, 0.25 mm wide), smooth, milky-white to buff, somewhat robust	OM: net prosenchyma; IM: net synenchyma (1.5-2 μm wide), mantle 10-20 μm thick, HN labyrinthic	EH: rare to frequent, 1.5-2 μm wide, septa often close together (banded), hyaline, clamped	none observed	CB-pl
Laccaria: single (1-3 mm long, 0.2- 0.25 mm wide), smooth, occasionally robust, buff, cream colour to tan, often hyaline at tip	OM: net prosenchyma; IM: net synenchyma (2-4 μm wide), mantle 11-18 μm thick, septa possibly thicker and closer together, mantle with debris or deposits, HN labyrinthic	EH: frequent, determinant (100-180 μm long) as well as indeterminant hyphae, 2-5 μm wide, thin-walled, smooth, infrequently branched, hyaline, flattened clamps	none observed	M-r, C-pl, CB-pl
<i>Piloderma</i> : single to pinnate (3 mm long, 0.2 mm wide), finely grainy, woolly, hyaline to white or pale to bright yellow	OM: felt to net prosenchyma (1.5-3 μm wide) with needle-like crystals, numerous parallel hyphae on surface; IM: net synenchyma (1.5-3 μm wide), mantle 15-34 μm thick, HN labyrinthic	EH: very abundant, 1-3 μm wide, abundant crystals (sometimes needle-like (2-4 μm long) or round), hyaline to pale tan-yellow, anastomosis septate, 'H' type or short 'H' or almost contact type, no clamps seen	strands frequent, loose, undifferen- tiated, 18-30 μm wide or more, branching, white to dark yellow	M-r, C-r
Russulaceae 1: single to pinnate (1-3 mm long, 0.2-0.25 mm wide), smooth to cottony, pale to dark brown, to pale yellow-cream, robust	OM: net prosenchyma to irregular (interlocking) synenchyma to net synenchyma (1-12 x 3-25 μm), thick walls; IM: irregular to net synenchyma (2-3 μm wide), mantle 15-20 μm thick, HN labyrinthic	EH: rare to abundant, 2-4 μm wide, thick- walled (to 1 μm), heavily verrucose (large encrustations), hyaline, hyphal attachment often towards base of mycorrhizae, anastomosis 'H', large round clamps at most septa	none observed	M-r, C-r, C-pl, CB-pl
Russulaceae 2: single (1-3 mm long, 0.2 mm wide), smooth, greyish- brown to reddish-brown, robust	OM: net prosenchyma to non-interlocking irregular synenchyma to regular synenchyma (2- 7 x 7-13 μm), IM: net synenchyma (2-3 μm wide), mantle 17-20 μm thick, HN labyrinthic	EH: rare, fusiform cystidia, ~2 wide x 6 μm long, as well as indeterminant, lightly verrucose, 2-3 μm wide, hyaline hyphae, clamped	none observed	M-r, C-pl, CB-pl

