Diversity of ectomycorrhizae on experimentally planted Douglas-fir seedlings in variable retention forestry sites on southern Vancouver Island

R.A. Outerbridge and J.A. Trofymow

Abstract: Studies were done on Vancouver Island of ectomycorrhizal (EM) communities at four distances (5–45 m) from isolated forest patches in three second-growth (SG) and three old-growth (OG) Douglas-fir sites subject to variable retention harvesting. We tested the hypothesis that retention of mature trees enhances colonization and diversity of EM fungi on seedlings planted in adjacent areas. In total 41 EM morphotypes were described, with mean diversity of 3.47 morphotypes and root colonization of 62% per seedling. Overall, root colonization declined with distance (72% at 5 m vs. 52% at 45 m), as did EM diversity (4.7 at 5 m vs. 2.9 at 45 m). For individual sites, the distance effect was significant for root colonization at four sites and for EM diversity at three to four sites. This suggests that variable retention is important for the recovery of ectomycorrhizal biota in harvested sites. Seedling root colonization was significantly lower in SG sites than in OG sites. Though EM diversity did not differ with stand age, OG sites had potentially more total (34) and unique (14) EM morphotypes than did SG sites (total 27, unique 7). Differences with stand age might be related to the relative abilities of EM fungi to disperse to regenerating second-growth forests.

Key words: variable retention silviculture, ectomycorrhizae ecology, Douglas-fir seedlings, old growth, second-growth forests.

Résumé : L'étude, conduite sur l'Île de Vancouver, a porté sur des communautés ectomycorhiziennes (EM) situées à quatre distances (5–45 m) de parcelles boisées isolées, venant sur trois sites de seconde venue (SG) et trois sites surannés (OG) de Douglas, sujets à des coupes de rétention variable (VR). Les auteurs ont vérifié l'hypothèse que le maintien d'arbres matures favorise la colonisation et la diversité par les champignons EM sur les semis plantées sur les surfaces adjacentes. Les auteurs décrivent un total de 41 morphotypes EM, avec une diversité moyenne de 3,47 morphotypes, et une colonisation moyenne de 62 % par plant. Dans l'ensemble, la colonisation racinaire diminue avec la distance (72 % à 5 m vs 52 % à 45 m), tout comme la diversité EM (4,7 à 5 m vs 2,9 à 45 m). Pour ce qui est des sites individuels, la distance affecte significativement la colonisation racinaire sur quatre sites et la diversité sur trois à quatre sites. Ceci suggère que la VR est importante pour réactiver le biote mycorhizien sur les sites récoltés. La colonisation racinaire des plantules est significativement plus faible pour les sites SG que pour les sites OG. Bien que la diversité ne diffère pas selon l'âge du peuplement, les sites OG abritent potentiellement un nombre plus grand de morphotypes EM totaux (34) et uniques (14), que les sites SG (totaux 27, uniques 7). La différence selon l'âge du peuplement pourrait être reliée aux capacités relatives des champignons EM à se disperser aux forêts de seconde venue.

Mots clés : sylviculture à rétention variable, écologie des ectomycorhizes, plantules de Douglas, forêt surannée, forêt de seconde venue.

[Traduit par la Rédaction]

Introduction

Old-growth forests are rapidly diminishing on Vancouver Island and are being replaced by early seral stage (regeneration) and second-growth forests. On eastern Vancouver Island, less than 2% of the drier forest type, dominated by coastal Douglas-fir (*Pseudotsuga menziesii*) and coastal western hemlock (*Tsuga heterophylla*), remains as old growth and much of the older second growth is currently being harvested (MacKinnon 2003; Trofymow et al. 2003). In 1998 Weyerhaeuser Coastal BC group (then known as MacMillan Bloedel) began phasing out clear-cut harvesting over a 5-year period to adopt a radically different approach. This would include a system of stewardship zones and variable retention harvesting and silviculture systems (Beese et al. 2003). Although variable retention (VR) of mature trees has been discussed in literature at least since 1997 (Franklin et al. 1997), it is newly adopted in British Columbia. It is an attempt to practice tree harvesting in such way as to maintain the biological diversity inherent in these forest ecosystems (Beese et al. 2003). One objective of VR is to maintain features of the original stand through retention of individual or groups of late succession trees through to the next harvesting. The assumption is that these retained trees act as a

Received 24 March 2004. Published on the NRC Research Press Web site at http://canjbot.nrc.ca on 3 December 2004.

R.A. Outerbridge and J.A. Trofymow.[1] Pacific Forestry Centre, Canadian Forest Service, Natural Resources Canada, 506 West Burnside Road, Victoria, BC V8Z 1M5, Canada.

[1]Corresponding author (e-mail: ttrofymow@pfc.forestry.ca).

 doi: 10.1139/B04-134

refuge for forest-dwelling species including well-known vertebrate species, as well as little known invertebrates, and provide an important habitat and recolonization source for plant and fungal species (Franklin et al. 1997; Kranabetter 1999; Kremsater et al. 2003).

Knowledge of the impacts of silvicultural systems, such as VR, on ectomycorrhizal fungi (EM fungi) is needed to conserve their diversity. EM fungi are important for tree growth and form vital links between tree roots and the soil. Almost all the nutrients taken up and used for tree growth pass through ectomycorrhizae (Smith and Read 1997). For example, a review and meta-analysis of literature on artificial inoculation trials (Trofymow and van den Driessche 1991) demonstrated that artificial inoculation of tree seedlings is important to the survival and growth of tree seedlings on severely burnt, pesticide-treated, or EM-impoverished reclamation sites.

Previous studies have shown that diversity of ectomycorrhizal fungi in Douglas-fir forests is quite high. It is speculated that Douglas-fir can form ectomycorrhizae with approximately 2000 fungal species worldwide (Trappe 1977). On southern Vancouver Island, in close proximity to some of the sites in the present study, Goodman (1995) recorded a total of 69 distinct ectomycorrhizal morphotypes in a mature and old-growth stands of Douglas-fir. In the same stands, Goodman and Trofymow (1998*b*) found that density of ectomycorrhizal roots was greater in the forest floor over mineral soil than in mineral soil or logs. Surveys of macrofungi known to form ectomycorrhizae confirm the importance of Douglas-fir as a symbiont on southern Vancouver Island (Outerbridge 2002; Countess 2001).

As stands age, so does the forest floor ecosystem. Changes in light and wind penetration, soil pH, and availability of mineral nutrients as organic matter accumulates likely contribute to species presence and abundance of ectomycorrhizal fungi (Read 1992). Silvicultural practices disrupt the natural course of the fungal succession. Understandably, ecological effects of the removal of all trees, clearcutting, has been under scientific scrutiny. Jones et al. (2003) reviewed mycorrhizae-related literature on the subject. They concluded that, following clearcut logging, the most evident and important effects on EM fungi are the changes in species composition, as opposed to loss of inoculum. *Picea* seedlings (Hagerman et al. 1999*a*; Jones et al. 2002) and soil cores (Hagerman et al. 1999*b*) were used to investigate effects of clear-cut logging on EM colonization and diversity across cut blocks of different sizes. It has been shown that partial cutting systems could allow some timber removal without significant reduction in ectomycorrhizal mushroom communities (Kranabetter and Kroeger 2001). Massicotte et al. (1999) performed inoculation trials and speculated that mycelium extending from adjacent stands may speed up regeneration of small forest openings. Kranabetter and Wylie (1998) observed decreased EM richness across forest openings on naturally regenerated western hemlock seedlings. Kranabetter and Friesen (2002) found that many of the mature-forest EM fungi were unable to maintain or continue root colonization in openings.

