

## Anatomical studies on ectomycorrhizas

### II. The ectomycorrhizas produced by *Amanita muscaria*, *Laccaria laccata* and *Suillus brevipes* on *Pinus patula*

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**Abstract.** Ectomycorrhizas of 5-month-old *Pinus patula* plants grown in autoclaved *shola* soil (forest soil) inoculated with basidiospores of *Amanita muscaria*, *Laccaria laccata* and *Suillus brevipes* are described. The main emphasis is on the organisation of the mantle tissue as seen in plan view and features of associated hyphae and strands. The different layers in the mantle produced by the different fungi are described and illustrated.

**Key words:** *Pinus patula* – *Amanita muscaria* – *Laccaria laccata* – *Suillus brevipes* – Ectomycorrhizas – Rhizomorphs – Mantle structure

#### Introduction

In this paper, the second in this series, detailed descriptions are given of the mantle structure of ectomycorrhizas in *Pinus patula* produced by *Amanita muscaria*, *Laccaria laccata* and *Suillus brevipes*. Details of earlier work on tissue types found in ectomycorrhizas produced by different fungi in other plants are given in the first paper in this series (Mohan et al. 1993).

#### Materials and methods

The methods for characterisation of ectomycorrhizas used in the present study are as given in Mohan et al. (1993).

#### Results

##### *Amanita muscaria*

The ectomycorrhizas produced by *A. muscaria* (L. per. Fr.) Hooker are illustrated in Figs. 1–5.

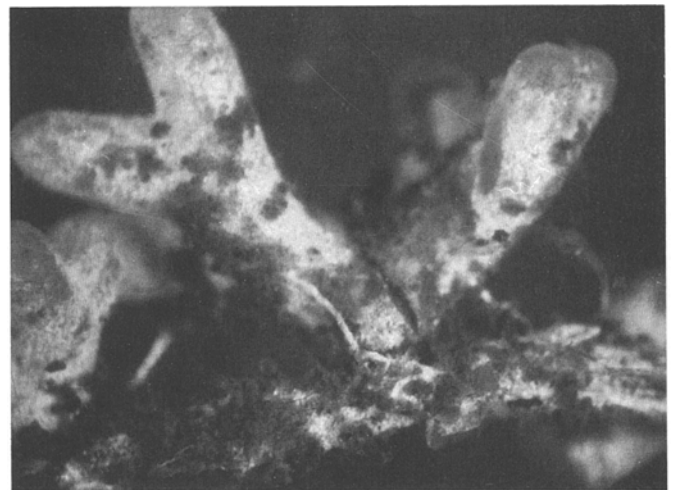


Fig. 1. Habit (morphology of mycorrhizas) of *Amanita muscaria*, ×10

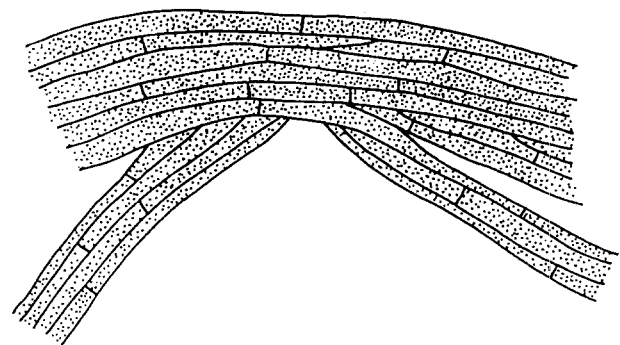
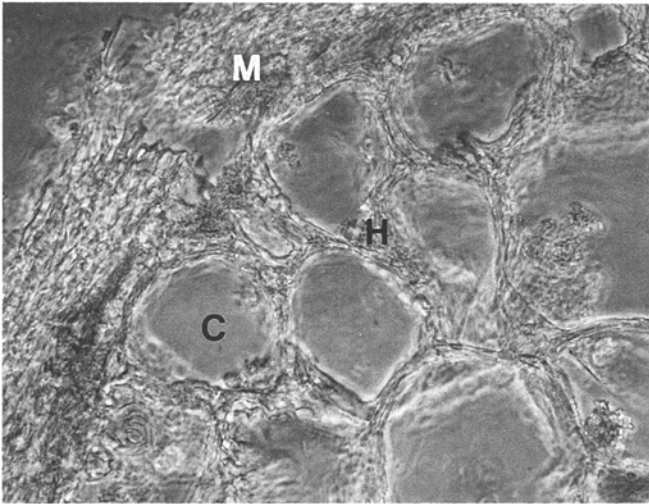


