

# Diversity and abundance of ericoid mycorrhizal fungi of *Gaultheria shallon* on forest clearcuts

Guoping Xiao and Shannon M. Berch

**Abstract:** Roots of salal (*Gaultheria shallon* Pursh) collected from forest clearcuts were examined by light and scanning electron microscopy, and the ericoid mycorrhizal fungi were isolated and identified. Heavy colonization of typical ericoid mycorrhizae was present in and restricted to the first of the two layers of root cortical cells. Neither ectomycorrhizae nor arbutoid mycorrhizae were observed. In the field, over 85% of the roots and 90% of the cortical cells within colonized roots were colonized. One hundred and seventy-five of the 278 fungal isolates from salal roots formed ericoid mycorrhizae with salal in the laboratory, and these isolates were grouped into four species based on spore formation and cultural characteristics: *Oidiodendron griseum* Robak, *Acremonium strictum* W. Gams, and two unidentified, nonsporulating fungal species. The association in the laboratory between *A. strictum* and salal was atypical in that the fungus improved the growth of salal seedlings but was slow to colonize roots and occasionally grew and even sporulated on the shoots. No differences in percent colonization or diversity of ericoid mycorrhizal fungi were found in salal growing on clearcuts from two different forest types.

**Key words:** *Gaultheria shallon*, *Oidiodendron griseum*, *Acremonium strictum*, ericoid mycorrhizal fungi.

**Résumé :** Les auteurs ont examiné, en microscopie photonique et électronique par balayage, des racines du salal (*Gaultheria shallon* Pursh) récoltées sur des sites forestiers coupés à blanc, et ils en ont isolé et identifié les champignons mycorrhiziens éricoïdes. On observe une colonisation typique importante par les champignons mycorrhiziens éricoïdes et cette colonisation se limite aux deux premières couches de cellules corticales. Aucune mycorrhize arbutoïde ou ectomycorhize n'ont été observées. Sur le terrain, plus de 85% des racines et 90% des cellules corticales des racines colonisées sont colonisées. Sur les 278 champignons isolés des racines de salal, 175 ont formé des mycorrhizes éricoïdes sur le salal au laboratoire et ces isolats ont pu être regroupés en 4 espèces, sur la base des spores formées et les caractéristiques culturelles : l'*Oidiodendron griseum* Robak, l'*Acremonium strictum* W. Gams, et deux espèces de champignons non-sporulants et non-identifiés. L'association obtenue au laboratoire entre l'*A. strictum* et le salal est atypique en ce que le champignon améliore la croissance des plantules de salal mais colonise lentement les racines et pousse occasionnellement et même sporule sur les tiges. Aucune différence n'a été observée quant au pourcentage de colonisation ou à la diversité des champignons mycorrhiziens éricoïdes du salal poussant sur des coupes à blanc, effectuées sur deux types forestiers différents.

**Mots clés :** *Gaultheria shallon*, *Oidiodendron griseum*, *Acremonium strictum*, champignons mycorrhiziens éricoïdes.

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## Introduction

There are about 103 genera and 3350 species in the Ericaceae, a family that is widespread in arctic, temperate, and tropical climates (Mabberley 1987). In some areas, ericaceous species form pure heathlands (Specht 1978). Important forest lands have been overtaken by ericaceous plants after logging and burning in Europe (Gimingham 1972; Ellenberg 1988) and in North America (Meades 1986; Weetman et al. 1989; Mallik 1991). Although the domination of ericaceous plants and their ericoid mycorrhizal fungi is generally recognized

to characterize soils with very low pH, low available nutrients, and high organic matter content, most of what is known is based on the study of one ericoid mycorrhizal fungus, *Hymenoscyphus ericae* (Read) Korf and Kernan, and a small number of plant species, including *Calluna vulgaris* L. and *Vaccinium macrocarpon* Ait. (Read 1983, 1987, 1991; Read and Bajwa 1985) in northern Europe.

There is a slowly growing literature on the mycorrhizae of salal (*Gaultheria shallon* Pursh), an ericaceous shrub important in North America. Ericoid, arbutoid, and ectomycorrhizae were reported on salal roots from northern California (Largent et al. 1980). Roots of salal grown with Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and western hemlock in pots containing field soil from the Oregon coast range developed mostly ericoid mycorrhizae, a small amount of ectomycorrhizae, but no arbutoid mycorrhizae (Smith 1993). This difference might be due to soil factors that are important in determining the nature of mycorrhizal associations (Dighton and Coleman 1992). We reported on a system adapted to synthesize ericoid mycorrhizae with salal and on the identity of one of the fungi, *Oidiodendron griseum*,

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that forms mycorrhizae in the field with salal (Xiao and Berch 1992). In addition, a number of the fungi known to form ericoid mycorrhizae with other of the Ericaceae, *Hymenoscyphus ericae*, *Oidiodendron flavum* Szilyinyi, *Oidiodendron maius* Barron, *Pseudogymnoascus roseus* Raullo, and *Scytalidium vaccinii* Dalpé, Litten & Sigler (a possible anamorph of *H. ericae*; Egger and Sigler 1993), were found to be capable of forming typical ericoid mycorrhizae with salal in the lab (Xiao 1994).

Salal is an important component of the understorey of coniferous forests on the west coast of North America. On northern Vancouver Island, British Columbia, there are two very different side by side forest ecosystems. The HA (hemlock-amabilis) forest type is characterized by western hemlock (*Tsuga heterophylla* (Raf.) Sarge.) and amabilis fir (*Abies amabilis* (Dougl.) Forbes), and the CH (cedar-hemlock) by western red cedar (*Thuja plicata* Donn) and western hemlock. The CH has a dense understorey cover of salal (*Gaultheria shallon* Pursh), while in the HA salal is a sparse understorey component. After clearcutting and slash-burning, salal remains a minor component of the vegetation on the HA but recovers the CH cutovers quickly and densely. Salal accomplishes this by sprouting from old rhizomes present prior to disturbance and subsequently occupies both aboveground and belowground environments by producing large amount of biomass (Messier and Kimmins 1991). Salal outcompetes other plants including commercially planted conifers for nutrients and dominates the nutrient-stressed sites in a few years, making regeneration of CH cutovers difficult (Weetman et al. 1989).