To test the hypothesis that retention of mature trees affects root colonization, species richness, and composition of ectomycorrhizal fungi on planted seedlings, we compared the EM communities at increasing distances from small forest patches in stands subjected to variable retention harvesting. Goodman (1995) and Goodman et al. (1996–2000) provided descriptions of various EM species from this area. We gained confidence in the experimental design and familiarity with the sites and EM morphotyping from a pilot study we did earlier (Outerbridge et al. 2001, unpublished results). We used old-growth and second-growth Douglas-fir dominated sites to test for differences in the EM inoculum potential of the adjacent clearcuts.

Materials and methods

Sites

The six sites examined in the present study were located in two areas of southern Vancouver Island: Shawnigan Lake (sites C1500 and H1200) and Nanaimo River (sites DAM1, TL, B1, and R23), between 48°40′N and 49°04′N latitude and 123°49′W and 124°08′W longitude (Table 1). This region is characterized by cool dry summers and mild wet winters, and the sites spanned two biogeoclimatic zones from the Coastal Douglas-fir zone (CDF; siteTL) to the Dry (CWH xm; sites DAM1 and B1) and Moist Maritime (CWH mm; sites C1500, H1220, and R23) Coastal Western Hemlock subzones (Green and Klinka 1994) (Table 1). The topography of the sites varies from flat (site TL), to shallow slope (sites B1, C1500, DAM1, and R23), to steep (site H1220). The elevation ranges from 37 to 1009 m (Table 1). The predominant soils are fluvial or moraine Duric Humo-Ferric Podzol (sites DAM1 and B1), colluvial shallow lithic Orthic Humo-Ferric Podzol (sites R23, C1500, and H1220), and fluvial Orthic Dystric Brunisol (site TL) (Jungen 1985). The sites were logged in 1998, no site preparation or treatment was done (a few piles of burned slash were noticed on the road sides).

The sites were characterized by low levels of woody vegetation (of interest here because of potential for mycorrhizal linkages), including seedlings of naturally regenerating amabilis fir (*Abies amabilis*) (site R23), Douglas maple (*Acer glabrum*) (site DAM1), bigleaf maple (*Acer macrophyllum*) (site H1220), red alder (*Alnus rubra*) (site DAM1), western white pine *(Pinus monticola*), Douglas-fir, willow sp., and western hemlock. The most prevalent shrubs, which occurred at all sites, were salal (*Gaultheria shallon*), Oregon grape (*Mahonia nervosa*), and trailing blackberry (*Rubus ursinus*). Many stations were also characterized by high densities of bracken fern (*Pteridium aquilinum*) or fireweed (*Epilobium angustifolium*).

The six sites were established in autumn 2000: three sites in second growth (one with group tree retention and two with small stands) and three sites in old growth (all with group tree retention) (Table 1). We used 1-year-old Douglas-fir seedlings, purchased from Pelton Reforestation Ltd., Maple Ridge, B.C. (the source of the operationally planted Douglas-fir stock), using appropriate seed-lots for lower elevation (second growth) and higher elevation (old growth) sites. The trees were planted in Vexar tubes along three transects at each site, at four distances from the forest patch: immediately adjacent (3–5 m), 15 m, 25 m, and 45 m. To increase power in statistical analysis, we originally planned on repeating the measurements in two subsequent sampling

Table 1. Second-growth and old-growth sites examined in the study of the edge effects on ectomycorrhizal diversity.

Site	Sampling: Pilot	Sampling: Experiment	Type of forest patch edge	Original stand composition	Elevation (m)	Latitude (N)	Longitude (W)	Biogeoclimatic zone
Second growth								
DAM1	2000	2002–2003	Other[a]	89% *P. menziesii*	250	49°04′	124°05′	CWH xm1
TL	2000	2002–2003	Other[a]	80% *P. menziesii*	37	49°04′	123°52′	CDF
B1	2000	2002–2003	Group[b]	45% *P. menziesii*; 40% *T. heterophylla*	300	49°03′	124°08′	CWH xm1
Old growth								
C1500	2000	2002–2003	Group[b]	75% *P. menziesii*	600	48°40′	123°50′	CWH mm1
R23	2000	2002–2003	Group[b]	50% *P. menziesii*; 45% *A. amabilis*	1009	49°00′	124°08′	CWH mm2
H1220	nd[c]	2002–2003	Group[b]	77% *P. menziesii*	550	48°40′	123°49′	CWH mm1

[a]Small stand edge.
[b]A distinct group of trees <0.4 ha in size surrounded by a cut area.
[c]nd, not determined because no seedlings were available to sample in pilot study.

years. Thus, a total number of 288 seedlings were planted (6 sites × 3 transects × 4 distances × 2 seedlings × 2 sampling times) in autumn 2000. A 2001 visit to the sites, however, revealed that mortality of the planted seedlings was too great, so a second sampling was impractical. Therefore all the measurements in this experiment are final and are based on 144 seedlings (6 sites × 3 transects × 4 distances × 2 seedlings), sampled in November 2002, 2 years after planting. A sample of 20 additional seedlings, randomly selected from each of the seed lots mentioned above, was examined for the presence of fungi on the roots to establish their mycorrhizal status prior to planting. We found no mycorrhizae on any of the seedlings. Consultation with other researchers working in this field (Dr. Shannon Berch, British Columbia Ministry of Forests, John Dennis, Canadian Forest Service, personal communications) confirmed the usual lack of mycorrhization on container-grown nursery seedlings of Douglas-fir, at least in the coastal regions of British Columbia.

Laboratory processing

The seedlings were stored at 2 °C until processing. Preparation of seedling root plugs for taxonomic identification of ectomycorrhizae and for abundance measurements were similar to those used by Goodman (1995) on soil cores and by Kranabetter and Friesen (2002) on tree seedlings. Seedling roots were washed to remove soil and debris and then a sample of roots, external to the container-grown root plug, were removed, cut to 3–5 cm in length and placed in distilled water in a grid-lined plastic tray. The roots were randomly dispersed throughout a grid-lined dish and, following the gridlines, a subsample of the first 100 root tips was examined for ectomycorrhizae for each seedling. This method provides consistent sampling intensity throughout the study. Both ectomycorrhizal and nonmycorrhizal root tips were recorded. Ectomycorrhizal morphological types were determined using a stereomicroscope. Some observations of cellular structures were made using an inverted compound Leitz–Labovert microscope under 400× magnification and 1000× oil immersion. Morphological types (or "species") were labelled according to colour or a set of distinguishing morphological features (Table 2). Some morphological types were later identified to fungal genus and (or) species using Goodman et al. (1996–2000), Agerer (1987–2002, 1996–2002), and Ingleby et al. (1990), and by searching the online Database of Descriptions of Ectomycorrhizae (Goodman et al. 2000) at <http://www.pfc.cfs.nrcan.gc.ca/biodiversity/ecto/index_e.html>. The sample roots and the remaining plug roots were then placed in a glass Petri dish and oven dried at 75 °C for 48 h; the dry weights were recorded. Some root tips with representative morphotypes were placed in sterile water at –20 °C for future reference.