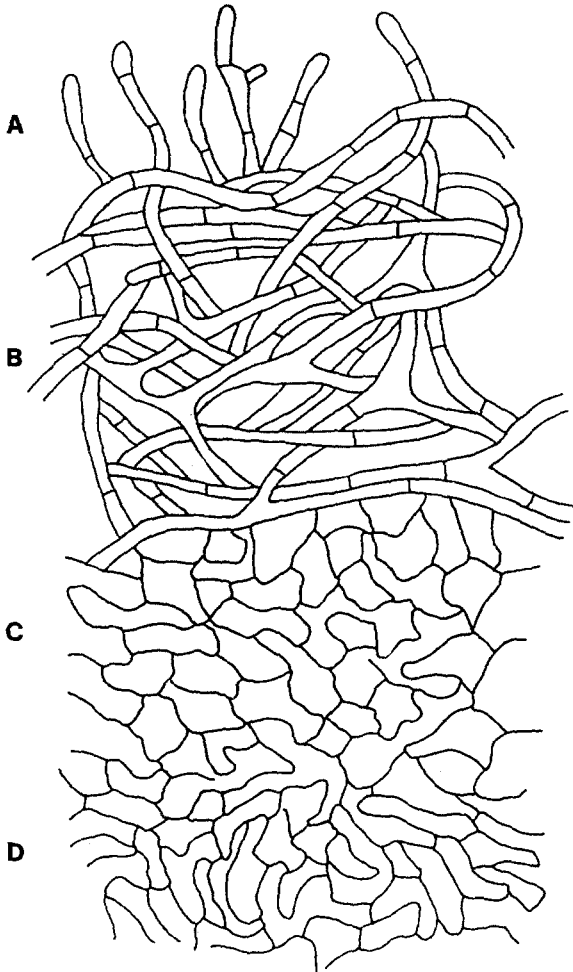
Fig. 2. Rhizomorph (*A. muscaria*), ×450

#### Morphological characteristics

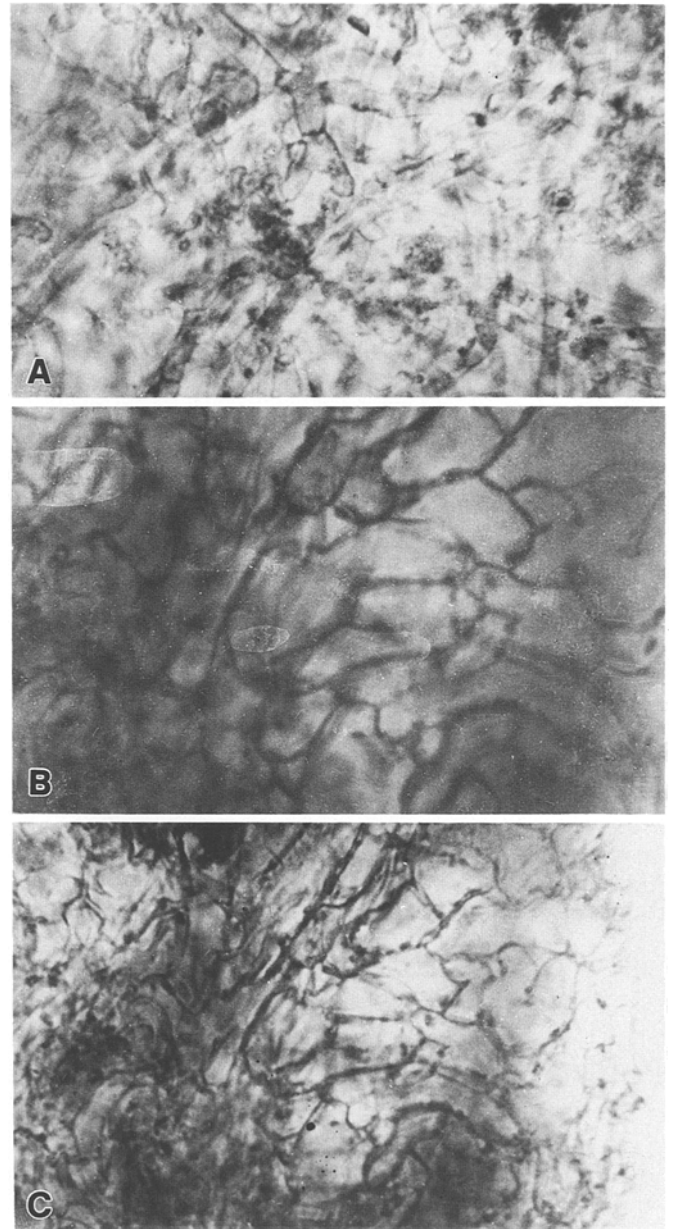
The mycorrhizas are mostly of the bipodial and dense-clustered forms (rarely monopodial). They are short and stubby when young and tortuous with frequent, irregularly spaced branches, often forming quite dense clus-



**Fig. 3.** Transverse section of root (*A. muscaria*),  $\times 480$ . C, Cortical cells; H, Hartig net cells; M, mantle