M-r, C-r, C-pl, CB-pl	Cr	M.r. C-r, CB-pl, CB-pl	M-r, C-pl, CB-pl	C-r	M-r, CB-pl	M-r, C-pl, CB-pl
strands not always seen, loose to smooth, undifferentiated, 18- 30 µm wide or greater, hyaline to tan	none observed	not always present, medium to dark brown, strands may have 'rope-like', knotted appearance, branching, compact, with hyphae ~2-5 μm wide, differentiated	strands infrequent, smooth, undifferentia- ted, 16-30 µm wide, dark brown	none observed	none observed	none observed
EH: rare to very abundant, bristle (common) as well as whip-like (infrequent) cystidia (60-400 μ m long), sometimes tapering, 1.5-5 μ m wide, thin (infrequent) to thick-walled (common), sometimes retraction septa, tan to hyaline, basal clamps, usually thinner walled below clamp	EH: few, 3-5 μm wide, some short mantle hyphae or slightly longer (to 50 μm), hyaline, smooth, no clamps seen	EH: rare to frequent, several types may be seen: 1) most abundant type, $4-7 \mu m$ wide, thick- walled, branched, dark brown, small clamps as well as $2-5 \mu m$ wide, thin-walled, unbranched, tan, no clamps; 2) pale to medium brown tapering cystidia (to 120 μm long, $5 \mu m$ wide at base, 1.5 μm at tip), thick-walled, mostly single but sometimes branching, some with basal clamp; 3) large dark brown EH (6 μm wide), thick walls ($\sim 1 \mu m$ wide), often present but attachment not always seen, no clamps seen	EH: abundant, 2.5-3.5 μm wide, thin-walled, branched, yellow-brown, round clamps	EH: few, 3-4 µm wide, some short (30-70 µm long) to longer, rounded hyphal tips, smooth, septate, brown, no clamps seen	EH: frequent, 1.5-3 μm wide, thin-walled, finely verrucose, olive-green, clamped	EH: rare, 1.5-2 μm wide, thin-walled, hyaline, branched, no clamps seen
OM: felt prosenchyma to net prosenchyma or interlocking synenchyma (2-10 x 12-30 μm); IM: net to non-interlocking irregular synenchyma (2-5 μm wide), mantle 14-23 μm thick, HN labyrinthic	OM: net synenchyma (or towards net prosenchyma), rather large, branching mantle cells (3-7 μm wide), some elongated but not as obvious as lacticifers, septate; 1M: net synenchyma, HN labyrinthic	OM: non-interlocking irregular synenchyma to regular synenchyma (4-11 x 9-26 μm, up to 40 μm on longest angle), sometimes forming clusters of raised roundish cells; IM: net synenchyma (2-4 μm wide), mantle 20-30 μm thick, HN labyrinthic	OM: non-interlocking irregular synenchyma to regular synenchyma (3-10 x 7-15 μm); IM: net synenchyma (3-5 μm wide), mantle 13-20 μm thick, black, HN labyrinthic	OM: variable, ret prosenchyma to regular isodiametric or angular synenchyma with radiating large cells (can be interlocking irregular synenchyma), IM: net synenchyma, HN labyrinthic	OM: net proserch)/ma to net synenchyma (2-3 μm wide), IM: net synenchyma (2-3 μm wide), mantle 10-15 μm thick, HN labyrinthic	OM: net prosenchyma, IM: net synenchyma (1.5-2 μm), 10 μm thick, HN labyrinthic
Thelephora: single to pinnate (1-5 mm long, 0.25 mm wide), smooth to long spiny, frequently robust, beige or fawn or grey to darker brown or rusty brown	Thelephoraceae-like: buff, cream, whitish-grey to slight yellowish, robust, dull, rough texture, possibly crystalline deposits, no EH or rhizomorphs obvious	<i>Tomentella</i> 1: single (1-5 mm long) to pinnate (6-10 mm long, 0.4-0.6 mm wide), sometimes densely clustered, grainy to warty, dark brown to black, some with silvery-fawn colour on darker brown, robust	<i>Tomentella</i> 2: single (2-7 mm long) to pinnate (11 mm long, 0.3-0.4 mm wide), finely grainy, red-brown, robust	Tomentella 3: single to pinnate, sometimes grainy surface charcoal to grey-greenish black, to dark black- brown	non-rhizomorphic olive-green: single (1-2 mm long, 0.25 mm wide), finely grainy, robust, olive-green, sometimes mottled black apically	non-rhizomorphic thin mantled: single (1-2 mm long, 0.2 mm wide) to pinnate, smooth, tan/yellow to brown

