The impacts of broadcast burning after clear-cutting on the diversity of ectomycorrhizal fungi associated with hybrid spruce seedlings in central British Columbia[1]

Karen Mah, Linda E. Tackaberry, Keith N. Egger, and Hugues B. Massicotte

Abstract: Morphological and molecular (polymerase chain reaction – restriction fragment length polymorphism) methods were used to assess ectomycorrhiza (ECM) diversity in naturally regenerating and planted *Picea engelmannii* Parry ex Engelm. × *Picea glauca* (Moench) Voss seedlings in two recently clear-cut sites, two clear-cut and broadcast burned sites, and two mature forests (>100 years) in central British Columbia. Based on 24 characterized ECM, burning did not affect overall diversity (Shannon, Simpson, Shannon evenness, and Margalef indices). However, the occurrence and relative abundance of some ECM morphotypes varied significantly. *Hebeloma* and a Russulaceae type 1 were more abundant and *Cenococcum* was less abundant in planted seedlings from cut–burned sites compared with those from clear-cut sites. E-strain, MRA, and *Amphinema* were more abundant in planted seedlings from both cut–burned and clear-cut sites compared with naturally regenerating seedlings from mature sites. ECM diversity of regenerating seedlings was significantly greater in mature forests compared with clear-cut sites and was greater in planted seedlings than naturally regenerating seedlings in clear-cut sites. Molecular analysis of the internal transcribed spacer region of the nuclear-encoded ribosomal RNA gene repeat showed no significant differences among treatments or seedling type. Twenty-two genotypes were identified from eight common morphotypes: *Cenococcum* (one), *Thelephora* (two), E-strain, *Tuber, Hebeloma,* and Russulaceae type 1 (three each), MRA (four), and *Amphinema* (six). *Hebeloma* genotypes matched three for *Amphinema*; genotypes from unidentified lightly colonized tips matched those for E-strain, MRA, and *Amphinema–Hebeloma*.

Résumé : Des méthodes morphologique et moléculaire (réaction en chaîne de la polymérase – polymorphisme de la longueur des fragments de restriction) ont été utilisées pour évaluer la diversité ectomycorhizienne (ECM) sur des semis d'épinette hybride (*Picea engelmannii* Parry ex Engelm. × *Picea glauca* (Moench) Voss) naturellement regénérés ou plantés dans deux sites après une coupe à blanc récente, dans deux sites après une coupe à blanc et un brûlage dirigé, et deux sites dans une forêt mature (>100 ans) dans le centre de la Colombie-Britannique. En se basant sur les 24 morphotypes ectomycorhiziens décrits, le brûlage n'a pas affecté la diversité totale (index de Shannon, Simpson, Shannon égalité, Margalef). Toutefois, la présence et l'abondance relative de quelques morphotypes variaient significativement. L'*Hebeloma* et le Russulacées type 1 étaient les plus abondants, et le *Cenococcum* le moins abondant sur les semis plantés dans les sites coupés à blanc et brûlés comparés aux semis des sites coupés à blanc. E-strain, MRA et *Amphinema* étaient plus abondants sur les semis plantés (sites coupés à blanc et brûlés et sites coupés à blanc) que sur les semis regénérés naturellement (sites matures). La diversité ectomycorhizienne sur les semis naturellement regénérés était significativement plus grande dans les sites matures comparée aux sites coupés à blanc, et plus grande aussi pour les semis plantés comparés aux semis naturellement regénérés dans les sites coupés à blanc. L'analyse moléculaire de la région ITS située sur l'ADNr n'a pas révélé de différence significative entre les traitements ou entre les types de semis. Vingt-deux génotypes ont été caractérisés pour les huit morphotypes les plus communs: un pour *Cenococcum*, deux pour *Thelephora*, trois pour chacun des morphotypes E-strain, *Tuber, Hebeloma*, et le Russulacées type 1, quatre pour le MRA et six pour *Amphinema*. Trois génotypes d'*Hebeloma* étaient identiques à trois génotypes d'*Amphinema*; tous les génotypes de champignons sur les racines mycorhiziennes faiblement colonisées étaient identiques à des génotypes du E-strain, du MRA et d'*Amphinema–Hebeloma*.

Introduction

In British Columbia, broadcast burning of clear-cut sites has been used as a method of site preparation prior to outplanting seedlings (Hawkes et al. 1990). Impacts of fire on soil physical and chemical properties vary according to fire intensity and severity (Wells et al. 1979; Agee 1993); however, little is known concerning fire effects on rhizosphere

Received April 17, 2000. Accepted November 14, 2000. Published on the NRC Research Press website on February 2, 2001.

K. Mah, L.E. Tackaberry, K.N. Egger, and H.B. Massicotte.[2] Faculty of Natural Resources and Environmental Studies, College of Science and Management, University of Northern British Columbia, 3333 University Way, Prince George, BC V2N 4Z9, Canada.

[1]Funding assistance provided by Forest Renewal British Columbia does not imply endorsement of any statements or information contained herein.

[2]Corresponding author. e-mail: hugues@unbc.ca

 DOI: 10.1139/cjfr-31-2-224

components such as ectomycorrhizae (ECM). Most studies that have examined ECM following burning and (or) clear-cutting found diminished mycorrhizal development in clear-cut and burned sites (Harvey et al. 1980; Perry et al. 1982; Schoenberger and Perry 1982; Parke et al. 1984). In contrast, Pilz and Perry (1984) reported increases in some ECM types. Comparisons and interpretations of these studies are difficult because of differences in methods of assessment, hosts examined, and fire intensity and severity.

The relationship between mycorrhizal diversity and disturbance is mostly undescribed (Staddon et al. 1996). It has been proposed that seedlings growing in disturbed soil environments could benefit from access to a diversity of fungi, since the most effective fungal symbionts may be favoured, providing a buffering capacity that would allow seedlings to adapt to environmental changes (Perry et al. 1987; Simard et al. 1997). In central British Columbia, ECM fungi associate with important commercial tree species including spruce and pine. Hybrid spruce (*Picea engelmannii* Parry ex Engelm. × *Picea glauca* (Moench) Voss) is commonly used in reforestation, yet we know of no studies that have examined ECM diversity of seedlings outplanted on sites that have been broadcast burned following clear-cutting. Fungal diversity studies following burning may be hindered by the complexity of the soil environment, difficulty in identifying fungal symbionts and the enormous variability of fire, both between fire sites as well as spatially within a fire area.

The resolution of morphological identification has improved because of the publication of standard descriptions (Ingleby et al. 1990; Agerer 1987–1998; Agerer et al. 1996–1998; Goodman et al. 1996), but characterization requires considerable skill and can be affected by phenotypic variation of ECM on different hosts and under changing environmental conditions (Egger 1995). In contrast, molecular characterization of ECM may be easier to learn, requires less time to process ECM tips, and is not affected by environmental variation (Egger 1995; Gardes and Bruns 1996). The internal transcribed spacer (ITS) region of the nuclear-encoded ribosomal RNA gene repeat (rDNA) has been widely used for molecular characterization and is suitable for identification to the species or species group for most fungi (White et al. 1990; Gardes and Bruns 1996).

This study was established to determine the effect of broadcast burning following clear-cutting on the diversity of ECM on planted and naturally regenerating (referred to as "regenerating") hybrid spruce seedlings growing in mature, clear-cut, and clear-cut plus broadcast burned (referred to as "cut–burned") sites in central British Columbia. The study explores differences in ECM abundance and diversity between planted and regenerating seedlings and compares molecular (polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP)) results with morphological assessments. The study examines the Phi index as a measure of diversity for molecular analyses in addition to traditional diversity indices.

Materials and methods

Study area

The study sites were located in the Aleza Lake Research Forest east of Prince George, in the central interior of British Columbia (54°07′N, 122°04′W, elevation 600–750 m (Jull 1992)). The area is in the Sub-Boreal Spruce (SBS) biogeoclimatic zone, Willow wet cool variant (wk1) (Delong et al. 1996) and has a continental climate with a mean annual precipitation of 930 mm and temperatures averaging between –12°C and 15°C (Jull 1992). The forests are uneven aged with multistoried canopies. Hybrid spruce and subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) are the climax species (Meidinger et al. 1991). Soils are Brunisolic Gray Luvisols and Gray Luvisols formed on loam to clay glaciolacustrine deposits (DeLong et al. 1996); humus forms are mor and moder types (Meidinger et al. 1991).

