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Structure and Function
Trees

Some distinctive types of spruce mycorrhizae

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Summary. For examination and recognition of mycorrhizal types, the structure of the hyphal mantle in tangential longitudinal sections has proved to be a valuable feature for investigation. Features of hyphal mantle structure have been used for establishing an identification key. The colour of the hyphal mantle is a helpful additional characteristic, but is not useful as a diagnostic feature because of variations in advanced age and under varying environmental conditions. Rhizomorphs yield good taxonomic characteristics. The Hartig net was uniform for all types examined. When differences were observed they had to be interpreted as different stages of development. Ultrastructural examination revealed further features of mycorrhizal types: namely the shape of septal pores, presence and structure of matrix material in the region of the hyphal mantle and characteristic deposits on cell walls of the hyphae. The ten types presented can be distinguished without any ultrastructural features. As far as the fungal partners involved are concerned, basidiomycetes are clearly dominant. For the first time two basidiomycetes with continuous parenthesomes of their dolipores were found to form ectomycorrhizae. Classification of these fungi with the heterobasidiomycetes is discussed.

Key words: *Picea abies* – Mycorrhizal types – Hyphal mantle structure

Introduction

In view of the multitude of mycorrhizal fungi it cannot be assumed that all fungi have the same effect on their hosts. Differences with regard to uptake, capacity of storage and transfer of nutrients, protection from pathogens and promotion of the host's growth may be expected and some have already been proved (Bowen and Theodorou 1967; Sinclair et al. 1975; Richard et al. 1971; Perrin and Garbaye 1983; Chu-Chou and Grace 1985).

It is therefore not only important for a tree that its roots are mycorrhizal but the partner with which it is associated is probably also significant. The identification of the mycorrhizal fungi involved is difficult, as their identification and characterization is normally carried out with the help of sexual stages, the fruiting bodies. Frequently, characterization by the asexual hyphal stage is not possible as it lacks distinctive features. Nevertheless, it is necessary to characterize types of mycorrhizae in order to understand differences in the association of mycorrhizal types in certain habitats, distribution and possible reactions of mycorrhizal types to environmental changes. Several investigations listing distinctive features suitable for the characterization of mycorrhizal types have already been published (Trappe 1967; Zak 1973; Agerer 1986). It is now, however, necessary to test these features to determine whether they are useful in describing a great number of mycorrhizal types.

This paper presents ten mycorrhizal types (nos. 8–17). Seven other types (nos. 1–7) have already been published (Haug et al. 1986). Characteristics used for description are discussed. A key for all 17 types is added to simplify identification.

Materials and methods

Root samples were taken from five stands of 60- to 80-year-old spruce trees (*Picea abies* (L.) Karst.) near Freudenstadt in the

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Northern Black Forest. Depths profiles about 1 m deep and 70 cm wide were dug in order to be able to investigate mycorrhizae from deeper strata of the soil. Roots and soil were kept in a plastic box at 4° C until they were further investigated. Soil and mycorrhizae were separated by wet-sieving. Pictures of the mycorrhizae were taken under a stereo microscope. Then the mycorrhizae were fixed in glutaraldehyde with phosphate or arsen buffer. Postfixation was carried out with osmium tetroxide and en bloc contrasting with uranyl acetate; ERL (Spurr 1969) was used as the embedding medium. Serial longitudinal semi-thin sections (0.5–2 μm) for light microscopy were cut from the outer region of the hyphal mantle to the middle of the mycorrhizae. For electron microscopy ultrathin median longitudinal sections (80–120 nm) were used. Semithin sections were stained with Neufuchsin-Kristallviolett and ultrathin sections were contrasted with lead citrate.

Results and discussion

Terminology for description of hyphal mantle structures

Chilvers' terminology (1968 a) is used in describing hyphal mantle structures (see also Zak 1973; Godbout and Fortin 1985). The term "prosenchyma" represents a moderatly compact tissue in which the individual hyphal elements are clearly distinguishable and relatively large interhyphal spaces exist. A "synenchyma", called pseudoparenchyma by some authors, is a tissue with little obvious interhyphal spaces in which wide hyphae are subdivided into short cells. Extensions of hyphae cannot be observed over long distances. The term "prosenchyma" and "synenchyma" present the outermost points and there are many transitional and intermediate stages. Thus it is difficult to work only with these two terms; further distinctions make it easier to give clear descriptions. A prosenchyma with distinct and large interhyphal spaces is called "loose prosenchyma". In a "compact prosenchyma" the normal shape of the hyphae is also distinct and their extensions can be followed, but there are hardly any interhyphal spaces. Subdivisions of "synenchyma" are related to the shape of the hyphal cells. In an „irregular synenchyma" they are oval or longish and vary much in size; in a "puzzle synenchyma" they have sinuate walls; and, finally, in a "polygon synenchyma" they are approximately isodiametric with fairly straight walls (Figs. 1–12).

Marking of the types

The types described are numbered consecutively (8–17). Striking features and characteristic differences between the individual types are used for nomenclature.

Discussion of features used for the characterization

Characterization by means of colours. The applicability of the colour of the hyphal mantle as a feature for differentiation is confusing due to two facts:
1. Mycorrhizae of the same colour, which cannot be distinguished with the stereo microscope, reveal distinctly different hyphal mantle structures in section (see types 1 and 2 with transparent hyphal mantle and surrounding white mycelium).
2. Types of the same hyphal mantle structure vary in colour, depending on their age.

Marks (1965) and Trappe (1967) comment on the problematic nature of colour and they consider the colours of the different stages of development vary too much to be useful.

In some cases cortex cells shine through the hyphal mantle. Their vacuolar deposits have an influence on the colours of mycorrhizae, which raises additional problems. Therefore Dominik (1969), who uses colour as the distinctive feature in his key, warns not to mistake the colour of the tannin layer for that of hyphal mantle.

Like Dominik, Zak (1973) does not consider colour too problematic. In his opinion, it is stable in most cases and clearly defined or subject to particular changes which can also be defined. Besides, it does not vary more than other biological material, and may be even less than certain fruiting bodies.

According to our experience, colour as a distinctive feature remains problematic as, in addition, each observer's visual impression is different and depends on the illumination used. The use of colour charts is very difficult. However, as a feature of description, colour or variations in colour should be noted.

Characterization of mycorrhizae by means of the mantle structures. Tangential longitudinal sections in various regions of the hyphal mantle are suitable to comprehend the structure of the hyphal mantle. Neither under various environmental conditions nor with ageing does the structure of the hyphal mantle change considerably (Haug 1987).

When we compare a loosely prosenchymatic hyphal mantle with a synenchymatic puzzle-shaped one, characterization with the help of these features seems to be promising. Limits become obvious, however, when two different forms with synenchymatic and puzzle-shaped hyphal mantle are compared. If the colour of the mycorrhiza is not taken into consideration, types 16 and 17 differ merely in their proportion of the synenchymatic layer in the hyphal mantle: while it makes up almost the whole hyphal mantle of type 16, it turns quite quickly into the prosenchymatic portion in type 17. The question arises whether such proportions are stable. For the mycorrhizae examined, great variations were not noted with these two types, but this result cannot be transferred automatically to other types. With the help of colour, these two types can be easily distinguished.

Characterization of the mycorrhizae by means of rhizomorphs. Rhizomorphs are useful for describing mycorrhizae (Haug et al. 1986, types 1, 2, 5). Various kinds of hyphal association, different diameters of the hyphae and shapes of septa (with or without clamp connections) offer many distinguishing features and allow delineation of many types. Godbout and Fortin (1985) describe seven types of rhizomorphs; Agerer et al. (1986) present six possible kinds of organization.

Characterization of mycorrhizae by means of the Hartig net structure. Various authors have tried to use the Hartig net for characterization of mycorrhizae (e.g., Marks 1965; Chilvers 1968a; Zak 1973). They refer to depth of penetration of the Hartig net between the cortical cells, to the diameter of hyphae and to intracellular penetration into cortex cells. The features listed above do not seem to be very useful, as they all depend on the stage of development of the Hartig net. With older stages of the Hartig net, it is often possible to observe hyphae with very large diameters in the outer cortex region, while they are much more narrow in the inner region of the cortex, near the endodermis. If cortex cells are dead, hyphae will often penetrate into them.

According to investigations of four mycorrhizal types by Blasius et al. (1986), the Hartig net is always structured in the same way. The main growth direction of hyphae is transversal to the root axis between the cortex cells. Examination of the Hartig net of the 17 types described here confirmed this structure.

Differences were found in the way the hyphae were septate in the section of the Hartig net. Frequently occurring septa made some of the types (e.g., type no. 9) stand out. Unfortunately, this is also a relative characteristic, as in ultrathin sections the course of an individual hypha can only be observed over very short distances.