Because of the parallels between the poor growth of conifers on sites in British Columbia dominated by salal and on sites in Europe dominated by *Calluna vulgaris* (Read 1983), it became clear that the mycorrhizae of salal on these sites should be investigated. The specific objectives of this study were to compare salal on CH and HA sites in relation to types of mycorrhizae, intensity of colonization, and the fungi that form these associations.

## Materials and methods

### Study area and sampling

The study area is located between Port McNeill and Port Hardy on northern Vancouver Island, British Columbia (50°60'N, 127°35'W). The area is undulating with elevations less than 300 m and annually receives an average precipitation of 1700 mm with daily temperatures varying from 3.0°C in January to 13.7°C in July. The soils are Ferro-Humic Podzols with a thick layer (20–60 cm but mostly >45 cm) of organic matter (Messier 1991) with an average pH ranging from 3.4–4.8 (Prescott et al. 1993) in CH sites and Ferro-Humic Podzols with a relatively thinner (10–40 cm) organic matter layer (Messier 1991) with an average pH of 3.2–4.0 in HA sites (Prescott et al. 1993). Nitrogen availability is lower in CH forest floors than in HA forest floors (Prescott et al. 1993). The forests of the study site were clear-cut in 1986 and broadcast burned in 1987.

For HA and CH, two root samples, each about 500 g (with rhizomes and soil), were collected with a shovel in September 1991 from each of 15 (16 × 16 m) plots, giving a total of 30 samples per clearcut type. The samples were kept in plastic bags in a cooler when collected and transported to The University of British Columbia and stored in a refrigerator in the laboratory until studied.

### Mycorrhizal colonization

Roots were subsampled by collecting them from different individual rhizomes, washed with running tap water, cut into 1-cm lengths, and transferred to a gridded tray (15 × 38 cm). A total of 50 pieces from each subsample was obtained by choosing 5 pieces from each of 10 randomly chosen grids. The root pieces were mounted on slides in FDA Blue No. 1 (Chapman 1992) and examined under a compound light microscope. Percent colonization was assessed in two ways: root colonization and cell colonization. For percent root colonization, 50 root pieces (each 1 cm long) per sample were counted as colonized or uncolonized. One hundred contiguous cortical cells within each colonized root were counted as colonized or uncolonized for the percent cell colonization. The data were analyzed by a *t*-test.

The same root pieces used to determine percent colonization were examined for the morphology of the colonization. Occasionally, additional cross and longitudinal free-hand sections were made for determination of detailed structure by freezing a piece of root on a freezing microtome stage and cutting it with a double-edged razor blade. Colonized roots of salal synthesized *in vitro* (see below) were also examined in this way.

Roots collected from the field, washed with tap water to remove soil particles, and two entire root systems from the mycorrhiza synthesis study were prepared for scanning electron microscopy by the methods of Tanaka and Mitsushima (1984) and Tanaka and Nagura (1981). The roots were first fixed with 0.5% paraformaldehyde with 0.5% glutaraldehyde in 0.1 M cacodylate with 0.475 M sucrose, pH 7.4, then postfixed in 1% osmium tetroxide in cacodylate buffer. After soaking in dimethylsulphoxide (DMSO) solution, the roots were frozen on blocks in liquid nitrogen, and fractured with a razor blade. The exposed cell matrices were digested in buffered osmium tetroxide. Before critical point drying, the specimens went through dehydration and finally were mounted on stubs using rapid setting araldite and coated lightly with gold-palladium at 2 kV 10 mA for 2 min. About 100 field roots and 20 cultivated roots were examined using a Cambridge 250 scanning electron microscope (SEM).

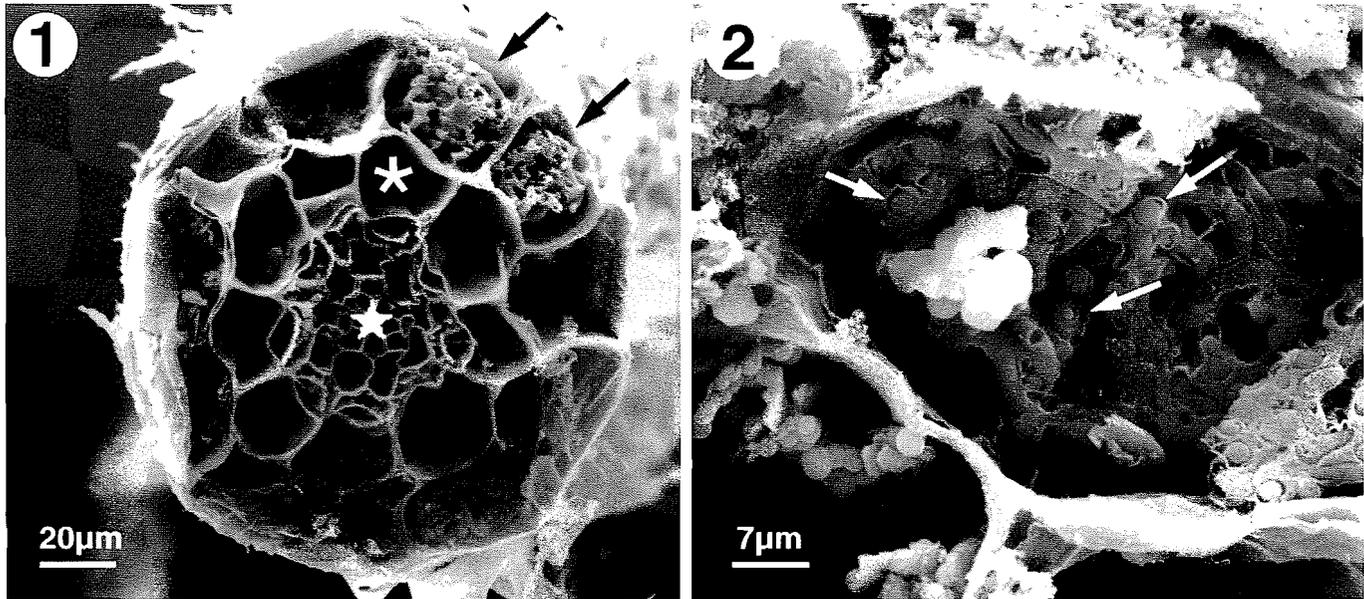
### Isolation of mycorrhizal fungi

The 60 root samples were subsampled, washed for 30 min in running tap water, and surface sterilized in 30% hydrogen peroxide for 1 min. For each subsample, approximately 0.5-cm lengths were excised from the disinfected roots, and 15–20 pieces (up to 10 pieces per Petri plate) were plated out for fungal isolation using the methods previously described (Xiao and Berch 1992). All fungal isolates from salal field roots, except for *Penicillium*, *Aspergillus*, and *Trichoderma* species, were tested in axenic culture for the formation of mycorrhizae. Isolates that formed mycorrhizae with salal in axenic culture were identified whenever possible, and some were sent out to specialists for identification. Vouchers of sporulating species are deposited in culture collections (UAMH or CBS).

