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Hartig net structure and formation in fully ensheathed ectomycorrhizas

D. Blasius, W. Feil, I. Kottke and F. Oberwinkler

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The structure and formation of the Hartig net was examined in four different types of spruce ectomycorrhizas from the field. The investigations confirmed that in the case of mycorrhizas with a fully developed sheath enclosing the root apex, the Hartig net is formed by a highly branched fingerlike or puzzlelike hyphal system.
It is shown that the hyphae penetrate and grow mainly in a transversal direction to the axis of the root, resulting in typical indentation patterns on some tangential walls and a fountainlike separation of hyphae when growing from the radial to the tangential intercellular spaces. Septae formation within the branched hyphal system of the Hartig net is rare. Structures which, in sections, appear to be incomplete septae are explained as the result of the branching pattern.
The alignement and the typical branching pattern of the Hartig net is also found around the outermost cortical cells. A description of the changing structure in the different layers of a pseudoparenchymatous hyphal mantle is given. Finally, details of the three-dimensional structure of the mantle and the Hartig net are represented in a block diagram.

D. Blasius, W. Feil, I. Kottke & F. Oberwinkler, Lehrstuhl Spezielle Botanik der Universität Tübingen, Auf der Morgenstelle 1, D-7400 Tübingen, Fed. Rep. of Germany.

Introduction

In recent years attention has been drawn to Hartig net formation of ectomycorrhizas. To study early infectional stages, most investigators synthesize ectomycorrhiza in vitro. The results on primary infection of roots in pure culture are concordant in the observation that hyphae penetrate intercellularly after a loose hyphal weft has been formed (Nylund & Unestam 1982, Piché et al. 1983b, Malajzuk et al. 1984, Duddridge & Read 1984b). In addition to this primary infection of roots, we have to consider the ontogeny of most of the mycorrhizas in the field, which are quickly ensheathed after emerging. They do not break through the well differentiated sheath but "both host and fungal tissues grow slowly in unison" (Harley & Smith 1983, p. 121). The Hartig net formation does not keep pace with the elongation of the ensheathed root when growth is renewed from dormancy and a "pre-Hartig net region" (Atkinson 1975) can be observed (Fig. 1).

With slowing down of root growth the Hartig net is established in this region up to the next metacutization layer. No information is available on this process.

The three-dimensional structure and development of the Hartig net within fully ensheathed apices was therefore studied in longitudinal and cross sections of field-collected mycorrhizas.

Material and methods

The mycorrhizas were collected in october 1983 from two 70 years old Norway spruce (*Picea abies* (L.) Karst. stands in the Northern Black Forest (testing grounds of the Forstliche Versuchsanstalt Baden-Württemberg) and from the 5-years-old seedlings growing beneath. The roots were washed thoroughly and examined under

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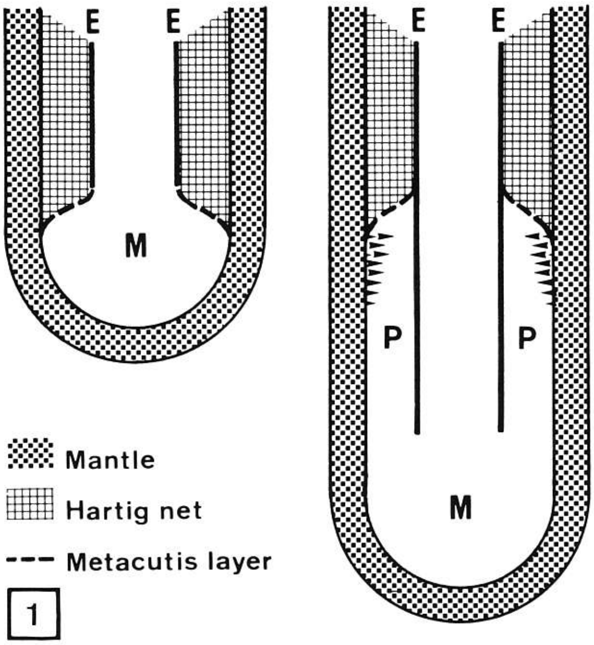