ion-rhizomorphic unclamped: single	OM: felt to net prosenchyma, IM: net	EH: rare, short mantle cells (15-60 um long), or	none observed	C-pl,
2-4 mm long, 0.2 mm wide), smooth	synenchyma (2-4 µm wide), mantle 20-26 µm	frequent, whip-like, bristle-like cystidia (60-		CB-pl
o finely grainy, reddish-brown at pase to vellow-brown at tip, as well	thick, HN labyrinthic	100 µm long), 2.5-5 µm wide, occasionally		
is patchy yellow or white		hyphae (2-4 µm wide), no clamps seen		
101-rhizomorphic white: single (2-4	OM: net prosenchyma, IM: net synenchyma (3-6	EH: frequent, 3-5 μm wide, thin-walled,	none observed	M-r,
nm long, 0.2 mm wide), smooth,	μm wide), mantle 13-15 μm thick, HN	restricted septa, branched, hyaline, flattened		C-pl,
ellow-brown to patchy-white	labyrinthic	clamps		CB-pl
hizomorphic brown unclamped:	OM: felt to net prosenchyma; IM: net	EH: frequent, 1-2.5 µm wide, thin-walled,	strands rare to	M-r,
ingle (5 mm long, 0.25 mm wide),	synenchyma (2-2.5 µm wide), mantle 10-20 µm	sometimes finely verrucose, rarely branched,	frequent, loose to	C-pl,
inely grainy, black-brown or pale	thick, sometimes mottled black appearance, HN	hyaline, no clamps seen	smooth, undifferentia-	CB-pl
olive-green	labyrinthic		ted, 8-20 µm wide,	
			brown-black	
hizomorphic orange unclamped:	OM: felt to net prosenchyma, IM: net	EH: frequent to abundant, tapering bristle-like	strands rare to	M-r,
ingle (2-3 mm long, 0.3 mm wide),	synenchyma (2-3 μm wide), mantle 15-22 μm	cystidia (20-70 µm long), also indeterminant	frequent, loose, to	CB-pl
inely grainy to short spiny, robust,	thick, HN labyrinthic	hyphae, 2-2.5 µm wide, tan colour, no clamps	smooth, undifferen-	
older tips patchy rusty orange/brown,		seen	tiated, 13-15 µm	
sclerotia			wide, light orange-	
			brown	
hizomorphic white: single (2-4 mm	OM: net prosenchyma (2-5 µm wide), IM: net	EH: frequent, 2-5 µm wide, thin-walled,	strands abundant,	M-r,
ong, 0.2 mm wide), woolly to	synenchyma (3-5 μm wide), mantle 10-20 μm	restricted septa, branched, hyaline, anastomoses	loose, undifferentia-	C-pl
cottony, robust, patchy to uniformly	thick, HN labyrinthic	present, clamped	ted, 20-30 µm wide,	
white			white	
*r=regenerating, pl=planted, M=matur	re forest site, C=clearcut site, CB=cut plus burned	site		

Appendix E. An example of calculations for richness (Margalef), evenness (Shannon) and composite (Shannon and Simpson) index measures using ectomycorrhizae morphological abundance data for seedlings growing in mature site 1 in the Aleza Lake Research Forest, Central Interior of British Columbia.

ECM fungus*				Seedl	ing			
	1	2	3	4	5	6	7	8
E-strain				0.011		0.010		
Cenococcum				0.315	0.167		0.144	0.098
MRA			0.106	0.054				
Tuber						0.016		
Ascomycete unknown	0.053							
Amphinema			0.137			0.021		0.063
Hebeloma			0.323	0.109		0.021	0.326	0.126
Inocybe								
Laccaria								0.098
Piloderma	0.404		0.118		0.183		0.209	
Russulaceae 1	0.388	0.030		0.250	0.478	0.575	0.112	0.017
Russulaceae 2						0.135		
Thelephora		0.970				0.135		0.092
Thelephoraceae-like								
Tomentella 1	0.085		0.273	0.054		0.021	0.048	0.011
Tomentella 2								0.167
Tomentella 3								
Non-rhizomorphic olive-green	0.027		0.043		0.028			
Non-rhizomorphic thin mantled				0.076	0.133	0.026	0.086	0.115
Non-rhizomorphic unclamped								
Non-rhizomophic white						0.041	0.005	
Rhizomorphic brown unclamped					0.011			
Rhizomorphic orange unclamped								
Rhizomorphic white	0.043			0.130			0.070	0.213
Total proportional abundance	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Number of tips (n) [†]	188	199	161	92	180	193	187	174
Number of species (S)	6	2	6	8	6	10	8	10
Margalef‡	0.955	0.189	0.984	1.548	0.963	1.710	1.338	1.744
Shannon§	1.330	0.135	1.617	1.779	1.381	1.438	1.787	2.108
Simpson	0.673	0.058	0.775	0.797	0.692	0.629	0.802	0.866
Shannon evenness#	0.742	0.195	0.903	0.856	0.771	0.624	0.859	0.915

*All types in this study are listed although they may not all be found on seedlings in this site.

†Lightly colonized tips were excluded from the original sample of ~200 tips assessed per seedling.

Margalef index = (S-1)/ln n, n being the total number of identified mycorrhizal tips.

