

## Original papers

# Mycorrhizae of *Entoloma saepium*: parasitism or symbiosis?

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**Abstract.** The mycorrhizae of *Entoloma saepium* on *Rosa* sp. are comprehensively described and compared to other mycorrhizae of the genus *Entoloma*, as well as to similar unidentified mycorrhizae known from the literature. *E. saepium* appears to be more a parasite than a symbiont, as it invades and almost completely destroys the root meristem and young root cells.

**Key words:** Characterization – Identification – *Entoloma saepium* – Mycorrhizae – Parasite – *Prunus domestica* – *Rosa* sp.

## Introduction

The genus *Entoloma* has often been considered at least in part as ectomycorrhizal (Becker 1956; Miller 1982; Noordeloos 1984, 1987; Strullu 1985), but mycorrhizal associations have only been proven for a few species (Antibus et al. 1981; Linkins and Antibus 1982; Modess 1941; Zerova and Rozhenko 1966).

*Entoloma saepium* is known to form its fruit bodies under shrubs and trees of the family Rosaceae (Kreisel 1987; Moser 1983; Noordeloos 1981). The same is true of *Entoloma clypeatum* (Kreisel 1987; Moser 1983; Noordeloos 1987), the mycorrhizal behaviour of which has already been shown (Zerova and Rozhenko 1966). Thus it was of interest to investigate *E. saepium* found under *Rosa* bushes and *Prunus* trees.

Methods to characterize ectomycorrhizae have been previously explained by Agerer (1986, 1987–1992, 1991), and a glossary of terms has been published (Agerer 1987–1992). The sections described below were made after embedding in Historesin.

## *Entoloma saepium* (Noulet-Dassier) Richon & Roze on *Rosa* sp.

Reference specimen: Germany, Hessen, Lkrs. Offenbach, Egelsbach, north end of Oberrheintalgraben in moder under turf-sward, podzolic soil with quaternary alluvial sands, leg. H. Zinth, 19 May, 1992; under *Rosa* sp., fruit bodies RA 11706 and mycorrhizae RA 11707 in Herb. R. Agerer (M).

### Morphological characters (Figs. 1a–c)

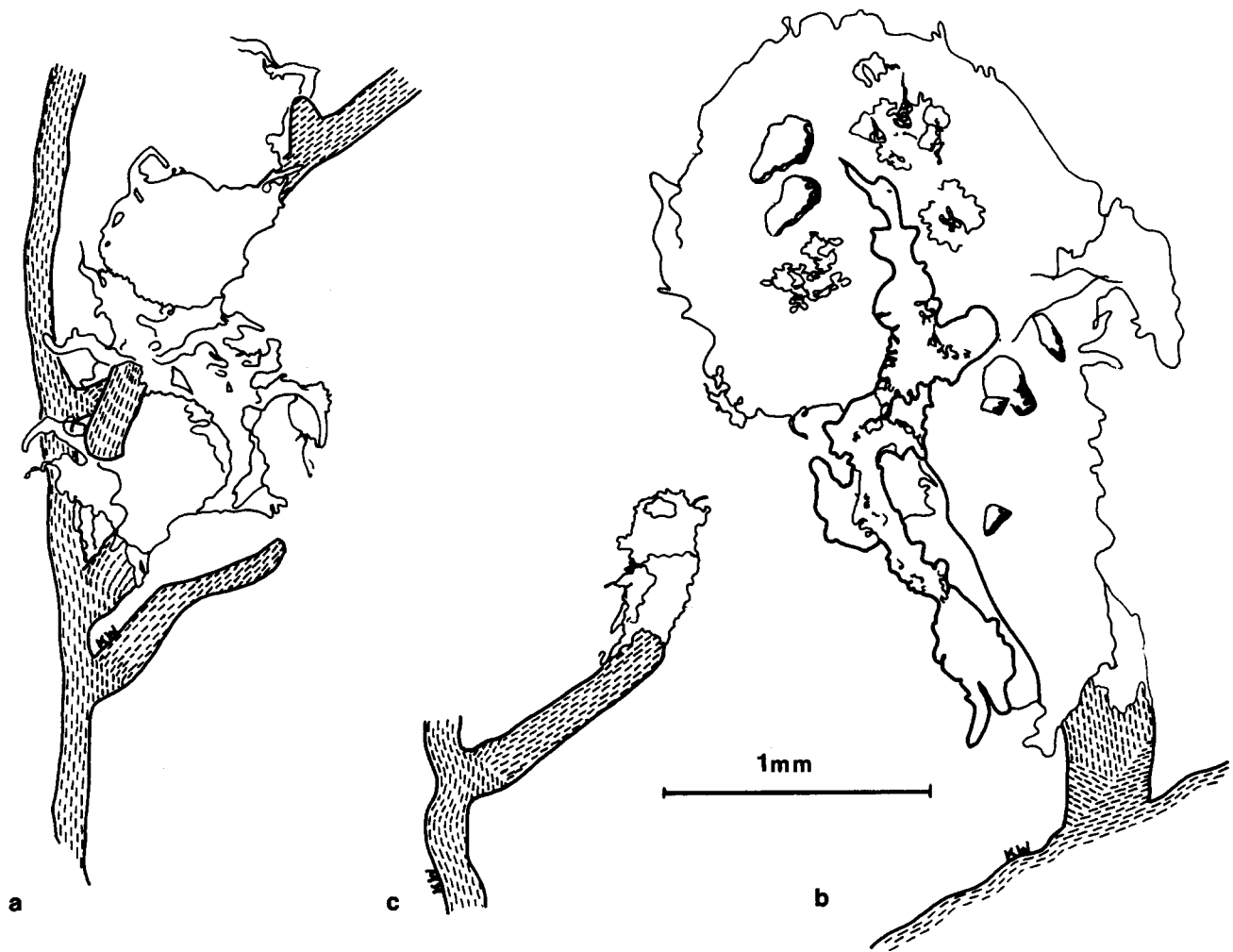
Unramified, drum-stick shaped, white to brownish-grey, young specimens white, surface woolly to felty, with adhering soil debris; 1–6 mm long, and 0.5–4 mm in diameter; rhizomorphs colourless, embedded in a thin mycelium, several mycorrhizae may be connected.

