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Comparative structure of vesicular-arbuscular mycorrhizas and ectomycorrhizas

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Abstract

During the establishment of vesicular-arbuscular mycorrhizas, fungal hyphae contact the root surface, form appressoria and initiate the internal colonization phase. Structural changes occur in the cell wall, the cytoplasm and the nucleus as the fungus progresses from a presymbiotic to a symbiotic phase. Nuclei in spores are in G_1 whereas in intraradical hyphae they are in G_1 and G_2. Changes in nuclear organization are evident in various stages in the colonization process. Dramatic changes in both symbionts occur as the nutrient exchange interface is established between arbuscules and root cortical cells. An interfacial matrix, consisting of molecules common to the primary wall of the cortical cell, separates the cortical cell plasma membrane from the fungal cell wall.

Ectomycorrhizas are characterized structurally by the presence of a mantle of fungal hyphae enclosing the root and usually an Hartig net of intercellular hyphae characterized by labyrinthine branching. As hyphae contact the root surface, they may respond by increasing their diameter and switching from apical growth to precocious branching. The site of initial contact of hyphae may be either the root cap or the 'mycorrhiza infection zone'. The mantle varies considerably in structure depending on both the plant and fungus genome. In some ectomycorrhizas, the mantle may be a barrier to apoplastic transport, and in most it may store polyphosphate, glycogen, lipids and perhaps protein.

Introduction

Structural characteristics of the various types of mycorrhizas have been documented extensively so that a basis now exists for correlating the anatomy with functional aspects of each mycorrhizal type. The symbionts in these mutualistic symbioses establish, by marked structural changes, an interface for nutrient exchange (Smith and Smith, 1990; Bonfante and Scannerini, 1992). In the resulting integrated system, the mycorrhiza, the host benefits ultimately by showing enhanced growth while the fungus may require the mycorrhizal association to complete its life cycle.

In this article the two major mycorrhizal types, vesicular-arbuscular mycorrhizas (VAMs) and ectomycorrhizas will be considered in terms of structure-function relationships. Recent reviews have summarized the anatomical aspects of VAMs (Bonfante, 1984) and ectomycorrhizas (Kottke and Oberwinkler, 1986).

Morphology to understand functioning in VAMs

VAM fungi cannot be maintained in axenic culture, do not produce sexual structures, and are obligate symbionts which usually limits studies of the morphology of VAMs to the mature colonization phase. The development of new

techniques, i.e. an in vitro dual culture system with *Agrobacterium rhizogenes* T-DNA-transformed carrot roots (Bécard and Piché, 1989), germination of VAM spores and production of a limited quantity of axenic mycelium, use of host mutants and non-host genotypes of normal myc$^+$ plants (Gianinazzi-Pearson et al., 1991; Bradbury et al., 1991), now allow morphological studies of the presymbiotic phase of VAM fungi. Moreover, the improvement of some techniques (cryo-fixation, freeze-substitution and affinity techniques) has offered important tools to study the symbiotic phase in more detail.

The presymbiotic phase of VAM fungi

Spores of many VAM fungal species may germinate and form limited hyphal growth in nutrient-poor media or even in deionized water but swollen or collapsed hyphae and frequent septation begin to appear after 15–20 days of culture, leading to the arrest of hyphal growth (Hepper, 1984). This saprophytic growth may involve flavonols which enhance independent growth of VAM fungi in vitro (Bécard et al., 1992).

Growing hyphae of in vitro produced mycelium are characterized by a subconical apex, whereas apices from arrested hyphae appear rounded or swollen. Ultrastructurally, they show a thin fibrillar wall, a cytoplasm rich in organelles, abundant glycogen and only a limited number of vacuoles (Figs. 1, 2). In *Gigaspora margarita,* the germinating hyphae lead to the development of larger and yellow hyphae (10–12 µm). From these, a special cell type, the auxiliary cell, is initiated which is rich in nuclei, organelles, membranes and glycogen particles (Figs. 3, 4).

