Shared compatibility of ectomycorrhizae on *Pseudotsuga menziesii* and *Betula papyrifera* seedlings grown in mixture in soils from southern British Columbia

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Abstract: Seedlings of *Pseudotsuga menziesii* (Mirb.) Franco and *Betula papyrifera* Marsh. were grown in the greenhouse in monoculture and dual culture in soils collected from a young mixed species plantation in the southern interior of British Columbia. The objectives of the study were (*i*) to evaluate the ability of *P. menziesii* and *B. papyrifera* to share compatible ectomycorrhizal fungi in order to assess their potential for hyphal linkages and (*ii*) to study the influence of neighboring seedlings on ectomycorrhizae occurrence. Eleven ectomycorrhizal morphotypes were recognized, seven of which *P. menziesii* and *B. papyrifera* seedlings shared in common over 90% of their root tips. The abundance and frequency of *Rhizopogon,* E-strain I, and *Tuber* on *P. menziesii*, and the frequency of *Lactarius*, *Hebeloma*, and *Cenococcum* on *B. papyrifera*, were affected by the presence of a neighboring seedling. The number of ectomycorrhizal morphotypes shared in common and colonization of root tips by common types were slightly greater when *P. menziesii* and *B. papyrifera* were grown in dual culture rather than in monoculture.

Résumé : Des semis de *Pseudotsuga menziesii* (Mirb.) Franco et de *Betula papyrifera* Marsh. ont été cultivés en serres, en monoculture ou en culture mixte, dans du sol prélevé dans une jeune plantation d'espèces mixtes dans le sud de la zone continentale en Colombie-Britannique. Cette étude avait pour objectifs (*i*) d'évaluer la capacité de *P. menziesii* et de *B. papyrifera* de partager des champignons ectomycorhiziens compatibles dans le but d'évaluer la possibilité qu'ils forment des liens fongiques et (*ii*) d'étudier l'influence des semis avoisinants sur l'occurence des ectomycorhizes. Onze types morphologiques furent identifiés dont sept que les semis de *P. menziesii* et de *B. papyrifera* partageaient sur plus de 90% de leurs extrémités racinaires. L'abondance et la fréquence de *Rhizopogon*, de la race E 1 et de *Tuber* sur *P. menziesii* et de *Lactarius*, *Hebeloma* et *Cenococcum* sur *B. papyrifera* étaient affectées par la présence d'un semis avoisinant. Le nombre de types morphologiques partagés et la colonisation des extrémités racinaires par les types les plus répandus étaient légèrement plus élevés lorsque *P. menziesii* et *B. papyrifera* étaient cultivés en culture mixte qu'en monoculture.
[Traduit par la Rédaction]

Introduction

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and paper birch (*Betula papyrifera* Marsh.) often are cohorts in mixed seral forests in the wet southern climatic region of interior British Columbia. Douglas-fir frequently is planted and paper birch quickly seeds or sprouts following either wildfire or clear-cutting with broadcast burning or mechanical site preparation (Simard and Vyse 1994). Paper birch competes with Douglas-fir for light and water in young plantations because of its prolific reproduction habits and rapid growth rates (Haeussler et al. 1990; Simard 1990). As a result, paper birch is commonly removed from plantations to promote rapid growth of more commercially valuable Douglas-fir. Although competition is an important process in shaping the structure of Douglas-fir plantations, paper birch may benefit conifers by providing mycorrhizal inoculum and improving site productivity (Hendrickson et al. 1987; Sachs 1995).

Plant community composition influences the mycorrhizal associations of seedlings planted into those communities (Amaranthus and Perry 1989; Borchers and Perry 1990; Eissenstat and Newman 1990; Deacon and Fleming 1992; Massicotte et al. 1994; Smith et al. 1995). Extramatrical hyphae emanating from ectomycorrhizal (EM) shrubs, for example, may contact neighboring conifer roots and induce colonization (Read 1987; Borchers and Perry 1990). The ubiquity of extramatrical hyphae and low host specificity of vesicular–arbuscular (VA) mycorrhizal fungi and many EM fungi (Molina et al. 1992) have led to the hypothesis that hyphal links between plants are common (Newman 1988). The ability of different plant species to form mycorrhizae with the same fungal species has additional

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ecological consequences, including interplant transfer of carbon or nutrients (e.g., Francis and Read 1984; Finlay and Read 1986; Hamel and Smith 1992; Newman and Eason 1993; Arnebrant et al. 1993), transfer of nutrients from dying to living roots (Ritz and Newman 1986; Eason and Newman 1990), and alteration of the balance of plant–plant interactions (Grime et al. 1987; Newman 1988; Perry et al. 1989*b*; Miller and Allen 1992; Perry et al. 1992). Groups of host plants that share common below ground mutualists (e.g., mycorrhizal fungi) have been referred to as "guilds," or associations that function "for mutual aid and the promotion of common interests" (Perry et al. 1989*a*).

Both Douglas-fir and paper birch form predominantly EM (Harley and Harley 1987), although minor amounts of VA mycorrhizae have been observed in roots of Douglas-fir (Cazares and Smith 1996) and paper birch (Malloch and Malloch 1981). Although paper birch and Douglas-fir are each exclusive hosts to some genera of EM fungi, they also may share several fungi in common (Molina et al. 1992) and hence potentially function as plant guilds based on their mycorrhizal associates and possibly other beneficial soil organisms. The EM common to Douglas-fir and paper birch growing in British Columbia soils include *Thelephora*, *Mycelium radicis atrovirens* Melin. complex, E-strain, *Laccaria*, *Cenococcum geophilum* Fr., *Lactarius*, *Hebeloma*, and *Tomentella* (M.D. Jones, unpublished results). Douglas-fir also is frequently colonized by *Rhizopogon* of the section *Villosuli* (Molina and Trappe 1982*b*, 1994; Massicotte et al. 1994; Smith et al. 1995). The EM genera commonly associated with *Betula* include *Russula*, *Leccinum*, *Boletus*, and *Cortinarius* (Fleming 1985; Deacon and Fleming 1992; Molina et al. 1992). Many of these EM fungi form abundant extramatrical hyphae, which function in the mobilization and capture of nutrients and water in the soil (Read 1992), and some form strands or rhizomorphs thought to function in the translocation of water, carbon, and nutrients between plants (Brownlee et al. 1983; Duddridge et al. 1980, 1988; Cairney 1992).