Statistical analysis

Diversity was defined as number of EM morphotypes per seedling found in a sample of 100 root tips. Percent root colonization was measured for each seedling (sample), that is, number of root tips colonized by one or more EM fungi divided by the total number of root tips examined (nb: several samples did not have sufficient root systems to yield 100 root tips). Average values and their standard deviations were calculated for all eight seedlings from each transect, and for each distance, within a site and overall, two-way Analyses of Variance (ANOVA) with replication and mean comparisons by Tukey–Kramer (honestly significant difference) test were performed using SAS, version 6.0. (SAS Institute Inc. 1989). Regression analyses were performed using Microsoft Excel, version 1997. We used $\alpha = 0.05$ to identify statistical significance. In addition to overall calculations using data for each distance pooled across all the sites, we compared data for each distance within each site as well as within age group of the original stand (i.e., second growth vs. old growth). We focused on distance from the forest patch as the source of variation for the two variables measured: % root colonization and number of EM morphotypes as a measure of diversity.

Results

Overall frequencies of individual EM morphotypes

In total, we examined 14 323 root tips from the sampled Douglas-fir seedlings, and identified 41 distinctly different ectomycorrhizal morphotypes. These are listed alphabetically by their code names, along with their proportion of

Table 2. Ectomycorrhizal morphotypes and their proportionate frequency of colonization (percentage of all colonized root tips).

EM morphotype code	Ectomycorrhizal morphotype brief description or fungal species	Percentage of EM root tips colonized by morphotype	
		Experimental study	Pilot study
Ambys*	*Amphinema byssoides*	2.42	3.41
BlkBr	Blackish brown, apices tapered, "*Piceirhiza chordata*"-like	3.83	
Blkwarty	Black-brown, irregular, rough, "*Piceirhiza nigra*"-like, also ITE.5-like	0.10	0.26
BrHumar	Medium brown, micaceous, *Humaria*-like	0.66	
BRmet	Brown, chocolate-metallic	1.05	1.37
BRpit	Brown, rich medium brown, honeycomb-like surface		0.07
BrPyram	Yellowish brown, pyramidal, thick, rounded, smooth	0.34	
Canth*	*Cantharellus formosus*-like	0.93	1.49
Cenoc*	*Cenococcum geophilum*	25.35	27.30
Copper	Light brown to copper, coralline, smooth to felty, shiny	1.18	
Crmcons	Cream to beige, short, simple, tips midway constricted, smooth	0.13	
DermCin	*Dermocybe cinamomea*? (syn. *Cortinarius cinnamomeus*)	0.05	
GoldWh	Golden white, bronze rhizomorphs	0.03	
Gomph	Purplish white, *Gomphidius*-like	0.42	0.84
GRmet	Green metallic		0.45
GTRonBr	Grayish translucent over brown, smooth white patches, aging to *Laccaria*-like	1.16	
GYBwoven	Greenish-yellow; later brown; woven	0.10	
Lactarub*	*Lactarius rubrilacteus*	1.15	0.45
OLcott	Olive to brown with cottony white mycelium	0.27	0.58
Olcyst	Olive to brown, with long cystidia		0.14
OLdkhy	Olive, thick, + dark hyphae	1.36	0.38
OLsm	Olive, smooth, simple	0.72	0.33
ORgroup	Orange; pale to dark, think to medium mantle, smooth to fibrous	1.35	9.88
Pbaculi*	"*Pseudotsugaerhiza baculifera*"	2.85	4.15
Pilo*	*Piloderma* sp.	0.62	0.51
Pink	Pink		0.03
Purple blue	Purple blue		0.04
RBrPub	Reddish brown, pubescent, monopodial pinnate to pyramidal in one plane	0.20	
Rhizo*	*Rhizopogon* sp.	43.08	27.81
SalmWh	Peachy-white, patchy, silvery salmon rhizomorphs	0.34	
SilBrCot	Silvery brown, cottony white mycelium	0.45	
SILspt	Silvery, thick, short, some with rusty spots	0.39	0.82
Thel	Purple-brown/fibrous mats, *Thelephora*-like		0.47
ThickRus	*Russula*-like, thick felty, pallid to yellow, irregular, staining purple	1.18	
ThickYel	Yellow thick, felty, monopodial pyramidal in one plane, white flecks	0.22	
Toment*	*Tomentella*-like	0.03	
TRfelt	Felty translucent white	2.31	3.13
TRfrost	Yellowish, frosted, thin, translucent, monopodial pinnate	0.04	
Trunc*	*Truncocolumella citrina*	1.42	0.43
Tuber	*Tuber*-like	0.14	1.67
Wbulb	White, bulbous tip		0.21
Wcott	White cottony (*A. byssoides*-like, but white strands, thicker mantle)	0.24	1.81
Wheb	White over light olive, felty, unbranched; *Hebeloma*-like	0.34	0.71
Wlila	White, pallid, lilac hue	2.67	1.77
Wshortrh	White over dark grey, short strands, coralloid	0.56	
Wtort	White, tortuous	0.03	6.94
Wtrip	White, simple three-tip branching pattern		0.28
Wyel	White, yellowish hue		2.00
Ybrhy	Yellow, bright, felty, speckled brown; emanating hyphae brown; rhizomorphs grey		0.26
YGrMet	Yellow-green metallic; *Tricholoma zelleri*?	0.23	
Yshort	Yellow, short, simple, smooth to shaggy	0.03	

Note: Pilot study data was obtained from an earlier survey of the same sites using a similar approach on operationally planted seedling (Outerbridge et al. 2001, unpublished results).

*Morphotypes reported in Goodman et al. (1996–2000).

Table 3. Ectomycorrhizal morphotypes found in the experimental study and their frequencies.

