

Russulaceous ectomycorrhizae of *Abies lasiocarpa* and *Picea engelmannii*

G. Kernaghan, R.S. Currah, and R.J. Bayer

Abstract: During a 3-year study of the ectomycorrhizal fungi of subalpine forests in the Front Ranges of the Canadian Rockies, species of *Russula* and *Lactarius* were conspicuous mycobionts of both erect and krummholz forms of *Abies lasiocarpa* (Hook.) Nutt. and *Picea engelmannii* Parry. Morphological identifications of Russulaceous mycorrhizae were confirmed by comparing polymerase chain reaction amplified ribosomal DNA (internal transcribed spacer region) with that of sporocarp tissue. Restriction fragment length polymorphism analysis using *AluI*, *HhaI*, *HinII*, and *RsaI* gave a distinctive profile for each of 14 Russulaceous sporocarps and facilitated the identification of five mycorrhizae. Mantles formed by *Lactarii* (*Lactarius alnicola*, *Lactarius caespitosus*, and *Lactarius deliciosus* var. *areolatus*) exhibit characteristic laticifers and pigments comparable to the associated sporocarp. Those formed by species of *Russula* (*R. brevipes* and *R. silvicola*) bear distinctive cystidia or sulphovanillin-reactive cells.

Key words: ITS, *Lactarius*, RFLP, *Russula*, subalpine, tree line.

Résumé : Au cours d'une étude de 3 ans sur les champignons ectomycorhiziens de forêts subalpines dans la région Front Ranges des Rocheuses canadiennes, des espèces de *Russula* et de *Lactarius* sont apparues comme mycobiontes des formes dressées aussi bien que krummholz de l'*Abies lasiocarpa* (Hook.) Nutt. et du *Picea engelmannii* Parry. L'identification morphologique des mycorrhizes de russulacées a été confirmée en comparant l'ADN ribosomal (région interne non transcrite) amplifié à la réaction polymérase en chaîne avec celui des tissus d'un sporocarp. L'analyse du polymorphisme des longueurs de restriction en utilisant *AluI*, *HhaI*, *HinII* et *RsaI* montre un patron distinctif pour chacun de 14 sporocarpes de russulacées et on a permis l'identification de cinq mycorrhizes. Les manchons formés par les lactaires (*Lactarius alnicola*, *Lactarius caespitosus* et *Lactarius deliciosus* var. *areolatus*) montrent des laticifères caractéristiques et des pigments comparables à ceux associés aux sporocarpes. Ceux formés par les russules (*Russula brevipes* et *Russula silvicola*) portent des cystides typiques ou des cellules réagissant à la sulphovanilline.

Mots clés : ITS, *Lactarius*, RFLP, *Russula*, subalpin, ligne des arbres.
[Traduit par la rédaction]

Introduction

Abies lasiocarpa (Hook.) Nutt. and *Picea engelmannii* Parry form an extensive band of near-climax forest in the subalpine zone of the central Rocky Mountains. In the Front Ranges of the Canadian Rockies, these species reach their upper elevational limit between 1900 and 2450 m, where they become stunted and multistemmed, taking on a krummholz growth form (Baig 1972). In these high-elevation environments, plants are especially dependent on mutualistic symbioses with mycorrhizal fungi to aid in water and nutrient acquisition (Moser 1967; Haselwandter 1987; Väre et al. 1997). Despite this, subalpine ectomycorrhizae have received little attention in Europe (Moser 1982; Treu 1990) and even less in North America (Cázares 1992).

During a study of the ectomycorrhizal communities in the subalpine forests of the Front Ranges of the Canadian Rockies we have found the Russulaceae to be common and conspicuous conifer associates. Ectomycorrhizae formed by species of *Lactarius* and *Russula* are relatively distinctive, and several

species of Russulaceous fungi have been shown to form mycorrhizae with members of the genus *Picea* (Alexander 1981; Molina and Trappe 1982; Agerer 1986; Treu 1990; Amiet and Egli 1991; Weiss 1991; Pillukat and Agerer 1992; Kraigher et al. 1995), whereas only two Russulaceous species, *Lactarius deliciosus* Fr. (Acsai and Largent 1983) and *Russula ochroleuca* (Pers.) Fr. (Pillukat and Agerer 1992), have been described from *Abies*. In general, *Abies* has received little attention as a mycorrhizal host, with relatively few mycorrhizae described on the genus (Masui 1926; Acsai and Largent 1983; Kottke and Oberwinkler 1990; Peña-Cabriales and Valdés-Hgo 1974; Pillukat and Agerer 1992). With respect to western North American subalpine conifers, there have been no published descriptions of ectomycorrhizae from either *Picea engelmannii* or *A. lasiocarpa*.

Until recently, the identification of the fungal taxa in ectomycorrhizal symbioses has been determined by in vitro resynthesis or by demonstrating that hyphae are continuous between mantle tissue and sporocarps. The polymerase chain reaction (PCR), in combination with fungus-specific primers, now makes identification of ectomycorrhizal fungi from root tips faster and more reliable (Gardes and Bruns 1993, 1996; Erland et al. 1994; Egger 1995). Restriction fragment length polymorphism (RFLP) analysis of PCR-amplified ribosomal DNA allows for the comparison of ectomycorrhizae with sporocarps collected at different sites and times, including herbarium specimens (Brunns et al. 1990).

Received January 28, 1997.

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In this study then, we use PCR-amplified fungal DNA in conjunction with mycorrhizal anatomy to identify and describe associations between the Russulaceae (*Russula* and *Lactarius*) and the erect and krummholz forms of *A. lasiocarpa* and *Picea engelmannii*.