 $Shannon = -\sum p_i \ln p_i$, p being the proportional abundance of each morphotype/n, or the number in each cell. $Simpson = 1 - \sum p_i^2$.

#Shannon evenness = Shannon/ In S.

Appendix F. Ectomycorrhizae morphotypes found on naturally regenerating (r) and planted (pl) hybrid white spruce seedlings in treated (clearcut, and cut plus burned) and mature sites in the Aleza Lake Research Forest. Table shows known or suspected genera or species from comparisons made with the published literature.

Morphotype	Code	Suspected Genera/species	Reference			
Amphinema spp.	9	Amphinema byssoides (Pers.:Fr.) Erikss.	Agerer 1987-1998 (plate 23); Ingleby <i>et al.</i> 1990; Danielson 1991; Goodman <i>et al.</i> 1996 (CDE 6),			
		Amphinema-like	Massicotte et al. 1998'. Hagerman et al. 1999 (OUC 020).			
Cenococcum spp.	2	Cenococcum geophilum Fr.	Agerer 1987-1998 (plate 11);			
			Ingleby et al. 1990; Danielson 1991;			
			Goodman et al. 1996 (CDE 10);			
		Cenococcum	Visser et al. 1998.			
			Hagerman et al. 1999 (OUC 030);			
			Massicotte et al. 1998.			
E-strain	1	Humaria hemisphaerica (Wigg.:Fr) Fuckel	Ingleby et al. 1990.			
		E-strain	Danielson 1982; Visser et al. 1998;			
			Massicotte et al. 1998.			
Hebeloma	I	Hebeloma mesophaeum (Pers.) Quél	Ingleby et al. 1990; Visser et al. 1998.			
		Hebeloma-like	Danielson 1991; Massicotte et al.			
			1998; Hagerman et al. 1999 (OUC			
			080).			
Inocybe	H	Inocybe petiginosa (Fr.:Fr.) Gillet	Ingleby et al. 1990.			
		Inocybe appendiculata Kühn.	Beenken et al. in Agerer et al. 1996-			
			98 ² ; Agerer 1987-1998 (plate 94).			
Laccaria	E	Laccaria proxima (Boud.) Pat.	Ingleby et al. 1990.			
		Gomphidius glutinosus (Schaeff.: Fr.) Fr.	Agerer 1987-1998 (plate 58).			
Mycelium radicis	3	MRA Melin	Massicotte et al. 1998; Visser et al.			
atrovirens		Type ITE.3	1998.			
		Piceirhiza bicolorata	Ingleby et al. 1990.			
			Agerer 1987-1998 (plate 73).			
Piloderma	Α	Piloderma byssinum (Karst.) Jül.	Visser et al. 1998.			
		Piloderma croceum	Agerer 1987-1998 (plate 62).			
		Piloderma-like	Hagerman et al. 1999 (OUC 200).			
Russulaceae 1	F	Piceirhiza gelatinosa	Agerer 1987-1998 (plates 30).			
		Piceirhiza guttata	Agerer 1987-1998 (plate 32).			
		Russula xerampelina	Agerer 1987-1998 (plate 2).			
Deres 1 and 2	C	Russula spp. Type 2	Visser et al. 1998.			
Russulaceae 2	G		Include (1000 Devictor 1001			
Inelephora	8	Thelephora terrestris (Enth.) Fr.	Ingleby <i>et al.</i> 1990; Danielson 1991.			
		Thelephora like	Agerer 1987-1998 (plate 48).			
		Lactarius datarrimus Groger	A gener 1087-1008 (plate 3)			
Thelephoraceae	P	n/a	Agerer 1987-1998 (plate 5).			
Tomentella 1	6	Type ITE 5	Ingleby at al 1000. Viscon at al			
Tomentena 1	0	Type IIL.5	1998.			
		Piceirhiza nigra	Agerer 1987-1998 (plate 19).			
		Tomentella-like	Goodman et al. 1996 (CDE 2).			
Tomentella 2	7	n/a				
Tomentella 3	0	n/a				
Tuber	4	Tuber sp.	Ingleby 1990; Massicotte et al. 1998; Visser et al. 1998.			
		Tuber puberulum Berk.	Agerer 1987-1998 (plate 22).			
olive-green	J	n/a				
rhizomorph, brown	D	n/a				
rhizomorph, gold	C	n/a				

rhizomorph, white	В	Cortinarius obtusus Fr.	Agerer 1987-1998 (plate 12).
unclamped, thin mantle	L	n/a	
unclamped, yellow	M	n/a	
unknown ascomycete	5	Genea verrucosa Vitt.	Jakucs et al. in Agerer et al. ³ 1996- 98; Agerer 1987-1998 (plate 120).

