portance is not in the productive ecosystems in early stages of the succession, although they contribute to nutrient acquisition in these stages, but in protective ecosystems in the final stages of succession, where they keep nutrient cycles closed and prevent loss of resources from the entire ecosystem.

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Comparative anatomy of the host-fungus interface in mycorrhizas

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Summary. There are several types of mycorrhizal symbiosis (ectomycorrhiza, endomycorrhiza, ectendomycorrhiza), and the interfaces between the host-plant and the fungal symbiont have different organizations. The interfaces between the partners are always limited on the one side by the fungal plasmalemma and on the other side by the plasmalemma of the host plant or the perisymbiont membrane derived from it. The cytoplasms of the partners are therefore separated by a mixed apoplast consisting of a fungal wall and a host wall or an apposition layer.

Key words. Ectomycorrhiza; endomycorrhiza; ectendomycorrhiza; interfaces; plasmalemma; matrix; perisymbiont membrane; mixed apoplast.

The confrontation of two organisms in a parasitic or symbiotic association leads to the formation of a contact zone or 'interface' composed of elements derived from both partners, through which most of the interactions and exchanges between the partners take place. Mycorrhizal symbioses, in particular, develop interfaces of widely different organization which are characteristic of the different types of mycorrhiza (ectomycorrhiza, endomycorrhiza, ectendomycorrhiza).

In this article we discuss the different mycorrhizal interfaces, firstly those of vesicular-arbuscular (VA) mycorrhizas, then those of other endomycorrhizas (ericoid mycorrhizas, orchid mycorrhizas, *Terfezia* mycorrhizas) and of ectomycorrhizas, and finally the intracellular structures of *Monotropa* ectendomycorrhizas.

Interfaces of vesicular-arbuscular mycorrhizas

Vesicular-arbuscular mycorrhizas are the most widespread among plant species. The fungi involved are Zygomycetes belonging to the family of Endogonaceae. The fine structure of these mycorrhizas is well known and there are numerous ultrastructural studies, as summarized in recent reviews[9, 30, 54, 89].

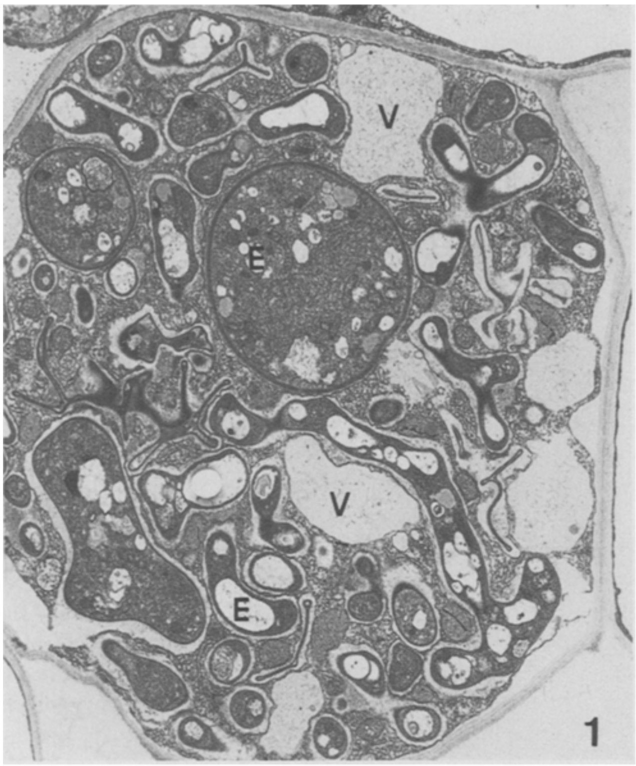
The fungus produces a network of intercellular hyphae within the root cortex. From the hyphae of this network, branches penetrate the cell wall and form ramified intracellular structures, the arbuscules (fig. 1). In some host-plants, cells of the superficial layers of the cortex contain coils of large hyphae. Often the hyphae dilate to produce ampoules with thickened walls, the vesicles.

