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Structure and histochemistry of mycorrhizae synthesized between *Arbutus menziesii* (Ericaceae) and two basidiomycetes, *Pisolithus tinctorius* (Pisolithaceae) and *Piloderma bicolor* (Corticiaceae)

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Abstract. Arbutoid mycorrhizae were synthesized in growth pouches between *Arbutus menziesii* Pursch. (Pacific madrone) and two broad host range basidiomycete fungi, *Pisolithus tinctorius* (Pers.) Coker and Couch and *Piloderma bicolor* (Peck) Jülich. *P. tinctorius* induced the formation of dense, pinnate mycorrhizal root clusters enveloped by a thick fungal mantle. *P. bicolor* mycorrhizae were usually unbranched, and had a thin or non-existent mantle. Both associations had the well-developed para-epidermal Hartig nets and intracellular penetration of host epidermal cells by hyphae typical of arbutoid interactions. *A. menziesii* roots developed a suberized exodermis which acted as a barrier to cortical cell penetration by the fungi. Ultrastructurally, the suberin appeared non-lamellar, but this may have been due to the imbedding resin. Histochemical analyses indicated that phenolic substances present in epidermal cells may be an important factor in mycorrhiza establishment. Analyses with X-ray energy dispersive spectroscopy showed that some of the granular inclusions present in fungal hyphae of the mantle and Hartig net were polyphosphate. Other inclusions were either protein or polysaccharides.

Key words: Arbutoid mycorrhizae - *Arbutus* - *Piloderma* - *Pisolithus* - Exodermis

Introduction

Species of *Arbutus* (Ericaceae) form mycorrhizae with a broad range of fungal partners (Molina and Trappe 1982; Acsai and Largent 1983a, b). Together with species of *Arctostaphylos* and *Pyrola*, this mycorrhizal interaction has been classified as arbutoid and is characterized by a Hartig net and intracellular penetration, both confined to the epidermis, and the presence of a variable fungal sheath (Harley and Smith 1983; Fusconi and Bonfante-Fasolo 1984). Many of the fungi involved in arbutoid relationships form ectomycorrhizae in association with other hosts (Molina and Trappe 1982; Heslin 1986).

Detailed structural studies are only available for mycorrhizae of *Arbutus unedo* L. (Rivett 1924; Fusconi and Bonfante-Fasolo 1984; Giovannetti and Lioi 1990). Rivett's (1924) pioneering study described the arbutoid clusters as "root tubercles" and discussed the colonization of roots in relation to root anatomy. Fusconi and Bonfante-Fasolo (1984) described mycorrhizae formed between *A. unedo* and an unknown ascomycete symbiont and found a typical Hartig net, intracellular colonization, and a thin fungal mantle. Similarly, Giovannetti and Lioi (1990) described mycorrhizae that developed between *A. unedo* and unknown fungi when grown in field soil in the greenhouse. Münzenberger (1991) described the anatomy and ultrastructure of synthesized *A. unedo* - *Laccaria amethystea* arbutoid mycorrhizae. She provided evidence for a labyrinthic Hartig net as well as lamellar suberin deposition in the hypodermal cells of the host. Except for the accounts of the mycorrhizae formed in pure culture (Zak 1974, 1976a, b; Molina and Trappe 1982), no similar detailed anatomical study is available for *Arbutus menziesii*.

Root cell wall composition is an important factor that may limit fungal colonization in mycorrhizal roots (Nylund 1987), but critical studies of cell wall composition are lacking. The objectives of this study were to document and compare the structural features of mycorrhizae formed in growth pouches between *A. menziesii* and the ectomycorrhizal fungi *Pisolithus tinctorius* and *Piloderma bicolor*, and to investigate the nature of cortical and epidermal cell walls in relation to root colonization.

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Materials and methods

Plant material and mycorrhizal synthesis

A. menziesii seeds (seed zone 252, Drain, Ore.) were surface-sterilized with 30% H_2O_2 for 20 min, and placed on sterile wet filter paper at 4° C in petri plates for 22 days, and then brought to room temperature for germination. Three weeks later, small germinating seedlings were transferred into growth pouches containing 10 ml of sterile distilled water. Sixty days later, seedlings with a root system 10–15 cm in length with short laterals were inoculated with mycelial plugs of *P. tinctorius* (isolate 76-1, see Grenville et al. 1985) or *P. bicolor* (isolate CBS 292.77) placed 5–10 mm from the growing laterals. The mycobionts were grown and introduced into the pouches as described previously (Massicotte et al. 1986).

Growth conditions

Seedlings were grown in a growth room under 70 μmol m^{-2} s^{-1} light on a 16:8 h light:dark cycle at 20:18° C day:night temperatures. Moderate levels of humidity (40–50%) were maintained by a humidifier. Seedlings received 5 ml of half-strength modified Melin-Norkrans nutrient solution (Marx and Bryan 1975) every 2 weeks and were watered as needed with sterile distilled water for the duration of the experiment.

Hand sectioning of fresh material

Roots from growth pouches and mycorrhizal roots from 2-year-old *A. menziesii* seedlings grown in a greenhouse in field soil were hand-sectioned by sandwiching them between small pieces of styrofoam and sectioning with a two-sided razor blade.

External morphology and light microscopy

The external morphology of roots and mycorrhizae was examined with a Zeiss DR photodissecting microscope at intervals of 2–3 days after inoculation. Samples of roots inoculated with *P. tinctorius* were collected 12 weeks after inoculation and samples of roots inoculated with *P. bicolor* 18 weeks after inoculation. Samples were fixed in 2.5% glutaraldehyde in 0.10 M HEPES buffer, pH 6.8 at ambient temperature for 4 h, rinsed in buffer several times, dehydrated in a graded series of ethanol and embedded in gelatin capsules using LR White Resin (London Resin Company Ltd.) after dehydration. Thick sections (1–1.5 μm) were cut with glass knives on a MT-1 microtome, stained for light microscopy (LM) with 1% (w/v) methylene blue/1% (w/v) azure B/1% (w/v) sodium tetraborate in distilled water, rinsed in distilled water, counterstained in a 0.5% (w/v) solution of basic fuchsin in distilled water, rinsed in distilled water, air dried, mounted in immersion oil and viewed on a Leitz photomicroscope. Twenty-five roots at various stages of development were examined for each fungus species.

Transmission electron microscopy and energy dispersive spectroscopy

Thin sections (150 nm) of samples fixed and embedded for LM were collected on formvar-coated copper grids and stained for 10 min with 2% ethanolic uranyl acetate and then counter-stained for 5 min with Reynold's lead citrate. Sections were examined on a JEOL 100CX scanning transmission electron microscope at 60 kV. For energy dispersive spectroscopy (EDS) microanalysis, unstained sections coated with carbon were examined with the same microscope equipped with a horizontal entry energy dispersive spectrometer interfaced to a Tracor Northern TN 5500 microanalyser. X-ray microanalyses were performed at the eucentric point in the microscope stage at a take off angle of 30° under standardized lens conditions for 100 s with a static spot.

