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The Structure and Fungal Associates of Mycorrhizas in *Leucopogon parviflorus* (Andr.) Lindl.

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Mycorrhizal root systems of *Leucopogon parviflorus* (Andr.) Lindl. were collected from wild populations at three sites on the coast of New South Wales, Australia and examined by light and electron microscopy. The structure of the hair roots is typical of the family, there being an epidermal layer in which ericoid mycorrhizas are formed, two cortical layers (an exodermis and endodermis) and a very small stele. The colour, size and coil structure of the fungal symbionts indicate that there were at least two different fungi that consistently formed ericoid mycorrhizal structures at these sites. Transmission electron microscopy of the endophytes showed only ascomycete fungi. Plants from two of the populations were used for fungal isolations. Fungi were isolated by incubating surface sterilized hair-root pieces in a solution of bovine serum albumin with penicillin and streptomycin. Twenty-one different culture types were obtained, four of which were common to both sites. Two of the common culture types were dark, sterile, slow-growing cultures similar to the ericoid endophyte *Hymenoscyphus ericae* (Read) Korf & Kernan.

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Key words: *Leucopogon parviflorus*, bearded heath, Epacridaceae, Ericaceae, mycorrhiza, ultrastructure, endophytes, callose.

INTRODUCTION

Members of the Epacridaceae possess very fine hair roots, which are reported to form mycorrhizas similar to those found in the Ericaceae (Brook, 1952; Allen *et al*., 1989). In the Ericaceae fungi invade through the outer walls of the epidermal cells and produce characteristic intracellular coils that are separated from the host cytoplasm by the host plasmalemma and an interfacial matrix through which nutrient exchange is thought to occur (Read, 1983). Fungi shown to form such coils include *Oidiodendron* (Robak) Udagawa & Toyazaki species (Dalpé, 1989) and *Hymenoscyphus ericae* (Read) Korf & Kernan (Read, 1974) although no enhancement of host growth has been demonstrated with the *Oidiodendron* associations. The ericoid endophyte *H. ericae* has been shown to mobilize nutrients from organic matter, greatly increasing nitrogen uptake (Read, 1991), and to increase iron and calcium uptake in alkaline conditions (Bajwa and Read, 1986; Leake, Shaw and Read, 1990). In contrast very little is known about the structure or function of epacrid mycorrhizas, or the fungal endophytes involved.

Leucopogon parviflorus (Andr.) Lindl. is an epacrid species with a wide distribution that includes the foredunes around much of Australia's coast. The soil in these dunes is poorly developed, with low nutrient levels. Nutrient input is almost entirely from decomposing plant material and salt spray. Under these conditions a fungal association with the nutritional characteristics of ericoid mycorrhizas would be a major advantage in successful establishment of the plant. This paper describes aspects of the ultrastructure and histochemistry of naturally occurring mycorrhizas in *L. parviflorus*, confirming that ascomycete fungi are involved. It also describes the characteristics of some of the fungi isolated from mycorrhizal roots of *L. parviflorus* and discusses their similarities with those from ericoid mycorrhizal roots.

MATERIALS AND METHODS

Collection of material

Leucopogon parviflorus plants less than 25 cm high were collected from each of three sites; Long Reef (33°45′ S 151°19′ E) (March 1993), Marley Beach (34°07′ S 151°07′ E) (October 1993), and Long Beach, Jervis Bay (35°02′ S 150°47′ E) (June 1994). Root segments bearing up to three hair roots of healthy appearance were removed and prepared for light microscopy, transmission electron microscopy and isolation of fungi.

Isolation of fungi

Fungi were isolated from infected hair roots by incubating surface-sterilized root pieces in a solution of bovine serum albumin with penicillin and streptomycin, as described by Williams (1990) for arbuscular mycorrhizal fungi. Two methods of surface sterilization were used. Half of the roots were treated with a 4% (v/v) solution of household bleach containing 4% available chlorine for 2 min (Williams, 1990) and the other half received a sequential washing treatment, modified from that of Pearson and Read (1973). This involved placing the roots in a nylon sack under running tap water for 48 h, followed by 24 h under running reverse