Mycorrhizae may affect plant–plant interactions in mixed species plantings by exchanging nutrients through interconnecting hyphae as well as improving general plant vigor through improved nutrition, improved water status, protection from pathogens, or detoxification of allelochemicals (Perry et al. 1989*b*). Perry et al. (1989*b*) found that competition between Douglas-fir and ponderosa pine (*Pinus ponderosa* Dougl. ex P. & C. Laws.) was reduced when they were inoculated with four different species of EM fungi compared with the single greenhouse contaminant *Thelephora*. Seedling biomass and nitrogen (N) and phosphorus (P) uptake were greater for both tree species with the inoculated mycorrhizal species than with the contaminant. Pioneer species, such as many early seral hardwoods that regenerate from surviving rootstocks, appear to retain a reservoir of soil organisms that benefit conifer establishment. Legacies of soil organisms provided by pioneer plant species following disturbance influence mycorrhizal composition and performance of conifers in field studies (Amaranthus and Perry 1989) and in greenhouse soil bioassays (Borchers and Perry 1990; Smith et al. 1995; Massicotte et al. 1994). For example, Massicotte et al. (1994) and Smith et al. (1995) found that some *Rhizopogon* and Dark Brown types colonized secondary hosts, such as western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), rhododendron (*Rhododendron macrophyllum* G. Don), and salal (*Gaultheria shallon* Pursh), when grown in mixture with a well-colonized primary host, ponderosa pine or Douglas-fir. The ability of paper birch to function as a reservoir of EM inoculum for neighboring Douglas-fir in southern British Columbia has not been evaluated.

To determine the potential for host plant species to be connected by compatible EM fungi, the occurrence of EM in single and dual species (i.e., common garden) bioassays can be examined. This study focuses on results from a greenhouse bioassay with Douglas-fir and paper birch seedlings inoculated with soils collected from a planted (to Douglas-fir) clearcut in the southern interior of British Columbia that was originally forested with paper birch, Douglas-fir, and other EM tree species. Our objective was to describe the type and abundance of EM formed on Douglas-fir and paper birch seedlings when grown alone and in mixture. The EM described in this study will contribute to a base-line database for future comparisons among other studies in similar ecosystems. The influence of host specificity and host species interactions on patterns of colonization are discussed in relation to interplant hyphal connections, nutrient transfer, and plant community dynamics.

Methods

Site description

Soil was collected in March 1993 from a clearcut site in the North Adams River valley in south central British Columbia (51°28′N, 119°24′W). The site occurs at 700 m elevation within the Thompson variant of the Moist Warm Interior Cedar–Hemlock biogeoclimatic subzone, which is characterized by warm, moist summers and cold, snowy winters (Lloyd et al. 1990). The soil type is a Humo-Ferric Podzol (Canada Soil Survey Committee 1978) formed over a granitic alluvial blanket. The soil surface layers (to 50 cm) are sandy loam to loamy sand, with coarse fragment content less than 10%. The original mixed forest of Douglas-fir, paper birch, western hemlock, western redcedar (*Thuja plicata* Donn ex D. Don.), and trembling aspen (*Populus tremuloides* Michx.) was clear-cut in 1987 and planted with Douglas-fir in 1988. Areas between the planted seedlings were occupied by native hardwoods.

Soil collection and preparation

Soil was collected from a 0.25-ha area located in the middle of the 13-ha clearcut. Soil was collected to 15 cm depth, including forest floor and buried organic material, from five sample points randomly located between Douglas-fir seedlings and paper birch sprouts. The five samples were combined to make one sample. The combined sample was placed in plastic bags, set on ice in a cooler, and then transported to the laboratory, where it was immediately sieved to 4 mm, homogenized, and mixed (3:1 v/v) with perlite to minimize compaction. Small woody debris, fine roots, and EM inoculum were not removed from the sample. The soil sample was split, and one portion was left untreated and the other autoclaved at 180°C for 3 h for preparation of controls to detect EM contaminants from the greenhouse. The soil mixtures were then distributed to eighty 600-mL (2-D) sterilized Leach tubes (Ray Leach 'Cone-tainer' Single Cell System, supplied by Stuewe and Sons, Corvallis, Oregon): 50 tubes were filled with untreated soil mixture and 30 with autoclaved mixture. The Leach tubes were set up in a greenhouse at Oregon State University, Corvallis.

Study design

The greenhouse bioassay consisted of tree species grown in monoculture and dual culture as "bait" for EM fungal inoculum in the untreated field soil. The three treatments differed in mycorrhizal host

species: Douglas-fir alone (1 seedling per tube), paper birch alone (1 seedling per tube), and Douglas-fir and paper birch in mixture (2 seedlings per tube). The three treatments were replicated 16 times in a completely randomized design. Because of seedling mortality during the growing period, however, the number of replicates actually harvested was 16 of Douglas-fir alone, 12 of paper birch alone, and 9 of Douglas-fir and paper birch in mixture. The autoclaved control tubes were also planted with the three species mixtures to detect EM contaminants from the greenhouse.

Seedling preparation and growth conditions

Douglas-fir seeds were surface sterilized and stratified by soaking first in 30% H_2O_2 for 2 min and then in 3% H_2O_2 for 5 h, rinsed with distilled water, and dried at room temperature for 24 h. Paper birch seed was soaked in aerated 10% H_2O_2 for 15 min, and rinsed and dried as above. Five seeds per species were planted per monoculture Leach tube on May 1, 1993. Douglas-fir seeds were buried to 1 cm depth, and paper birch were barely covered with sterile silica sand to stabilize the surface and reduce mortality due to damping-off fungi. Ten seeds were planted in the dual culture tubes (5 seeds per species) and covered with 0.25 cm silica sand. Most seeds germinated within 2 weeks, and germinants were thinned to one per species per tube after 4 weeks. Every 6 months, each Leach tube received 50 mL of Peters solution (20:20:20 N–P–K) to maintain seedling growth. The seedlings were grown for 16 months.

Seedlings were grown in the greenhouse under sodium-vapor lamps with a 16 h : 8 h light:dark cycle complementing natural light and providing a minimum intensity of 280 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Air temperature ranged between 24°C (light) and 18°C (dark). Leach tubes were watered on alternate days and relocated on the greenhouse bench monthly to reduce environmental differences.

Mycorrhiza assessments

Leach tubes were randomly harvested and root systems examined over a 1-month period when seedlings were 16 months old. Seedlings were removed from Leach tubes, roots of different species were carefully separated, and then thoroughly washed with tap water. The EM were examined according to guidelines of Agerer (1987) and Ingleby et al. (1990). Root squashes and hand sections were examined microscopically to characterize fine details and determine the presence of a Hartig net.

Abundance of each EM type was determined by placing the entire root system over a clear plastic grid with numbered (2.5-cm^2) squares (Smith et al. 1995). Root tips were sampled in randomly selected squares to count at least 100 tips per seedling. The proportion of root tips colonized by each of the various EM types was calculated for each seedling root system. For each treatment, abundance of a particular EM was the average percentage of root tips per seedling per species colonized by that type (based on all replicates per treatment). Frequency was the number of seedlings per species on which a particular type was present. After mycorrhizae were identified and enumerated, root tissue was oven-dried at 80°C for 48 h and weighed.