The distribution of nuclei within spores and germinating hyphae has been of particular interest, since a hypothesis to explain the limited growth of the fungus involves the absence of nuclear DNA replication during spore germination (Burggraaf and Beringer, 1989). A static cytofluorimetric analysis has confirmed that nuclei extracted from spores of *G. margarita* and *Glomus versiforme* are rather homogeneous with regard to DNA quantity (0.75 and 0.25 pg of DNA respectively). Peaks with a double quantity of DNA were not found, suggesting that duplication events, if any, are very rare; nuclei in the spore may be blocked at the G_1 phase (Bianciotto and Bonfante, 1992). On the contrary, in the intraradical mycelium a small population of nuclei with a double quantity of DNA exists even if the quantity of DNA per nucleus does not change. This result is particularly evident in the nuclei of *Glomus mosseae.*

A combination of morphological observations and cell biology techniques may lead to a better understanding of the cell cycle of the fungus.

The contact phase

The in vitro system for culturing VAM fungi has demonstrated that the root is required for fungal growth since it produces not only the signal molecules which may trigger the infection process, but provides the right surface on which the fungus can develop (Bonfante and Perotto, 1992). The fungal germ tubes or extraradical mycelium make contact with the root, branching and growing on the root surface. Appressoria, lens shaped structures 20–40 µm long, are formed usually between adjacent epidermal cells as a result of the contact (Garriock et al., 1989). *G. mosseae* requires living root surfaces to produce appressoria in about 42 hours, while they are not formed either when roots are killed or a non-host plant is used (Giovannetti et al., 1991). In addition, in pea mutants which do not form either nodules or mycorrhizas (nod$^-$ and myc$^-$ phenotype), normal appressoria were not observed (Gianinazzi-Pearson et al., 1991) and wall thickenings, similar to the papillae formed in response to pathogens, were present at the point where the VAM fungus attempted penetration. In seedlings of non-nodulating alfalfa which exhibited resistance to VAM colonization, appressoria varying in size and shape, but typically larger than those on nod$^+$fix$^+$ genotypes, developed on the root surface but failed to form internal structures (Bradbury et al., 1991). The formation of appressoria and penetrating hyphae are the first morphological