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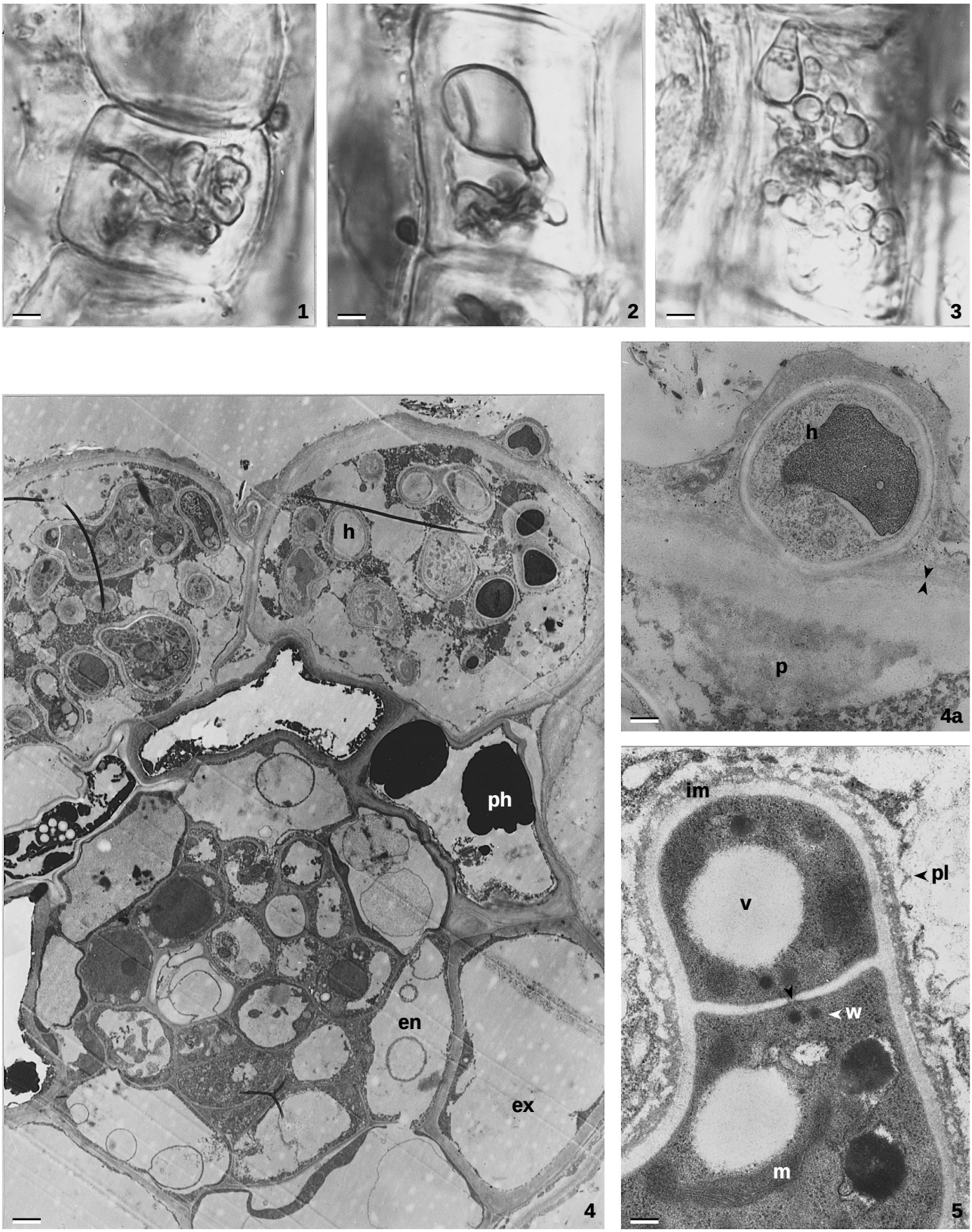

FIGS 1, 2 and 3. Fungal structures formed in the epidermal cells of *L. parviflorus* hair roots. Fig. 1. A fine, hyaline fungus forming regular coils. Fig. 2. A thick, brown pigmented fungus forming a large vesicle. Fig. 3. A fine hyaline fungus forming small vesicles. Bars = 3·5 μm.

FIG. 4. Transverse section of a mycorrhizal hair root of *L. parviflorus*. The endodermis (en) and exodermis (ex) containing electron dense deposits (ph) are extremely vacuolate. Many hyphal profiles (h) can be seen in the epidermal cells. Bar = 2 μm.

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osmosis water. All roots were then sectioned into 1–2 mm long segments and incubated according to the method of Williams (1990).

Four hundred and five root segments from two plants from Marley Beach were surface sterilized and incubated. Two hundred and twenty-six were considered to be producing actively growing hyphae. Eighty-seven of these segments were chosen randomly and transferred to modified Melin Norkans (MMN) agar (Marx, 1969), water agar (WA), or 1/4 strength potato dextrose agar (PDA) (Singleton, Mihail and Rush, 1992) and incubated in the dark at 26 °C. Fungal growth was monitored for 16 weeks at which time those cultures which appeared to be identical on a particular medium were grouped on the basis of culture characteristics. Sixteen culture types were recognized on MMN agar, six on WA and 20 on PDA. One example of each of the 42 culture types was sub-cultured on to the three agar media given above and incubated as above for 16 weeks. After 16 weeks cultures were again grouped into culture types based on the culture characteristics on the three media, and a stock culture of each type established on MMN agar. The same procedure was followed with the Long Beach material. Four hundred and seven root segments of the Long Beach material were incubated. Three hundred and nineteen segments were considered to be producing actively growing hyphae; 180 of these were transferred to agar. After 16 weeks five culture types were differentiated on MMN agar, 14 on WA and eight on PDA.