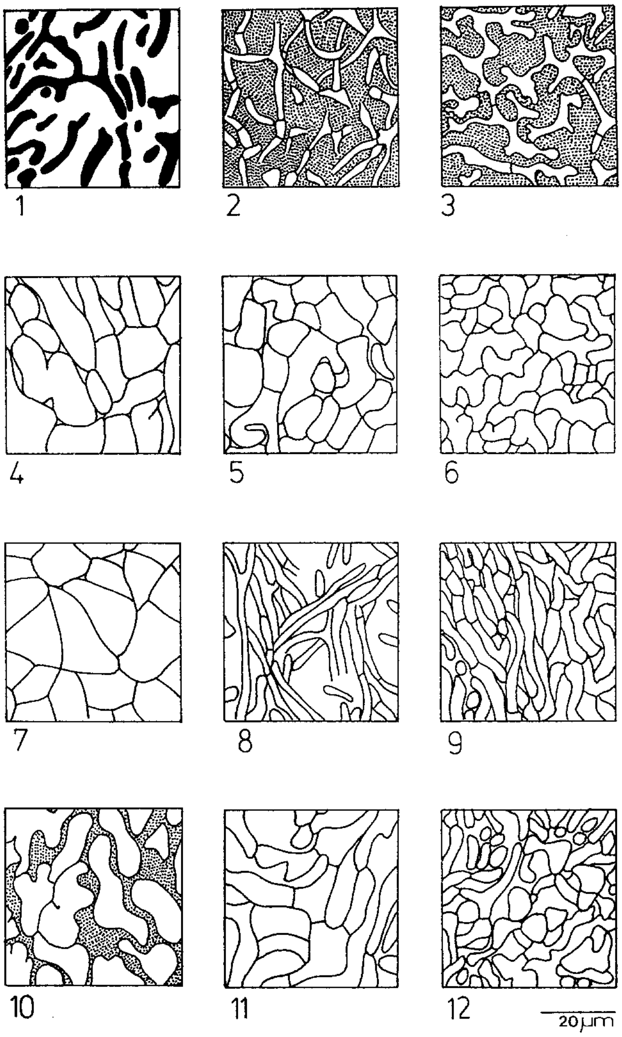
Characterization of the mycorrhizae by means of ultrastructural features. Since the beginning of ultrastructural examinations of ectomycorrhizae in the late 1960s (Chilvers 1968b; Scannerini 1968; von Hofsten 1969), these investigations have made considerable progress. The results, however, were scarcely used for classification of ectomycorrhizae. Many mycorrhizae were ultrastructurally examined in the course of investigations of differently damaged stands of spruces in the Black Forest (Haug et al. 1986; Haug 1987). Thus the possibilities as well as limits of transmission electron microscopic investigation with regard to "new" features can be assessed. The advantages of recognizing details such as septal pores, matrices, deposits on cell walls and states of cytoplasm are obvious. According to investigations by Khan and Kimbrough (1982) and Oberwinkler (1985) the shape of septal pores makes systematic classification possible. Small, but very characteristic deposits on cell walls (Haug et al. 1986, type 2) can only be recognized with transmission electron microscopy. Information on the plasmatic state of hyphae and cortex cells indicates the age and physiological activity of the mycorrhizae. As these stages depend very much on their state of development, they are not suitable for the characterization of mycorrhizae. If Zak's (1973) criteria were to be applied, costly preparation and cutting technique would be a drawback, as Zak particularly favours features which can be examined quickly and without any problems. Thus the question arises whether the mycorrhizal types described can be clearly distinguished without any ultrastructural features. This is possible, but every additional clear feature makes identification and recognition easier.

Key for described mycorrhizae (types 1–7 described in Haug et al. 1986)

1 mycorrrhizae with rhizomorphs — 2
1* mycorrhizae without rhizomorphs — 4

2 hyphal mantle compactly prosenchymatous, hyphal diameter range 3.5–7 µm; rhizomorphs smooth, hyphal walls sticking tightly together, large-diameter hyphae in the middle of the rhizomorphs — **Type 2**
2* hyphal mantle wholly composed of loose prosenchyma — 3

3 hyphae of rhizomorphs and hyphal mantle without clamp connections; rhizomorphs with intensive yellow colouring, hyphal mantle yellow or white **Type 1** (= *Picea abies* – *Piloderma croceum* (Haug 1987)



Figs. 1–12. Longitudinal sections within hyphal mantle

Figs. 1–3. Loose prosenchymas, whose interhyphal spaces are filled with slimy matrix material. **Fig. 4.** Compact prosenchyma with large-diameter hyphae. **Figs. 5–7.** Synenchymatous hyphal mantle structures. **Fig. 5.** Irregular synenchyma. **Fig. 6.** Puzzle synenchyma. **Fig. 7.** Polygon synenchyma. **Fig. 8.** Loose prosenchyma. **Fig. 9.** Compact prosenchyma. **Fig. 10.** Puzzle structures with matrix-filled interhyphal spaces. **Fig. 11.** Loose prosenchyma with large diameter hyphae. **Fig. 12.** Relatively compact prosenchyma with oval to longish shapes

3* hyphae of rhizomorphs and hyphal mantle with clamp connections and little needle-like appositions; hyphal mantle greenish yellow **Type 5**

4 hyphae of the whole hyphal mantle embedded in slimy matrix material 5
4* hyphae of the hyphal mantle embedded only partly in slimy matrix material or without any matrix 7

5 hyphae with bright colouring in darker matrix, arrangement of hyphae clearly visible 6
5* hyphae with dark colouring in brighter matrix material (Fig. 1), often branched in Y-like way, wound in circle-like shape forming spiral to labyrinthic patterns, surface of mycorrhizae smooth, wax-like, colour of mycorrhizae varying between white, yellow and ochre-brown

Piceirhiza gelatinosa (Gronbach and Agerer 1986; Haug 1987)

6 Hyphal mantle overlaid by a net-like hyphal layer; hyphae in the middle and inner regions of the mantle branched in an antler-like way (Fig. 3), hyphal mantle transparent with slightly silvery coating; ascomycete **Type 6**
6* hyphal mantle surrounded by a compact layer of slime, hyphae branched in a star-like manner (Fig. 2); mycorrhizae yellowish to transparent; basidiomycete with non-perforate parenthesomes **Type 10**

7 hyphal mantle with clearly synenchymatous section (Figs. 5–7) 8
7* hyphal mantle prosenchymatous throughout (Figs. 8, 9) 12

8 hyphal mantle with polygon-synenchyma (Fig. 7) 9
8* hyphal mantle with puzzle-synenchyma (Fig. 6) 10

9 hyphal mantle yellowish-orange (in advanced age yellowish-green), with brighter protuberances of dead hyphae **Type 3**
9* hyphal mantle dark brown, rough surface; with few hair-like and rigid hyphae leaving the mycorrhizae **Type 4**

10 outer puzzle hyphae embedded in matrix material (Fig. 10), region of root tips with cystidia, ascomycete **Type 15**
10* puzzle-synenchyma without any matrix material, no cystidia, basidiomycete 11

11 hyphal mantle, except for the inner layer, with puzzle structure; overlaid by a net-like layer of dead hyphae, which may form protuberances; just above the epidermal layer puzzle synenchyma merges into a compact prosenchyma; mycorrhizae whitish to transparent **Type 16**
11* outer third of the hyphal mantle with puzzle structure, inner regions loosely prosenchymatous, hyphae interwoven with each other; puzzle synenchyma changing quickly to prosenchymatous tissue **Type 17**

12 hyphal mantle with cystidia 13
12* hyphal mantle without cystidia 14

13 hyphae in the compact prosenchymatous region of the same diameter, straight, rarely septate, forming weaving patterns, cystidia with thick walls **Type 12**
13* hyphae in the compact prosenchymatous region often septate, hyphae wide or narrow in diameter, slight tendency to puzzle synenchyma **Type 13**

14 outer region of hyphal mantle loosely prosenchymatous (Fig. 8), inner region compactly prosenchymatous (Fig. 9) 16
14* hyphal mantle loosely prosenchymatous, thin 15
14** hyphal mantle compactly prosenchymatous, outer hyphae embedded in matrix, mycorrhizae transparent, many transparent hyphae with spherical wall appositions emerge from the hyphal mantle **Type 7**

15 some hyphae wide in diameter (up to 11 μm), some lobed, richly branched (Fig. 11), hyphal mantle transparent, dolipore septa with non-perforate parenthesomes **Type 8**
15* hyphae equal in diameter (3.5–4.5 μm), moderately septate, straight, hyphal mantle transparent, many dolipore septa with perforate parenthesomes in the Hartig net region **Type 9**

16 hyphae in the loosely prosenchymatous outer region embedded in matrix, hyphae interwoven, only a few hyphal layers thick; hyphal slices in the compact prosenchymatous region oval to longish (Fig. 12) **Type 14**
16* loosely prosenchymatous region without any matrix, hyphal mantle many hyphal layers thick, consisting of vital hyphae, hyphae with clamp connections; hyphal mantle white **Type 11**

Description of types

Type 8: Prosenchymatous type with large-diameter, short septate hyphae

Macroscopic characteristics (Fig. 13)
Colour: hyphal mantle hyaline, cortex cells shining through, therefore mycorrhiza light at the root tip, brownish to the base; surface slightly woolly; hyphal mantle thin and inconspicuous.

Microscopic characteristics
Hyphal mantle without differentiation into distinct layers, wholly composed of loose prosenchyma (Fig. 23d), hyphal diameters range 3.5–11 μm, hyphae subdivided into short cells by septa without clamp connections (Fig. 23b, c); hyphae with darkly pigmented walls near surface of the hyphal mantle and a diameter of about 3.5 μm (Fig. 23a); hyphal mantle typically 20 μm thick;
Hartig net in the region of the outer cortex cells with large-diameter hyphae.

Ultrastructural characteristics
Dolipores with non-perforate parenthesomes (Fig. 23e); no matrix in the region of the hyphal mantle (Fig. 23g); partly intracellular infections of cortex cells (Fig. 23f).

Type 9: Prosenchymatous type

Macroscopic characteristics (Fig. 14)
Colour: hyphal mantle colourless, hyaline;
surface slightly glossy, hyphal mantle thin and inconspicuous, a lot of loose hyphae surrounding the mycorrhizae.