### Synthesis of mycorrhizae

Fungal isolates from salal field roots were maintained on modified Melin-Norkrans agar (MMN) and used as inoculum for the synthesis experiments in this study. The approach used here was the same as that described in Xiao and Berch (1992). The basal medium used in the synthesis chambers was MMN without mineral nitrogen, malt extract, or glucose. The pH was 4, which is within the pH range (3.2–4.8) of the soils on the CH and HA sites. This medium was poured into Petri plates and later half of the agar disc was removed. Salal seeds were surface sterilized with 30% hydrogen peroxide and germinated on water agar. One germinant per Petri dish was planted at the center of the cut edge of the agar and incubated. When a real leaf emerged, five replicates per isolate were inoculated with about 2 mm<sup>3</sup> of colonized medium cut from the edge of a fungal colony.

**Fig. 1.** SEM cross section of a fine root of salal from the field showing the outer colonized layer of cortical cells (arrows), the inner uncolonized layer (\*) of cortical cells, and the simple stele (★). **Fig. 2.** SEM of salal cortical cell containing hyphal complex. Hyphae surrounded by host cell membrane (arrows).



The plates were then sealed with parafilm and placed vertically in a growth chamber at 25°C, 18 h light at  $310 \text{ mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  illumination, and 6 h dark. Fourteen days after inoculation, we began checking the plants for colonization by placing the unopened chamber on the stage of a light microscope and examining the whole root system intact at 50–100×. Forty-five days after inoculation, roots were harvested by gently pulling them out of the agar, stained with FDA Blue No. 1, and examined at 400–1000× to confirm colonization. All isolates were reisolated from the roots of these plants. Representatives of the isolates reisolated from synthesized salal roots were retested to form mycorrhizae with salal in the same axenic culture system.

## Results

### Morphology of salal fine roots and mycorrhizae

Both field and cultivated fine roots of salal are about 100–200 μm in diameter and very simple in anatomy, consisting of five to six layers of cells radially arranged in cross section (Fig. 1), and lack root hairs. Using the Petri plate culture system, it was possible to observe undisturbed roots throughout their development (Figs. 3–5). Typical ericoid mycorrhizal colonization was observed from both field and in vitro roots. The apical region of in vitro nonmycorrhizal and mycorrhizal roots was covered by rounded root cap cells that sloughed off as the root proceeded (Fig. 3). The apical cells were small and very dense. Often, the apical region in vitro was surrounded by a continuous layer of mucigel (Fig. 3). The stele was enclosed by two layers of cells and only the outer layer was colonized (Fig. 1) by mycorrhizal fungi.

In some cases, a mantle-like structure of hyphae (Fig. 7) was observed in field roots, but no Hartig net was ever seen. The hypha became narrow when penetrating the outermost wall of the host cell (Fig. 6). Once inside the cell, it proliferated around the nucleus (Fig. 8), developing a hyphal complex and occupying all the cell space (Fig. 9). As seen under SEM, in colonized cortical cells hyphae were constantly sep-

arated from the host cytoplasm by a continuous host plasmalemma (Fig. 2). No difference between the roots of the CH and HA sites or from the culture chambers when inoculated with many of the test isolates was detected in terms of morphology of the salal mycorrhizae. Nothing that fits the definition of ectomycorrhiza or arbutoid mycorrhiza was seen.

The only atypical association that was regularly observed occurred in axenic culture in roots colonized by *Acremonium strictum*. As in the typical ericoid mycorrhizae, this fungus formed a web of hyphae on the surface of the roots and eventually penetrated some of the outermost cortical cells, but it did not produce typical hyphal complexes inside the cortical cells. Within the colonized cells, the hyphae of the fungus were loosely arranged. Colonization of cortical cells took much longer than it did for the typical ericoid mycorrhizal fungi, although in other studies positive host-growth responses were observed early on (Xiao 1994). In addition, this fungus occasionally grew and sporulated on the shoots of the salal seedlings.