EM morphotype code	Rank	No. of root tips observed	No. of sites (out of 6)	No. of stations (out of 72)	No. of seedlings (out of 144)	Location of EM type limited to 1 seedling	Location of EM type limited to 1 site	Stand age
Rhizo	1	4045	6	72	63			OG, SG
Cenoc	2	2380	6	71	137			OG, SG
BlkBr	3	360	3	7	11			OG, SG
Pbaculi	4	268	6	24	28			OG, SG
Wlila	5	251	4	10	12			OG, SG
Ambys	6	227	5	23	28			OG, SG
TRfelt	7	217	2	8	9			OG, SG
Trunc	8	133	5	18	20			OG, SG
OLdkhy	9	128	3	13	15			OG, SG
ORgroup	10	127	3	4	5			OG, SG
Copper	11	111	3	4	6			OG, SG
ThickRus	12	111	1	2	4		**R23**	OG
GTRonBr	13	109	1	2	2		B1	SG
Lactarub	14	108	3	6	8			OG, SG
BRmet	15	99	5	6	7			OG, SG
Canth	16	87	1	3	4		B1	SG
OLsm	17	68	1	3	3		**C1500**	OG
BrHumar	18	62	1	3	3		**H1220**	OG
Pilo	19	58	5	11	12			OG, SG
Wshortrh	20	53	1	1	1	B1	B1	SG
SilBrCot	21	42	2	3	4			OG, SG
Gomph	22	39	1	1	1	**R23**	**R23**	OG
SILspt	23	37	3	4	4			OG, SG
BrPyram	24	32	1	2	2		**H1220**	OG
Salm Wh	25	32	1	1	1	**R23**	**R23**	OG
Wheb	26	32	3	4	4			OG, SG
OLcott	27	25	2	3	3			OG
Wcott	28	23	1	6	7		**H1200**	OG
YGrMet	29	22	1	1	2		DAM1	SG
ThickYel	30	21	1	1	1	**R23**	**R23**	OG
RBrPub	31	19	3	4	4			OG, SG
Tuber	32	13	2	2	2			OG, SG
Crmcons	33	12	2	2	2			OG
Blkwarty	34	9	2	2	2			OG, SG
GYBwoven	35	9	1	1	2		B1	SG
DermCin	36	5	1	1	1	**R23**	**R23**	OG
TRfrost	37	4	1	1	1	B1	B1	SG
GoldWh	38	3	1	1	1	**H1220**	**H1220**	OG
Toment	39	3	1	1	1	**R23**	**R23**	OG
Wtort	40	3	1	1	1	DAM1	DAM1	SG
Yshort	41	3	1	1	1	**H1220**	**H1220**	OG

Note: Old-growth sites are indicated with boldface type.

colonized root tips (relative frequency) and total frequency of root colonization in Table 2. Table 3 lists the same morphotypes ranked according to the total number of root tips colonized and shows their frequency patterns by summarizing their presence by site, sampling station (distance), and total number of seedlings. The seven most frequent morphotypes were *Rhizopogon* sp. (28% of all root tips examined), *Cenococcum geophilum* (17%), BlkBr (3%), Pbaculi (2%), Wlila (2%), Ambys (2%), and TRfelt (2%).

Three morphotypes (*Cenococcum geophilum*, *Rhizopogon* sp., and "*Pseudotsugaerhiza baculifera*") were found at all six sites (these species were also among the ones with highest colonization frequency and the widest distribution pattern). Other common morphotypes were *Amphinema byssoides*, BlkBr, Wlila, Trfelt, Trunc, and OLdkhy. Ten morphotypes were found on a single seedling only.

On average, ectomycorrhizal fungi colonized 62% (±21% SD) of the root tips and ranged from 44% in TL at 45 m to 86% in B1 at 5 m (Table 4). The survey yielded an average of 3.47 ectomycorrhizal morphotypes per seedling (±1.63

Table 4. Mean % root colonization per seedling, number of morphotypes per seedling, and total number of morphotypes by distance for each site and across all sites.

Site	Distance (m)	% root colonization[a]	No. of morphotypes[a]	Total no. of morphotypes
Second-growth sites				
DAM1	5	82 (6.9)a	4.33 (0.62)a	11
	15	51 (9.6)b	2.67 (0.21)b	4
	25	50 (3.2)b	3.33 (0.42)ba	9
	45	47 (7.3)b	3.00 (0.26)b	7
TL	5	50 (5.4)a	2.83 (0.48)a	6
	15	55 (5.6)a	2.83 (0.31)a	5
	25	58 (6.5)a	3.00 (0.26)a	5
	45	44 (6.4)a	2.67 (0.33)a	5
B1	5	86 (3.6)a	6.67 (0.76)a	17
	15	79 (8.4)a	4.33 (0.21)ba	11
	25	58 (7.9)ba	3.67 (0.67)b	8
	45	46 (5.5)b	3.17 (0.54)b	10
Old-growth sites				
R23	5	74 (8.7)a	4.33 (0.84)a	14
	15	54 (10.7)a	2.33 (0.33)b	5
	25	61 (4.2)a	2.50 (0.22)b	7
	45	51 (10.5)a	2.67 (0.33)b	6
C1500	5	83 (5.1)a	4.17 (0.70)a	9
	15	62 (8.4)b	3.67 (0.56)a	9
	25	76 (4.0)ba	3.33 (0.33)a	9
	45	69 (6.6)ba	3.17 (0.31)a	7
H1220	5	78 (5.0)a	5.83 (1.60)a	17
	15	71 (6.2)a	2.83 (0.31)a	6
	25	49 (9.7)b	2.83 (0.31)a	7
	45	59 (7.0)ba	2.83 (4.00)a	6
All	5	75a	4.69a	32
	15	62b	3.11b	22
	25	59b	3.14b	20
	45	52b	2.90b	19

Note: Values sharing the same letter within a site are not statistically different from each other.
[a]SE are given in parentheses.

SD) with the highest average number of morphotypes per seedling at 5 m in B1 (6.67), and the lowest at 15 m in R23 (2.33) (Table 4).

Distance effects on EM root colonization and diversity

Examination of the % root colonization and diversity data by distance (Table 4) shows that it was typically higher at 5 and (or) at 15 m, than at 25 and (or) 45 m, with the most visible distinction between 5 m and all other stations. ANOVA based on all 144 seedlings, grouped by distance versus site, showed that distance from the forest patch was a significant source of variation in % root colonization and diversity ($P < 0.0001$ for both), and there was no interaction of site with distance for % root colonization ($P = 0.5150$) or diversity ($P = 0.5098$) (Table 5*a*). Regression analysis also detected a strong negative overall effect of distance from the forest patch on % root colonization and diversity ($P < 0.0001$ for both) (Table 6).

Based on separate ANOVA for each site, the effects of distance on % root colonization and number of morphotypes were significant for four sites, B1, DAM 1, C1500, and H1220, but not significant for TL and R23 (Table 5*b*). Regression analysis of the data indicated a decline in % root colonization with increasing distance from the forest patch at all the sites but TL. However, the regressions were statistically significant at only two sites DAM1 ($P = 0.0114$) and B1 ($P < 0.0001$) (Table 6). Individual ANOVA analyses also showed that distance from the forest patch was a significant source of variation in EM diversity at three sites (B1, DAM1, and R23) but not at others (C1500, H1220, and TL) (Table 5*b*). Tukey's HSD tests indicated where the differences were (Table 4). Regression analysis shows a definite decrease in EM diversity with the increase in distance from the forest patch at three of the six sites (B1, C1500, and H1220), a weaker downward trend in DAM1 and R23, but almost no change in TL. However, only one of these regressions, at B1, is statistically significant ($P = 0.0012$) (Table 6).

Table 5. Results of analysis of variance (ANOVA) testing for effect of distance from the retained trees on % root colonization and number of morphotypes for the overall experiment (*a*), for each site (*b*), and for each age group (*c*).

Source of variance	% root colonization *P* value	Number of morphotypes *P* value
(*a*) Overall: ANOVA testing for overall effects of site or age, and distance ($n = 144$)		
Site	0.0013*	0.0013*
Distance	<0.0001*	<0.0001*
Site × distance	0.5150	0.2079
Age	0.0333*	0.5410
Distance	<0.0001*	<0.0001*
Age × distance	0.5098	0.8480
(*b*) Site: ANOVA testing for effect of distance at each site ($n = 24$ per site)		
DAM 1	0.0106*	0.0044*
TL	0.3985	0.9519
B1	0.0041*	0.0047*
R23	0.2734	0.0141*
C1500	0.0196*	0.4547
H1220	0.0490*	0.0519
(*c*) Stand age: ANOVA testing for effect of distance for each age group ($n = 72$ per age group)		
Second growth	<0.0001*	0.0002*
Old growth	0.0130*	0.0011*

Note: $\alpha = 0.05$.
*Statistically significant.

