

Original papers

Anatomical studies on ectomycorrhizas

III. The ectomycorrhizas produced by *Rhizopogon luteolus* and *Scleroderma citrinum* 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 *Rhizopogon luteolus* and *Scleroderma citrinum* are described. The main emphasis is on the organisation of the mantle tissue as seen in plan view and the 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* – *Rhizopogon luteolus* – *Scleroderma citrinum* – Ectomycorrhizas – Rhizomorphs – Mantle structure

Introduction

In this paper, the third in this series, detailed descriptions are given of the mantle structure of ectomycorrhizas in *Pinus patula* produced by *Rhizopogon luteolus* and *Scleroderma citrinum*. 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 this present study are as given in Mohan et al. (1993).

Results

Rhizopogon luteolus

The ectomycorrhiza produced by *R. luteolus* Fr. em Tul. are illustrated in Figs. 1–5.

Morphological characteristics

The mycorrhizas are usually highly dichotomously branched, complex coralloid forms, rarely bipodial or monopodial. They are stubby (short, thick and finger-like) and compactly branched (Fig. 1). The main axis is up to 2.0 mm in length and up to 0.6 mm in diameter. The mycorrhizas are whitish- to brownish-yellow (5C8) when young and light brown (5D8) when old. The surface is pubescent with fine sepia-coloured (dark brown) hyphae. Rhizomorphs are abundant, long, rope-like, compactly arranged, thick (up to 0.3 mm in diameter), and light yellow (4A5) to brownish-yellow (5C8). They are directly connected to the mantle layer of the mycorrhiza system.

Anatomical characteristics

Rhizomorphs (Fig. 2). The rhizomorphs are 30–155 µm wide and composed of closely packed, thin-walled, septate and non-clamped hyphae. Individual hyphae are