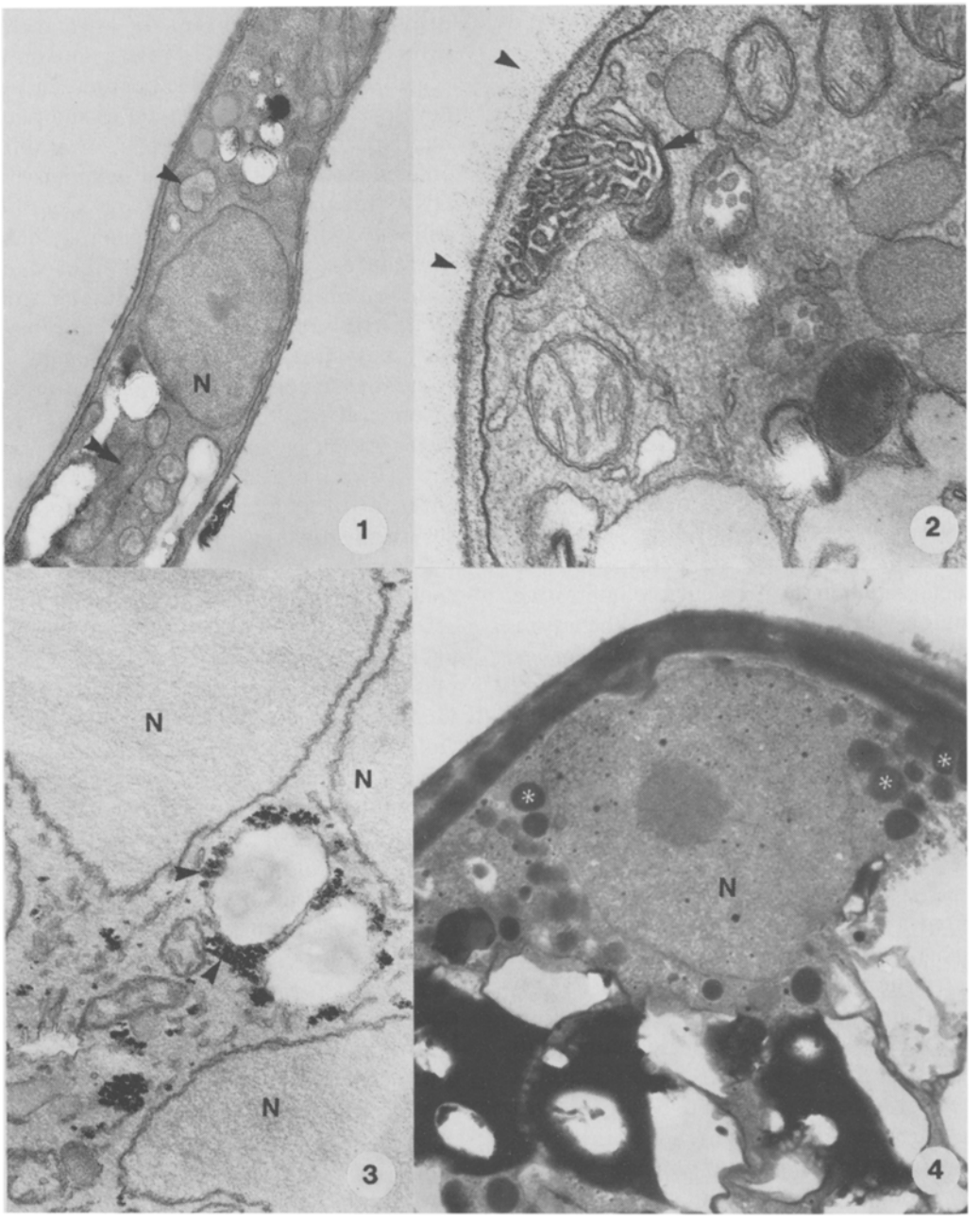

Plate 1. Figs. 1–4. Ultrastructural features of vesicular-arbuscular fungi in the presymbiotic phase.
Fig. 1. Presymbiotic hyphae of *Glomus mosseae,* showing a nucleus (N) with diffuse chromatin, abundant mitochondria (double arrowhead), a small bacterium-like organelle (BLO) (arrowhead) and a thin cell wall. ×11,000
Fig. 2. Enlargement of a presymbiotic hypha, showing details of the thin wall, which is covered by fine radiating fibrils (arrowheads). Vesicles (double arrowheads), rich in electron dense material, are associated with the plasma membrane. ×36,000
Fig. 3. Detail of a young auxiliary cell from a germinating spore of *Gigaspora margarita.* Nuclei (N) with diffuse chromatin and glycogen granules (arrowheads) which surround small lipid globules are evident. ×30,000
Fig. 4. Detail of a mature auxiliary cell. The nucleus (N) occupies a peripheral position and is surrounded by a highly vacuolated cytoplasm. Abundant electron dense granules (probably pigment) (*) are found near the cell wall. ×30,000

signs of the compatibility between plant and fungus.

The penetration events are influenced by root characteristics since the outer layers of the root often possess thickened walls containing abundant cellulose, phenols, and UV autofluorescent components (Bonfante and Perotto, 1992). These components may act as structural barriers to the penetration of the fungus, which may penetrate through junctions between the epidermal cells, directly through tangential walls, or root hairs only before the deposition of such substances. The deposition of suberin on the radial cell walls of the hypodermal layer may channel fungal growth and development inside the root. Characteristics of the root surface together with root anatomical features (Brundrett and Kendrick, 1990) may, therefore, influence the first steps of the plant–fungal interactions. It would be interesting to determine whether the nod^-/myc^- phenotype found in peas and alfalfa is the result of changes in the regulatory control of cell wall synthesis, leading to an abnormal deposition of wall components which blocks fungal penetration.

The colonization process

Once the fungus overcomes the barrier represented by the outer root layers it mostly develops intercellular hyphae and highly branched structures called arbuscules. Each arbuscule begins as a trunk with few branching hyphae and with subsequent repeated branching completes its development in 4–5 days (Brundrett et al., 1985). Arbuscules rapidly collapse forming clumps which are initially limited to the apical part of the arbuscular branches, but quickly extend to the whole complex.