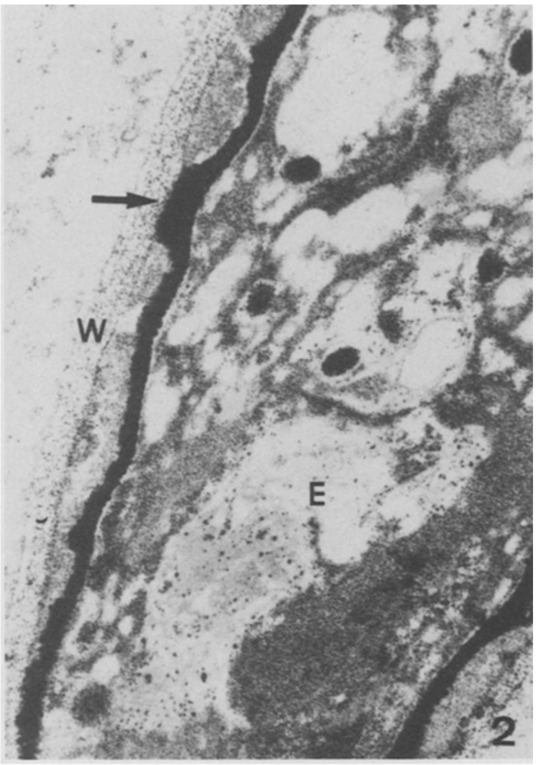
Both partners of the symbiosis may, to a certain extent, modulate mycorrhizal morphology[9, 16, 49, 66, 67]. For example a given endophyte associated with different host-plants may form a mycorrhiza with or without coils, and with or without a network of intercellular hyphae. Components of the host cell wall and in particular phenolic compounds appear to be responsible for this modulation[10].

Interfaces of the intercellular hyphae

In most VA mycorrhizas, the hyphae of the intercellular network are located in the spaces between the cortical cells where they are in close contact with the outer surface of the walls of the cells and sometimes penetrate between two cells by separating the middle lamella. This network presents an organization similar to the one of the ectomycorrhizal Hartig net[67].

In some mycorrhizas (Jeanmaire, personal communication), the outer surface of the walls of the cortical cells is coated with a layer of a dense polysaccharide which prevents any direct contact between the hyphae and the walls. The endophyte cell walls produce notched protuberances which pervade this layer to anchor the hyphae to the wall (fig. 2).





Abbreviations: c, cement; CC, cortical cell; E, endophyte; F, fungus; HN, Hartig net; M, matrix, Ma, mantle; N, nucleus; P, peg; p, fungal wall; V, vacuole; W, host wall.

Figure 1. Vesicular-arbuscular mycorrhiza. Overall view of a host cell with a living arbuscule. The cytoplasm occupies a large part of the cell volume and the vacuolar apparatus is reduced to several isolated elements. × 5,500. (From C. Jeanmaire.)

Figure 2. Vesicular-arbuscular mycorrhiza. Intercellular hypha attached by its notched protuberances (arrow) to the host cell wall. × 22,500. (From C. Jeanmaire.)

Figure 3. Vesicular-arbuscular mycorrhiza. The isolation layer (star) is well organized around the large branch and diffuse around the fine branch. The plasmalemma formations are well expressed in the matrix. × 30,000.

The interfaces between host root cells and intercellular hyphae thus have the following organization: fungal plasmalemma/fungal cell wall/host cell wall/host plasmalemma.

Interfaces of intracellular structures

The intracellular structures are arbuscules (fig. 1) and sometimes, in the outer cortical cells, coils[16, 22, 55, 59, 60, 66, 90].

In the host cell, there is an important increase of the cytoplasmic volume and a decrease of the vacuolar apparatus[24].

The fine structure of the cytoplasm is modified[35]. Dictyosomes remain rare except in a few mycorrhizas[86]. The endoplasmic reticulum develops and there are numerous contacts between its cisternae and the plasmalemma of the interface, as discussed in detail below[32, 67]. Plastids are either proplastids[61, 93] or chromoplasts[87], secretory leukoplasts[36] or small amyloplasts[8]. The presence in most mycorrhizas of plastids without starch has been interpreted as an indication of a fungus-induced change in the carbon metabolism of the host-plant[89]. The differentiation of secretory leukoplasts in some species is the expression of a defense reaction of the host cell[36].

Arbuscular hyphae are surrounded by the host cell plasmalemma[22, 26, 57 – 62, 66, 67, 85, 89, 95] which develops a very large surface area[24, 102]. This surface can be further increased by numerous plasmalemma formations[31]. The plasmalemma of the interface has ATPase activities[71] and neutral phosphatase activities[56] which are absent from the peripheral plasmalemma of the same cell[28].

It has been shown recently[50], using immunocytochemical techniques, that this membrane contains an oligosaccharide fraction also found in the peripheral plasmalemma but lacks a glycoprotein component present on the latter.

The plasmalemma surrounding the fungus therefore presents different structural and functional properties from the peripheral plasmalemma. It should be called the 'perisymbiont membrane'[50] by analogy with the peribacteroid membrane in the root nodules of leguminous plants.