### Anatomical characters in plan views (Figs. 2, 3a–c, 4a–f, 6b)

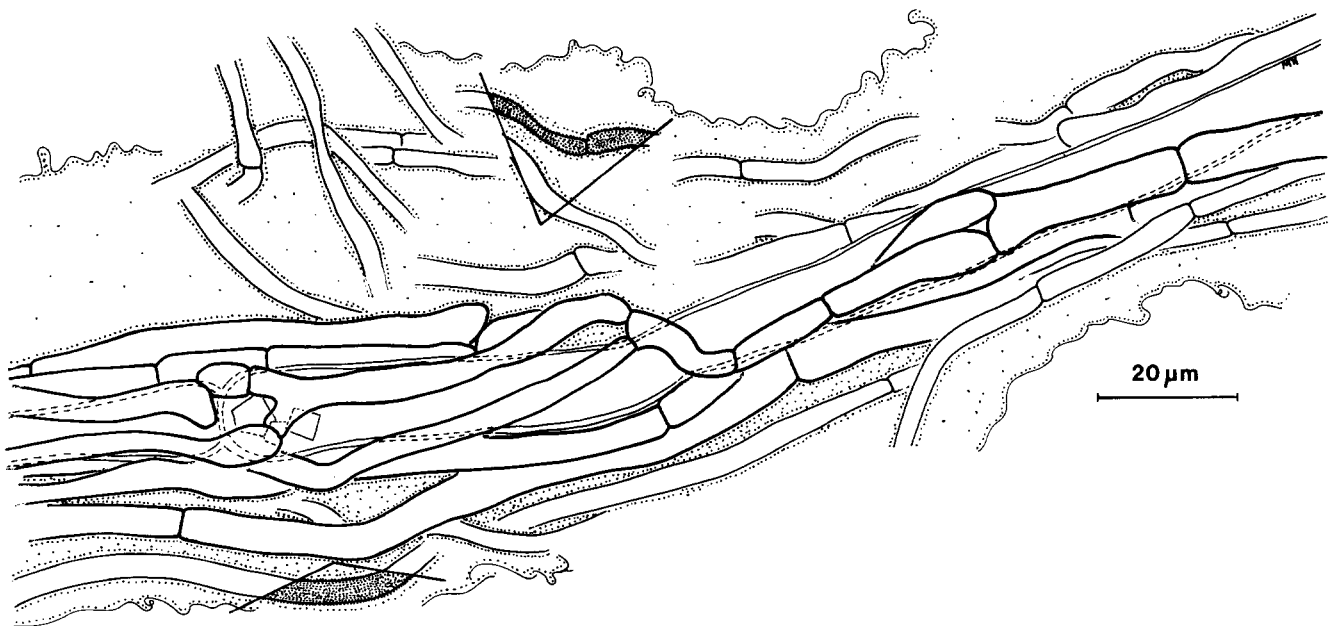
*Outer surface of mantle* (Fig. 3a) on intact root (viz G in Fig. 8) plectenchymatous, hyphae over rather long distances growing in parallel, hyphae colourless, 3–6  $\mu\text{m}$  in diameter, cells 12–45  $\mu\text{m}$  long, clamps lacking, hyphae in part inflated, especially at points of ramification, walls gelatinous. *Middle layers of mantle* (Fig. 3b) on intact root not abruptly delimited from the outer parts, hyphae colourless, clamps lacking, growing in parallel, 5–6.5  $\mu\text{m}$  in diameter, cells 13–45  $\mu\text{m}$  long, in deeper layers a little bit shorter (10–35  $\mu\text{m}$ ), roundish cells intermixed of diameter 6–10  $\mu\text{m}$ , roundish cells more frequent towards inner parts of mantle. *Inner surface of mantle* (Figs. 3c, 4e) on intact root not abruptly delimited from outer parts, pseudoparenchymatous, cells roundish, often rather thick at the corners of neighbouring cells (Fig. 4e), colourless, 1.2–6  $\mu\text{m}$  in diameter, walls distinctly gelatinous; 32–37 cells in a square of  $20 \times 20 \mu\text{m}$ . *Emanating hyphae* (Fig. 4d)

Considered as part IL of the series “Studies on ectomycorrhizae”; part XLVIII: Waller and Agerer (1993)

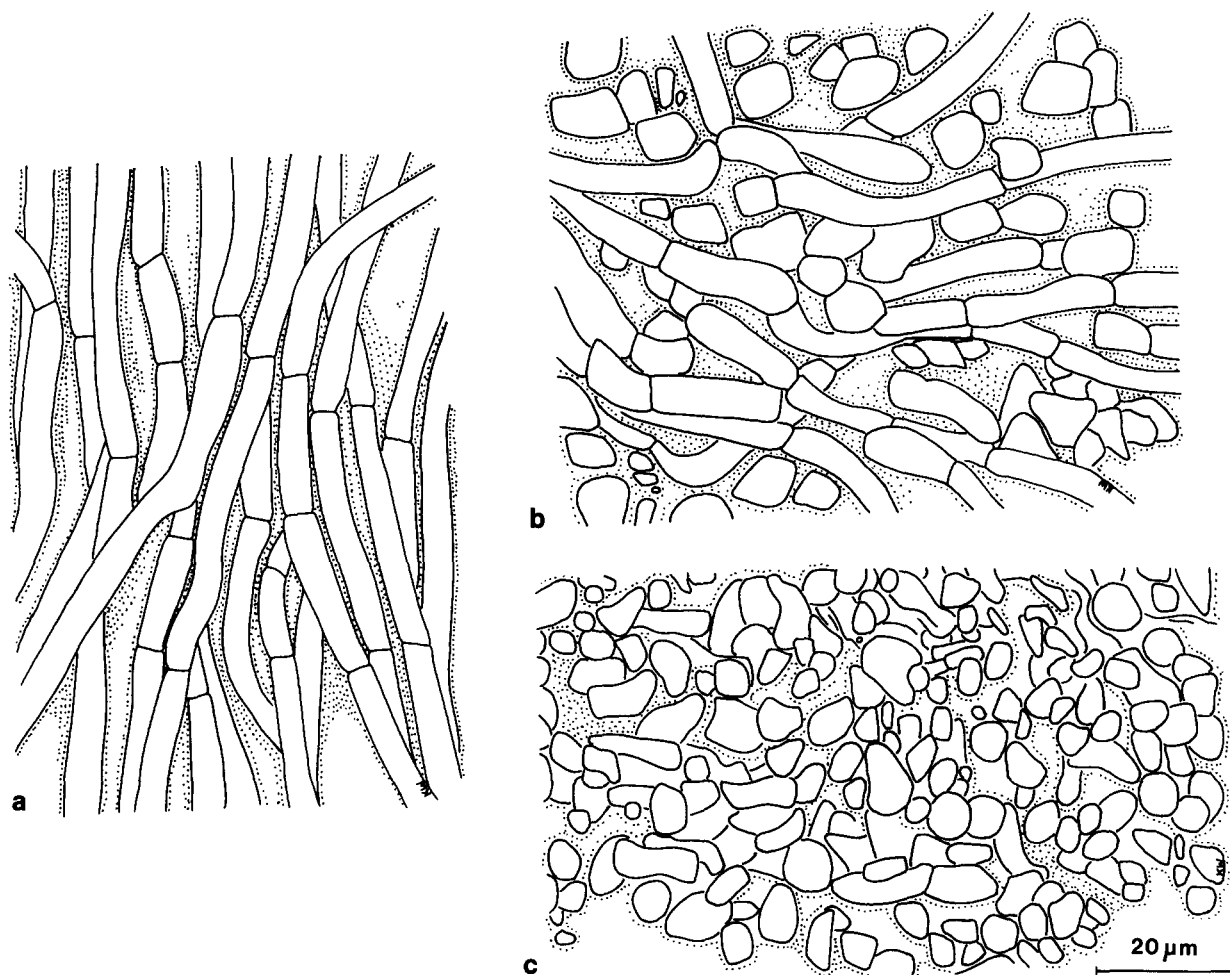
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**Fig. 1.** a Habit, two mycorrhizae glued together by soil particles. b Mycorrhiza in higher magnification. c Habit, young mycorrhiza. From RA 11707