Statistical analysis

The ability of host tree species to share compatible EM fungi was evaluated based on composition of EM on seedling root tips. Abundance of each EM morphotype (percentage of root tips per tree species) and seedling root biomass were compared among host species (Douglas-fir, paper birch) and host species associations (grown alone, grown in mixture) in a 2 × 2 factorial structure of treatments using analysis of variance (ANOVA) for unbalanced data in a completely randomized design ($n = 16$ for Douglas-fir alone, $n = 12$ for paper birch alone, $n = 9$ for Douglas-fir in mixture, $n = 9$ for paper birch in mixture). Where host species × host species association interactions occurred in the ANOVA, abundance of EM morphotypes and seedling root biomass were compared between paper birch and Douglas-fir hosts using *t*-tests ($n = 21$ for paper birch, $n = 25$ for Douglas-fir). Chi-square tests were used to compare frequency of EM (number of root tips colonized by each type per seedling) between host species associations (Douglas-fir or paper birch grown alone versus in mixture). Small cell counts were avoided by testing frequencies (number of colonized tips) rather than abundance (percentage of colonized tips) of EM and by including in the host association comparisons only those EM that were associated with the tested host species ($n = 9$ for paper birch, $n = 8$ for Douglas-fir). Statistical analysis was conducted using SAS Institute Inc. (1989).

Results

Ectomycorrhizae

Eleven EM morphotypes were identified on paper birch and Douglas-fir hosts (Table 1). Seven types associated with both Douglas-fir and paper birch to varying degrees, one was unique to Douglas-fir and three were unique to paper birch. Morphological characteristics allowed for identification to the genus level for all EM and the species level for some. The types are described below in order of abundance.

The EM common to Douglas-fir and paper birch

MRA: Those formed by the *Mycelium radicis atrovirens* complex of fungi colonized 96% of Douglas-fir and paper birch seedlings, but was more dominant on the roots of paper birch (mean abundance 67%) than Douglas-fir (mean abundance 45%, Table 1). The EM were cylindrical, unbranched, dark brown to black, with a loose mantle. The seedling root tip was usually uncolonized because it grew through the mantle. The mantle surface was an irregular locking synenchyma, or fine jigsaw pattern, with large (10 μm wide), pillowy hyphal cells. Extramatrical hyphae were 2–4 μm wide, light brown, septate, without clamps. Strands were absent.

E-strain I: E-strain I colonized 91% of Douglas-fir and paper birch seedlings, but was more dominant on the roots of Douglas-fir (mean abundance 36%) than paper birch (mean abundance 15%, Table 1). Fruiting bodies of the genus *Wilcoxina* were found in several Leach tubes with paper birch and Douglas-fir. Features of *Wilcoxina* corresponded closely with those described by Danielson (1982) for E-strain on *Pinus banksiana* Lamb. The EM were branched, smooth, pale to reddish brown with a light tip. The mantle was a discontinuous, irregular synenchyma lock, with large (2–8 μm wide) thick-walled hyphal cells. Extramatrical hyphae were rare, light brown, 3–10 μm wide, branched, without clamp connections. Loose, reddish brown strands were rare.

Cenococcum: *Cenococcum geophilum* Fr. colonized 20% of Douglas-fir and 90% of paper birch seedlings, but usually (88% of those colonized) occupied less than 2% of the root systems (Table 1). The EM were cylindrical, unbranched, up to 5 mm long, black, with a smooth texture under abundant emanating hyphae. The mantle was compact, black, net synenchyma, with a radiating isocentric pattern (Trappe 1971). Emanating hyphae were bristly, fragile, black, smooth, 3–5 μm wide, septate, without clamp connections. Strands were absent.

Tuber: Tuber was present on 62% of paper birch and only 4% of Douglas-fir seedlings, and was more abundant on the root

Table 1. Abundance of EM on Douglas-fir and paper birch seedlings grown in potted soil from a mixed Douglas-fir and paper birch stand.

EM type[†]	Host	No. of seedlings colonized (controls)[‡]	No. of colonized seedlings with <5% colonization (controls)[‡]	Mean abundance (%) of EM type on host[§]
MRA complex	Douglas-fir	23/25 (0)	5/23 (na)	45.0 (6.1)*b*
	Paper birch	21/21 (0)	0/21 (na)	66.5 (3.8)*a*
	Host total	44/46 (0)	5/44 (na)	*p*=0.006
E-strain I	Douglas-fir	22/25 (77)	3/22 (0)	35.8 (6.2)*a*
	Paper birch	20/21 (35)	0/20 (0)	15.3 (2.3)*b*
	Host total	42/46 (56)	3/42 (0)	*p*=0.006
Cenococcum	Douglas-fir	5/25 (0)	3/5 (na)	0.7 (0.3)*a*
	Paper birch	19/21 (23)	18/19 (67)	1.8 (0.5)*a*
	Host total	24/46 (12)	21/24 (67)	*p*=0.053
Tuber	Douglas-fir	1/25 (0)	1/1 (na)	0.1 (0.1)*b*
	Paper birch	13/21 (0)	6/13 (na)	4.2 (1.3)*a*
	Host total	14/46 (0)	7/14 (na)	*p*=0.001
Thelephora	Douglas-fir	3/25 (73)	1/3 (5)	1.0 (0.7)*a*
	Paper birch	5/21 (77)	2/5 (0)	1.3 (0.7)*a*
	Host total	8/46 (75)	3/8 (3)	*p*=0.749
E-strain II	Douglas-fir	3/25 (0)	0/3 (na)	1.1 (0.7)*a*
	Paper birch	5/21 (0)	2/5 (na)	5.6 (3.2)*a*
	Host total	8/46 (0)	2/8 (na)	*p*=0.145
Laccaria	Douglas-fir	2/2 (5)	2/2 (na)	0.1 (0.0)*a*
	Paper birch	2/21 (81)	2/25 (0)	0.2 (0.1)*a*
	Host total	4/46 (40)	4/4 (5)	*p*=0.416
Rhizopogon	Douglas-fir	23/25 (0)	2/23 (na)	15.7 (2.1)*a*
	Paper birch	0/21 (0)	0/0 (na)	0.0 (0.0)*b*
	Host total	23/46 (0)	2/23 (na)	*p*=0.000
Hebeloma	Douglas-fir	0/25 (0)	0/0 (na)	0.0 (0.0)*b*
	Paper birch	12/21 (0)	8/12 (na)	1.6 (0.6)*a*
	Host total	12/46 (0)	8/12 (na)	*p*=0.007
Lactarius	Douglas-fir	0/25 (0)	0/0 (na)	0.0 (0.0)*b*
	Paper birch	10/21 (0)	4/10 (na)	3.1 (1.4)*a*
	Host total	10/46 (0)	4/10 (na)	*p*=0.020
Tuber-like	Douglas-fir	0/25 (0)	0/0 (na)	0.0 (0.0)*a*
	Paper birch	2/21 (0)	1/2 (na)	0.4 (0.3)*a*
	Host total	2/46 (0)	1/2 (na)	*p*=0.164

[†]In descending order of abundance.