¹Massicotte, H.B., Tackaberry, L.E., Ingham, E.R., and Thies, W.G. 1998. Ectomycorrhizae establishment on Douglas-fir seedlings following chloropicrin treatment to control laminated-root rot disease: assessment 4 and 5 years after outplanting. Applied Soil Ecology 10: 117-125. ²Beenken, L., Agerer, R. and Bahnweg, G. 1996. *Inocybe appendiculata* Kühn + *Picea abies* (L.) Karst. *In*

white rhizomorph-like

K n/a

²Beenken, L., Agerer, R. and Bahnweg, G. 1996. *Inocybe appendiculata* Kühn + *Picea abies* (L.) Karst. *In* Descriptions of ectomycorrhizae. *Edited by* R. Agerer, R.M. Danielson, S. Egli, K. Ingleby, D. Luoma, and R. Treu. Einhorn-Verlag, Schwäbisch Gmünd, Germany. pp. 35-40.

³ Jakucs, E., Bratek, Z., and Agerer, R. 1998. *Genea verrucosa* Vitt + *Quercus* spp. *In* Descriptions of ectomycorrhizae. *Edited by* R. Agerer, R.M. Danielson, S. Egli, K. Ingleby, D. Luoma, and R. Treu. Einhorn-Verlag, Schwäbisch Gmünd, Germany. pp. 19-23. Plant hosts for all other morphotypes are reported to be *Picea* spp.

the Aleza Lake Re-	search Forest, Central Interio	r of British Columbia.	monty occurring ectomycorr	IIIZAC associated with hy u	in write spruce growing in
Morphotype	Clearcut-planted	Mature-regenerating	Mature-regenerating	Mature-regenerating	Clearcut-regenerating
	Cut plus burned-planted p-value†	Clearcut-planted p-value‡	Cut plus burned-planted p-value‡	Clearcut-regenerating p-value§	Clearcut-planted p-value:
Cenococcum	4.06 (0.94)	5.55 (1.65)	5.55 (1.65)	5.55 (1.65)	1.59 (0.64)
	1.13 (0.52)	4.06 (0.94)	1.13 (0.52)	1.59 (0.64)	4.06 (0.94)
	0.002	0.758	0.006	0.072	0.045
E-strain	6.26 (1.30)	0.11 (0.07)	0.11 (0.07)	0.11 (0.07)	14.11 (7.01)
	12.73 (3.37)	6.26 (1.30)	12.73 (3.37)	14.11 (7.01)	6.26 (1.30)
	0.333	0.001	0.001	0.013	0.411
MRA	26.16 (2.78)	4.34 (2.52)	4.34 (2.52)	4.34 (2.52)	11.22 (3.59)
	23.27 (4.13)	26.16 (2.78)	23.27 (4.13)	11.22 (3.59)	26.16 (2.78)
	0.242	0.001	0.001	0.075	0.001
Amphinema	17.69 (4.70)	2.01 (0.87)	2.01 (0.87)	2.01 (0.87)	14.66 (6.41)
	13.89 (2.83)	17.69 (4.70)	13.89 (2.83)	14.66 (6.41)	17.69 (4.70)
	0.559	0.006	0.004	0.089	0.420
Hebeloma	6.19 (1.46)	7.38 (2.37)	7.38 (2.37)	7.38 (2.37)	4.72 (2.62)
	14.34 (2.30)	6.19 (1.46)	14.34 (2.30)	4.72 (2.62)	6.19 (1.46)
	0.005	0.638	0.057	0.122	0.160
Russulaceae 1	1.02 (0.56)	35.38 (6.44)	35.38 (6.44)	35.38 (6.44)	3.79 (3.27)
	5.09 (1.71)	1.02 (0.56)	5.09 (1.71)	3.79 (3.27)	1.02 (0.56)
	0.008	0.001	0.001	0.001	0.451
Thelephora	2.68 (1.36)	8.31 (5.95)	8.31 (5.95)	8.31 (5.95)	34.85 (6.81)
	4.02 (1.69)	2.68 (1.36)	4.02 (1.69)	34.85 (6.81)	2.68 (1.36)
	0.431	0.200	0.431	0.006	0.001
lightly colonized	25.79 (2.94)	11.04 (3.05)	11.04 (3.05)	11.04 (3.05)	7.92 (1.78)
	19.39 (2.13)	25.79 (2.94)	19.39 (2.13)	7.92 (1.78)	25.79 (2.94)
	0.106	0.001	0.006	0.020	0.001
()	110 0-v jo motion como internet	and in hold and and an house	in al anot data land	more a in anomatican a more	hoting chindrand Moone

