Ectomycorrhiza formation in *Eucalyptus*.

IV. Ectomycorrhizas in the sporocarps of the hypogeous fungi *Mesophellia* and *Castorium* in Eucalypt forests of Western Australia

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**SUMMARY**

*Mesophellia* and *Castorium* are common hypogeous macrofungi in the karri (*Eucalyptus diversicolor* F. Muell.) and jarrah (*Eucalyptus marginata* Donn ex Sm.) forests of south-western Australia. Sporocarps of *Mesophellia* and *Castorium* develop 5–20 cm below the soil surface in close association with eucalypt roots. During differentiation of the sporocarps, eucalypt roots become trapped within the peridium where they branch profusely and form a dense ectomycorrhizal layer. Mature sporocarps of *M. trabalis* nom. ined. contain approximately 5 m of roots of 45 cm² surface area. Anatomical studies have shown that these roots have Hartig nets penetrating to the hypodermis and are similar to the superficial eucalypt ectomycorrhizas formed in soil and litter. The association of *Mesophellia* and *Castorium* sporocarps with tree roots suggests that these are important mycorrhizal fungi in forests of southern Australia.

Key words: *Mesophellia*, *Castorium*, ectomycorrhizas, eucalypt forest, hypogeous fungi, sporocarps.

**INTRODUCTION**

Previous papers in this series have described the morphology and anatomy of eucalypt ectomycorrhizas synthesized in the laboratory or taken from feeder roots near the surface in forest soil (Malajczuk, Molina & Trappe, 1982; Malajczuk, Dell & Bougher, 1987). Amongst the fungi which form symbiotic associations with roots of eucalypts are many hypogeous fungi, those producing sporocarps below ground. Recently, Malajczuk, Trappe & Molina (1987) have drawn parallels between hypogeous fungi in eucalypt forests of Australia and related taxa in coniferous forests of North America. However, many of the genera are unique to vegetation in each of the respective continents.

Both *Mesophellia* and *Castorium* are predominately associated with eucalypts in Australia. The sporocarps are found at depth in the soil profile intertwined amongst the tree roots. Although the sporocarps of one of these genera are reported to contain eucalypt roots (Beaton & Weste, 1983, 1984) little is known of the mycorrhizal status of these roots. In this paper we describe the structure of *Mesophellia* and *Castorium* sporocarps and their internal ectomycorrhizas.

**MATERIALS AND METHODS**

Sporocarps for histological examination were collected from karri (*Eucalyptus diversicolor* F. Muell.) and jarrah (*Eucalyptus marginata* Donn ex Sm.) forests in south-west Australia. After dissection small pieces were fixed in 3% glutaraldehyde in 25 mM potassium phosphate buffer (pH 7.1) or Karnovsky's fixative. Sections were prepared from epoxy embedded material. Except for the Masson Fontana method (Bancroft & Stevens, 1977) techniques were the same as described previously (Malajczuk et al., 1987).

**RESULTS**

**Field observations**

The most common species found during the early summer sampling period (December) were *Mesophellia*...
Figure 1. Structure of the sporocarp of *Mesophellia trabalis*. (a) Immature sporocarp with central glebal core (C) prior to differentiation of trabeculae within the outer gleba (G). The root layer (arrows) is present in the peridium (P). (b) Mature sporocarp dissected to expose the multilayered peridium (P) surrounding the spore mass (S). P<sub>1</sub>, ectoperidium, P<sub>2</sub>, mesoperidium, P<sub>3</sub>, endoperidium. (c) Detail of mature peridium with prominent living root layer (R). The spore mass has been removed exposing the trabeculae (T). The heavily pigmented main ectoperidial layer (P<sub>1</sub>) is surrounded by a distinct layer of cottony hyphae and soil particles (P<sub>1b</sub>). (d) Mature sporocarp cut open exposing the peridium (P), spore mass (S) and columella (C) in a live specimen. (e) Section through the immature outer gleba with differentiating hymenium (HY). (f) Section through the mature hymenium with basidia bearing spores (arrows). (e, f) Stained with Schiff’s Reagent/toluidine blue.
Figure 2. For legend see p. 453.
Figure 3. For legend see facing page.
phellia trabalis nom. ined., M. labyrinthina nom. ined. and Castorium camphoratum nom. ined. Sporocarps were readily recognised from gravel pisoliths because of their characteristic round shape and low density. Sporocarps were abundant in regrowth and mature forests and were located below the litter layer in the mineral soil to a depth of 20 cm. As many as 34 sporocarps/m² were observed at some sites.