Fixation, embedding and microscopy

Branched root systems with up to three hair roots were removed and cut into segments in 0·0125 M PIPES buffer (Salema and Brandão, 1973). Segments for transmission electron microscopy were then fixed for 4 h at room temperature in 2·5% glutaraldehyde with 1% caffeine in the same buffer, and rinsed three times before post fixation in 2% OsO_4 in the same buffer for 2 h. They were then rinsed as before, dehydrated in a graded ethanol series (10% steps) over 2·5 h at room temperature and embedded in Spurr's resin (Spurr, 1969). For light microscopy, post fixation with OsO_4 was omitted and root segments were embedded in glycol methacrylate by the method of Feder and O'Brien (1968), except that the resin was polymerised by UV at room temperature. Some samples were freeze substituted. Specimens with one to two hair roots were plunge frozen in a mixture of methyl cyclohexane and isopentane (1:1 v/v) and substituted in acetone for 5 d at −70 °C. They were brought to room temperature in three 24 h stages at −20, −4 and 4 °C, then transferred via ethanol to LR White resin for infiltration and embedding.

Sections were cut on a Reichert-Jung Ultracut microtome at 90–150 nm for transmission electron microscopy, stained with uranyl acetate followed by lead citrate and viewed on an Hitachi 7000 transmission electron microscope at 100 kV. For light microscopy, sections cut at 1 μm were stained with PAS reagents, 0·5% toluidine blue in 0·1 M acetate buffer, pH 4·4, or 0·5% aniline blue (aqueous), according to O'Brien and McCully (1981) and photographed on Kodak Ektachrome 64 ISO (T) for bright field, or Kodak Elite 400 ISO film for fluorescence, on a Leitz Orthoplan Photomicroscope.

Whole unfixed roots were mounted in reverse osmosis water and photographed as above.

RESULTS

Whole *L. parviflorus* hair roots showed high levels of infection in all plants examined. Hyphal coils of the ericoid type (Fig. 1) occurred in nearly 100% of mature, fully expanded epidermal cells. Infections were confined to individual cells with single entry points. Infection of root apices occurred only in roots with no meristematic tissue evident. At least two types of fungi appeared to form mycorrhizal coils in the epidermal cells. One type was a thick-walled, brown-pigmented fungus with hyphae 1·3 μm in diameter which formed coils and large vesicles within the cell (Fig. 2). The other was a thin-walled hyaline fungus of hyphal diameter 0·9 μm, which formed tight coils and in some instances small vesicles (Fig. 3).

A total of 149 isolates were obtained from the 267 root segments transferred to agar from the Marley Beach and Long Beach isolations. Forty-nine of these were identified as *Penicillium*, *Trichoderma* or *Fusarium* species, and were discarded. The remaining 100 were sterile and had slow growth rates (between 0·1 and 1·0 mm d^{-1}). These isolates were grouped on the basis of culture characteristics into 21 different culture types which are described in Table 1. Only four culture types, CW6, CM11, HM6 and HM7, were common to both sites. The isolation technique influenced the range of culture types obtained; only three of the 21 (CP3, HM6, HM7) were isolated by both sterilization techniques. The agar medium on which the root segments were placed was clearly selective in only one instance (CM21). Of the seven culture types where there were three or more isolates only CM21 was not found on all three media. The 13 isolates of CM21 were found only on MMN agar and PDA.

The majority of isolates were dark brown or olivaceous green. Dark fungi with strong concentric banding and an irregularly dissected hyaline margin were common (HM1 to CP18 in Table 1). CP3, illustrated in Fig. 6, is an example of these culture types. Dark, slow growing green/olivaceous brown isolates with inflated submerged cells and filamentous aerial hyphae were also common. CM9 (Fig. 7) and CM11 (Fig. 8) represent the extremes in the range of olivaceous

FIG. 4a. Hypha (h) on the surface of the epidermal cell shown in Fig. 4 (Fig. 4 is the adjacent section). Note the epidermal cell wall has a two-layered structure (opposing arrows indicate outer layer) and is depressed beneath the hypha. Immediately beneath the hypha a papilla (p) has formed on the inside of the host cell wall. Bar = 400 nm.

FIG. 5. Section of part of an intracellular hypha in an epidermal cell of a *L. parviflorus* hair root. The hyphal septum has a simple pore (arrow) with associated Woronin bodies (w), characteristic of an ascomycete fungus. Several mitochondrial profiles (m), vacuoles (v) , and an interfacial matrix (im) separating the fungus from the host plasmalemma (pl) can also be seen. Bar = 300 nm.