Materials and methods

Site description

Sporocarps and ectomycorrhizae were collected at approximately 2100 m asl on the southeast slope of Mount Tripoli, in the Nikanassin Range, Alberta (117°17'W, 52°52'N), and approximately 2300 m asl on the southwestern slope of Mount Rae, Peter Lougheed Provincial Park, Alberta (114°59'W, 50°36'N). Sites are dominated by *Picea engelmannii* and *A. lasiocarpa*, with scattered *Larix lyallii* Parl. Stands are at least 100 years old (based on increment cores), with closed canopies and sparse understorey vegetation. With increasing elevation, *Picea* and *Abies* grow as stunted multistemmed clones that form discrete islands or krummholz, separated by low-growing woody plants such as species of *Salix*, *Dryas*, *Phyllococe*, and *Cassiope*. Soils are Dystric and Eutric Brunisols and Orthic and Orthic Humic Regosols (Trottier 1972; Mortimer 1978).

Growing seasons are short, cool, and moist. During the snow-free period (June–September) mean daily temperatures range from 6 to 10°C and mean monthly precipitation ranges from 23 to 110 mm (Environment Canada, Archive of Climatological Data).

Collection

Ectomycorrhizae were collected from the organic soil horizon with a 3.5-cm soil corer. Samples were collected each month during the snow-free season of 1994 along transects through the subalpine forest and the krummholz zone. Soil cores were placed in sections of plastic pipe to maintain their integrity during transport. Russulaceous sporocarps were collected monthly from both sites during 1994, 1995, and 1996. Soil cores and sporocarps were stored on ice until examined.

Morphological characterization and photography

Sporocarps were identified, air-dried, and deposited in the University of Alberta Cryptogamic Herbarium (ALTA). Ectomycorrhizae were separated from soil by wet sieving samples through an 850- μ m soil sieve placed over a 600- μ m sieve. The morphology of ectomycorrhizae deemed to be Russulaceous (based on the presence of smooth mantles, laticifers, cystidia, or other distinctive cells and the absence of clamp connections) was described using a dissecting photomicroscope with fibre optic lighting. Host identity was determined either by tracing the root system from the mycorrhizae to the tree or by analysis of cross-field pitting in attached secondary root tissue (Core et al. 1979).

Anatomical descriptions were made using the oil-immersion objective of an Olympus photomicroscope with bright-field and Nomarsky interference. For light microscopy, portions of fungal mantle were peeled from root tissue and mounted in 5% KOH and in Ponceau-S (Daughtridge et al. 1986). Fresh mantle tissue was also mounted in sulphovanillin (Singer 1986), which gives a dark blue colour reaction in laticifers and other specialized cells of Russulaceous mycorrhizae (Miller et al. 1991) (designated here as SV+). This reaction occurred in fresh material but not in mycorrhizae that had been frozen.

Scanning electron microscopy (SEM) used a Jeol JSM6301FXV equipped with an Emitec K1200 cryosystem. Samples were quick-frozen in liquid nitrogen slush, placed on a cryosublimation stage for 10 min at -40°C, and then placed on the SEM cold stage at -40°C and examined at low voltage until surface ice had been

removed. Samples were then cooled to -155°C and transferred to a cryochamber for gold sputtering and then returned to the SEM cold stage for examination at 2.5 kV. Images were digitally edited in Adobe Photoshop 3.0.

DNA extraction and amplification

DNA was extracted from one or two sporocarps of each of the nine Russulaceous species collected on the subalpine sites, and from five herbarium specimens characteristic of spruce–fir forests. DNA was also extracted from fresh, frozen, or lyophilized subsamples of Russulaceous ectomycorrhizae. Total genomic DNA was extracted from 2–5 mg lyophilized ectomycorrhizae (10–25 mg if fresh or frozen) and 5 mg dried lamellae by the method outlined by Gardes and Bruns (1993). When amplification (see below) was unsuccessful, DNA was extracted following Jobes et al. (1995), modified by using 5% of the recommended amounts of tissue and reagents.

Amplification protocols were modified from Gardes and Bruns (1993). DNA extracts were diluted 1:25, 1:125, 1:625, and 1:3125 with water, and a 25- μ L aliquot of each dilution was added to an equal volume of PCR cocktail containing 400 μ M of each dNTP (Canadian Life Technologies Inc., Burlington, Ont.), 100 mM KCl, 5 mM MgCl₂, 20 mM Tris-HCl (pH 9), 2.5% Triton X-100 (Sigma-Aldrich Canada, Mississauga, Ont.), 1 unit *Taq* DNA polymerase (Promega Corp., Madison, Wis.), and 2 μ M of each oligonucleotide primer, namely ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). Temperature cycling used a Techné Gene-E programmable thermocycler as follows: 70°C while DNA was added to cocktail, 94°C for 2 min, then 30 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 2 min, and finally 72°C for 10 min.

RFLP analysis

Ten-microlitre aliquots of PCR-amplified product were combined with 7 μ L water; 1 μ L restriction endonuclease; either *AluI*, *HhaI*, *HinI*, or *RsaI* (Pharmacia Biotech Inc., Baie D'Urfe, Que.); and 2 μ L buffer (supplied by manufacturer). These restriction enzymes recognize 4-bp sequences common in fungal internal transcribed spacer (ITS; Egger 1995). After 3–5 h at 37°C, the digested DNA was loaded onto 2% agarose gels (ICN Biomedicals, Aurora, Ohio) and separated by electrophoresis for 4 h at 75 V in a 1% TBE buffer (90 mM Tris-borate, 1 mM EDTA, pH 8.3).