**Sporocarp structure**

**Peridium.** The sporocarps of Mesophellia and Castorium form from localized aggregations of mycelia within the soil horizon. As the peridium and gleba begin to differentiate, eucalypt roots grow and become embedded within the peridium (Fig. 1a). The sporocarps are thus connected to host trees by roots. Hyphae from the sporocarps ramify through the soil and form ectomycorrhizal associations with eucalypt roots in the mineral soil. The mature sporocarps are characterized by a firm, crusty peridium surrounding a central core (Mesophellia) and spore bearing cavities (Mesophellia and Castorium) [Figs 1d; 2(a, c)]. The fully differentiated peridium is three-layered in Mesophellia and Castorium (Beaton & Weste, 1983, 1984). In this paper we designate the exoperidium as P₁, mesoperidium as P₂ and endoperidium as P₃. In M. trabalis the P₁ and P₃ each consist of two layers [P₁a, P₁b; P₃a, P₃b; Fig. 1(a, b)] and in M. labyrinthina (Fig. 2b) P₁ is two-layered. In C. camphoratum [Fig. 2(b, d)] the P₃ is two-layered. Eucalypt roots are confined to a single white, rubbery layer within P₂ [Figs. 1c; 2(b, e)]. The bulk of the peridial tissue consists, however, of closely packed hyphae (Fig. 2h). Occasional small woody roots (1–1.5 mm diam) pass from the soil through the outer dark brown or black-pigmented peridial layers (P₁b in Mesophellia, P₁ in Castorium) and connect with the fine root clusters within the peridium. Many of the fully mature sporocarps sampled were lying unattached in the soil. It is not clear at what stage of development root connections with the host become detached. Live roots have, however, been observed in sporocarps with spores present.

In the two Mesophellia spp. the outermost layers (P₁) of the peridium consist of a loose hyphal tomentum containing soil debris and scattered eucalypt ectomycorrhizas. In Castorium the outermost peridial layer (P₁) turns black and hardens as the fruiting body matures [Fig. 2(e, g)]. Both the P₁ layer and the inner endoperidium (Fig. 2f, P₃b) reacted with ammoniacal silver nitrate in the absence of an external reducing agent suggesting melanization had occurred.

**Gleba.** In M. trabalis the outermost part of the gleba differentiates into wedge shaped trabeculae which connect the rubbery core to the peridium [Fig. 1(c, d)]. The basidia develop between the trabeculae within elongated channels [Fig. 1(e, b)]. In M. labyrinthina the basidiospores form within labyrinthine channels which transect the gleba (Fig. 2b). Spores in Castorium camphoratum occur in numerous locules through the gleba (Fig. 2c).

**Sporocarp ectomycorrhizas**

Eucalypt roots which develop within the peridium of Mesophellia and Castorium [Fig. 3(a–e)] are closely packed and in their dimensions resemble fine roots found in soil or litter. In M. trabalis each sporocarp has on average 5 m of short roots, giving a surface area of approximately 45 cm². All of the fine roots are ectomycorrhizal since they have a well developed Hartig net penetrating between unextended epidermal cells to the hypodermis [Fig. 3(d, e)]. Externally, the epidermis is completely surrounded by closely packed fungal hyphae thus making it impossible to distinguish a mantle from other peridial tissue.

**Figure 2.** Structure of the sporocarps of Mesophellia labyrinthina (a, b) and Castorium camphoratum (c–h). (a) Mature sporocarp cut open exposing the peridium (P) and gleba (G). (b) Mature sporocarp showing the 2-layered exoperidium (P₁) and the cartilaginous core (C) of the gleba penetrated by labyrinthine locules (arrows). The mesoperidium (P₂) which lies internal to the dark-brown pigmented layer (P₁) is densely packed with roots. (c, d) Mature sporocarp cut open exposing the peridium (P) and inner gleba (G). The melanized exoperidium (P₁) surrounds the root-filled mesoperidium (P₂). The endoperidium (P₃) is defined internally by a narrow pigmented zone (Φ). (e, f) Sections through the mature peridium: P₁, outer crusty, thick melanised layer; P₂, root occupied zone, P₃a, rubbery pseudo-parenchymatous layer; P₃b, inner melanised layer of the endoperidium; R, roots. (g) Junction of ector- and meso-peridial layers. Scattered hyphae (arrows) can be seen in the crusty layer. (h) Hyphal morphology of endoperidium (P₃b). (e, g, h) Stained with toluidine blue, (f) Masson-Fontana.