**Fig. 2.** Rhizomorph, hyphae embedded in gelatinous matrix, outer hyphae in part rough (drawn only in a sector). From RA 11707



**Fig. 3.** **a** Plan view of mantle surface on intact root (zone G in Fig. 8), layer of hyphae growing in parallel, cell walls gelatinous. **b** Plan view of mantle on intact root, middle layer, consisting of hyphae growing in parallel, some are roundish, cell walls consid-

erably gelatinous. **c** Plan view of inner mantle surface on intact root, pseudoparenchyma of roundish to oval cells, cell walls considerably gelatinous. From RA 11707

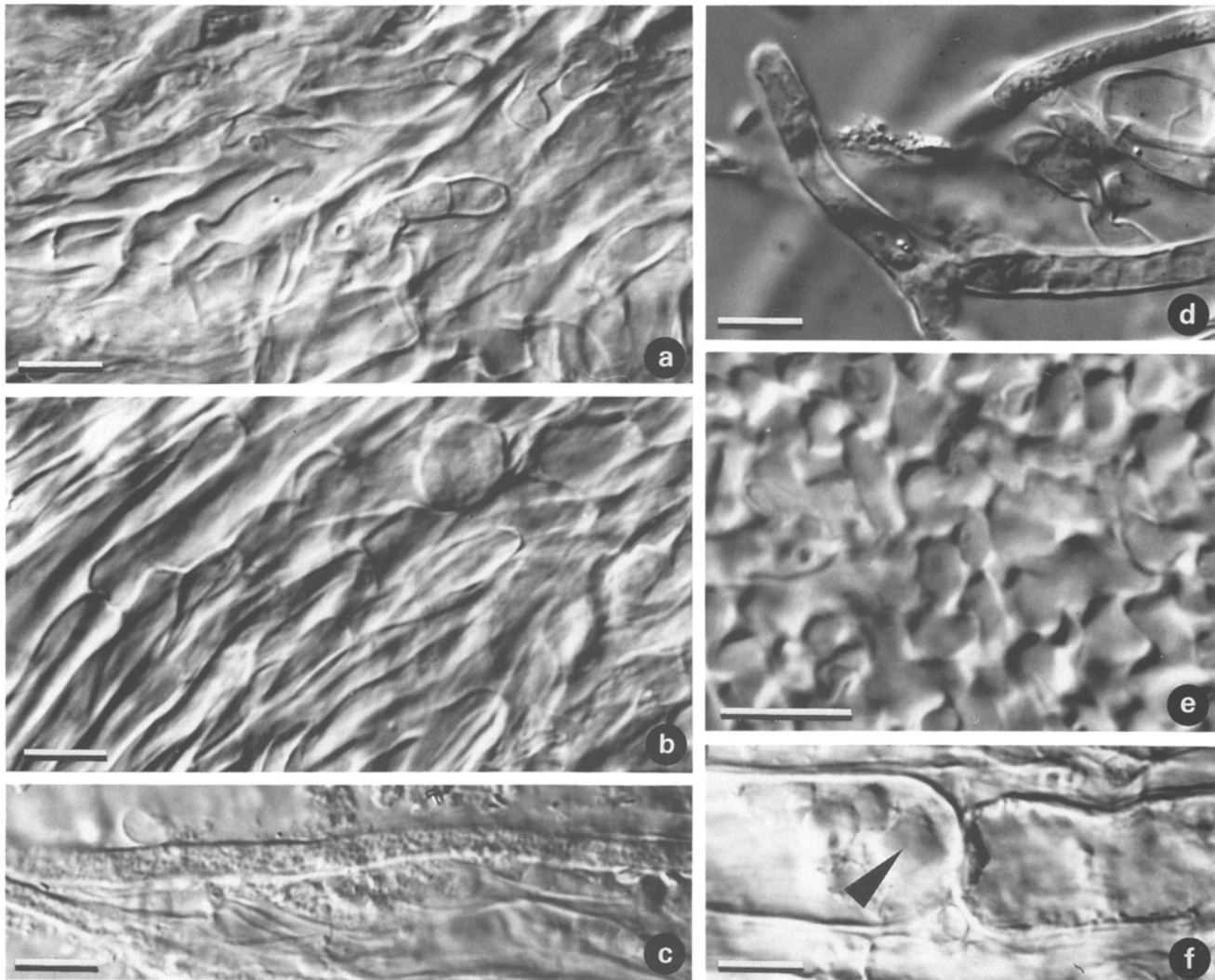
clampless, 7–9  $\mu\text{m}$  in diameter, consisting of 1–3 cells 25–50  $\mu\text{m}$  in length, wall 0.7  $\mu\text{m}$  thick. *Rhizomorphs* (Figs. 2, 4c, f, 6b) embedded in a mat of hyphae which envelope soil debris and sand grains; no distinct junction between rhizomorphs and mantle; hyphal mats with colourless, 5- to 16- $\mu\text{m}$ -thick hyphae, cells 50–190  $\mu\text{m}$  long, walls 0.5–1  $\mu\text{m}$  thick, cytoplasm with strongly light-reflecting crystalloid structures, septa mostly complete, some pores enlarged, remaining hyphae forming a loose envelope around the thick hyphae, colourless, 2–5  $\mu\text{m}$  in diameter, wall 0.3  $\mu\text{m}$  thick. *Cystidia* not found.