The fact that the perisymbiont membrane is never broken makes any direct contact between the fungus and the host cell cytoplasm impossible. The fungus, by perforating the wall, penetrates into the intracellular space but remains outside the cytoplasm in what may be considered to be an extension of the host apoplast, and this constitutes the matrix of the interface. Transfers between the fungus and the host cell therefore occur exclusively by an apoplastic pathway[54, 66, 96, 97, 101].

The modifications induced by the symbiosis do not concern the host plant alone but also the endophyte. The walls of the arbuscular hyphae are much thinner than the walls of the intercellular hyphae and they have an amorphous structure[10, 14]. It has been proposed that these transformations of the fungal wall and the extensive branching of the fungus during arbuscule formation are induced by interactions between the host cell and the endophyte and, in particular, the production of a chitinase by the host plant[10, 19]. However, a recent immunocytochemical study has shown that plant chitinase does not come into contact with the fungal cell wall in mycorrhizal roots of *Allium*[91].

The trunk and sometimes the major branches of the arbuscule are lined with a continuous polysaccharide isolation layer[27, 88, 93] (fig. 3). Its structure is comparable to the host wall appositions induced by some parasitic infections in plants[26, 58]. Around the fine branches, this isolation layer takes the form of a more or less dense layer of polysaccharide fibrils around the hyphae and in the matrix (fig. 3). There is a progressive reduction of the isolation layer from the trunk to the small branches[26].

The isolation layer and the matrix fibrils are synthesized by the host cell as shown by conventional cytochemical methods[22, 26, 66, 88] or immunoaffinity techniques[20].

The fibrils of the isolation layer are polymerized on the perisymbiont membrane running along the interface[27, 67] and the numerous contacts between the cisternae of the endoplasmic reticulum and the plasmalemma of the interface (perisymbiont membrane) are thought to function in the transfer of precursors to the sites of polysaccharide synthesis[32, 67].

The extensive branching of the arbuscule which considerably increases the area of contact between the partners[67] and the possible interference of the endophyte with the host cell wall synthesis process[27] (lysis or inhibition of polymerization) may explain why a structured isolation layer is not formed around the fine branches of the arbuscule.

In the arbuscule, the interfaces are organized as follows: for the large branches or the trunk – perisymbiont membrane/matrix (extension of the apoplastic space)/isolation layer (produced by the host cell and comparable to a cell wall)/fungal cell wall/fungal plasmalemma; and for the small branches – perisymbiont membrane/matrix/isolation layer reduced to polysaccharide fibrils/fungal cell wall/fungal plasmalemma. The only difference between these two types of interface is in the structure of the isolation layer.

In cells with a coil, as in those with an arbuscule, the plasmalemma is not broken and surrounds all the intracellular hyphae. The isolation layer is always present. However, there is little increase in the cytoplasmic volume which forms a thin film lining the wall and part of the fungal hyphae. The vacuolar system is well developed. No ATPase activity has been demonstrated on the perisymbiont membrane around coils[90]. There appears to be only a limited interaction between partners in cells with a coil[90]. The interface is of the same type as that observed around the large branches of arbuscules (see above).

Arbuscules have a limited life span, estimated to be only a few days in some cases[7, 24]. The hyphae lose their