Site effects on EM root colonization and diversity

ANOVA showed that site was a significant source of variation in % root colonization ($P = 0.0013$) and number of morphotypes ($P = 0.0013$) (Table 5*a*). Mean % root colonization ranged from 52% (6.9) in TL to 72% (9.5) in C1500, each of the two sites differing significantly from the three other sites. Mean number of morphotypes ranged from 2.83 (0.07) in TL to 4.46 (0.64) in B1, and B1 was significantly most different from all the other sites (Table 7). Overall, TL had the most impoverished mycoflora, while B1 and H1220,

Table 6. Results of regression analyses testing for the effect of distance on % root colonization and number of morphotypes for each site and stand age group.

	% root colonization				No. of morphotypes			
Site or age	Intercept	Slope	R^2	P value	Intercept	Slope	R^2	P value
DAM1	73.75	–0.730	0.2576	0.0113*	3.85	–0.023	0.0935	0.1462
TL	55.51	–0.169	0.0310	0.4160	2.92	–0.004	0.0050	0.7434
B1	90.88	–1.056	0.5095	<0.0001*	6.23	–0.079	0.3860	0.0012*
Second growth	73.38	–0.651	0.2213	<0.0001*	4.33	–0.035	0.3860	0.0029*
R23	69.94	–0.450	0.0932	0.1470	3.65	–0.031	0.1119	0.1102
C1500	76.96	–0.214	0.0387	0.3568	4.12	–0.024	0.0880	0.1593
H1220	76.07	–0.520	0.1550	0.0570	4.96	–0.060	0.1413	0.0702
Old growth	74.32	–0.394	0.0856	0.0126*	4.25	–0.038	0.1053	0.0054*
Overall	73.85	–0.523	0.1425	<0.0001*	4.29	–0.037	0.1110	<0.0001*

*Statistically significant.

though quite different in habitat, supported the highest diversity of EM fungi.

Examination of root dry weights revealed that root plugs from TL were significantly smaller, in comparison with the other two second-growth sites DAM 1 and B1 (means = 13.53, 17.06, and 23.92 g, respectively) but not in comparison with the old-growth sites (means = 10.48, 11.90, and 13.93 g, in R23, C1500, and H1220, respectively). Regression analysis based on all 144 seedlings confirmed the correlation of dry root weights and % root colonization (R = 0.049, P = 0.00751). Overall, root weights were lower in the old-growth sites (12.10 g ± 0.86 g SE) than in the second-growth sites (18.17 g ± 0.86 g SE), with the lowest mean of 10.48 g in R23.

Second-growth versus old-growth forests

A total of 27 morphotypes were found in second growth and 34 in old growth forests (Table 7); 20 were common to both second-growth and old-growth sites, 7 were unique to second growth, and 14 were unique to old growth (Table 3). Of the 14 morphotypes unique to old growth, 12 (BrHumar, BrPyram, DermCin, GoldWh, Gomph, OLsm, SalmWh, ThickRus, ThickYel, Toment, Wcott, and Yshort) were limited to a single site, R23 or H1220. Each of the seven morphotypes (Canth, GTRonBr, GYBwoven,TRfrost, Wshortrh, Wtort, YGrMet) unique to second growth was found at a single site only (either DAM 1 or B1) (Table 3).

Root colonization in the old-growth sites was higher (65%) than in the second-growth sites (59%) (Table 7) and the difference was significant (P = 0.0333) (Table 5*a*). Mean number of morphotypes was 3.54 for second growth and 3.39 for old growth; however, differences in EM diversity between the age groups were not statistically significant (P = 0.5410) (Table 5*a*).

Within each age group, distance from the forest patch was a significant source of variation in % root colonization (second growth $P < 0.0001$, old growth P = 0.013) and number of morphotypes (second growth P = 0.0002, old growth P = 0.0011) (Table 5*c*).

Discussion

Edge effects

The main focus of this project was the examination of diversity and colonization of ectomycorrhizae on seedlings planted in previously harvested areas at four different distances from a retained patch of trees. The results provided support for our hypothesis, showing an overall strong effect of distance on both % root colonization and number of morphotypes. Thus, it would appear that the further away from the retained mature trees, the lower the fungal inoculum potential in the harvested area. In most sites, this effect was reflected in both EM diversity (number of morphotypes) and % root colonization. The limited effect of edge on some sites could be owing to a number of causes, most likely, related to site characteristics such as soil structure and moisture holding capacity, site elevation, topography and aspect, and how they may have affected seedling growth (we noticed, for example, poor seedling growth at TL, which also had comparatively low % colonization and species richness).

It is important to realize the limitation of studies like this one, in which nursery-grown seedlings are used as bait for EM fungi in a disturbed habitat. The degree of site disturbance (relatively minimal in this case), the host species used, the timing of clearcutting, planting, and sampling, as well as an array of natural environmental factors, can potentially strongly influence the outcome of such research. This is reflected in the inconsistency of results in related studies. The negative effect of distance from the forest edge on the number of EM morphotypes was apparent on naturally regenerating *Tsuga heterophylla* seedlings (Kranabetter and Wylie 1998) but was only slight on precolonized nursery seedlings (Durall et al. 1999). Hagerman et al. (1999*a*) reported significantly reduced richness beyond 2 m from the clearcut–forest boundary and suggested that "proximity to overstory trees may be more important than cut block size for patterns of ectomycorrhizal diversity and colonization". However, Jones et al. (2002) observed no edge effects in a follow up study of the same clearcuts, but with seedlings planted in mechanically site-prepared mounds. Soil cores are sometimes used instead of seedlings to investigate EM inoculum level. Based on this method, Hagerman et al. (1999*b*) did not see reduced diversity of ectomycorrhizae in clearcuts until two and tree growing seasons after tree removal. We chose not to use soil cores in the present study. In the harvested areas, the numbers of live roots in general, and especially active mycorrhizal roots, would likely have been very low (Hagerman et al. 1999*b*), and the ectomycorrhizae in soil cores taken from within the forest patches would probably differ considerably from those able to persist in the changed

Table 7. Mean % root colonization per seedling, number of morphotypes per seedling, and total number of morphotypes by transect for each site. Values sharing the same letter are not statistically different from each other.

