Mycorrhiza (1993) 3:39–42

Mycorrhiza

Anatomical studies on ectomycorrhizas

I. The ectomycorrhizas produced by *Telephora terrestris* 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 *Thelephora terrestris* 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.

Key words: *Pinus patula* – *Thelephora terrestris* – Ectomycorrhizas – Rhizomorphs – Mantle structure

Introduction

It is now generally felt that there is a need for a proper classification of the ectomycorrhizas. This applies more to the fungal partner since it is easy to identify the host plant. Efforts in this direction have been made e.g. by Dominik (1956, 1961, 1969), Chilvers (1968) and Zak (1971, 1973), and more recently by Agerer and his coworkers (1986a, b; 1987a, b, c; 1988a, b; Agerer and Weiss 1989) and by Ingleby et al. (1990). Hundreds of species of ectomycorrhizal fungi are involved and this task is not very easy. Most of the classification is based on the gross morphology of the mycorrhizas and additional anatomical details, with earlier workers relying mainly upon cross sections of the ectomycorrhizas. More recently, the plan or surface view of the mantle has also been studied using various staining and squashing techniques. These details, together with the sterile structures found on the outer surface of the mantle, e.g. cystidia and setae, are of additional value. Thus a new classification system is evolving through the work of several people with different plant species. In the present work, an attempt has been made to study the structure of the mantle in the ectomycorrhizas produced by *Thelephora terrestris* on *Pinus patula* seedlings growing in forest nurseries in the Nilgiri Hills, Tamilnadu, India. This is the first detailed description of the mantle structures of ectomycorrhizas in *P. patula*.

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Materials and methods

Isolation and selection of distinct ectomycorrhizas

The ectomycorrhizal samples were collected from 5-month-old, nursery-raised *P. patula* seedlings grown in autoclaved *shola* soils inoculated with basidiospores of *T. terrestris*. The seedlings with mycorrhizas were soaked in water and the soil debris removed with fine needles, forceps and brushes. Based mainly on the colour of the mycorrhizal mantle and form of branching, morphologically distinct mycorrhiza pieces were selected, detached from the root system of the seedlings and separated into groups under the dissecting microscope.

Photographs of fresh mycorrhizas were taken using a Wild M3 stereo microscope. Some fresh mycorrhizas were placed in sterile distilled water and stored at 4° C for studying the plan or surface views of the mantle layers. Other samples were preserved in formalin/acetic acid/alcohol for the preparation of transverse sections.

Characters used in the identification of ectomycorrhizas

Morphological features of the mycorrhizas, i.e. colour, form, surface texture of the mantle, attached mycelium and rhizomorphs, were examined under the dissecting microscope. Anatomical features of the ectomycorrhizas were studied by taking transverse sections. The plan or surface view was studied using squashing and peeling techniques. The methods and terminologies of Chilvers (1968), Zak (1973), Agerer (1986a, 1988b) and Ingleby et al. (1990) were followed for the study of both macroscopic and microscopic features and for descriptions of the mantle layers. The colour terminology used is that of Kornerup and Wanscher (1978).

Results

The ectomycorrhiza produced by *T. terrestris* Pers.: Fr. are illustrated in Figs. 1 to 5.

Morphological characteristics

The mycorrhizas are mostly bipodial (Fig. 1), rarely monopodial when young and becoming compactly clus-

Fig. 1. Habit (morphology of mycorrhizas) ×10

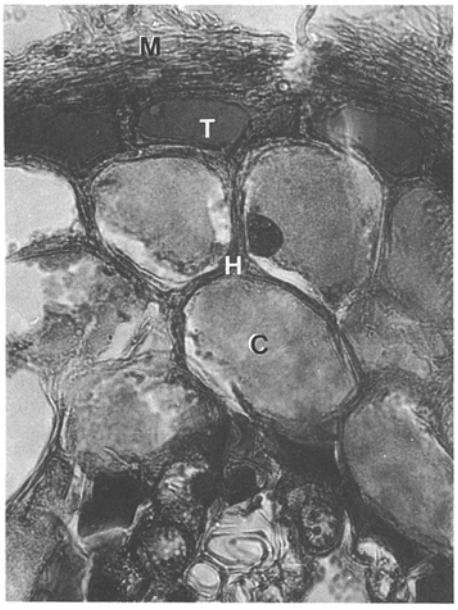

Fig. 3. Transverse section of root ×445. *C*, Cortical cells; *H*, Hartig net cells; *M*, mantle; *T*, tannin cells