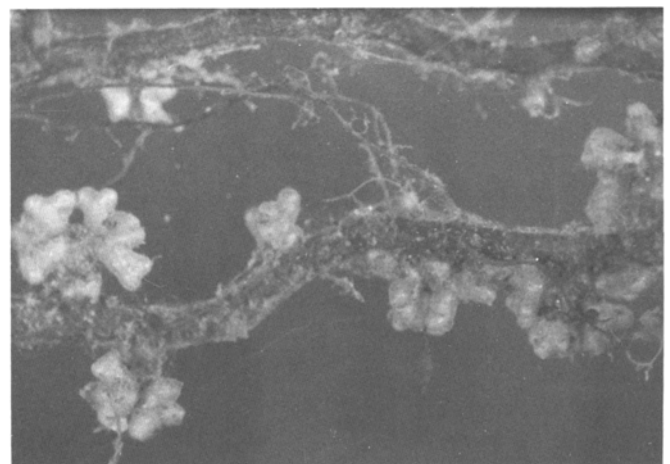


Fig. 1. Habit (morphology of mycorrhizas) of *Rhizopogon luteolus*, ×10

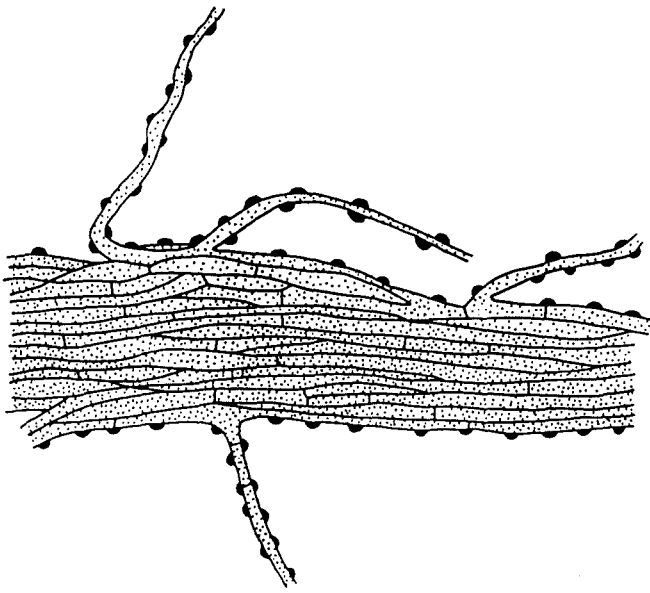


Fig. 2. Rhizomorph (*R. luteolus*), $\times 3$

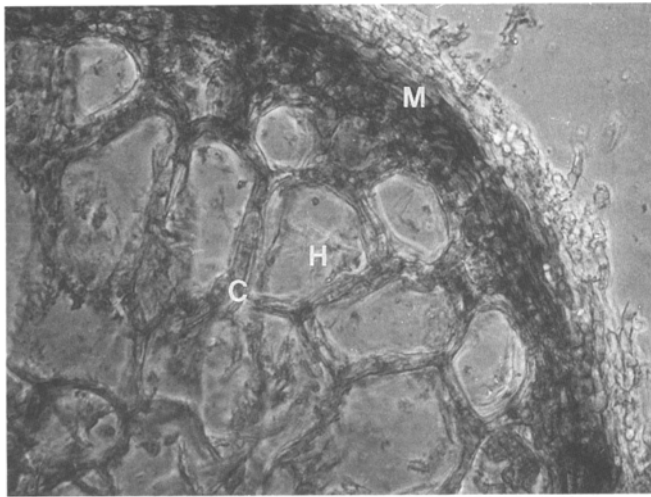


Fig. 3. Transverse section of root (*R. luteolus*), $\times 460$. C, Cortical cells; H, Hartig net cells; M, mantle

pale yellow (3A3) to yellowish-brown (5D8) in colour. Anastomoses between hyphae are occasionally present. Prominent, warty encrustations are present on the outer surface of the rhizomorph hyphae.

Mycorrhiza (cross section) (Fig. 3). The *mantle edge* is 20–45 μm deep and consists of plectenchymatous tissue. Pale whitish to yellowish-white emanating hyphae radiate from the surface of the mantle. The emanating hyphae are 20–110 μm long, mildly tortuous, thin-walled, septate and without clamp connections. There are no cystidia, setae or sclerotia.

Tannin cells are mostly in a single row, elongate, 25–45 \times 5–15 μm in size and yellowish-brown (5D8) to dark-brown (6F5) in colour. The Hartig net is composed of a single row of rectangular hyphal cells 3–4 μm thick.

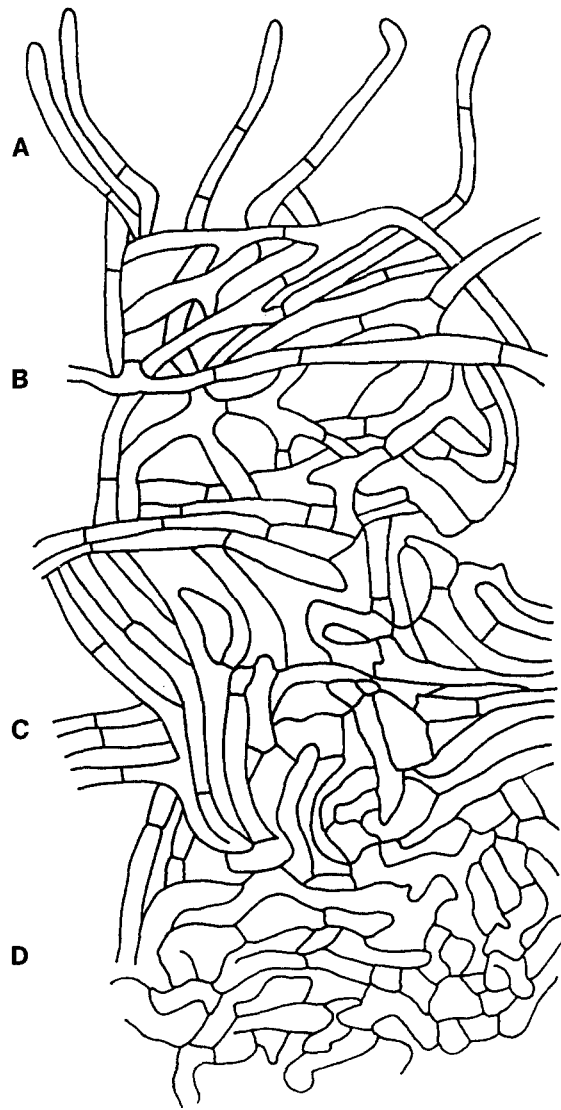


Fig. 4A–D. Diagrammatic plan or surface view details of mantle layers (*R. luteolus*). A Emanating hyphae, $\times 700$; B outer layer, $\times 880$; C middle layer, $\times 990$; D inner layer, $\times 770$