**Fig. 4A-D.** Diagrammatic plan or surface details of mantle layers (*A. muscaria*). A Emanating hyphae,  $\times 515$ ; B outer layer,  $\times 440$ ; C middle layer,  $\times 1450$ ; D inner layer,  $\times 880$



**Fig. 5A-C.** Plan or surface view details of mantle layers (*A. muscaria*). A Outer layer,  $\times 660$ ; B middle layer,  $\times 1760$ ; C inner layer,  $\times 930$

ters, when old (Fig. 1). The main axis is 2.0–4.0 mm in length and 0.4–0.6 mm in diameter. The mycorrhizas are white to silver-white when young and greyish-white (1B1) to light yellowish-white (4A2) when old. The surface of the mycorrhiza has silver-white tints. When the mycorrhizas are bruised or kept in formalin/acetic acid/alcohol (FAA), the white tints disappear and they become greyish-white to yellowish-white. The surface of the mycorrhizas are smooth when young and slightly roughened and distinctly granular in appearance when old. The rhizomorphs are milky-white to greyish-white (1B1) in colour. They grow on the surface of the mycorrhizal system, a few being directly connected to the mantle layer of the mycorrhizas.

### Anatomical characteristics

*Rhizomorphs* (Fig. 2). The rhizomorphs are 50–125  $\mu\text{m}$  wide and composed of closely packed hyphae which are parallel, thin-walled, septate and without clamp connections. The hyphae are white to greyish-white (1B1) when mounted in water and pale brown to greyish-brown (7E2) in lactophenol. Anastomoses between hyphae are infrequent.

*Mycorrhiza (cross section)* (Fig. 3). The *mantle edge* is 15–30  $\mu\text{m}$  deep and consists of a simple, prosenchymatous tissue. Hyaline to greyish-white (1B1) emanating hyphae emerge from the surface of the mantle. The emanating hyphae are up to 90  $\mu\text{m}$  in length, slightly tortuous, slender, thin-walled, septate and without clamp connections. The distal ends of the emanating hyphae are slightly broader (3–5  $\mu\text{m}$  wide) than those of the basal region (2–3  $\mu\text{m}$  wide). The blunt, broader hyphal tips look like cystidia; there are no setae or sclerotia.

*Tannin cells* are mostly in a single row, elliptical to slightly elongate, 25–50  $\times$  10–30  $\mu\text{m}$  in size and pale brown (5D6) to yellowish-brown (5D8) in colour. The Hartig net is composed of a single row of oval to globose hyphal cells 3–5  $\mu\text{m}$  in thickness.

*Cortical cells* are elliptical and 30–50  $\times$  20–30  $\mu\text{m}$  in size. The Hartig net is composed of a single row of oval to globose hyphal cells 3–5  $\mu\text{m}$  in thickness and penetrates up to three cortical cell layers deep.

*Mycorrhiza mantle (plan or surface view)* (Figs. 4, 5). The mycorrhizas in plan view have three different layers of mantle. The outer layer is composed of a felt prosenchymatous tissue. The individual hyphae of this layer are loosely interwoven, septate, 2–5  $\mu\text{m}$  in diameter and without clamp connections. The middle layer consists of a densely packed, net prosenchymatous tissue with individual hyphae similar to those of the outer layer. The inner layer is composed of a net synenchymatous tissue, the hyphae of which are septate and 5–7  $\mu\text{m}$  in diameter. The distinguishing features of the mycorrhizas produced by *A. muscaria* are as follows:

1. The mycorrhizas produced are short, stubby and dichotomously branched to complex clustered.
2. The mycorrhizas are milky-white to silver-white. Silver-white tints seen on the surface of the mycorrhizas disappear when bruised or when the mycorrhizas are kept in FAA.
3. The mycorrhizas are smooth to slightly granular. This granular appearance is due to the presence of distinctive cystidium-like emanating hyphae on the surface of the mantle layer. The emanating hyphae are septate and without clamp connections.

### *Laccaria laccata*

The ectomycorrhizas produced by *L. laccata* (Scop.) Berk. & Br. var. *laccata* Sing. are illustrated in Figs. 6–9.



Fig. 6. Habit (morphology of mycorrhizas) of *Laccaria laccata*,  $\times 10$

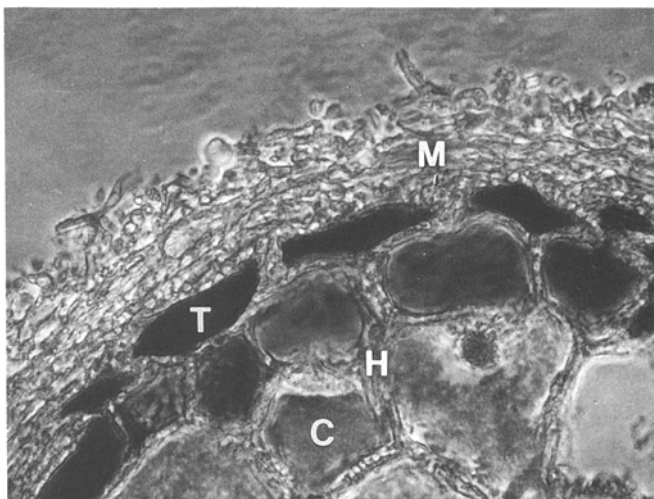


Fig. 7. Transverse section of root (*L. laccata*),  $\times 460$ . C, Cortical cells; H, Hartig net cells; M, mantle; T, tannin cells