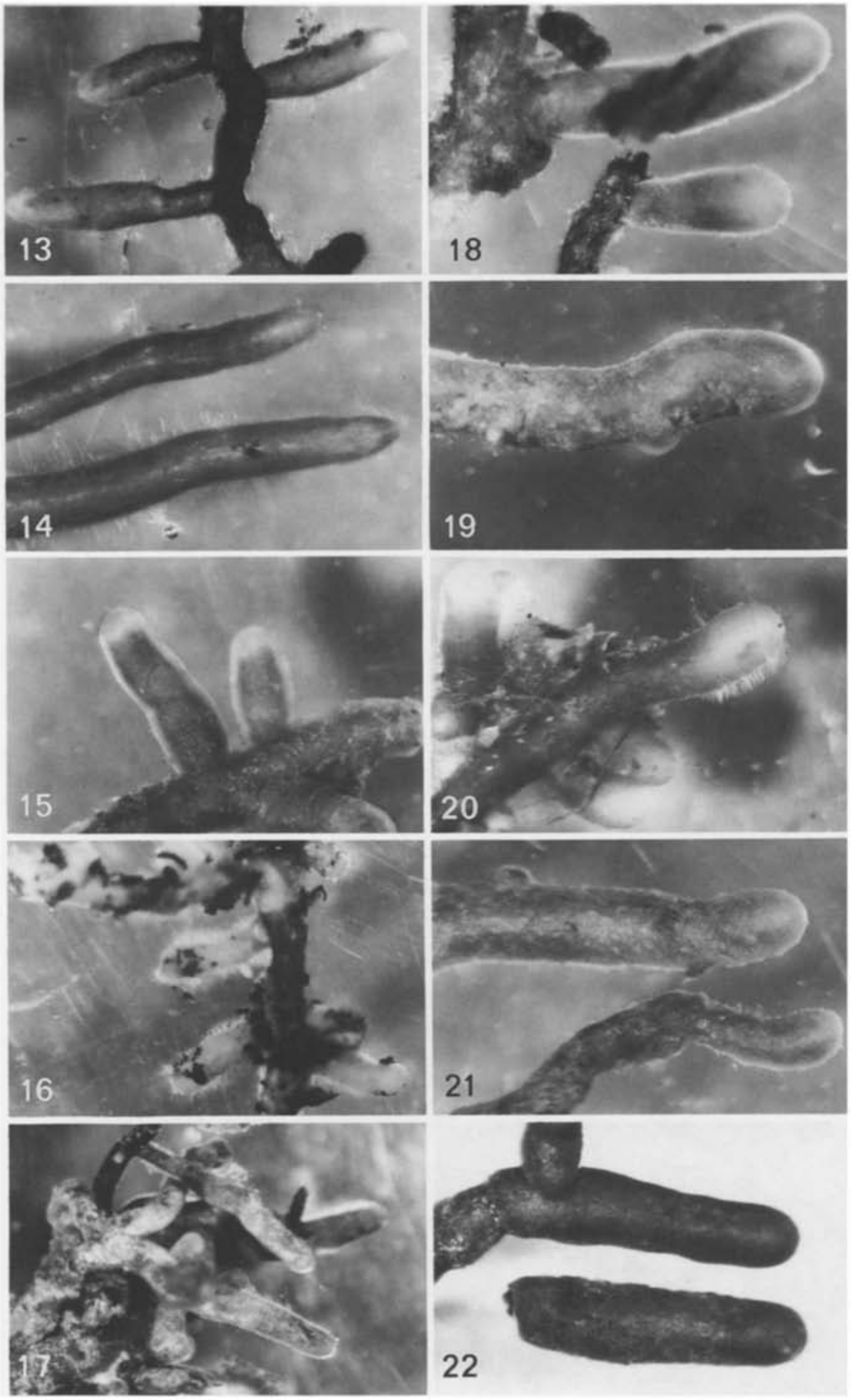
Microscopic characteristics
Hyphal mantle without differentiation into distinct layers, very loose, clearly prosenchymatous (Fig. 24c), hyphae septate without clamp connections, rarely branched, arranged parallel to the longitudinal axis of the root; diameter of hyphae: 3.5–4.5 μm (Fig. 24a, b);
hyphal mantle typically 20 μm thick.

Ultrastructural characteristics
Dolipores with perforate parenthesomes (Fig. 24d); hyphae of the outer hyphal mantle dead or heavy vacuolized, inner hyphae and Hartig-net hyphae with dense cytoplasm (Fig. 24e); no matrix material between the hyphae of the hyphal mantle (Fig. 24e); Hartig net with many septa (Fig. 24d).

Type 10: Slimy type with non-perforate parenthesomes

Macroscopic characteristics (Fig. 15)
Hyphal mantle nearly colourless, only slightly yellowish to white, transparent;
hyphal mantle compact, thick, smooth surface.

Microscopic characteristics
Hyphal mantle strongly slimy, surrounded by a compact layer of slime, in which few hyphae are visible (Fig. 25a, d); hyphae in the inner regions of the hyphal mantle often branched in a star-like way, hyphae often septate, without clamp connections, diameter of the hyphae: 1.2–2.3 μm (Fig. 25b, c);
hyphal mantle typically 40 μm thick.



Figs. 13–22. Photographs of mycorrhizae types 8–17

Ultrastructural characteristics
Dolipores with non-perforate parenthesomes (Fig. 25e, f); hyphae of the hyphal mantle embedded in a fine structured electron-transparent matrix material, outermost layer of slime electron dense (Fig. 25g); hyphae of Hartig net not embedded in matrix material.

Type 11: Loosely and compactly prosenchymatous type with clamp connections

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Abbreviations: c cortical cell; *cy* cystidia; *hm* hyphal mantle; *hn* Hartig net; *ih* intracellular hyphae; *m* matrix material; *n* nucleus; *p* parenthesomes; *t* tannins in cortical cells; *wb* Woronin bodies

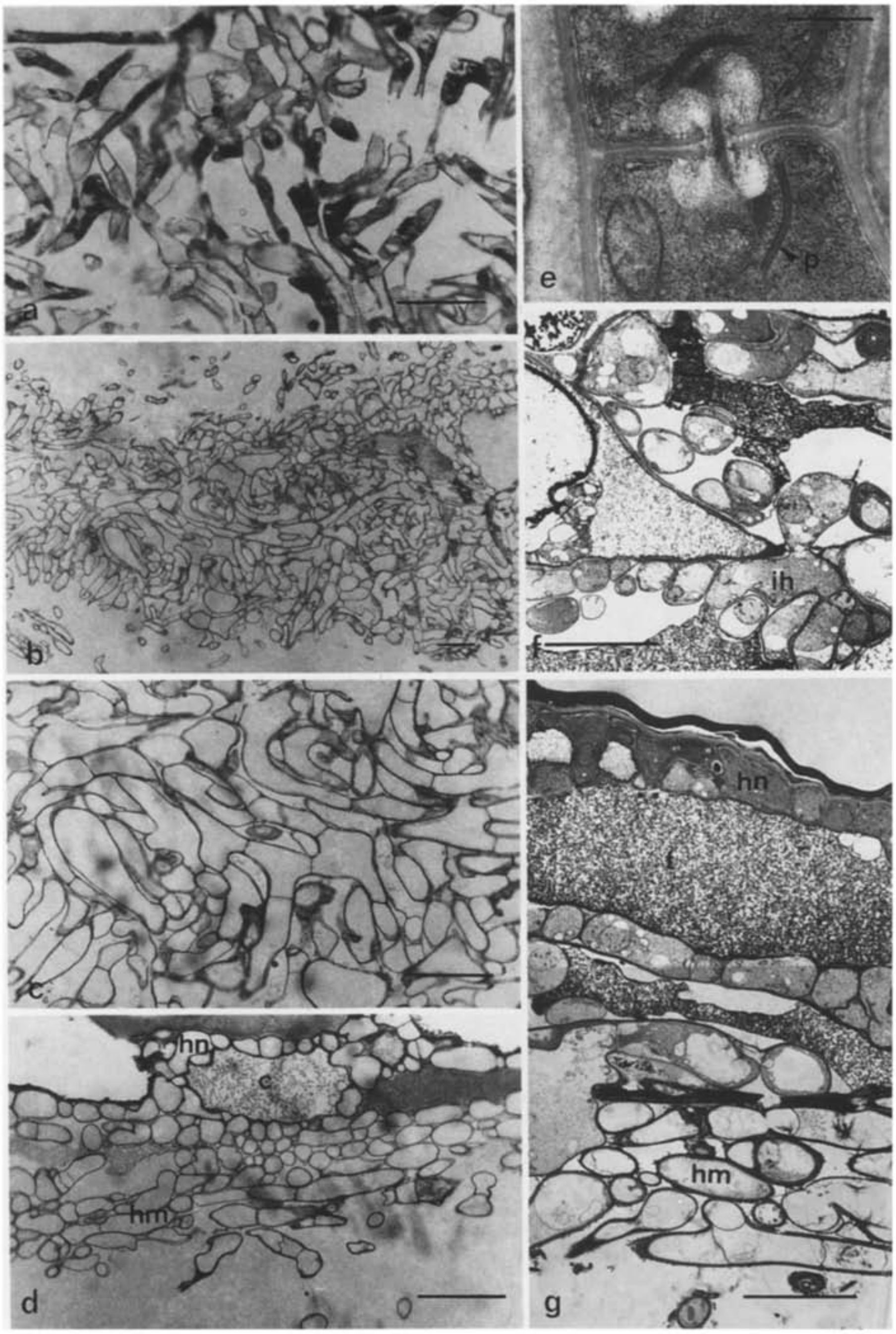

Fig. 23a–g. Type 8. Prosenchymatous type with large-diameter, short septate hyphae. **a–d** Light micrographs of longitudinal sections. **a** Surface layer of mantle, showing hyphae with darkly pigmented walls; **b, c** loose prosenchyma within hyphal mantle; **d** median section through mycorrhiza: hyphal mantle and adjacent tissues. **e–g** Transmission electron micrographs. **e** Dolipore septum with non-perforate parenthesomes; **f** cortical cells with intracellular hyphae; **g** hyphal mantle and cortical cells with Hartig net (*scale:* **a–d** 20 µm; **e** 0.5 µm; **f–g** 10 µm)

Macroscopic characteristics (Fig. 16)
Colour: hyphal mantle white or light yellow; surface woolly, downy; mycorrhizae enveloped in dense white mycelium.

