

The potential for woody understory plants to provide refuge for ectomycorrhizal inoculum at an interior Douglas-fir forest after clear-cut logging

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Abstract: Clear-cut logging can decrease the amount of inoculum for some ectomycorrhizal fungi. Ectomycorrhizal plants that are not selected for harvest (refuge plants) may, therefore, be important for the maintenance of ectomycorrhizal fungal inoculum in clearcuts following logging. The purpose of this study was to identify refuge plants that could provide ectomycorrhizal fungal inoculum for outplanted seedlings. The ectomycorrhizal status of selected plants was assessed in 1.6-ha clearcuts and in adjacent forest. Over 3 years, 17 and 14 morphotypes were described for *Pseudotsuga menziesii* (Mirb.) Franco (Douglas-fir) and *Arctostaphylos uva-ursi* (L.) Spreng, respectively. Ten morphotypes (six of these confirmed with restriction fragment length polymorphism patterns) were shared by both species. Anatomical and molecular analyses revealed that, for the morphotypes observed, ectomycorrhizal fungi formed ectomycorrhizal anatomy when associated with Douglas-fir and arbutoid anatomy with *A. uva-ursi*. Because advanced regeneration seedlings of Douglas-fir and *A. uva-ursi* are well distributed throughout this site, these two species have a high potential to provide ectomycorrhizal fungal inoculum for outplanted seedlings. There was no difference in mycorrhizal richness between *A. uva-ursi* plants sampled from the clearcut and forest in the latter 2 years of the study. Conversely, the richness of ectomycorrhizae associated with Douglas-fir sampled from the forest was significantly greater than for seedlings sampled from the clearcuts.

Résumé : La coupe à blanc peut entraîner une diminution de la quantité d'inoculum de certains champignons ectomycorhiziens. Les plantes ectomycorhizées qui ne sont pas récoltées (plantes-refuges) peuvent par conséquent jouer un rôle important dans le maintien de l'inoculum des champignons ectomycorhiziens dans les coupes à blanc après la récolte. Le but de cette étude consistait à identifier les plantes-refuges pouvant servir de source d'inoculum pour les semis transplantés. La mycorrhization de plantes sélectionnées a été évaluée dans une coupe à blanc de 1,6 ha et dans la forêt adjacente. Sur une période de trois ans, respectivement 17 et 14 types morphologiques ont été décrits chez *Pseudotsuga menziesii* (Mirb.) Franco (douglas de Menzies) et *Arctostaphylos uva-ursi* (L.) Spreng. Dix types morphologiques, dont six sont confirmés par les patrons du polymorphisme de longueur des fragments de restriction, sont communs aux deux espèces. Des analyses anatomique et moléculaire ont révélé que, dans le cas des types morphologiques observés, les champignons ectomycorhiziens forment des ectomycorhizes avec le douglas de Menzies et des mycorhizes arbutoides avec *A. uva-ursi*. Étant donné que la régénération préétablie de douglas de Menzies et de *A. uva-ursi* est bien distribuée partout dans ce site, ces deux espèces ont un potentiel élevé comme source d'inoculum des champignons ectomycorhiziens pour les semis transplantés. Il n'y avait pas de différence dans la richesse des mycorhizes entre les plants de *A. uva-ursi* échantillonnés dans la coupe à blanc et dans la forêt adjacente au cours des deux dernières années de l'étude. Par contre, la richesse des ectomycorhizes associées au douglas de Menzies était significativement plus grande en forêt que dans la coupe à blanc.

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Introduction

Ectomycorrhizal fungi take up and transport essential nutrients and water to their host plants (Harley and Smith 1983; Parke et al. 1983). In addition, ectomycorrhizal fungi can provide plant resistance to pathogens (Barham et al. 1974) and herbivory (Gehring and Whitham 1991). The ectomycorrhizal association is, therefore, considered important

for adequate growth and survival of many tree species at both early and late stages of development (Christy et al. 1982; Perry et al. 1987; Villeneuve et al. 1991). However, silvicultural systems such as clear-cut logging can reduce the quantity and diversity of ectomycorrhizal fungal inoculum (Harvey et al. 1980; Parsons et al. 1994; Kranabetter and Wylie 1998; Durall et al. 1999; Hagerman et al. 1999). Thus, seedlings grown in clear-cut soils often form fewer ectomycorrhizae than seedlings grown in undisturbed soils (Harvey et al. 1980; Perry et al. 1982; Parke et al. 1984). Furthermore, the dispersal of spores into disturbed areas may be insufficient for providing inoculum at levels comparable with the uncut forest (Perry et al. 1987).

Most ectomycorrhizal fungal species form ectomycorrhizae with many different tree species (Molina et al. 1992). For example, field and greenhouse studies have shown that, within a

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stand, many of the ectomycorrhizal fungi associated with *Pseudotsuga menziesii* (Mirb.) Franco (Douglas-fir) also associate with *Betula papyrifera* Marsh. (paper birch) (Simard et al. 1997a; Jones et al. 1997) and *Pinus muricata* D. Don. (bishop pine) (Horton and Bruns 1998). From the perspective of stand regeneration, a mycorrhizal community shared among plant species has important implications for the maintenance of ectomycorrhizal fungal inoculum on a site and the establishment of mycorrhizae with outplanted seedlings (Molina and Trappe 1982; Amaranthus and Perry 1989, 1994; Borchers and Perry 1990). After clear-cut logging, some plant species previously present underneath the canopy persist and frequently proliferate throughout an opening. Many of these early seral plant species form ectomycorrhizae and have the potential to maintain ectomycorrhizal fungal inoculum that would otherwise die because of the lack of a plant associate. These plant species have been referred to as refuge plants, and various studies have discussed their importance (Dahlberg 1990; Visser 1995; Massicotte et al. 1999).