### Morphological characteristics

The mycorrhizas are mostly dichotomously branched (bipodial) and rarely mono-, tri- and tetrapodial. They are slightly tortuous and fairly long with frequent, irregularly spaced, short branches (Fig. 6). The main axis is up to 5.5 mm in length and up to 0.6 mm in diameter. The mycorrhizas are white when young and whitish-orange to greyish-brown (6D3) when old. Loose, hyaline hyphae are associated with the surface of the mycorrhizal system and are just visible under the dissecting microscope. The surface of the mycorrhiza is pubescent. There are no rhizomorphs or hyphal strands.

### Anatomical characteristics

*Mycorrhiza (cross section)* (Fig. 7). The *mantle edge* is 10–50  $\mu\text{m}$  deep and consists of a simple, felt prosenchy-

matous tissue overlying net prosenchymatous tissue. White or pale orange (5A3) to pale brown (6D4) emanating hyphae emerge from the mantle surface. The emanating hyphae are up to 138  $\mu\text{m}$  long, mostly tortuous, thin-walled, 2–5  $\mu\text{m}$  wide and septate with abundant clamp connections. Branches of the emanating hyphae are rarely present. There are no cystidia, setae or sclerotia.

*Tannin cells* are mostly in a single row, subglobose to elongate, 20–45  $\times$  10–30  $\mu\text{m}$  in size and pale orange-yellow (4A7) to brownish-orange (6C6). The Hartig net is composed of one or two rows of oval to globose hyphal cells 3–8  $\mu\text{m}$  thick.

*Cortical cells* are subglobose to broadly elliptical and measure 25–60  $\times$  20–50  $\mu\text{m}$ . The Hartig net is composed of one or two rows of oval to globose hyphal cells 3–8  $\mu\text{m}$  thick. The Hartig net penetrates up to four cortical cell layers deep.

*Mycorrhiza mantle (plan or surface view)* (Figs. 8, 9). The mycorrhizas in plan view have three different layers of mantle. The outer layer is composed of a felt prosenchymatous tissue. The individual hyphae of this layer are 3–8  $\mu\text{m}$  wide and septate with frequent clamp connections. The middle layer consists of a net prosenchymatous tissue. The hyphae are similar to those of the outer layer with few clamp connections. The inner layer is composed of a net synenchymatous tissue with hyphae 3–5  $\mu\text{m}$  in diameter. The distinguishing features of the mycorrhizas produced by *L. laccata* are as follows:

1. The mycorrhizas are typically short and dichotomously branched structures. They are white to yellowish-white when young, turning greyish-brown (6D3) when old. The surface is pubescent with hyaline, loose hyphae. Morphologically it is not easy to differentiate this mycorrhiza from young mycorrhizas produced by *Telephora terrestris*.

2. Rhizomorphs or hyphal strands are not present, unlike the mycorrhiza formed by *T. terrestris*.

### *Suillus brevipes*

The ectomycorrhiza produced by *S. brevipes* (Peck) Kuntze var. *brevipes* (Peck) Kuntze are illustrated in Figs. 10–13.

#### Morphological characteristics

The mycorrhizas are mostly monopodial and bipodial and a few are tri- and tetrapodial (Fig. 10). They are generally straight or slightly tortuous. The mycorrhizas are slightly broader at the base than at the tip. The main axis is 1.2–8.4 mm in length and 0.2–0.4 mm in diameter. The mycorrhizas are pale yellow (4A3) to yellowish-brown (5D8) when young and pale brownish-yellow (5C8) to reddish-brown (8E8) when old. The surface is smooth when young and striations are seen when old. Loose hyphae are attached to the surface of the mycor-