Scanning electron microscopy

Ectomycorrhizal roots and clusters were fixed as for LM, and then post-fixed with 2% OsO_4 on ice for 2 h, followed by a treatment with thiocarbohydrazide and a subsequent post-fixation with OsO_4 (Kelley et al. 1973). Specimens were then washed in distilled water, dehydrated in a graded series of ethanol, critical-point dried, mounted on aluminum stubs, and observed with a JEOL JSM-35C scanning electron microscope. Twenty ectomycorrhizae of *P. tinctorius* were examined.

Fluorescence microscopy

Hand sections of fresh roots were examined, either unstained or after staining with berberine-aniline blue-ferric chloride (Brundrett et al. 1988) and were viewed on a Leitz epifluorescence microscope using UV (340–380 nm excitation wavelength) light. Resin-embedded material was stained with either calcofluor (O'Brien and McCully 1981) or acriflavine-HCl (Culling 1974) and viewed under UV and blue light (350–460 excitation wavelength), respectively.

Histochemistry

Phenolic compounds. Hand sections of roots were stained with either 0.01% toluidine blue in 0.1 M acetate buffer, pH 4.4, saturated vanillin in concentrated HCl, or the Hoepfner-Vorsatz reagent, all according to the procedures in Ling-Lee et al. (1977).

Proteins. Sections of LR White-embedded roots were stained with either 1% aniline blue black in 7% acetic acid or 0.25% Coomassie brilliant blue in 7% acetic acid for 10 min at 50° C (O'Brien and McCully 1981).

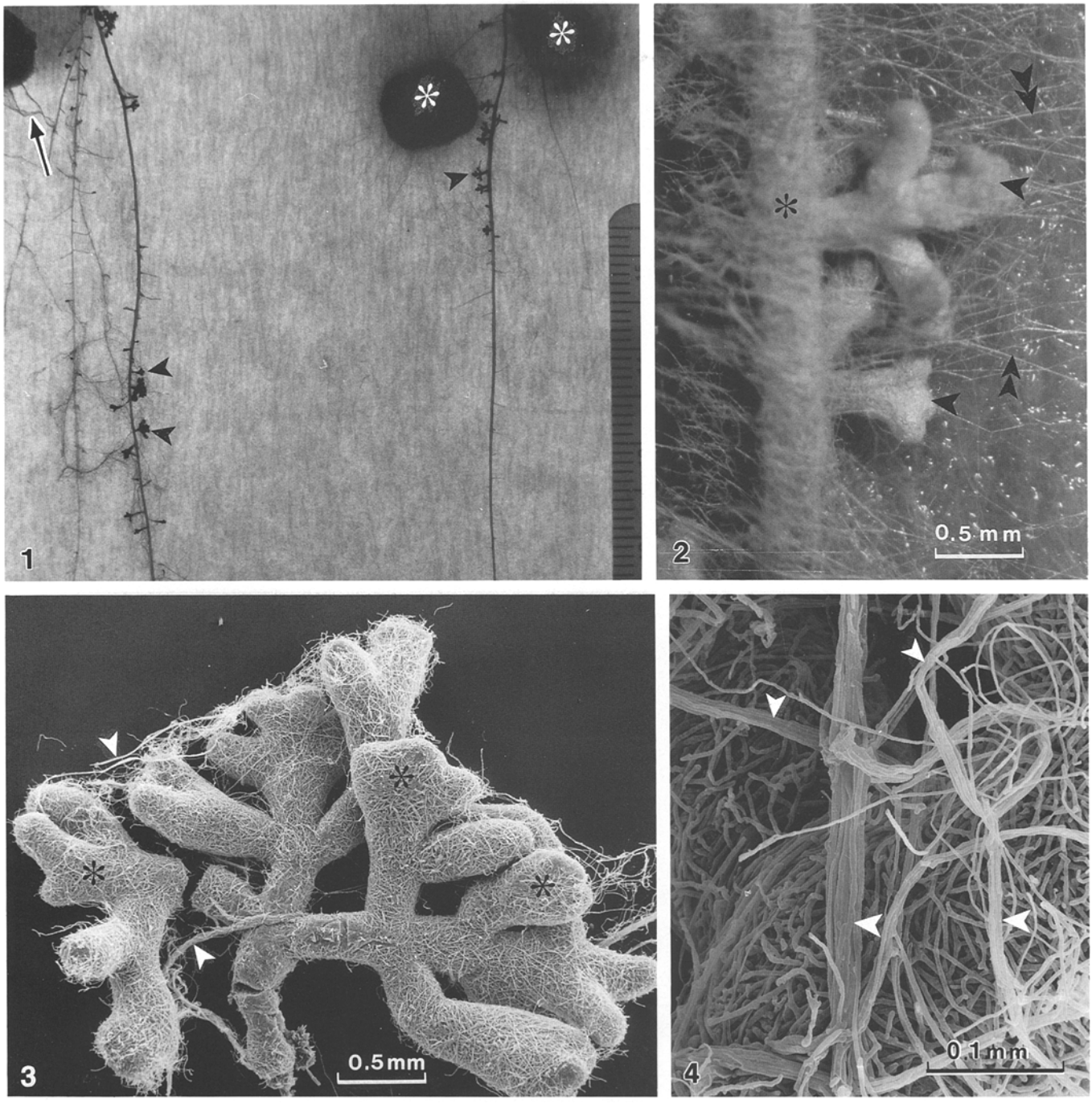
Polyphosphate. Sections of LR White-embedded roots were stained with 0.05% toluidine blue O adjusted to pH 1.0 with 1 N HCl (Ashford et al. 1975).

Results

Morphology

A. menziesii grew well in pouches, albeit very slowly, and produced a root system with many laterals (Fig. 1). Mycorrhizal apices formed with both fungal species within 10 days of inoculation, although *P. tinctorius* began to form a mantle within 4 days. Small clusters of mycorrhizae proliferated on second-order laterals (Fig. 2) 15–21 days after inoculation with *P. tinctorius*. Seedlings inoculated with *P. bicolor* did not show this branching pattern. New mycorrhizae developed in small numbers up to 6 months after inoculation with either fungus.

P. tinctorius mycorrhizae had well-developed, pale yellow to yellow-brown mantles covering first- and second-order lateral roots (Fig. 3). Hyphal strands were