### Percent colonization

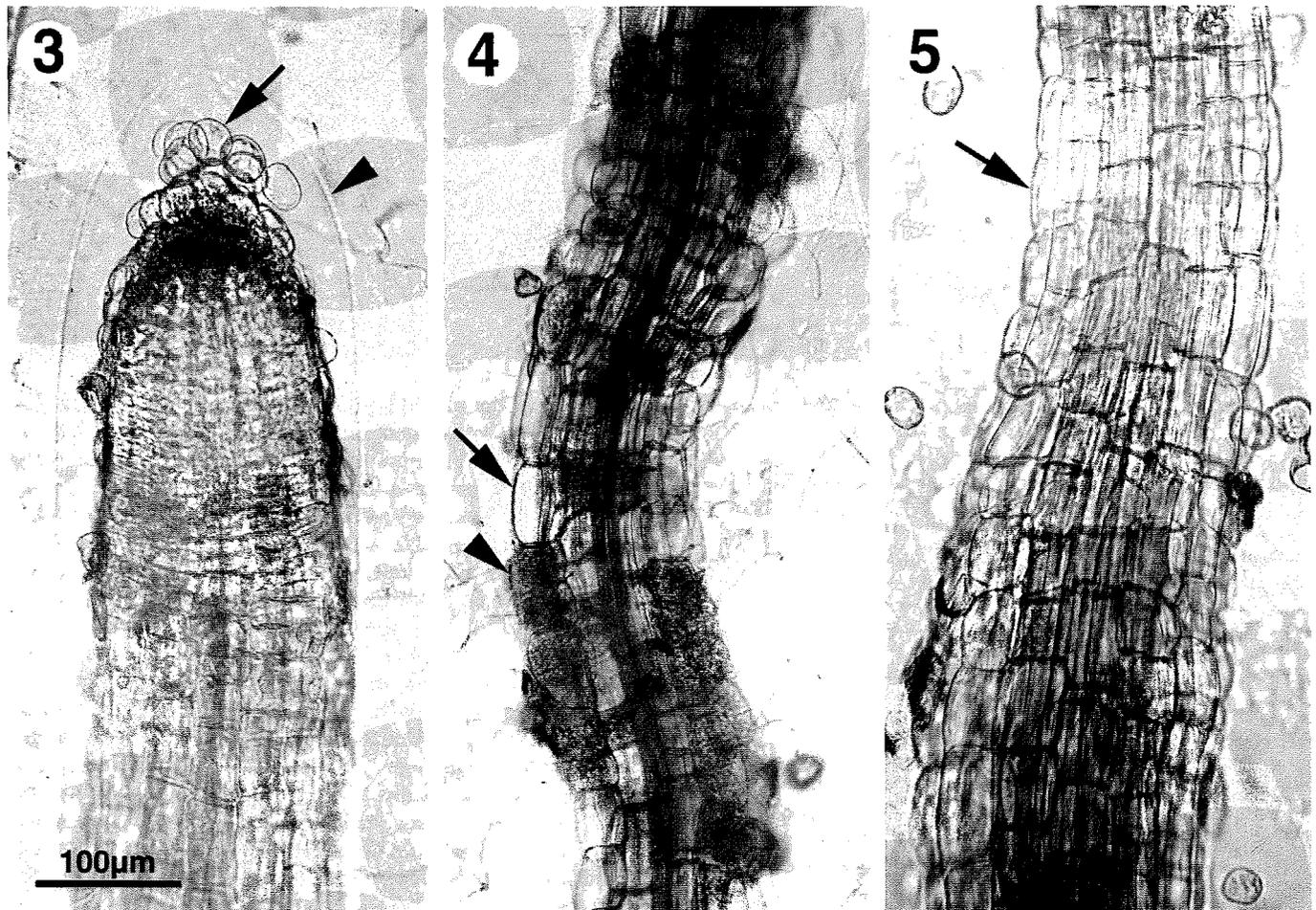
The field roots of salal were highly colonized by the ericoid mycorrhizal fungi in terms of root colonization and cell colonization. The colonization rate was as high as 87% for root colonization and 91% for cell colonization within the colonized roots. No statistically significant difference (at  $\alpha = 0.01$ ) of mycorrhizal colonization between the two forest types was found (Table 1).

### Mycorrhizal fungi

From the 560 root pieces per forest type, a total of 278 fungal isolates were retained. Among them, 175 colonized salal roots in axenic culture, forming more or less typical ericoid mycorrhizae. These mycorrhizal fungal isolates were grouped by the morphology of their colonies and asexual fruiting

**Fig. 6.** Cross section of field salal root showing hypha narrowing as it penetrates wall of host cortical cell (arrow) and then forming a hyphal complex (\*). **Fig. 7.** Mantle-like accumulation of hyphae (arrow) on surface of ericoid mycorrhiza of salal from field. **Fig. 8.** Colonized cortical cell of a salal field root with hypha (arrow) proliferating around host nucleus (arrowhead) after penetrating thickened host cell wall (\*). **Fig. 9.** Dense hyphal complexes (★) within thick-walled (\*), outer cortical cells of salal root from the field.

**Fig. 3.** Root tip of salal viewed through wall of culture chamber, showing the sloughed root cap cells (arrow) and mucigel (arrowhead). **Fig. 4.** Inoculated root of salal viewed through wall of culture chamber, showing colonized (arrowhead) and uncolonized (arrow) cells. **Fig. 5.** Uninoculated salal root viewed through wall of culture chamber, showing uncolonized cortical cells (arrow).