Cortical cells are broadly elliptical and measure 25–45 \times 20–40 μm in size. The Hartig net mainly consists of a single row of rectangular hyphal cells 3–4 μm thick 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 plectenchymatous tissue in which the hyphae are loosely woven. The individual hyphae of this layer are superimposed on each other and may be branched or unbranched. They are 3–6 μm in diameter, septate and without clamp connections. The middle layer is composed of a plectenchymatous tissue which is more compact than that of the outer layer. The cell walls of individual hyphae are up to 1 μm in thickness, and the hyphae are 3–6 μm in diameter, septate and without clamp connections. The inner layer consists of a net spongy tissue with elon-

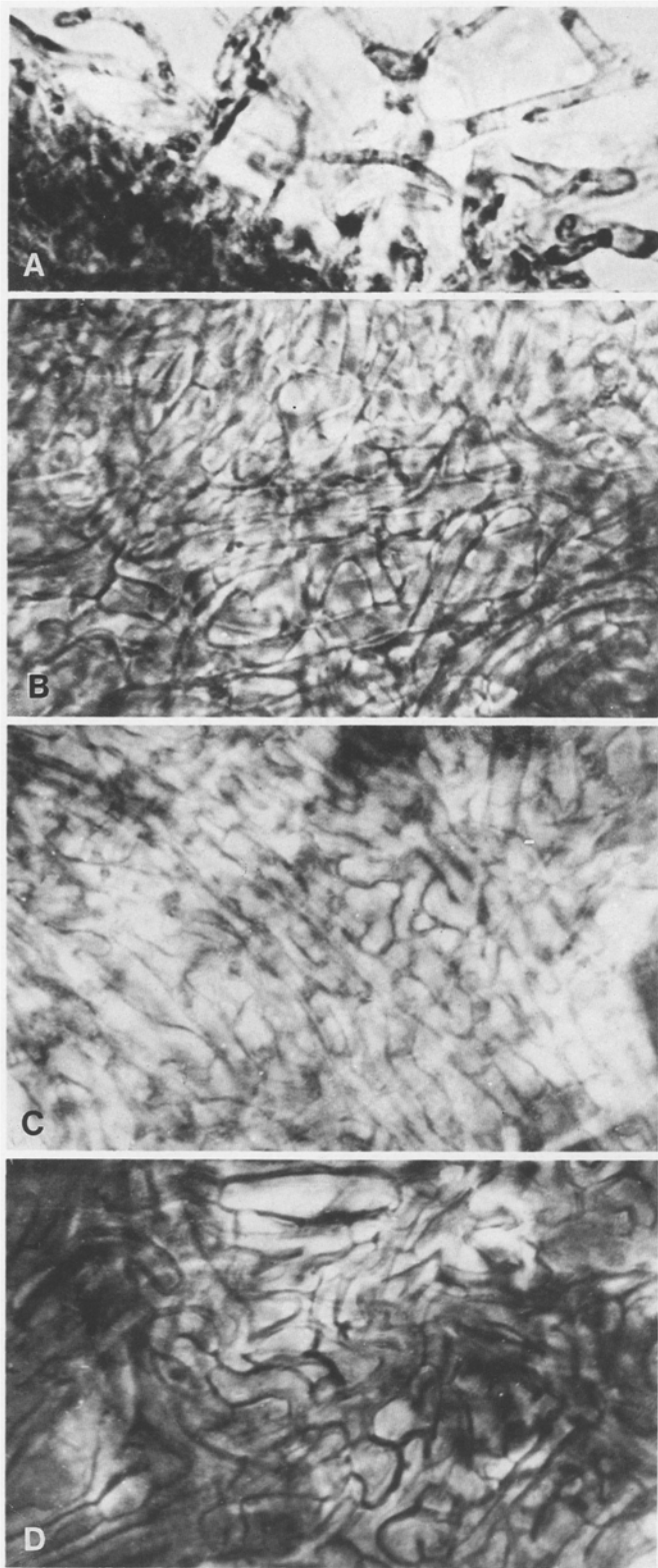


Fig. 5A-D. Plan or surface view details of mantle layers (*R. luteolus*). **A** Emanating hyphae, $\times 850$; **B** outer layer, $\times 1200$; **C** middle layer, $\times 1350$; **D** inner layer, $\times 1050$

gated, more densely packed hyphae 3–5 μm in diameter.