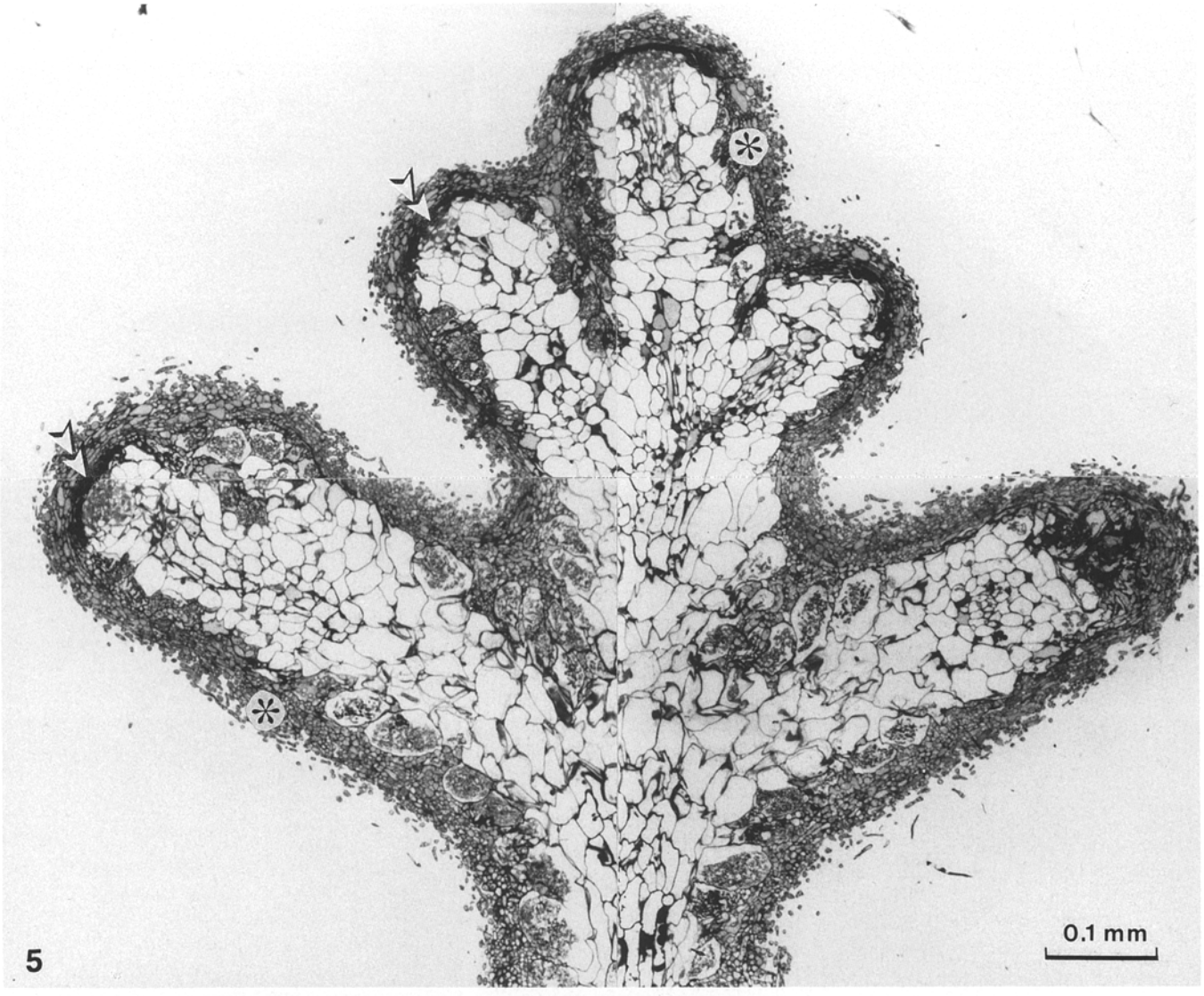


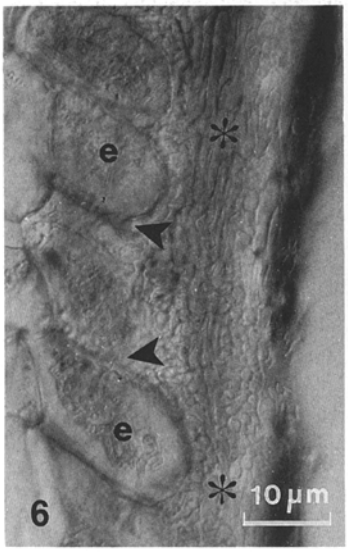
Figs. 1–4. *Arbutus menziesii – Pisolithus tinctorius* mycorrhizae. **Fig. 1.** Portion of root systems of two seedlings in a growth pouch showing mycorrhizal roots at different stages of development (*arrowheads*), plugs of mycelium (*), and hyphal strands (*arrow*). Scale is in millimeters. **Fig. 2.** Higher magnification of mycorrhizal clusters of lateral roots with well-developed mantle (*arrowheads*) attached to the primary root (*). Numerous extraradical hyphae are present (*double arrowheads*). **Fig. 3.** Scanning electron micrograph (SEM) of root cluster showing three-dimensional pinnate branching. Each lateral is covered by a mantle (*) of intermingling hyphae. Hyphal strands (*arrowheads*) are present. **Fig. 4.** SEM of a group of hyphal strands of varying diameter (*arrowheads*). Portions of the fungal mantle are present in the background

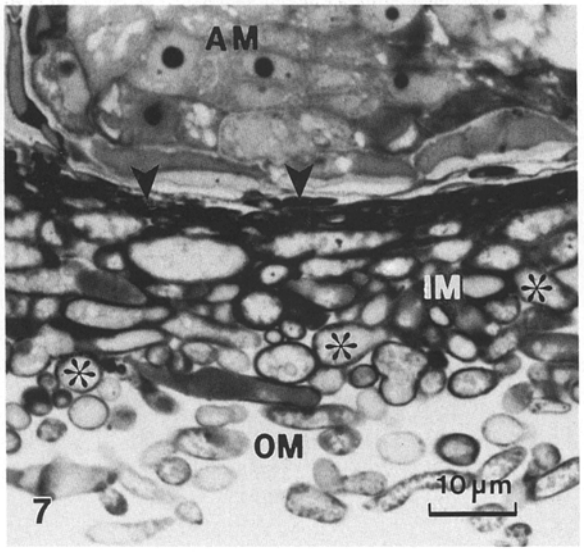
prevalent 3–5 weeks after inoculation (Fig. 4), and occasionally linked two adjacent seedlings. *P. bicolor* produced single, unbranched mycorrhizae with a thin mantle comprised of bright yellow hyphae embedded in a mucilaginous matrix.

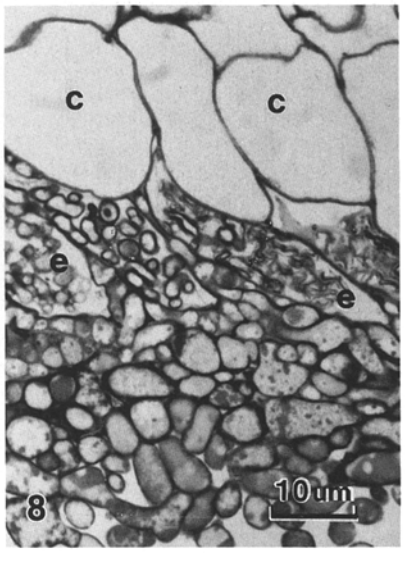
Light microscopy

Arbutus – P. tinctorius. Hand sections of fresh mycorrhizal roots showed a mantle comprised of several layers of fungal hyphae, a para-epidermal Hartig net and epidermal intracellular hyphae (Fig. 6). Sections through