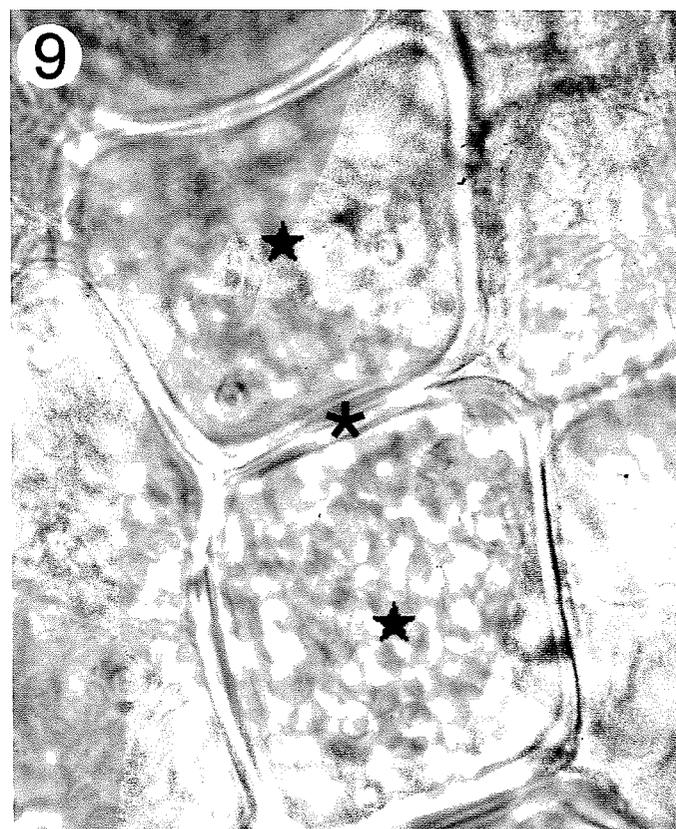
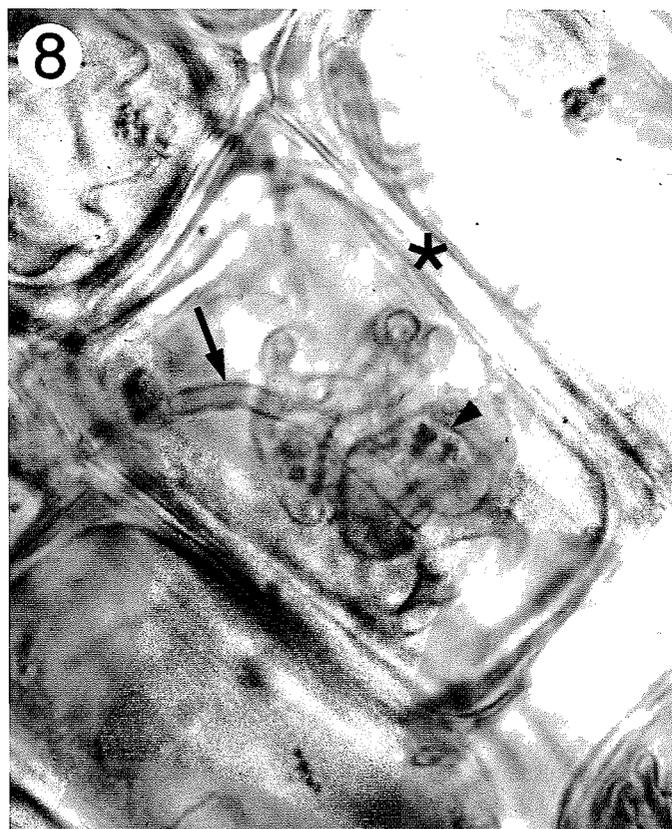
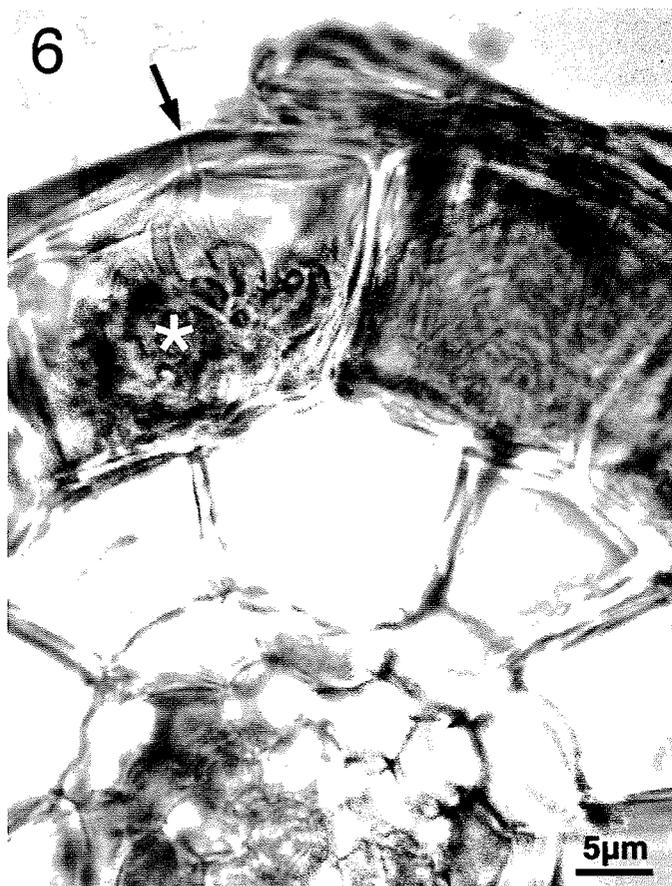
bodies when present and probably belong to four different species, two of which sporulated in culture and two of which did not. The most common was identified as *O. griseum* (Xiao and Berch 1992). The second sporulating species was identified as *Acremonium strictum* W. Gams by W. Gams, Centraalbureau voor Schimmelcultures, Baarn, Netherlands. The other two "species" were not identified owing to the lack of any fruiting bodies. These fungi were isolated from field salal roots with a similar frequency on HA and CH cut-blocks (Table 2).

#### *Oidiodendron griseum* Robak

Figs. 10, 11  
Colonies on MMN reached 2 cm in diameter in 14 d and 5 cm in diameter in 30 d at 25°C. They are white at first, later olive-greenish, distinctly zonate (Fig. 11), and sporu-

late with long-stalked conidiophores usually arising from hyphae at the agar surface (Fig. 10). Colonies on potato dextrose agar (PDA) reached 2 cm in diameter in 14 d and 4 cm in diameter in 30 d at 25°C. They are whitish grey, cottony with a mealy appearance due to abundant production of conidia, domed, wrinkled. On malt extract agar (MEA), colonies reached a diameter of 2 cm in 14 d and 3.5 cm in 30 d at 25°C. Colonies are flat and hyaline. No conidiophores or conidia are produced. Conidiophores on MMN are 161–390 × 2–4.5 µm, brown, smooth, cylindrical, unbranched in the lower part. Conidia are grey-green, smooth to finely roughened, subglobose, ovoid or cylindrical, commonly about 2.0–4.0 × 1.5–2.5 µm.

Isolates S4, S18, S45, and S80 were examined and deposited in the Microfungus Collection at the University of Alberta Herbarium (UAMH).



**Figs. 10 and 11.** *Oidiodendron griseum*. Fig. 10. Apex of conidiophore (arrow) with fragmenting conidia (arrowhead). Fig. 11. Colony on MMN. **Figs. 12 and 13.** *Acronium strictum*. Fig. 12. Conidiophore (arrow) with apical conidium (arrowhead). Fig. 13. Colony on MMN. **Fig. 14.** Colony of Unknown 1 on MMN. **Fig. 15.** Colony of Unknown 2 on PDA.

**Table 1.** Comparison of colonization intensity of salal roots from two different forest clearcuts, hemlock–amabilis (HA) and cedar–hemlock (CH).