[‡]Data are expressed as fraction of seedlings colonized by the indicated EM type. Numbers in parentheses are the mean percent abundance on root tips of control seedlings. na, not applicable because type absent from all control host seedlings.

[§]The average percent of root tips colonized. Numbers in parentheses are standard errors. Values followed by the same letter are not significantly different at the 5% level. *p*-value denotes significance of *t*-test ($n = 21$ for Douglas-fir, $n = 25$ for paper birch; df = 1).

systems of paper birch (mean abundance 4%) than Douglas-fir (mean abundance < 1%, Table 1). The EM were single to infrequently branched, stout, smooth, creamy buff colored, with abundant, needlelike, tapering setae bristling out from the mantle surface. The setae were 50–150 µm long and 3–4 µm wide. The mantle was a smooth, compact, irregular interlocking synenchyma, 10–20 µm wide. Emanating hyphae were rare, hyaline, smooth, rarely branched, 2–5 µm wide, septate, without clamp connections. Strands were absent.

Thelephora: *Thelephora* occurred on 17% of paper birch and Douglas-fir and occupied only 1% of their root systems (Table 1). The EM were pinnately branched, light to medium brown. The compact mantle was smooth to rough textured, with infrequent emanating hyphae. The emanating hyphae were smooth, hyaline, 4 µm wide, infrequently branched, with clamp connections. Cystidia were hyaline, 4 µm wide and 100 µm long, with basal clamp connections. Light brown strands were occasionally present.

E-strain II: E-strain II was present on 17% of paper birch and Douglas-fir seedlings, and was more abundant on root systems of paper birch (mean abundance 6%) than Douglas-fir (mean abundance 1%, Table 1). The EM were single, rough, dark brown with a pale tip. The mantle was a discontinuous, compact, irregular synenchyma lock, with large (up to 8 μm wide) hyphal cells. Emanating hyphae were few, hyaline, 4–5 μm wide, minimally branched, without clamp connections. Strands were absent.

Laccaria: Laccaria occurred on 9% of paper birch and Douglas-fir seedlings, and occupied less than 1% of their root systems (Table 1). *Laccaria laccata* sensu *latu* fruited in some of the Leach tubes where this occurred. The EM were single, 8–10 mm long and 3–4 mm wide, whitish to dark brown, cottony textured. The loose, cottony mantle was a net prosenchyma of variable density. Abundant emanating hyphae were hyaline to light brown, 3–4 μm wide, moderately branched, tortuous, verrucose, with abundant clamp connections 2–4 μm wide. Loose cottony strands were present.

The EM unique to Douglas-fir

Rhizopogon: Rhizopogon in the section *Villosuli* occurred on 92% of Douglas-fir seedlings, where it occupied on average 16% of the root systems (Table 1). The EM were single to pinnately branched to clumped, dark brown to light brown to white. The mantle was a rough, crusty, felt prosenchyma with abundant external hyphae. Emanating hyphae were hyaline to light brown, 2–3 μm wide, smooth, with infrequent branching and without clamp connections. Cystidia were up to 10 μm wide and 3 mm long, and frequently bent. Brown strands were abundant, 25 μm wide.

The EM unique to paper birch

Hebeloma: Hebeloma occurred on 57% of paper birch seedlings, where it generally occupied <2% of the root systems (Table 1). The EM were single to pinnately branched, yellow to blackish, crusty textured, with abundant adhering soil. The compact mantle was rough, with yellowish hyaline hyphae up to 8 μm wide. Emanating hyphae were rare, hyaline, smooth, 4 μm wide, with very infrequent branching and without clamp connections. Loose strands were present.

Lactarius: Lactarius occurred on 48% of paper birch seedlings, where it occupied on average 3% of the root system (Table 1). The EM were single to pinnately branched, smooth, swollen, long, white to yellowish. The compact, smooth mantle was a net synenchyma with lactiferous hyphae up to 10 μm wide. Emanating hyphae were 2 μm wide, hyaline, smooth, unbranched, with rare, flattened clamp connections. Compact, white–yellow strands were present.

Tuber-like: Tuber-like type occurred on 10% of paper birch seedlings, where it occupied <1% of the root system (Table 1). The EM were single to infrequently branched, smooth, brown, with few, very fine, hyaline cystidia emanating from the mantle surface. The cystidia were approximately 50 μm long and 2 μm wide. The dark color of the mantle as well as scarcity and small size of cystidia were the main features distinguishing *Tuber*-like type from *Tuber*. The mantle was a smooth, compact, irregular interlocking synenchyma, 10–15 μm wide. Emanating hyphae were rare, hyaline, smooth, rarely branched, 2–3 μm wide, without clamp connections. Strands were absent.

Greenhouse contaminants

The greenhouse contaminants on Douglas-fir control seedlings were E-strain I and *Thelephora* (Table 1), which each colonized 75% of the seedlings and occupied 65% and 58% of their root tips, respectively. E-strain I also occurred on the majority of Douglas-fir in unsterilized soil, but occupied on average only 36% of the root systems. *Thelephora* was detected on <20% of Douglas-fir in unsterilized soil and occupied on average only 1% of the root systems. The greenhouse contaminants on paper birch control seedlings were mainly *Thelephora*, *Laccaria*, E-strain I, and occasionally, *Cenococcum* (Table 1). *Thelephora* and *Laccaria* each colonized >75% of control paper birch seedlings, and occupied 65% and 54% of their root tips, respectively. Both were detected on <25% of paper birch seedlings in unsterilized soil and occupied <2% of their root systems. E-strain I and *Cenococcum* each colonized <35% of control paper birch seedlings and occupied only 4% and 1% of their root tips, respectively. Both increased in frequency and abundance in unsterilized soils. The dramatic difference in abundance of E-strain I, *Thelephora*, and *Laccaria* on Douglas-fir or paper birch between control and unsterilized soils suggests that the importance of their presence as contaminants in the greenhouse was diminished in the company of fungi local to Adams Lake.

Comparison of EM between Douglas-fir and paper birch

Douglas-fir and paper birch shared in common MRA, E-strain I, *Cenococcum*, *Tuber*, *Thelephora*, E-strain II, and *Laccaria* (Table 1). The EM were determined "in common" where their morphological characteristics matched between tree species. *Rhizopogon* was unique to Douglas-fir and *Hebeloma*, *Lactarius*, and *Tuber*-like were unique to paper birch. The dominant EM on paper birch were MRA (mean abundance 67%), E-strain I (15%), E-strain II (6%), *Tuber* (4%), and *Lactarius* (3%). The dominant EM on Douglas-fir were MRA (mean abundance 45%), E-strain I (36%), and *Rhizopogon* (16%). The percentage of root tips colonized by common EM averaged 84% for Douglas-fir and 96% for paper birch.

Of the shared EM, all were of similar frequency between the two tree species, except that *Cenococcum* and *Tuber* were more frequent on paper birch (>60% of seedlings) than on Douglas-fir (<20% of seedlings). Mean abundance of MRA, *Cenococcum*, and *Tuber* was greater on paper birch than on Douglas-fir ($p < 0.05$), and mean abundance of E-strain I was greater on Douglas-fir than on paper birch ($p < 0.01$, Table 1).