Some mycorrhizal fungal species form more than one category of mycorrhizae depending on the plant associate involved. For example, some basidiomycetous and ascomycetous fungi that form ectomycorrhizae with trees form arbutoid mycorrhizae in association with ericaceous plants in the genera *Arbutus* and *Arctostaphylos*. Arbutoid formation by many species of ectomycorrhizal fungi has been observed in the laboratory (Zak 1976; Molina and Trappe 1982), but detailed anatomical investigation of field samples is limited to a few studies (Zak 1974; Mejsstrik and Hadac 1975; Largent et al. 1980; Acsai and Largent 1983). Plants that commonly form ericoid mycorrhizae can also form associations with ectomycorrhizal fungi. Smith et al. (1995), reported colonization of the ericaceous plants *Rhododendron macrophyllum* G. Don and *Gaultheria shallon* Pursh by ectomycorrhizal fungi in a greenhouse experiment using soils sampled from a Douglas-fir stand in the Oregon Coast Range. Although the incidence of ectomycorrhizal colonization of these ericoid plants was low, the occurrence of this association in natural ecosystems may have important implications for the retention of mycorrhizal inoculum under certain conditions. Similarly, Largent et al. (1980), reported ectomycorrhizae on *Vaccinium scoparium* Leib., *Vaccinium ovatum* Pursh, *Vaccinium arbuscula* (Gray) Merriam, *Vaccinium parvifolium* Smith, *Ledum glandulosum* ssp. *glandulosum* Nutt., *Leucothoe Davisiae* Torr., *R. macrophyllum*, *G. shallon*, *Chimaphila umbellata* (L.) Bart., and numerous species of *Arctostaphylos* sampled from northern California.

The alternative silvicultural systems trial at Opax Mountain was originated to gain a better understanding of the impact of various harvesting methods on the ecology of interior dry Douglas-fir forests in British Columbia. The objectives of this part of the Opax study were (i) to identify understory plants that could support ectomycorrhizal fungal inoculum for Douglas-fir seedlings outplanted in the clearcuts, (ii) to describe the community of ectomycorrhizal fungi associated with refuge plants sampled from the clear-cut openings and the same species in adjacent uncut forest, and (iii) to quantify the diversity (expressed as richness) of ectomycorrhizae associated with refuge plants amongst each other and at clear-cut and forest locations.

Materials and methods

Site characteristics

The Opax Mountain Silvicultural Systems Trial is located approximately 20 km northwest of Kamloops in the southern interior of British Columbia (51°35'N, 120°74'W) and ranges in elevation from 950 to 1370 m (Bealle-Statland 1998). The site was harvested in the winter of 1993–1994. The alternative silvicultural systems implemented included individual tree selection (20, 35, and 50% volume removal) as well as 0.1-, 0.4-, and 1.6-ha patch cuts in a randomized block design. The upper elevation block (1200–1370 m) is classified as Interior Douglas-fir Dry Cool (IDFdk1) biogeoclimatic subzone variant and the lower elevation block (950–1100 m) is classified as IDF Very Dry Hot (IDF xh2) variant. This study was conducted at the three upper elevation 1.6-ha patch cut treatment units and at the three lower elevation 1.6-ha patch cut treatment units.

The study area is a mixed stand of Douglas-fir, hybrid spruce (*Picea engelmannii* Parry × *Picea glauca* (Moench) Voss) and lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.) ranging in age from 70 to 220 years old (Bealle-Statland 1998). The composition of the stand varies between upper and lower elevations, but in general, Douglas-fir is the dominant overstory species. Trembling aspen (*Populus tremuloides* Michx.) and paper birch are minor components of the stand (Bealle-Statland 1998). Common understory plants include *Arctostaphylos uva-ursi* (L.) Spreng., *C. umbellata*, *Juniperus communis* L., *Mahonia aquifolium* (Pursh) Nutt., *Paxistima myrsinites* (Pursh) Raf., *Shepherdia canadensis* (L.) Nutt., *Spirea betulifolia* Pall. var. *lucida* (Dougl.) C.L. Hitchc., *Vaccinium caespitosum* Michx., and *Vaccinium membranaceum* Dougl.:Hook. (Table 1).

At lower elevations of the research site, soils are Orthic Gray Luvisols and Orthic Eutric Brunisols with a Hemimor humus layer of 3.5 cm (average thickness). Higher elevations are characterized by soils that are Brunisolic Gray Luvisols with a Hemimor humus layer of 2.6 cm (average thickness). Soil textures are a thin layer of silt loam over loam and clay loam textured glacial till (G. Hope, personal communication). The mean annual air temperature in this biogeoclimatic zone is between 1.6 and 9.5°C. Mean annual precipitation ranges from 300 to 750 mm with 20–50% of that falling as snow (Hope et al. 1991).

Sampling

Potential refuge plants

Root leaders containing fine root tips were sampled from 16 species of common woody understory plants (Table 1) in July 1995 to assess their ectomycorrhizal status. Roots were traced back to the mother plant to ensure they were attached to the plant being sampled. Roots from 10 individuals of each plant species were sampled from the undisturbed forest both at the upper and lower elevations of the site. The roots were placed in soil in plastic bags, brought to the laboratory, and stored at 5°C for approximately 4 months during processing. Soil and debris were gently washed from the roots over a 1-mm sieve. Roots of an individual plant were cut into approximately 1-cm pieces and randomized in a baking dish containing deionized water. Up to 200 ectomycorrhizal and arbutoid mycorrhizal root tips per individual plant were randomly selected, counted, and described according to specific morphological features. In addition, counts of active nonmycorrhizal root tips and inactive root tips were recorded. No distinction could be made between inactive nonmycorrhizal and inactive mycorrhizal root tips.

Distinction between active and inactive ectomycorrhizal fine root tips followed the criteria described by Harvey et al. (1976). As observed under the stereomicroscope, inactive roots were character-

Table 1. Ectomycorrhizal status of 16 understory species sampled from the Opax Mountain site in July 1995.