Colonization and site	Mean % colonization	SD	<i>t</i> -test ( <i>p</i> )
Cell			
HA	89.2	2.76	0.013
CH	90.8	1.86	
Root			
HA	86.1	4.37	0.321
CH	87.1	2.65	

***Acronium strictum* W. Gams** Figs. 12, 13 Colonies reached 2 cm in diameter in 14 d and 4 cm in 30 d at 25°C on MMN. Colonies are white-creamy with a pinkish tint, flat, usually moist, with slight radial wrinkling (Fig. 13). Phialides are simple and arise from submerged or slightly fasciculated aerial hyphae (Fig. 12). Conidia are mostly cylindrical, 3 × 1 μm. On PDA, colonies reached a diameter of 3 cm in 14 d and 5 cm in 30 d at 25°C. On MEA, colonies reached a diameter of 2.8 cm in 14 d and 4.5 cm in 30 d at 25°C. On both these media, colonies are very thin, flat, moist, and hyaline with a pinkish tint.

Isolates examined were S214, S217, S220, and S232. S232 was deposited at the Centraalbureau voor Schimmelcultuur (CBS).

#### Unknown 1

Fig. 14 Colonies reached a diameter of 2 cm on MMN in 14 d and 4 cm in 30 d at 25°C. They are olive-brown, distinctly zonate with a lustrous surface (Fig. 14). On PDA, colonies reached 2 cm in 14 d and 5 cm in 30 d at 25°C. They are raised in the center, wrinkled, and distinctly zonate. Colonies are grey, black, and white from the center to the edge, with a powdery look. No spores and no pigment are produced on MMN and PDA. On MEA, colonies reached a diameter of 2 cm in 14 d and 4 cm in 30 d at 25°C. Violaceous brown pigment was released into the medium so that the whole agar disc was stained brown-purple. Colonies are olive-greenish, flat, and smooth.

Isolates examined were S9, S219, S234, and S245.

#### Unknown 2

Fig. 15 On MMN, colonies reached a diameter of 2 cm in 14 d and 3 cm in 30 d at 25°C. Two types of zonated colonies were produced. One was creamy with a dark center and droplets of cream-colored liquid scattered over the surface. The other was grey with four zones, alternating floccose and light grey, fuzzy and dark grey, olive green and off-white from the center to the edge. A marked radial wrinkling is exhibited. Colonies reached 1.5 cm in diameter in 14 d and 3 cm in diameter in 30 d on PDA at 25°C. They are creamy, moist, domed and funiculose with ropes radiating out (Fig. 15). On

**Table 2.** Number of isolates of four different mycorrhizal fungi isolated from 560 salal root pieces each from two different forest clearcuts, cedar–hemlock (CH) and hemlock–amabilis (HA).

Fungal species	CH	HA
<i>Oidiodendron griseum</i>	28	31
<i>Acronium strictum</i>	21	20
Unknown 1	18	18
Unknown 2	19	22
Total	86	91

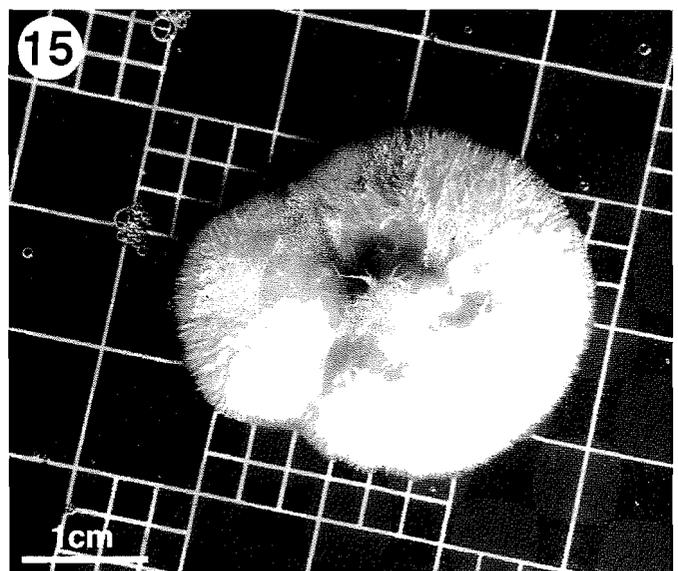
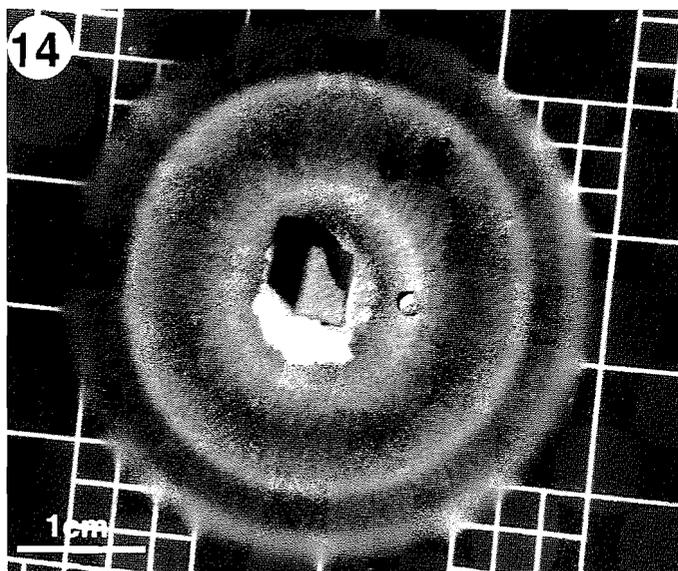
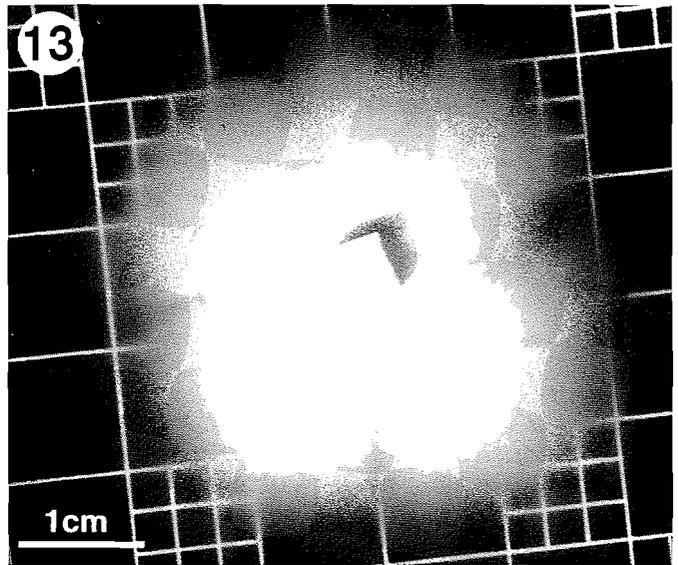
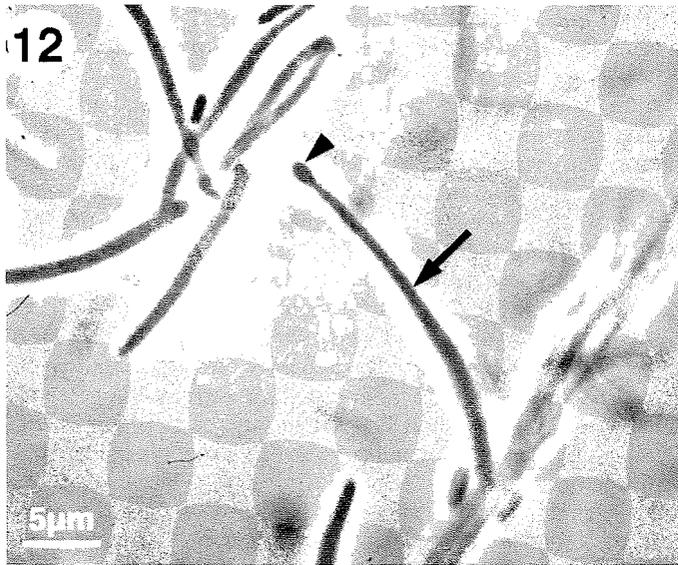
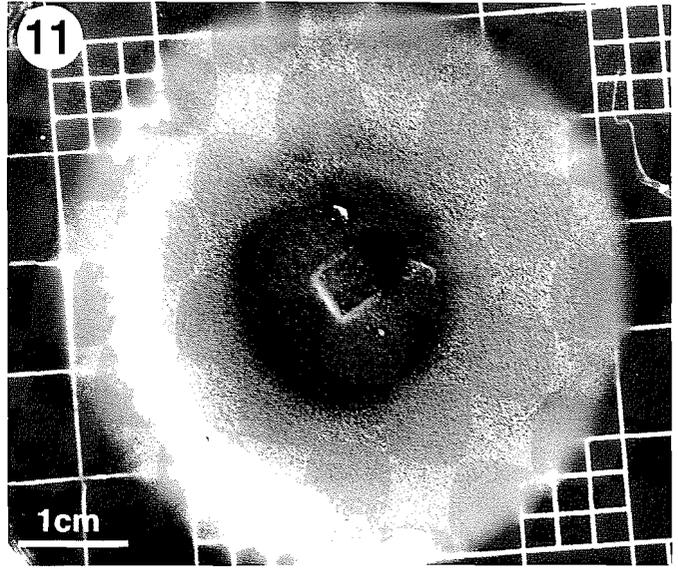
MEA, colonies reached a diameter of 2 cm in 14 d and 3.5 cm in 30 d at 25°C. They are funiculose and oliveaceous-black. No pigment or spores were produced on any medium.