Site descriptions

Four treated sites (two clear-cut and two cut–burned) and two adjacent mature forest sites (approximately 100 and 200 years old) were selected. Northwood Pulp and Timber Ltd. held the license for the clear-cut and cut–burned sites. Two sites were clear-cut in the winter of 1992 and planted in the spring of 1993–1995; two additional sites were clear-cut in the winter of 1993–1994, broadcast burned in the fall of 1994, and planted in the spring of 1995. In all plots the planted seedlings were 2-year-old nursery stock, hybrid spruce. The forest floors were mor and moder humus types with average LFH depth of 2.6 cm, pH 4.7, and C/N ratio of 28.68. The mineral soils had an average pH 4.2, a C/N ratio of 15.18, and were silt-loam to clay-loam. Fire intensity and severity was not measured but was estimated as moderate (R. Jansen, personal communication).

Seedling sampling

In June 1997, a 50 × 50 m block was established in each site resulting in two clear-cut, two cut–burned, and two mature stand replicates. Blocks were at least 10 m inside site boundaries to reduce edge effects. A total of 88 seedlings were randomly selected: 14 planted seedlings from each clear-cut and cut–burned site and eight regenerating seedlings from each mature site. Half of the seedlings were harvested in late June and the remaining in late August. In August, eight additional regenerating seedlings were harvested from each clear-cut site; almost no regenerating spruce were found on the cut–burned sites, although seedlings were growing on landings and roads leading to these areas. This precluded sampling of regenerating seedlings on cut–burned sites. Seedlings were harvested with the soil, bagged, transported in plant pots to avoid root disturbance, and stored at 5°C until ECM characterization.

The age of each seedling was estimated by counting stem bud scars as well as growth rings on cross-sections cut above the root collar (using a dissecting microscope, Olympus SZ-30). Regenerating seedlings from clear-cut sites were aged by bud scars only. The age of the mature stands was estimated by coring and counting growth rings of the five largest diameter hybrid spruce and subalpine fir trees at each site.

Ectomycorrhiza characterization

Root systems were soaked in cold water and carefully washed free of soil and organic debris. Entire root systems of regenerating seedlings were sampled; only lateral and egressed roots growing from soil plugs were sampled for planted seedlings. Roots were floated in water over a grid of 2-cm^2 cells and segments 2 cm long were randomly sampled until 200 tips were selected. If a cell contained more than 20 root tips, a subcell (1 cm^2 in size) was randomly sampled. Only turgid root tips with intact meristems were selected. An unbranched tip was considered as one mycorrhiza. Initial ECM characterization was made using the dissecting microscope. Subsequently, root squashes were prepared and viewed using a compound microscope (Olympus CH-2, 100–1000×). ECM features such as fungal mantle, presence of rhizomorphs, emanating hyphae and other distinguishing characteristics were docu-

mented (detailed descriptions of morphotypes are in Mah (1999) or can be obtained from the corresponding author).

Root tips were categorized as mycorrhizal, non-mycorrhizal, or lightly colonized – unknown (unidentifiable because of weakly developed ECM features). Morphological descriptions were made with reference to Agerer (1987–1998), Ingleby et al. (1990), and Goodman et al. (1996). Morphotypes not readily identified to genus or species were given type names based on conspicuous features. For each seedling, the number of ECM morphotypes and their relative abundance were calculated. Site occurrence and the frequency of occurrence were recorded for each morphotype; morphotypes not found on a seedling were assigned a value of 0. Non-mycorrhizal and lightly colonized tips were combined when calculating overall ECM abundance and percent colonization.

DNA extraction, amplification, restriction endonuclease digestion, and gel electrophoresis

A proportional number of tips (10% of the ECM abundance value) was collected for each morphotype, placed in 1-mL microcentrifuge tubes, and stored at –20°C for comparative molecular analysis. For example, if one morphotype represented 120 of 200 tips on a seedling, 12 tips (10%) were selected. For types having only 1–10 tips, one tip was saved for molecular characterization. In total, approximately 1800 ECM tips were selected for molecular analysis. Extraction of fungal DNA used a hexadecyltrimethylammonium bromide (CTAB) extraction protocol based on the miniprep protocol of Zolan and Pukkila (1986). Root tips were stored at –80°C for approximately 10–15 min, then crushed with micropestles (Mandel Scientific) in 350 mL of 2× CTAB buffer (1.4 M NaCl, 100 mM Tris-HCl (pH 8.0), 20 mM ethylenediaminetetraacetic acid (EDTA) (pH 8.0), 2% CTAB, 0.2% β-mercaptoethanol), and incubated in a 60°C water bath for approximately 45 min, followed by addition of 350 mL of chloroform – isoamyl alcohol (24:1). The mixture was vortexed briefly, then centrifuged at 12 000 × *g* for 10 min at room temperature. The aqueous phase was transferred to a new centrifuge tube, and the DNA was precipitated by the addition of an equal volume of cold isopropanol. The solution was gently mixed, then precipitated DNA was collected by centrifugation at 12 000 × *g* for 10 min at room temperature. The pellet was washed twice with 70% ice-cold ethanol, the excess ethanol removed, and the DNA pellet resuspended in 50 mL of 8 mM NaOH.

A Perkin-Elmer Cetus thermocycler was used for PCR amplification (Mullis and Faloona 1987) at two settings. For robust, well-colonized roots: denaturation at 94°C for 45 s, annealing at 48°C for 45 s, and extension at 72°C for 130 s increasing 1 s per cycle for 35 cycles. For lightly colonized roots or roots which were weakly amplified using the first protocol, cycles were extended to 40, and annealing temperature was decreased to 46°C to reduce annealing stringency. Pure UltraTherm™ DNA polymerase (BIO/CAN Scientific) was used for lightly colonized tips; UltraTherm diluted 1:1 with PCR buffer provided by the manufacturer was used for robust tips. The primers used were ITS1 (White et al. 1990) and NL6Bmun (Egger 1995). For digestion of PCR products, three restriction endonucleases were used: *Alu*I, *Hin*fI, and *Rsa*I (Pharmacia Biotech). Resulting fragments were run on 2.5% high-resolution gel (1.0% NuSieve agarose, 1.5% agarose, 10× TBE buffer) at approximately 90 mV and visualized by staining with ethidium bromide. Digital images of gels were taken using a Gel Print 2000i photodocumentation system (BioPhotonic Corp.).

Analysis of molecular data

Fragment patterns were analyzed using the RFLP analysis application software RFLPscan Plus, version 3.0 (©1990–1996 Scanalytics). Fragment size was calibrated using the desmile calibration method with log piecewise linear curve fitting. Fragments in all lanes of a gel were matched simultaneously at a 2% tolerance level; fragment patterns from different gels were compared at a 6% variation level to compensate for differences between gels. Fragments that varied by less than 6% were considered to be homologous. Fragment patterns that differed in at least one enzyme were considered to be different genotypes. Fragments less than 75 base pairs were not counted to reduce the possibility of including PCR artifacts and primer–dimer products. Fourteen data bases were created using RFLPscan data base, versions 2.1 and 3.0 (©1990–1996 Scanalytics), separated by seedling type (planted vs. regenerating), replicate, site, and season (June vs. August) for each treatment. A data base for each of eight common morphotypes (*Amphinema*, *Cenococcum*, E-strain, *Hebeloma*, *Mycelium radicis atrovirens* Melin (MRA), Russulaceae type 1, *Thelephora*, and *Tuber*) and for the lightly colonized – unknown group was also created. Separate data bases were prepared initially to permit pairwise comparisons of all banding patterns to be compiled for each data base; pairwise comparisons of larger groups were obtained by merging data bases. Restriction fragment patterns were matched using Dice's index (Dice 1945) [(2 × the number of common bands)/(2 × the number of common bands plus the number of polymorphic bands)], then the index was converted to a distance value (i.e., 1 – Dice's index) for calculation of the Phi index.