*Anatomical characters, longitudinal section*  
(Figs. 5, 6c, 7, 8)

Mycorrhiza consisting of three different parts from distal to proximal:

1. Distally there is a thick club-shaped portion. Here the meristem, the root cap cells and the former adjoining cortical cells up to the vessels are destroyed and

almost completely digested. At this point the club-shaped portion is readily detached from the rest of the root. It consists of five different structures (cf. cross-section): the outer layer consisting of tangentially oriented as well as cross-sectioned hyphae, 3–7  $\mu\text{m}$  in diameter, turning into emanating hyphae, walls 0.5  $\mu\text{m}$  thick with soil debris adhering to the outer surface, cells at the apex are more roundish (A in Fig. 8); in deeper regions, the outer middle layer, (B in Fig. 8) strongly light reflecting, large crystal-like complex structures are interspersed; the adjoining layer, the inner middle layer (C in Fig. 8), is composed of approximately perpendicularly oriented hyphae with small, often rhomboid crystals lining the hyphal walls, and with remnants of root cell walls in between; inner layer (D in Fig. 8) is also composed of approximately perpendicularly oriented hyphae but crystals are lacking; the central part (E in Fig. 8) consists of irregularly shaped hyphae interspersed with cortical cell wall remnants. At the border to the undestroyed part of the root, the crystal-like structures and the rhomboid crystals were not found (F in Fig. 8).



**Fig. 4.** **a** Normarski's interference contrast plan view of middle layer of mantle on intact root (zone G in Fig. 8). **b** Plan view of mantle surface on intact root, hyphae growing in parallel. **c** Rhizomorph, surface view, hyphae in part with granular contents. **d** Surface view, emanating hyphae. **e** Plan view of the inner mantle

surface, strongly gelatinous pseudoparenchyma of angular cells with thickened corners. **f** Rhizomorph, thick central hypha with crystal-like structure near septum. **a**, **c**, **e**, **f** from RA 11707; **b**, **d** from RA 11708; bar = 10  $\mu$ m

2. The more proximal part (Figs. 5a, b and G in Fig. 8) is composed of two or even three hyphal layers which envelope the root: the inner layer is made up of hyphae mostly oriented perpendicularly to root surface (M in Fig. 8), the outer layers are formed by irregularly shaped or sometimes tangentially oriented hyphae.

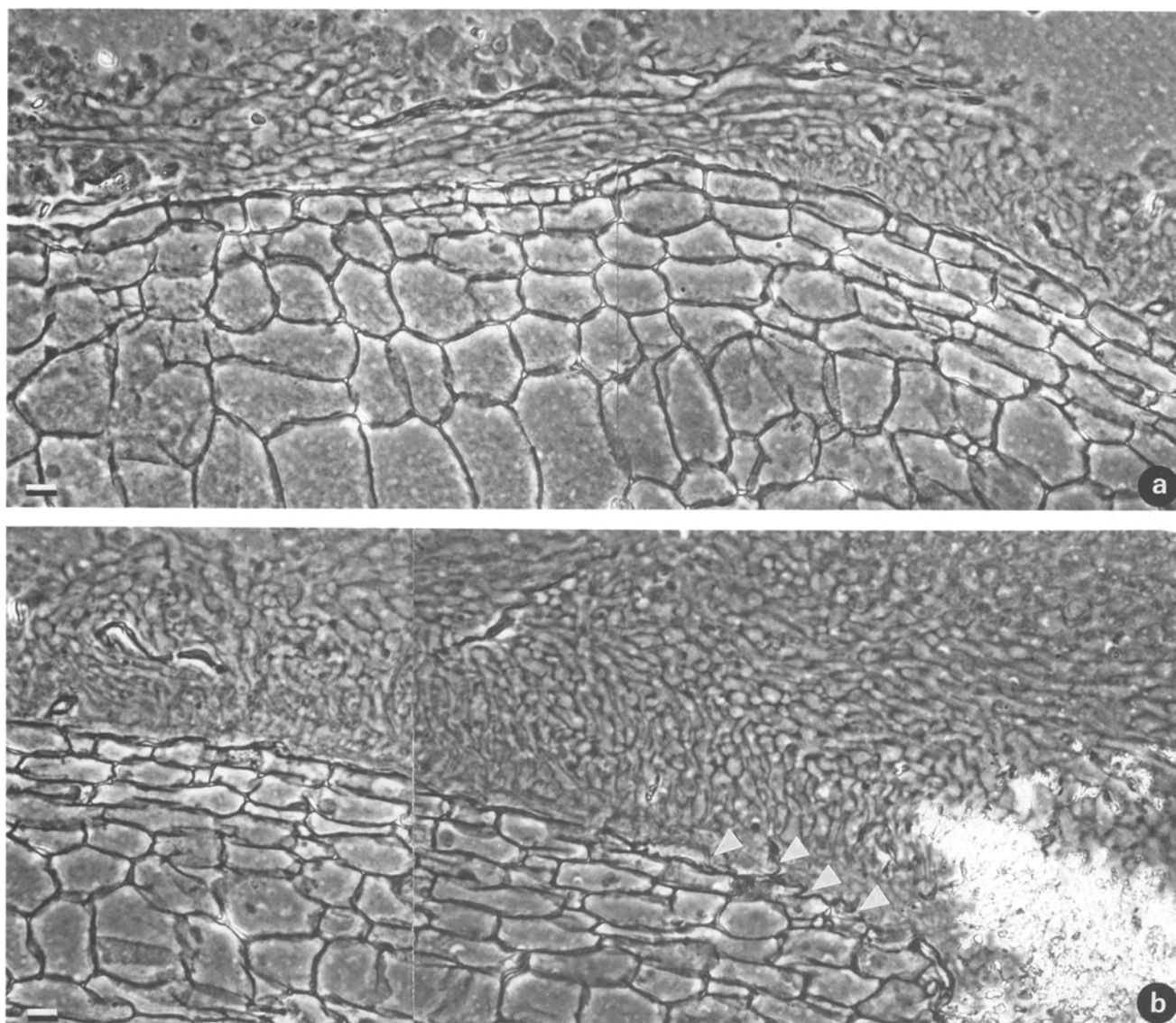
3. At the most proximal parts (Fig. 5a and F in Fig. 8) tangentially oriented hyphae dominate.