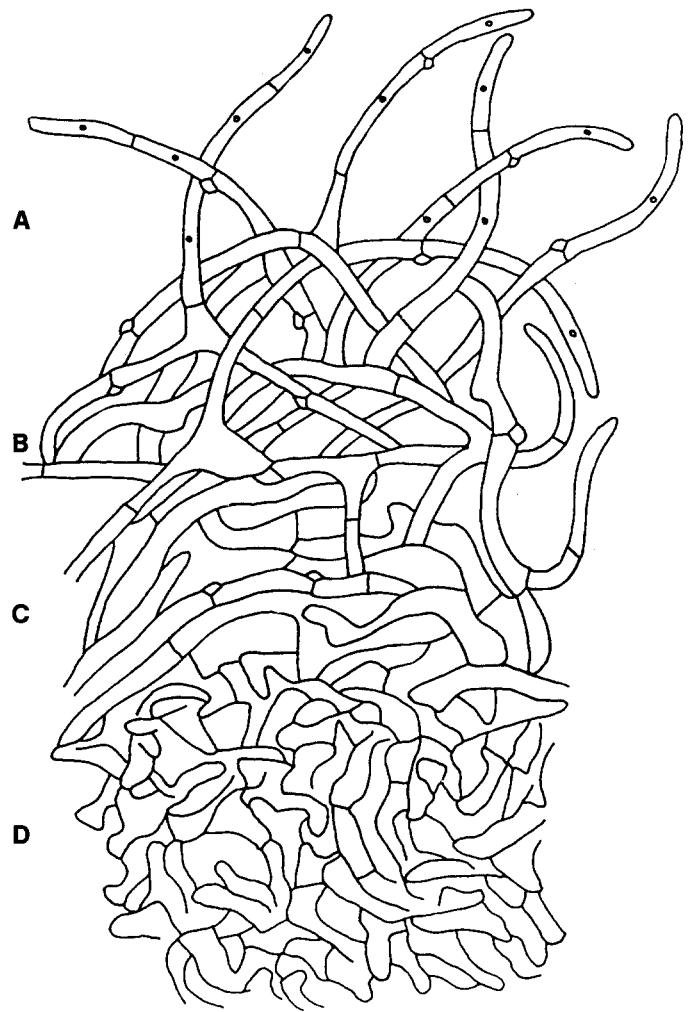


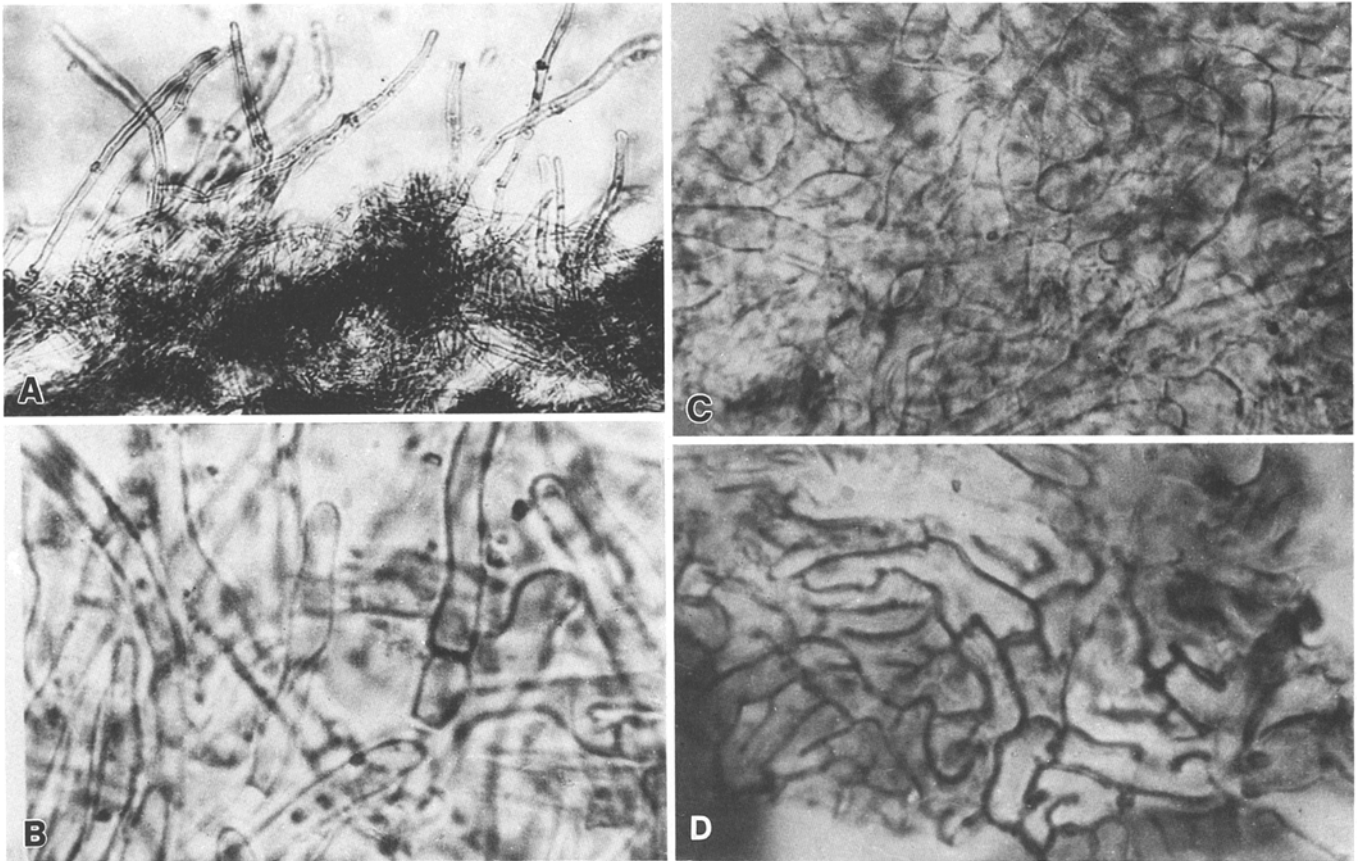
Fig. 8A–D. Diagrammatic plan or surface view details of mantle layers (*L. laccata*). A Emanating hyphae,  $\times 260$ ; B outer layer,  $\times 560$ ; C middle layer,  $\times 645$ ; D inner layer,  $\times 940$