Statistical analysis of morphotype abundance and diversity indices

Seedling characteristics were compared using ANOVA (STATISTICA for Windows release 5.1 G 1997 edition, Statsoft, Inc.). Morphotype abundance data were transformed by the arcsine square root function (Sokal and Rohlf 1987); replicate site and season data were pooled (as determined by Student's *t* test, Bonferroni correction of $\alpha = 0.004$ (α/ number of comparisons, e.g., 0.05/13)). Treatment and seedling type differences based on morphotype abundance for each of seven common ECM (*Cenococcum*, E-strain, MRA, *Amphinema*, *Hebeloma*, *Thelephora* and Russulaceae type 1) were determined using a one-way ANOVA. The Shannon and Simpson composite indices, Shannon evenness, and Margalef (richness) measures were used to assess diversity (Magurran 1988). For diversity indices, morphotype data for lightly colonized – uncolonized tips were excluded, and data were not transformed; a one-way ANOVA was used to determine treatment and seedling effects on diversity. Five planned comparisons were made (Bonferroni correction of $\alpha = 0.01$ (0.05/5): (*i*) among planted seedlings in clear-cut and cut–burned sites to test for burning effects; (*ii*) among regenerating seedlings in mature and planted seedlings in clear-cut sites to test for cutting and seedling effects; (*iii*) among regenerating seedlings in mature and planted seedlings in cut–burned sites to test for treatment and seedling effects; (*iv*) among regenerating seedlings in mature and clear-cut sites to test for cutting effects; and (*v*) among planted and regenerating seedlings in clear-cut sites to test for seedling differences.

To assess genetic diversity for the above planned comparisons, the Phi, Shannon, and Simpson indices were calculated using fragment patterns from all successfully amplified and digested tips. Shannon and Simpson indices use the proportional abundance of the different genotypes. The Phi index, derived by Egger (see Baldwin 1999), uses pairwise distances (obtained from the Dice index distance matrices) for ECM restriction fragment patterns to calculate the index value, instead of proportional abundance data on which traditional diversity indices are based. For a data matrix with *i* rows and *j* columns, the pairwise distances (*d*) for each individual root tip were squared, summed, then divided by $n - 1$ to give an average squared distance for each column, where *n* equals the total number of tips in the matrix. The Phi (Φ) index was calculated by summing the average squared distances for all columns divided by *n*:

Table 1. Characteristics of naturally regenerating seedlings on mature forest sites and planted seedlings in clear-cut and clear-cut plus burned sites sampled in the Aleza Lake Research Forest.

Seedling characteristic	Mature 1	Mature 2	Clear-cut 1	Clear-cut 2	Cut plus burned 1	Cut plus burned 2
Age (years)	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*

Note: Values are means with SE given in parentheses. For each mature site, $n = 8$; for each clear-cut and cut–burned site, $n = 14$. Data does not include measurements for regenerating seedlings on clear-cut sites; these seedlings were estimated to be 4 years old. Within rows, means followed by the same letters are not significantly different ($p \leq 0.05$) (one-way ANOVA).

*Leader growth values were log transformed. Values presented here are not transformed.

$$\Phi = \frac{\sum_{j=1}^{n}\left(\frac{\sum_{i=1}^{n} d_{ij}^2}{n-1}\right)}{n}$$

where $i = j = n$

The index ranges between 0, when all distances are equal to zero (i.e., all fragment patterns identical) and 1, when all distances are equal to 1 (i.e., no fragments shared among any of the pairs of samples). For molecular analyses, replicate site and season data were pooled and a one-way ANOVA was used to determine significant differences. To assess genetic variation within morphotypes, Phi, Shannon, and Simpson values were also calculated for each of eight common morphotypes.

Results

Seedling characteristics

Seedling age ranged from 4 to 7 years (at sampling). Planted seedlings in cut–burned site 2 and regenerating seedlings (estimated to be 4 years old; data not shown) in clear-cut sites were the youngest. Height, leader growth, and basal diameter were significantly greater in the planted seedlings from the clear-cut and cut–burned sites than in regenerating seedlings from the mature sites (Table 1).

ECM morphotype occurrence, frequency, and abundance

Overall, 24 ECM morphotypes were described, with four types occurring on fewer than 5% of seedlings (Table 2). More putative basidiomycete (19) than ascomycete (5) fungi were described. Fourteen types had morphological features that matched descriptions in the literature; the remaining types were more difficult to confirm. Regenerating seedlings from mature sites had the most morphotypes (20) and regenerating seedlings in clear-cut sites had the fewest (12). Planted seedlings from clear-cut and cut–burned treatments had 17 and 18 morphotypes, respectively. Lightly colonized tips represented 18% of approximately 17 000 tips analyzed and were more numerous on planted seedlings in treated sites.

The relative abundance and frequency of occurrence for all ECM morphotypes, and treatment and seedling differences for seven common ECM are shown in Table 2. For planted seedlings, *Hebeloma* and Russulaceae type 1 were significantly more abundant, and *Cenococcum* was significantly less abundant, in cut–burned sites compared with clear-cut sites. E-strain, MRA, and *Amphinema* were significantly more abundant in planted seedlings on both cut–burned and clear-cut sites compared with regenerating seedlings on mature sites. With respect to regenerating seedlings, *Cenococcum* was significantly more abundant in regenerating seedlings on mature sites than in planted seedlings on cut–burned sites, and Russulaceae type 1 was significantly more abundant in regenerating seedlings on mature sites than in seedlings on any other site. *Thelephora* was significantly more abundant on regenerating seedlings in clear-cut sites than any other site, including planted seedlings in clear-cut sites. *Piloderma* was only found on regenerating seedlings (Table 2).

Amplification and fragment pattern variation for common ECM morphotypes

Sixty-nine percent (1276) of selected tips were successfully amplified and digested for RFLP analysis. Fragment patterns for eight common morphotypes (the seven described above plus *Tuber*) and the lightly colonized category were examined further. These comprised 91% (1155) of all amplified tips. Amplification success was highest for *Cenococcum*, E-strain, *Amphinema*, *Hebeloma*, and Russulaceae type 1. Unsuccessful amplifications and tips displaying weak or double bands precluded further examination. *Thelephora*, MRA, E-strain, and lightly colonized tips had the most double bands (9, 8, 5, and 7%, respectively).

In total, 22 genotypes were identified for the eight common ECM morphotypes plus the lightly colonized group (Tables 3 and 4). *Cenococcum* produced one, and *Thelephora* had two genotypes. E-strain, *Tuber*, *Hebeloma*, and Russulaceae type 1 each exhibited three genotypes, and MRA produced four. *Amphinema* produced the most genotypes (six). The three genotypes of *Hebeloma* matched three that were identified as *Amphinema*. The five genotypes resulting from the lightly colonized group matched one genotype each of E-strain, MRA, and the nonrhizomorphic thin-mantled type and two genotypes of *Amphinema–Hebeloma* (Table 3). Most genotypes occurred on both regenerating and planted seedlings and in more than one site type. Exceptions included genotype 2 of MRA and genotypes 4, 5, and 6 of *Amphinema*, which were not detected on regenerating seedlings; MRA genotype 4 as well as *Tuber* genotypes 2 and 3 were never found on planted seedlings (Table 4). For the common morphotypes, regenerating seedlings in mature sites had the least number of genotypes, while planted seedlings in clear-cut and cut–burned sites had the most; regenerating seedlings on clear-cut sites had intermediate numbers (Table 4).

Table 2. ECM morphotype abundance (mean percent with SE given in parentheses) and frequency of occurrence (%) for planted and regenerating hybrid spruce seedlings in treated (clear-cut and cut–burned) and mature sites in the Aleza Lake Research Forest.

	Mature (regenerating) (n = 16)		Clear-cut (regenerating) (n = 16)		Clear-cut (planted) (n = 28)		Cut–burned (planted) (n = 28)	
ECM morphotype	Abundance*	Frequency	Abundance*	Frequency	Abundance*	Frequency	Abundance*	Frequency
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 type 1	35.4 (6.4)*a*	94	3.8 (3.3)*bc*	19	1.0 (0.6)c	21	5.1 (1.7)*b*	57
Russulaceae type 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)	94	7.9 (1.8)	94	25.8 (2.9)	100	19.4 (2.1)	100

Note: Within rows, means followed by the same letter are not significantly different (Bonferroni correction of $\alpha = 0.01$) as determined by separate one-way ANOVA comparisons for treatments and two seedling types. Arcsine square root transformed morphotype abundance values were used for ANOVA calculations. Means (±SE) presented are not transformed.