Microscopic characteristics
Hyphal mantle consisting of two layers (Fig. 26d); in the outer region hyphae rarely septate, septa with clamp connections, large interhyphal spaces (Fig. 26a); towards the root surface decreasing interhyphal spaces (Fig. 26b), hyphae just above the epidermal layer lying side by side, hyphae subdivided into short cells by septa with clamp connections (Fig. 26c), arranged parallel to the longitudinal axis of the root, diameter of hyphae: 2.3–3.5 µm;
hyphal mantle typically 50 µm thick.

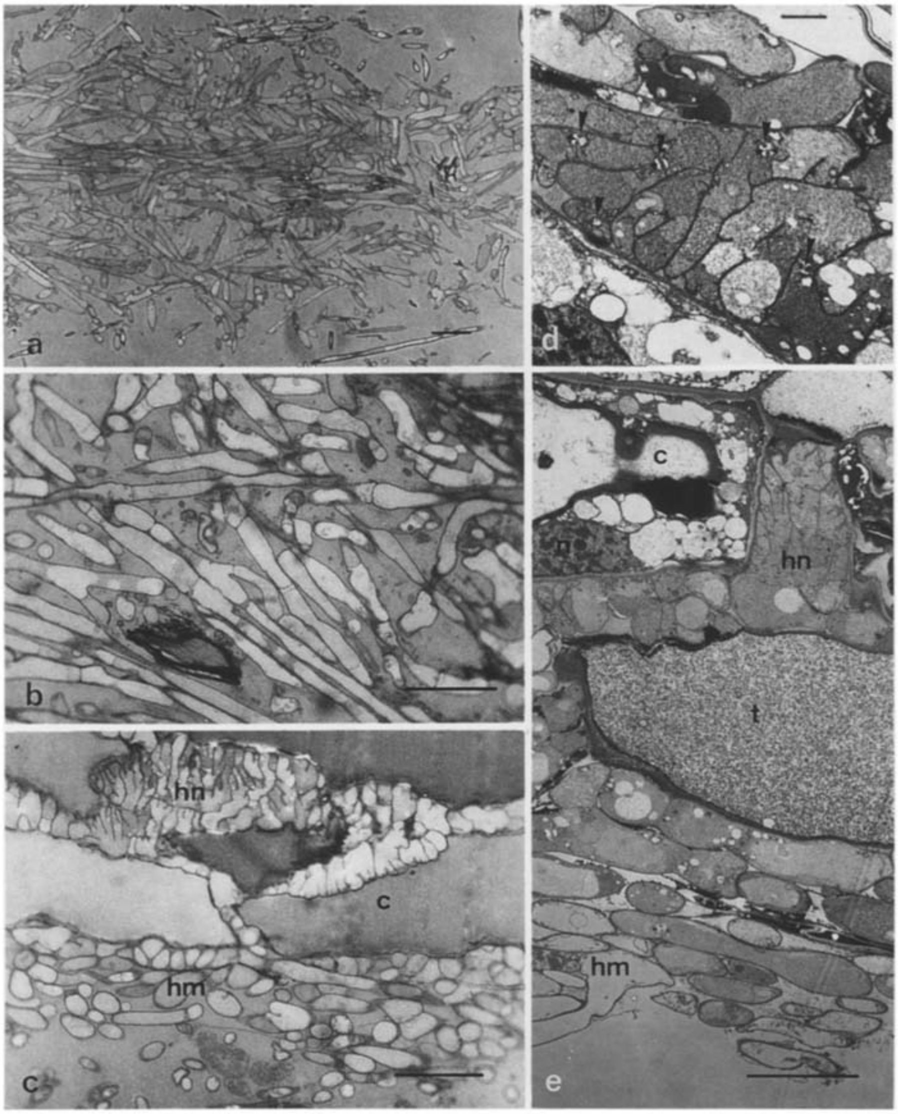

Fig. 24a–e. Type 9. Prosenchymatous type. **a–c** Light micrographs of longitudinal sections. **a, b** Loose prosenchyma within hyphal mantle; **c** median section through mycorrhiza: hyphal mantle and adjacent tissues. **d–e** Transmission electron micrographs. **d** Hartig net with many dolipore septa (▶); **e** hyphal mantle and cortical cells with Hartig net (*scale:* **a–c** 20 μm; **d** 2 μm; **e** 10 μm)

Ultrastructural characteristics

Dolipores with perforate parenthesomes (Fig. 26e); inner hyphae of the hyphal mantle with dense cytoplasma (Fig. 26f), outer hyphae highly vacuolated; walls of the adjacent hyphae melt into one another (Fig. 26g).

Type 12: Loose and compact prosenchymatous type with weaving pattern and cystidia

Macroscopic characteristics (Fig. 17)

Colour: hyphal mantle yellowish-brown to light yellow, partly with lighter spots;
velvety surface; hyphal mantle compact; surface covered with short, needle-like hyphae.

Microscopic characteristics

Hyphal mantle clearly septate in an outer loose region and an inner compact region (Fig. 27d); large interhyphal spaces in the outer region (Fig. 27a); inner layer compact prosenchymatous, hyphae arranged parallel, forming weaving patterns (Fig. 27b, c); hyphae of small diameter (1.1–2.3 μm) with thin walls; cystidia: thick-walled, dark-coloured, tapered, broad base (Fig. 27c, d);
hyphal mantle typically 35 μm thick.

Ultrastructural characteristics

Dolipores with perforate parenthesomes (Fig. 27e); hyphae of the hyphal mantle embedded in electron-transparent matrix material (Fig. 27g); thick-walled cystidia separated by a septum from the thin-walled basal part (Fig. 27f).