Plant species	Colonization (%)*	Morphotypes observed	Morphotypes shared with Douglas-fir
<i>Acer glabrum</i>	0	0	0
<i>Alnus viridis</i> ssp. <i>sinuata</i>	48.12±7.60	8	8
<i>Amelanchier alnifolia</i>	17.11±9.53	5	4
<i>Arctostaphylos uva-ursi</i>	34.99±6.33	17	10
<i>Betula papyrifera</i>	56.01±10.04	13	8
<i>Chimaphila umbellata</i>	0	0	0
<i>Juniperus communis</i>	0	0	0
<i>Mahonia aquifolium</i>	0	0	0
<i>Paxistima myrsinites</i>	1.03±1.03	1	1
<i>Populus tremuloides</i>	44.52±6.80	16	11
<i>Pseudotsuga menziesii</i>	46.78±9.24	15	15
<i>Salix commutata</i>	25.38±5.99	14	7
<i>Shepherdia canadensis</i>	15.16±7.79	8	4
<i>Spiraea betulifolia</i>	0.99±0.99	2	2
<i>Vaccinium caespitosum</i>	0.97±0.97	1	1
<i>Vaccinium membranaceum</i>	0	0	0

*Values are means ± SE.

ized as having a dark apex and wrinkled texture. Most active ectomycorrhizae had a pale apex and were turgid although certain morphotypes such as *Cenococcum*-like and *Tomentella*-like were turgid and smooth yet had dark apices because of complete coverage by darkly pigmented fungal tissue. Mycorrhizal roots were observed under 400× or 1000× magnification either as whole mounts (entire root tip) or as a mantle peel (only the fungus). Mantle peels were made by separating the fungal tissue from the root with fine forceps. Classification of mycorrhizal roots followed the detailed procedure described by Goodman et al. (1996). Over 50 characters including colour of the mycorrhizae, features of the extramatrical hyphae, mantle pattern, and reaction to specific chemicals were used to separate the active mycorrhizal roots into distinct morphological categories. These groupings were then compared with published descriptions (Agerer 1987–1998; Ingelby et al. 1990; Goodman et al. 1996) to suggest the identity of the fungal symbiont. Photographs and frozen specimens are stored at the North Kelowna campus of Okanagan University College.

In September 1997 and 1998, plant species that had the highest potential to provide refugia for ectomycorrhizal inoculum on clear-cut sites (based on extensive mycorrhizal colonization and regular distribution throughout the openings observed in 1995) were selected for further study. These species were identified as *Arctostaphylos uva-ursi* and advanced regeneration seedlings of Douglas-fir. Five individuals from each species were sampled from each of the six 1.6-ha openings (three upper elevation and three lower elevation) and in the adjacent uncut forest ($n = 120$ in each year). Equal number of plants were sampled from openings and adjacent forest. Root leaders and tips were sampled and assessed for mycorrhizal colonization as previously described.

Root sectioning

A freezing microtome (Physitemp Instruments, Clifton, N.J.) was used to prepare 5- to 20-μm cross sections of mycorrhizae formed by *Arctostaphylos uva-ursi* and Douglas-fir. Cross sections of the four mycorrhizal types most commonly associated with both advanced regeneration seedlings of Douglas-fir and *Arctostaphylos uva-ursi* (E-strain-like, *Piloderma* sp., *Amphinema*-like and *Cenococcum*-like) were observed at 400× and 1000× to characterize the type of mycorrhizae (arbutoid or ectomycorrhizae).

DNA isolation

Total genomic fungal DNA was isolated from frozen root tips according to the method of Baldwin and Egger (1996) with some modifications. The extraction buffer contained 3% CTAB, 0.5–1% β-mercaptoethanol, and 1% polyvinylpyrrolidone. Ground root tips were suspended in extraction buffer and incubated at 65°C for 90 min to 2 h. After incubation, samples were extracted twice with one volume chloroform – isoamyl alcohol (24:1). DNA was precipitated with two-thirds volume isopropanol overnight at –20°C. The DNA was then washed two times with two volumes of ice-cold wash buffer (76% ethanol, 10 mM ammonium acetate) and dried at room temperature for 10–15 min. The pellet was resuspended in 50 mL 8 mM NaOH and stored at 4°C. For long-term storage, DNA samples are kept at –20°C.

Polymerase chain reaction (PCR) amplification

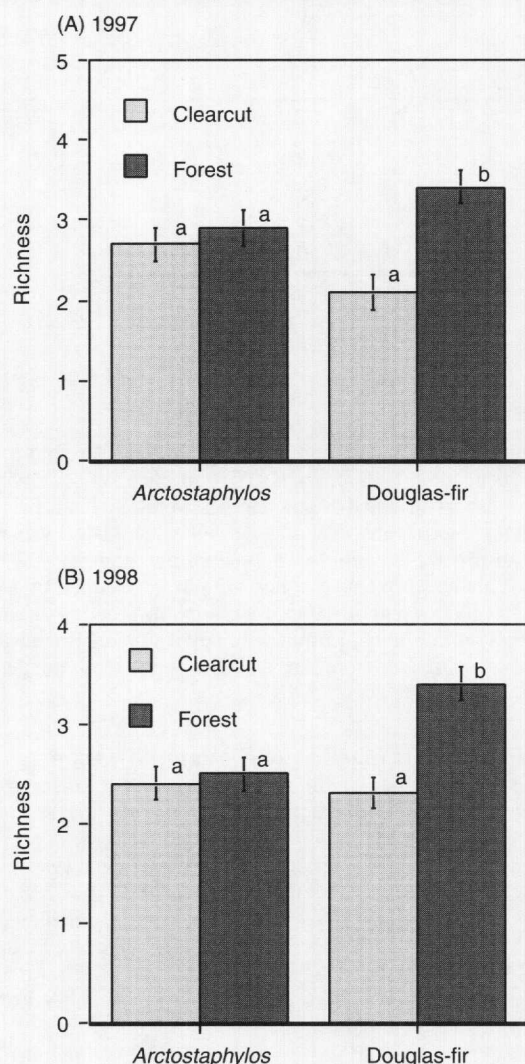
In general, DNA preparations were used in PCR reactions either undiluted or diluted by one-half. Following extraction, the internal transcribed spacer (ITS) region of the fungal DNA was specifically amplified by the primers ITS1 and NL6bmun (Egger 1995). A typical PCR amplification reaction consisted of the following components; 4 mL template DNA, 17.2 mL sterile distilled water, 0.188 mM deoxyribonucleotides (Amersham), 3 mL 10× PCR buffer (100 mM Tris-HCl pH 8.3, 500 mM KCl), 1.875 mM MgCl₂, 0.375 mM each primer, and 0.8 U Expand high-fidelity PCR system (Boehringer-Mannheim).