Mycorrhizal and sporocarp DNA, digested by the same enzyme, were loaded side by side for comparison and a 123-bp DNA ladder (Sigma, St. Louis, Mo.) used to determine fragment size. Gels were stained with ethidium bromide and viewed under UV light. Images were captured with a Mitsubishi gel documentation system and analyzed with Gel Pro analyzer software (Media Cybernetics, Silver Springs, Md.).

Results

All Russulaceous taxa from the subalpine sites and other locations (Table 1) could be differentiated by RFLP analysis of the amplified ITS region (Table 2). Russulaceous ectomycorrhizae from the subalpine sites were matched (Fig. 1; Table 2) with *Lactarius alnicola* Smith, *Lactarius caespitosus* Smith & Hesler, *Lactarius deliciosus* Fr. var. *areolatus* Smith, *Russula brevipes* Peck, and *Russula silvicola* Shaffer and are described in detail below. Fragment sizes given (Table 2) are accurate to $\pm 3\%$. Fragments of less than 75 bp could not be resolved.

The size of the ITS regions amplified varied among the genera analyzed (Table 2), from 720 bp in *Russula* (slightly larger in *Russula nigricans*) to 738 bp in *Macowanites* and between 746 and 775 bp in *Lactarius*.

Table 1. Collecting locations and accession numbers of sporocarps used for RFLP analysis.

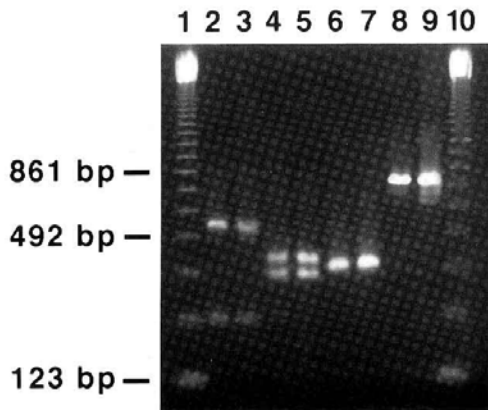
Fungus	Location	Accession No.
<i>Lactarius alnicola</i> Smith	Mount Tripoli, Alta.	ALTA 9870
<i>Lactarius caespitosus</i> Smith and Hesler	Mount Rae, Alta.	ALTA 9871
<i>Lactarius deliciosus</i> var. <i>areolatus</i> Smith	Mount Tripoli, Alta.	ALTA 9872
<i>Lactarius pubescens</i> Fr.	Mount Tripoli, Alta.	ALTA 9874
<i>Lactarius</i> affin. <i>luculentus</i> Burl.	Mount Tripoli, Alta.	ALTA 9875
<i>Lactarius uvidus</i> (Fr.) Fr.	Kananaskis valley, Alta.	ALTA 9873
<i>Macowanites americana</i> Singer & Smith	Cypress Bowl, B.C.	ALTA 9876
<i>Russula brevipes</i> Pk.	Mount Tripoli, Alta.	ALTA 9878
<i>Russula fragilis</i> (Pers.: Fr.) Fr.	Devon, Alta.	ALTA 9879
<i>Russula integra</i> Fr.	Mount Tripoli, Alta.	ALTA 9880
<i>Russula torulosa</i> Bres.	Mount Rae, Alta.	ALTA 9877
<i>Russula silvicola</i> Shaffer	Mount Tripoli, Alta.	ALTA 9881
<i>Russula nigricans</i> Fr.	Prince Albert, Sask.	ALTA 9882
<i>Russula xerampelina</i> Fr.	Devon, Alta.	ALTA 9883

Table 2. Restriction fragment sizes and nondigested ITS region sizes.

Fungus	Enzyme				ITS region
	<i>AluI</i>	<i>HhaI</i>	<i>HinfI</i>	<i>RsaI</i>	
<i>Lactarius alnicola</i>	550	398	368	700	775
	219	361	245	75	
<i>Lactarius caespitosus</i>	536	406	382	764	764
	234	350	382		
<i>Lactarius deliciosus</i> var. <i>areolatus</i>	529	392	378	670	758
	222	348	378	90	
<i>Lactarius</i> affin. <i>luculentus</i>	486	320	364	669	746
	133	222	258		
	81	110	110		
<i>Lactarius pubescens</i>	520	389	382	746	746
	176	316	364		
<i>Lactarius uvidus</i>	503	329	382	694	746
	188	198	364		
		165			
<i>Macowanites americana</i>	460	738	342	661	738
	201		233		
			166		
<i>Russula brevipes</i>	497	364	369	655	720
	227	262	282		
<i>Russula fragilis</i>	491	363	373	570	720
	227	209	339	149	
		149			
<i>Russula integra</i>	441	355	350	680	720
	218	360	340		
<i>Russula nigricans</i>	510	412	383	632	727
	214	322	344		
<i>Russula silvicola</i>	481	380	351	514	720
	209	339	271	144	
			96		
<i>Russula torulosa</i>	441	326	347	368	720
	218	272	320	340	
		90			
<i>Russula xerampelina</i>	466	354	375	720	720
	203	218	326		

Note: The values in bold are common between sporocarps and mycorrhizae.

Fig. 1. RFLP patterns of the ITS region amplified from *Lactarius caespitosus* sporocarp tissue compared to *Lactarius caespitosus* mycorrhizae on *Abies*. Lanes 1 and 10, 123-bp size markers; lanes 2 and 3, treated with *AluI*; lanes 4 and 5, treated with *HinfI*; lanes 6 and 7, treated with *HhaI*; lanes 8 and 9, treated with *RsaI*.