Ultrastructural observations have revealed important modifications of both partners during the symbiotic phase. Intraradical hyphae are rich in nuclei, mitochondria, secretion granules, glycogen particles and lipid globules. Cell wall structure has been studied in detail since there is a dramatic reduction in thickness (from about 12 µm in the spore wall to 20–30 nm in the arbuscular walls) and changes in chitin organization. Nuclei of extraradical or intercellular hyphae show a chromocentric organization with dispersed chromatin and a well developed nucleolus. On aging and particularly during arbuscule collapse, the chromatin condenses and the nucleus becomes pycnotic. DNA localization by using a monoclonal antibody, which binds to single and double DNA molecules, shows a strong decrease in the number of gold particles during this stage (Balestrini et al., 1992a). Arbuscules are not only key sites for nutrient exchange, but also represent a dead end for VAM fungi. Their ephemeral life, branching pattern and nuclear degeneration pattern suggest that arbuscules are the first step in a programmed death. Intercellular hyphae might represent the surviving structures in roots.

Morphological analysis shows that concomitant with arbuscule development, there is also a dramatic modification of the host cell architecture: invagination of the plant plasmalemma, fragmentation of the vacuole and increase in the number of organelles, such as Golgi bodies. The effects of the fungus over the plant nucleus are of particular interest. The nucleus increases in size without changing its ploidy (Blair et al., 1988; Berta et al., 1990), and shows an unfolding of its chromatin. In addition, there is a positional effect caused by the fungus: the nucleus occupies a median position in the infected cells, differently from the peripheral position shown in the non-infected cells (Balestrini et al., 1992b). This suggests that the plant cytoskeleton may be involved in the response of the plant to fungal penetration and in exchange between the partners.

The interface as a new apoplastic compartment

When the fungus penetrates root cells, either to form coils or arbuscules, the host plasmalemma invaginates and proliferates around the fungus. This creates a new compartment, an interface where interfacial material is laid down. Affinity probes, such as enzymes, lectins and antibodies, have been used to characterize this compartment as a space of high molecular complexity (Bonfante et al., 1992). These methods revealed

the presence of structural and enzymatic molecules such as ß(1–4) glucans, non-esterified polygalacturonans and HRGP molecules which are common to the host primary wall. On the contrary, callose or chitin were not found.

The apoplastic space, which is a structural expression of the symbiotic status, prevents direct contact between the plant and fungus, but does not represent a drawback for nutrient exchange between the partners (Smith et al., Chapter 10).

Structural features of ectomycorrhizas

Initial colonization of roots

The earliest stages of root colonization by ectomycorrhizal fungi have been studied infrequently partly because many of the synthesis methods used are not conducive to the recognition of the first contact of hyphae with the root. This problem has been largely overcome by using methods such as growth pouches (Fortin et al., 1980), the paper-sandwich technique (Chilvers et al., 1986) and simple in vitro methods (Malajczuk et al., 1990). Piché and Peterson (1988) timed colonization of *Pinus strobus* by *Pisolithus tinctorius* using the growth pouch system, and found fungal attachment to roots 1–2 days after inoculation, mantle and Hartig net establishment 2–4 days post inoculation, and root dichotomy after 5 days. Alterations in ultrastructure and response to the PATAG reaction for polysaccharides as hyphae contacted the root surface were described earlier (Piché et al., 1983a, b). Similar results have been obtained with *Eucalyptus urophylla* roots colonized by *P. tinctorius* using a simple in vitro method (Lei et al., 1990). Also Lei et al. 1991 have characterized the surface mucilage as containing a-D-glucose and a-D-mannose residues using the lectin concanavalin A.

Dramatic alterations in branching and diameter of hyphae occur in the earliest stages of colonization of *Eucalyptus pilularis* roots by *P. tinctorius* (Jacobs et al., 1989) and *Picea abies* roots colonized by *Hebeloma crustuliniforme* (Brunner and Scheidegger, 1992); it was suggested by Jacobs et al. (1989) that these early morphogenetic changes might be a reliable indicator of a compatible interaction between symbionts.

Recognition phenomena, the initial site of root contact by hyphae, and the subsequent establishment of the mantle and Hartig net are still in question. Horan et al. (1988) suggested that initial colonization of *Eucalyptus globulus* roots occurs at the root cap and that substances emanating from the root apex are responsible for a chemotropic response of the hyphae (Horan and Chilvers, 1990). This is an important observation since the dogma in the ectomycorrhiza literature is that there is a 'mycorrhiza infection zone' located proximal to the root apical meristem. There is evidence that colonization does appear to begin in such a region in some ectomycorrhizas (Melville et al., 1987).