Samples were amplified using a Perkin-Elmer DNA thermal cycler. A 7-min hot start was followed by PCR cycling as follows: 1 min at 95°C followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 48°C for 45 s, ramping to 72°C for 55 s with a 1-s extension after each cycle, and extension at 72°C for 130 s. A final extension step was added for 7 min at 72°C, and then the temperature was held at 4°C. The PCR products were visualized on 1.5% agarose gels.

Restriction enzyme digests

Three restriction enzymes, *AluI*, *HinfI*, and *MboI* were used. Digests were performed in a total volume of 20 mL, consisting of 17 mL of PCR product, 2 mL of REact buffer, and 10 U of enzyme, then resolved on a 2.5% agarose gel consisting of 1% agarose and 1.5%

Fig. 1. (A) Richness of mycorrhizae per plant associated with Douglas-fir and *Arctostaphylos uva-ursi* between clear-cut and forest locations in 1997. *Arctostaphylos uva-ursi* (ANOVA: $P = 0.4540$; $n = 30$ samples from clear-cut plots, $n = 30$ samples from forest plots), Douglas-fir (ANOVA: $P < 0.0001$; $n = 30$ samples from clear-cut plots, $n = 30$ samples from forest plots). (B) Richness of mycorrhizae per plant associated with Douglas-fir and *Arctostaphylos uva-ursi* between clear-cut and forest locations in 1998. *Arctostaphylos uva-ursi* (ANOVA: $P = 0.5919$; $n = 29$ samples from clear-cut plots, $n = 30$ samples from forest plots), Douglas-fir (ANOVA: $P < 0.0001$; $n = 30$ samples from clear-cut plots, $n = 30$ samples from forest plots).



NuSieve (FMC Bio Products) by electrophoresing at 80 V for 4 h. Gels were stained for 45 min in ethidium bromide, destained in distilled water for 20 min, and photographed on an ultraviolet transilluminator. Restriction fragment length polymorphism (RFLP) band sizes were estimated by comparison to a standard 100 base pair molecular weight ladder (Gibco BRL). Banding patterns were manually compared with RFLPs previously generated from sporocarps collected in the Interior Cedar-Hemlock biogeoclimatic zone of British Columbia and from ectomycorrhizal root tips from other studies (Hagerman et al. 1999; S.E. Sakakibara, unpublished data).

Data analysis

The relative abundance of ectomycorrhizal types for each year

Table 2. ANOVA model and results for the number of morphotypes (richness) associated with selected refuge plants sampled from the Opax site in 1997 and 1998.

Source	df	SS	F	p > F
1997				
Block (elevation)	1	0.21	0.15	0.70
Plant species	1	0.008	0.006	0.94
Clearcut vs. forest	1	16.9	12.3	0.0006
Clearcut vs. forest × plant species	1	8.0	5.8	0.017
1998				
Block (elevation)	1	0.24	0.30	0.58
Plant species	1	4.2	5.3	0.23
Clearcut vs. forest	1	6.7	8.5	0.004
Clearcut vs. forest × plant species	1	10.4	13.1	0.0004

of study is expressed as a percentage of the total number of active ectomycorrhizae or arbutoid mycorrhizae of a specific type averaged over the total mycorrhizal community. Percent colonization was calculated by dividing the number of active ectomycorrhizal fine roots by the total number of active fine roots (mycorrhizal and nonmycorrhizal). Diversity is expressed as richness, where richness is the number of morphotypes encountered. Richness was calculated at the scale of the individual plant ($n = 200$ mycorrhizae).

The effect of block (elevation), treatment (clearcut vs. forest), and plant species on the richness of the mycorrhizal community was tested by three-factor ANOVA. Individual effects were subsequently tested by one-way analysis of variance. All statistical tests were performed using JMP (version 3.1, SAS Institute Inc., Cary, N.C.).

Results

Initial survey of potential refuge plants (1995)

Colonization by ectomycorrhizal fungi was observed for 11 of the 16 plant species surveyed (Table 1). While the roots of some of these species were sparsely colonized, six plant species had greater than 25% of their fine roots colonized by ectomycorrhizal fungi. These species included *B. papyrifera*, *Alnus viridis* ssp. *sinuata* (Regel) Ä. Löve & D. Löve, *Pseudotsuga menziesii*, *Populus tremuloides*, *Arctostaphylos uva-ursi*, and *Salix commutata* Bebb. In this initial year of study, a total of 27 distinct mycorrhizal types were encountered on the roots of the 11 ectomycorrhizal and arbutoid mycorrhizal plant species. All 27 types were observed in samples of the six most extensively colonized species. Moderate levels of mycorrhizal colonization were observed for *Amelanchier alnifolia* Nutt. and *S. canadensis* and extremely low levels were found on *P. myrsinites*, *S. betulifolia*, and *V. caespitosum*. In the case of the latter three plant species, means were based on one or two roots from one sample. It is highly likely that the results were due to sampling error. Colonization by ectomycorrhizal fungi was not observed for *C. umbellata*, *J. communis*, *M. aquifolium*, *Acer glabrum* Torr., and *V. membranaceum*.

Diversity of ectomycorrhizae associated with selected refuge species in clear-cut and forest areas 1997–1998

There was no difference in mycorrhizal richness between *Arctostaphylos uva-ursi* plants sampled from the clearcut and forest in 1997 and 1998 (Figs. 1A and 1B). Conversely, the richness of ectomycorrhizae associated with Douglas-fir sampled from the forest was significantly greater than for

Table 3. Relative abundances (with SE given in parentheses) of the morphotypes formed by ectomycorrhizal fungi encountered on the roots of *Arctostaphylos uva-ursi* and advanced regeneration *Pseudotsuga menziesii* sampled from both clear-cut and forested areas at the Opax site in the fall of 1997 and 1998.