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TABLE 1. *Culture characteristics of fungi isolated from mycorrhizal hair roots of* Leucopogon parviflorus

Culture type	Source*	Sterililization method†	No. isolates	Isolation media‡	Description of cultures on MMN after 6 weeks at 26 °C§
HM1	M	H	1	MMN	mostly submerged;fuscous black; irregularly dissected hyaline margin. Hyphae 2 μm diam, inflated cells 11 μm.
CP3	M	C,H	6	MMN PDA	mostly submerged; banded; greyish sepia or dark brick centre; fuscous black band; irregularly dissected hyaline margin; hyphal diam. 2·5 μm, inflated cells 14 μm.
CP6	M	C	17	MMN WA PDA	mostly submerged; buff to hazel with sparse aerial hyphae, irregularly dissected hyaline margin.
CM6	M	C	1	MMN	banded; iron grey centre; buff band; greenish glaucous band, hyaline margin.
CP18	M	C	1	MMN	banded; buff centre; bands of citrine green and smoke grey repeated after an olivaceous band; irregularly dissected hyaline margin.
CW6	M,L	C	2	WA	mostly submerged; buff, irregularly dissected margin; hyphal diam. 1·75 μm, inflated cells 3·5 μm.
CM11	M,L	C	5	MMN WA PDA	felty; pale olivaceous grey centre becoming olivaceous and submerged towards margin; irregularly septate; hyphal diam. 3·6 μm, inflated cells 6·6 μm.
HW35	L	H	1	WA	felty; greenish black; irregularly septate.
HM6	M,L	C,H	7	MMN WA PDA	felty; olivaceous.
HM36	L	H	1	MMN	floccose with distinct concentric ridges in aerial hyphae; grey olivaceous becoming olivaceous at margin.
CM21	L	C	13	MMN PDA	mostly submerged; black; paler tufts of aerial hyphae in centre.
CW33	L	C	2	WA	mostly submerged; greenish black; strong deformation of agar.
CM9	M	C	1	MMN	mostly submerged; olivaceous black; sparse aerial hyphae. hyphal diam. 1·1 μm, inflated cells 7·7 μm.
CW14	L	C	1	WA	felty; greenish black.
HM7	M,L	C,H	6	MMN WA PDA	velvety; fuscous black; forms chains of sclerotia. hyphal diam. 1 μm, inflated cells 1·7 μm.
HP46	L	H	1	PDA	banded olivaceous grey/white centre becoming brown vinaceous towards margin.
HP29	L	H	1	PDA	sepia colony becoming honey towards margin; large, irregular, hyphal cells (11·5 μm). Regular hyphae 2 μm diam.
HM13	M	H	2	MMN	as for HP29; produces gold pigment in agar.
HP4	M	H	1	MMN	buff, irregular yeast like cells. 2·2–10·5 μm diam.
CP14	M	C	1	PDA	mostly submerged with powdery surface; buff.
HP28	L	H	29	MMN WA PDA	submerged with waxy surface; buff.

* Source: plants collected from: M, Marley Beach; L, Long Beach.
† Sterilisation method: C, roots treated with 4% bleach for 2 min; H, roots washed in running water for 72 h.
‡ Culture type was isolated on: MMN, modified Melin Norkrans agar; WA, water agar; PDA, $\frac{1}{4}$ strength potato dextrose agar.
§ Colours refer to the colour chart in Rayner (1970).

culture types (CM11 to CW14) described in Table 1. Cultures CM21, CW33 and CM9 were similar in gross characteristics to the *Oidiodendron* species which form ericoid mycorrhizal structures (e.g. Currah, Tsuneda and Murakami, 1993), but did not produce conidia on MMN. The inflated cells in some isolates may be arthrospores. CM11 and HW35 were olivaceous and felty, and were similar to the ericoid endophyte *H. ericae* in these characters, but lacked the hyaline margin characteristic of this fungus. Although CM11 was clearly distinct from CM9 some of the intermediate culture types distinguished (HW35, HM36, HM6, CM21, CW33) may prove to be genetically very similar. Loss of pigmentation occurred following sub-culturing in some isolates. This indicates that colour is not a reliable culture characteristic. For this reason the number of culture types distinguished in Table 1 may be an overestimate.

The basic anatomy of the hair roots of *L. parviflorus* was similar to that described in *Lysinema ciliatum* R.Br. by Allaway and Ashford (1996). Roots had a small stele with profiles of one to two xylem elements surrounded by an endodermis, exodermis and epidermis. The endodermis and exodermis became highly vacuolate close behind the apex (Fig. 4). The vacuoles stained strongly blue with toluidine blue (Fig. 9), light pink following the PAS reaction (Fig. 10), and the material they contained was electron-opaque (Fig. 4). Taken together these observations indicate that the vacuoles have accumulated phenolic compounds.