The distinguishing features of the mycorrhizas produced by *R. luteolus* are as follows:

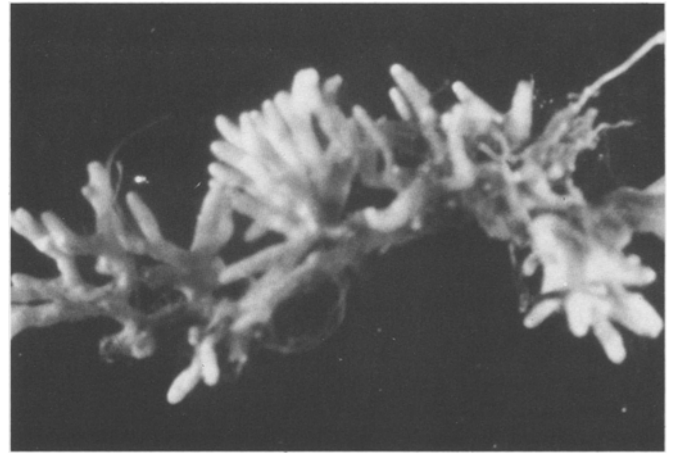


Fig. 6. Habit (morphology of mycorrhizas) of *Scleroderma citrinum*, $\times 10$

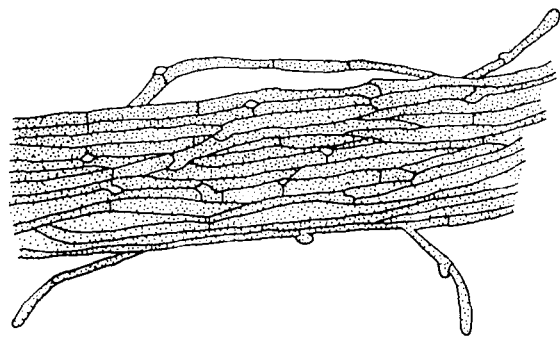


Fig. 7. Rhizomorph (*S. citrinum*), $\times 425$

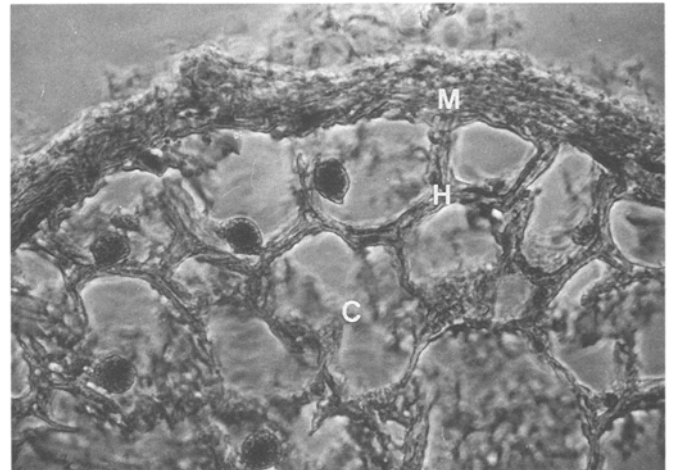


Fig. 8. Transverse section of root *S. citrinum*, $\times 500$. **C**, Cortical cells; **H**, Hartig net cells; **M**, mantle

1. The mycorrhizas are typically short, stubby and compactly branched coralloid forms. The surface is pubescent with fine sepia-coloured hyphae.
2. Rhizomorphs are abundant, directly connected to the mantle layer of the mycorrhizas and of the same colour as the mycorrhiza system. Prominent, warty encrusta-

tions are visible on the outer surface of the rhizomorphs when the hyphae are mounted in cotton blue.

3. The emanating hyphae radiating from the mantle surface are septate and without clamp connections.

Scleroderma citrinum

The ectomycorrhiza produced by *S. citrinum* Pers. are illustrated in Figs. 6–10.