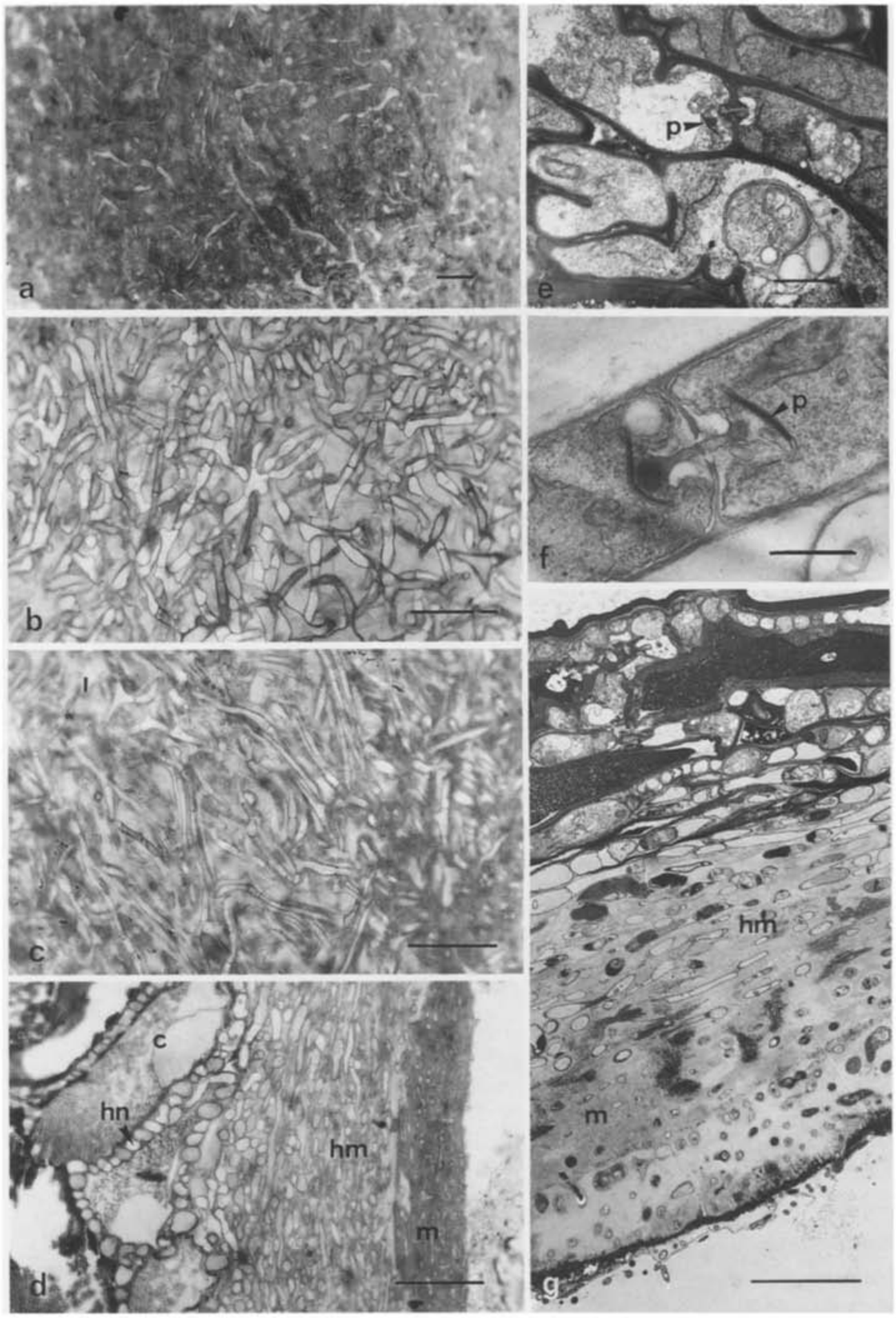

Fig. 25a–g. Type 10. Slimy type with non-perforate parenthesomes. **a–d** Light micrographs of longitudinal sections. **a** Surface layer of mantle, consisting of slime, in which few hyphae are visible; **b, c** loose prosenchyma within hyphal mantle, star-like branched hyphae, embedded in matrix material; **d** median section through mycorrhiza: hyphal mantle and adjacent tissues. **e–g** Transmission electron micrographs. **e** Hartig net with dolipore septum; **f** dolipore septum with non-perforate parenthesomes; **g** hyphal mantle and adjacent epidermal cells (*scale:* **a–d** 20 μm; **e** 1 μm; **f** 0.5 μm; **g** 10 μm)

Type 13: Loose and compact prosenchymatous type with cystidia

Macroscopic characteristics (Fig. 18)
Colour: hyphal mantle pale yellow to brown, at the root tip nearly white;
velvety, slightly glossy surface;
surface is covered with a large number of needle-like cystidia.

Microscopic characteristics

Mantle structure is complex (Fig. 28d) and may exhibit up to three arrangements at various depths within it: the surface layer consists of cystidia and hyphae emerging from the mantle; further down this merges first into a loose weft of rarely branched hyphae without clamp connections (Fig. 28a), and with depth in a compact prosen-

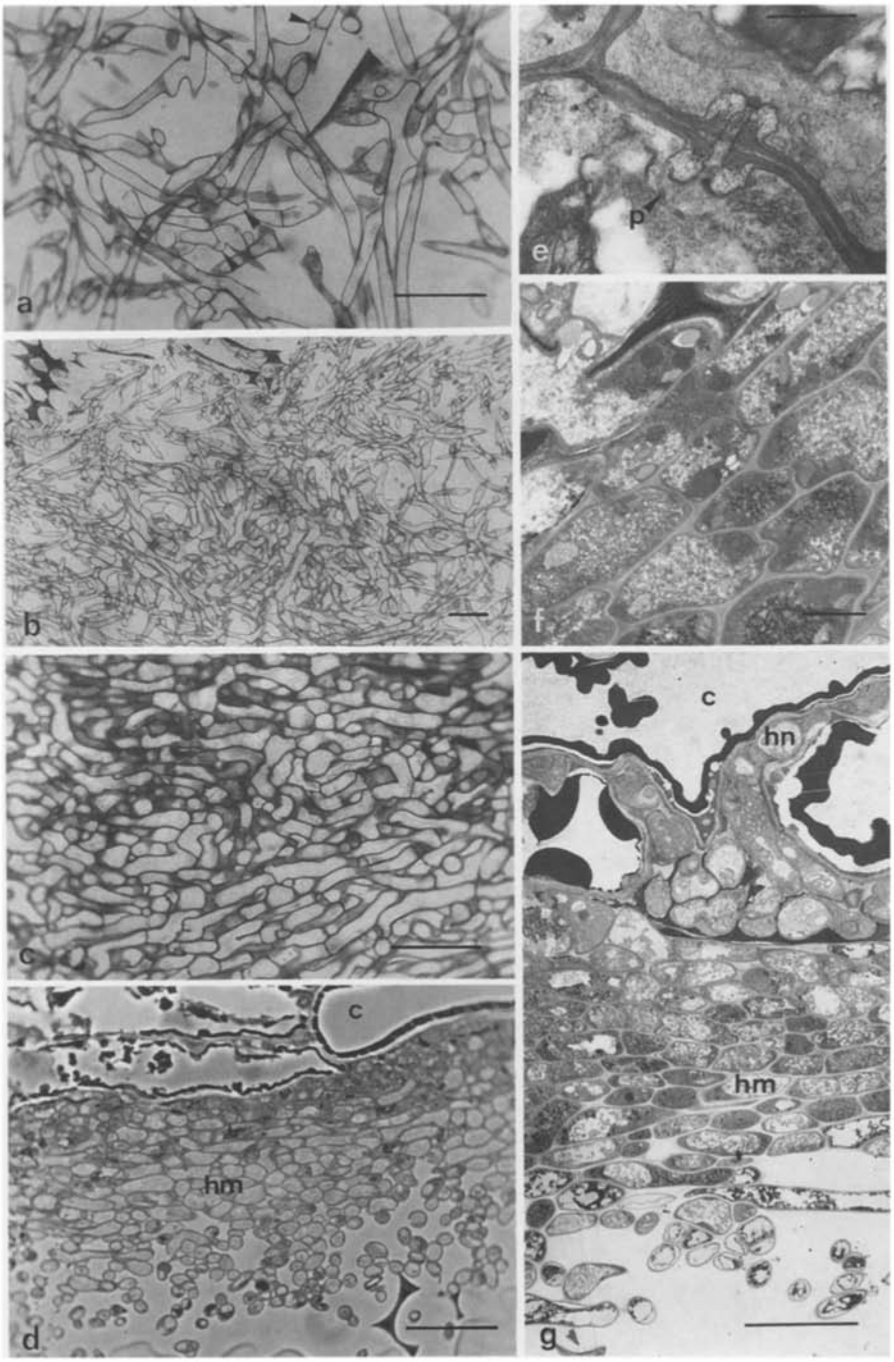

Fig. 26a–g. Type 11. Loose and compact prosenchymatous type with clamp connections. **a–d** Light micrographs of longitudinal sections. **a** Outer region of the hyphal mantle – loose prosenchyma, hyphae with clamp connections (►); **b, c** compact prosenchyma within hyphal mantle; **d** median section through mycorrhiza: hyphal mantle and adjacent tissues. **e–g** Transmission electron micrographs. **e** Dolipore septum with perforate parenthesomes; **f** hyphae of the inner mantle region with dense cytoplasm; **g** hyphal mantle and cortical cells with Hartig net (*scale:* **a–d** 20 μm; **e** 0.5 μm; **f** 2 μm; **g** 10 μm)

chyma of frequently branched hyphae with a diameter of 3.5 μm (Fig. 28b, c);
mantle typically 50 μm thick.

Ultrastructural characteristics
Dolipores with perforate parenthesomes (Fig. 28e); outer layers of hyphae dead or highly vacuolated, inner layers of hyphae embedded in electron-transparent matrix material (Fig. 28f); outgrowing hyphae and cystidia thick-walled.

Type 14: Type with a net-like surface layer

Macroscopic characteristics (Fig. 19)
Colour: hyphal mantle yellowish-brown to white; surface smooth, hyphal mantle compact;
hyphal outgrowths from the mantle are rare.

Microscopic characteristics
Hyphal mantle: outer layers formed of a loose, net-like prosenchyma, hyphae embedded in slimy