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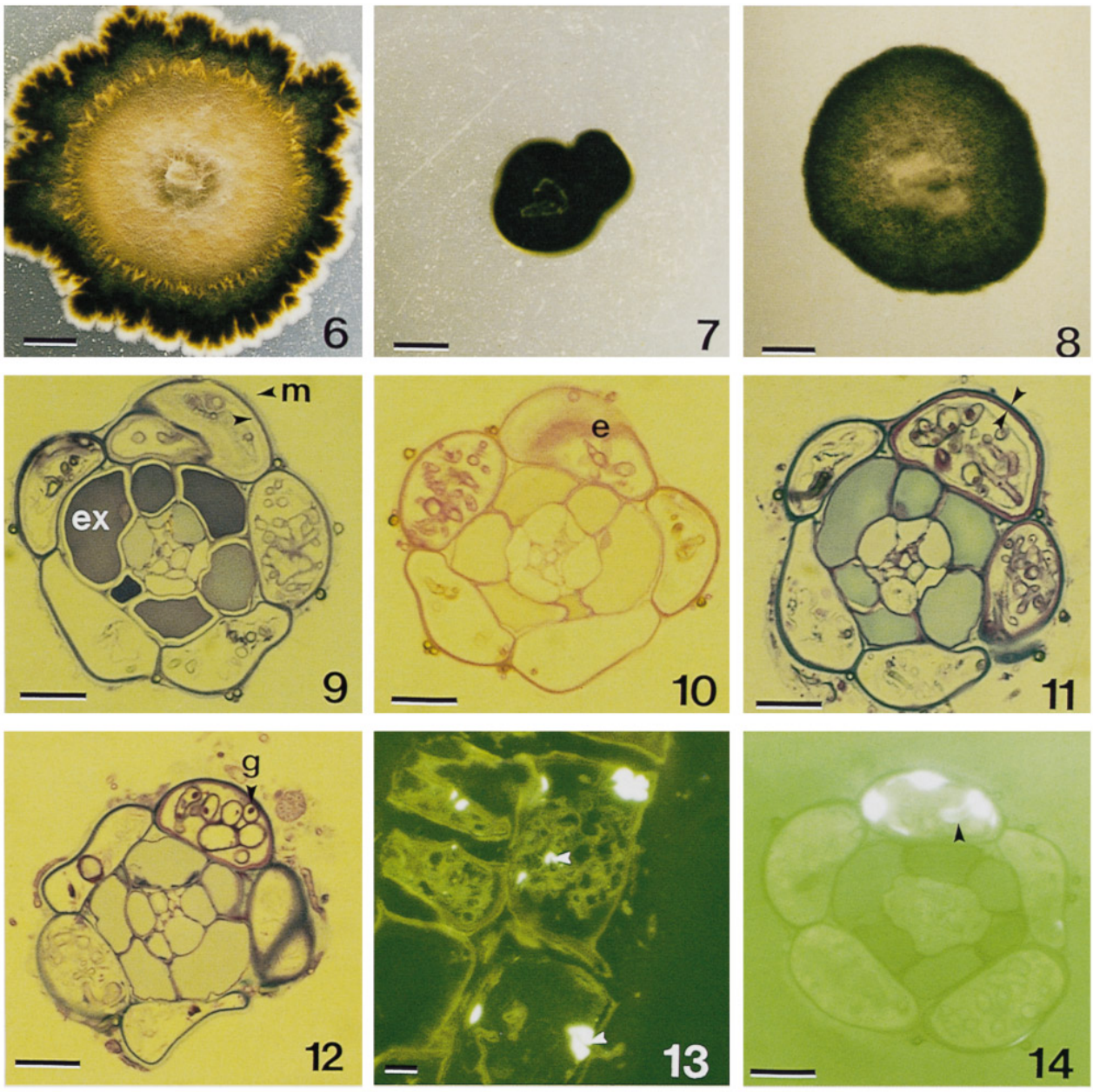

FIGS 6, 7 and 8. Cultures of fungi isolated from mycorrhizal hair roots of *L. parviflorus*, grown for 6 weeks on modified Melin Norkrans agar. Fig. 6. Culture type CP3. Fig. 7. Culture type CM9. Fig. 8. Culture type CM11. Bars = 8 mm.

FIGS 9–12. Transverse sections through the same region of a chemically fixed hair root of *L. parviflorus*. Fig. 9. Stained with toluidine blue. Hyphal profiles are present within and on the surface of most epidermal cells. A lightly staining mucilage layer (m) overlies the epidermal cells. Several epidermal cells have a two-layered cell wall: the thick inner wall stains pale purple (arrow), the outer wall dark purple. The exodermis (ex) and endodermis have large central vacuoles that stain strongly blue indicating phenolic compounds are present. Fig. 10. Stained with PAS reagents; the outer wall layer of the epidermal cells (e) is strongly PAS positive; hyphal wall profiles within the epidermal cells are also strongly stained. Figs 11 and 12. Stained with PAS reagents and counterstained with toluidine blue. The two layers of some epidermal cell walls (arrows) stain differentially. Granules (g) within the hyphae can be seen. Bars = 10 μm.