Establishment and structure of the mantle

Mantle morphology, which is the result of the interaction between mycobiont and phycobiont genomes (Massicotte et al., 1987), plays a major role in identifying the mycobiont species of field-collected ectomycorrhizas. Recently, Wong et al. (1990) have shown that the extent of mantle development by closely related genotypes of *Laccaria bicolor* on *Pinus banksiana* roots depends on whether sib-monokaryotic or dikaryotic mycelium is used as the inoculum. This study emphasized that there is considerable intraspecific genetic variability in mantle formation. The control by various abiotic factors on mantle formation has received little attention, but it is reasonable to suggest that it is the interaction between the environment, the fungal genome and the host genome that controls mantle morphology.

The organization of hyphae within the mantle and the nature of the hyphal walls obviously play a major role in determining the mechanism of transport of water and nutrients from the soil solution to root tissue. Ashford et al. (1988, 1989) showed that the mantle of *Pisonia* and *Eucalyptus pilularis* ectomycorrhizas is impermeable to cellufluor, a fluorochrome used to test for apoplastic permeability. The presence of a barrier to apoplastic transport in the mantle

would necessitate the uptake of substances from the soil solution into the fungal symplast and set up a shared enclosed apoplastic nutrient exchange compartment (Ashford et al., 1989). Recently, however, Behrmann and Heyser (1991) have shown that the mantle of *Pinus sylvestris–Suillus bovinus* ectomycorrhizas is permeable to the apoplastic tracers, sulphorhodamine G and lanthanum nitrate, suggesting that mantle permeability may be molecule or species specific.

Hyphae within the mantle are capable of synthesizing storage reserves thought to include glycogen and polyphosphate. The presence of the metabolic machinery for nitrogen assimilation, the rapid accumulation of free amino acids in ectomycorrhizas (Chalot et al., 1991), and evidence that ectomycorrhizal fungi have a role in the uptake of nitrogen compounds, suggest that the mantle could serve as a depository for storage protein.

Further research is needed to determine the role and variability of these storage reserves and mantle permeability in the overall functioning of mycorrhizas.

Establishment and structure of the Hartig net

The usual criteria used in defining an ectomycorrhiza are the presence of a mantle and an Hartig net, although Warcup (1980) suggests that only a mantle is required. The majority of ectomycorrhizas do, however, develop an intercellular network of hyphae, the Hartig net, which is confined to the epidermis in most angiosperm species (Fig. 10), but pervades the cortex in most conifer species (Figs. 6, 7). A number of papers have provided detailed accounts of Hartig net structure (see Kottke and Oberwinkler, 1986). Hyphae in the Hartig net show labyrinthic branching which increases the apoplastic and symplastic exchange interface between the symbionts. In non-senescent regions of an ectomycorrhiza the cytoplasm of these highly branched hyphal tips often contains large numbers of mitochondria and rough endoplasmic reticulum cisternae (Massicotte et al., 1990; Kottke and Oberwinkler, 1986), suggesting an enhancement of an energy-requiring step in the uptake or release of nutrients in this interface. Recently, Lei and Dexheimer (1988) have shown the localization of ATPase activity along the Hartig net hyphal plasma membranes and plasma membranes of contiguous living cortical cells and suggest that this provides evidence for the existence of a mechanism for bidirectional transport between the symbionts.

Since there is no direct symplastic continuity between root cells and Hartig net hyphae, materials must pass from the symplast to the apoplast and vice versa, facilitated by permeability of the walls of either or both symbionts in the Hartig net region. There is some evidence that wall changes do occur in this interface (Duddridge, 1986; Paris et al., 1991), but whether these are related to transport phenomena has not been determined.

As the Hartig net is established it is reasonable to suppose that the symplastic continuity between root cells via plasmodesmata may be altered. Nylund (1980) describes regions of plasmodesmata between cortical cells in the Hartig net region of Norway spruce ectomycorrhizas. Fungal hyphae were apparently unable to penetrate between these regions so that they were maintained until cortical cells senesced. The author was uncertain as to whether the Hartig net hyphae eventually separated cell walls in these regions. In a recent study of *Dryas integrifolia–Hebeloma cylindrosporum* ectomycorrhizas, Melville et al. (1988) described primary pitfields with electron-lucent thickenings and plasmodesmata which impeded Hartig net hyphal growth initially (see Fig. 8); these were eventually breached to form a continuous Hartig net. The question still remains as to whether in the functional portion of an ectomycorrhiza, epidermal and cortical cells retain their symplastic continuity. Experiments using fluorochromes of known size could be used to test for symplastic continuity.