Site	Transect	% root colonization[a]	No. of morphotypes[a]	Total no. of morphotypes
Second-growth sites				
DAM1	1	65.3	3.75	10
	2	53.4	2.75	6
	3	53.4	3.50	8
	Average	57.3 (6.9)bc	3.33 (0.52)ba	15
TL	1	53.1	2.88	6
	2	44.3	2.88	5
	3	57.8	2.75	6
	Average	51.7 (6.9)c	2.83 (0.07)b	7
B1	1	61.5	4.63	15
	2	75.3	5.00	12
	3	64.6	3.75	9
Second-growth average	Average	67.1 (7.2)ba	4.46 (0.64)a	20
		58.7 (9.1)A	3.54 (0.83)A	27
Old-growth sites				
R23	1	62.7	2.75	8
	2	46.9	2.50	7
	3	69.9	3.63	11
	Average	59.8 (11.8)bac	2.96 (0.59)b	19
C1500	1	66.2	3.63	10
	2	67.1	4.00	9
	3	83.1	3.13	9
	Average	72.2 (9.5)a	3.58 (0.44)ba	15
H1220	1	66.2	2.88	7
	2	65.4	3.75	14
	3	61.6	4.25	13
	Average	64.4 (2.4)bac	3.63 (0.69)ba	21
Old-growth average		65.5 (9.4)B	3.39 (0.60)A	34

Note: Values sharing the same letter are not statistically different from each other.
[a]SD are given in parentheses.

environment of the clearcuts (Kranabetter and Friesen 2002). Repeated surveys and measurement of site-specific conditions are needed to provide more data to draw firmer conclusions.

Stands of second-growth vs. old-growth origin.

From a mycological point of view, as stands age following clear-cutting or fire, the diversity of ectomycorrhizal fungi change and early-stage genera, for example *Thelephora*, *Laccaria*, or *Paxillus*, are replaced or joined by late-stage ones, such as *Cortinarius* and *Russula* (Dighton and Mason 1985; Visser 1995). These changes in the fungal community may be caused by two factors: changes in the physical and biochemical soil properties as the stand ages, as well as by the amount and dispersal of fungal propagules from the surrounding forest.

Our study provided some indication that the two age groups might vary in their inoculum potential in a variable retention harvesting setting. Although no significant differences were found between second growth and old growth for EM diversity (number of morphotypes per seedling), % root colonization and total numbers of morphotypes were consistently higher in the old growth than in the second growth. As many as one-third of the morphotypes were unique to old growth, suggesting that stand age had an important effect on species composition. Visser (1995) used both macrofungus survey and root tip data and found no decrease in ectomycorrhizal colonization of roots with stand age, but she reported a significant increase in species richness between the 6- and 41-year-old stands of jack pine following a wildfire. The seedlings in our study were only 3 years old at the time of sampling, and there were no macrofungal fruit bodies associated with them that we could add to our root tip examination data, which is likely the reason for the contrasting results.

It is possible that ectomycorrhizal dispersal into the cut areas might be more effective from the old-growth trees than from the second-growth trees. The reasons for this are still unknown but could potentially be owing to phoretic agents such as mushroom-eating squirrels or molluscs present in the old-growth and not in second-growth patches. Presence of small trees left in the cut area could also act as a source of inoculum (refuge plants) (Kranabetter 1999; Hagerman et al. 2000). Natural regeneration, though not significant overall, could account for some of the differences between the old-growth and second-growth sites in the present study (Outerbridge and Trofymow 2003, unpublished data). Bruns (1995) argues that late stage fungi are not very effective in

colonizing new substrates via spores, dispersing primarily via vegetative mycelium. This argument is based on evidence in the literature of their higher nitrogen and protein requirements, which seems to correlate with greater investment in extrametrical mycelium, especially cords.

Our finding of the effects of previous stand age contrast with a study of paired old-growth – second-growth stands of Douglas-fir on southern Vancouver Island that found EM abundance did not differ with stand age (Goodman and Trofymow 1998*a*). However, in that study, sites had been carefully chosen to minimize site type differences, and old-growth and second-growth stands were immediately adjacent to each other. This would allow for easier dispersal of EM fungi from the old-growth stands to the regenerating second-growth stands. In the present study, the only suitable VR sites containing old growth were in the higher elevation subzones, and those with second growth were at lower elevation, which forced us to select correspondingly different Douglas-fir seed lots, both factors potentially contributing to the observed stand age differences. Notwithstanding, could these contrasting findings of residual stand age be due to differences in rates of colonization of EM fungi into regenerating stands? Perhaps older second-growth stands provide suitable habitat for EM fungi but the large size of older clear-cuts, and limited dispersal by some EM fungal species meant that second-growth stands were never adequately recolonized by all EM fungal species by the time of the next harvest.

It is too early to draw firm conclusions from our observations about the rarity or successional classification of EM fungi, but based on other literature on the subject (Carroll and Wicklow 1992), it is possible that forest age might play a significant role in determining species composition of ectomycorrhizal mycoflora. If, as the present study suggests, second-growth stands do contain reduced numbers and abundance of EM fungal species, then it will be important to understand what forestry practices (e.g., variable retention in particular) can be used to prevent the loss of additional species with subsequent harvest. If the old-growth Douglas-fir forests in this area do contain species not found in second growth, then how these forests are managed becomes even more important as the remaining area of this type of old-growth forest is extremely limited on Vancouver Island.

Percent root colonization

The results of the present study were similar but not identical to the pilot study we did earlier at the same sites using a similar approach on operationally planted seedlings (Outerbridge et al. 2001, unpublished data). For example, average overall frequency of root colonization (62%) was twice that in the pilot study (31%) (individual morphotypes are listed in Table 2) and more consistent with the work of other researchers. Parke et al. (1984) conducted a greenhouse bioassay with Douglas-fir to compare abundance of ectomycorrhizal fungus propagules in soils from clear-cuts and adjacent undisturbed forests. Root colonization ranged from 60% to 80% for seedlings grown in soil from clear-cut sites. Roth and Berch (1992) out-planted nursery-grown Douglas-fir seedlings and found that after one season 72% to 93% of new roots were mycorrhizal. Pilz and Perry (1984) reported a mean root tip colonization of about 40%–80% for Douglas-fir seedlings from the unburned clear-cut treatment. Jones et al. (1997) compared mycorrhizal colonization on Douglas-fir seedlings outplanted in monocultures with that on mixed cultures with paper birch and found them statistically similar, ranging from 56% to 78%.

Number and identity of individual EM morphotypes

In the present study, we found a total of 41 EM morphotypes. Many of the morphotypes were the same as those we encountered in the pilot study (Outerbridge et al. 2001, unpublished), while 19 were new, as indicated in Table 2 (which includes 10 additional species found only in the pilot study). By comparison, Kranabetter et al. (1999) report an average of 52 morphotypes per conifer species in a similar study in northwestern British Columbia. Jones et al. (1997) obtained 32 ectomycorrhizal types on Douglas-fir seedlings 28 months after outplanting, and Mah et al. (2001) characterized 24 morphotypes in a study of naturally regenerating and planted *Picea* seedlings. Two studies of the chronosequence of Douglas-fir stands on southeastern Vancouver Island, in which Goodman (1995) studied ectomycorrhizae in soil cores and Countess (2001) surveyed macrofungus sporocarps in the same plots, yielded 69 EM morphotypes and 148 ectomycorrhizal fungal species, respectively.

Rhizopogon and *Cenococcum* were the most frequent EM fungi observed. These findings were consistent with those of other researchers. *Cenococcum*, *Rhizopogon*, and *Amphinema byssoides* are frequently found on Douglas-fir roots, both in mature stands (Goodman 1995; Goodman and Trofymow 1998*a*) and on young seedlings (Hunt 1991; Berch and Roth 1993). They frequently colonize roots of other conifer species as well, and are sometimes found in significant numbers on root plugs of nursery-grown seedlings.