FIG. 13. Longitudinal section of a freeze-substituted mycorrhizal hair root of *L. parviflorus* stained with aniline blue. Fluorescent material is visible around infecting hyphae and adjacent to hyphal profiles within the cell (arrows). Bar = 10 μm.

FIG. 14. Transverse section of a chemically fixed hair root of *L. parviflorus* stained with aniline blue. Fluorescence is present in one of the infected cells, where a sheath of fluorescent material surrounds the infecting hypha (arrow). Other infected cells do not react. Bar = 10 μm.

Collapsed epidermal cells (Fig. 12) were common even in young roots. Intact epidermal cells were highly vacuolate and had a cell wall that was characteristically two-layered. This was best shown in cells containing dense hyphal coils. In PAS-stained sections counterstained with toluidine blue a thin outer layer stained dark blue and a (wider) inner layer

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a strong magenta (Figs 11 and 12). An inner layer was also detected in uninfected cells and cells with less distinct coils, but it was very thin and stained lightly. With toluidine blue alone the outer layer stained blue and the inner pale pink, while with PAS the outer layer stained an orange-pink and the inner magenta (Fig. 10). In electron micrographs two cell wall layers were evident in all epidermal cells, the outer layer being the narrower and more electron opaque (Fig. 4a). A thin layer of mucilage containing bacteria, fungi and debris surrounded most roots (Figs 9, 11 and 12). This mucilage stained pale blue with toluidine blue. An uneven, often discontinuous and variably stained layer of material overlying the wall surface in electron micrographs was interpreted as remnants of mucilage (Fig. 4).

Both elongate and large rounded hyphal profiles were seen in densely infected cells. These profiles were a strong magenta in sections stained with PAS counterstained with toluidine blue (Figs 11 and 12). Hyphae in less densely infected cells stained only faintly. In electron micrographs sections of healthy endogenous hyphae contained dense cytoplasm and many mitochondrial profiles (Figs 4 and 5). Septal pores were simple and were flanked by Woronin bodies (Fig. 5). An electron opaque interfacial matrix was present between the hyphae and the host plasmalemma (Fig. 5). The host cell cytoplasm was concentrated around the hyphae and contained large numbers of organelles (Fig. 4).

External hyphae were closely appressed to epidermal cell walls and surrounded by a layer of material continuous with mucilage overlying the epidermal cell wall. In Fig. 4a a disruption in the continuity of the outer cell wall layer beneath the hypha suggested that digestion of the cell wall had occurred. On the inner surface of the host wall beneath the surface hypha there was a papilla (Fig. 4a) which had somewhat greater electron opacity than the inner wall. Papillae were commonly observed, although they were not present beneath all surface hyphae.

In sections from both chemically fixed and freeze-substituted hair roots, a tubular structure which fluoresced when stained with aniline blue was observed around many penetrating hyphae (Figs 13 and 14). Fluorescent material was also evident around some coils, although many infected cells showed no fluorescence with aniline blue. The fluorescence was not seen in high pH controls, indicating that callose may be present.

DISCUSSION

Hair roots in *L. parviflorus* are structurally similar to those found in other members of the Epacridaceae (Allaway and Ashford, 1996), and in the Ericaceae (Read, 1983). The small stele is surrounded by a two-layered cortex, consisting of an endodermis and exodermis, both containing phenolics, and an epidermis of large, highly vacuolate cells in which mycorrhizal coils occur. The infected epidermal cells have a partially thickened wall that is similar to the thick wall in *Lysinema ciliatum* in the early stages of its development (Ashford, Allaway and Reed, 1996)

The endophytes in *L. parviflorus* roots show intracellular coiling typical of ericoid mycorrhizas. The fungi invade individual epidermal cells, the host plasmalemma is invaginated around the hyphae, and an electron-opaque interfacial matrix occurs as in typical ericoid mycorrhizas (Bonfante-Fasolo and Gianinazzi-Pearson, 1979; Duddridge and Read, 1982; Bonfante-Fasolo and Perotto, 1988). Based on the frequent occurrence of simple septa with pores and associated Woronin bodies the *L. parviflorus* endophytes all appear to be ascomycetes, as are the majority of fungi observed in naturally occurring ericoid mycorrhizas (e.g. Bonfante-Fasolo and Gianinazzi-Pearson, 1979; Duddridge and Read, 1982).