Recent results using microautoradiography (Warner and Heyser, 1991) have shown that the Hartig net is a major sink for ^{14}C labelled assimilates but the transport route across the cortex was not shown.

Changes in root morphology and structure

The most obvious morphological changes that

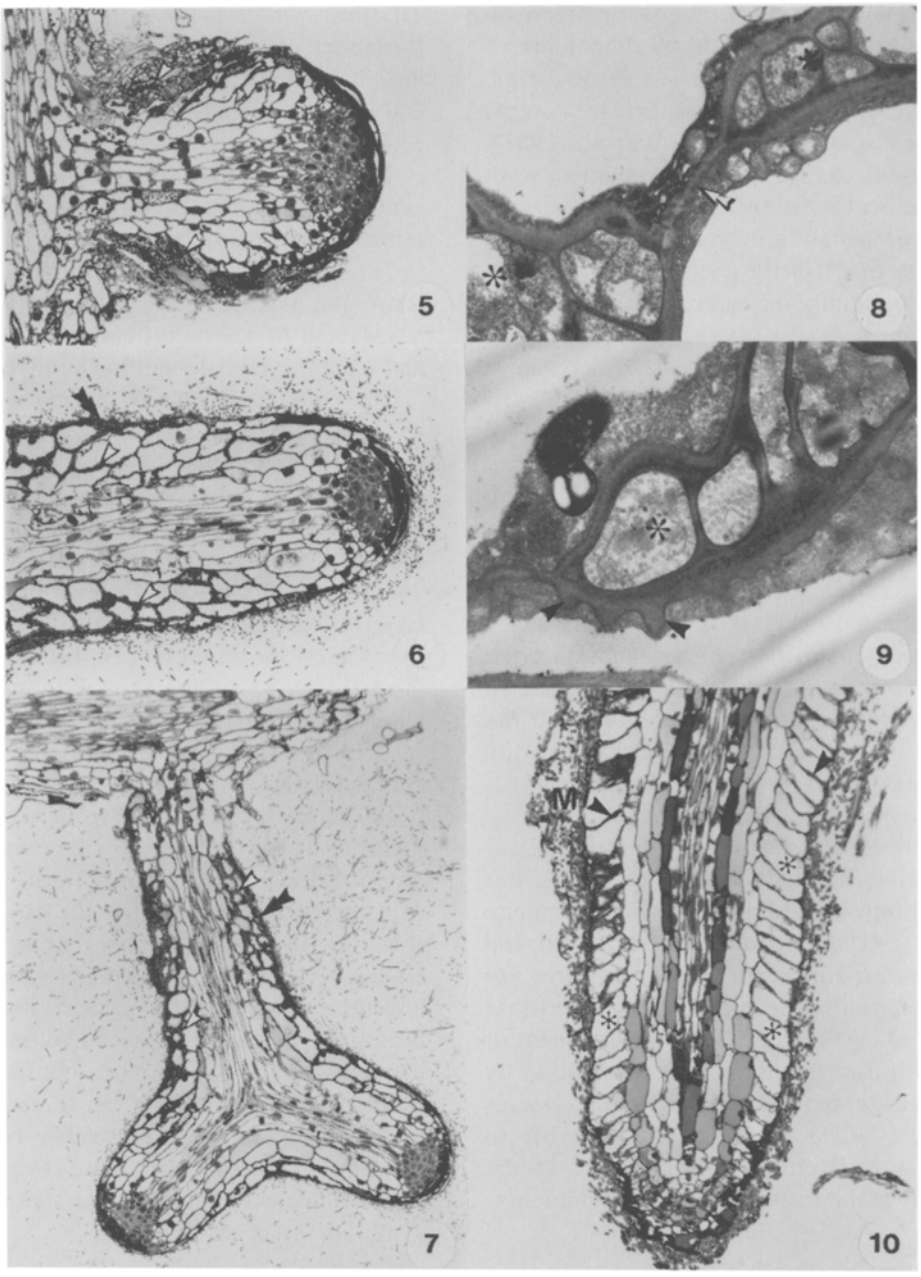

Plate 2. Figs. 5–7. First-order lateral roots of *Pinus resinosa* colonized by *Piloderma bicolor* showing change from monopodial to dichotomous branching. Hartig net hyphae (arrowheads) and a thin mantle (double arrowheads) are evident.
Fig 5. ×340. Fig 6. ×340. Fig 7. ×200. Figs. 8–9. *Dryas integrifolia–Hebeloma cylindrosporum* ectomycorrhizae.
Fig. 8. Hartig net hyphae (asterisks) between cortical cells. A primary pit field with plasmodesmata (double arrowhead) has not been breached by hyphae. ×9,000. Fig. 9. Wall ingrowths (arrowheads) in cortical cell adjacent to Hartig net hyphae (asterisk). ×11,000. Fig. 10. Non-median L.S. *Betula alleghaniensis* root colonized by *Piloderma bicolor*. Marked radial elongation of epidermal cells (*) is apparent. Arrowheads indicate Hartig net hyphae. Mantle–M. ×300.

occur in roots as a result of colonization by ectomycorrhizal fungi are induced dichotomy of the apical meristem in the genus *Pinus* (Figs. 5–7) and the precocious formation of lateral roots in many species (Massicotte et al., 1987). Other structural changes are more subtle. Root epidermal cells of most angiosperm species show a dramatic reorientation of growth subsequent to the initiation of a Hartig net (Fig. 10). These cells elongate radially instead of axially (Clowes, 1951; Massicotte et al., 1990) but apparently, at least in *Fagus,* without any overall increase in cell volume as compared to epidermal cells of control roots (Clowes, 1951). The effect of ectomycorrhizal fungi on reorientation of cortical microtubules and cellulose microfibrils in epidermal cells is currently being explored; methods to study the cytoskeleton of ectomycorrhizal partners have been developed (Timonin et al., 1991). Allaway et al. (1985) calculated from published micrographs that the Hartig net caused the epidermal cell surface in contact with the fungus to be greater than the area of the outer face of the epidermal cells by about 13-fold in *Fagus sylvatica* and about 10-fold in *Eucalyptus fastigata.*