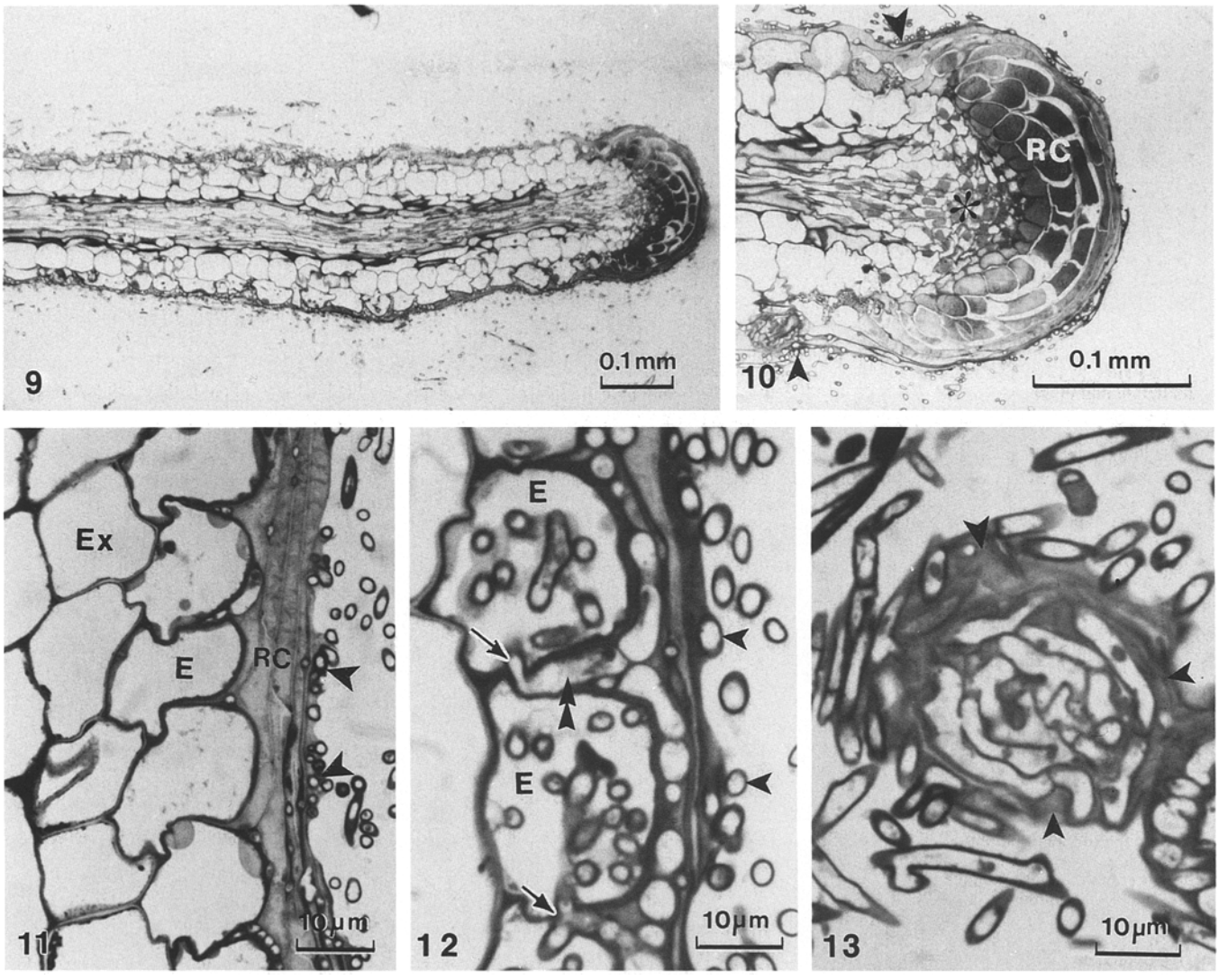








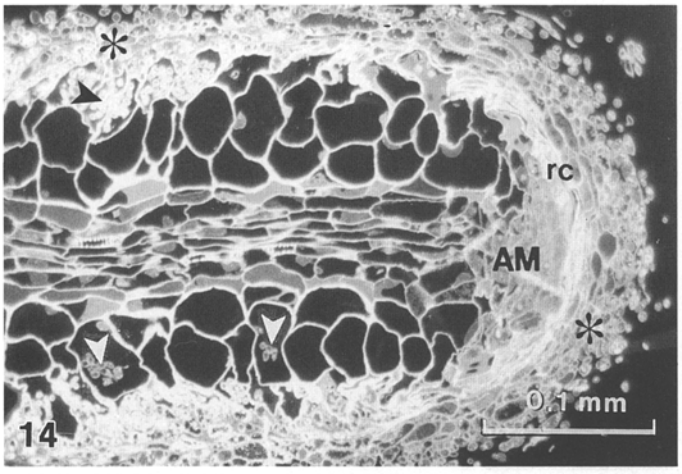
Figs. 5–8. Light microscopy of *A. menziesii* – *P. tinctorius* mycorrhizae. **Fig. 5.** Longitudinal section showing tuberculate-like root branching, somewhat flattened apices (*double arrowheads*) and thick mantle (*). Many epidermal cells have intracellular hyphae. **Fig. 6.** Hand section of mycorrhiza showing well-developed mantle (*), Hartig net (*arrowheads*) and intracellular hyphae within epidermal cells (*e*). **Fig. 7.** Enlargement of a sectioned mycorrhiza apex showing apical meristem cells (*AM*), necrotic root cap cells (*arrowheads*) and mantle hyphae (*). Outer mantle hyphae (*OM*) are more loosely organized than inner mantle (*IM*) hyphae. **Fig. 8.** Section of mycorrhiza taken proximal to the apical meristem. Note the variable shape and cytoplasmic inclusions of hyphae. Epidermal cells (*e*) containing intracellular hyphae are somewhat distorted. Intracellular hyphae in the epidermal cells on the right have degenerated. *c*, Cortical cells



Figs. 9–13. Light microscopy of *A. menziesii* – *P. bicolor* mycorrhizae. **Fig. 9.** Median longitudinal section of mycorrhizal lateral root. **Fig. 10.** Higher magnification of apical portion. The small apical meristem (*), layers of root cap cells (*RC*) and limited mantle development (*arrowheads*) are evident. **Fig. 11.** Portion of root prior to epidermal cell penetration. A few hyphae are found within mucilage (*arrowheads*) and adjacent to and within collapsed root cap (*RC*) cells. The radial walls of exodermal cells are more lightly stained than radial walls of epidermal cells. *E*, Epidermis; *Ex*, exodermis. **Fig. 12.** Region of root proximal to that shown in Fig. 11. A few mantle hyphae (*arrowheads*), Hartig net hyphae (*double arrowheads*) and intracellular hyphae within epidermal cells (*E*) are evident. Note the hyphal peg penetration of the radial epidermal cell wall (*arrows*). **Fig. 13.** A glancing tangential section of a group of surface hyphae within the mucilagenous layer above the epidermis (*arrowheads*). The surface hyphae show jigsaw puzzle-like configurations. Extraradical hyphae are mainly tubular in shape

mycorrhizal clusters showed tuberculate-like root growth, a continuous mantle enveloping each cluster, flattened apices of individual lateral roots, and vacuolation of root cells (Fig. 5). The apical meristems of lateral roots were small, and root cap cells were collapsed (Figs. 5, 7). Inner mantle hyphae were more compactly arranged than outer mantle hyphae and inclusions of varying sizes were present (Figs. 7, 8). Intracellular hyphae, some of which had degenerated, were confined to the radially enlarged epidermal cells which showed some distortion (Fig. 8).

