Symbiosis, 3 (1987) 249–268
Balaban Publishers, Philadelphia/Rehovot

Review article

Vesicular-Arbuscular Mycorrhizae: Fungus-Plant Interactions at the Cellular Level

PAOLA BONFANTE-FASOLO

Dipartimento di Biologia Vegetale and Centro di Studio sulla Micologia del Terreno del CNR — Viale Mattioli 25
10125 Torino, Italy
Tel. 011-6699884

Received May 18, 1987; Accepted September 10, 1987

Abstract

Vesicular-arbuscular (VA) mycorrhizae are symbiotic associations between soil fungi belonging to Endogonaceae and plant roots. VA mycorrhizae occur in about 80% of plants ranging from bryophytes to angiosperms with a general lack of host specificity. Cellular interactions between the partners are mediated by cell surface properties, (of the cell walls and/or plasmamembranes), which play an important role during the development of the symbiosis: (1) the fungal cell wall becomes thinner and changes from a fibrillar into an amorphous texture, associated by different three-dimensional arrangements of the chitin chains; (ii) the response of the host cells to fungal colonization varies in the different layers of the root. Coils occur mainly in epidermal or outer cortical layers and do not provoke a strong host response, while arbuscules colonizing inner cortical cells, stimulate cytoplasm and plasmamembrane modifications of the host cells.

Keywords: mycorrhizae, vesicular-arbuscular mycorrhizae, cell wall, chitin, cell surface, cellular interactions, host, nucleus

This paper is a modified version of that presented as an invited lecture at the International Congress of Microbiology, Manchester, 1986

0334-5114/87/$03.00

1. Introduction

Mycorrhizae are symbiotic associations between plant roots and soil fungi. They occur in the majority of plants, from bryophytes to monocotyledons and give rise to a number of morphologically distinct root-fungus associations. Plants under natural conditions are therefore usually mycorrhizal plants (Harley and Harley, 1987; Harley and Smith, 1983). Mycorrhizae have been studied extensively, because of their ecological and agricultural importance (Schenck, 1982; Powell and Bagyaraj, 1984; Gianinazzi and Gianinazzi-Pearson, 1986). The fungal components act as fertilisers by improving the mineral nutrition of the host, mainly by an increased phosphate uptake. This has been referred to as the "plant growth effect" (Powell and Bagyaraj, 1984).

The term mycorrhiza is related to a variety of interactions between different groups of plants and fungi. Mycorrhizae are usually divided into many morphologically distinct types, reflecting differences in anatomy, physiology and the different taxonomic positions of the partners. The classification of Harley and Smith (1983) stresses the importance of separating between ectomycorrhizae, where the fungus does not penetrate the host cells and endomycorrhizae with intracellular fungal intrusion. Other classifications consider mainly the ecological distribution (Read, 1986), the nutritional exchange (Lewis, 1986) and the cytological features of mycorrhizal types (Scannerini and Bonfante-Fasolo, 1983).

These classifications comprise different levels of integration between the symbionts — from that of the plant community to that of molecular interaction. The organismal level appears as the most significant, since mycorrhizal plants develop much better than non-mycorrhizal plants and are more resistant to heavy metal contamination and pathogens (Gianinazzi-Pearson and Gianinazzi, 1986; Read, 1986).

The mycorrhizal roots undergo anatomical and histological modifications. According to Clowes (1981) the root apex of ectomycorrhizal roots is rounded, without cap cells, has a reduced meristem and a low rate of mitosis. Recent observations have revealed modifications also in the root development of some endomycorrhizae, (Berta and Bonfante-Fasolo, 1983; Berta and Gianinazzi-Pearson, 1986; Fusconi et al., 1986). It has been demonstrated that VA mycorrhizae depress the meristem activity of the root, decreasing the mean mitotic index and slow down cell division in the metaphase, producing parenchymatous root tips (Fusconi et al., 1986) (Fig. 1). These effects appear to be the result of cellular interactions between the partners.

In this article I describe the structure and function of some cellular interactions related to the physical contact between the cell surfaces of the partners.