*Abundance values are percentages calculated as follows: (number of root tips for each fungal type)/(total number of root tips sampled per seedling) × 100.

Treatment effects on ECM morphotype and molecular diversity

There were no significant differences in ECM morphotype diversity on planted seedlings between clear-cut, and cut–burned sites for any of the indices measured (Table 5). However, the ECM diversity of regenerating seedlings in mature sites and of planted seedlings in clear-cut sites was significantly higher than for regenerating seedlings in clear-cut sites (Shannon (p = 0.008) and Margalef (p = 0.001)); Simpson indices were low (p = 0.059 and p = 0.022) but not significant (Table 5).

With respect to molecular diversity, there were no significant differences between clear-cut, cut–burned and unburned mature stands, or for seedling type, using the Phi, Shannon, and Simpson indices (data not shown). Diversity indices indicated that variation within morphotypes was lowest for *Cenococcum* and *Tuber* (Shannon and Simpson indices) and for *Tuber*, *Thelephora*, and *Cenococcum* (Phi index). *Hebeloma* and *Amphinema* (Shannon), *Hebeloma* and MRA (Simpson), and MRA and E-strain (Phi) resulted in the highest index values (Table 4).

Discussion

ECM morphotype occurrence and abundance

The 24 ECM described in our study compare well with other reports for spruce. Danielson and Pruden (1989) reported 25 morphotypes for young to mature urban white spruce (*Picea glauca* (Moench) Voss) and blue spruce (*Picea pungens* Engelm.) growing in Calgary, Alta. Seventeen morphotypes were recently described for planted and regenerating hybrid spruce seedlings, and 20 for regenerating spruce seedlings, on sites originating from wildfires that occurred in 1992 in central British Columbia (K.N. Egger and H.B. Massicotte, unpublished data). The major morphotypes described are considered to have a broad host range and are similar to those reported for spruce and other conifers. Danielson and Pruden (1989) reported E-strain to be the most common morphotype found on urban white and blue spruce, followed by *Amphinema byssoides* (Fr.) J. Erikss, *Hebeloma*, *Tuber*, and *Tomentella*. Morphotypes on jack pine (*Pinus banksiana* Lamb.) in Alberta 6 years following wildfire also included *Cenococcum*, E-strain, MRA, and *Russula*

Table 3. Approximate restriction fragment sizes (bp) of the amplified ITS region for hybrid spruce ECM.

ECM morphotype*	No. of tips amplified	Undigested	Approximate band sizes (bp)		
			*Alu*I	*Hin*fI	*Rsa*I
Cenococcum					
Genotype 1	45	920	435, 150, 110	270, 160, 130, 100	270, 185, 140, 100
E-strain					
Genotype 1	36	995	685, 185, 115	495, 170, 150	930
Genotype 2*a*	29	995	685, 185, 115	495, 170, 150	985
Genotype 3	30	980	675, 185, 115	495, 170, 160, 150	880, 95
MRA					
Genotype 1*b*	108	900	635, 150, 115	435, 250, 160	560, 175
Genotype 2	40	900	635, 150, 115	435, 250, 160	440, 160, 145, 120
Genotype 3	5	900	370, 265, 150, 115	435, 250, 160	560, 175
Genotype 4	8	900	370, 265, 150, 115	435, 250, 160	440, 160, 145, 120
Tuber					
Genotype 1	10	1020	575, 185, 145, 115	330, 310, 180, 125	365, 305, 260, 90
Genotype 2	4	1020	445, 185, 145, 130, 115	330, 310, 180, 125	365, 305, 260, 90
Genotype 3	3	1020	575, 185, 145, 115	330, 310, 180, 125	455, 305, 260
Ascomycete unknown			nd†		
Amphinema					
Genotype 1*c*	42	960	560, 190, 115, 95	320, 290, 160, 150	780, 180
Genotype 2*d*	60	960	370, 190, 115	320, 290, 160, 150	780, 180
Genotype 3*e*	15	960	655, 190, 115	320, 290, 160, 150	780, 180
Genotype 4	24	960	370, 190, 115	320, 290, 160, 150	960
Genotype 5	18	960	560, 190, 115, 95	320, 290, 160, 150	325, 295, 150, 135
Genotype 6	19	960	370, 190, 115	320, 290, 160, 150	325, 295, 150, 90
Hebeloma					
Genotype 1*d*	31	960	370, 190, 115	320, 290, 160, 150	780, 180
Genotype 2*e*	26	960	655, 190, 115	320, 290, 160, 150	780, 180
Genotype 3*c*	20	960	560, 190, 115, 95	320, 290, 160, 150	780, 180
Inocybe	4		nd		
Laccaria	14		nd		
Piloderma					
Genotype 1	3	990	365, 265, 110, 95	320, 185, 165, 160	990
Genotype 2	5	990	365, 265, 190, 115	320, 185, 165, 160	810, 180
Russulaceae type 1					
Genotype 1	78	970	525, 185, 150, 110	310, 255, 165, 150	795, 175
Genotype 2	30	970	525, 185, 150, 110	260, 220, 165, 150	795, 175
Genotype 3	23	970	675, 185, 110	335, 290, 165, 150	565, 205, 175
Russulaceae type 2	14		nd		
Thelephora					
Genotype 1	45	1000	585, 185, 120, 110	315, 260, 165, 150, 110	795, 205
Genotype 2	56	1040	585, 185, 150, 120	315, 260, 165, 150, 110	835, 205
Thelephoraceae-like	1		nd		
Tomentella 1	1		nd		
Tomentella 2	5		nd		
Tomentella 3	1		nd		
Non-rhizomorphic olive-green	2	1005	425, 190, 150, 95	325, 215, 165, 150	830, 175
Non-rhizomorphic thin mantled*f*	7	1000	425, 270, 190, 115	315, 220, 165, 155	800, 180
Non-rhizomorphic unclamped	2		nd		
Non-rhizomorphic white	7		nd		
Rhizomorphic brown	2	1000	300, 245, 190, 110, 85	335, 240, 165, 150	590, 240, 170
Rhizomorphic orange	4	1000	420, 190, 120, 110, 95	320, 220, 165, 150	825, 175
Rhizomorphic white	8		nd		

Table 3 (*concluded*).

ECM morphotype*	No. of tips amplified	Undigested	Approximate band sizes (bp)		
			*Alu*I	*Hin*fI	*Rsa*I
Lightly colonized or unknown					
Genotype 1*b*	60	900	635, 150, 115	435, 250, 160	560, 175
Genotype 2*a*	11	995	685, 185, 115	495, 170, 150	985
Genotype 3*d*	11	960	370, 190, 115	320, 290, 160, 150	780, 180
Genotype 4*e*	9	960	655, 190, 115	320, 290, 160, 150	780, 180
Genotype 5*f*	7	1000	425, 270, 190, 115	315, 220, 165, 155, (116)	800, 180

Note: Band sizes presented are before (undigested) and after digestion with three restriction endonucleases. RFLP patterns: primers ITS1 and NL6Bmun.

*Genotypes sharing the same letter were considered to be the same.

†nd, not determined.

spp. and, in a 122-year-old pine stand, *Cenococcum*, *Hebeloma*, MRA, *Piloderma*, *Russula* spp., and *Tomentella* spp. (Visser 1995).