Arbutus – *P. bicolor*. First-order lateral roots were elongated, unbranched and had a rounded apex with several layers of root cap cells (Figs. 9, 10). Few mantle hyphae were present at the root apex and along the root axis (Figs. 9, 10). Several layers of collapsed root cap cells and mucilage persisted along the root surface (Figs. 10, 11). Proximal to the layers of root cap cells, fungal hyphae contacted the epidermis (approx. 1–2 mm from the root apex) forming a thin mantle, a para-epidermal Hartig net and intracellular hyphae within epidermal cells (Fig. 12). Lateral hyphal pegs, which give rise to intracellular hyphae, were present in the radial walls of the epidermis (Fig. 12). Surface hyphae were embedded in mucilage and showed a highly modified pattern of growth (Fig. 13).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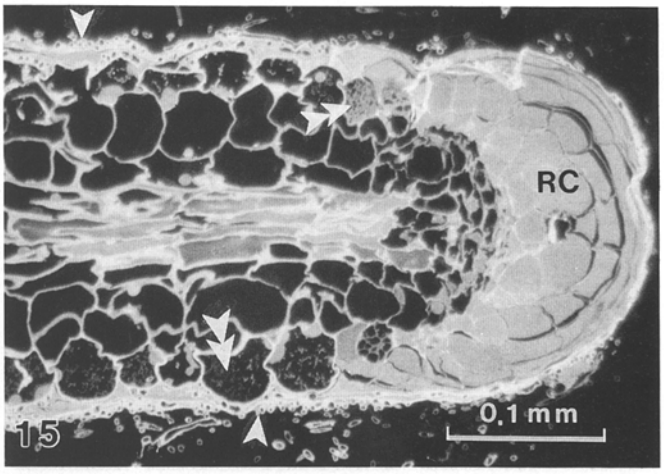
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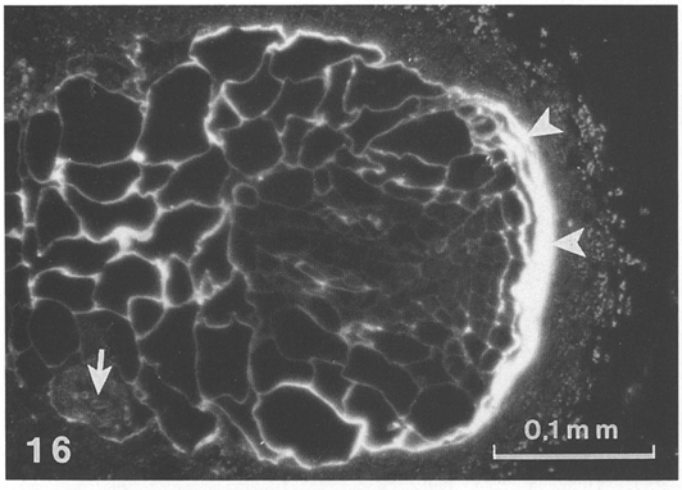
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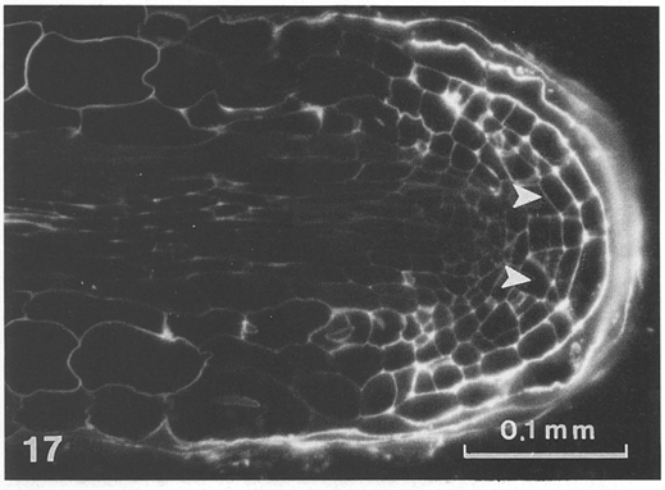
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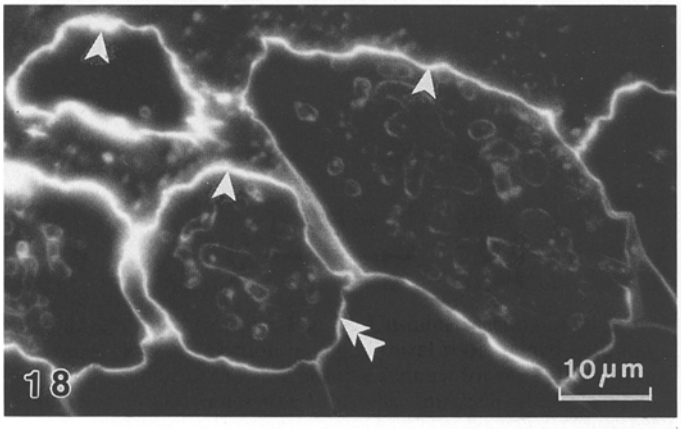
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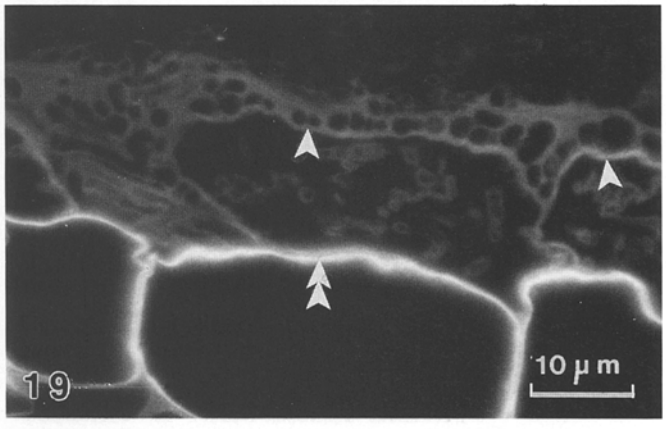
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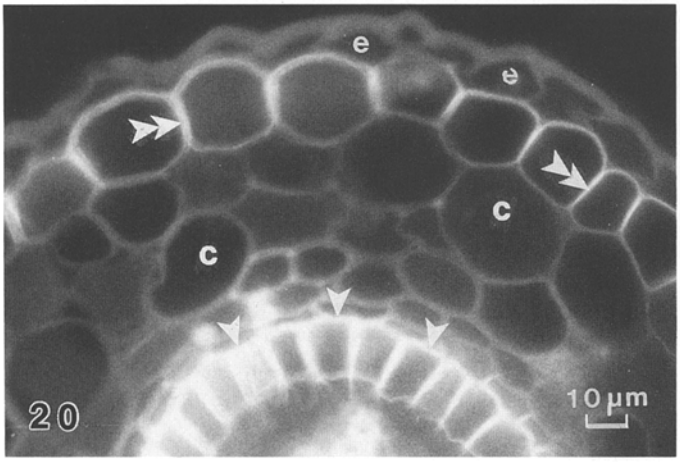
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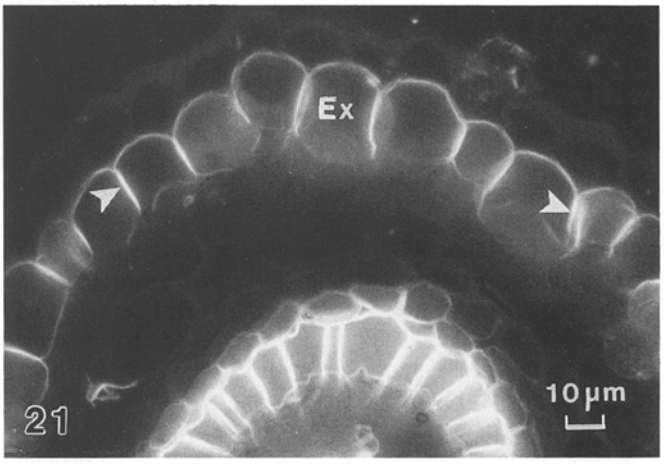
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Fluorescence microscopy