Isolates examined were S203, S227, S246, and S255. Colonies resembled pure culture of isolates 100 and 101 from D.J. Read of *H. ericae* in overall appearance and growth rate.

## Discussion

Salal formed two kinds of root–fungus associations in axenic culture: typical ericoid mycorrhizae with *O. griseum* and two unknown species, and atypical mycorrhizae with *Acronium strictum*. The morphology and structure of salal roots, whether colonized or uncolonized, are in general similar to the roots of other ericaceous plants. The roots are very fine, as thin as 100 μm in diameter, typical of the ‘hair roots’ in ericaceous plants (Beijerinck 1940), and lack root hairs. Read (1983) characterized the fine roots of ericaceous plants as having one to three layers of cells, the number varying according to species, surrounding a narrow central stele. Salal roots differ somewhat from the fine roots of *C. vulgaris* (Bonfante-Fasolo and Gianinazzi-Pearson 1982), *Rhododendron ponticum* (Duddridge and Read 1982), and *Rhododendron brachycarpum* G. Don. (Currah et al. 1993) that have a single layer of cortical cells surrounding a simple stele, because in salal there are two layers of cells outside the endodermis. In mycorrhizae, only the outer layer of cells is colonized by fungi and the colonization does not extend into the stele or meristematic region of the root. Bonfante-Fasolo et al. (1981) found that there were one or two layers of cells surrounding the stele in the fine roots of *Vaccinium myrtillus* L. and that some of the cells were colonized by a fungus; however, it is not clear if any cells of the second layer were colonized.

Cells appeared collapsed behind the apical region of non-mycorrhizal roots of *C. vulgaris* (Bonfante-Fasolo and Gianinazzi-Pearson 1982) and *V. myrtillus* (Bonfante-Fasolo et al. 1981). The collapsed appearance was also observed in this study but might be due to processing for electron microscopy because no sign of collapse was found on live



and intact mycorrhizal and nonmycorrhizal roots of salal growing in the culture chambers.

To classify their mycorrhizal types, Largent et al. (1980) defined ectomycorrhizae and arbutoid mycorrhizae as follows: mycorrhizae with a mantle and a Hartig net are called ectomycorrhizae, and mycorrhizae with a mantle, a Hartig net, and intracellular hyphal penetration are arbutoid mycorrhizae. They found ericoid, arbutoid, and ectomycorrhizae on field salal roots from northern California. In another study, roots of salal grown in pots containing field soil developed ericoid mycorrhizae, trace amounts of ectomycorrhizae, but no arbutoid mycorrhizae (Smith 1993). Neither ectomycorrhizae nor arbutoid mycorrhizae were observed from either field or synthesized salal roots in this study. However, a mantle-like fungal structure was occasionally found on the surface of field salal roots with or without intracellular colonization. Having a mantle can give the superficial impression of an ectomycorrhiza, but without a Hartig net a colonized root cannot be considered a true ectomycorrhiza. Similarly, without a Hartig net, a mycorrhizal root with a mantle and intracellular colonization cannot be considered a true arbutoid mycorrhiza either. Under what conditions salal and other ericaceous plants might form typical ectomycorrhizae or arbutoid mycorrhizae in the field merits further consideration.