rhizas, sometimes surrounding them as a loose web. These hyphae are thin, undulating and pale yellow (1A2) to yellowish-brown (5D8). There are no rhizomorphs or hyphal strands.

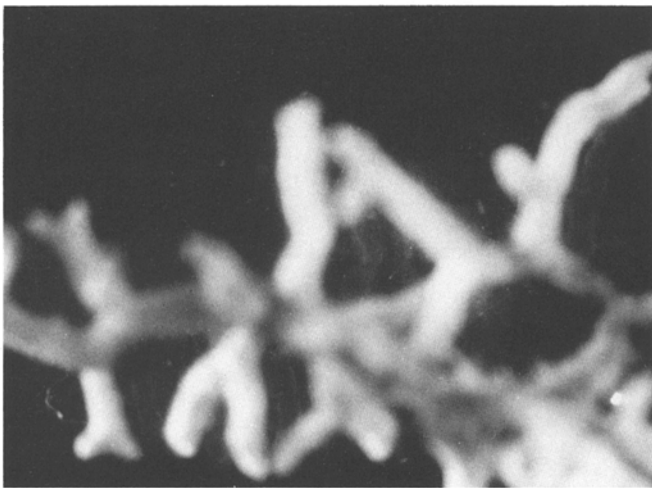
#### Anatomical characteristics

*Hyphae surrounding the mycorrhizas.* These hyphae are thin-walled, 5–7  $\mu\text{m}$  wide, septate, without clamp connections and are pale yellow (1A2) to yellowish-brown (5D8). Anastomosing is infrequent.

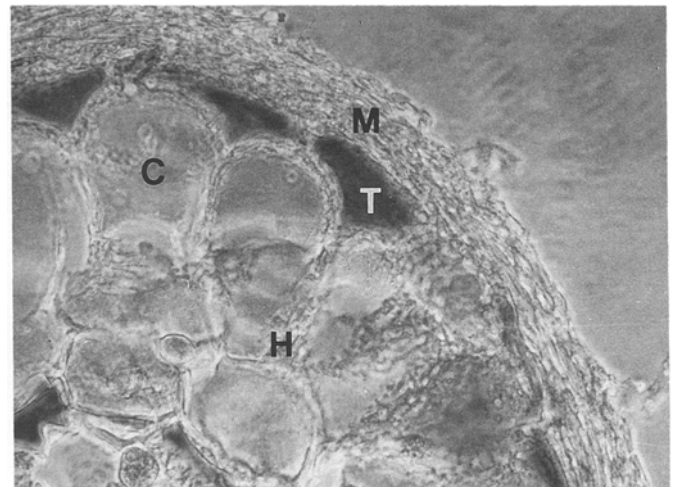
*Mycorrhiza (cross section)* (Fig. 11). The *mantle edge* is 10–50  $\mu\text{m}$  deep and consists of simple, prosenchymatous tissue. White to pale yellowish-brown, emanating hyphae radiate from the surface of the mantle layer. The emanating hyphae are 30–140  $\mu\text{m}$  long, mostly tortuous, 3–4  $\mu\text{m}$  wide, septate and without clamp connections. The surfaces of the emanating hyphae and the mantle hyphae are slightly encrusted. There are no cystidia, setae or sclerotia.