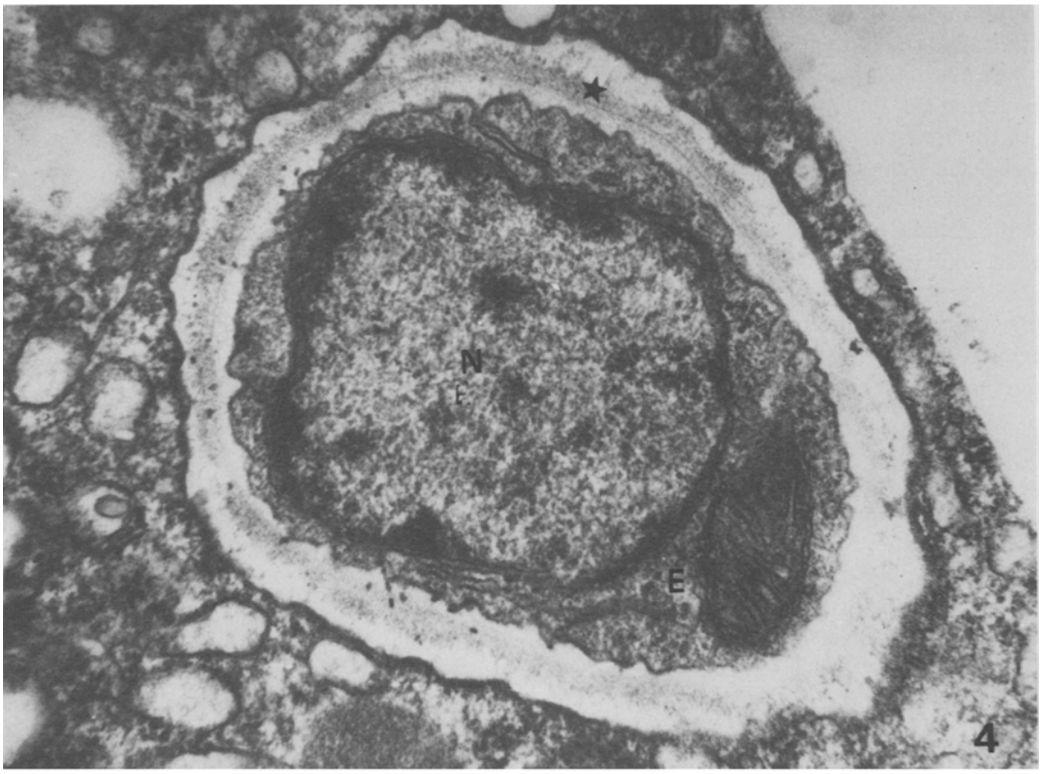


Figure 4. Ericoid mycorrhiza. Section of a hypha of the coil. The isolation layer (star) is clearly visible. × 40,000. (From P. Bonfante-Fasolo.)

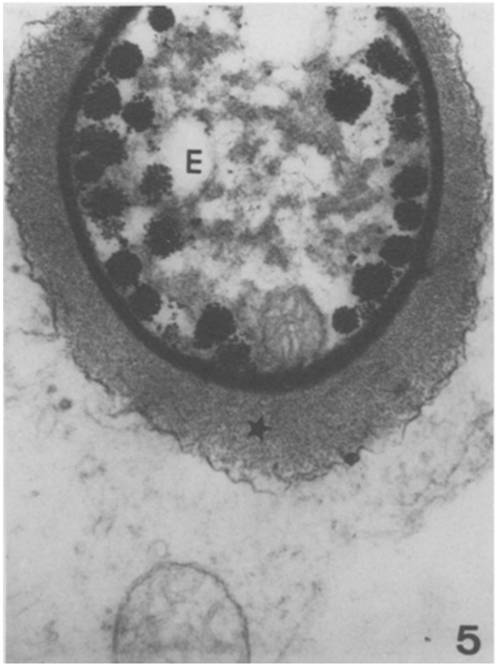


Figure 5. Orchid mycorrhiza. Section of an intracellular hypha surrounded by a thick isolation layer (star). × 32,000. (From M. S. Pais.)

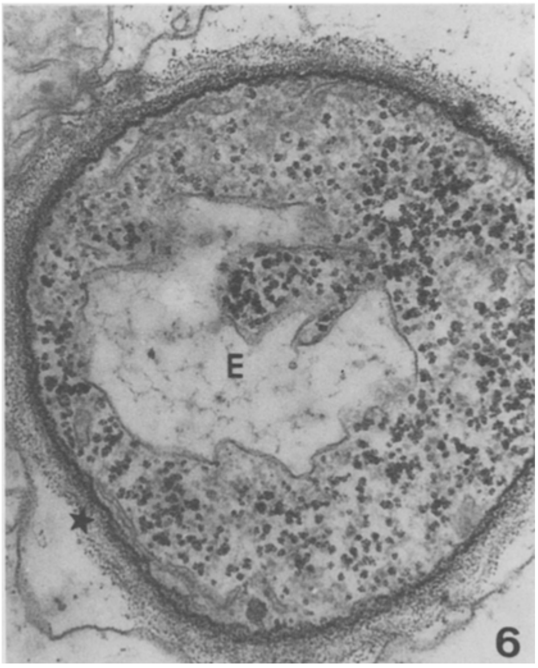


Figure 6. Endomycorrhiza of *Terfezia*. The isolation layer (star) is clearly visible. × 30,000. (From Z. Fortas.)

cytoplasmic contents and the whole arbuscule collapses into a compact mass[23, 58, 62, 85] embedded in a common matrix and coated with a fibrillar polysaccharide substance[26, 88]. Except in a few cases, the lysis of the fungus is incomplete.

The material coating the dead hyphae has a denser structure than the fibrils surrounding the living branches. This indicates that the fungal hyphae inhibit the formation of the isolation layer as long as they are alive. Its constituents remain dispersed in the matrix in the form of fibrils or even soluble carbohydrates. After the death of the arbuscule, the antagonist action of the endophyte disappears and the precursors contained in the matrix polymerize around the fungal remains[27,66,67].

The death of the arbuscule induces considerable modifications in host cell organization[22,66]. The vacuolar apparatus increases until it occupies most of the volume of the cell and the volume of the cytoplasm decreases. Carbohydrates accumulate as starch in the plastids[36,66].