The differences in ECM abundance between clear-cut and cut–burned sites, and between clear-cut (with and without burning) and mature sites may have depended on structures or propagules of fungi capable of surviving burns or tolerating conditions such as moisture stress in post-fire and disturbed environments. Baar et al. (1999) found *Rhizopogon*, *Wilcoxina* (E-strain), and *Tomentella* spp. colonizing bioassay seedlings grown in soil that had been collected immediately after fire. *Cenococcum geophilum* Fr. sclerotia were found up to 2 years after fire on sites in Wyoming (Miller et al. 1994). The ECM that produce rhizomorphs or extended mycelial networks may have an advantage in disturbed sites with greater access to or storage of soil nutrients (Harley and Smith 1983). They may also act as important sources of inoculum for outplanted seedlings, possibly existing on residual roots of pre-fire trees or resprouting vegetation (Baar et al. 1999; Hagerman et al. 1999). *Amphinema*, *Thelephora*, and *Hebeloma* were common rhizomorphic fungi in treated sites on both planted and regenerating seedlings in our study.

E-strain morphotypes are predominantly formed by fungi in the genus *Wilcoxina* (Egger 1996), but other taxa have been hypothesized to form this type of mycorrhiza (Danielson 1982; Ingleby et al. 1990), including post-fire ascomycetes belonging to the order Pezizales (Egger and Paden 1986). Some E-strain fungi (e.g., *Wilcoxina mikolae* (Yang & Wilcox) Yang & Korf) possess thick-walled chlamydospores that may enhance survival in soil after disturbance (Thomas et al. 1983). The species that comprise MRA are uncertain, but candidates include several ascomycete groups. E-strain and some MRA types have limited mantle development and may not compete as effectively in mature sites against other fungi; however, both are often identified on sites following disturbance. E-strain has been commonly found after fire (Baar et al. 1999), in coal spoils (Danielson 1991), and in clearcuts (Hagerman et al. 1999). MRA has been reported to dominate trenched sites (Simard et al. 1997) and amended oil sands (Danielson 1991) and has also been identified on post-fire seedlings (Baar et al. 1999) and following clear-cutting (Hagerman et al. 1999).

The Russulaceae type 1 was the most abundant ECM on mature sites. Both Russulaceae and *Piloderma* spp. seem to prefer to fruit in decaying wood in conifer forests (Schaffer 1975 in Kernaghan et al. 1997; Visser et al. 1998). *Piloderma* was also abundant in mature sites and only present as a minor component on regenerating seedlings in clear-cut sites. In a 100-year-old Norway spruce (*Picea abies* (L.) Karst.) stand in Sweden, *Piloderma croceum* Erikss. & Hjortst. accounted for 19% of all mycorrhizal tips (Dahlberg et al. 1997); in mixedwood stands of similar age in Alberta, *Piloderma byssinum* (Karst.) Jül. was equally abundant (Visser et al. 1998). Removal of woody debris and litter through burning and clear-cutting most likely contributed to the limited presence of these fungi on disturbed sites.

Genetic variation of ECM morphotypes

The 22 genotypes identified for common ECM of hybrid spruce is similar in number to other reports. Varga (1998) found 14 and 31 distinct ECM RFLP variants for Sitka alder (*Alnus crispa* var. *sinuata* (Regel) Hulten) and lodgepole pine (*Pinus contorta* Dougl. ex Loud.), respectively, while Mehmann et al. (1995) reported 23 RFLP variants from fungal sporocarps and cultures originating from a 40-year-old Norway spruce stand in Switzerland. In two studies on Bishop pine (*Pinus muricata* D. Don), 14 ECM molecular variants were identified on seedlings 5 months after wildfire (Horton et al. 1998) and 11 mycorrhizal taxa were observed on 1-year-old field and bioassay post-fire seedlings (Baar et al. 1999).

Cenococcum and *Tuber* produced one and three genotypes, respectively, and had low diversity values, suggesting a high degree of similarity within each morphotype. Others have reported different levels of genetic variation for *Cenococcum*. LoBuglio et al. (1991), examining isolates of *Cenococcum* over a large geographic area, found high genetic variation. Studies more limited in geographic range (Varga 1998; Baldwin 1999) report two and four genotypes. Fragment patterns for *Cenococcum* in our study were similar to those reported by Hagerman et al. (1999) for OUC 30 *Cenococcum*. Although *Thelephora* produced only two genotypes, indicating low heterogeneity in this fungus, diversity values varied (Phi: low; Shannon: intermediate; Simpson: high). Hagerman et al. (1999) identified one genotype for *Thelephora* (OUC 240 *Thelephora*-like from four samples) which was similar to our *Thelephora* genotype 2, except we observed additional fragments in both *Alu*I and *Hin*fI. All indices for MRA and E-strain were in the higher range, supporting other evidence that these ECM types are phylogenetically diverse. E-strain genotypes 1 and 2 corresponded to OUC 60 E-strain (Hagerman et al. 1999), and MRA geno-

Table 4. Occurrence (+, presence; –, absence) of molecular genotypes and diversity values (Shannon, Simpson, and Phi indices) for ECM commonly found on regenerating and planted hybrid spruce seedlings growing in treated (clear-cut and cut–burned) and unburned (mature) sites in the Aleza Lake Research Forest.

ECM morphotype	Mature (regenerating)	Clear-cut (regenerating)	Clear-cut (planted)	Cut–burned (planted)	Shannon	Simpson	Phi
Cenococcum					1.665	0.703	0.056
Genotype 1	+	–	+	+			
E-strain					2.913	0.888	0.135
Genotype 1	–	+	+	+			
Genotype 2	–	+	+	+			
Genotype 3	–	+	+	+			
MRA					3.032	0.915	0.158
Genotype 1	+	+	+	+			
Genotype 2	–	–	+	+			
Genotype 3	–	+	+	–			
Genotype 4	+	–	–	–			
Tuber					2.282	0.886	0.036
Genotype 1	+	+	+	–			
Genotype 2	–	+	–	–			
Genotype 3	–	+	–	–			
Amphinema					3.056	0.912	0.098
Genotype 1	+	–	+	+			
Genotype 2	+	+	+	+			
Genotype 3	–	+	–	–			
Genotype 4	–	–	–	+			
Genotype 5	–	–	+	+			
Genotype 6	–	–	+	+			
*Hebeloma**					3.173	0.938	0.063
Genotype 1	+	–	+	+			
Genotype 2	+	–	+	+			
Genotype 3	+	–	+	+			
Russulaceae 1					2.903	0.896	0.074
Genotype 1	+	–	+	+			
Genotype 2	+	+	–	+			
Genotype 3	+	+	+	+			
Thelephora					2.734	0.913	0.055
Genotype 1	–	+	+	+			
Genotype 2	–	+	–	+			
Lightly colonized[†]					3.937	0.963	0.267
Genotype 1	+	+	+	+			
Genotype 2	–	–	+	+			
Genotype 3	+	–	+	+			
Genotype 4	–	–	+	+			
Genotype 5	+	–	+	–			
Total genotypes[‡]	11	14	17	17			

Note: Fragment pattern details for genotypes presented, including those that were considered matches for *Hebeloma–Amphinema* and the lightly colonized group, are found in Table 3. All amplified tips were included in diversity analysis to assess variation within each morphotype.

*All *Hebeloma* fragment patterns matched those for *Amphinema*.

[†]Fragment patterns for lightly colonized tips matched those for E-strain, MRA, *Amphinema–Hebeloma*, and the non-rhizomorphic thin-mantle type.

[‡]Genotype totals exclude patterns that were considered the same.

types 1 and 2 were similar to their OUC 179 MRA. Varga (1998) reported three RFLP variants for MRA on lodgepole pine; two of these were similar to our MRA patterns for *Hin*fI and *Rsa*I.

Some Russulaceae species are difficult to separate morphologically due to similar features. To resolve differences, DNA analysis has been used (Kernaghan et al. 1997). Horton and Bruns (1998) identified three Russulaceae ECM RFLP variants from Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and bishop pine and Kernaghan et al. (1997) described three *Lactarius* and two *Russula* ECM taxa from white spruce and subalpine fir on subalpine sites in Alberta. In an assessment of prescribed fire on the community structure of ponderosa pine (*Pinus ponderosa* Dougl ex Laws.) ECM, Stendell et al. (1999) found Russulaceae and Thelephoraceae species to be among the most frequent and abundant ECM prior to burning; however, these levels were reduced greatly on post-fire sites. The Russulaceae type 1 in

Table 5. ECM diversity (Shannon, Simpson, Shannon evenness, and Margalef indices) comparing mean values by ANOVA.