The restriction enzymes also varied in their ability to cleave the ITS region. On average *RsaI* cleaved less frequently than the other enzymes (average number of resulting fragments: *RsaI* = 1.36, *AluI* = 2.01, *HhaI* = 2.21, *HinfI* = 2.21). Although *RsaI* recognized no restriction sites in some species, e.g., *Lactarius caespitosus*, *Lactarius pubescens*, and *Russula xerampelina* (Table 2; *Lactarius caespitosus* in Fig. 1, lanes 8 and 9), the data were still informative and differentiated *Lactarius caespitosus* from *Lactarius deliciosus* var. *areolatus*.

In some cases (*Lactarius caespitosus* and *Lactarius deliciosus*) *HinfI* cleaved the ITS region into two equal fragments (Table 2; *Lactarius caespitosus* in Fig. 1, lanes 6 and 7). The two fragments then overlapped on the gel, resulting in a band with twice the normal fluorescence.

Descriptions of ectomycorrhizae

Lactarius alnicola (on *Picea engelmannii*) (Figs. 2, 3)

Monopodial–pinnate to pyramidal. Mantle smooth, yellow–ochre (concolorous with sporocarp). Hyphal strands (50–100 μm wide) differentiated into inner, \pm parallel, hyaline hyphae (2.5–3.5 μm wide) and an outer prosenchymous layer of cells similar in size and shape to those of outer mantle. Emanating hyphae uncommon, hyaline (3.0–3.5 μm wide). Outer mantle prosenchymous, cells short, blocky (sometimes rounded) (4.5–10 μm wide \times 17–35 μm long), often with short branches. Cells relatively thick-walled and finely encrusted, containing yellow pigment. Central mantle synnenchymous, cells becoming shorter and thinner walled (6.0–9.0 μm wide \times 10–19 μm long), some forming rosettes, isolated clusters of cells strongly SV+. Laticifers abundant, obtusely branched (4.5–10 μm wide), strongly SV+. Inner mantle a reticulum formed by obtusely branched, \pm cylindrical hyphae (2.0–9.0 μm wide).

Lactarius caespitosus (on *Abies lasiocarpa*) (Fig. 4)

Monopodial–pinnate. Mantle smooth, covered with a thin layer of mucilaginous material (< 1 μm), hyaline to olivaceous (similar to pigment suffused throughout the sporocarp). Hyphal strands (30–200 μm wide) olivaceous, differentiated into outer pigmented hyphae (2.0–3.5 μm wide) and inner hyaline hyphae (3.5–6.0 μm wide). Emanating hyphae uncommon (2.5–3.5 μm wide). Outer mantle a prosenchyma of elongated, noninterlocking epidermoidal cells (1.5–5.0 μm wide \times 17–45 μm long). Laticifers abundant in inner mantle (4.0–11 μm wide), obtusely branched, SV weakly positive, contents appearing granular in 5% KOH. Inner mantle a tightly woven synnenchyma of narrow, obtusely branched, \pm cylindrical hyphae (2.5–4.5 μm wide).

Lactarius deliciosus var. *areolatus* (on *Abies lasiocarpa*)

Monopodial–pinnate. Mantle smooth, pale orange, becoming green where bruised or with age (i.e., concolorous with sporocarps). Hyphal strands \pm cylindrical (150–200 μm wide), pale yellow but soon becoming green, hyphae parallel (2.0–3.0 μm wide) but often contorted and wider at surface of larger strands. Emanating hyphae uncommon, similar to inner hyphae of strands. Outer mantle prosenchymous, formed from cylindrical, obtusely branched hyphae (3.0–4.5 μm wide), often running parallel and forming narrow sheets. Laticifers common (3.5–7.0 μm wide), greenish in sulphovanillin, contents separated by wall material every 4.0–10 μm . Inner mantle hyphae becoming shorter, wider, and more branched (4.5–5.5 μm wide).

Russula silvicola (on *Abies lasiocarpa*) (Fig. 4)

Monopodial–pinnate. Mantle smooth, hyaline (assuming colour of underlying root tissue). Emanating hyphae not seen. Outer mantle composed of subangular–polygonal cells, 3- to 4-sided (some sides rounded) (4.5–7.5 μm \times 7.0–11 μm wide), often forming rosettes of 4 or 5 cells, isolated cells strongly SV+, grading through a layer of short, noninterlocking, epidermoidal cells (5.5–11 μm wide \times 9.0–22 μm long) to an inner synnenchymous mantle, composed of narrow, obtusely branched, cylindrical hyphae (1.5–4.0 μm wide).

Russula brevipes (Figs. 6–8)