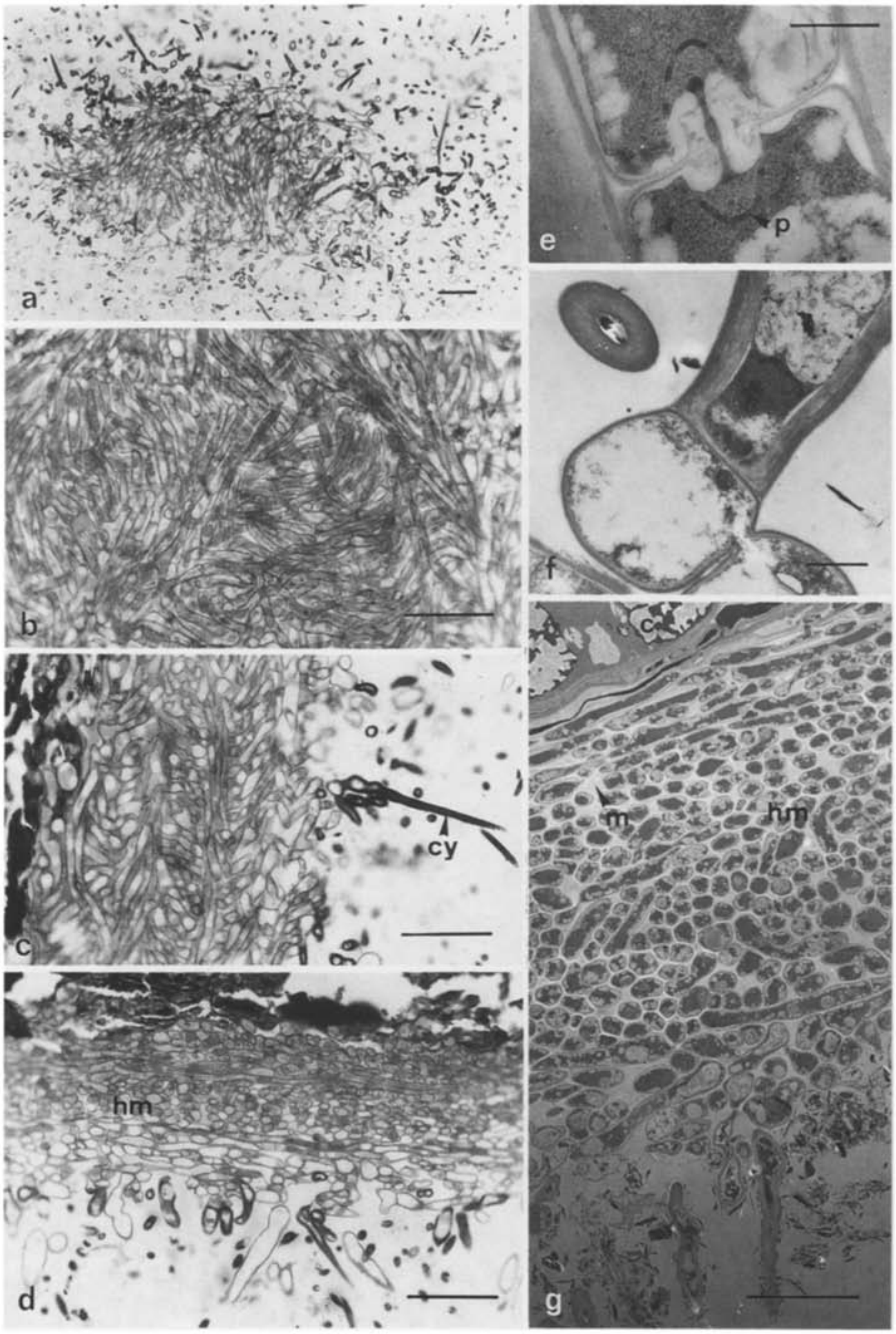

Fig. 27 a–g. Type 12. Loose and compact prosenchymatous type with weaving pattern and cystidia. **a–d** Light micrographs of longitudinal sections. **a** Outer loose prosenchymatous region of the hyphal mantle; **b** inner compact prosenchymatous region of the hyphal mantle with weaving pattern; **c, d** median section through mycorrhiza: hyphal mantle with cystidia. **e–g** Transmission electron micrographs. **e** Dolipore septum with perforate parenthesomes; **f** thin-walled basal part and thick-walled upper part of a cystidium; **g** hyphal mantle, hyphae embedded in electron-transparent matrix (*scale:* **a–d** 20 µm; **e** 0.5 µm; **f** 1 µm; **g** 10 µm)

matrix material (Fig. 29a, b); diameter range 2.5–3.5 µm, hyphae interwoven, branched in Y-like way; this merges into a compact prosenchyma, hyphae of larger diameter (3.5–7 µm), frequently branched, hyphal slices oval to longish (Fig. 29c);
mantle typically 35 µm thick.

Ultrastructural characteristics
Dolipores with perforate parenthesomes (Fig. 29e); mantle hyphae wholly embedded in electron-dense fibrous matrix material (Fig. 29f, g).

Type 15: Puzzle-type with cystidia

Macroscopic characteristics (Fig. 20)
Colour: hyphal mantle yellowish-brown to yellow, at the root tip nearly white;
surface is covered with a large number of cystidia at the apex, becoming smooth some distance fur-

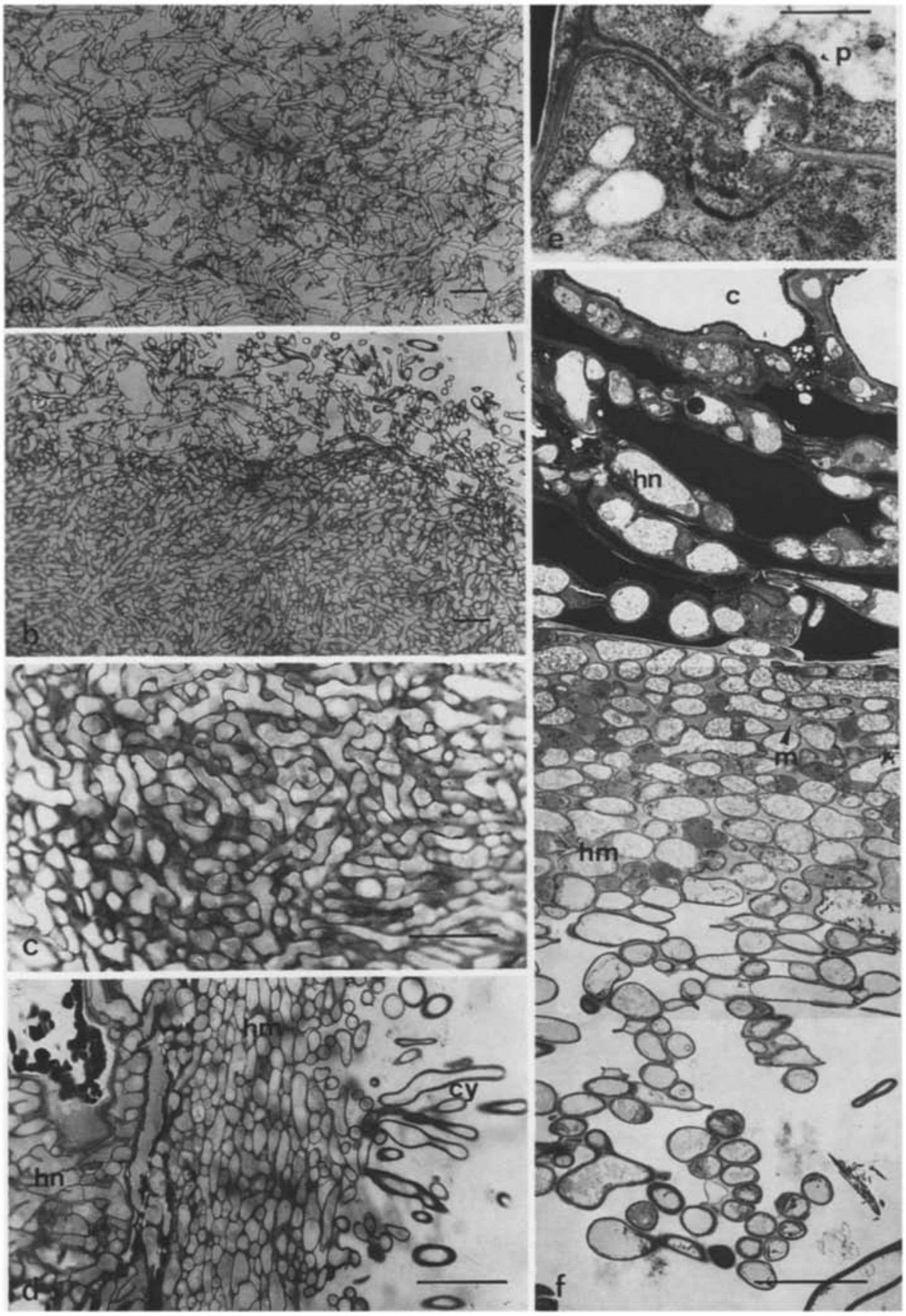

Fig. 28a–g. Type 13. Loose and compact prosenchymatous type with cystidia. **a–d** Light micrographs of longitudinal sections. **a** Outer loose prosenchymatous region of the hyphal mantle; **b** change from loose prosenchyma to compact prosenchyma with depth; **c** compact prosenchyma within hyphal mantle; **d** median section through mycorrhiza: hyphal mantle and adjacent tissues. **e–f** Transmission electron micrographs. **e** Dolipore septum with perforate parenthesomes; **f** hyphal mantle and epidermal cells filled with tannins (*scale:* **a–d** 20 µm; **e** 0.5 µm **f** 10 µm)

ther back; few hyaline hyphae radiate from the mycorrhizal surface.

Microscopic characteristics

Cystidia are growing out from a bulbous base, tapered, thick-walled, diameters range from 3.5–4.5 µm, length 125 µm (Fig. 30c, f);

hyphal mantle wholly composed of puzzle-synenchyma (Fig. 30c), large diameter hyphae: 4.5–7 µm, embedded in dark-colouring matrix material, much matrix material between the surface hyphal elements (Fig. 30a, b); hyphae adjacent to epidermis smaller, building up prosenchymatic tissue;

mantle typically 25 µm thick.

Ultrastructural characteristics

Simple pores with Woronin bodies (Fig. 30d); mantle hyphae embedded in electron-dense matrix material, which decreases with depth (Fig. 30f).