The presence of thin hyaline hyphae in some cells and thick darkly pigmented hyphae in other cells suggest that more than one kind of fungal endophyte colonizes roots of *L. parviflorus*. Similar hyphal forms have been described in *Pernettya macrostigma* Col., a New Zealand member of the Ericaceae (Brook, 1952; McNabb, 1961). The two hyphal forms were interpreted by these authors to represent different developmental stages of the same fungus. In *L. parviflorus* the differences in size and pigmentation of the hyphae show no relationship to the extent of infection in a cell, and are unlikely to be accounted for by differences in developmental stage of the same infection. Our findings agree more with those of Burgeff (1961) for the Ericaceae, where two different types of coils were produced in aseptic culture by a fine hyaline fungus and a broad dark fungus.

Some histochemical evidence suggests that there is a variation in host response to infection in *L. parviflorus*. The formation of callose around only some infection pegs and hyphal coils may represent a response to different endophytes. Callose-containing papillae and plugs around infection pegs are characteristic of a resistant response to attempted fungal invasion (Sherwood and Vance, 1976; Hinch and Clarke, 1982; Currah *et al*., 1993), and their formation in *L. parviflorus* mycorrhizas may indicate partial incompatibility in some associations. Similar structures around some infection pegs have been reported in ericoid mycorrhizas (Brook, 1952; Douglas, Heslin and Read, 1989). A detailed description of their appearance in *Vaccinium myrtillus* is given by Burgeff (1961), who described them as cellulose sheaths that occurred only in older cells infected by a large robust fungus which was distinct from the fine hyphae of the fungus infecting younger cells.

Without synthesis trials it is not possible to know how many of the *L. parviflorus* isolates are mycorrhizal endophytes. It is likely that synthesis experiments will prove a number of the isolates to be mycorrhizal. This assumption is based primarily on the successful synthesis of mycorrhizas in ericaceous plants using fungi isolated by almost identical methods. For example, of 83 isolates obtained from *Gaultheria shallon* Pursh (Ericaceae) 24 were shown to be mycorrhizal in synthesis experiments (Xiao and Berch, 1992). A second factor suggesting that some of the *L. parviflorus* isolates will prove to be mycorrhizal is their morphological similarity to the known ericoid mycorrhizal species *H. ericae* (Read, 1974) and *Oidiodendron* spp, (Couture, Fortin and Dalpé, 1983; Stoyke and Currah, 1991; Xiao and Berch, 1992). The morphology of mycorrhiza forming isolates of *H. ericae* from a variety of European habitats was very consistent (Perotto *et al*., 1990).

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However, the relationship between the European *H. ericae* isolates and morphologically similar isolates from the Southern hemisphere is not clear. Pectic zymogram analysis by Hutton, Dixon and Sivasithamparam (1994) showed no relationship between European isolates of *H. ericae* and Western Australian epacrid isolates of similar appearance. Polymerase chain reaction analysis of these isolates might be of value in clarifying these relationships.

On the basis of the variation in the gross appearance of the hyphal coils in *L. parviflorus* and what appears to be partial incompatibility in some infected cells, it is proposed that more than one fungal endophyte form mycorrhizas in these roots. The ultrastructure of septa in the mycorrhizas examined suggests that most, if not all, of the endophytes in *L. parviflorus* are ascomycetes. The cultural characteristics of fungi isolated from *L. parviflorus* mycorrhizas are sufficiently similar to those of ericoid mycorrhizal fungi to suggest a close relationship between the ericoid and epacrid endophytes. Comparison of the ability of epacrid and ericoid mycorrhizal isolates to form mycorrhizas with *L. parviflorus*, and a molecular analysis of the fungi involved, would help to clarify the relatedness of these endophytes and to demonstrate any functional relevance of morphological or genetic similarity.

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LITERATURE CITED

Allaway WG, Ashford AE. 1996. Structure of hair roots in *Lysinema ciliatum* R. Br. and its implications for their water relations. *Annals of Botany* **77**: 383–388.

Allen WK, Allaway WG, Cox GC, Valder PG. 1989. Ultrastructure of mycorrhizas of *Dracophyllum secundum* R. Br. (Ericales: Epacridaceae). *Australian Journal of Plant Physiology* **16**: 147–153.

Ashford AE, Allaway WG, Reed ML. 1996. A possible role for the thick-walled epidermal cells in the mycorrhizal hair roots of *Lysinema ciliatum* R. Br. and other Epacridaceae. *Annals of Botany* **77**: 375–381.

Bajwa R, Read DJ. 1986. Utilization of mineral and amino N sources by the ericoid mycorrhizal endophtye *Hymenoscyphus ericae* and by mycorrhizal and non-mycorrhizal seedlings of *Vaccinium*. *Transactions of the British Mycological Society* **87**: 269–277.

Bonfante-Fasolo P, Gianinazzi-Pearson V. 1979. Ultrastructural aspects of endomycorrhiza in the Ericaceae. I. Naturally infected hair roots of *Calluna vulgaris* L. Hull. *New Phytologist* **83**: 739–744.