Morphological characteristics

The mycorrhizas are generally bipodial and complex clustered coralloid structures, rarely mono- or tripodial. They are elongated, compactly branched and mildly tortuous, occasionally straight (Fig. 6). The main axis is 1.0–6.0 mm in length and 0.2–0.5 mm in diameter. The mycorrhizas are pale white when young and dull brown to brownish-orange (6C8) when old. The surface of the mycorrhizas is smooth when young and slightly granular to woolly or velvety when old. Rhizomorphs are abundant, long and tread-like, pure milky white to dull white in colour. They grow on the surface of the mycorrhizas with a few attached directly to the mantle layer. Some loose hyphae radiate from the surface of the rhizomorphs.

Anatomical characteristics

Rhizomorphs (Fig. 7). The rhizomorphs are 25–90 μm wide and composed of closely packed hyphae which are parallel, undulating, thin-walled and septate with frequent clamp connections. Individual hyphae are yellowish-white (4A2) to yellowish-grey (4B2). Anastomoses between hyphae are frequent. Some hyphae radiate from the main branch of the rhizomorphs and have distal ends swollen like cystidia. These radiating hyphae are similar in shape to the emanating hyphae of the mantle layer.

Mycorrhiza (cross section) (Fig. 8). The *mantle edge* is 15–50 μm deep and consists of a loosely woven, felt prosenchymatous tissue. Numerous hyaline to greyish-white (1B1) emanating hyphae emerge from the surface of the mantle. The emanating hyphae are 50–130 μm long, mostly straight (a few slightly tortuous) thin-walled and septate with clamp connections. The distal ends of the emanating hyphae are broader (4–7 μm in diameter) than those of the basal region (2–4 μm in diameter) and these swollen tips resemble cystidia. Otherwise there are no cystidia, setae or sclerotia.

Tannin cells are mostly in a single row (in a few cases in one or two rows), cylindrical to slightly elongate, 25–65 \times 10–30 μm in size and greyish-yellow (1B5) to yellow (2A6) in colour. The Hartig net is composed of a single row of oval to spherical hyphal cells 2–4 μm thick.

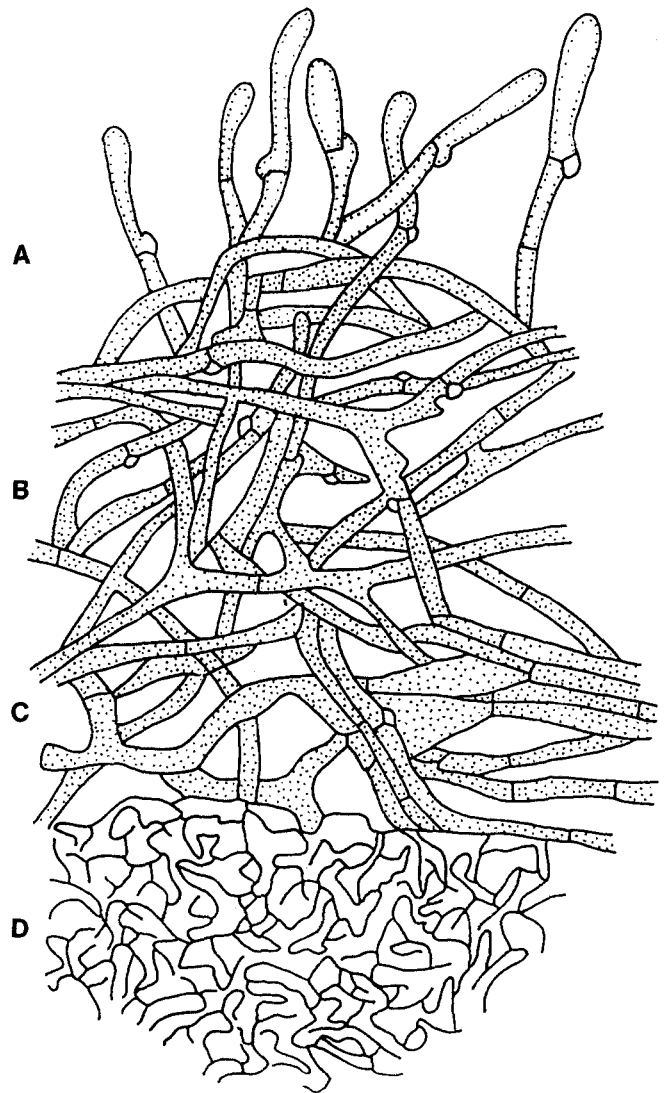


Fig. 9A–D. Diagrammatic plan of surface view details of mantle layers (*S. citrinum*). A Emanating hyphae, $\times 520$; B outer layer, $\times 725$; C middle layer, $\times 660$; D inner layer, $\times 680$

Cortical cells are broadly elliptical to slightly elongate, 30–70 \times 15–50 μm . The Hartig net is composed of a single row of oval to spherical hyphal cells 2–4 μm thick and penetrates up to three cortical cell layers deep.