Diversity index	Comparison of treatment and seedling type*			
	First value	Second value	*F*	*p*
	Clear-cut, planted	**Cut–burned, planted**	**df = 1, 54**	
Shannon	1.19 (0.08)	1.16 (0.06)	0.067	0.797
Simpson	0.61 (0.03)	0.61 (0.03)	0.003	0.954
Evenness	0.71 (0.03)	0.71 (0.03)	0.000	0.988
Margalef	0.90 (0.06)	0.90 (0.06)	0.000	0.978
	Mature, regenerating	**Clear-cut, regenerating**	**df = 1, 30**	
Shannon	1.30 (0.52)	0.87 (0.08)	8.174	0.008*
Simpson	0.61 (0.22)	0.48 (0.04)	3.844	0.059
Evenness	0.69 (0.05)	0.62 (0.04)	1.355	0.254
Margalef	1.07 (0.12)	0.59 (0.05)	14.567	0.001*
	Mature, regenerating	**Clear-cut, planted**	**df = 1, 42**	
Shannon	1.30 (0.52)	1.19 (0.08)	0.579	0.451
Simpson	0.61 (0.22)	0.61 (0.03)	0.000	0.991
Evenness	0.69 (0.05)	0.71 (0.03)	0.058	0.810
Margalef	1.07 (0.12)	0.90 (0.06)	2.244	0.142
	Mature, regenerating	**Cut–burned, planted**	**df = 1, 42**	
Shannon	1.30 (0.52)	1.16 (0.06)	1.054	0.311
Simpson	0.61 (0.22)	0.61 (0.03)	0.004	0.952
Evenness	0.69 (0.05)	0.71 (0.03)	0.082	0.776
Margalef	1.07 (0.12)	0.90 (0.06)	1.954	0.169
	Clear-cut, planted	**Clear-cut, regenerating**	**df = 1, 42**	
Shannon	1.19 (0.08)	0.87 (0.08)	7.849	0.008*
Simpson	0.61 (0.03)	0.48 (0.04)	5.618	0.022
Evenness	0.71 (0.03)	0.62 (0.04)	2.591	0.115
Margalef	0.90 (0.06)	0.59 (0.05)	13.453	0.001*

Note: Values for indices are means with SE given in parentheses. Pairs of indices were assessed using one-way ANOVA to test for treatment effect (clearcut, cut–burned, and unburned mature) and to test for seedling differences (regenerating (n = 16) versus planted (n = 28)) of hybrid spruce seedlings growing in the Aleza Lake Research Forest. Means are pooled for both replicate sites and season.

*Significantly different (p = 0.01) after applying a Bonferroni correction for planned comparisons; p values <0.0015 are given as 0.001.

our study had the same number of genotypes as E-strain but a lower Phi value, suggesting that Russulaceae genotypes were more genetically similar than those of E-strain.

Amphinema and *Hebeloma* ECM are similar morphologically (Ingleby et al. 1990), which was supported by the molecular data that showed that all three fragment patterns identified for *Hebeloma* were identical to genotypes of *Amphinema*. It is possible that *Hebeloma* was not found or, more likely, that *Hebeloma* tips were mistaken for *Amphinema*; this would result in fewer genotypes for *Amphinema* and affect both abundance and richness measures. Hagerman et al. (1999) identified three band patterns for *Hebeloma* and one for *Amphinema*. *Hebeloma* genotype 2 (and *Amphinema* genotype 3) had similar band patterns to OUC 80 *Hebeloma*-like (Hagerman et al. 1999); *Amphinema* genotypes 1 and 5 and *Hebeloma* genotype 3 were similar to OUC 20 *Amphinema*-like. One band pattern was determined for a *Hebeloma* sp. on Bishop pine field seedlings by Baar et al. (1999) one year after wildfire. Varga (1998) reported two RFLP variants for *Hebeloma* on Sitka alder.

The five genotypes resolved for the lightly colonized tips were all characterized on other ECM, suggesting these tips were early or weak colonization stages of more common ECM. Scoring these morphologically affected abundance measures but not richness. Overall, the number of genotypes of common ECM appeared to increase with increased disturbance (from mature to clear-cut and cut–burned), whereas more ECM morphotypes were identified from the unburned mature sites.

Morphological and genetic diversity due to treatment and seedling effects

Based on abundance values, broadcast burning did not appear to affect the ECM diversity of planted seedlings following clear-cutting. Visser (1995) found that ECM species richness was significantly lower in 6-year-old compared with 41-year-old jack pine post-fire stands, at which time ECM levels seemed to stabilize. In a recent study examining a chronosequence of low-intensity wildfires in Scots pine (*Pinus sylvestris* L.) stands, Jonsson et al. (1999) noted increased dominance in common types on fire-disturbed sites, suggesting a shift in frequencies of ECM fungi rather than replacement. Species richness did not seem to be affected. Our study also showed changes in the abundance and frequency of some ECM following burning but no difference in species diversity. Pilz and Perry (1984) reported differences

in ECM frequency (increases in two common morphotypes) but no differences in total numbers of ECM root tips.

The reduction in ECM diversity of regenerating seedlings on clear-cut sites compared with planted seedlings on the same sites and to regenerating seedlings on mature sites may reflect stand and seedling age differences. Most seedlings in the mature sites were rooted in woody substrates and were possibly inoculated by ECM fungi that had active, established fungal reservoirs on adjacent mature trees. Tree seedlings have been shown to form ECM with a greater range of fungi when growing close to other trees already colonized by these fungi (Massicotte et al. 1994; Simard et al. 1997) or when adjacent to the edge of intact forests (Kranabetter and Wylie 1998; Durall et al. 1999). In mature SBS stands in central British Columbia, 48% of hybrid spruce seedlings were growing on woody substrates (Kneeshaw and Burton 1997). In an old-growth Douglas-fir – larch forest in western Montana, Harvey et al. (1980) found more active ECM root tips in humus and decayed wood than in other soil fractions. Woody substrates provide moisture (Harvey et al. 1978) and a haven for possible animal vectors that disperse fungal inocula (Maser et al. 1978). Regenerating seedlings in mature sites were approximately 2 years older than regenerating seedlings on clear-cut sites. Planted seedlings in clearcuts were also about 2 years older because of initial greenhouse growth. These seedlings were bigger and supported larger root systems, possibly exploiting larger soil volumes and accessing more or different fungal propagules than regenerating seedlings. Decreased species richness on clearcut regenerating seedlings appeared to be due to the absence of ECM that were identified on other seedlings infrequently.

No significant differences were found in the molecular diversity of spruce ECM resulting from treatment or seedling type. Baldwin (1999) also found no difference in ECM molecular diversity (using the Phi, Shannon and Simpson indices) for regenerating black spruce (*Picea mariana* (Mill.) BSP) seedlings growing in clear-cut and cut–burned sites (of low and high intensity burns) in a paper birch (*Betula papyrifera* Marsh.) – black spruce forest. The Phi index was expected to be less affected by intraspecific variation, as unique genotypes with small pairwise distance values contribute less to the average squared genetic distance than to proportional abundance indices. Conversely, unique genotypes with large pairwise distance values contribute more to the average squared genetic distance, which would make it more sensitive to diverse genotypes such as E-strain, MRA, and *Amphinema*. However, there was little difference in outcomes of the Shannon, Simpson, and Phi indices. Exploring the Phi index using other data sets should further clarify its value as a measure for molecular diversity.

The observation of few regenerating seedlings on cut–burned sites was not likely related to available fungal inoculum (since planted seedlings were colonized) but rather to seed production and disturbance. Regeneration likely occurred after 1993 when the seed crop was rated good (J. Revel, personal communication) and when exposed mineral soil on clear-cut sites was potentially ideal for regeneration (Feller 1998). However, cones and seedlings on cut–burned sites were subjected to clear-cutting in the winter 1993 and then burning in 1994, effects that may have reduced seed and (or) seedling survival on these sites. Sites cut in 1992 and not burned had more regenerating spruce seedlings.