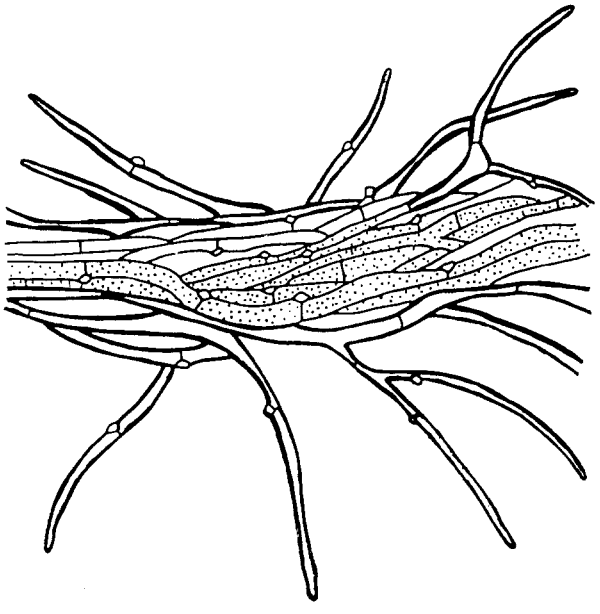

Fig. 2. Rhizomorph ×640

tered when old. Both mycorrhizal types are straight to slightly bent. Monopodial forms are slightly shorter and thinner (up to 6.7×0.6 mm) when compared to the bipodial mycorrhizas (up to 9.0×1.0 mm). The mycorrhizas are pale yellowish-brown (5D8) to pale-brown (7D8) in colour when young and dark-brown (7F8) when old. They are smooth with loose, straight hyphae emerging over the whole surface. Rhizomorphs are abundant, thread-like and growing on the surface of the mycorrhizal system; a few are attached directly to the mantle layer of the mycorrhizas. Rhizomorphs are similar in colour to the mycorrhiza system. Individual hyphae of the rhizomorphs are compactly arranged and are pale-brown (7D6) to dark-brown (7D8) in colour. Some loose hyphae radiate from the surface of the rhizomorphs.

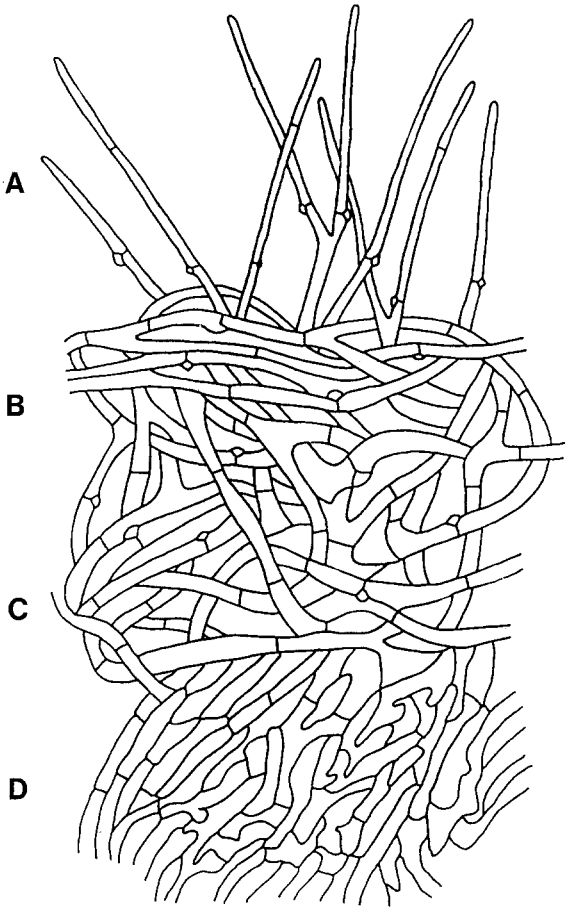

Fig. 4A–D. Diagrammatic plan or surface view details of mantle layers. **A** Emanating hyphae, ×480; **B** outer layer, ×1320; **C** middle layer, ×1280; **D** inner layer, ×1980