The majority of fungi in our study still only bear descriptive names. We were able to identify to genus or species only 8 of the 41 morphotypes found. In fact, it is possible that the total number of species could be lower or higher if some of the morphotypes discerned as two or more species are in fact one, or vice versa. It is well known, for example, that the appearance of the mycorrhizal mantle changes with its development (Agerer 1987–2002; Goodman et al. 1996–2000). This is certainly the case with GYBwoven, which turns from vivid greenish-yellow to rich brown (personal observation) or *Lactarius rubrilacteus* in which the orange tips turn green as they age (Goodman et al. 1996–2000). There was also some variation in colour and texture of the mantle depending on the substrate in which the root was embedded, especially between the roots located in rotted wood versus those found in sandy soil. It was for these reasons that we placed all the orange-coloured EM fungi in Orgroup as one morphotype, rather than separating them into several morphotypes. Preliminary anatomical study indicates that despite the apparent morphological differences they are probably the same or closely related. Conversely, although we list only one *Rhizopogon* sp., for the time being, it is not certain that we are dealing with only one species at these sites. Over 100 species of *Rhizopogon* have been mentioned in the literature (Grubisha et al. 2002), although our knowledge about their diversity and distribution in British Columbia is still very scant (Massicotte et al. 1994). Perhaps future surveys will lead us to some conclusions about this dominant

ectomycorrhizal genus. More detailed morphological studies (Goodman et al. 1996–2000), semidetailed descriptions (Jones et al. 1997), and classification based on recent molecular techniques (Mah et al. 2001; Sakakibara et al. 2002) are needed to improve the efficiency of ectomycorrhizal research in North America.

Future research

We suggest that studies of ectomycorrhizae using transects in clear-cuts of increasing age adjacent to older uncut stands might give further insight into the rate at which EM fungi disperse into surrounding newly growing stands. We hypothesize that, as clear-cuts age, the edge effect should diminish as EM fungi from edge trees disperse and colonize seedlings further and further from the edge. Work should also be done to see how different VR practices could potentially affect EM fungi diversity. In particular, past work has focused on edge effects in group variable retention. However, considerable differences exist among sites and operations on how VR is being practiced, including retention of individual versus groups of trees, as well as variation in the percentage retention of the original stand. At this point it is unclear what role individual trees play as refugia or sources of EM fungi for trees regenerating the harvest area. Nor is it clear what level of retention is needed to ensure maintenance of EM species diversity on a site. Examination of sites, preferably in replicated experimental blocks, with VR settings containing single trees and groups of trees and sites with increasing levels of retention would allow researchers to assess which VR practice is best for maintaining EM fungal diversity.

Conclusions

We demonstrated strong edge effects on both EM root colonization and diversity, with the greatest decline occuring within 15–20 m of the patch edge. Significant differences in root colonization and total number of EM morphotypes were observed in old-growth versus second-growth sites and may reflect either differences in habitat suitability or, more likely, differences in dispersal of EM fungal species during the regeneration of second-growth stands. Retention of trees in harvest settings (as compared with clearcut harvest) does, however, appear beneficial in maintaining EM fungal diversity on a site.

Acknowledgements

This study was funded and supported by Weyerhaeuser Ltd. Coastal BC Group, Nanaimo, B.C., by a BC Forest Investment and Innovation grant to Weyerhaeuser, and by the Canadian Forest Service Biodiversity Network node at the Pacific Forestry Centre, Victoria, B.C. We would like to thank Bill Beese and Glen Dunsworth, who initiated this cooperation, as well as other Weyerhaeuser staff members who offered us help with the site selection, especially Jim Loftus, Jeff Sandford, Linda MacDonald, and Paul Harris. Bob Ferris (Pacific Forestry Centre) assisted us in site selection and in the field, Harry Kope and Joanne Davidson assisted us in the laboratory, and Terry Mahoney performed some of the statistical tests. We thank Laurie Kremsater, Mike Preston, and Nick Stanger for reviewing the manuscript before submission.

References

Agerer, R. (*Editor*). 1987–2002. Colour atlas of ectomycorrhizae. Vols. 1–2. Einhorn–Verlag, Munich.

Agerer, R. (*Editor*). 1996–2002. Descriptions of ectomycorrhizae. Vols. 1–4. Einhorn–Verlag, Munich.

Beese, W.J., Dunsworth, B.G., Zielke K., and Bancroft, B. 2003. Maintaining attributes of old-growth forests in coastal B.C. through variable retention. For. Chron. **79**: 570–578.

Berch, S.M., and Roth, A.L. 1993. Ectomycorrhizae and growth of Douglas-fir seedlings preinoculated with *Rhizopogon vinicolor* and outplanted on eastern Vancouver Island. Can. J. For. Res. **23**: 1711–1715.

Bruns, T.D. 1995. Thoughts on the processes that maintain local species diversity of ectomycorrhizal fungi. Plant Soil, **170**: 63–73.

Carroll, G.C., and Wicklow, D.T. 1992. (*Editors*). The fungal community: its organization and role in the ecosystem. 2nd ed. Marcel Dekker, Inc., New York.

Countess, R. 2001. Macrofungal diversity and community structure in Douglas-fir dominated chronosequences of logging origin. M.Sc. thesis, Biology Department, University of Victoria, Victoria, B.C.

Dighton, J., and Mason, P. 1985. Mycorrhizal dynamics during forest tree development. *In* Developmental biology of higher fungi. *Edited by* D. Moore, L.A. Casselton, D.A. Wood, and J.C. Frankland. Cambridge University Press, Cambridge, UK. pp. 117–139.

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.

Franklin, J.F., Berg, D.R., Thornburgh, D.A., and Tappeiner J.C. 1997. Alternative silvicultural approaches to timber harvesting: Variable retention harvest systems. *In* Creating a forestry for the 21st century: The science of ecosystem management. *Edited by* K.A. Kohn and J.F. Franklin. Island Press, Washington, D.C. pp. 111–139.

Goodman, D.M. 1995. Diversity of ectomycorrhizae in old growth and mature stands of Douglas- fir (*Pseudotsuga menziesii*) on southeastern Vancouver Island. Ph.D. dissertation, Biology Department, University of Victoria, Victoria, B.C.

Goodman, D.M., and Trofymow, J.A. 1998*a*. Comparison of communities of ectomycorrhizal fungi in old growth and mature stands of Douglas-fir at two sites on southern Vancouver Island. Can. J. For. Res. **28**: 574–581.

Goodman, D.M., and Trofymow, J.A. 1998*b*. Distribution of ectomycorrhizae in micro-habitats in mature and old-growth stands of Douglas-fir on southern Vancouver Island. Soil Biol. Biochem. **30**: 2127–2138.

Goodman, D.M., Durall, D.M., Trofymow, J.A., and Berch, S.M. (*Editors*). 1996–2000. A manual of concise descriptions of North American ectomycorrhizae: including microscopic and molecular characterization. Copublished by Mycologue Publications, Canadian Forest Service and B.C. Ministry of Forests, Victoria, B.C.

Goodman, D.M., Trofymow, J.A., and Thomson, A.J. 2000. Developing an online database of descriptions of ectomycorrhizae. B.C. J. Ecosyst. Manag. **1**: 1–8.