Examining the relation of hyphae to the root cortex cells, no Hartig net and no tannin cells were found. However, in the more proximal parts of the mycorrhiza, single hyphae in transverse section were seen within cell walls of outer cortex cells (Fig. 7c). Intracellular hyphae occur (Fig. 7d) but are very infrequent. Most deleterious for the root is the action of the hyphae at the border between the club-shaped portion and the

normal root. Here the hyphae invade obviously living root cells (Fig. 7b) and destroy them, leaving only numerous cell wall remnants (Fig. 6e and C in Fig. 8). Cells with intact nuclei are present close to the invaded cells (Fig. 7a). Evidence for the digesting activities of the fungus can also be seen in the more proximal parts of the root. This includes obliquely oriented, abruptly ending cortex layers, together with sloughed off cortical cell remnants (Fig. 5), which are included in the mantle.

#### *Anatomical characters, cross-section (Figs. 6a–d, 7)*

*Mantle* at the club-shaped part (viz. longitudinal section) 90–140  $\mu$ m thick, several distinct layers discernable; outer mantle layer 20–50  $\mu$ m thick, towards outer parts more loosely woven and turning into emanating



**Fig. 5.** Longitudinal section through a mycorrhiza from the proximal end (left side of **a**) up to the digestion zone (right side of **b**). The left side of **b** continues to the right side of **a**. From RA

11707; *arrowheads* indicate obliquely oriented cortex cell layers as remnants of sloughed-off layers; normal phase contrast; *bar* = 10  $\mu\text{m}$

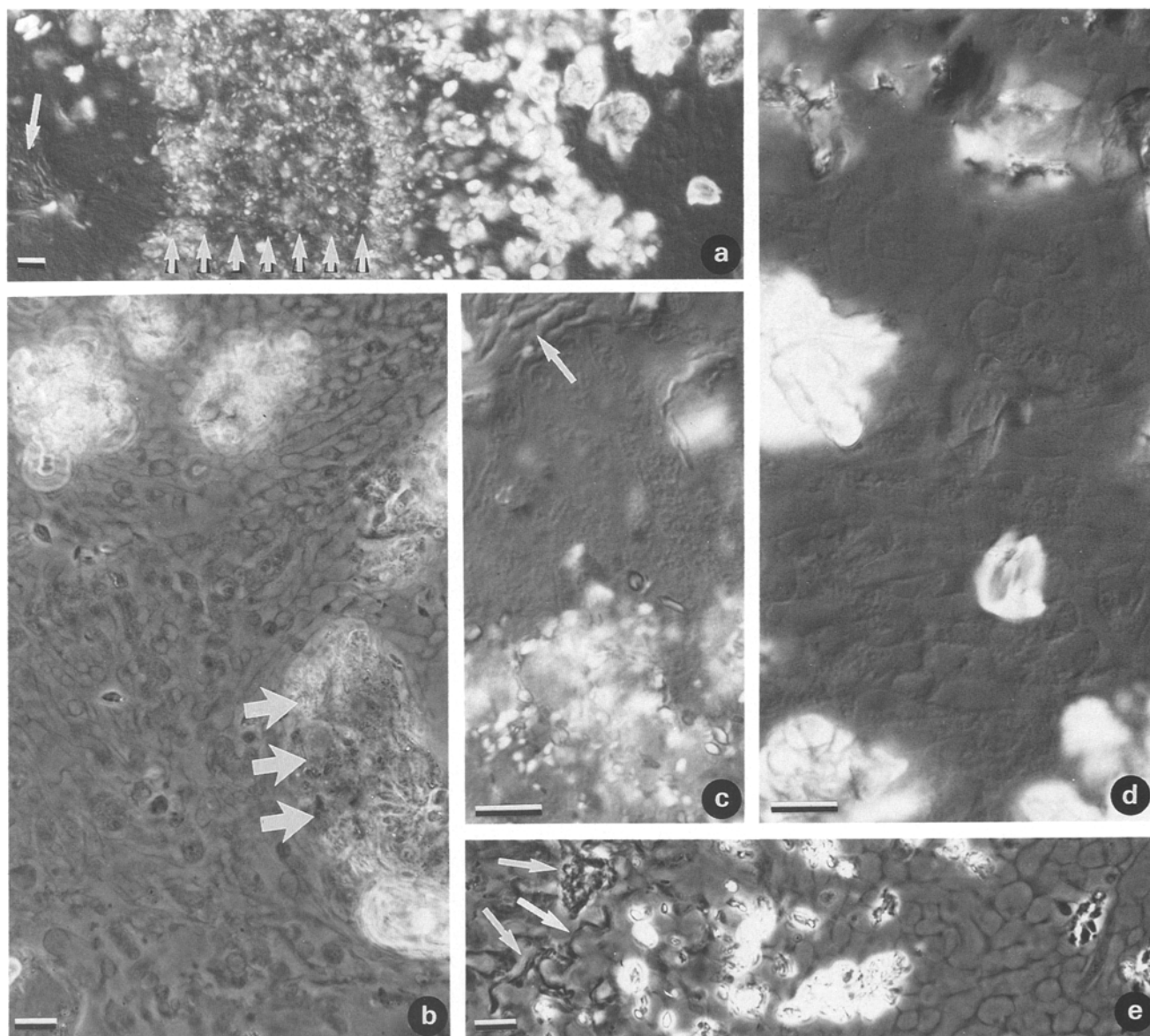
hyphae, with adhering soil debris; outer middle layer 20–40  $\mu\text{m}$  thick, rather compact, consisting of tangentially oriented hyphae intermixed with roundish cells (3–7  $\mu\text{m}$  in diameter) and strongly light-reflecting large crystal-like complex structures; inner middle layer 30–40  $\mu\text{m}$  thick, of hyphae oriented approximately perpendicularly to root surface, with small, strongly light-reflecting, rhombic crystals or crystal-like structures, in between a zone of brown cortex cell wall remnants; innermost layer 20–30  $\mu\text{m}$  thick, with hyphae oriented also approximately perpendicularly but crystals lacking; cortical cell wall remnants also interspersed between hyphae in the inner middle layer and in the innermost layer; root cell wall remnants most densely arranged in the centre.