Anatomical characteristics

Rhizomorphs. The rhizomorphs (Fig. 2) are 150–650 µm wide, and composed of closely packed, parallel hyphae covered with radiating hyphae. Two types of hyphae are present: The hyphae of the central part are slightly wider (6–10 µm) compared to those in the outer part (3–6 µm). The cell walls of the central hyphae are thinner (less than 0.5 µm) than those of the outer, surrounding, thick-walled hyphae (1–2 µm). Anastomoses between hyphae are frequent. Both the thin-walled central hyphae and thick-walled outer hyphae are septate with clamp connections. Septa and clamp connections are closer in the central, thin-walled hyphae than in the outer thick-walled hyphae. The hyphae radiating from the surface of the rhizomorphs are pale-brown (7D6) to dark-brown (7D8) and are similar in shape to the emanating hyphae of the mantle layer.

Mycorrhiza (cross section) (Fig. 3). The *mantle edge* is 10–30 µm deep and consists of a simple prosenchymatous tissue. Numerous, hyaline to pale-brownish, emanating hyphae radiate from the surface. The emanating hyphae are up to 150 µm long, mostly straight, with the distal ends sometimes slightly or distinctly bent. The emanating hyphae are typically branched near the mantle layer and are septate with clamp connections mostly in the basal part. The surface of the hyphae is smooth. The hyphae are 3–5 µm in diameter basally and 1–2 µm in diameter distally. There are no cystidia, setae or sclerotia.

Tannin cells are mostly in a single row, elliptical to elongate, pale-yellow (3A3) to yellowish-brown (5D8) and are 25–55 µm × 10–30 µm in size. The Hartig net is composed of a single row of oval to rectangular hyphal cells 3–6 µm thick.

Cortical cells are round or slightly elliptical and 20–70 µm × 15–50 µm in size. The Hartig net is composed of a single row of oval to rectangular hyphal cells 3–6 µm and penetrates to a depth of up to three cortical cell layers.

Mycorrhiza mantle (plan or surface view) (Figs. 4, 5). The mycorrhizas in plan view have three mantle layers. The outer layer is composed of a net prosenchymatous tissue with loosely interwoven hyphae 3–8 µm in diameter and septate with clamp connections. The middle layer consists of a net prosenchymatous tissue with densely packed hyphae 4–10 µm in diameter and septate with infrequent clamp connections. The inner layer is composed of an irregular synenchymatous tissue with compactly arranged hyphae 2–6 µm in diameter.