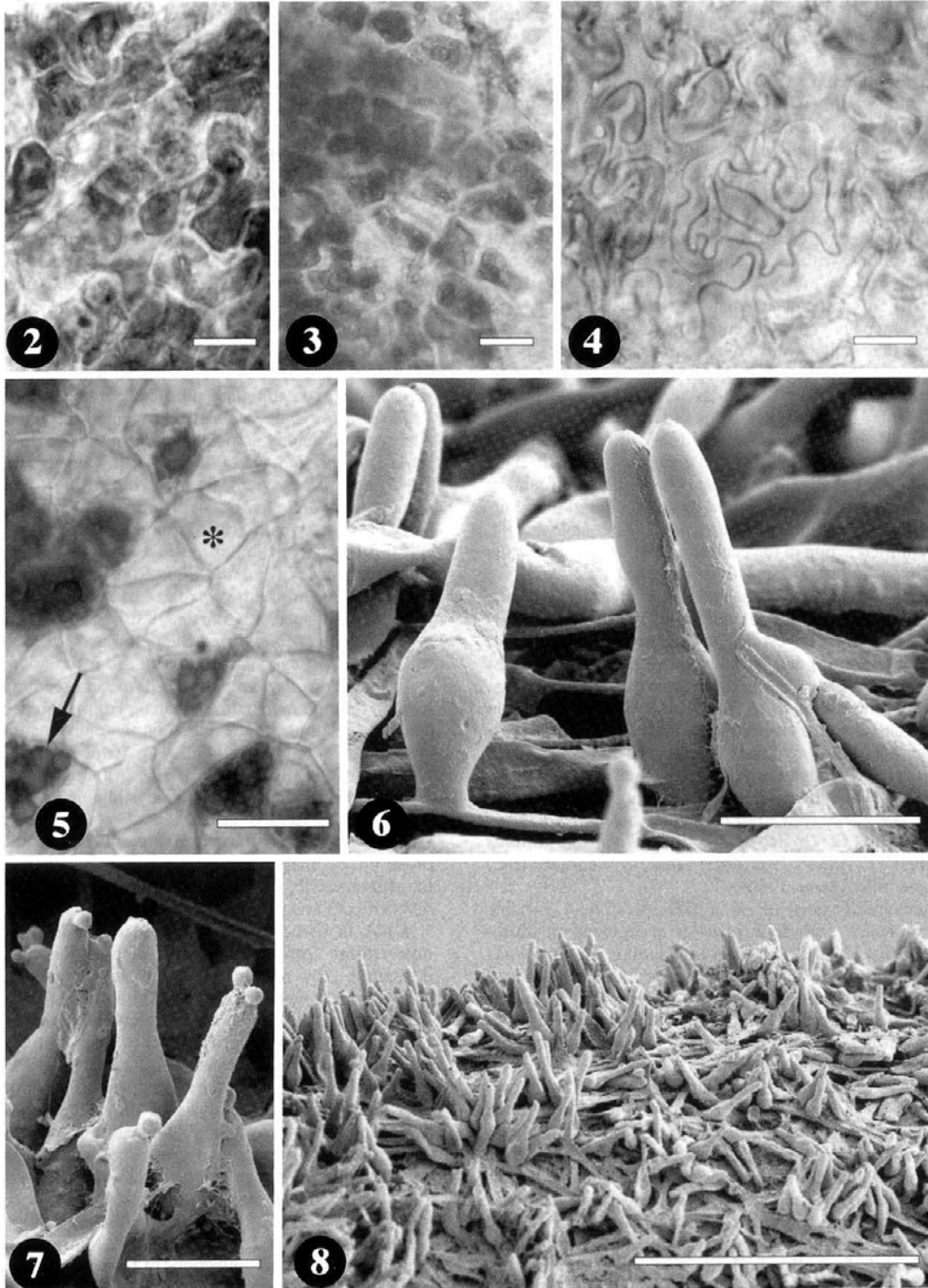
Monopodial–pinnate. Mantle fuzzy (due to many erect cystidia), hyaline to fuscous–copper. Emanating hyphae not seen. Outer mantle a loose reticulum of cylindrical hyphae (2.5–5.0 μm wide) with two-, three-, and four-way intersections, giving rise to a dense layer of ampule-shaped (rarely cylindrical) cystidia (3.5–6.5 μm wide \times 13–30 μm long) with two or three small, rounded apical projections. Inner mantle synnenchymous, a mixture of cylindrical hyphae and partially interlocking epidermoidal cells (3.0–8.0 μm wide \times 16–38 μm long).

Although no secondary root tissue was collected with the *R. brevipes* mycorrhizae, the morphology of the fine roots, as well as the proximity of host plants, strongly indicates that *A. lasiocarpa* is the host.

Discussion

The symbioses identified in this study are morphologically distinct and common at the subalpine sites studied. We

Figs. 2–8. Mantle cells of Russulaceous ectomycorrhizae. Figs. 2, 3. Subangular cells in central mantle of *Lactarius abnicola*. Scale bar = 10 μm . Fig. 4. Elongated epidermoidal cells in outer mantle of *Lactarius caespitosus*. Scale bar = 10 μm . Fig. 5. Outer mantle cells of *Russula silvicola* in sulphovanillin. Note reactive polygonal cells (arrow) and rosette (asterisk on uppermost cell). Scale bar = 10 μm . Figs. 6–8. Ampule-shaped cystidia on *R. brevipes* mantle. Scale bar = 10 μm in Figs. 6 and 7 and 100 μm in Fig. 8.



describe them on either *Picea engelmannii* or *A. lasiocarpa*, but some may be capable of concurrent symbioses with other woody plants (e.g., species of *Larix*, *Betula*, *Salix*, or *Dryas*). We expect that these fungi may be recognizable on other hosts, as anatomical characters of the mantle and emanating elements are often determined by the mycobiont (Brand 1991; Pillukat and Agerer 1992).

Lactarius alnicola, now considered a conifer associate (Hesler and Smith 1979), was originally collected under *Alnus*, resulting in its misleading epithet. It has been frequently collected in the Rocky Mountains (Hesler and Smith 1979) where it is associated with *P. engelmannii* (a subalpine species), and at lower elevations under *Picea glauca* (Moench.) Voss. (Currah et al. 1989). It is then expected that *Lactarius alnicola* forms ectomycorrhizae with *Picea engelmannii* at higher elevations, with *Picea glauca* at lower elevations, and with the hybrid of the two (*Picea glauca* var. *albertiana* (S. Brown) Sarg.) at intermediate elevations.