The interface of dead arbuscules has the following organization: perisymbiont membrane/common matrix/polysaccharide encapsulation material equivalent to the isolation layer/remains of fungus.

Interfaces of other endomycorrhizas

There are several other types of endomycorrhiza: the ericoid mycorrhiza, the orchid mycorrhiza, and the endomycorrhiza of some Cistaceae formed with fungi belonging to the family of the Terfeziaceae. In these endomycorrhizas, the fungal partners are either Basidiomycetes or Ascomycetes; they form hyphal coils instead of arbuscules inside the host cells.

The organization of the interface of these mycorrhizas, although they have been less studied than VA mycorrhizas, is nevertheless well known[5,6,11-13,15,21,29,37,38,46,52,53,75,98].

In all these endomycorrhizas, the plasmalemma of the host cell is never broken and surrounds the intracellular hyphae. Although the fungus penetrates inside the cell, it remains confined to the apoplast which constitutes the matrix, and the exchanges take place via the apoplastic pathway.

The walls of the fungi are modified in the symbiosis[17,18,33]. They are always lined with a layer of polysaccharide material distinct from the fungal wall and similar to an isolation layer (figs 4-6). In fact, the interfaces of these mycorrhizas have the same organization as that of VA mycorrhizas in that they comprise host plasmalemma/matrix/isolation layer/fungal cell wall/fungal plasmalemma.

However, there are important differences in the course of mycorrhizal development. In ericoid mycorrhizas, the host cell dies soon after infection and the hyphae of the fungus continue to live for some time in the dead host cell. In orchid mycorrhizas, it is the fungus which dies soon after infection and the cortical cells of the host which continue to live with a mass of dead hyphae inside. In this case, the interface is similar to those of host cells in VA mycorrhizas which contain dead arbuscules.

In the endomycorrhizas produced in Cistaceae by fungi of the Terfeziaceae family, the endophyte and the host cells follow a synchronous development, and dead host cells only contain dead or degenerating hyphae. Al-

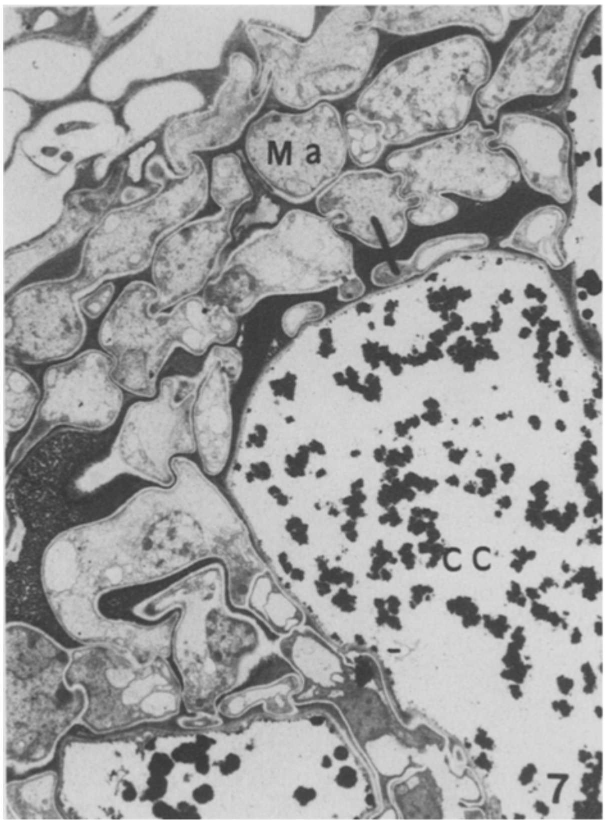


Figure 7. Ectomycorrhizal mantle. The internal mantle may be easily distinguished from the external mantle (upper left). × 3,000.