Acriflavine-stained sections of LR White-embedded material indicated structural differences between mycorrhizae formed by the two fungal species. Mycorrhizae formed with *P. tinctorius* had thick mantles, abundant intracellular hyphae in epidermal cells, and a root cap of collapsed cells (Fig. 14) whereas mycorrhizae formed with *P. bicolor* had thin mantles, less intracellular hyphae and a root cap consisting of intact cells filled with insoluble polysaccharides (Fig. 15). Roots stained with calcofluor showed a distinct difference in root cap structure and root apex shape between *P. tinctorius* (Fig. 16) and *P. bicolor* mycorrhizae (Fig. 17). Calcofluor staining of roots colonized by *P. tinctorius* showed a differential response of epidermal and cortical cells, the former staining positively and the latter negatively (Fig. 18). The converse was observed for *P. bicolor* mycorrhizae (Fig. 19). Hand sections of fresh *Arbutus* roots showed autofluorescence of the exodermis, endodermis and xylem (Fig. 20). Berberine-aniline blue staining intensified the fluorescence of Casparian strips in exodermal cells (Fig. 21).

Histochemistry

Epidermal cell walls stained positively for phenolic compounds with low pH toluidine blue O (Fig. 23), the Hoepfner-Vorsatz reaction (Fig. 24) and vanillin (Fig. 25) when compared to unstained controls (Fig. 22). Cortical cell walls, with the exception of the endodermis (Figs. 23, 25), were not stained.

Figs. 14, 15. Acriflavine-HCl-stained sections of mycorrhizae embedded in LR White and viewed with blue light. **Fig. 14.** *Arbutus - P. tinctorius*. Median longitudinal section showing thick mantle (*), intracellular hyphae (*arrowheads*), small apical meristem (*AM*) and collapsed root cap cells (*rc*). **Fig. 15.** *Arbutus - P. bicolor*. Median longitudinal section showing thin mantle (*arrowheads*), intracellular hyphae (*double arrowheads*) and several layers of root cap cells (*RC*) with acriflavine-positive contents

Figs. 16–19. Calcofluor-stained sections of mycorrhizae embedded in LR White and viewed with UV light. **Fig. 16.** *Arbutus - P. tinctorius*. The flattened root cap cells (*arrowheads*) and epidermal cell walls fluoresce intensely. Intracellular hyphae (*arrow*) are present. **Fig. 17.** *Arbutus - P. bicolor*. Layers of intact root cap cells (*arrowheads*) and flattened root cap cells are intensely fluorescent. **Fig. 18.** *Arbutus - P. tinctorius*. Epidermal cell walls (*arrowheads*) are intensely fluorescent whereas exodermal cell walls (*double arrowheads*) are not. **Fig. 19.** *Arbutus - P. bicolor*. Epidermal cell walls (*arrowheads*) are faintly fluorescent whereas exodermal cell walls (*double arrowheads*) fluoresce intensely

Fig. 20. Hand section of nonmycorrhizal root unstained viewed with UV light. Endodermal cell walls (*arrowheads*) and exodermal cell walls with a distinct Casparian strip (*double arrowheads*) show intense primary fluorescence. Epidermal (*e*) cell walls and other cortical (*c*) cell walls also show some fluorescence

Fig. 21. Hand-sectioned nonmycorrhizal root stained with berberine-aniline blue and viewed with UV light. The Casparian strip (*arrowheads*) within the exodermal cells (*Ex*) is enhanced with berberine-aniline blue. Casparian strips in endodermal cells are also obvious

Protein staining. Mantle and intracellular hyphae in the *Arbutus - P. tinctorius* ectomycorrhizae contained bodies which stained positively for protein (Fig. 26).

Polyphosphate staining. Many small inclusions in the mantle and Hartig net of the *Arbutus - P. tinctorius* mycorrhizae stained positively with low pH toluidine blue O (Fig. 27) indicating polyphosphate; this was confirmed by EDS analysis (Fig. 28).

Transmission electron microscopy

Sections of *Arbutus - P. tinctorius* mycorrhizae showed the various inclusions in mantle hyphae: the small, vacuolar inclusions of polyphosphate shown by EDS and the larger, amorphous bodies, presumably the bodies staining positively for protein (Fig. 29). A dense, interhyphal matrix was also evident (Fig. 29). Modifications of the exodermal cell wall, interpreted as suberin deposition, were evident along the outer tangential wall (Fig. 30) and radial walls (Fig. 31).