OUC No.	Mycorrhizae type	Year	<i>A. uva-ursi</i>	<i>P. menziesii</i> (advanced regeneration)
20	<i>Amphinema</i> -like	1997	11.4 (2.7)	8.8 (3.1)
		1998	16.6 (4.9)	6.6 (1.6)
30	<i>Cenococcum</i> -like	1997	20.9 (3.3)	12.1 (3.2)
		1998	31.1 (4.0)	13.4 (3.1)
40	<i>Cortinarius aff. laniger</i> *	1997	8.4 (2.2)	2.8 (1.4)
		1998	2.8 (1.9)	1.1 (0.6)
50	<i>Dermocybe</i> -like	1997	0.25 (0.2)	—
		1998	—	—
60	E-strain-like	1997	7.0 (3.0)	10.9 (4.1)
		1998	11.8 (3.4)	5.7 (2.5)
95	<i>Inocybe aff. albidodisca</i>	1997	0.58 (0.58)	—
		1998	—	—
143	<i>Lactarius vietus</i> *	1997	—	9.0 (3.9)
		1998	—	9.3 (3.1)
140	<i>Lactarius</i> -like	1997	—	1.2 (1.2)
		1998	—	2.4 (1.5)
170	<i>Mycelium radicans atrovirens</i>	1997	7.8 (2.8)	4.3 (2.3)
		1998	2.8 (1.3)	0.2 (0.2)
200b	<i>Piloderma</i> sp.*	1997	14.9 (7.8)	6.9 (3.7)
		1998	15.8 (5.4)	9.7 (3.4)
210	<i>Rhizopogon</i> sp. A section <i>Fulviglebae</i> *	1997	—	13.3 (3.9)
		1998	—	7.4 (1.9)
230	<i>Rhizopogon</i> sp. B section <i>Villosuli</i> *	1997	—	20.0 (5.9)
		1998	—	37.4 (7.5)
142	<i>Russula aff. puellaris</i>	1997	—	—
		1998	—	0.7 (0.7)
220a	<i>Russula nigricans</i> *	1997	2.8 (2.8)	6.7 (2.8)
		1998	4.8 (3.2)	2.9 (1.9)
240a	<i>Thelephora</i> -like I	1997	2.2 (2.2)	1.2 (0.9)
		1998	—	1.8 (1.0)
240b	<i>Thelephora</i> -like II	1997	1.3 (0.9)	0.58 (0.4)
		1998	4.1 (2.2)	—
251	<i>Tomentella</i> -like II	1997	14.9 (4.3)	0.8 (0.6)
		1998	8.8 (3.4)	1.5 (1.2)
270	<i>Truncocolumella citrina</i> *	1997	—	0.5 (0.5)
		1998	—	0.2 (0.2)
OX97 No. 20	Unknown	1997	7.3 (3.2)	—
		1998	—	—
OX97 No 160	Unknown	1997	0.5 (0.5)	1.0 (0.6)
		1998	1.7 (0.8)	0.08 (0.08)
Total richness			14	17

*Confirmed by PCR-RFLP analysis.

advanced regeneration Douglas-fir seedlings sampled from the clearcuts (Figs. 1A and 1B). In these 2 years of study, where only *Arctostaphylos* and Douglas-fir were assessed, there was no block (elevation) effect (Table 2). Advanced regeneration Douglas-fir seedlings had lower numbers of active fine roots in the clearcuts as compared with *Arctostaphylos uva-ursi* sampled from the clearcuts. This trend was statistically significant in 1997 (1997: ANOVA, $P = 0.0009$; 1998: ANOVA, $P = 0.1269$).

Mycorrhizal community

A comparison between the community of ectomycorrhizal

fungi associated with *Arctostaphylos uva-ursi* and advanced regeneration Douglas-fir seedlings was made using the data from 1997 and 1998. Over the two sampling seasons a total of 14 morphotypes were encountered on the roots of *Arctostaphylos uva-ursi* and 17 morphotypes were associated with advanced regeneration Douglas-fir seedlings (Table 3). Ten morphotypes (six of these confirmed by RFLP patterns) were shared by both species. The morphotypes that were common to both plant species were *Amphinema*-like, *Cenococcum*-like, *Cortinarius* c.a. *laniger* Fr., E-strain-like, *Mycelium radicans atrovirens* Melin., *Piloderma* sp., *Russula nigricans* (Bull.) Fr., *Tomentella*-like type II, and OX97 No. 160 (Table 4).

Table 4. Morphological characteristics of the morphotypes occurring with a relative abundance of >6% in any one year and (or) the morphotypes matched to sporocarps by molecular analyses.

	Mantle type(s)	Emanating hyphae	Mycelial strands	Cystidia
<i>Amphinema</i>-like				
OUC 020: Light brown to orange mycorrhiza, rough, with loose yellowish mycelial strands and fine yellow and white emanating hyphae	Outer: felt prosenchyma, hyphae 3–4 µm wide; inner: net synenchyma, 3–4 µm wide	4 µm in diameter; very abundant clamps; clear contents; smooth to finely verrucose; stain yellow in 10% KOH	Loosely organized; only 3 or 4 hyphae wide; abundant clamps	Absent
<i>Cenococcum</i>-like				
OUC 030: Black, rough mycorrhiza; moderate to abundant black, straight emanating hyphae; strands absent	Outer: net synenchyma, hyphae 4–5 µm; "stained glass" pattern; inner: net synenchyma or non-interlocking irregular synenchyma, 3–6 µm	3–4 µm in diameter; smooth; no clamps	Absent	Absent
<i>Cortinarius</i> aff. <i>laniger</i>*				
OUC 040: White mycorrhiza; rough; abundant white emanating hyphae and mycelial strands	Outer: felt prosenchyma; inner: net synenchyma or irregular synenchyma	3–5 µm in diameter; frequent clamp connections; hyphae can have both clear and granular contents	Compact; white; 1 mm in diameter	Absent
<i>E-strain</i>-like				
OUC 060: Light brownish-orange mycorrhiza with mantle becoming lighter towards apex; smooth; rare emanating hyphae	Outer: incomplete felt prosenchyma, hyphae 3–8 µm; inner: net prosenchyma, 1.5–2.5 µm	4–7 µm in diameter; no clamps; smooth to verrucose	Absent	Absent
<i>Inocybe</i> aff. <i>albidodisca</i>*				
OUC 095: White-grey-silver mycorrhiza; smooth; no emanating hyphae; thick septa stain darkly in toluidine blue	Outer: net prosenchyma-net synenchyma, hyphae 4 µm; inner: net synenchyma, 4 µm	Absent	Absent	Absent
<i>Lactarius</i> <i>vietus</i>*				
OUC 143: Beige-teal mycorrhiza; smooth; laticifers abundant	Outer: net prosenchyma – net synenchyma, hyphae 3–6 µm, laticifers common; inner: net synenchyma 4–5 µm	Absent	Loose, undifferentiated	Absent
<i>Mycelium</i> <i>radicis</i> <i>atrovirens</i>-like				
OUC 170: Black-brown or grey mycorrhiza; very narrow (less than 0.5 mm) with rough texture; rare to common emanating hyphae	Outer: felt prosenchyma, hyphae 3 µm, dark brown hyphae; inner: net synenchyma, hyaline hyphae, 2 µm	2 µm in diameter; no clamps; smooth to finely verrucose	Absent	Absent
<i>Piloderma</i> sp.*				
OUC 200: White mycorrhiza; large crystals, no clamps	Outer: net prosenchyma, hyphae 3–4 µm; inner: net synenchyma, hyphae 3–4 µm	3–4 µm in diameter; abundant crystalline ornamentation; no clamps	Loose, undifferentiated	Absent
<i>Rhizopogon</i> sp. A section <i>Fulviglebae</i>*				
OUC 210: White and brown-black mycorrhiza; abundant and matted emanating hyphae form a tubercle around mycorrhizas; strands common	Outer: net prosenchyma, hyphae 2 µm; inner: net synenchyma, hyphae 2 µm when visible	2 µm in diameter; no clamps, smooth, densely packed	Loosely organized, hyphae 2 µm in diameter	Absent
<i>Rhizopogon</i> sp. B, section <i>Villosuli</i>*				
OUC 230: light brown mycorrhiza covered with abundant white hyphae and strands	Outer: felt prosenchyma, hyphae 3 µm; inner: net synenchyma, not readily visible	3 µm in diameter; crystalline ornamentation and no clamps	Compact and frayed looking with crystalline ornamentation on the hyphae	Absent