Comparison between morphology and molecular characterization

Morphological and molecular characterization methods presented different advantages for identifying ECM. High levels of morphological variation can result in a degree of subjectivity, especially for categories involving ubiquitous characteristics (white mantle, clamps, rhizomorphs, etc.). Morphologically similar types can be difficult to resolve taxonomically; some ECM can be identified to species level and others only to genus or family. Molecular analysis increased the resolution for some morphotypes but still left some uncertainty with respect to species. Morphology likely underestimated diversity in ECM with multiple genotypes (e.g., E-strain, *Amphinema*, Russulaceae type 1, MRA) but identified some rare types that were few in numbers and that did not amplify successfully. Lightly colonized tips and thin-mantled ECM were problematic for both morphological characterization and molecular amplification. Interestingly, based upon the molecular analysis, these tips appeared to be comprised of identified types, suggesting that failure to identify or amplify them did not affect assessment of species richness.

Molecular protocols, primers, or restriction endonucleases can vary between studies making it difficult to compare band patterns. The primers ITS1-F, which is fungus specific (Gardes and Bruns 1991), and ITS4 (White et al. 1990) have been frequently used by others (Jonsson et al. 1999; Kårén et al. 1997; Kernaghan et al. 1997; Horton and Bruns 1998). The primer NL6Bmun was used in our study, because it is fungus specific and, at the annealing temperatures used, amplifies both basidiomycetes and ascomycetes efficiently. Pairing NL6Bmun with the universal primer ITS1 eliminates an intron that occurs in some ascomycetes and that may be amplified using ITS1-F (Egger 1995).

Summary

Significant differences due to treatment and seedling type were found for some common ECM suggesting a shift in the abundance and frequency of these symbiotic fungi. Sometimes differences occurred between clear-cut and cut–burned sites, and sometimes between mature and treated sites (clear-cut and cut–burned). A number of genotypes were identified within some ECM morphotypes; variation was greatest for planted seedlings in both treatments compared with regenerating seedlings. Despite differences at the morphotype level, ECM diversity (both morphological and molecular) did not appear to be affected by burning or clear-cutting. An exception was lower ECM diversity on regenerating seedlings in clear-cut sites, which was partly attributed to fewer rare and uncommon ECM identified on these seedlings. The study results are limited to burns of moderate severity. A combination of morphological and molecular techniques provided a comprehensive view of ECM on seedlings following clear-cutting and broadcast burning. Morphological characterization included rare types that may have been missed or not amplified by molecular methods while molecular analysis mostly validated morphotype accuracy and provided a sense

of the variation within and between morphotypes. Further improvements and standardization in methods are necessary to facilitate assessment of the responses of ECM symbiotic associations to disturbances.

Acknowledgements

The authors thank Dr. Paul Sanborn and Sharon Dow (B.C. Ministry of Forests) for site maps and data, Ron Jansen (Northwood Pulp and Paper Limited) for site treatment information, and Dr. Chris Opio, Dr. Brad Hawkes, and Dr. Dan Durall for input on fire and molecular issues. We thank Dr. Lito Arocena for help with soil analysis and Dr. Bruno Zumbo and Dr. Mike Walters for statistical aid. Quentin Baldwin, Tamara Bereck, and Brent Young assisted with seedling harvesting and with molecular analysis. We are grateful to Dr. Paul Sanborn and Dr. Art Fredeen who read an earlier version of the manuscript and to two anonymous reviewers for helpful comments. Funding for this research was provided by Forest Renewal British Columbia.

References

Agee, J.K. 1993. Fire ecology of Pacific Northwest forests. Island Press, Washington, D.C.

Agerer, R. (*Editor*). 1987–1998. Colour atlas of ectomycorrhizae. Einhorn-Verlag Eduard Dietenberger, Schwäbisch Gmünd, Germany.

Agerer, R., Danielson, R.M., Egli, S., Ingleby, K., Luoma, D., and Treu, R. (*Editors*). 1996–1998. Descriptions of ectomycorrhizae. Einhorn-Verlag, Schwäbisch Gmünd, Germany.

Baar, J., Horton, T.R., Kretzer, A.M., and Bruns, T.D. 1999. Mycorrhizal colonization of *Pinus muricata* from resistant propagules after a stand-replacing wildfire. New Phytol. **143**: 409–418.

Baldwin, Q.F. 1999. Effects of prescribed burning upon mycorrhizal fungal diversity inhabiting the roots of two and a half-year-old black spruce (*Picea mariana*): molecular characterization of ectomycorrhizal fungi via PCR/RFLP. M.Sc. thesis, Memorial University of Newfoundland, St. John's.

Dahlberg, A., Jonsson, L., and Nylund, J.-E. 1997. Species diversity and distribution of biomass above- and below-ground among ectomycorrhizal fungi in an old-growth Norway spruce forest in south Sweden. Can. J. Bot. **75**: 1323–1335.

Danielson, R.M. 1982. Taxonomic affinities and criteria for identification of the common ectendomycorrhizal symbiont of pines. Can. J. Bot. **60**: 7–18.

Danielson, R.M. 1991. Temporal changes and effects of amendments on the occurrence of sheathing (ecto-) mycorrrhizas of conifers growing in oil sands tailings and coal spoil. Agric. Ecosyst. Environ. **35**: 261–281.

Danielson, R.M., and Pruden, M. 1989. Ectomycorrhizal status of urban spruce. Mycologia, **81**: 335–341.

DeLong, C., Jenvey, S., and Mcleod, A. 1996. Draft field guide insert for site identification and interpretation for the southeast portion of the Prince George Forest Region. B.C. Ministry of Forests, Prince George. pp. 108–109.

Dice, L.R. 1945. Measures of the amount of ecologic association between species. Ecology, **26**: 297–302.

Durall, D.M., Jones, M.D., Wright, E.F., Kroeger, P., and Coates, K.D. 1999. Species richness of ectomycorrhizal fungi in cutblocks of different sizes in the Interior Cedar–Hemlock forests of northwestern British Columbia: sporocarps and ectomycorrhizae. Can. J. For. Res. **29**: 1322–1332.

Egger, K.N. 1995. Molecular analysis of ectomycorrhizal fungal communities. Can. J. Bot. **73**(Suppl. 1): S1415–S1422.

Egger, K.N. 1996. Molecular systematics of E-strain mycorrhizal fungi: *Wilcoxina* and its relationship to *Tricharina* (Pezizales). Can. J. Bot. **74**: 773–779.

Egger, K.N., and Paden, J.W. 1986. Biotrophic associations between lodgepole pine seedlings and postfire ascomycetes (Pezizales) in monoxenic culture. Can. J. Bot. **64**: 2719–2725.

Feller, M.C. 1998. Influence of ecological conditions on Engelmann spruce (*Picea engelmannii*) and subalpine fir (*Abies lasiocarpa*) germinant survival and initial seedling growth in south-central British Columbia. For. Ecol. Manage. **107**: 55–69.

Gardes, M., and Bruns, T.D. 1991. Identification of indigenous and introduced symbiotic fungi in ectomycorrhizae by amplification of nuclear and mitochondrial ribosomal DNA. Can. J. Bot. **69**: 180–190.

Gardes, M., and Bruns, T.D. 1996. ITS-RFLP matching for identification of fungi. *In* Methods in molecular biology. Vol. 50. *Edited by* J. Clapp. Humana Press Inc., Totowa, N.J. pp. 177–186.

Goodman, D.M., Durall, D.M., Trofymow, J.A., and Berch, S.M. (*Editors*). 1996. A manual of concise descriptions of North American ectomycorrhizae. Mycologue Publications, Sidney, B.C.

Hagerman, S.M., Jones, M.D., Bradfield, G.E., Gillespie, M., and Durall, D.M. 1999. Effects of clear-cut logging on the diversity and persistence of ectomycorrhizae at a subalpine forest. Can. J. For. Res. **29**: 124–134.

Harley, J.L., and Smith, S.E. 1983. Mycorrhizal symbiosis. Academic Press, London.

Harvey, A.E., Jurgensen, M.F., and Larsen, M.J. 1978. Seasonal distribution of ectomycorrhizae in a mature Douglas-fir/larch forest soil in western Montana. For. Sci. **24**: 203–208.

Harvey, A.E., Larsen, M.J., and Jurgensen, M.F. 1980. Partial cut harvesting and ectomycorrhizae: early effects in Douglas-fir – larch forests of western Montana. Can. J. For. Res. **10**: 436–440.