#### *Colour reaction in different reagents*

*Mantle and rhizomorph preparations:* acetic-fuchsin: no reaction (=nr); aniline: nr; brilliant cresyl blue: violet; cotton blue: nr; ethanol 70%: nr; formol: nr; guaiac: blue; iron sulfate: nr; KOH 15%: nr; lactic acid: nr; phenol: nr; phenol-aniline: nr; ruthenium red: nr; sudan IV: nr; sulpho-vanillin: red; toluidine blue: outer and middle layers irregularly patchy violet, inner layers dark blue to violet, layer with root cell remnants green. *Whole mycorrhizae:* guaiac: blue; sudan IV: slightly red; sulpho-vanillin: red.

#### *Autofluorescence*

*Whole mycorrhiza:* UV 254 nm: nr; UV 366 nm: nr. *Sections:* UV-filter 340–380 nm: mantle slightly bluish;



**Fig. 6a–e.** Sections through the club-shaped distal part of the mycorrhiza (cf. Fig. 8). **a** Cross-section, overview from the centre (left) to the outer layers (cf. Fig. 8); *small arrows* indicate inner middle layer with small crystals; *large arrows* indicate central part with undigested root cell walls. **b** Cross-section, transition zone of a rhizomorph (below) and the mantle (above); *arrows* indicate adhering soil particles. **c** Cross-section between centre with undi-

gested root cell remnants (*arrow*), through the inner layer to inner middle layer with small crystals. **d** Cross-section, outer middle layer with large, irregularly shaped crystal-like structures (outer parts below). **e** Longitudinal section from root cell remnants (*arrows*) of inner middle layer to outer middle layer. From RA 11707; **b**, **e** in normal phase contrast; **a**, **c**, **d** in Normarski's interference contrast; *bar* = 10  $\mu\text{m}$

blue filter 450–490 nm: slightly yellow; green filter 530–560 nm: red.

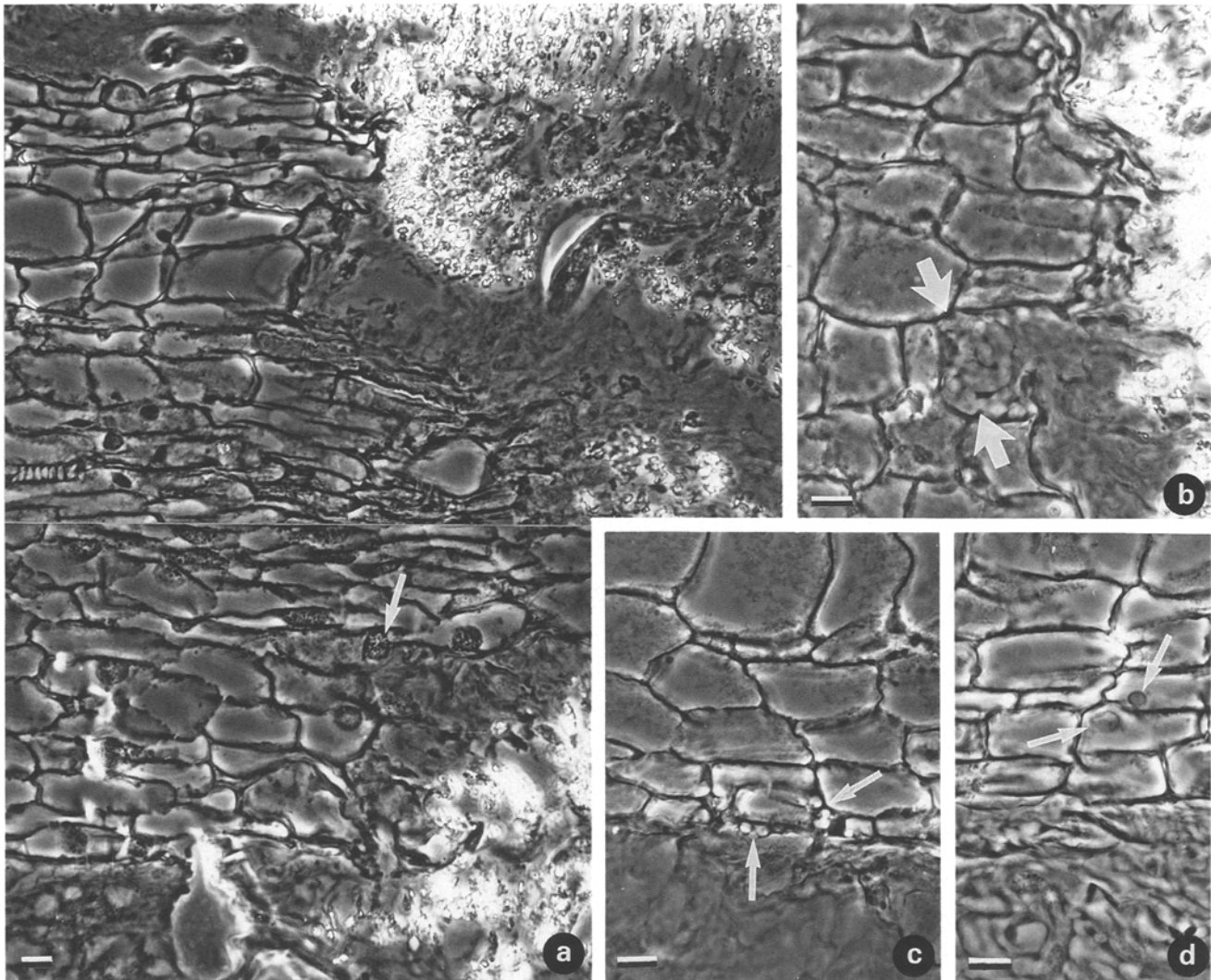
#### Staining of nuclei

Aceto-carmin: two round or roundish nuclei per cell, 2–3  $\mu\text{m}$  in diameter.