Comparison of EM between seedlings grown alone and in mixture

Tuber was absent from Douglas-fir when grown alone, but occurred on 11% (1/9) of the Douglas-fir seedlings grown in mixture with paper birch; it colonized on average 62% of paper birch seedlings in mixture and monoculture. Additional EM were not detected on root systems of paper birch when grown in mixture compared with when grown alone; however, frequency (number of seedlings colonized) and abundance (average

Table 2. Comparisons of frequency of EM on root systems of Douglas-fir and paper birch between those grown alone and those in mixture.

EM type	Douglas-fir alone	Douglas-fir in mixture	Effect of mix[†]	Paper birch alone	Paper birch in mixture	Effect of mix[‡]
MRA complex	16/16 (100)	9/9 (100)		12/12 (100)	9/9 (100)	
E-strain I	13/16 (81)	9/9 (100)	+	11/12 (92)	9/9 (100)	
Cenococcum	3/16 (19)	2/9 (22)		10/12 (83)	9/9 (100)	+
Tuber	0/16 (0)	1/9 (11)	+	7/12 (58)	6/9 (67)	
Thelephora	3/16 (19)	0/9 (0)	—	4/12 (33)	1/9 (11)	—
E-strain II	3/16 (19)	0/9 (0)	—	3/12 (25)	2/9 (22)	
Laccaria	2/16 (13)	0/9 (0)	—	2/12 (17)	0/9 (0)	—
Rhizopogon	16/16 (100)	7/9 (78)	—	0/12 (0)	0/9 (0)	
Hebeloma	0/16 (0)	0/9 (0)		5/12 (42)	7/9 (78)	+
Lactarius	0/16 (0)	0/9 (0)		2/12 (17)	8/9 (89)	+
Tuber-like	0/16 (0)	0/9 (0)		1/12 (8)	1/9 (11)	
χ^2 test[§]		$p<0.001$[‖]			$p<0.001$[¶]	

Note: Data are expressed as number of seedlings colonized. Numbers in parentheses are percent of seedlings.

[†]Difference of ±10% between Douglas-fir in mixture and Douglas-fir alone.

[‡]Difference of ±10% between paper birch in mixture and paper birch alone.

[§]Level of significance for differences in frequency of EM (number of tips colonized by each type per seedling, data not shown) between host species associations (Douglas-fir or paper birch grown alone versus in mixture) was determined by χ^2 test.

[‖]χ^2 test for Douglas-fir included only those EM types that occurred on Douglas-fir (df = 7).

[¶]χ^2 test for paper birch included only those EM types that occurred on paper birch (df = 8). *Tuber* and *Tuber*-like were combined because of their low frequency and morphological similarity.

percentage of root tips colonized per seedling) of several EM on paper birch and Douglas-fir were affected by the association. On Douglas-fir, E-strain I and *Tuber* occurred on >10% more seedlings in mixture (9/9 and 1/9, respectively) than monoculture (13/16 and 0/16, respectively), while *Rhizopogon* (7/9 in mixture versus 16/16 in monoculture), *Thelephora* (0/9 versus 3/16), E-strain II (0/9 versus 3/16), and *Laccaria* (0/9 versus 2/16) occurred on >10% fewer seedlings (Table 2). On paper birch, *Cenococcum*, *Hebeloma*, and *Lactarius* increased in frequency by >10% in mixture (9/9, 7/9, and 8/9, respectively) versus monoculture (10/12, 5/12, and 2/12, respectively), whereas *Thelephora* (1/9 in mixture versus 4/12 in monoculture) and *Laccaria* (0/9 versus 2/12) decreased in frequency by >10%. The frequencies of EM on Douglas-fir and paper birch grown alone were different from those grown in mixture ($p < 0.001$, Table 2).

The EM abundance was affected by host species association (i.e., grown in isolation versus in mixture) for 2 of the 11 types: E-strain I ($p = 0.091$) and *Rhizopogon* ($p = 0.050$, Table 3). E-strain I increased in abundance from 25% when Douglas-fir was grown alone to 54% when grown in mixture with paper birch. Similarly, it increased in abundance from 13% to 19% on paper birch when mixed with Douglas-fir. Abundance of *Rhizopogon* decreased from 20% when Douglas-fir was grown alone to 9% when in mixture with paper birch. There were no interactions between host species and host species association in abundance per seedling for any of the EM.

Influence of seedling competition on EM colonization

We observed that paper birch grew faster and shaded Douglas-fir seedlings in the mixture Leach tubes. As a result, root biomass of Douglas-fir in mixture with paper birch was only 15% of that grown in monoculture ($p = 0.001$, Table 4). Conversely, paper birch growth appeared to benefit from the association with Douglas-fir; its root biomass was 1.7 times greater in mixture than in monoculture ($p = 0.079$). Shoot biomass was not measured for either species; as a result, we cannot determine whether biomass differences between mixture and monoculture seedlings resulted from uniform changes in whole seedling growth or from shifts in allocation between roots and shoots. The lower root biomass of Douglas-fir in mixture corresponded to reduced frequency and abundance of *Rhizopogon*, as well as reduced frequency of *Thelephora*, E-strain II, and *Laccaria*. Note, however, that frequency of *Thelephora* and *Laccaria* also decreased among paper birch in spite of its increased root biomass in mixture versus isolation. In contrast, greater root biomass of paper birch in mixture corresponded with increased frequency of *Cenococcum*, *Hebeloma*, and *Lactarius*.

Discussion

Patterns of host specificity

The soil bioassay resulted in formation of EM on Douglas-fir and paper birch in monoculture and mixture with 11 fungal types: 8 EM types occurred on Douglas-fir (in order of abundance: MRA, E-strain I, *Rhizopogon*, E-strain II, *Thelephora*, *Cenococcum*, *Tuber*, and *Laccaria*) and 10 on paper birch (in order of abundance: MRA, E-strain I, E-strain II, *Tuber*, *Lactarius*, *Cenococcum*, *Hebeloma*, *Thelephora*, *Tuber*-like, and *Laccaria*). Patterns of host specificity were similar to those observed by M.D. Jones (unpublished results), who examined roots of 2-year-old outplanted Douglas-fir and paper birch seedlings on the same site where soils were collected for the present study. All of the EM identified in our bioassay appeared to correspond to those found on the outplanted seedlings. An additional 19 EM occurred on the outplanted seedlings, which may have been a function of the diversity of native EM plant species accessible to seedling roots, a wider range of

Table 3. Abundance of EM on Douglas-fir and paper birch alone and in mixture.