**Figure 3.** Structure of Eucalyptus roots from within the peridium of Mesophellia trabalis (a, f), M. labyrinthina (b) and Castorium camphoratum (c–e). (a) Surface view of root clusters (arrows) after removal of the outer peridial layers. (b, c) Transverse sections of root clusters showing the unbranched superficial ectomycorrhizas (R) embedded in fungal tissue (★). (d) Detail of ectomycorrhizal anatomy. The Hartig net (arrows) extends between the unexpanded epidermal cells (E) as far as the outer cortex (H). (e) Section of senescing ectomycorrhizas showing invasion of cortical cells (CX) by fungal tissue (arrows). (f) Portion of ectomycorrhiza showing the Hartig net (HN) penetrating to the hypodermis (H). Many fungal hyphae (★) lie external to the epidermis (E) in the region where the mantle occurs in soil-dwelling superficial ectomycorrhizas. Electron-dense material is present in interhyphal areas (arrows). (b–e) Stained with toluidine blue.
Figure 4. Structure of ectomycorrhizas. (a) Portion of the Hartig net (HN) and mantle (M) in a mature ectomycorrhiza taken from the peridium of _Castorium camphoratum_, showing the barrier zone within the hypodermis due to the deposition of electron-dense material (*) and suberization of the hypodermal cell wall (arrows). (b) Hypodermal cell wall with suberized lamellae (small arrows) adjacent to a vacuolated hyphal component of the Hartig net (HN) and two epidermal cells (E). The middle lamella between the hypodermal and epidermal cells has separated (large arrows). (c–e) Mature _Eucalyptus diversicolor_ ectomycorrhizas taken from forest soil adjacent to sporocarps of _Mesophellia trabalis_. (c) Transverse section showing the narrow sheathing mantle (M) and well developed Hartig net (arrows) extending between vacuolated epidermal cells as far as the partially collapsed hypodermis (H). The cortex (CX) has lignified cell walls. (d) Electron-dense material (*) in the inner mantle (M) adjacent to the epidermis (E). (e) Partially vacuolated hyphae in the Hartig net (HN) between two epidermal cells (E). (c) Stained with toluidine blue.
As in similar superficial ectomycorrhizas (Malajczuk et al., 1987), the hypodermis develops chemical (polyphenol accumulation) and physical (suberized wall lamellae) barriers to further penetration by fungal tissue [Fig. 4(a, b)]. However, in mature sporocarps, the cortex may become invaded intracellularly, presumably during senescence of the roots.

**Comparison of sporocarp and soil ectomycorrhizas**

Eucalypt fine roots synthesized in the laboratory and from beneath *M. trabalis* in the karri forest were examined for comparison with ectomycorrhizas observed in the sporocarps. The soil-borne roots are simple or weakly pyramidal branched and are enclosed in a loosely packed, thin mantle [Fig. 4(c, d)]. Anatomical features of these superficial ectomycorrhizas [Fig. 4(d, e)] are similar to those in the ectomycorrhizas observed within the peridial layers (Fig. 3).

**Discussion**

*Mesophellia* and *Castorium* sporocarps were always associated with first and second order lateral roots of eucalypts. These roots were enveloped in sheaths of mycelium which extended into the soil, thus forming aggregations of soil, mycelia, fruit bodies and tree roots. During differentiation of the sporocarps of *Mesophellia* and *Castorium* eucalypt roots become trapped within the peridial layers where they branch profusely and form a dense ectomycorrhizal layer. In contrast to Beaton & Weste (1983), most of the short roots enclosed in the mesoperidia had an Hartig net even where external deposits of phenolic materials were present. Since the sporocarps probably take several months to mature before all root connections with the host are severed, these ectomycorrhizas may play a role in nutrient relationships between the host and tree. The fungus could for example stimulate root development within the peridium thus enhancing additional carbohydrate flow into the sporocarp. In addition, uptake of inorganic nutrients by the tree may be enhanced by placement of roots within the fungal tissue. In other genera such as *Hysterangium* which also cohabit the eucalypt forest, tree roots are excluded from the sporocarps, presumably due to chemical as well as physical barriers. In *Mesophellia* and *Castorium* the roots fail to penetrate the gleba and are restricted to a narrow zone within the peridium [e.g. Fig. 2(e, f)]. The lack of penetration of adjacent layers of the peridium (e.g. *P. a.,* Fig. 2d) is puzzling given the absence of any obvious barrier zone. Sporocarps at the stage of gleba and peridium differentiation were not available in this study so it remains unclear how the timing of sporocarp development relates to root growth.

Beaton & Weste (1983) did not observe roots in immature sporocarps and concluded that roots invade near maturity. More likely, peridial development and root extension are synchronized events.

*Mesophellia* and *Castorium* are unusual in that sporocarps are abundant in early summer when most of the other fleshy hypogeous and epigeous groups have finished fruiting (Christensen, 1980). Two factors appear to be important: firstly, the sporocarps possess a crusty outer peridium which probably resists dessication, and secondly, they are found at depth (some 5–20 cm below the soil surface).

Malajczuk et al., (1987) have identified interactions between eucalypts, hypogeous mycorrhiza-forming fungi such as *Mesophellia*, and small mycophagous mammals. Much of the sporocarp tissue is consumed by small marsupials. The hypogeous fungi are particularly dependent on animals for their dispersal (Trappe & Maser, 1977). Spores of *Mesophellia* that pass through the digestive tracts of mycophagous marsupials remain viable and have been shown to form ectomycorrhizas with eucalypt seedlings in glasshouse trials (Lamont, Ralph & Christensen, 1985).

The frequent occurrence of *Mesophellia* and *Castorium* sporocarps in eucalypt forests in southern Australia (Malajczuk, unpublished observations) suggest that these are important mycorrhizal fungi in natural forest stands. Detailed samplings are required to obtain information on the magnitude of inorganic and organic nutrients turned over by the sporocarps.

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**References**


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