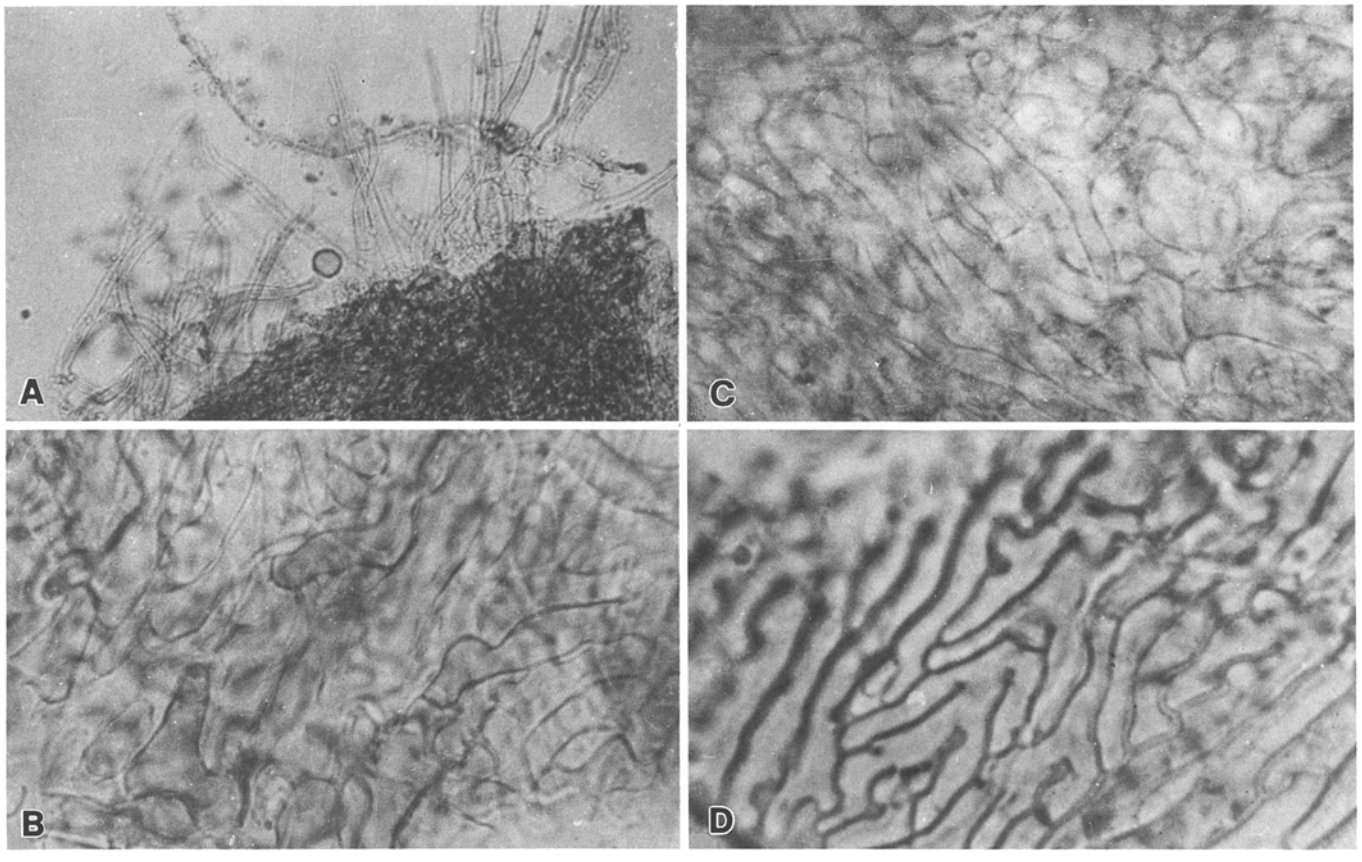

Fig. 5A–D. Plan or surface view details of mantle layers. **A** Emanating hyphae, ×370; **B** outer layer, ×1150; **C** middle layer, ×980; **D** inner layer, ×1520

Discussion

The structure of the ectomycorrhizas in *P. patula* produced by *T. terrestris* in artificially inoculated plants found in the present study agrees in several respects with earlier studies on the ectomycorrhizas produced by this fungus in other tree species.

The rhizomorphs of the mycorrhizas produced by *T. terrestris* are very characteristic and are made up of two distinct types of hyphae. The hyphae present in the central core are thin-walled and wide whereas the hyphae forming the outer layers are thick-walled and narrow (Fig. 2). The hyphae radiating from the outer surface of the rhizomorphs are very similar to the emanating hyphae present on the surface of the mycorrhizas, i.e. they are thin-walled, septate and have abundant clamp connections. This type of rhizomorph with a very characteristic structure was described earlier by Agerer and Weiss (1989) for mycorrhizas produced by *T. terrestris* on *Picea abies*, and by Chu-Chou and Grace (1983b) for mycorrhizas produced by *T. terrestris* on *Pinus radiata*. These authors also found a central core of wide, thin-walled hyphae and an outer covering of narrow, thick-walled hyphae. The similarity between the radiating hyphae in the rhizomorphs and the mantle was also noticed by Agerer and Weiss (1989) and Thomas and Jackson (1979) in the case of mycorrhizas produced by *T. terrestris* on *Picea sitchensis*.

Apart from the rhizomorph structure, another feature characteristic of the mantle of the mycorrhizas produced by *T. terrestris* is the presence of branched emanating hyphae on the surface, each branch possessing a basal clamp connection (Fig. 4A). Similar emanating hyphae in the mantles of mycorrhizas produced by *T. terrestris* have been described by Chu-Chou and Grace (1983a) in *Pseudotsuga menziesii*. Thomas and Jackson (1979) found two types of emanating hyphae in the mantle of the mycorrhizas of *T. terrestris* with *Picea sitchensis*, one long type which had clamp connections in the distal septa and the other shorter with a single clamp connection. These types of hyphae are absent from *P. patula*. Similarly, Agerer and Weiss (1989) found cystidia on the surface of the mantle in mycorrhizas of *Picea abies* which were also not observed in the present study.

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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