**Fig. 9A-D.** Plan or surface view details of mantle layers (*L. laccata*). **A** Emanating hyphae,  $\times 350$ ; **B** outer layer,  $\times 860$ ; **C** middle layer,  $\times 1040$ ; **D** inner layer,  $\times 1600$



**Fig. 10.** Habit (morphology of mycorrhizas) of *Suillus brevipes*,  $\times 10$



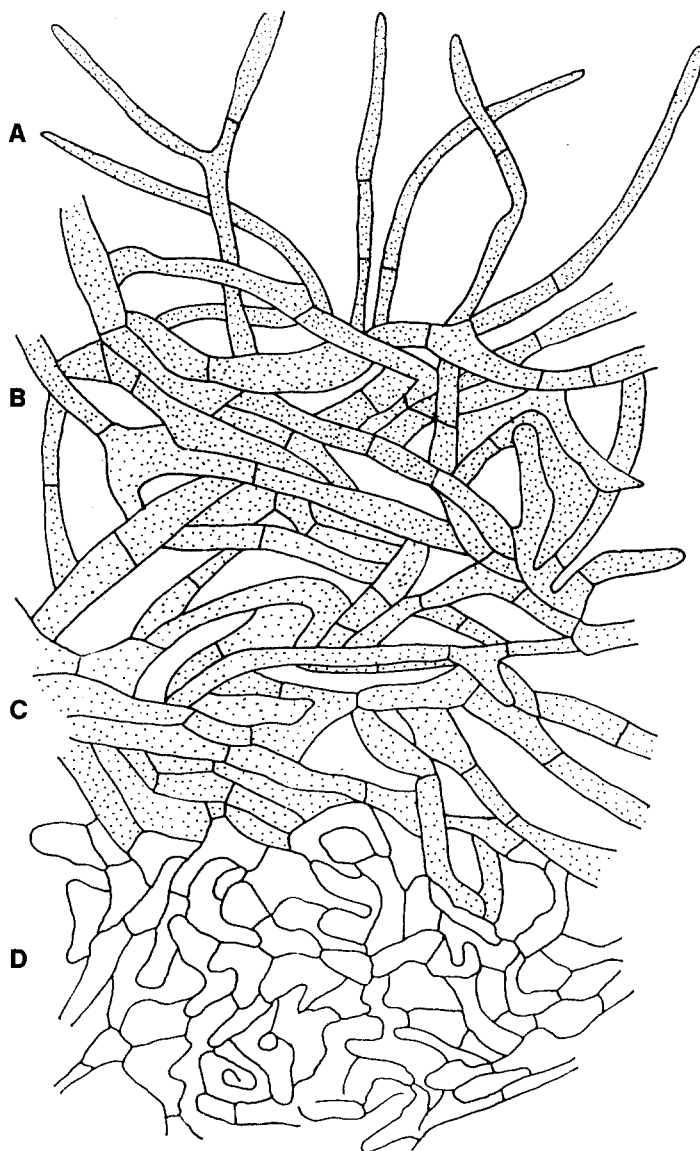
**Fig. 11.** Transverse section of root (*S. brevipes*),  $\times 560$ . **C**, Cortical cells; **H**, Hartig net cells; **M**, mantle; **T**, tannin cells

*Tannin cells* are mostly in a single row, elliptical,  $20-55 \times 10-40 \mu\text{m}$  in size. The Hartig net is composed of a single row of rectangular hyphal cells  $2-4 \mu\text{m}$  thick.

*Cortical cells* are slightly oval to elliptical and measure  $20-50 \times 10-20 \mu\text{m}$ . The Hartig net is composed of a

single row of rectangular hyphal cells  $2-4 \mu\text{m}$  thick and penetrates up to three cortical cell layers deep.

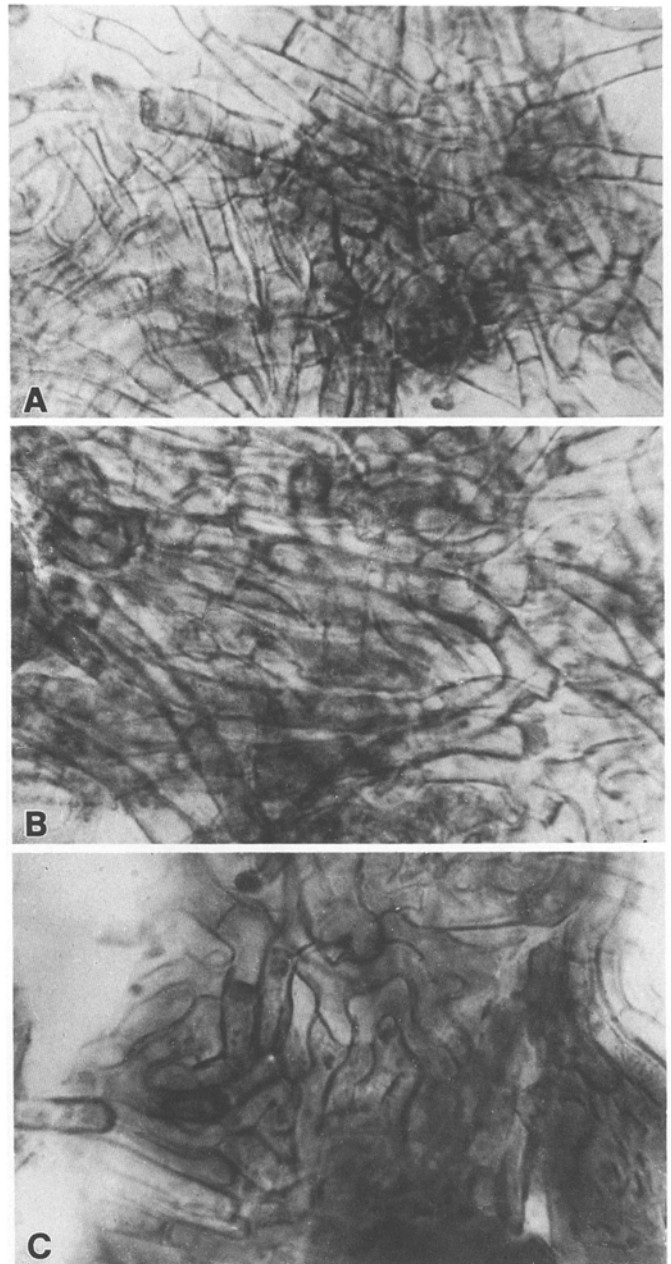
*Mycorrhiza mantle (plan or surface view)* (Figs. 12, 13). The mycorrhizas in plan view have three different layers