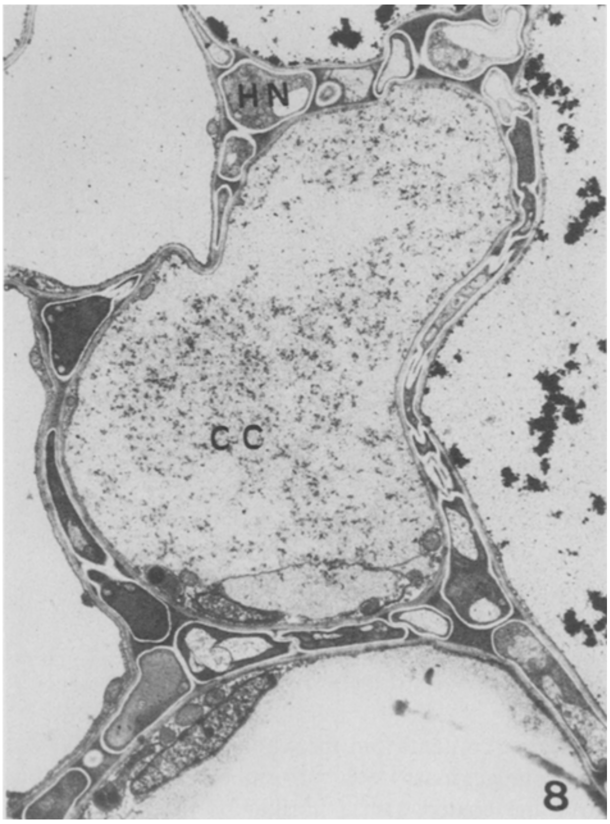


Figure 8. Hartig net of the same mycorrhiza. The cortical host cells are vacuolated. × 3,000.

though the interface has the usual organization in these endomycorrhizae[34], the colonization of a cell by the fungus does not induce an increase of the cytoplasmic volume, the vacuolar apparatus always remains very important and the tonoplast of the vacuoles is often close to the plasmalemma running along the interface. Interestingly, although some hyphae are intracellular, they remain joined to the internal face of the host cell wall[29, 34]. This represents a new type of interface, comprising the fungal plasmalemma/fungal wall/host cell wall.

Interfaces of ectomycorrhizas

Ectomycorrhizas are common in trees of temperate regions. The fungal partners are mainly Basidiomycetes and Ascomycetes and, much more rarely, Endogonaceae[54]. Ectomycorrhizas comprise a mantle of hyphae around the root (fig. 7) and a network of intercellular hyphae, the Hartig net (fig. 8), in which the symplastic continuity between the cortical host cells is maintained [76]. The Hartig net constitutes an important area of contact between the fungus and the root cortical cells. ATPase activities, demonstrating active transport, have been localized on the plasmalemma of both partners[69], and most exchanges are considered to take place in this part of the mycorrhiza.

The fungal hyphae, both in the mantle and in the Hartig net, are more or less coated with a cement (fig. 9) which has been described as a coating layer[84], an interfacial matrix[89], and apposition layer[40, 41, 77, 92, 99] or the external layer of the hyphal wall[97].

In mycorrhizas formed by Ascomycetes, the cement is electron-dense[79, 84, 99, 100] whereas it is transparent in Basidiomycete mycorrhizas[89, 93, 94, 99]. However, for some authors, this property depends more on the stage of mycorrhizal development than on the type of fungal symbiont[64].

Host cells surrounded by the Hartig net, unlike those containing endomycorrhizae, do not show any extensive modification of their organization. Most of their volume is occupied by a large vacuole, and the cytoplasm is reduced to a thin parietal film (fig. 8). The nuclei are not deformed or hypertrophied[93, 96]. However, the amyloplasts, when present, are smaller than those in uninfected roots[96].

In the most simple interfaces, the fungal cell walls and the walls of the cortical cells are in direct contact[4, 39, 47], though more often they are separated by a layer of cement of variable width. The interfaces have the following structure: fungal plasmalemma/fungal cell wall/cement layer (which may be very thin or absent)/host cell wall/host cell plasmalemma.

Various studies have attempted to determine the nature and origin of the cement. It appears to consist of polysaccharides[40, 41, 68, 72, 77, 79] (fig. 10) associated with proteins[34, 79].

The pectic material originating from the middle lamella of the host cell wall[25, 72], and chitin, which is characteristic of fungal walls, are not present in large quantities[78] (fig. 11). Some constituents are of fungal origin whereas others may be produced by a superficial alteration of the host cell walls[43, 44, 77, 92, 99].

Although the walls of both partners are clearly visible in the young parts of mycorrhizas[44, 47, 92], their structure is modified in the older parts to a variable extent depending on the mycorrhiza[39, 74]. These transformations may correspond to biochemical changes[72, 73, 74] leading to the formation of an interface adapted to nutritional ex-

Figure 9. Detail of a part of the Hartig net showing the cement, which is much denser than the fungal walls. × 20,000.

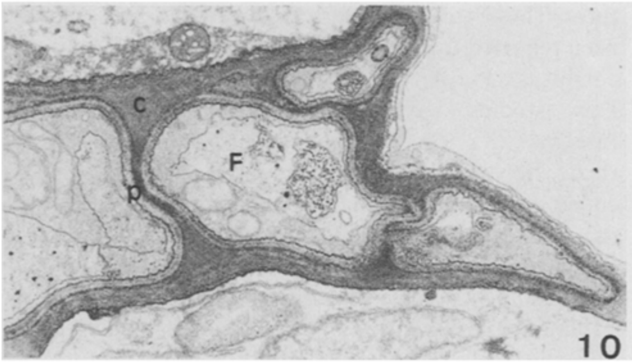


Figure 10. Hartig net. PATAg test for polysaccharides. The boundary between the cortical cell walls and the cement is indistinct. × 20,000.