Fig. 1. Diagram of the different developmental stages of fully ensheathed mycorrhizas. Left: dormant mycorrhiza, Hartig net reaches metacutis layer; right: slowly growing mycorrhiza, pre-Hartig net zone visible, Hartig net initation distal to the metacutis (region under investigation, arrows). – E = endodermis; M = meristem; P = pre-Hartig net zone.

the stereo microscope. Four different types were selected and either fixed in FAA and embedded in paraplast or fixed in glutaraldehyde and embedded in Histo-resin (LKB 2218–500). Sections were stained with Chlorazol black E and Pianese IIIb (Wilcox & Marsh 1964).

Results

The four types of mycorrhizas which were distinguished from outer appearance could be clearly differentiated by their mantle structures. They were classified with the key given by Dominik (1969) as subtype H, genus Ha (pseudoparenchymatous, hyphae colourless, hyphal diameter 4–7 μm, hyphal strands present); subtype F, genus Fg (pseudoparenchymatous, hyphae brown, hyphal diameter 3–5 μm, mantle surface bearing small papillae); subtype B, genus Bp (prosenchymatous, hyphae colourless, 2–3 μm in diameter) and subtype I, genus Ia (prosenchymatous to pseudoparenchymatous, hyphae colourless, 4–6 μm in diameter, bristles on mantle surface).

Because the investigated mycorrhizas could be differentiated by the hyphal mantle structure, the four types are probably formed by different fungal species, although species cannot be named up to now.

In contrast to the distinct differences within the structure of the mantles, no differences could be revealed in Hartig net structure of the four mycorrhiza types. Micrographs of the Hartig net will be given therefore only from subtype F and H. To reconstruct the three-dimensional development of the Hartig net we examined sections of different sectional planes as illustrated in Fig. 2.

The basic structure of the Hartig net is especially visible in radial sections and partly in tangential and cross sections (Fig. 2, plane A). Extremely fingerlike branched hyphae penetrate transversally to the axis of the root (Fig. 3). It must be emphasized, that hyphae do not grow in a longitudinal direction through the intercellular spaces in valuable degree. It is also important to notice that large areas of the branched hyphal system lack septae.

The radially penetrating hyphae envelope the cortical cells like hands from all sides. Especially tangential sections therefore, show two different aspects. Sections drawn alongside a tangential wall (Fig. 2, plane B), show a typical indentation pattern of hyphae contacting each other in the intercellular space. The indentation pattern may be more fingerlike (Fig. 4) and sometimes puzzlelike (Fig. 5).

Sections drawn through the point where the radial intercellular space runs into the tangential intercellular space (Fig. 2, plane C), show a fountainlike separation of the hyphae (Fig. 6).

When observed in cross sections, intercellularly penetrating hyphae appear to be wedge-shaped, frequently