Wall ingrowths may occur in epidermal or cortical cells (Fig. 9) adjacent to Hartig net hyphae (Ashford and Allaway, 1985; Massicotte et al., 1986). These modifications of wall and plasma membrane increase the surface area for nutrient exchange between the symbionts (Allaway et al., 1985). With the development of freeze substitution techniques accompanied by microautoradiography for mycorrhizal systems (Paris et al., 1991), it should be possible to explore how nutrients are exchanged at the cellular level in the interface between symbionts.

Conclusions

Notwithstanding many morphological differences, vesicular-arbuscular mycorrhizas and ectomycorrhizas share some substantive structural features which allow them to become functioning symbioses. In this brief review, the following points have been recognized as common and relevant to function: the extraradical, contact, and the colonization phase. The first, which is essential for the uptake and transport of water and nutrients, consists of morphologically heterogenous hyphae in VAMs and in well-organized hyphae and rhizomorphs in ectomycorrhizas.

The contact phase plays an important role for recognition and subsequent acceptance or rejection between symbionts in both mycorrhizal types. Acceptance is manifested by the formation of appressoria and infection hyphae in VAMs and by the modification of hyphal growth and mantle establishment in ectomycorrhizas.

The colonization phase, during which nutrient exchange occurs, involves the formation of intercellular hyphae, intercellular hyphae and arbuscules in VAMs and Hartig net hyphae in ectomycorrhizas. Arbuscules and Hartig net hyphae provide a substantial increase in the contact surface area between fungus and host. Events leading to the establishment of these interfaces are closely regulated and involve modifications to the morphogenesis of both symbionts. The interfacial zone, particularly in VAMs, has well defined molecular characteristics.

In the complex interaction between fungi and plant roots which forms mycorrhizas, modifications in the morphogenesis and structure of each symbiont may provide clues as to how the system functions physiologically. Understanding the role of the presymbiotic phase and the changes which occur in both symbionts during the earliest stages of hyphal contact with the root surface still requires considerable work. Plant mutants which are being used to explore these early events in VAMs represent an important approach in studying the signals involved and the mechanisms regulating this symbiotic association.

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