Salal roots from both CH and HA sites were extensively colonized by ericoid mycorrhizal fungi, and differences between the two sites in terms of root colonization or cell colonization intensity could not be detected. Studies show that low availability of mineral nitrogen and high organic nitrogen content can result in high intensity of colonization of ericoid mycorrhizae (Reed 1987) and that application of inorganic nitrogen can suppress ericoid mycorrhiza formation (Brook 1952; Morrison 1957; Stribley and Read 1976; Moore-Parkhurst and Englander 1982). Most of the work on colonization intensity of ericoid mycorrhizal fungi has centered on mycorrhizal plants in the laboratory. Stribley and Read (1976) found that 70% of the root cortical cells of *V. macrocarpon* were colonized by *H. ericae*. In axenic culture, 9% of the root cortical cells of *Vaccinium angustifolium* were colonized by *Oidiodendron rhodogenum*, 12% by *Oidiodendron cerealis*, and 21% by *O. griseum* (Dalpé 1986). No information is available on mycorrhiza colonization intensity of ericaceous plants in relation to nitrogen availability and organic nitrogen concentration in the field, so no comparison can be made to this study.

A number of species of root-associated fungi of salal were isolated from both CH and HA sites with similar frequency. *Acremonium strictum* is first reported here as an atypical ericoid mycorrhizal fungus. The genus *Acremonium* contains over 100 species, many of which are soil-borne fungi (Domsch et al. 1980). The species placed in *Acremonium* sect. *Albolanosa* Morgan-Jones & Gams, which includes *Acremonium strictum*, live in grasses and sedges (Morgan-Jones and Gams 1982). Many species of grasses infected by *Acremonium* are reported to be toxic to livestock and insects (Siegel et al. 1987; Clay 1989) and nematodes (West et al. 1987). In addition, infection by *Acremonium* is also reported to be beneficial to the host by increasing drought tolerance (Belesky et al. 1987) and allelopathy (Quigley et al. 1990). *Acremonium strictum* is a ubiquitous fungus that occurs in soil, plant

wheatfield debris, rhizosphere, plant surfaces, excrement, hay, stained wood, atmosphere, iron ore tailings, fuel, and fuel filters (Domsch et al. 1980; Wong et al. 1978). It has an extremely wide range of activities: plant pathogen (Hesseltine and Bothast 1977; Chase 1978; Chase and Munnecke 1980; Seemueller 1976; Natural et al. 1982), parasite on eggs of the sugar beet cyst nematode (Nigh 1979), mycoparasite on fungi such as rusts, powdery mildews, agarics, *Scleroderma*, polypores (Domsch et al. 1980), and inhibitor of fungi (McGee et al. 1991). Since *Acremonium strictum* has the least number of differential characters among *Acremonium* species (Domsch et al. 1980), it is difficult to identify. Comparison of colony descriptions of our isolates of *Acremonium strictum* identified by W. Gams with the original species description (Domsch et al. 1980) and those of the same species used by McGee et al. (1991) revealed that our description is consistent with the original but different from the description by McGee et al. (1991). Colonies of our and the original isolates were pink, moist, and smooth, while the colonies of McGee's (McGee et al. 1991) isolates were velutinous to cottony. It is possible that McGee et al. (1991) either worked with a very different isolate of *A. strictum* or some other species.

In axenic culture with salal, *O. griseum* and the two unknowns formed typical ericoid mycorrhizae, i.e., a light web of hyphae on the root surface and extensive and dense hyphal complexes in root cortical cells (Read 1983). *Acremonium strictum*, though also forming a hyphal web on the root surface, colonized the outer layer of salal root cortical cells less quickly and less densely and formed only loose hyphal complexes inside the cortical cells. Although positive host responses were observed (Xiao 1994), other differences, and the evidence that this species is poorly circumscribed, polyphyletic, or very broad in its metabolic activity, suggest that the association between this fungus and salal is fundamentally different from that between salal and typical ericoid mycorrhizal fungi.

Twenty years ago, two distinct groups of ericoid mycorrhizal fungi were isolated from a number of ericaceous species including *C. vulgaris* (Pearson and Read 1973) and one of these was later identified as *H. ericae*. No other ericoid mycorrhizal fungi have since been reported from *C. vulgaris*. Couture et al. (1983) isolated a single ericoid mycorrhizal fungus, *O. griseum*, from *V. angustifolium*. Douglas et al. (1989) isolated a single species of ericoid mycorrhizal fungus, *O. maius*, from *Rhododendron*. Currah et al. (1993) isolated only *Oidiodendron periconioides* from *R. brachycarpum*. This might give the impression that an ericaceous species forms ericoid mycorrhizae with no more than one species of fungus, and if this were to be true, that this fungus plays a very important role in the survival and growth of its host. Although others have reported that fungi isolated from one species can form mycorrhizae with others (Dalpé 1991; Xiao 1994), this is the first report that several ericoid mycorrhizal fungi can be isolated from a single ericaceous species. Our work indicates that in fact many fungi form mycorrhizae with an ericaceous plant in the field, a situation more similar to that observed for most other mycorrhizal types and plant species.

The diversity of mycorrhizal fungi and high intensity of colonization suggest that ericoid mycorrhizal fungi are

involved in the dominance of salal on CH clearcuts, although the dense mat of rhizomes inherited from the CH forests also plays a role. *Oidiodendron griseum* was isolated from a variety of substrates (Burgeff 1961; Barron 1962; Wong et al. 1978; Domsch et al. 1980; Couture et al. 1983; Dalpé 1986; Douglas et al. 1989; Stoyke and Currah 1991), demonstrated to be antagonistic to the saprophytic fungi isolated from its original habitat (Dickinson and Boardman 1970), and shown to be able to use chitin as a nitrogen source (Leake and Read 1989). *Acremonium strictum* is mycoparasitic (Gandy 1979). This suggests that the mycorrhizal fungi of salal could be able to utilize organic nitrogen, as demonstrated for *H. ericae*, and directly inhibit the growth of the ectomycorrhizal fungi of the planted trees growing around salal. These abilities would help to explain how salal can come to dominate CH clearcuts to the detriment of planted conifers.

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