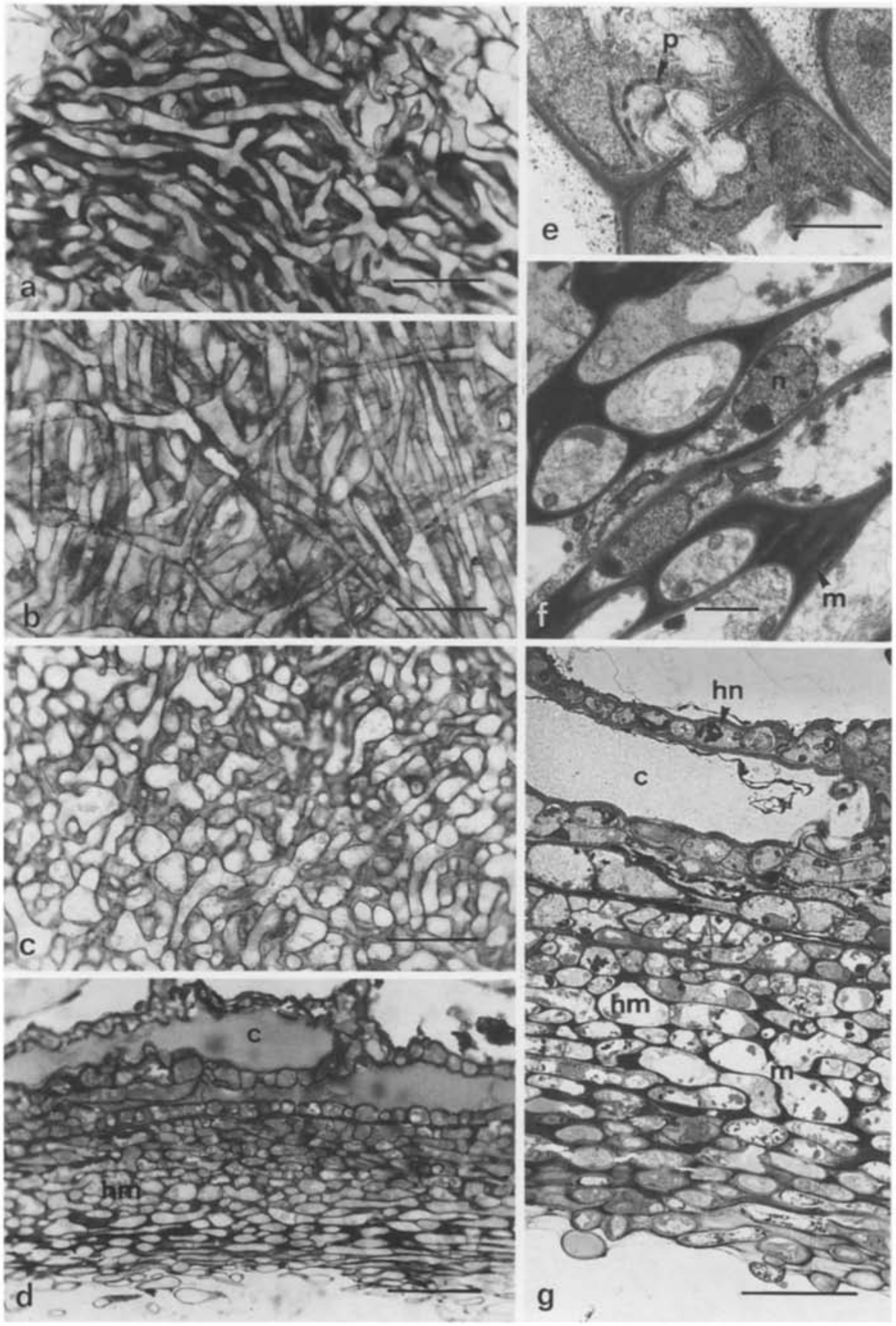

Fig. 29a–g. Type 14. Type with a net-like surface layer. **a–d** Light micrographs of longitudinal sections. **a, b** Surface layers of mantle, composed of a loose net-like prosenchyma, hyphae embedded in slimy matrix material; **c** compact prosenchyma within hyphal mantle; **d** median section through mycorrhiza: hyphal mantle and adjacent tissues. **e–g** Transmission electron micrographs. **e** Dolipore septum with perforate parenthesomes; **f** hyphae embedded in electron-dense fibrous matrix material; **g** hyphal mantle and cortical cells with Hartig net (*scale:* **a–d** 20 μm; **e** 0.5 μm; **f** 1 μm; **g** 10 μm)

Type 16: Puzzle-type with protuberances

Macroscopic characteristics (Fig. 21)
Colour: hyphal mantle white to transparent, cortex cells visible;
surface smooth, interrupted by numerous protuberances; hyphal outgrowths from the mantle are rare.

Microscopic characteristics
Mantle structure exhibits three arrangements at various depths within it (Fig. 31c); mantle consists of a puzzle-synenchyma (Fig. 31a), occasionally this is overlaid by a thin layer of dead hyphae forming protuberances; just above the epidermal layer hyphae become smaller (1.7–3 μm), forming a prosenchyma; hyphae partly parallelly arranged, septate without clamp connections, embedded in dark-stained matrix material (Fig. 31b);
mantle typically 30 μm thick.

Ultrastructural characteristics
Dolipores with perforate parenthesomes

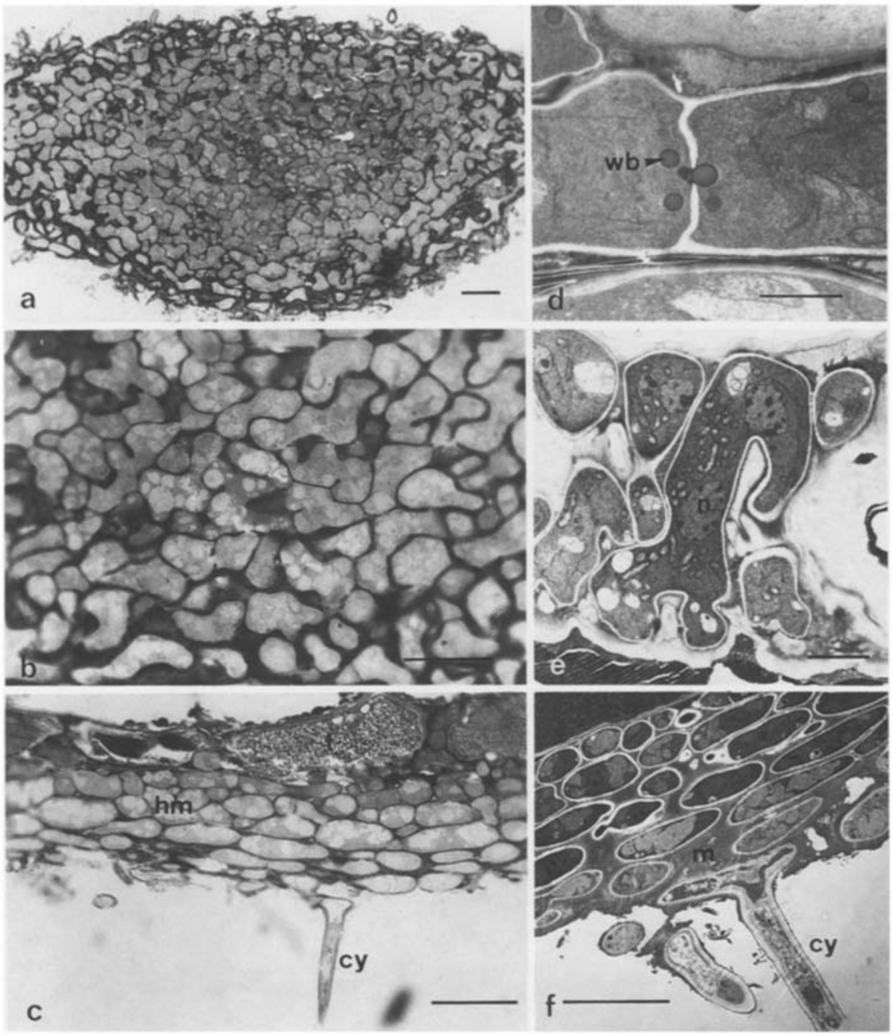

Fig. 30a–f. Type 15. Puzzle-type with cystidia. **a–c** Light micrographs of longitudinal sections. **a, b** Puzzle synenchyma within hyphal mantle; **c** median section through mycorrhiza: hyphal mantle with cystidia. **d–f** Transmission electron micrographs. **d** Simple septum of a hypha with Woronin bodies; **e** Hartig net; **f** hyphal mantle with outgrowing cystidia (*scale:* **a–c** 20 µm; **d** 0.5 µm; **e** 1 µm; **f** 10 µm)

(Fig. 31 d); surface hyphal elements dead (Fig. 31 e); inner layers of hyphae embedded in electron-dense matrix material (Fig. 31 e).

Type 17: Puzzle-type with smooth surface

Macroscopic characteristics (Fig. 22)

Colour: Hyphal mantle yellowish-brown to brown; surface smooth, slightly glossy, mantle compact; hyphal outgrowths from the mantle are rare.

Microscopic characteristics

Hyphal mantle consisting of three layers (Fig. 32 d); brief net prosenchyma (Fig. 32 a), with rarely septate hyphae, branched in a star-like way, overlying puzzle-synenchyma (Fig. 32 b), which soon merges into a loose prosenchyma (Fig. 32 c); synenchyma hyphae range in width from 5.8–9.2 µm, prosenchyma hyphae are 1.2–3.5 µm thick;
mantle typically 30 µm thick.

Ultrastrctural characteristics

Dolipores with perforate parenthesomes (Fig. 32 e); outer hyphal elements embedded in a few, moderately electron-dense homogen matrix material (Fig. 32 g, h), inner hyphal elements in electron-transparent, fine granulated matrix material (Fig. 32 f, h).

Systematic assignment of mycorrhizal types

Based on electron microscopic examinations of pore structures it was possible to classify the fungal partners with either the ascomycetes or with the basidiomycetes. The latter clearly stand out as the main partners in forming mycorrhizae (15 out of 17), as Marks and Forster (1973) have already mentioned. This can also be seen from a list of mycorrhizal fungi by Trappe (1962). Only two fungal partners have simple pores with Woronin bodies and have thus to be classified with the ascomycetes.