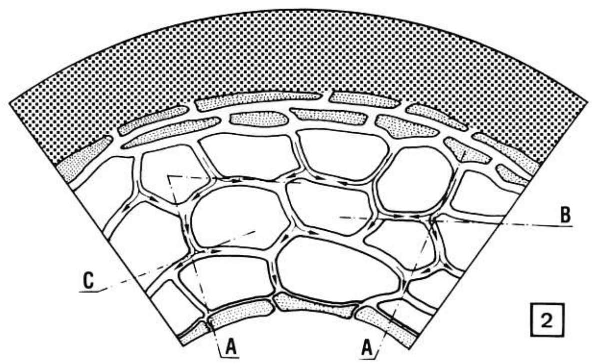
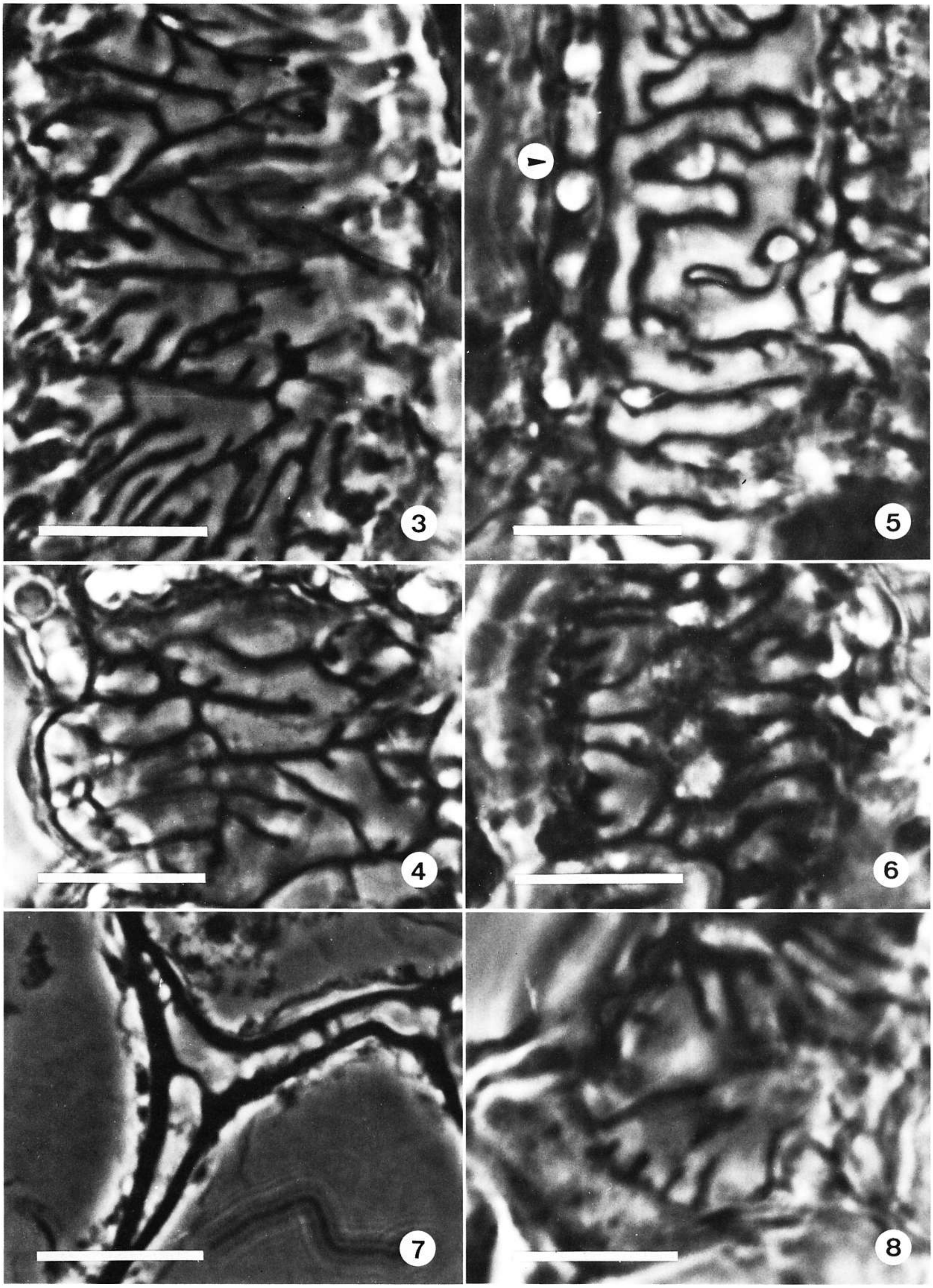


Fig. 2. Diagram showing the different planes of longitudinal sections under investigation. A: radial and partly tangential (high branched hyphal system); B: tangential (indentation pattern); C: tangential (fountainlike pattern).