<i>Russula nigricans</i>* OUC 220a: Creamy tan mycorrhiza; velvety texture; emanating hyphae absent	Outer: net prosenchyma or synenchyma, hyphae 3 µm; inner: not visible	Absent	Absent	Abundant, 10–20 µm in length; 3–5 µm diameter; bottle shaped; multilobed
<i>Tomentella</i>-like type II OUC 251: Black mycorrhiza; very grainy and swollen; abundant emanating hyphae	Outer: nonlocking or regular synenchyma, hyphae 5–10 µm, cells rounded; inner: not visible	3–4 µm in diameter; abundant clamps; dark brown; smooth	Absent	Absent
<i>Truncocolumella citrina</i>* OUC 270: Yellow-brown mycorrhiza; very grainy, bruises brown when touched, yellow emanating hyphae and strands	Outer: net prosenchyma, hyphae 4–5 µm; inner: net synenchyma 4–9 µm	4–5 µm in diameter; clamps rare but present; smooth	Loose, undifferentiated	Absent
Unknown OPAX 97 20: Tan-white mycorrhiza; smooth	Outer: felt prosenchyma, hyphae 3 µm wide; inner: net synenchyma, oriented in circular groupings, hyphae 4 µm wide	3 µm in diameter; smooth, no clamps, hyaline, clear contents, thick walls	Absent	Absent

*Confirmed by PCR-RFLP analysis

Five morphotypes were found only on Douglas-fir: *Lactarius vietus* Fr., *Truncocolumella citrina* Zeller, *Rhizopogon* sp. A, section *Fulviglebae*, *Rhizopogon* sp. B, section *Villosuli* and *Russula* c.a. *puellaris* Fr. Three morphotypes were found only on *Arctostaphylos uva-ursi*: *Dermocybe*-like, *Inocybe* c.a. *albidodisca* Kuehn, and unknown OX97 No. 20.

In addition to *Arctostaphylos uva-ursi*, many of the other plants surveyed also shared a substantial component of their mycorrhizal community with that of Douglas-fir. For instance, 73% of the mycorrhizal types associated with *Populus tremuloides*, also associated with Douglas-fir, and *B. papyrifera*, *Alnus viridis* ssp. *sinuata* and *S. commutata* each shared 53, 53, and 47% of their morphotypes, respectively, with Douglas-fir (Table 1).

Root sectioning

Prior to sectioning, specific mycorrhizal morphotypes looked very similar between Douglas-fir and *Arctostaphylos uva-ursi*, because both plant species formed fungal mantles. However, observation of cross sections under 400× and 1000× magnification revealed that, although the mycobiont was the same (determined by PCR-RFLP analysis), the structure of the mycorrhizae was different. Mycorrhizae formed by *Arctostaphylos uva-ursi*, as has been well documented, were characterized by intracellular colonization of the epidermal layer and the absence of a Hartig net. The same fungus colonizing Douglas-fir roots formed ectomycorrhizae with a fungal mantle, a Hartig net, and no intracellular penetration.

PCR-RFLP analysis

RFLP patterns were generated from 15 of the most common ectomycorrhizal types associated with *Arctostaphylos uva-ursi* and Douglas-fir root samples from 1997 and 1998 (Table 5). Eight of these 15 morphotypes each gave rise to a single, unique RFLP pattern. Seven morphotypes generated more than one pattern, but the variable patterns for four of these morphotypes differed only at one of the three enzymes (within morphotypes) suggesting that they are closely related. Of the eight monomorphic morphotypes, six types generated RFLP patterns that matched sporocarps or root tips sampled from a range of forest types in the southern interior of British Columbia (Table 5). A more detailed report of these results is planned for future publication (S.M. Sakakibara, S.M. Hagerman, S.M.K. Gillespie, M.D. Jones, M.E. Forrest, and D.M. Durall, unpublished data).

Nine of the 15 morphotypes subjected to PCR-RFLPs were shared by both Douglas-fir and *Arctostaphylos uva-ursi* (Table 5). While several of these gave rise to more than one RFLP pattern each, all of these morphotypes with >6% colonization exhibited different banding patterns on both Douglas-fir and *Arctostaphylos uva-ursi*. This indicates that, while there may be more variation at the DNA level than can be identified morphologically, this variation is not confined to a specific host. Both plant species are able to form symbioses with these types of ectomycorrhizal fungi.