Mycorrhiza mantle (plan or surface view) (Figs. 9, 10). 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, elongated, thin-walled, 3–5 μm in diameter and septate with frequent clamp connections. The middle layer consists of felt prosenchymatous tissue. The individual hyphae of this layer are loosely interwoven, 3–5 μm in diameter and septate with few clamp connections. The inner layer is composed of an irregular synenchymatous tissue with septate hyphae 2–4 μm in diameter.

The distinguishing features of the mycorrhizas produced by *S. citrinum* are as follows:

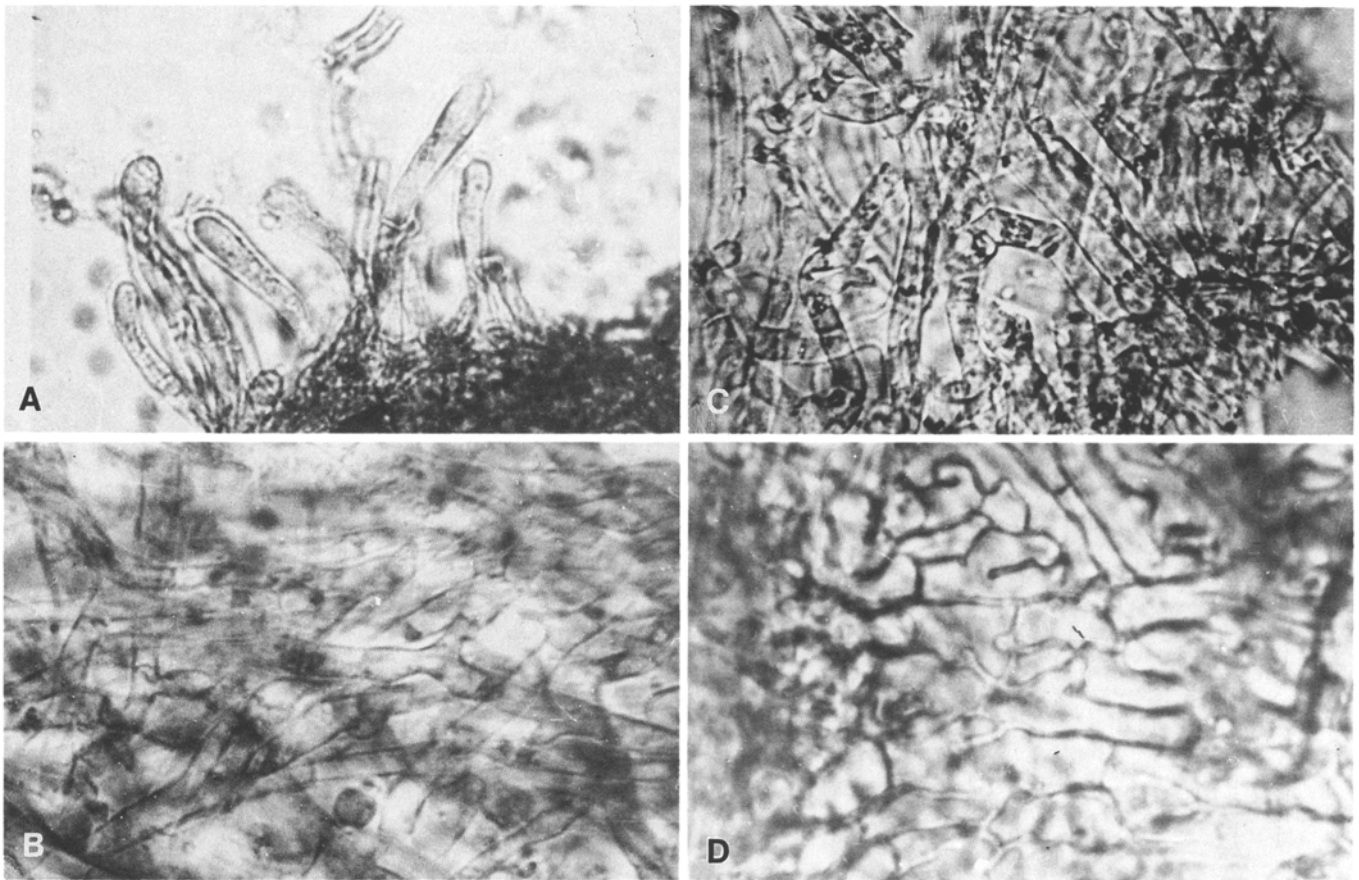


Fig. 10A-D. Plan or surface view details of mantle layers (*S. citrinum*). A Emanating hyphae, $\times 800$; B outer layer, $\times 1080$; C middle layer, $\times 990$; D inner layer, $\times 1090$

1. The mycorrhizas are usually dichotomously branched to complex clustered forms, pale white to brownish-orange.

2. Rhizomorphs are abundant, white and grow on the surface of the mycorrhizal system with a few directly connected to the mantle layer. The hyphae radiating from the surface of the rhizomorphs have distinct, swollen, cystidia-like distal ends, and are similar in shape to the emanating hyphae of the mantle layer.

Discussion

This is the first detailed study of the mantle structure and associated hyphae of ectomycorrhizas produced by *S. citrinum* in any tree species.

The rhizomorphs of the mycorrhizas produced by *R. luteolus* and *S. citrinum* are very characteristic. In the case of *R. luteolus*, the most characteristic feature of the rhizomorphs is the prominent, warty encrustation of the outer surface of the hyphae (Fig. 2). In the case of *S. citrinum*, the most characteristic feature is the cystidia-like radiating hyphae (Fig. 7). These radiating hyphae are similar to the emanating hyphae of the mantle (Figs. 9A, 10A).

Pachlewski and Pachlewska (1974) and Uhl (1988) carried out detailed studies of the mycorrhizas produced