Green, R.N., and Klinka, K. 1994. A field guide to site identification and interpretation for the Vancouver forest region. Research Branch, B.C. Ministry of Forests, Victoria, B.C.

Grubisha, L.C., Trappe, J.M., Molina, R., and Spatafora, J.W. 2002. Biology of the ectomycorrhizal genus *Rhizopogon*. VI. Re-examination of infrageneric relationships inferred from phylogenetic analyses of ITS sequences. Mycologia, **94**: 607–619.

Hagerman, S.M., Jones, M.D., Bradfield, G.E., and Sakakibara, S.M. 1999*a*. Ectomycorrhizal colonization of *Picea engelmannii* × *Picea glauca* seedlings planted across cut blocks of different sizes. Can. J. For. Res. **29**: 1856–1870.

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

Hagerman, S.M., Sakakibaras, S., and Durall, D. 2000. Potential of woody understorey plots to provide refuge for ectomycorrhizal inoculum of an Interior Douglas-fir forest after clearcut logging. *In* From science to management and back: A science forum for southern interior ecosystems of British Columbia. *Edited by* C. Hollstedt, K. Sutherland, and T. Innes. Southern Interior Forest Extension and Research Partnership, Kamloops, B.C. pp. 131–135.

Hunt, G.A. 1991. Ectomycorrhizal fungi in British Columbia container nursery. FRDA Handbook 009. Canada – British Columbia Forest Resource Development Agreement. Pacific Forestry Centre, Canadian Forest Service, Victoria, B.C.

Ingleby, K., Mason, P.A., Last, F.T., and Fleming, L.V. 1990. Identification of ectomycorrhizae. Institute of Terrestrial Ecology, Merlewood, UK. Natural Environment Research Council, Her Majesty's Stationery Office, London, UK.

Jones, M.D., Durall, D.M., Harniman, S.M.K., Classen, D.C., and Simard, S.W. 1997. Ectomycorrhizal diversity on *Betula papyrifera* and *Pseudotsuga menziesii* seedlings grown in the greenhouse or outplanted in single-species and mixed plots in southern British Columbia. Can. J. For. Res. **27**: 1872–1889.

Jones, M.D., Hagerman, S.M., and Gillespie, M. 2002. Ectomycorrhizal colonization and richness of previously colonized, containerized *Picea engelmannii* does not vary across clearcuts when planted in mechanically site-prepared mounds. Can. J. For. Res. **32**: 1425–1433.

Jones, M.D., Durall, D.M., and Cairney, J.W.G. 2003. Ectomycorrhizal fungal communities in young forest stands regenerating after clearcut logging. New Phytol. **157**: 399–422.

Jungen, J.R. 1985. Soils of southern Vancouver Island: Soil Survey Report 44. Available as Technical Report 17 from Survey and Resource Mapping Branch, B.C. Ministry of Environment, Victoria, B.C.

Kranabetter, J.M. 1999. The effect of refuge trees on a paper birch ectomycorrhiza community. Can. J. Bot. **77**: 1523–1528.

Kranabetter, J.M., and Friesen, J. 2002. Ectomycorrhizal community structure on western hemlock (*Tsuga heterophylla*) seedlings transplanted from forests into openings. Can. J. Bot. **80**: 861–868.

Kranabetter J.M., and Kroeger P. 2001. Ectomycorrhizal mushroom response to partial cutting in a western hemlock/western redcedar forest. Can J. For. Res. **31**: 978–987.

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.

Kranabetter, J.M., Hayden, S., and Wright, E.F. 1999. A comparison of ectomycorrhizal communities from three conifer species planted on forest gap edges. Can. J. Bot. **77**: 1193–1198.

Kremsater, L., Bunnell, F., Huggard, D., and Dunsworth, G. 2003. Indicators to assess biological diversity: Weyerhaeuser's coastal British Columbia forest project. For. Chron. **79**: 590–601.

MacKinnon, A. 2003. West-coast, temperate, old-growth forests. For. Chron. **79**: 475–484.

Mah, K., Tackaberry, L.E., Egger, K.N., and Massicotte, H.B. 2001. The impacts of broadcast burning after clearcutting on the diversity of ectomycorrhizal fungi associated with hybrid spruce seedlings in central Brittish Columbia. Can. J. For. Res. **31**: 224–235.

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.

Massicotte, H.B., Molina, R., Tackaberry, L.E., Smith, J.E., and Amaranthus, M.P. 1999. Diversity and host specificity of ectomycorrhizal fungi retrieved from three adjacent forest sites by five host species. Can. J. Bot. **77**: 1053–1076.

Outerbridge, R.A. 2002. Macrofungus ecology and diversity under different conifer monocultures on southern Vancouver Island. Ph.D. dissertation, Biology Department, University of Victoria, Victoria, B.C.

Outerbridge, R., and Trofymow, J.A. 2003. Diversity of ectomycorrhiza on planted seedlings in variable retention forestry sites: Results of vegetation survey. Report to Weyerhaeuser Coastal BC Group, Nanaimo, B.C., Forest Project – Silviculture and Adaptive Management Program. Unpublished.

Outerbridge, R., Trofymow, J.A., and Kope, H. 2001. Diversity of ectomycorrhizae on planted seedlings in variable retention forestry sites: Results of pilot study and description of design of future experiment. Final report to Weyerhaeuser Coastal BC Group, Nanaimo, B.C., Forest Project – Silviculture and Adaptive Management Program and to Department of Forest Science, University of British Columbia. Unpublished.

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.

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.

Read, D.J. 1992. The mycorrhizal fungal community with special reference to nutrient mobilization. *In* The fungal community. Its organization and role in the ecosystem. 2nd ed. *Edited by* G.C. Carroll and D.T. Wicklow. Marcel Dekker, Inc., New York. pp. 631–652.

Roth, A.L., and Berch, S.M. 1992. Ectomycorrhizae of Douglas-fir and western hemlock seedlings out-planted on eastern Vancouver Island. Can. J. For. Res. **22**: 1646–1655.

Sakakibara, S.M., Jones, M.D., Gillespie, M., Hagerman, S.M., Forrest, M.E., Simard, S.W., and Durall, D.M. 2002. A comparison of ectomycorrhiza identification based on morphotyping and PCR-RFLP analysis. Mycol. Res. **106**: 868–878.

SAS Institute Inc. 1989. SAS/STAT User's guide, version 6. Vol. 2. 4th ed. SAS Institute Inc., Cary, N.C.

Smith, S.E., and Read, D.J. 1997. Mycorrhizal symbiosis. 2nd ed. Academic Press, Harcourt Brace & Company, New York.

Trappe, J.M. 1977. Selection of fungi for ectomycorrhizal inoculation in nurseries. Annu. Rev. Phytopathol. **15**: 203–22.

Trofymow, J.A., and van den Driessche, R. 1991. Mycorrhizas. *In* Mineral nutrition of conifer seedlings. *Edited by* R. van den Driessche, CRC Press, Boca Raton, Fla. pp. 183–227.

Trofymow, J.A., Addison, J., Blackwell, B.A., He, F., Preston, C.A., and Marshall, V.G. 2003. Attributes and indicators of old growth and successional Douglas-fir forests on Vancouver Island. Environ. Rev. **11**: S187–S204.

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