#### Material studied and method of identification

Reference specimen for *Rosa*. – Additional material: Germany, Hessen, Lkrs. Offenbach, Egelsbach, in moder, on *Prunus domestica*, leg. H. Zinth, 13 May 1992, Mycorrhiza RA 11704 (reference specimen for *Entoloma saepium* on *Prunus*, in M). – Germany, Hessen, Lkrs. Offenbach, Egelsbach, in moder, on *Prunus domestica*, leg. H. Zinth, June 1992, Mycorrhiza RA 11708 (in M).





**Fig. 7.** **a** Longitudinal section through the zone of root digestion, conical undigested part of vascular bundle protrudes into the club-shaped part of the mycorrhiza, densely arranged, strongly light-reflecting crystals are shown by white areas (*arrows* indicate nuclei of obviously living cells). **b** Longitudinal section, digestion zone with hyphae growing within a root cell (*arrows*). **c** Longitu-

dinal section, outer cortex cells on intact root (zone G in Fig. 8) with intercellular hyphae (*arrows*). **d** Longitudinal section, cortex cells on intact root (zone G in Fig. 8) with some intracellular hyphae (*arrows*). From RA 11707; normal phase contrast; *bar* = 10  $\mu\text{m}$

## Discussion

The *E. saepium* mycorrhizae on *Prunus domestica* are very similar to those on *Rosa* sp. They differ in that they are more ovoid than round and the hyphal cells of the inner surface of the mantle in plan view are rounder and do not show thickened corners at the borders to the adjacent hyphal cells.

The destruction of the distal parts of the root, including differentiating meristem and cortex cells, indicate the parasitic behaviour of *E. saepium*, and the hyphae obviously invade apparently living cells, leaving only cell remnants. The outermost cell layers seem to be sloughed off and digested. Although hyphae also grow within some cells of the more proximal parts of the roots, their development is limited. There are also

some hyphae growing intercellularly, reminiscent of an incomplete Hartig net.

The tightly packed mantle at the most proximal parts of the roots without formation of a Hartig net is similar to the ectomycorrhiza-like habit described for several Asteraceae (Warcup and McGee 1983), but digestion of cortex cells does not occur in Asteraceae (Warcup and McGee 1983).

Unidentified, tuberculate mycorrhizae of similar appearance, but combined with the formation of an epidermal Hartig net, are known from *Castanopsis borneensis* and *Engelhardtia roxburghiana* from Taiwan (Haug et al. 1991). Although the tuberculate mycorrhizae unify several mycorrhizal tips to one ovoid body, some individual mycorrhizae of the tubercles behave like the mycorrhizae of *E. saepium*. They have dead