Discussion

Refuge plants at Opax Mountain

Colonization by ectomycorrhizal fungi was observed for 11 of 16 plant species surveyed at the Opax site. Although

Table 5. Summary of ectomycorrhizae analyzed by PCR-RFLP.

OUC No.	Mycorrhizae type	No. of tips analyzed	Percentage tips from <i>P. menziesii</i>	Percentage tips from <i>A. uva-ursi</i>	No. of different RFLP patterns	Sporocarp matches
20	<i>Amphinema</i> -like	12	58	42	2 ^a	
30	<i>Cenococcum</i> -like	11	64	36	1	
40	<i>Cortinarius</i> sp.	7	86	14	2 ^a	<i>C. armillatus</i> (Fr.) Fr. – <i>C. laniger</i> Fr. ^d and others
60	E-strain-like	17	59	41	2 ^b	
95	<i>Inocybe</i> sp.	6	33	67	1	<i>Inocybe</i> aff. <i>albidodisca</i> Kuehn.
142	<i>Russula</i> sp.	5	100	0	1	<i>R. placita</i> – <i>R. puellaris</i> Fr. – <i>R. bicolor</i> ^d
143	<i>Lactarius</i> sp.	14	100	0	1	<i>L. rubrilacteus vietus</i> Fr. and others ^c
200	<i>Piloderma</i> sp.	15	33	67	4 ^a	
210	<i>Rhizopogon</i> sp. A	11	100	0	2 ^b	<i>Rhizopogon</i> spp. section <i>Fulviglebae</i> ^e
230	<i>Rhizopogon</i> sp. B	14	100	0	1	<i>Rhizopogon</i> spp. section <i>Villosuli</i> ^e
220a	<i>Russula</i> sp.	11	73	27	1	<i>R. nigricans</i>
240a	<i>Thelephora</i> I-like	5	60	40	2 ^c	
240b	<i>Thelephora</i> II-like	5	20	80	2 ^c	
251	<i>Tomentella</i> -like	1	100	0	1	
270	<i>Truncocolumella</i> sp.	3	100	0	1	<i>Truncocolumella citrina</i>

^aRFLP patterns differed only in the digest by a single enzyme.

^bAn aberrant RFLP pattern was observed infrequently in these morphotypes. This is probably due to the presence of additional fungus on the root tip.

^cRFLP patterns differed in the digest by two enzymes.

^dMore than one sporocarp gave rise to the same RFLP banding pattern.

^eRFLPs matched root tips which were sequenced and found to group with these sections (L. Grubisha, personal communication).

many of the plants assessed were extensively associated with ectomycorrhizal fungi, *Arctostaphylos uva-ursi* and advanced regeneration Douglas-fir seedlings were abundant throughout the study site in addition to having a high proportion of roots colonized by ectomycorrhizal fungi. Therefore, these two particular plant species are likely good candidates to provide refuge for ectomycorrhizal fungi after logging. Additionally, the growth habit of *Arctostaphylos uva-ursi* makes it particularly effective as refugia, because it forms extensive mats throughout the openings and yet does not shade out young conifer seedlings. Other studies have reported the potential importance of *Arbutus* spp., and *Arctostaphylos* spp. including *Arctostaphylos uva-ursi* as refuge plants in northern temperate forests (Danielson 1984; Visser 1995; Horton et al. 1999; Massicotte et al. 1999). In the present study, many of the most common morphotypes associated with *Arctostaphylos uva-ursi* were also the common types associated with Douglas-fir. Some of these morphotypes included *Cenococcum*-like, *Amphinema*-like, E-strain, and *Piloderma* sp. This observation illustrates the broad receptivity of arbutoid plants and provides support for the statement that *Arbutus* and *Arctostaphylos* are "mycorrhizal generalists" (Molina and Trappe 1982).

The proximity of regeneration Douglas-fir seedlings to *Arctostaphylos* may contribute to the success of this conifer species by means of a shared ectomycorrhizal community. This was the suggestion of Horton et al. (1999), who investigated Douglas-fir regeneration at a chaparral community on the central coast of California. The researchers observed that Douglas-fir seedlings growing near patches of *Arctostaphylos glandulosa* ssp. *glandulosa* Eastw. had better survival than

seedlings growing near *Adenostoma fasciculatum* H. & A. (a predominately arbuscular mycorrhizal plant). Soil temperature, light, and allelopathy were found to be equal between the two shrub species, and the researchers concluded that the ectomycorrhizal community shared by *Arctostaphylos glandulosa* ssp. *glandulosa* and Douglas-fir was an important factor for Douglas-fir establishment at that site. Although mycorrhizal anatomy was not investigated, these researchers used molecular techniques to determine that the fungal species forming ectomycorrhizae with Douglas-fir were the same as the fungi associated with *Arctostaphylos glandulosa* ssp. *glandulosa*.

Although the study presented here ultimately focussed on two potential refuge species (*Arctostaphylos uva-ursi* and advanced regeneration Douglas-fir seedlings), other plant species hosted a diverse community of ectomycorrhizal fungi and they may also be important refuge plants at specific locations throughout the site. In particular, *B. papyrifera*, *Populus tremuloides*, *Alnus viridis*, and *S. commutata* were all highly colonized by ectomycorrhizal fungi but were sparsely distributed throughout the site. Both *S. canadensis* and *Amelanchier alnifolia* had moderate colonization. There is a paucity of information in the mycorrhizal literature concerning the degree to which ectomycorrhizae colonize these two species. *Paxistima myrsinites*, *S. betulifolia*, and *V. caespitosum* all had less than 1.04% of their roots colonized by ectomycorrhizae. Since these values were based on one or two roots from one sample, it is highly likely that the results were due to sampling error. Thus, it may have been that a mycorrhiza from another host other than the one being sampled was morphotyped. It is generally thought that

P. myrsinites and *S. betulifolia*, associate with arbuscular mycorrhizae and *V. caespitosum* associates with ericoid mycorrhizae.