by *R. luteolus* in *Pinus sylvestris*. The observations in the present study agree with their findings in both the morphology and anatomy of the mycorrhizas and rhizomorphs with minor variations. They found warty encrustations like those of the rhizomorphs also on the mantle hyphae, whereas in the present study the mantle hyphae were smooth. Further, Uhl (1988) found that the hyphae of the rhizomorphs were differentiated into outer, central and inner layers with different diameters and cell-wall thicknesses. The rhizomorphs associated with the mycorrhizas produced by *R. luteolus* in *P. patula* showed no such hyphal differentiation into different layers.

Apart from the characteristic rhizomorph structure, the mantle layer in the mycorrhizas produced by *R. luteolus* in *P. patula* consists of three distinct layers: an outermost felt prosenchymatous tissue, a middle layer of net prosenchymatous tissue and an inner net synenchymatous tissue (Figs. 4 and 5). Pachlewski and Pachlewska (1974) and Uhl (1988) in their studies on *P. sylvestris* mycorrhizas produced by *R. luteolus* found only a two-layered mantle with an outer, densely packed, net-like plectenchymatous layer and an inner, loosely woven hyphal layer. The same type of two-layered mantle was also found by Fontana and Centrella (1967) in the mycorrhizas of *P. sylvestris* produced by *Rhizopogon rubescens*.

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

References

- Fontana A, Centrella E (1967) Ectomycorrhizae produced by hypogeous fungi. *Allionia* 13:149-176
- Mohan V, Natarajan K, Ingleby K (1993) Anatomical studies on ectomycorrhizas. I. The ectomycorrhizas produced by *Telephora terrestris* on *Pinus patula*. *Mycorrhiza* 3:39-42
- Pachlewski R, Pachlewska J (1974) Studies on symbiotic properties of mycorrhizal fungi of pine (*Pinus sylvestris* L.) with the aid of the method of mycorrhizal synthesis in pure cultures on agar. Forest Research Institute, Warsaw
- Uhl M (1988) Studies on mycorrhizae. XV. Mycorrhizae formed by *Rhizopogon luteolus* on *Pinus sylvestris*. *Persoonia* 13:449-458