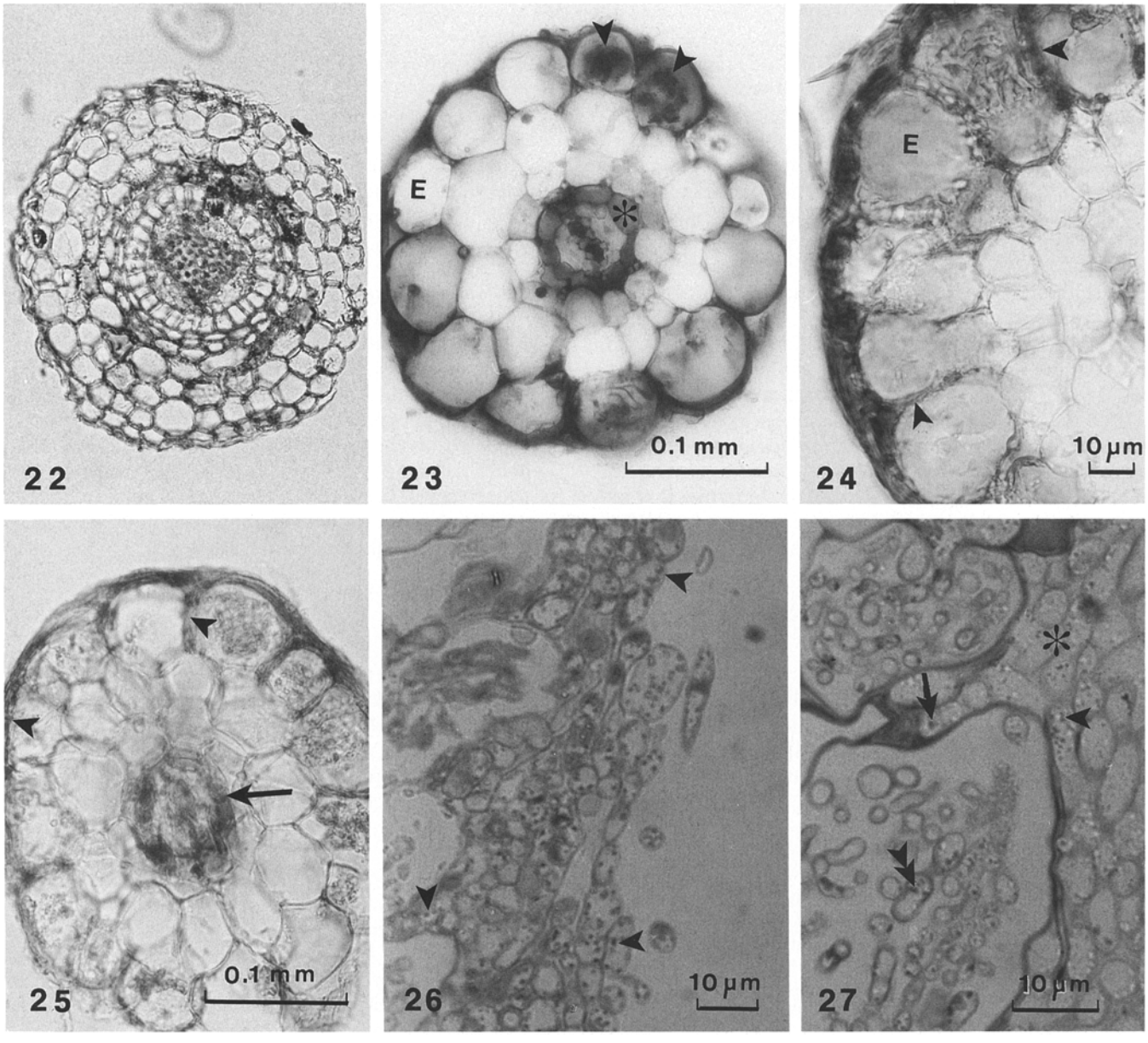
Discussion

The ability of the two ectomycorrhizal fungi *Pisolithus tinctorius* and *Piloderma bicolor* to form mycorrhizal associations with *A. menziesii* is consistent with the view that *Arbutus* is able to form mycorrhizal associations with a number of fungal species (Zak 1976b; Molina and Trappe 1982) which are rarely genus specific (Molina et al. 1992).

The difference in mycorrhiza morphology observed between *P. tinctorius* and *P. bicolor* is also consistent with the variation noticed among a number of ectomycorrhizal fungi and *A. menziesii* (Zak 1976b; Molina and Trappe 1982; Acsai and Largent 1983a) and emphasizes the role played by the mycobiont in mycorrhiza morphology (Massicotte et al. 1987b). The pinnate clusters of mycorrhizae observed with *P. tinctorius* in this study, and with other mycobionts previously (Molina and Trappe 1982; Giovannetti and Lioi 1990), arise from precocious lateral root initiation rather than a dichotomy of the root apical meristem as detailed for *Pinus strobus* (Piché et al. 1982).

The multi-layered mantle formed by *P. tinctorius* which envelops the entire root is typical of ectomycorrhizae. The mantle of *P. bicolor*, consisting of sparse hyphae embedded in a considerable amount of mucilage, is similar to ectendomycorrhizae formed by *Wilcoxina* species on pine (Piché et al. 1986; Scales and Peterson 1991). Rhizomorphs similar to those differentiating from the outer mantle of *P. tinctorius - Arbutus* mycorrhizae, have been recorded for mycorrhizal associations between *Arbutus* and several ectomycorrhizal fungal species (Zak 1974; Zak 1976b; Molina and Trappe 1982).

All of the mycorrhizae described for *Arbutus* develop a Hartig net from which intracellular hyphae are initiated. Our results concur with those of Rivett (1924),



Figs. 22–25. Hand sections. **Fig. 22.** Unstained section of *A. menziesii* root. **Fig. 23.** Section of mycorrhizal root stained with low pH toluidine blue O showing positive staining for phenolics within epidermal (*E*) and endodermal (*) cells. Intracellular hyphae (*arrowheads*) are evident. **Fig. 24.** Section of mycorrhizal root stained with the Hoepfner-Vorsatz reaction showing positive staining for phenolics in epidermal (*E*) cell walls (*arrowheads*). **Fig. 25.** Section of mycorrhizal root stained with vanillin-HCl. Positive staining for phenolics is evident in the epidermal cell walls (*arrowheads*) and within endodermal cells (*arrow*)

Figs. 26, 27. LR White-embedded tissues. **Fig. 26.** Section of *Arbutus – P. tinctorius* mycorrhiza stained with coomassie blue. Positive staining for protein bodies is present in mantle and Hartig net hyphae (*arrowheads*). **Fig. 27.** Similar material to that in Fig. 26 stained with low pH toluidine blue O. Polyphosphate granules (*arrowheads*) are present in the mantle (*), Hartig net (*arrow*) and intracellular hyphae (*double arrowheads*)

Fusconi and Bonfante-Fasolo (1984) and Münzenberger (1991), wherein the Hartig net is described as being para-epidermal and only the epidermal cells contain intracellular hyphae. In contrast, the outer cortical cell layer has been reported to be the site of the Hartig net and intracellular hyphae (Zak 1974, 1976b; Molina and Trappe 1982; Heslin 1986; Giovannetti and Lioi 1990). The latter interpretation could be a sectioning artifact due to the overlapping of radially enlarged epidermal cells or more likely an incorrect interpretation of root cell layers. Information concerning the suberization of the wall of the outer cortical cell layer would allow distinction of cortical and epidermal cells. The majority of angiosperm species forming ectomycorrhizae have the Hartig net confined to the epidermis (Harley and Smith 1983), commonly delimited by an exodermis (Brundrett