Appendix G. Statistical summation (one-way ANOVA*) for treatment (mature, clearcut and cut plus burned) and seedling effect (naturally regenerating (n=16)

*significant values (Bonferroni correction of $\alpha=0.01$) are in bold and are based on transformed data (arcsin \sqrt{p} , where p is proportional morphotype abundance). Means are presented as non-transformed data. P-values <0.0015 have been designated 0.001.

†df (1, 54). ‡df (1, 42). §df (1, 30).

Appendix H. An example of a Phi index calculation for ectomycorrhizae molecular data (PCR-RFLP) after PHYLIP analysis.

Tip	1	2	3	4	5	6	7	8	9	10
1	0.000	0.086	0.086	0.039	0.644	0.644	0.600	0.545	0.528	0.590
2	0.086	0.000	0.048	0.000	0.609	0.609	0.630	0.662	0.581	0.492
3	0.086	0.048	0.000	0.048	0.758	0.758	0.630	0.662	0.644	0.556
4	0.039	0.000	0.048	0.000	0.609	0.609	0.630	0.662	0.581	0.492
5	0.644	0.609	0.758	0.609	0.000	0.000	0.630	0.719	0.481	0.481
6	0.644	0.609	0.758	0.609	0.000	0.000	0.630	0.867	0.630	0.481
7	0.600	0.630	0.630	0.630	0.630	0.630	0.000	0.300	0.073	0.202
8	0.545	0.662	0.662	0.662	0.719	0.867	0.300	0.000	0.257	0.321
9	0.528	0.581	0.644	0.581	0.481	0.630	0.073	0.257	0.000	0.270
10	0.590	0.492	0.556	0.492	0.481	0.481	0.202	0.321	0.270	0.000
Α	2.131	2.166	2.718	2.160	3.108	3.508	2.479	3.136	2.142	1.821
В	0.237	0.241	0.302	0.240	0.345	0.390	0.275	0.348	0.238	0.202
С	0.282									

n = 10

D = cell matrix value

 $A = \Sigma (D^2)$

 $\mathbf{B} = \mathbf{A} / (\mathbf{n} - 1)$

 $C = \Sigma B / n$

Protocol

- Databases from RFLP patterns were created for each treatment or morphotype using all tips that were successfully amplified and digested. The software packages used included RFLP analysis application RFLPscan Plus, Version 3.0, (©1990-1996 Scanalytics) and RFLPscan Database Versions 2.1 and 3.0 (©1990-1996 Scanalytics).
- Pairs of tips were matched for shared and unique bands at a 2% tolerance level within gels and a 6% variation level between gels to compensate for gel differences. The modified Dice's index (1-Dice's index) was used (sum (polymorphic bands) / (shared bands + total bands) / 3 restriction enzymes) to convert the resulting matrix of similarity values to distances).
- Clustal analysis using the unweighted pair-group method with arithmetic means (UPGMA) of the distance matrix was done using the Neighbor-Joining/UPGMA module in PHYLIP (Phylogeny Inference Package) Version 3.5c (©1986-1995 Joseph Felsenstein).
- 4. Each cell (D) in the matrix was squared and the columns were added (A), then divided by the sample size (n) 1. The resulting value (B) for each column (tip) was summed and the final value was divided by n. Databases of various sizes can be compared as the sample size is taken into account in the calculation. Higher phi values represent a more genetically diverse site: smaller distances represent more closely related organisms.

Appendix I. Sample phenogram of MRA



Genotypes 1 and 2 are indicated by clusters contained within the lines. All other clusters were excluded.