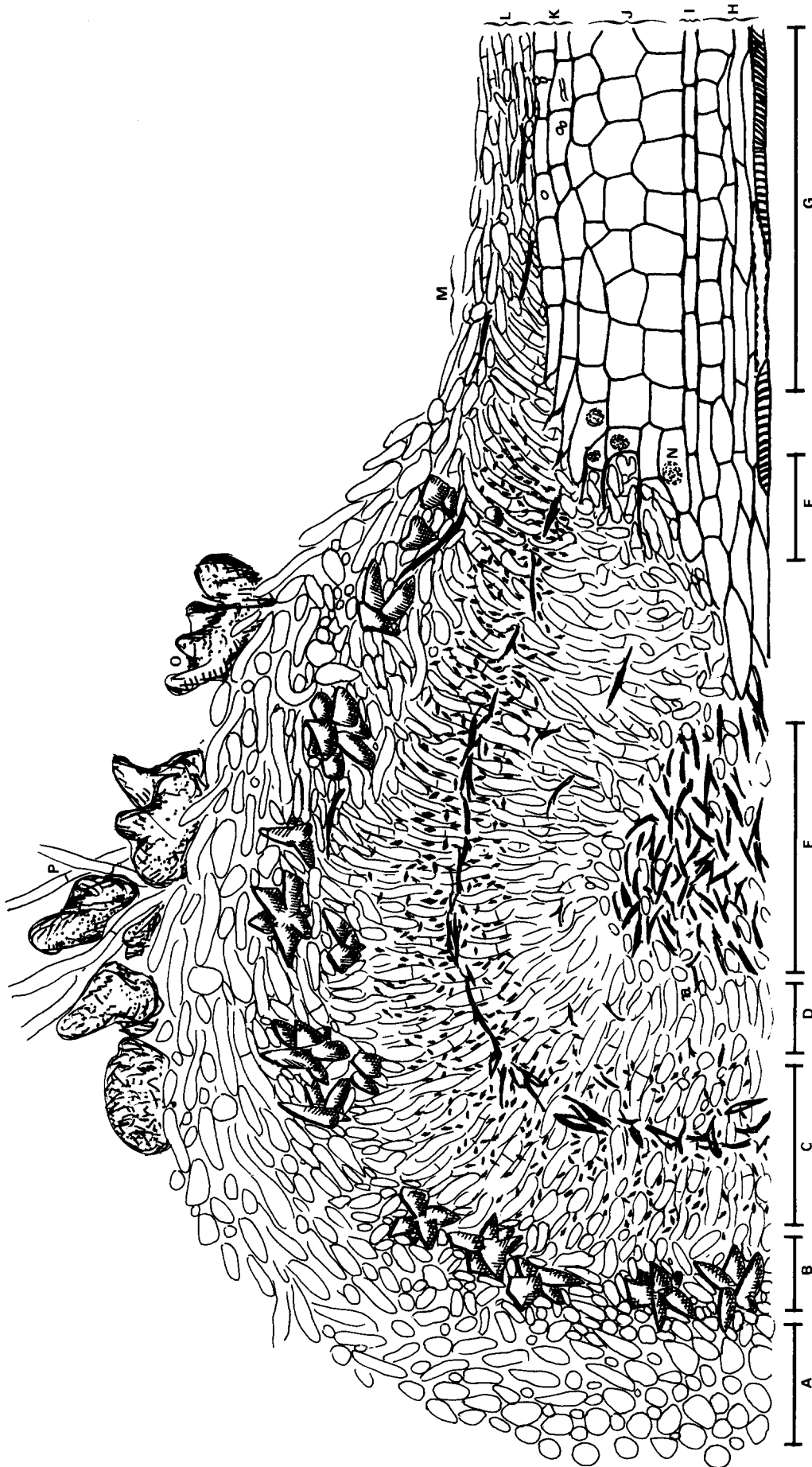


Fig. 8. Schematic drawing of the mycorrhiza of *Enteloma saepium* on *Rosa* sp. A, Outer layer with more rounded cells at the tip. B, Outer middle layer with large, crystal-like, irregularly shaped structures. C, Inner middle layer with preferentially perpendicularly oriented hyphae and small crystals, with a zone of undigested root cell remnants in between. D, Inner layer with preferentially perpendicularly oriented hyphae. E, Central part with undigested root cell remnants. F, Zone of digestion with intracellular hyphae. G, Intact root. H, Vascular tissue. I, Endodermis. J, Inner root cortex. K, Outer root cortex with few intercellular and intracellular hyphae. L, Hyphal mantle. M, Initial formation of perpendicularly oriented hyphae. N, Nucleus. O, Soil particles. P, Emanating hyphae.



tips and are embedded in a very thick mantle; immediately behind the dead apex, the cortex cells are replaced by centripetally growing hyphae, giving a so-called 'strangulation-zone'. Only a few cortical cells were found with intracellular hyphae; they occur neither at the dead apex nor more proximally. The thinner root structure at the 'strangulation-zone' with less layers of cortex cells is apparently caused by compression of root cells rather than by digestion, unlike in *E. saepium* mycorrhizae described here. A typical Hartig net was formed in the tuberculate mycorrhizae. A similar development of mycorrhizae is described by Masui (1926) on *Quercus pausidentata*. This 'compound mycorrhiza form A' also forms a 'strangulation-zone', with a thick centripetally growing mycelium, where the hyphae are approximately perpendicularly oriented to the root surface, and it also forms compressed root cortex cells. There is no Hartig net between the epidermal cells.

The centripetally growing hyphae in the mycorrhizae described by Haug et al. (1991) and by Masui (1926) are very similar to the perpendicularly oriented hyphae in the mycorrhizae of *E. saepium* on *Rosa* and *Prunus*. These layers perhaps do not belong to the mycorrhizal mantle in a narrow sense but appear as secondarily formed structures in connection with either digestion or strangulation of the root.

The ontogeny of the tuberculate mycorrhizae on *Castanopsis* and *Engelhardtia* (Haug et al. 1991) or on *Quercus pausidentata* (Masui 1926) or that of the simple mycorrhiza of *Entoloma saepium* on *Prunus* or *Rosa* has not been studied. In the tuberculate mycorrhizae, several individual tips form typical ectomycorrhizae. These are perhaps only an early stage of development, with the zone of strangulation developing later. Besides the difference in the Hartig net, the only basic difference between the individual tip with the 'strangulation-zone' in the tuberculate mycorrhiza and that of *E. saepium* is the dead but undigested root apex present in the former.

In *E. saepium*, young stages of mycorrhizal development without intracellular infection could possibly be found which were still distinguishable from other *Entoloma* species due to the lack of a typical Hartig net (see below). The similarity of *E. saepium* mycorrhizae to the tuberculate mycorrhizae described on *Engelhardtia roxburgiana*, *Castanopsis borneensis* (Haug et al. 1991), and on *Quercus pausidentata* (Masui 1926) suggests that the mycorrhizae are formed by related fungi, although their habits are different. The ability of ectomycorrhizal fungi to form tubercles is determined not only by the plant but also by the fungus. For example, tuberculate or non-tuberculate mycorrhizae can be formed by different species of the same fungal genus (e.g. *Suillus*, Treu 1990).

Modess (1941), in synthesis experiments with *Entoloma rhodopolium* (Fr.) Kummer and *Pinus sylvestris* and *P. montana*, found mycorrhizae without a true mantle but with a very prominent Hartig net with 15–20  $\mu\text{m}$  thick layers of hyphae.

Zerova and Rozhenko (1966) isolated mycorrhizae of *Entoloma erophilum* (Fr.) Karst and *E. sericeum* (Bull.) Quél. under oak from the soil and of *E. clypeatum* (L.) Quél. under apricot trees and traced hyphal connections to the fruit bodies. Only the mycorrhizae of *E. sericeum* were described, including the formation of dense clumps of mycorrhizae, 1–1.5 mm in diameter; young mycorrhizae are whitish, becoming dark and even black if aged; there is a very thick mantle of up to 200  $\mu\text{m}$  and rhizomorphs 100–224  $\mu\text{m}$  in diameter.

Linkins and Antibus (1982) and Antibus et al. (1981) synthesized mycorrhizae between *E. sericeum* and *Salix rotundifolia*. They found long, simple or profusely branched, white to yellowish-white, slightly tortuous, smooth to short tomentose ectomycorrhizae often with a felty appressed layer, a thick mantle of hyphae with clamps which run parallel to the root axis; they also found an epidermal Hartig net without any intracellular hyphae.

The mycorrhizae of *Entoloma* species so far described do not show the special features of *E. saepium* noted above. The unusual development of the Hartig net in the mycorrhizae of *E. sericeum* on pines (Modess 1941) is reminiscent of the perpendicularly protruding hyphae in the mycorrhizae in *E. saepium*. The thick mantle of *E. sericeum* (Zerova and Rozhenko 1966) is also reminiscent of *E. saepium*, but no description of the interaction between hyphae and root cells are available.

In summary, *E. saepium* seems to be a parasite on *Rosa* and *Prunus* rather than a symbiont, although some advantages to the plants from the fungus cannot be excluded. The genus *Entoloma* appears to be considerably diverse in its mycorrhizal behaviour.

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