The ochraceous-yellow mycorrhizae of *Lactarius alnicola* exemplify the similarity in colouration between the sporocarp and mycorrhizal mantles of all three *Lactarius* species identified. Microscopically, the tissue of the central mantle (Figs. 2, 3) is similar to that of the outer mantle of *R. silvicola* (Fig. 5) in that the cells are highly differentiated and may or may not be sulphovanillin reactive. They differ in shape and in distribution of reactive cells, which are found in isolated clusters in *Lactarius alnicola* and scattered individually throughout the mantle in *R. silvicola*. It is possible that the sulphovanillin reaction in the central mantle cells of *Lactarius alnicola* may be due to latex from damaged laticifers.

Lactarius caespitosus may be endemic to the subalpine zone of the Central Rockies (Hesler and Smith 1979). Because it is not reported from southwest British Columbia (G. Kernaghan, personal observation) and is rare west of the Cascade Crest in the United States (Hesler and Smith 1979), its range appears to follow that of *A. lasiocarpa*. This distribution suggests that *Lactarius caespitosus* and *A. lasiocarpa* have a high degree of host-symbiont specificity.

The narrow, elongate epidermoidal cells of the outer mantle (Fig. 4) are characteristic of the genus *Lactarius*, and have also been described from the mycorrhizae of *Lactarius glycosmus* (Fr.) Fr. (Ingleby et al. 1990), *Lactarius pallidus* (Pers.: Fr.) Fr. (Brand 1991), and *Lactarius vellereus* (Fr.) Fr. (Brand and Agerer 1986).

Lactarius deliciosus s.l. is a widespread and variable conifer associate with five varieties recognized in North America (Hesler and Smith 1979), including var. *areolatus* Smith, which is abundant throughout the Rockies. In North America, mycorrhizae formed by *Lactarius deliciosus* s.l. have been described from nature on *Abies concolor* (Gord. et Glend.) Lindl. (Acsai and Largent 1983) and from pure culture synthesis with species of *Larix*, *Picea*, *Pinus*, *Pseudotsuga*, and *Tsuga* (Molina and Trappe 1982). Agerer (1986) gives a thorough description of the naturally occurring mycorrhizae of *Lactarius deterrimus* Gröger (= *Lactarius deliciosus* var. *deterrimus* Hesler & Smith) on *Picea abies* (L.) Karst. Although differences between *Lactarius deliciosus* var. *deterrimus* and *Lactarius deliciosus* var. *areolatus* include spore size, degree of zonation of the pileus, and intensity of green staining at maturity, the mycorrhizae of the two are morphologically similar.

Russula silvicola is widely distributed throughout North American coniferous forests where it generally fruits in rotten wood or deep humus (Shaffer 1975). The distinctive subangular, sulphovanillin-reactive mantle cells have also been described from the mycorrhizae of a similar taxon, *Russula emetica* (Schaeff.: Fr.) Pers.: Fr. var. *silvestris* Singer on *Fagus*, and from *Russula mairei* Singer on *Fagus*, *Russula nana* Killerm. on *Polygonum* (Brand 1991), and *Russula fragilis* (Pers.: Fr.) Fr. on *P. glauca* (G. Kernaghan, unpublished data), all of which are included in subsection *Emeticinae*, a group delimited in part by the presence of sulphovanillin-reactive pileocystidia (Shaffer 1975). Other, more epidermoidal, sulphovanillin-reactive mantle cells are described from *Russula firmula* Schäffer (subsection *Urentinae*) on *Pinus* and *Russula laricina* Velen. (subsection *Laricinae*) on *Larix* (Treu 1990). The sulphovanillin-reactive mantle cells of *Russula* mycorrhizae may be analogous to reactive pileocystidia in that they may both act as storage sites of stearyl-velutinal (responsible for the sulphovanillin reaction), a precursor of the toxic antifeedants velleral and isovelleral, also found in the laticifers of *Lactarius* spp. and the cystidia of *Lentinellus* spp. (Camazine and Lupo 1984).

Russula brevipes is widely distributed throughout North America and mainly associated with species of *Abies*, *Picea*, *Tsuga*, and *Pseudotsuga* (Stanis 1979). Yamada and Katsuya (1995) describe a symbiosis involving *R. nigricans* (also in section *Compactae*) formed in vitro with *Pinus densiflora* Sieb. et Zucc. which also has ampule-shaped cystidia with apical projections. Similar cystidia have also been found on the mycorrhizae of *Russula illota* Romagn. (section *Ingrate*) (Brand 1991), on the mycorrhizae of *Monotropa* associated with *Russula* species (Martin 1986), and on unidentified mycorrhizae of *Abies firma* Sieb. et Zucc. (Masui 1926).

Comparable cystidia are absent from the sporocarps of *R. brevipes*, *R. nigricans*, or *R. illota*, and seem more common on *Russula* mycorrhizae than on fruiting bodies. Similar apical projections are described on the more fusiform pileocystidia of *Russula grisea* Gill. var. *iodes* Romagn. (section *Heterophyllae*) (Romagnesi 1967). Although it seems that distinctive structures such as the cystidia of *R. brevipes*, the sulphovanillin-reactive cells of *R. silvicola*, or the elongated epidermoidal cells of *Lactarius caespitosus* should in time prove to be diagnostic features for certain stirps within the Russulaceae, more matches between sporocarps and mycorrhizae are needed.

DNA is especially well suited to the identification of mycorrhizae formed by genera, such as *Russula*, that are difficult to grow in culture (Taylor and Alexander 1989) and possess scanty basal hyphae, making in vitro resynthesis and hyphal tracing difficult. More specifically, the ITS region has provided the appropriate level of resolution for the taxa studied, as all species tested had distinctive RFLP profiles. This may not have been the case given different taxa, however, because RFLP analysis of the ITS region may not discriminate between sibling biological species (Egger 1995).