EM type†	Douglas-fir alone	Douglas-fir in mixture	Paper birch alone	Paper birch in mixture	*p*-values‡
MRA complex	50.3 (8.3)	35.7 (8.1)	70.8 (4.8)	60.8 (5.9)	S, p=0.016** A, p=0.297 S×A, p=0.820
E-strain I	25.4 (7.6)	54.4 (7.6)	12.6 (1.9)	19.0 (4.8)	S, p=0.030** A, p =0.091* S×A, p=0.230
Cenococcum	0.7 (0.4)	0.7 (0.6)	1.9 (0.8)	1.8 (0.5)	S, p=0.079* A, p=0.639 S×A, p=0.940
Tuber	0.0 (0.0)	0.3 (0.3)	4.0 (1.6)	4.4 (2.1)	S, p=0.001** A, p =0.360 S×A, p=0.381
Thelephora	1.5 (1.1)	0.0 (0.0)	2.1 (1.2)	0.1 (0.1)	S, p=0.944 A, p=0.199 S×A, p=0.975
E-strain II	1.8 (1.0)	0.0 (0.0)	3.4 (3.3)	8.6 (6.2)	S, p=0.371 A, p=0.935 S×A, p=0.623
Laccaria	0.1 (0.1)	0.0 (0.0)	0.3 (0.2)	0.0 (0.0)	S, p=0.471 A, p=0.287 S×A, p=0.634
Rhizopogon	19.5 (2.6)	8.9 (2.7)	0.0 (0.0)	0.0 (0.0)	S, p=0.000** A, p=0.050** S×A, p=0.204
Hebeloma	0.0 (0.0)	0.0 (0.0)	2.0 (1.0)	1.1 (0.5)	S, p=0.007** A, p=1.000 S×A, p=1.000
Lactarius	0.0 (0.0)	0.0 (0.0)	2.5 (2.4)	4.0 (0.8)	S, p=0.061* A, p=0.562 S×A, p=0.581
Tuber-like	0.0 (0.0)	0.0 (0.0)	0.5 (0.5)	0.2 (0.2)	S, p=0.170 A, p=0.980 S×A, p=0.980

Note: Mean abundance is the average percent of root tips colonized. Numbers in parentheses are standard errors.

†In descending order of abundance.

‡ANOVA of 2 × 2 factorial structure of treatments in a completely randomized design (n = 16 for Douglas-fir alone, n = 9 for Douglas-fir in mixture, n = 12 for paper birch alone, n = 9 for paper birch in mixture; df = 1).

*ANOVA detected significant difference at $p < 0.10$.

**ANOVA detected significant difference at $p < 0.05$.

environmental niches available for a greater diversity of EM, the greater seedling age in field soils than greenhouse bioassay soil, as well as the larger number of outplanted than greenhouse seedlings examined. Specifically, root systems of outplanted Douglas-fir were dominated by *Rhizopogon*, E-strain, MRA, *Thelephora*, and *Cenococcum*, with lower abundance of *Tuber*, *Laccaria*, and 14 other minor types. Paper birch root systems were dominated by *Thelephora,* MRA*,* E-strain, and *Cenococcum*, with lower abundance of *Lactarius*, *Hebeloma*, *Laccaria*, and 14 other minor types.

Common EM on young seedlings in greenhouses and disturbed sites include *Thelephora* (Molina and Trappe 1984), MRA (Danielson et al. 1985; Ingleby et al. 1990), E-strain (Danielson et al. 1985; Smith et al. 1995), and *Laccaria* (Molina and Trappe 1984). Occurrence (frequency, abundance) of these EM on paper birch and Douglas-fir seedlings in our soil bioassay was similar to that on outplanted seedlings (M.D. Jones, unpublished results), suggesting that greenhouse contaminants had a minor effect on mycorrhizal composition of our seedlings. All of the EM common to both paper birch and Douglas-fir in this study (MRA, E-strain I, *Cenococcum*, *Tuber*, *Thelephora*, E-strain II, and *Laccaria*) generally are common on a broad range of coniferous and deciduous hosts (Molina et al. 1992; Smith et al. 1995; Ingleby et al. 1990).

Table 4. Root dry weight of Douglas-fir and paper birch seedlings grown alone and in mixture in treatment soil.

Species	Dry weight (g) when alone	Dry weight (g) when in mixture	*t*-test *p*-values[†]
Paper birch	1.06 (0.17)	1.80 (0.35)	0.079
Douglas-fir	0.54 (0.10)	0.08 (0.02)	0.001

Note: Numbers in parentheses are standard errors.

[†]*t*-test comparison between associations (alone versus mixture) for each species. Degrees of freedom and probability level are based on Satterthwaite's approximation for unequal variances (df = 11.7 for paper birch, df = 16.4 for Douglas-fir). The *t*-test comparison was made because of a significant interaction ($p < 0.001$) in the two host species × two host association ANOVA.

Unique to paper birch were *Lactarius* and *Hebeloma*. *Lactarius* and *Hebeloma* have been identified as common EM genera of *Betula* species in Great Britain (e.g., Ford et al. 1980; Fox 1983; Watling 1984; Mason et al. 1984; Last et al. 1984; Deacon and Fleming 1992). Several species of *Lactarius* and *Hebeloma* are listed as having a broad range and several a narrow range of hosts by Molina et al. (1992). *Lactarius* and *Hebeloma* in our study were not identified to species, but were found only on paper birch. In contrast with our results, M.D. Jones (unpublished results) found two types each of *Lactarius* and *Hebeloma* on outplanted seedlings of both paper birch and Douglas-fir, indicating that Adams Lake soil has inoculum potential for broad host ranging species of *Lactarius* and *Hebeloma*. These field results indicate that our soil bioassay did not fully reflect the overlap in EM between the two tree species growing in Adams Lake soil.

Most EM associates identified in our study have "early-stage" characteristics (MRA, E-strain I, *Cenococcum*, *Thelephora*, E-strain II, *Hebeloma* and *Laccaria*) and some have "late-stage" characteristics (*Tuber*, *Lactarius*, and *Rhizopogon*) described by Deacon and Fleming (1992). Early-stage fungi are characterized by low carbon demands, ready establishment from spores or mycelium, and a ruderal life history. Conversely, late-stage fungi are characterized by high carbon demands, establishment on older root systems or in older soil, poor establishment from spores, and usually advanced development of rhizomorphs or strands (Deacon et al. 1983; Last et al. 1984; Fleming 1983, 1985; Deacon and Fleming 1992). The predominance of early-stage versus late-stage fungi in our study may have resulted from the young age of host seedlings, the growth restrictions imposed by the bioassay environment (e.g., small soil volume in Leach tubes), and the type of propagules in the greenhouse bioassays (e.g., predominantly spores and some vegetative mycelium rather than predominantly vegetative mycelium associated with older roots). Both classes of fungi potentially can form hyphal connections and facilitate nutrient or water transfer between plants. The probability that fungi form hyphal connections between paper birch and Douglas-fir is greatest among the early-stage EM because most have a broad range of hosts (Newman 1988; Molina et al. 1992). However, the amount of material transferred between paper birch and Douglas-fir potentially is greatest among late-stage fungi because strands or rhizomorphs are thought to play a greater role in translocation of water, nutrients, or carbon than simple extramatrical hyphae (Cairney 1992).