**Fig. 12A–D.** Diagrammatic plan of surface view details of mantle layers (*S. brevipes*). **A** Emanating hyphae,  $\times 1300$ ; **B** outer layer,  $\times 1820$ ; **C** middle layer,  $\times 1740$ ; **D** inner layer,  $\times 1620$

of mantle. The outer layer is composed of a simple, prosenchymatous tissue, the hyphae of which are hyaline, septate, 2–4  $\mu\text{m}$  in diameter and without clamp connections. The middle layer is composed of a net prosenchymatous tissue with hyphae similar to those of the outer layer. The inner layer consists of an irregular, synenchymatous tissue with hyphae 2–3  $\mu\text{m}$  in diameter. The distinguishing features of the mycorrhizas produced by *S. brevipes* are as follows:

1. Rhizomorphs or hyphal strands are not seen on the mycorrhizal system. Instead, loose hyphae sometimes surround the mycorrhizal system as a loose web.
2. The emanating hyphae that radiate from the mantle edge are septate and without clamp connections.
3. The surface of the emanating hyphae and the mantle hyphae are slightly encrusted with granulations.



**Fig. 13A–C.** Plan or surface view details of mantle layers (*S. brevipes*). **A** Outer layer,  $\times 500$ ; **B** middle layer,  $\times 480$ ; **C** inner layer,  $\times 400$

## Discussion

In contrast to the mycorrhizas produced by *L. laccata* and *S. brevipes*, the mycorrhiza produced by *A. muscaria* had rhizomorphs. Rhizomorphs similar in colour to the mycorrhizal system produced by *A. muscaria* have also been described earlier by other workers (Chu-Chou and Grace 1983; Godbout and Fortin 1985; Last et al. 1985). Riffle (1973) found no mycelial strands or rhizomorphs in a pure culture study of *Pinus ponderosa* with *A. muscaria*.

The structure of the mantle of ectomycorrhizas produced by *A. muscaria* was studied by Riffle (1973) in

*Pinus ponderosa*, by Chu-Chou and Grace (1983) in *Pseudotsuga menziesii* and by Godbout and Fortin (1985) in *Populus tremuloides*. In the present study, the most characteristic feature of the mantle of *A. muscaria* mycorrhizas is the granular appearance caused by the presence of distinctive cystidium-like emanating hyphae on the surface. These hyphae are septate and without clamp connections. Among the earlier studies with *A. muscaria* mycorrhizas, only Godbout and Fortin (1985) found abundant, cystidium-like multi-septate, sometimes branched hyphae in *Populus tremuloides*.

The structure of the emanating hyphae seems to be different in the mycorrhizas produced by other species of *Amanita*. For example, Bakshi (1974) described the presence of clamped and incrustated hyphae in mycorrhizas produced by *A. hemibapha*; in the case of *A. verna*, the hyphae were setose, short and cylindrical.

*L. laccata* is an ectomycorrhizal fungus frequently associated with many trees. Surprisingly, there are only a few detailed studies of the structure of the mycorrhizas produced by this fungus on different host plants (Fassi and Fontana 1966; Thomas and Jackson 1979, 1982; Chu-Chou and Grace 1983). The three-layered mantle structure and the absence of rhizomorphs mycorrhizas produced by this fungus in *Pinus patula* were also found in the earlier studies.

The only previous study of the structure of the mycorrhizas produced by *S. brevipes* is that of Palm and Stewart (1984) for *Pinus resinosa* and *Pinus strobus*; their results more or less agree with the observations made in the present study of *P. patula*.

*Acknowledgement.* One of us (V. M.) is grateful to the University Grants Commission, New Delhi, India for the award of a Research Fellowship.

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