Diversity of the mycorrhizal community

As the diversity of some types of ectomycorrhizal fungal inoculum can decrease after clear-cut logging, refuge plants may be particularly important for maintaining a diverse ectomycorrhizal community that will be available to outplanted seedlings. Although the functional diversity of ectomycorrhizal fungi is poorly understood, it is known that ectomycorrhizal fungi have different physiological characteristics in culture. For example, ectomycorrhizal fungi differ in their ability to take up various forms and types of nutrients (Abunzinadah and Read 1986; Dighton 1991), in their rates of nutrient uptake (Langlois and Fortin 1984), in their tolerance to water stress in pure culture (Mexal and Reid 1973; Dieblot and Mudge 1984) and in the field (Parke et al. 1983), as well as in their tolerance to temperature extremes (Slankis 1974). It has therefore, been hypothesized that seedlings having access to a range of ectomycorrhizal fungi will be colonized by those mycobionts best adapted to the soil conditions present (Perry et al. 1987).

In 1997 and 1998 *Arctostaphylos uva-ursi* exhibited similar levels of mycorrhizal diversity in both forest and opening plot locations (Figs. 1A and 1B). These findings illustrate that, even 3 years after logging, *Arctostaphylos uva-ursi* maintains a community of mycorrhizal fungi in the openings similar in diversity to that found in the undisturbed forest. This observation is important, because many researchers suggest that seedlings that associate with a diverse array of ectomycorrhizal fungi may be better able to adapt to changes in the environment (Perry et al. 1987; Simard et al. 1997a).

In contrast to *Arctostaphylos uva-ursi*, which was found to have similar levels of morphotype richness irrespective of sampling location, advanced regeneration Douglas-fir seedlings sampled from the openings associated with a significantly less diverse assemblage of ectomycorrhizal fungi than did Douglas-fir seedlings sampled from the forest. This trend was true for 1997 and 1998 (Figs. 1A and 1B). The change in the aboveground environment (tree removal) may have had a more significant impact on Douglas-fir mycorrhizae than of mycorrhizae associated with *Arctostaphylos uva-ursi*. It may be that the water stress that often develops in these biogeoclimatic variants had a more significant influence on the rate of root senescence for Douglas-fir than for *Arctostaphylos uva-ursi*, which is tolerant of very dry and nutrient-poor sites (Ringius and Sims 1997). An increase in root senescence would translate into the resultant loss of ectomycorrhizal diversity because of the smaller number of fine roots. Additionally, advanced regeneration Douglas-fir seedlings may be unable to support some species of ectomycorrhizal fungi in the absence of fungal connections with mature trees (Simard et al. 1997b).

Ecology and morphology of arbutoid mycorrhizae

The mycorrhizae associated with *Arctostaphylos uva-ursi* sampled from the Opax study site had arbutoid morphology and shared the same characteristics previously described by Zak (1974) and Molina and Trappe (1982) for mycorrhizae

of *Arctostaphylos* and *Arbutus*. Analysis of cross sections revealed that the mycorrhizae formed by *Arctostaphylos uva-ursi* had a well-developed mantle (except for E-strain), and intracellular penetration of the epidermal cells. Contrary to Mejsstrik and Hadac (1975) and Largent et al. (1980) who reported ectomycorrhizal formation by various species of *Arctostaphylos*, we observed only arbutoid mycorrhizae for the mycorrhizal roots of *Arctostaphylos uva-ursi* investigated.

Subsequent to the laboratory studies performed by Zak (1974, 1976) and Molina and Trappe (1982), additional field studies have confirmed that the formation of arbutoid mycorrhizae by ectomycorrhizal fungi is a common occurrence in natural ecosystems (Largent et al. 1980; Acsai and Largent 1983) of Oregon and California. Observations made in the field study presented here corroborates previous findings and provides a broader range of observation of arbutoid formation by ectomycorrhizal fungi to include interior dry Douglas-fir forests in the southern interior of British Columbia. Furthermore, the molecular analysis of fungi forming mycorrhizae with *Arctostaphylos* and Douglas-fir presented in this study clearly demonstrates that the same species of fungus forms both arbutoid and ectomycorrhizal morphologies. Our findings support the suggestion by Molina and Trappe (1982), and Molina et al. (1992) that arbutoid mycorrhizae may be best described as a specific type or form of ectomycorrhiza.

The 1997 and 1998 results support the idea that *Rhizopogon* species are generally specific to plants in the Pinaceae (Molina et al. 1992). *Rhizopogon* sp. A section *Fulviglebae* and *Rhizopogon* sp. B section *Villosuli* were observed only on Douglas-fir and not on *Arctostaphylos uva-ursi*. Molina et al. (1997) grew *Arctostaphylos uva-ursi* with Douglas-fir in the same pot and found that *Rhizopogon* sp. B section *Villosuli* did not develop on *Arctostaphylos uva-ursi*, but Douglas-fir was extensively colonized by these fungal species. There were three relatively rare morphotypes found only on *Arctostaphylos uva-ursi*: *Dermocybe*-like, *Inocybe* c.a. *albidodisca* Kuehn, and unknown OX97 No. 20. However, the occurrence of rare morphotypes on one host does not necessarily mean that the morphotype is specific to that host (see Horton et al. 1999).

PCR-RFLP analysis

The molecular analysis of root tips collected in 1997 and 1998 indicated that, in most cases, morphotyping was an accurate method of distinguishing between mycorrhizae formed by different species of fungi. Additionally, the PCR-RFLP analysis demonstrated that the same fungi that formed ectomycorrhizae with Douglas-fir formed arbutoid mycorrhizae with *Arctostaphylos uva-ursi*.

Management implications

The proliferation of woody angiosperms (including *Betula* spp. and *Populus* spp.) after clear-cutting is a concern at some sites, because these trees are fast growing and can compete with outplanted seedlings for light and other nutrients (see Simard 1990). For this reason, many silviculturalists prescribe vegetation management regimes such as herbicide application or manual brushing and thinning. Although these tree species may indeed compete with young seedlings, they may also benefit a plantation by providing protection from

root disease such as *Armillaria* sp. (Morrison et al. 1988), increasing the structural diversity of a stand (Simard and Vyse 1994), and improving the mycorrhizal status of outplanted seedlings (Jones et al. 1997). As the quantity and diversity of ectomycorrhizal fungal inoculum can be reduced by clear-cut logging, these refuge species and others such as *Arctostaphylos uva-ursi*, are considered important for the maintenance of ectomycorrhizal inoculum in these ecosystems and for successful stand regeneration.

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