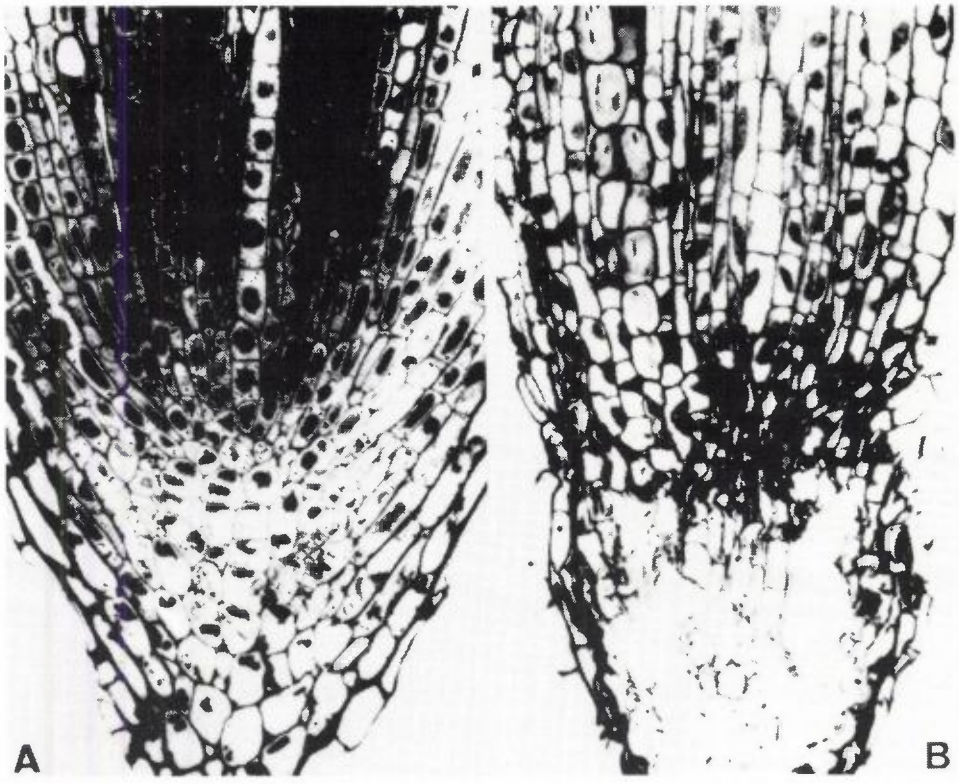

Figure 1. Plant-mycorrhizal fungus interaction: the organ level. A mycorrhizal plant of *Ornithogalum umbellatum* colonized by *Glomus* spp. (B) has a parenchymatous root tip and a reduced meristem, when compared with the non-mycorrhizal root of the same age (A). (Courtesy of Dr. G. Berta and Dr. A. Fusconi).

in vesicular-arbuscular mycorrhizae, the most common type of endomycorrhizae. According to Heslop-Harrison and Linskens (1984), these interactions fall into the category of the so-called "short range interactions" between different species. Particular attention will be given to the mycorrhizal partner cell walls, through which exchanges occur (Fig. 2). It is now clear that the cell wall is not a passive envelope but an active structure, affected by the environment. Recent results show that some components of the cell wall, the oligosaccharins, may play a regulative role in microorganism/plant systems (Albersheim and Darwill, 1985).

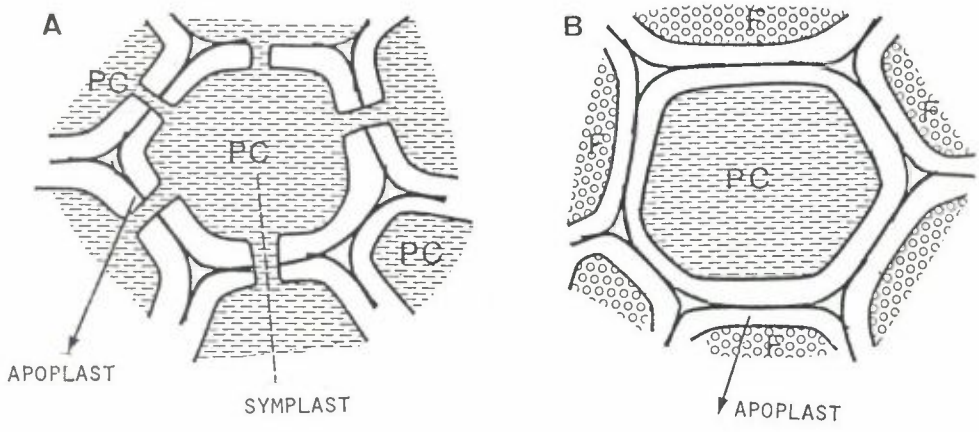

Figure 2. Scheme showing (A) the intracellular compartment or symplast and the extracellular compartment or apoplast in plant cells (PC); (B) the extracellular compartment between plant and fungal (F) cells.