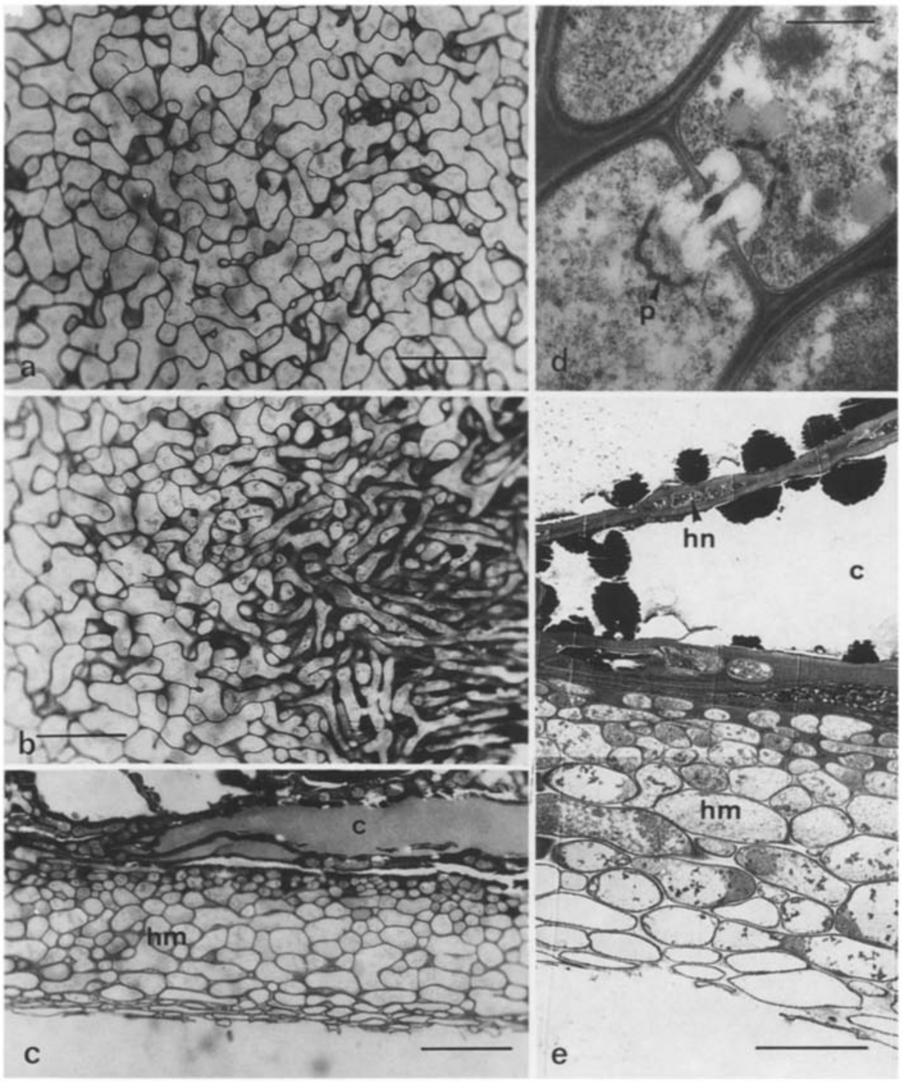

Fig. 31 a–f. Type 16. Puzzle-type with protuberances. **a–c** Light micrographs of longitudinal sections. **a** Puzzle synenchyma within hyphal mantle; **b** change from puzzle synenchyma to compact prosenchyma just above epidermal layer; **c** median section through mycorrhiza: hyphal mantle. **d–e** Transmission electron micrographs. **d** Dolipore septum with perforate parenthesomes; **e** hyphal mantle and cortical cells with Hartig net (*scale:* **a–c** 20 μm; **d** 0.5 μm; **e** 10 μm)

The fact that the dolipores of two basidiomycetous mycorrhizae have continious parenthesomes is interesting and is described here for the first time. The dolipores of the other basidiomycetous mycorrhizae have perforated parenthesomes; thus they must be classified with the homobasidiomycetes. According to Oberwinkler (1985), dolipores with continuous parenthesomes are characteristic of the following heterobasidiomycetes: the Hirneola group within the Auriculariales, the Tremellales s. ampl. (exclusive of the types with cone-shaped parenthesome elements), the Tulasnellales and the Dacrymycetales. Among the Homobasidiomycetes, the Hymenochaetales, Hirschioporus species, *Botryobasidium subcoronatum, Paullicorticium pearsonii* and *Clavulicium macounii* are exceptions, for they possess continuous parenthesomes. Genera of the Tulasnellales (Ceratobasidium, Tulasnella) and Tremellales (Sebacina) are known as endomycorrhizal partners with orchids (Harley and Smith 1983), but not as ectomycorrhizal partners. The fungus of type no. 10 with the feature combination of very mucilagenous hyphae and continous parenthesomes possibly belongs to the Tulasnellales. Membership of the homobasidiomycetes of these two fungi is relatively unlikely, as dolipores with perforated parenthesomes are here almost constant (Khan and Kimbrough 1982) and the genera mentioned above (Hymenochaete, Hirschioporus, Botryobasidium, Paullicorticium, Clavulicium) are supposed to be saprophytic. Thus this might be the first indication of heterobasidiomycetes as partners in ectomycorrhizae. Both types with continuous parenthesomes (types 8 and 10) are remarkable due to the fact that the intracellular

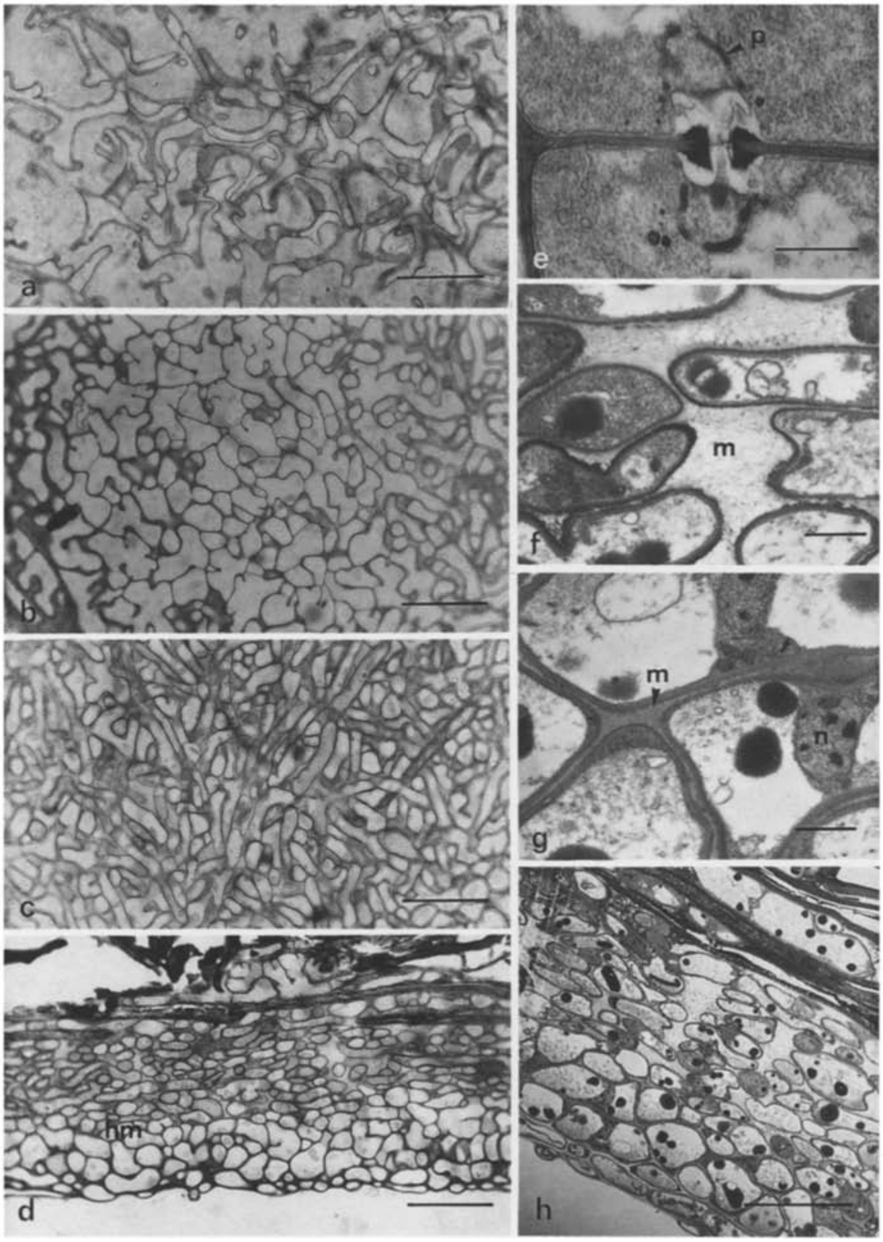

Fig. 32a–h. Type 17. Puzzle-type with smooth surface. **a–d** Light micrographs of longitudinal sections. **a** Surface layer consisting of loose prosenchyma; **b** puzzle synenchyma in the outer regions of the hyphal mantle; **c** compact prosenchyma within the hyphal mantle; **d** median section through mycorrhiza: hyphal mantle. **e–h** Transmission electron micrographs. **e** Dolipore septum with perforate parenthesomes; **f** inner region of the hyphal mantle, hyphae embedded in electron-transparent matrix material; **g** outer region of the hyphal mantle, hyphae embedded in a few, moderately electron-dense matrix material; **h** hyphal mantle (*scale:* **a–d** 20 μm; **e** 0.5 μm **f–g** 1 μm; **h** 10 μm)

stages occur more frequently, along with normal hyphal stages.

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