Fig. 3. Radial section showing a highly-branched hyphal system growing transversally to the root axis in the direction of the endodermis. – Fig. 4. Tangential section through plane B (Fig. 2) showing fingerlike indentation pattern. – Fig. 5. Tangential section showing puzzlelike indentation and ladderlike structures (arrow) as a result of cross sectioning of hyphae. – Fig. 6. Tangential section through plane C (Fig. 2) showing fountainlike separation of hyphae. – Fig. 7. Cross section showing wedge-shaped "septated" hyphae, seperating fountainlike from radial to tangential intercellular spaces. – Fig. 8. Longitudinal section showing penetration of hyphae in a broad lobed front. – Scale marks: 10 μm.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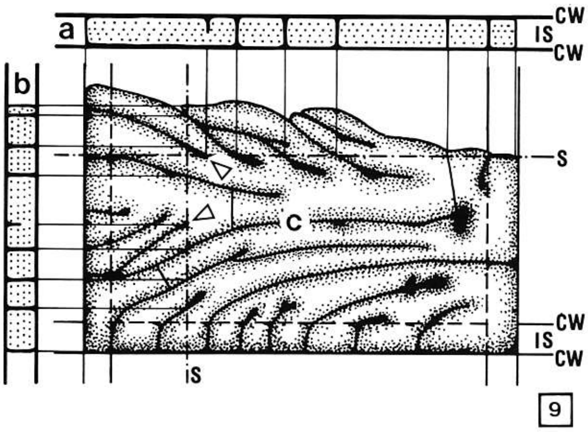


Fig. 9. Diagram showing the different aspects of the Hartig net from a superficial view (C) and from cross sections through the intercellular spaces (a transversal, b longitudinal to the root axis). "Complete septae" in cross sections are the walls of neighbouring hyphae or real septae, "incomplete septae" the beginning of branches (arrows). – CW = cortex-cell wall; IS = intercellular space; S = sectional plane.

septated and nonramified (Fig. 7). However, face views show that the penetrating hyphal system is a broad-lobed hyphal front with rare septation (Fig. 8).

Fig. 9 shows the different aspects of the same region of the Hartig net given from face view and cross view. Cross sections through intercellular spaces, either transversal (a in Fig. 9) or longitudinal (b in Fig. 9) to the root axis show a ladderlike structure looking very similar to septated or incomplete septated hyphae. A superficial view (c in Fig. 9) of the hyphal structure reveals that the "septae" are mostly neighboured branches of hyphae in close contact and the "incomplete septae" just the beginning of branches. Therefore these structures have a double-wall.

The two main principles of Hartig net structure described in this investigation, the transversal orientation of hyphae to the root axis and the high ramification with rare septation already occur on the surface of the outermost root cells. The pseudoparenchymatous structure of the mantle of subtype F (Fig. 10) is altered in the inner layers into a tubular cross texture (Fig. 11) and with direct contact to the root cells into a branched hyphal system (Fig. 12). However the ramification is not that abundant on the outermost root cell walls as in the deeper layers of the cortex.

The results from the investigations are summarized in a block diagram (Fig. 14). The outer and middle layers of the mantle show a pseudoparenchymatous structure with large isodiametric fungal cells. This structure is altered into a cross-textured hyphal pattern in the inner layer. Simultaneously the diameter of the cells is reduced to the diameter of the free hyphae (Fig. 13).

As the hyphae contact the outer root cells, they are orientated transversally to the root axis and begin to branch irregularly. This pattern continues within the Hartig net as demonstrated above.