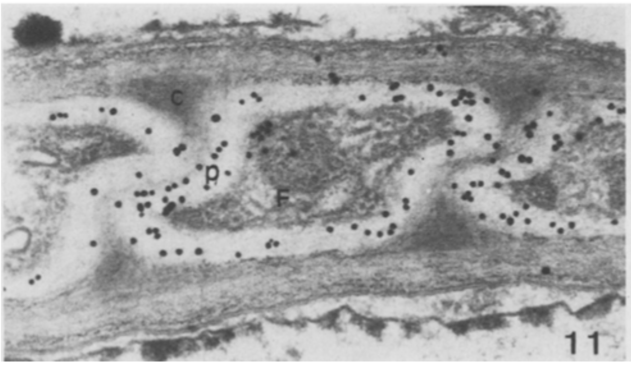


Figure 11. Hartig net. Localization of chitin with WGA labelled with colloidal gold. × 60,000.

changes[4,99]. They may also represent changes caused by the formation of elicitors, in the form of polysaccharide fragments derived from the root walls or in the form of chitosan or chitin fragments[73] originating from the fungal walls.

In some ectomycorrhizas, protuberances develop on the walls of the cortical cells in contact with the mantle and with the first hyphae of the Hartig net causing an important increase in the parietal surface area[1,2,45,63-65,72,82,103]. Their formation seems to be induced by the presence of the mycorrhizal fungus on the root[2]. However, their frequency may depend on the nutritional conditions of the fungus. Hence, in synthetic mycorrhizas, these protuberances only develop in media with high glucose concentrations[45]. They have a polysaccharide nature[45] and are similar to parietal outgrowths of transfer cells[2].

In old mycorrhizas, phenolic compounds are incorporated in these outgrowths, modifying their properties, in particular by reducing the permeability of the wall[2,3].

Interfaces of pegs of Monotropa hypopitys ectendomycorrhizas

Ectendomycorrhizas have both an ectomycorrhizal organization (mantle and Hartig net) and intracellular structures. They represent a rare type of mycorrhiza, having been reported from pines, some Ericaceae (arbutoid mycorrhizas), Pyrolaceae and Monotropaceae[54].

Few studies have investigated their fine structure[42,48,70,80,81,83]. In most cases, an ectendomycorrhiza shows typical ectomycorrhizal organization and coiled intracellular hyphae. The interfaces have the same structure as those described above for ecto- and endomycorrhizas, respectively. The ectendomycorrhizas of *Monotropa*[42,70] have the conventional ectomycorrhizal structure with a mantle and a Hartig net in the first layers of cortical cells. However, the intracellular structures are quite specific.

A massive hypha, shaped more or less like a wedge, grows from the intercellular hyphae and penetrates without branching into a living cortical cell (fig. 12), forming a 'peg'[42]. However, this is not a true intracellular structure as the cell wall is not broken but invaginated by the peg[42].

Finger-like protuberances with fibrillar contents develop from the invaginated part of the cell wall (fig. 12). The whole structure closely resembles that of a wall of a transfer cell[51]. These structures seem to be related to the transfer of large amounts of metabolites and in particular carbohydrates, from the fungus to the nonchlorophyllous host plant[42]. Analogous structures have already been described in the ectomycorrhizas[1,65].

The interface of the peg has the following organization: fungal plasmalemma/fungal cell wall/invaginated cell wall of the host cell/finger-like structures/host plasmalemma.