Rhizopogon was abundant on Douglas-fir but absent from all paper birch seedlings in our greenhouse bioassay. *Rhizopogon* species are numerous in western coniferous forests of North America and are considered host genus specific with Pinaceae (Molina and Trappe 1994). There are a few experimentally confirmed exceptions, however; *Rhizopogon* has been shown to form EM with ericaceous hosts *Arbutus menziesii* Pursh and *Arctostaphylos uva-ursi* (L.) Spreng. (Molina and Trappe 1982*a*), and *G. shallon* and *Rhododendron macrophyllum* in mixed plantings with well-colonized Douglas-fir in seedling microcosms (Smith et al. 1995). Read and Finlay (1985) also found that *Rhizopogon roseolus* Corda sensu Smith spread from *Pinus* to *Betula* roots in seedling microcosms. Some have suggested the possibility of *Rhizopogon* links between conifers and companion plants, and subsequent facilitation of interplant nutrient transfer (e.g., Read and Finlay 1985; Smith et al. 1995). *Rhizopogon* in the section *Villosuli* specifically, however, are specialized towards mycorrhizal association with Douglas-fir (Molina and Trappe 1994). In our study, *Rhizopogon* in the section *Villosuli* did not form EM with paper birch when in dual culture with well-colonized Douglas-fir seedlings. Members of the section *Villosuli* have had limited mycorrhizal development on western hemlock in pure culture syntheses (Molina and Trappe 1994), but not in dual culture soil bioassays where western hemlock was grown in mixture with well-colonized Douglas-fir hosts (Massicotte et al. 1994). R. Molina (unpublished data) also found that several *Rhizopogon* in the section *Villosuli* were unable to spread from well-colonized Douglas-fir to either *Arctostaphylos uva-ursi* or *Arbutus menziesii* in polycultures. In contrast, Smith et al. (1995) found in another polyculture soil bioassay that EM of *Rhizopogon* in the section *Villosuli* repeatedly occurred on western hemlock, and they suggested that the length of time in the presence of a well-colonized primary host (Douglas-fir) may have influenced hemlock's colonization with the host-specific fungus. In the present study, Douglas-fir and paper birch were grown in mixture for 16 months before their mycorrhizae were assessed, which is longer than other dual culture experiments where *Rhizopogon* inoculation of companion plants occurred (e.g., Read and Finlay 1985; Smith et al. 1995).

Effect of mixture

The change in EM colonization of Douglas-fir and paper birch when grown together in mixture compared with monoculture included both appearance of an additional type on Douglas-fir as well as variation in frequency and abundance of several types on Douglas-fir and paper birch. *Tuber* associated only with paper birch in single-species tubes, but colonized both paper birch and Douglas-fir in one of nine dual culture tubes. *Tuber* was more abundant on paper birch (20% of root tips colonized) in that particular dual culture tube than in the others where it was present (on average 4% of root tips colonized), suggesting that it may have spread from well-colonized paper birch to Douglas-fir roots. More extensive colonization of paper birch with *Tuber* increases the probability that its hyphae contact and initiate mycorrhizae on the secondary host, Douglas-fir. The result also appears to support the view that variation in colonization potential depends on whether a mycorrhizal fungus is already linked to a compatible host (Massicotte et al. 1994). The carbon requirements of some fungi to form EM may not be satisfied by some less compatible hosts until it is supplemented

by photosynthate from a linked more compatible host. Massicotte et al. (1994) drew a comparison with mycorrhizal succession on *Betula pendula* Roth (e.g., Fleming 1983; Fleming et al. 1984), where late-stage fungi, such as *Lactarius* and *Tuber*, formed EM with birch seedlings grown either in mycorrhizal soil (i.e., soil from immediately beneath a birch tree) in the greenhouse or with access to roots of mature birch trees in the field. Conversely, early-stage fungi, such as *Thelephora* and *Hebeloma*, formed EM with birch seedlings grown either in nonmycorrhizal soil (i.e., soil taken far away from a birch tree) or in isolation from mature birch trees in the field.

Rhizopogon abundance on Douglas-fir was significantly lower when it was grown in mixture with paper birch (9%) than when grown alone (20%). The reduction in *Rhizopogon* abundance corresponded to a dramatic reduction in Douglas-fir root biomass (0.08 g in mixture versus 0.54 g in isolation), presumably as a result of resource competition with paper birch. *Rhizopogon* produced prolific rhizomorphs on Douglas-fir seedlings in this study, which is typical of many *Rhizopogon* spp. (Molina and Trappe 1994). Extensive rhizomorph (strand or chord) networks are thought to be largely responsible for enhanced nutrient uptake (Read 1992), water uptake (Duddridge et al. 1980; Brownlee et al. 1983), drought stress tolerance (Dosskey et al. 1990), and carbon, nutrient, and water transfer between linked plants (e.g., Read et al. 1985; Duddridge et al. 1988). They also form the main linkages between a plant's root system and diffuse mycelial front (Read 1992), which has been shown as a major sink for photosynthate (Finlay and Read 1986). Based on fungal biomass (Deacon and Fleming 1992) and mycorrhizal stimulation of photosynthesis (Dosskey et al. 1990), the carbon drain on plants imposed by EM with extensive rhizomorph networks (e.g., *Rhizopogon* in this study) would be much greater than those with weak sheath and extramatrical hyphal development (e.g., MRA or E-strain I in this study). Considerable quantities of current photosynthate are allocated to extramatrical hyphae (e.g., Miller et al. 1989), which suggests that smaller Douglas-fir in mixture with paper birch would have lower capacity to support abundant *Rhizopogon* than would more robust, healthier Douglas-fir growing in isolation. Interestingly, larger root biomass of paper birch growing in mixture with Douglas-fir (1.80 g) than in isolation (1.06 g) corresponded to a >70% increase in frequency of *Lactarius* and >35% increase in frequency of *Hebeloma*, both of which were characterized in this study by the presence of rhizomorphs.

E-strain I abundance on Douglas-fir was significantly greater when it was grown in mixture with paper birch (54%) than when grown alone (25%). It also was more abundant on paper birch in mixture than isolation; however, the difference between associations was likely too small to be of ecological significance (19% in mixture versus 13% in isolation). E-strain mycorrhizae are prolific colonizers on burned sites and in nurseries (e.g., Danielson 1982; Wilcox et al. 1983) and are characterized by thin mantles and infrequent extramatrical hyphae (Ingleby et al. 1990). That they are host generalists, have low vegetative capacity (Wilcox et al. 1983), and are readily replaced by other more aggressive fungi is suggestive of the early-stage descriptions by Deacon and Fleming (1992). On healthy, robust Douglas-fir seedlings, abundance of E-strain I would normally decrease and abundance of EM such as *Rhizopogon* would increase with seedling age. The photosynthate food base provided by Douglas-fir in mixture with paper birch may have been too small, however, for *Rhizopogon* and other robust fungi to colonize extensively, thereby allowing E-strain I to persist.