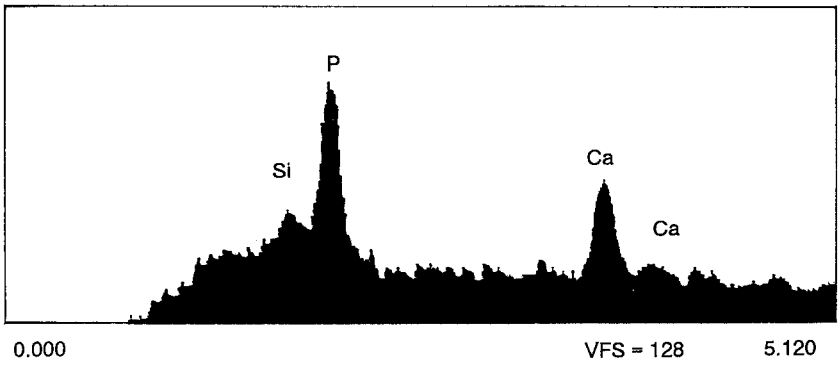
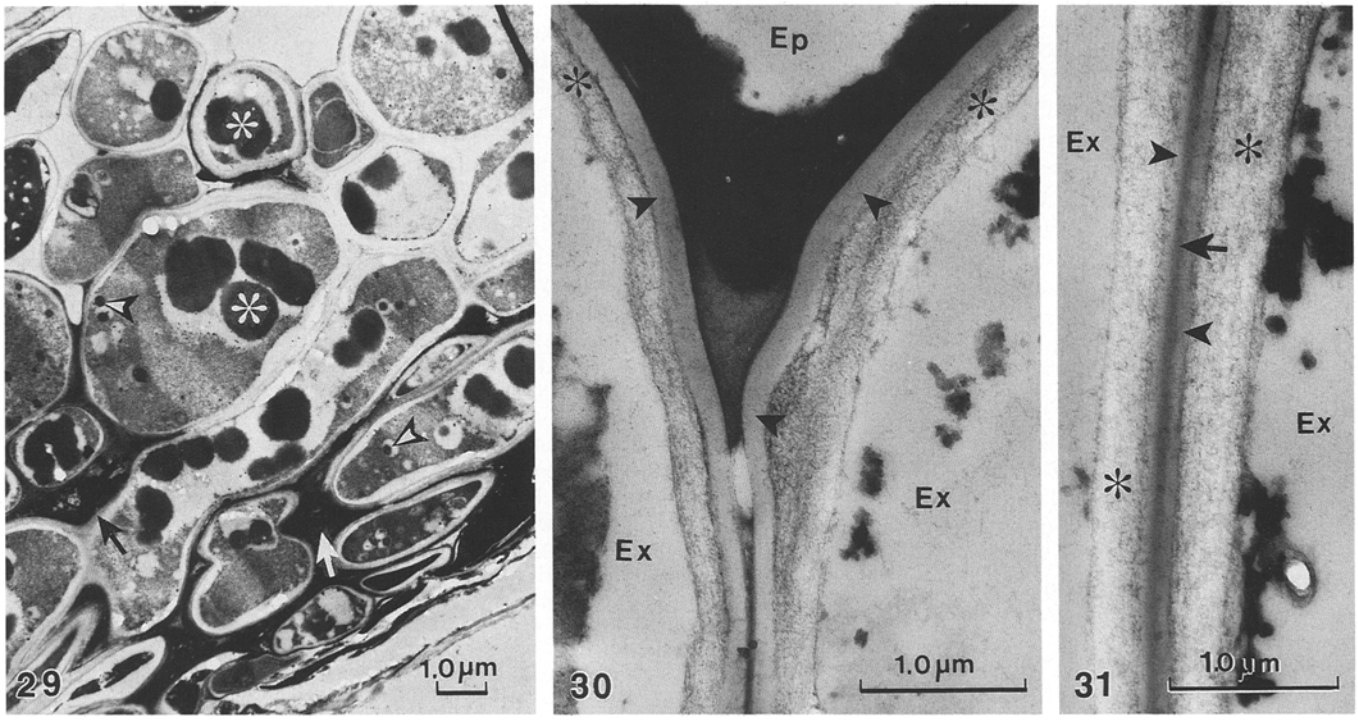


Fig. 28. X-ray spectrum of a dense, vacuolar inclusion in mantle hypha showing peaks for phosphorus and calcium. The silicon peak is from the microscope column



Figs. 29–31. Transmission electron micrographs of *A. menziesii* – *P. tinctorius* mycorrhizae. **Fig. 29.** Mantle hyphae in a longitudinal section showing inclusions interpreted as protein bodies (*) and polyphosphate bodies (*arrowheads*). Electron dense interhyphal material (*arrows*) is evident in the inner portion of the mantle. **Fig. 30.** Two exodermal cells (**Ex**) and a necrotic epidermal cell (*Ep*) in a transverse section. The outer tangential (*) and radial exodermal cell walls contain suberin (*arrowheads*). **Fig. 31.** Radial walls of two exodermal cells (*Ex*) in transverse section. Each wall has a dense non-lamellar suberin band (*arrowheads*) adjacent to the middle lamella (*arrow*) and a thicker, less dense portion (*) adjacent to the cytoplasm

et al. 1990). *Dryas integrifolia*, an angiosperm with a cortical Hartig net, is an exception (Melville et al. 1987). A detailed analysis of arbutoid mycorrhizae formed in several species of *Pyrola* also showed that the Hartig net and intracellular penetration are restricted to the epidermis (Robertson and Robertson 1985). It is assumed that fungal contact is restricted to the epidermal layer in *Arbutus*.

Reports indicating the presence of ectomycorrhizae on arbutoid hosts such as *Arbutus* and *Arctostaphylos* (Largent et al. 1980) should be regarded with caution unless anatomical data are provided (Münzenberger 1991).

Labyrinthic growth of the fungal symbiont in the Hartig net, a feature believed to be of critical diagnostic importance in ectomycorrhizal associations (Massicotte et al. 1987a) is also found in Arbutus and in the ectendomycorrhizae of *Pinus banksiana* (Scales and Peterson 1991), both of which also have intracellular hyphae. The Hartig net may provide a minimal but constant interface for nutrient exchange, whereas the intracellular interface may occur only at peak activity levels. There is no experimental evidence for nutrient exchange sites in arbutoid mycorrhizae.

A possible explanation for the confinement of mycorrhizal fungi to the epidermis of *Arbutus* roots comes from the observations comparing epidermal and cortical cell wall histochemistry. The wall of the outer tier of cortical cells differentiates a Casparian strip and suberin lamellae and, therefore, can be classified as an exoder-

mis (Peterson and Perumalla 1990); epidermal cell walls contain phenolic substances but lack suberin. Similarly, Münzenberger (1991) confirmed the presence of suberin in the hypodermis of *A. unedo* - *L. amethystea* mycorrhizae. It is of interest that Rivett (1924) recognized that the first cortical cell layer in *A. unedo* is suberized and that he described mycorrhizal hyphae as being confined to the epidermis. Suberin is known to impede the ingress of various fungi (Peterson 1989) including vesicular-arbuscular mycorrhizal fungi (Smith et al. 1990), and the wide occurrence of an exodermis in angiosperm roots (Perumalla et al. 1990; Peterson and Perumalla 1990) no doubt influences the pattern of mycorrhizal colonization in these roots. The phenolic content of epidermal cell walls does not prevent fungal colonization of this cell layer; it remains to be shown that these phenolic compounds are conducive to fungal colonization.

Arbutoid mycorrhizae may be a modified type of ectomycorrhiza (Molina and Trappe 1982) with structural differences determined largely by the host reaction to colonization by typical ectomycorrhizal fungi. In contrast, Fusconi and Bonfante-Fasolo (1984) suggest, based on the consistent intracellular phase, that arbutoid mycorrhizae should be considered a subtype of the ericoid mycorrhizal type. Arbutoid mycorrhizae also share characteristics with ectendomycorrhizae such as those described by Scales and Peterson (1991), and with mycorrhizae formed on *Pyrola* (Robertson and Robertson 1985). This difficulty in classifying mycorrhizal types is based partly on the artificial nature of some of the present categories and the limited number of broad comparative structural studies on many plant groups. Further study of diverse mycorrhizal associations will provide the information necessary to develop a definitive classification system.

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