Intraspecific variation may also be detected, but differs between groups of ectomycorrhizal fungi. No variation in ITS was observed among isolates of *Tylospora fibrillosa* Donk (Erland et al. 1994), and 1–2% variation was seen between isolates of *Laccaria bicolor* (Maire) Ort. (Gardes et al. 1991), whereas collections of *Cantharellus cibarius* Fr.

showed relatively large length variations (Feibelman et al. 1994). Very few studies involving Russulaceae have utilized DNA analysis, and the level of variation in the ITS region is not yet known. Using RFLP analysis of amplified ITS, Kraigher et al. (1995) distinguished between mycorrhizae of *Lactarius lignyotus* Fr. and *Lactarius picinus* Fr. on *Picea abies*, and Gardes and Bruns (1996) identified the mycorrhizae of *Russula amoenolens* Romagn., *R. brevipes*, and *R. xerampelina* s.l. on *Pinus muricata* D. Don. Unfortunately, RFLP data from these studies are not directly comparable with ours because of differences in PCR primers.

Given the level of anatomical and biochemical similarity between mycorrhizal mantles and their associated sporocarps, initial identifications, based on anatomical characters with subsequent confirmation using DNA, will expedite ectomycorrhizal identification and allow for the processing of the large sample sizes necessary for ecological investigations.

Acknowledgments

This research was supported by National Sciences and Engineering Research Council of Canada (NSERC) Operating grants to R.S.C. and R.J.B., an NSERC post-graduate scholarship to G.K., the Canadian Circumpolar Institute, and the Alberta Department of Environmental Protection. We thank the staff at Kananaskis Field Stations and Lisa Cuthbertson for field assistance, George Braybrook for help with imaging, and Markus N. Thormann for translations.

References

- Acasai, J., and Largent, D.L. 1983. Ectomycorrhizae of selected conifers growing in sites which support dense growth of bracken fern. *Mycotaxon*, **16**: 509–516.
- Agerer, R. 1986. Studies in ectomycorrhizae III. Mycorrhizae formed by four fungi in the genera *Lactarius* and *Russula* on spruce. *Mycotaxon*, **27**: 1–59.
- Alexander, I.J. 1981. The *Picea sitchensis* + *Lactarius rufus* mycorrhizal association and its effect on seedling growth and development. *Trans. Br. Mycol. Soc.* **76**: 417–423.
- Amiet, R., and Egli, S. 1991. Die Ectomycorrhizen des Grubigen Milchlings (*Lactarius scrobiculatus* (Scop.:Fr.) Fr.) an Fichte (*Picea abies* Karst.). *Schweiz. Z. Forstwes.* **142**: 53–60.
- Baig, M.N. 1972. The ecology of timber line vegetation in the Rocky Mountains of Alberta. Ph.D. dissertation, Department of Biology, University of Calgary, Calgary, Alta.
- Brand, F. 1991. Ectomycorrhizen an *Fagus sylvatica*—Charakterisierung und Identifizierung, ökologische Kennzeichnung und unsterile Kultivierung. *Libri Bot.* **2**: 1–228.
- Brand, F., and Agerer, R. 1986. Studien an Ektomykorrhizen VII. Die Mykorrhizen von *Laccaria amethystina*, *Lactarius subdulcis* und *L. vellereus* an Buche. *Z. Mykol.* **52**(2): 287–320.
- Bruns, T.D., Fogel, R., and Taylor, J.W. 1990. Amplification and direct sequencing from fungal herbarium specimens. *Mycologia*, **82**: 175–184.
- Camazine, S., and Lupo, A. 1984. Labile toxic compounds of the Lactarii: the role of the laticiferous hyphae as a storage depot for precursors of pungent dialdehydes. *Mycologia*, **76**: 355–358.
- Cázares, E. 1992. Mycorrhizal fungi and their relationship to plant succession in subalpine habitats. Ph.D. dissertation, Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oreg.
- Core, H.A., Côté, W.A., and Day, A.C. 1979. Wood structure and identification. Syracuse University Press, Syracuse, N.Y.
- Currah, R.S., Sigler, L., Abbott, S., and Flis, A. 1989. Cultural and taxonomic studies of ectomycorrhizal fungi associated with lodgepole pine and white spruce in Alberta. Alberta Forest Development Research Trust Report, Devonian Botanic Garden, University of Alberta, Edmonton, Alta.
- Daughtridge, A.T., Boese, S.R., Pallardy, S.G., and Garrett, H.E. 1986. A rapid staining technique for assessment of ectomycorrhizal infection of oak roots. *Can. J. Bot.* **64**: 1101–1103.
- Egger, K. 1995. Molecular analysis of ectomycorrhizal communities. *Can. J. Bot.* **73** (Suppl. 1): S1415–S1422.
- Erland, S., Henrion, B., Martin, F., Glover, L.A., and Alexander, I.J. 1994. Identification of the ectomycorrhizal basidiomycete *Tylospora fibrillosa* Donk by RFLP analysis of the PCR-amplified ITS and IGS regions of ribosomal DNA. *New Phytol.* **126**: 525–532.
- Feibelman, T., Bayman, P., and Cibula, G.W. 1994. Length variation in the internal transcribed spacer of ribosomal DNA in chanterelles. *Mycol. Res.* **98**: 614–618.
- Gardes, M., and Bruns, T. 1993. ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. *Mol. Ecol.* **2**: 113–118.
- Gardes, M., and Bruns, T.D. 1996. Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: above- and below-ground views. *Can. J. Bot.* **74**: 1572–1583.
- Gardes, M., White, T.J., Fortin, J.A., Bruns, T., and Taylor, J.W. 1991. Identification of indigenous and introduced symbiotic fungi in ectomycorrhizae by amplification of nuclear and mitochondrial ribosomal DNA. *Can. J. Bot.* **69**: 180–190.
- Haselwandter, K. 1987. Mycorrhizal infection and its possible ecological significance in climatically and nutritionally stressed alpine plant communities. *Angew. Bot.* **61**: 107–114.
- Hesler, L.R., and Smith, A.H. 1979. North American species of *Lactarius*. University of Michigan Press, Ann Arbor, Mich.
- Ingleby, K., Mason, P.A., Last, F.T., and Fleming, L.V. 1990. Identification of ectomycorrhizas. Institute for Terrestrial Ecology, Natural Environment Research Council, London, U.K. Research Publication No. 5.
- Jobes, D.V., Hurley, D.L., and Thien, L.B. 1995. Plant DNA isolation: a method to efficiently remove polyphenolics, polysaccharides, and RNA. *Taxon*, **44**: 379–386.
- Kottke, R.B., and Oberwinkler, F. 1990. Ascomycete mycorrhizas from pot grown silver-fir seedlings (*Abies alba* Mill.). *New Phytol.* **115**: 471–482.
- Kraigher, H., Agerer, R., and Javornik, B. 1995. Ectomycorrhizae of *Lactarius lignyotus* on Norway spruce, characterized by anatomical and molecular tools. *Mycorrhiza*, **5**: 175–180.
- Martin, J. 1986. Mycorrhization de *Monotropa uniflora* L. par des Russulaceae. *Bull. Soc. Mycol. Fr.* **102** (Fasc. 2): 155–159.
- Masui, K. 1926. A study on the mycorrhiza of *Abies firma*, S. et Z., with special reference to its mycorrhizal fungus, *Cantharellus floccosus*, Schw. *Mem. Coll. Sci. Kyoto Imp. Univ. Ser. B.* **2**: 65–73.
- Miller, S.L., Koo, C.D., and Molina, R. 1991. Characterization of red alder ectomycorrhizae: a preface to monitoring belowground ecological responses. *Can. J. Bot.* **69**: 516–531.
- Molina, R., and Trappe, J.M. 1982. Patterns of ectomycorrhizal host specificity and potential among pacific northwest conifers and fungi. *For. Sci.* **28**: 423–458.
- Mortimer, P. 1978. The alpine vascular flora and vegetation of Prospect Mountain, Front Range, Rocky Mountains, Alberta. M.Sc. thesis, Department of Botany, University of Alberta, Edmonton, Alta.
- Moser, M. 1967. Die ektotrophe Ernährungsweise an der Waldgrenze. *Mitt. Forstl. Bundes-Versuchsanst. (Wien)*, **75**: 357–380.
- Moser, M. 1982. Mycoflora of the transitional zone from subalpine forests to alpine tundra. *In Arctic and alpine mycology*. Vol. 1.