2. Vesicular-Arbuscular Mycorrhizae at the Cellular Level

Vesicular-arbuscular mycorrhizae (VAM) occur in about 80% of plants, and are characterized by a general lack of host specificity. Host plants may range from bryophytes through pteridophytes and gymosperms to angiosperms. All VAM fungi belong to a single family of Zygomicotina, the Endogonaceae (Peyronel, 1924). Their classification is still in dispute, mainly because we do not know yet to grow VAM fungi in pure culture. Their sexual stages are unknown and their life cycles are imperfectly understood (Walker, 1985). Their morphological development is highly constant inside the roots of different hosts (Bonfante-Fasolo, 1984). VAM fungi usually form an extraradical phase represented by chlamydospores and extramatrical mycella, and a very complex intraradical phase consisting of hyphal coils, intercellular hyphae, inter- and intracellular vesicles and arbuscules (Fig. 3).

During development of the symbiosis, two structural changes are characteristic: (1) the fungal wall is extensively modified and (2) the host response to fungal colonization differs in the different layers of the root.

Fungal wall modifications

In many VAM species, chlamydospores have a thick and complex wall that represents an important diagnostic feature (Walker, 1985). Electron mi-

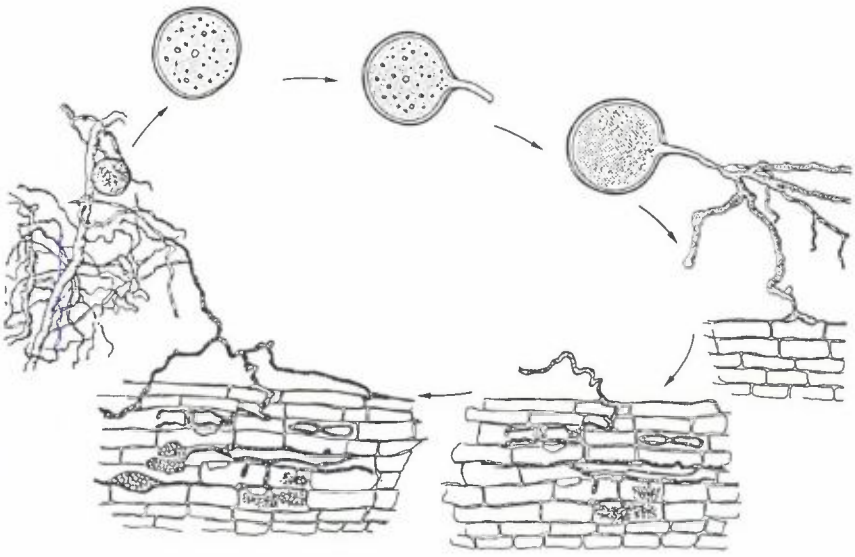

Figure 3. Scheme showing the life cycle of a VAM fungus: chlamydospores and extramatrical mycelia form during the extraradical phase, while coils, intercellular hyphae, arbuscules and vesicles form during the intraradical phase.

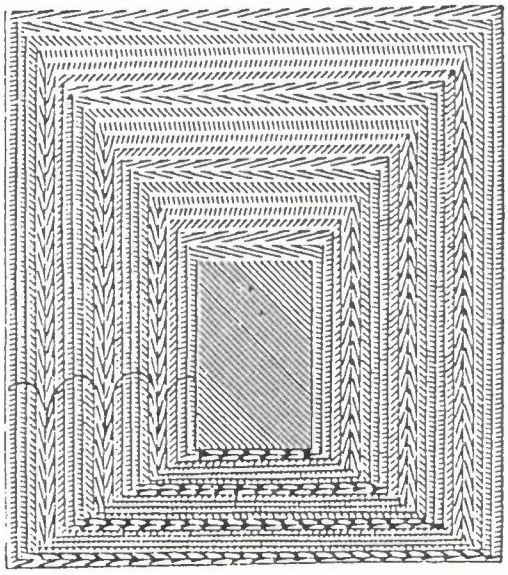

Figure 4. Pyramid model for helicoidal systems. A series of rectangles where parallel and equidistant straight lines have been drawn is arranged in the form of a truncated pyramid. Oblique sections give rise to bow shaped fibrils (from Bouligand, 1972).

croscopy shows that the wall of mature *Glomus versiforme* chlamydospores consists of an outer and inner layer separated by an electron-dense line (Bonfante-Fasolo and Vian, 1984). The inner layer is formed of arcs arranged in ranks, an arrangement called "helicoidal organization" (Neville, 1985), interpreted for the first time in insect cuticle by Bouligand (1972). According to Bouligand's pyramidal model (Fig. 4), there are planes formed by parallel and straight fibrils, each plane being laid down rotated by a small angle, with respect to the others, like the steps of a spiral staircase. The whole forms a ply structure, with laminae constantly changing in orientation. Oblique sections generate the pattern of arcs seen in the spore; however, the arc is an illusion, since it consists of short segments of parallel fibrils from each plane. This type of architecture is very unusual in fungi generally, and appears only in VAM fungi. It has also been found recently in *G. macrocarpum, Gigaspora* and *Acaulospora laevis* (Bonfante-Fasolo and Schubert, 1987; Mosse, 1986). In order to assure that the arcs are actually caused by a helicoidal arrangement, it is important to tilt the specimen and observe sections at different angles. This has so far been done for *G. versiforme* and *G. macrocarpum* (Bonfante-Fasolo and Vian, 1984 and unpublished results).