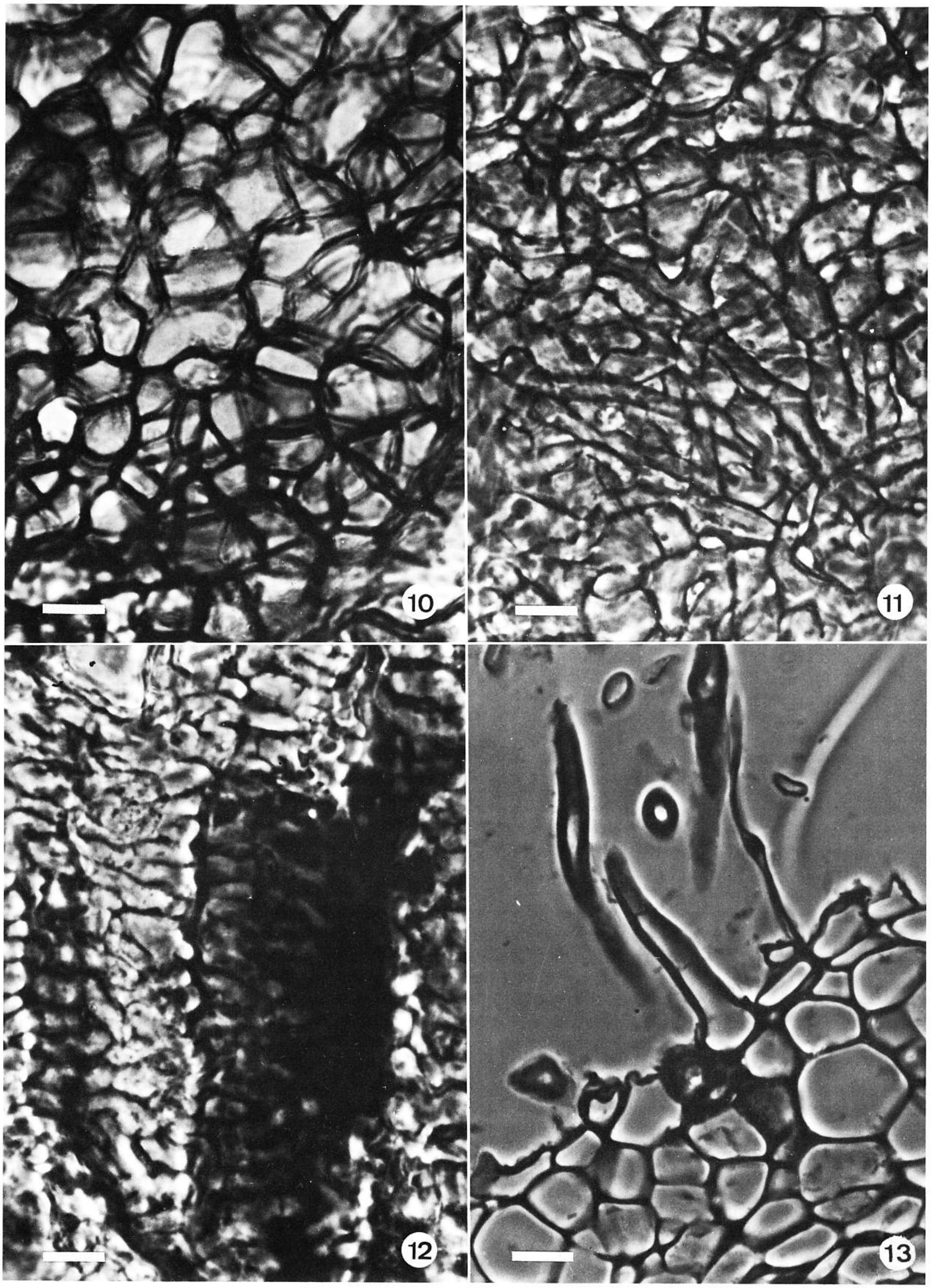
Discussion

Mangin (1910) first recognized that the Hartig net is built up by a highly branched hyphal system which he named "palmettes". Nylund and Unestam (1982) confirmed the branched nature of the hyphae within the Hartig net, "...producing a ladderlike effect in cross section and a labyrinth pattern in face view". This ladderlike structure of the Hartig net was already shown by Marks and Foster (1973) but it has been misinterpreted as short septate hyphae (Piché et al. 1983a) and the origin of branches as incomplete or developing cross walls (Hofsten 1969; Marks & Foster 1973; Atkinson 1975). Atkinson (1975, p. 49) mentioned that the presence of the incomplete cross walls "...appears to be a consistent feature of the fine structure of ectomycorrhizas". He compared the structure with that of transfer cells and interpreted these structures also as "...invaginations of the fungal cell wall" (p. 165). Duddridge and Read (1984a) gave an alternative interpretation that these structures "...are formed as the result of complex infoldings of the fungal wall, brought about by branching under compression of the fungal elements making up the Hartig net".