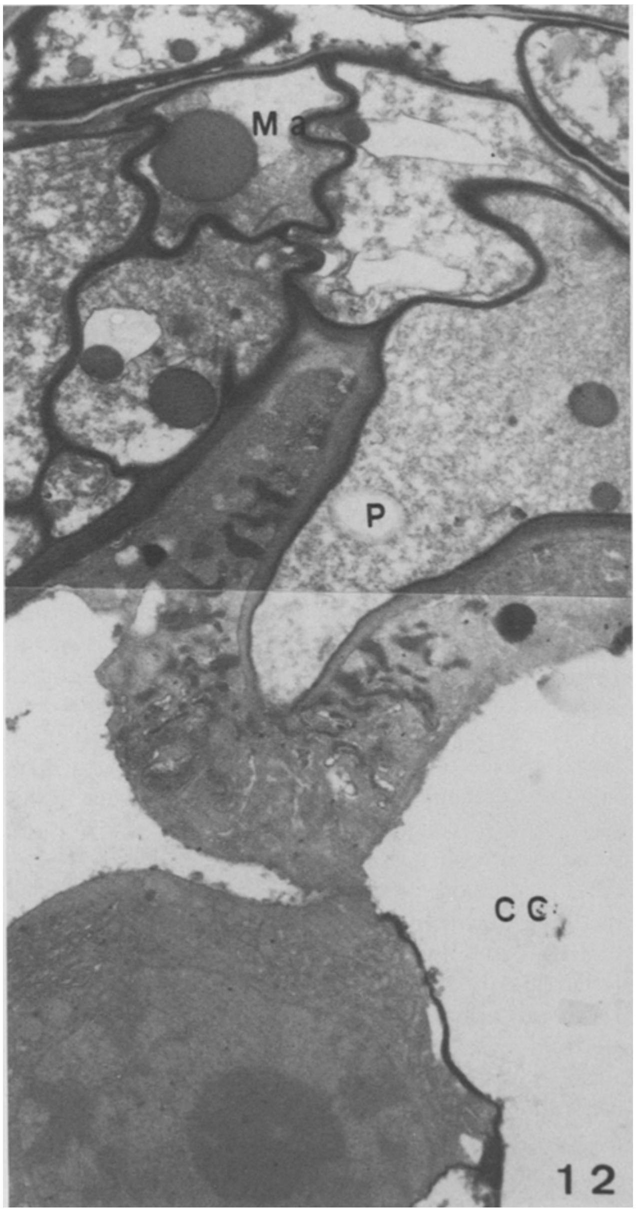


Figure 12. Ectendomycorrhiza of *Monotropa hypopitys*. Finger-like projections in the cell cytoplasm around the peg. × 11,000.

Conclusion

Although there are different types of mycorrhiza, the interfaces between the symbiotic fungi and the cells of the host plants are always bordered on the one side by the fungal plasmalemma, and on the other by the plasmalemma of the host plant or the perisymbiont membrane derived from it. The cytoplasms of the two partners never come into direct contact and are separated by a mixed apoplast comprising a fungal wall and a part originating from the host plant (wall or isolation layer)[97,101]. However, the interfaces of endomycorrhizas and ectomycorrhizas are not exactly identical.

In ectomycorrhizas, the part derived from the host plant is the cell wall, which sometimes presents delayed organizational modifications, whereas in endomycorrhizas it is the isolation layer with an architecture and formation different from those of the cortical cell wall.

In VA endomycorrhizas, ericoid endomycorrhizas and orchid endomycorrhizas, the walls of the fungus are transformed by the symbiosis. In ectomycorrhizas, the changes in the fungal wall occur late and vary from one association to another.

In VA endomycorrhizas, the host plasmalemma around the interface (perisymbiont membrane) presents different properties from the peripheral plasmalemma, and there are no known changes in the plasmalemma of cortical cells related to ectomycorrhizal symbiosis.

Finally, the cement which surrounds all the hyphae in ectomycorrhizas has no equivalent in endomycorrhizas. It should also be pointed out that although structures analogous to the arbuscules and intracellular coils of endomycorrhizas are known from parasitic associations of fungi with plants, no parasitic fungus produces structures similar to the mantle and the Hartig net which are so typical of ectomycorrhizas. In comparison with endomycorrhizas, ectomycorrhizas are the most highly specific and well-developed type of mycorrhizal symbiosis.

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Morphological, biochemical and molecular changes during ectomycorrhiza development

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Summary. An ectomycorrhiza, a specialized root organ, is the result of a complex interaction leading to a finely-tuned symbiosis between a plant and a compatible ectomycorrhizal fungus. Ultrastructural observations combined with cytochemical and biochemical studies reveal that structural and metabolic changes in the symbiont cells lead to the final phenotype of the active ectomycorrhiza. In the present review these changes are interpreted as changes in gene expression and discussed within the context of ectomycorrhiza development. Recent genetic data indicate that the continued vegetative growth of the ectomycorrhizal hyphae and the root tissues, and their ability to switch to symbiotic organ formation, is basically controlled by developmentally critical genes. The activity of these 'symbiotic genes' during the differentiation of ectomycorrhizas is associated with extensive changes in the concentration of particular polypeptides and protein biosynthesis. The present state of knowledge about the developmental biology of ectomycorrhizas allows only speculation about the events during their development.

Key words. Symbiosis; ectomycorrhiza; ectomycorrhiza development; gene expression; ectomycorrhizins; protein patterns.

> *"Puisant mes forces aux sources des galaxies*
> *En buvant la sève des arbres"*
> M. Jonasz