The effect of species mixtures on mycorrhizal occurrence of host plants expands earlier observations in dual culture and polyculture experiments (e.g., Read and Finlay 1985; Massicotte et al. 1994; Smith et al. 1995). These polyculture studies showed that secondary host species (e.g., hemlock, birch) became mycorrhizal with atypical fungal associates (e.g., *Rhizopogon*) when grown in the presence of well-colonized primary host species (e.g., Douglas-fir, pine). Our study showed (*i*) that Douglas-fir formed mycorrhiza with an additional fungus (but only 1/9 seedlings in mixture) and (*ii*) that the frequency and abundance of several other EM changed in mixture relative to monoculture. More specifically, Douglas-fir became mycorrhizal with *Tuber*, was under-represented by *Rhizopogon*, and over-represented by E-strain I in mixture with paper birch relative to monoculture. At the same time, paper birch was more frequented by *Lactarius*, *Hebeloma*, and *Cenococcum* in mixture versus monoculture.

Potential for interspecific hyphal linkages

The low host specificity of VA mycorrhizal and many EM fungi can result in physical hyphal connections between host plants of different species (Molina et al. 1992), which has been demonstrated using ^{14}C labelling and autoradiography (Brownlee et al. 1983; Francis and Read 1984; Finlay and Read 1986). Hyphal connections between plants have been demonstrated to facilitate interspecific transfer of carbon, nutrients, and water (e.g., Chiariello et al. 1982; Francis and Read 1984; Read et al. 1985; Finlay and Read 1986; Bethlenfalvay et al. 1991; Hamel and Smith 1992; Frey and Schuepp 1992; Arnebrant et al. 1993). Our study showed extensive overlap between Douglas-fir and paper birch in EM associates, suggesting a potential for interspecific hyphal connections. Douglas-fir and paper birch growing in monoculture shared in common six EM morphotypes over 80% and 95% of their root tips, respectively. When grown in mixture, the number of common EM increased to seven and proportion of root tips colonized by those types increased to 91% and 98% for Douglas-fir and paper birch, respectively. These results suggest that neighbors can influence transfer and feedback pathways within plant communities. However, it is possible that some of the shared EM morphotypes in this study were genetically dissimilar, which would affect the potential for functional links between species. This hypothesis could be tested by developing DNA profiles of these EM using molecular genetic tools.

Carbon and nutrients are thought to translocate through hyphal connections along electrochemical potential gradients from source to sink plants that differ in some way such as nutrient status or photosynthetic rate (Read et al. 1985; Newman 1988; Bethlenfalvay et al. 1991; Arnebrant et al. 1993). Rhizomorphs are viewed as important conduits for transfer (Duddridge et al. 1980, 1988; Brownlee and Jennings 1982; Brownlee et al. 1983), and their spatially separated inner vessel hyphae and outer living cortex hyphae are thought to function in bidirectional transfer of carbon compounds and nutrient ions, respectively (Cairney 1992). Of the common EM morphotypes identified in the present study, strands were frequently observed on *Thelephora*, occasionally on *Laccaria*, and rarely on E-strain I (note that the terms rhizomorph and strand are

used interchangeably by Read (1992)). Occasionally a few extramatrical hyphae on MRA mycorrhizae appeared loosely aggregated (Ingleby et al. 1990). The strongest strand formers, *Thelephora* and *Laccaria*, usually colonized <5% of the root tips on both Douglas-fir and paper birch. These results suggest that carbon or nutrient transfer between Douglas-fir and paper birch via interconnecting EM strands is possible, but the low frequency of the strongest strand-forming common fungi indicate that the magnitude of such exchange pathways may be limited.

Other strand-forming EM fungi that were specific to only one host species were *Rhizopogon* associated with Douglas-fir, and *Lactarius* and *Hebeloma* associated with paper birch. Because of the restricted host range of *Rhizopogon* in the section *Villosuli*, it is unlikely to form interspecific linkages between Douglas-fir and paper birch. Intraspecific *Rhizopogon* in the section *Villosuli* linkages may occur among Douglas-fir individuals, however, as demonstrated with host family specific *Suillus bovinus* (L. ex Fr.) O. Kuntze linkages among *Pinus sylvestris* L. individuals (Read et al. 1985). The advanced and frequent strand formation among *Rhizopogon* spp. section *Villosuli* implies potential for substantial direct exchange pathways between Douglas-fir hosts. *Lactarius* and *Hebeloma* only associated with paper birch in our study, but two common EM morphotypes of both genera were identified on outplanted Douglas-fir and paper birch seedlings by M.D. Jones (unpublished results). Her study provides further evidence for potential linkages between Douglas-fir and paper birch by strand-forming EM in the field.

Extramatrical hyphae of EM should not be discounted as unimportant to interspecific linkages and nutrient transfer, however. Read et al. (1985) used autoradiography after feeding *P. sylvestris* donor seedlings $^{14}CO_2$ to show that carbon isotope had translocated through the entire fan-shaped mycelial network (extramatrical hyphae in addition to strands) of *Suillus bovinus* interconnecting donor and receiver seedlings. In addition, carbon and nutrient transfer has repeatedly been demonstrated to occur between plants interconnected by VA mycorrhizae (e.g, Hirrel and Gerdemann 1979; Francis and Read 1984; Newman and Ritz 1986; Ritz and Newman 1986; Eissenstat 1990; Bethlenfalvay et al. 1991; Frey and Schuepp 1992; Hamel and Smith 1992; Newman and Eason 1993), which are more delicate in structure and do not form hyphal aggregates as seen in EM systems (Read 1992). Given demonstration of interplant carbon and nutrient transfer through extramatrical hyphae in previous studies, the large number and abundance of EM shared in common by paper birch and Douglas-fir, as well as the occurrence of some shared strand-forming EM, we expect that interspecific hyphal linkages are likely between Douglas-fir and paper birch. Hyphal linkages between species in Pinaceae and Betulaceae already have been documented by others. For example, Read and Finlay (1985) found that *Rhizopogon roseolus* spread from EM formed with *Pinus* spp. (Pinaceae) to roots of *Betula* sp. (Betulaceae) in seedling microcosms. Arnebrant et al. (1993) found that 5–15% of $^{15}N_2$ fixed by the association between *Alnus glutinosa* (L.) Gaertn. (Betulaceae) and *Frankia* was transferred to *Pinus contorta* Dougl. ex Loud. (Pinaceae) via *Paxillus involutus* (Batsch.) Fr. connections, and that approximately 20% of the N found in pine was derived from N_2- fixation by alder. Finally, Simard (1995) found that 4–6% of $^{13}CO_2$ and $^{14}CO_2$ fixed by paper birch and Douglas-fir seedlings growing in the field were transferred from paper birch to Douglas-fir, which she estimates occurred through EM connections.

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