According to our findings the development of "incomplete septae" cannot be explained by infolding of fungal cell walls: they are formed as the result of outgrowth of fungal branches as can be seen when sectioning at the origin of the branches.

Nylund and Unestam (1982) also give a model on the process of the establishment of the Hartig net and the hyphal mantle during primary infection. Their results on *Piloderma croceum* with *Picea abies* in pure culture differ considerably from our results on fully ensheathed mycorrhizas from the field. Nylund and Unestam observed the "change in fungal morphology into labyrinthic tissue formation leading to Hartig net formation" (p. 63) after intercellular penetration by single hyphae. Subsequently the mantle is formed. Our investigations showed that the development of the Hartig net starts from the innermost layer of a fully differentiated mantle and that penetration takes place in a broad lobed front and not by single hyphae. Penetrating hyphae are orientated transversally to the root axis. Nylund et al. (1982) described the orientation as "predominantly radial in relation to the root" (p. 105). The ori-

Fig. 10. Tangential section through the middle layer of the hyphal mantle of subtype F, showing a pseudoparenchymatous structure with isodiametric cells. – Fig. 11. Tangential section through an inner layer of the mantle with tubal cross texture. – Fig. 12. Tangential section through the mantle revealing the branched hyphal system on the outermost cortex-cells and it's orientation transversally to the root axis. – Fig. 13. Cross section with isodiametric cells and free hyphae. – Scale marks: 10 μm. Orientation of the micrographs: root axis vertically.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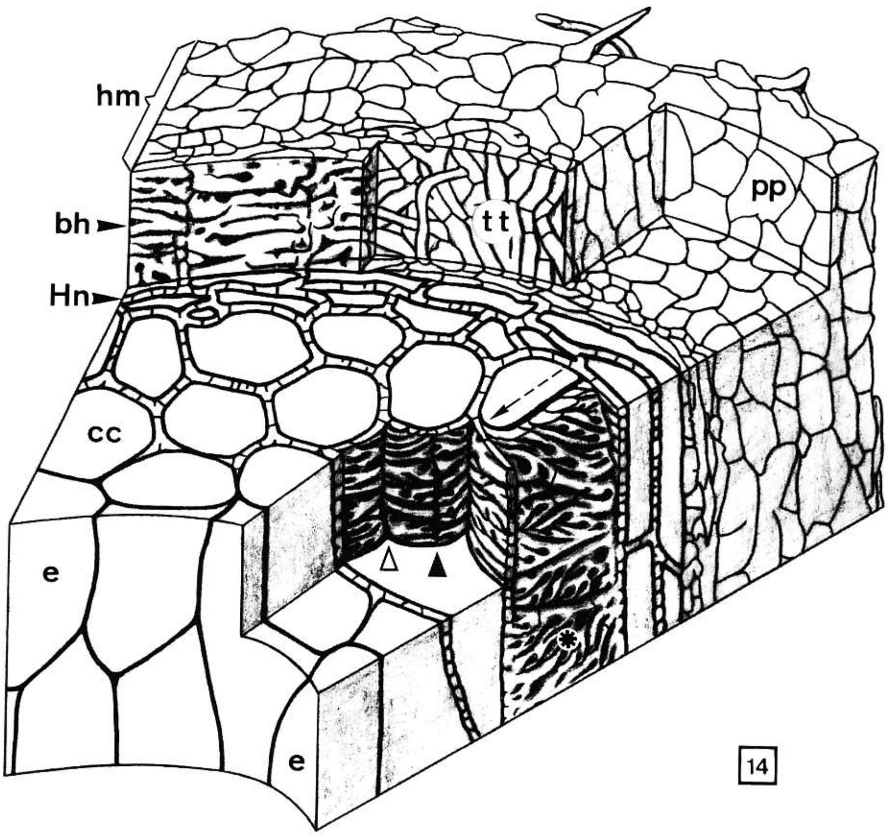

Fig. 14. Block diagram of hyphal mantle (hm) and Hartig net (Hn) showing the different aspects of sections as explained before: main growth direction of hyphae transversal to the root axis (intermitted arrow), branching of hyphae with rare septation (asterix), indentation pattern (black arrow), fountainlike separation of hyphae (open arrow), changes of structures within the mantle (pp=pseudoparenchymatous, tt=tubal cross texture, bh=branched hyphae on the outermost cortex cells). cc=cortex cell, e=endodermis.

entation of the hyphae also can be derived from the drawings of Mangin (1910), the micrographs of Strullu (1976, Fig. 4 b) and Nylund and Unestam (1982, Fig. 5) and a block diagram from Strullu (1979), but it is not described in the text.