Bonfante-Fasolo P, Perotto S. 1988. Ericoid mycorrhizae: new insights from ultrastructure allied to cytochemistry. In: Ghiara G, ed. *Cell interactions and differentiation*. Naples: University of Naples, 27–38.

Brook PJ. 1952. Mycorrhiza of *Pernettya macrostigma*. *New Phytologist* **51**: 388–397.

Burgeff H. 1961. *Mikrobiologie des Hochmoores mit besonderer berücksichtigung der Erikatzeen-Pilz-Symbiose*. Stuttgart: Fischer Verlag.

Couture M, Fortin JA, Dalpé Y. 1983. *Oidiodendron griseum* Robak: an endophyte of ericoid mycorrhiza in *Vaccinium* spp. *New Phytologist* **95**: 375–380.

Currah RS, Tsuneda A, Murakami S. 1993. Conidiogenesis in *Oidiodendon periconioides* and ultrastructure of ericoid mycorrhizas formed with *Rhododendron brachycarpum*. *Canadian Journal of Botany* **71**: 1481–1485.

Dalpé Y. 1989. Ericoid mycorrhizal fungi in the Myxotrichaceae and Gymnoascaceae. *New Phytologist* **113**: 523–527.

Douglas GC, Heslin MC, Reid C. 1989. Isolation of *Oidiodendron maius* from *Rhododendron* and ultrastructural characterization of synthesised mycorrhizas. *Canadian Journal of Botany* **67**: 2206–2212.

Duddridge J, Read DJ. 1982. An ultrastructural analysis of development of mycorrhizas in *Rhododendron ponticum*. *Canadian Journal of Botany* **60**: 2345–2356.

Feder N, O'Brien TP. 1968. Plant microtechnique: some principles and new methods. *American Journal of Botany* **55**: 123–142.

Hinch JM, Clarke AE. 1982. Callose formation in *Zea mays* as a response to infection with *Phytophthora cinnamomi*. *Physiological Plant Pathology* **21**: 113–124.

Hutton BJ, Dixon KW, Sivasithamparam K. 1994. Ericoid endophytes of Western Australian heaths (Epacridaceae). *New Phytologist* **127**: 557–566.

Leake JR, Shaw G, Read DJ. 1990. The biology of mycorrhiza in the Ericaceae. XVI. Mycorrhiza and iron uptake in *Calluna vulgaris* (L.) Hull in the presence of two calcium salts. *New Phytologist* **114**: 651–657.

McNabb RFC. 1961. Mycorrhiza in the New Zealand Ericales. *Australian Journal of Botany* **9**: 57–61.

Marx DH. 1969. The influence of cytotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. *Phytopathology* **59**: 159–163.

O'Brien TP, McCully ME. 1981. *The study of plant structure: principles and selected methods*. Melbourne: Termarcarphi Pty. Ltd.

Pearson V, Read DJ. 1973. The biology of mycorrhiza in the Ericaceae. I. The isolation of the endophyte and the synthesis of mycorrhizas in aseptic culture. *New Phytologist* **72**: 371–383.

Perotto S, Peretto R, More D, Bonfante-Fasolo P. 1990. Ericoid fungal strains from an alpine zone: their cytological and cell surface characteristics. *Symbiosis* **9**: 167–172.

Rayner RW. 1970. *A mycological colour chart*. Kew, Surrey: Commonwealth Mycological Institute.

Read DJ. 1974. *Pezizella ericae* sp. nov., the perfect state of a typical mycorrhizal endophyte of the Ericaceae. *Transactions of the British Mycological Society* **63**: 381–383.

Read DJ. 1983. The biology of mycorrhiza in the Ericales. *Canadian Journal of Botany* **61**: 985–1004.

Read DJ. 1991. Mycorrhizas in ecosystems. *Experientia* **47**: 376–391.

Salema R, Brandão I. 1973. The use of PIPES buffer in the fixation of plant cells for electron microscopy. *Submicroscopic Cytology* **5**: 79–96.

Singleton LL, Mihail JD, Rush CM. 1992. *Methods for research on soilborne phytopathogenic fungi*. St Paul: APS Press.

Spurr AR. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructural Research* **26**: 31–43.

Sherwood RT, Vance CP. 1976. Histochemistry of papillae formed in reed canarygrass leaves in response to non infecting pathogenic fungi. *Phytopathology* **66**: 503–510.

Stoyke G, Currah RS. 1991. Endophytic fungi from the mycorrhizae of alpine ericoid plants. *Canadian Journal of Botany* **69**: 347–352.

Williams PG. 1990. Disinfecting vesicular-arbuscular mycorrhizas. *Mycological Research* **94**: 995–997.

Xiao G, Berch SM. 1992. Ericoid mycorrhizal fungi of *Gaultheria shallon*. *Mycologia* **84**: 470–471.

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