Hawkes, B.C., Feller, M.C., and Meehan, D. 1990. Site preparation: fire. *In* Regenerating British Columbia's forests. *Edited by* D.P. Lavender, R. Parish, C.M. Johnson, G. Montgomery, A. Vyse, R.A. Willis, and D. Winston. University of British Columbia Press, Vancouver. pp. 131–149.

Horton, T.R., and Bruns, T.D. 1998. Multiple-host fungi are the most frequent and abundant ectomycorrhizal types in a mixed stand of Douglas-fir (*Pseudotsuga menziesii*) and bishop pine (*Pinus muricata*). New Phytol. **139**: 331–339.

Horton, T.R., Cázares, E., and Bruns, T.D. 1998. Ectomycorrhizal, vesicular–arbuscular and dark septate fungal colonization of bishop pine (*Pinus muricata*) seedlings in the first 5 months of growth after wildfire. Mycorrhiza, **8**: 11–18.

Ingleby, K., Mason, P.A., Last, F.T., and Fleming, L.V. 1990. Identification of ectomycorrhizas. Institute of Terrestrial Ecology, Natural Environment Research Council. Her Majesty's Stationery Office, London. Inst. Terr. Ecol. Res. Publ. 5.

Jonsson, L., Dahlberg, A., Nilsson, M-C., Zackrisson, O., and Kårén, O. 1999. Ectomycorrhizal fungal communities in late-successional Swedish boreal forests, and their composition following wildfire. Mol. Ecol. **8**: 205–215.

Jull, M. 1992. Aleza Lake Research Forest Management and Working Plan 1. B.C. Ministry of Forests, Prince George Forest Region, Prince George.

Kårén, O., Högberg, N., Dahlberg, A., Jonsson, L., and Nylund, J-E. 1997. Inter- and intraspecific variation in the ITS region of rDNA of ectomycorrhizal fungi in Fennoscandia as detected by endonuclease analysis. New Phytol. **136**: 313–325.

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

Kneeshaw, D.D., and Burton, P.J. 1997. Canopy and age structures of some old sub-boreal *Picea* stands in British Columbia. J. Veg. Sci. **8**: 615–626.

Kranabetter, J.M., and Wylie, T. 1998. Ectomycorrhizal community structure across forest openings on naturally regenerated western hemlock seedlings. Can. J. Bot. **76**: 189–196.

LoBuglio, K.F., Rogers, S.O., and Wang, C.J.K. 1991. Variation in ribosomal DNA among isolates of the mycorrhizal fungus *Cenococcum geophilum*. Can. J. Bot. **69**: 2331–2343.

Magurran, A.E. 1988. Ecological diversity and its measurement. Princeton University Press, Princeton, N.J.

Mah, K. 1999. 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. M.Sc. thesis, University of Northern British Columbia, Prince George.

Maser, C., Trappe, J.M., and Nussbaum, R.A. 1978. Fungal – small mammal interrelationships with emphasis on Oregon coniferous forests. Ecology, **59**: 799–809.

Massicotte, H.B., Molina, R., Luoma, D.L., and Smith, J.E. 1994. Biology of the ectomycorrhizal genus, *Rhizopogon,* II. Patterns of host–fungus specificity following spore inoculation of diverse hosts grown in monoculture and dual culture. New Phytol. **126**: 677–690.

Mehmann, B., Egli, S., Braus, G.H., and Brunner, I. 1995. Coincidence between molecularly or morphologically classified ectomycorrhizal morphotypes and fruitbodies in a spruce forest. *In* Biotechnology of ectomycorrhizae. *Edited by* V. Stocchi, P. Bonfante, and M. Nuti. Plenum Press, New York. pp. 41–52.

Meidinger, D., Pojar, J., and Harper, W.L. 1991. Sub-Boreal Spruce zone. *In* Ecosystems of B.C. *Edited by* D. Meidinger and J. Pojar. B.C. Ministry of Forests, Victoria. pp. 209–221.

Miller, S.L., Torres, P., and McClean, T.M. 1994. Persistence of basidiospores and sclerotia of ectomycorrhizal fungi and *Morchella* in soil. Mycologia, **86**: 89–95.

Mullis, K.B., and Faloona, F.A. 1987. Specific synthesis of DNA *in vitro* via a polymerase-catalysed chain reaction. Methods Enzymol. **155**: 335–350.

Parke, J.L., Linderman, R.G., and Trappe, J.M. 1984. Inoculum potential of ectomycorrhizal fungi in forest soils of southwest Oregon and northern California. For. Sci. **30**: 300–304.

Perry, D.A., Meyer, M.M., Egeland, D., Rose, S.L., and Pilz, D.P. 1982. Seedling growth and mycorrhizal formation in clearcut and adjacent, undisturbed soils in Montana: a greenhouse bioassay. For. Ecol. Manage. **4**: 261–273.

Perry, D.A., Molina, R., and Amaranthus, M.P. 1987. Mycorrhizae, mycorrhizospheres, and reforestation: current knowledge and research needs. Can. J. For. Res. **17**: 929–940.

Pilz, D.P., and Perry, D.A. 1984. Impact of clear-cutting and slash burning on ectomycorrhizal associations of Douglas-fir seedlings. Can. J. For. Res. **14**: 94–100.

Schoenberger, M.M., and Perry, D.A. 1982. The effect of soil disturbance on growth and ectomycorrhizae of Douglas-fir and western hemlock seedlings: a greenhouse bioassay. Can. J. For. Res. **12**: 343–353.

Simard, S.W., Perry, D.A., Smith, J.E., and Molina, R. 1997. Effects of soil trenching on occurrence of ectomycorrhizas on *Pseudotsuga menziesii* seedlings grown in mature forests of *Betula papyrifera* and *Pseudotsuga menziesii*. New Phytol. **136**: 327–340.

Sokal, R.R., and Rohlf, F.J. 1987. Introduction to biostatistics. 2nd ed. W.H. Freeman & Co., New York.

Staddon, W.J., Duchesne, L.C., and Trevors, J.T. 1996. Conservation of forest soil microbial diversity: the impact of fire and research needs. Environ. Rev. **4**: 267–275.

StatSoft, Inc. 1997. STATISTICA for Windows—computer program manual. StatSoft, Inc., Tulsa, Okla.

Stendell, E.R., Horton, T.R., and Bruns, T.D. 1999. Early effects of prescribed fire on the structure of the ectomycorrhizal fungus community in a Sierra Nevada ponderosa pine forest. Mycol. Res. **103**: 1353–1359.

Thomas, G.W., Rogers, D., and Jackson, R.M. 1983. Changes in the mycorrhizal status of Sitka spruce following outplanting. Plant Soil, **71**: 319–323.

Varga, A.M. 1998. Characterization and seasonal ecology of ectomycorrhizae associated with Sitka alder and lodgepole pine from naturally regenerating young and mature sub-boreal spruce forests. M.Sc. thesis, University of Northern British Columbia, Prince George.

Visser, S. 1995. Ectomycorrhizal fungal succession in jack pine stands following wildfire. New Phytol. **129**: 389–401.

Visser, S., Maynard, D., and Danielson, R.M. 1998. Response of ecto- and arbuscular mycorrhizal fungi to clear-cutting and the application of chipped aspen wood in a mixedwood site in Alberta, Canada. Appl. Soil Ecol. **7**: 257–269.

Wells, C.G., Campbell, R.E., DeBano, L.F., Lewis, C.E., Fredriksen, R.L., Franklin, E.C., Froelich, R.C., and Dunn, P.H. 1979. Effects of fire on soil: a state-of-knowledge review. USDA For. Serv. Gen. Tech. Rep. WO-7.

White, T.J., Bruns T.D., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* PCR protocols: a guide to methods and applications. *Edited by* M.A. Innis, D.H. Gelfand, J.J. Snisky, and T.J. White. Academic Press, Inc., New York. pp. 315–322.

Zolan, M., and Pukkila, P.J. 1986. Inheritance of DNA methylation in *Coprinus cinereus*. Mol. Cell Biol. **6**: 195–200.