Our observations revealed that the mature Hartig net is not built up by a "sheet of anastomosing fungal elements" (Duddridge & Read 1984 a, p. 571), though anastomosa can occur sporadically. It also cannot be interpreted as an unordered labyrinthine system but must be described as a highly ordered system with specific pattern formation.

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References

Atkinson, M. A. 1975. The fine structure of mycorrhizas. – D. Phil. Thesis, Oxford. (Unpubl.).

Dominik, T. 1969. Key to ectotrophic mycorrhizae. – Folia Forest. Pol. Ser. A, 15.

Duddridge, J. A. & Read, D. J. 1984a. The development and ultrastructure of ectomycorrhizas, I. Ectomycorrhizal development on Pine in the field. – New Phytol. 96: 565–573.

– & Read, D. J. 1984b. The development and ultrastructure of ectomycorrhizas, II. Ectomycorrhizal development on Pine in vitro. – New Phytol. 96: 575–582.

Harley, J. L. & Smith, S. E. 1983. Mycorrhizal symbiosis. – Academic press, London, New York.

Hofsten, A. 1969. The ultrastructure of mycorrhiza. I. Ectotrophic and ectendotrophic mycorrhiza of *Pinus silvestris*. – Sv. Bot. Tidskr. 63: 455–463.

Malajczuk, N., Molina, R. & Trappe, J. M. 1984. Ectomycorrhiza formation in *Eucalyptus*. II. The ultrastructure of compatible and incompatible mycorrhizal fungi and associated roots. – New Phytol. 96: 43–53.

Mangin, L. 1910. Introduction á l'étude des mycorrhizes des arbres forestières. – Nouv. Arch. Mus. Hist. Nat., Paris, Série 5,2: 245–260.

Marks, G. C. & Foster, R. C. 1973. Structure, morphogenesis and ultrastructure of ectomycorrhizae – In: Marks, G. C. & Kozlowski, T. T. (eds), Ectomycorrhizae, their ecology and physiology, London, New York, pp. 1–41.

Nylund, J. E. & Unestam, T. 1982. Structure and physiology of ectomycorrhizae. I. The process of mycorrhiza formation in Norway Spruce in vitro. – New Phytol. 91: 63–79.

– , Kasimir, A., Strandberg Arveby, A. & Unestam, T. 1982. Simple diagnosis of ectomycorrhiza formation and demonstration of the architecture of the Hartig net by means of a clearing technique. – Eur. J. For. Path. 12: 103–107.

Piché, Y., Peterson, R. L., Howarth, M. J. & Fortin, A. J. 1983a. A structural study of the interaction between the ectomycorrhizal fungus *Pisolithus tinctorius* and *Pinus strobus* roots. – Can. J. Bot. 61: 1185–1193.

– , Peterson, R. L. & Ackerley, C. A. 1983b. Early development of ectomycorrhizal short roots of pine. – Scanning electron microsc. 1983/III: 1467–1474.

Strullu, D. G. 1976. Contribution à l'étude ultrastructurale des ectomycorrhizes à basidiomycètes de *Pseudotsuga menziesii* (Mirb.). – Bull. Soc. Bot. France 123: 5–16.

– 1979. Ultrastructure et représentation spatiale du manteau fongique des ectomycorrhizes. – Can. J. Bot. 57: 2319–2324.

Wilcox, H. & Marsh, L. C. 1964. Staining plant tissues with chlorazol black E and pianese III-b. – Stain Technol. 39: 81–86.