- Edited by G.A. Larsen and J.F. Ammirati. University of Washington Press, Seattle, Wash. pp. 371–384.
- Peña-Cabriaes, J.J., and Valdés-Hgo, M. 1974. Rhizosphère du sapin (*Abies religiosa*). II. Mycorrhizes: isolement et culture. *Can. J. Microbiol.* **20**: 49–54.
- Pillukat, A., and Agerer, R. 1992. Studien an Ektomykorrhizen XL. Vergleichende Untersuchungen zur baumbezogenen Variabilität der Ektomykorrhizen von *Russula ochroleuca*. *Z. Mykol.* **58**: 211–242.
- Romagnesi, H. 1967. Les Russules d'Europe et d'Afrique du Nord. Bordas, Paris, France.
- Shaffer, R.L. 1975. Some common North American species of *Russula* subsect. *Emeticinae*. *Beih. Nova Hedwigia*, **51**: 207–237.
- Singer, R. 1986. The Agaricales in modern taxonomy. Koeltz Scientific Books, Koenigstein, Germany.
- Stanis, V.F. 1979. *Russula* species occurring in the boreal region of Ontario and Québec, Canada. M.Sc. thesis, Department of Botany, University of Toronto, Toronto, Ont.
- Taylor, A.F.S., and Alexander, I.J. 1989. Ectomycorrhizal synthesis with an isolate of *Russula aeruginea*. *Mycol. Res.* **92**: 103–107.
- Treu, R. 1990. Charakterisierung und Identifizierung von Ektomykorrhizen aus dem Nationalpark Berchtesgaden. *Bibl. Mycol.* **134**: 1–236.
- Trottier, G.C. 1972. Ecology of the alpine vegetation at Highwood Pass, Alberta. M.Sc. thesis, Department of Biology, University of Calgary, Calgary, Alta.
- Väre, H., Vestberg, M., and Ohtonen, R. 1997. Shifts in mycorrhiza and microbial activity along an oroarctic altitudinal gradient in northern Fennoscandia. *Arct. Alp. Res.* **29**: 93–104.
- Weiss, M. 1991. Studies on ectomycorrhizae. XXXIII. Description of three mycorrhizae synthesized on *Picea abies*. *Mycotaxon*, **40**: 53–77.
- White, T.J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR protocols: a guide to methods and applications*. Edited by M.A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White. Academic Press, New York. pp. 315–322.
- Yamada, A., and Katsuya, K. 1995. Mycorrhizal association of isolates from sporocarps and ectomycorrhizas with *Pinus densiflora* seedlings. *Mycoscience*, **36**: 315–323.