Quantitative analyses of the cell wall of the *G. versiforme* spores have shown that chitin is the most prominent component, representing 27% of the cell wall fraction, while only a small quantity of the aminosugar occurs in a non-acetylated form (Bonfante-Fasolo and Grippiolo, 1984). Chitin is difficult to locate ultrastructurally, since it is electron-transparent, and non-reactive to the PATAg test for polysaccharide (Thiery, 1987). However, new techniques based on affinity between molecules such as lectins and sugars or enzymes and their substrates, coupled with colloidal gold (Horisberger, 1985, Roth, 1983; Bendayan, 1985; Benhamou et al., 1987) have recently permitted chitin to be identified as well as other carbohydrates.

In order to detect chitin in thin sections of the *G. versiforme* spores, we complexed gold (G) with chitinase and gold with wheat germ agglutinin (WGA), the lectin specific for N-acetyl glucosamine residues, (Bonfante-Fasolo et al., 1986a). The two methods demonstrated a different distribution of the gold label in the two layers of the wall. Gold label occurred on the inner and outer layers of the wall with WGA/G, but the labelling of the secondary wall was very weak by the chitinase/G test. This difference is presumably the result of a different accessibility to the substrate by the two probes and the different distribution of the chitin molecule in the two layers. Since the chitinase is an endochitinase, it binds mainly to the chitin chains.

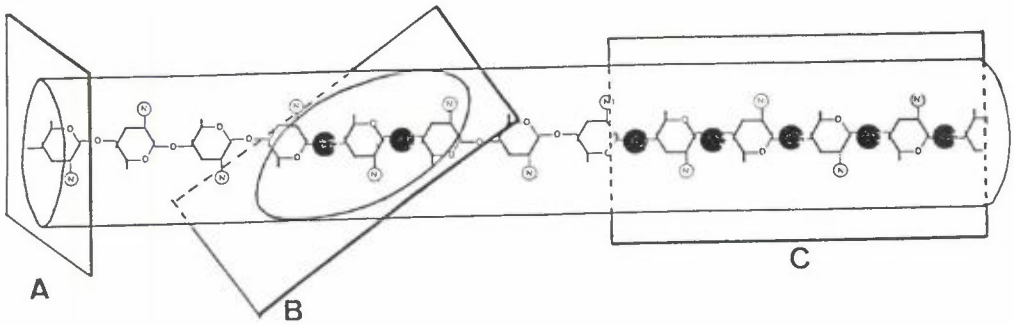

Figure 5. Distribution of binding sites (•) of a chitinase gold complex for different sectioning planes (A, B, C, transverse, oblique, longitudinal respectively of a hypothetical chitin molecule. The binding sites correspond to the covalent linkages accessible to chitinase (from Bonfante-Fasolo et al., 1986a).

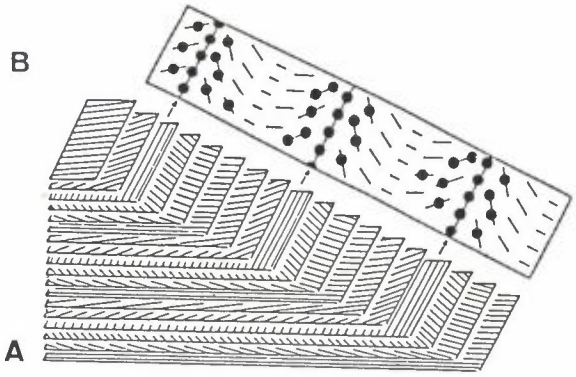

Figure 6. Variability in distribution of label with chitinase-gold in an oblique section (B) through a helicoidal system (A). The helicoidal system is drawn by a succession of parallel and equidistant planes. The straight lines within each plane represent the alignment of chitin molecules. The dark spots (•) represent the binding sites between the chitinase-gold and the substrate. The covalent linkages accessible to the probe are limited to fibrils cut more or less longitudinally (from Bonfante-Fasolo et al., 1986a).

On thin sections the probe binds strongly to the microfibrils lying parallel to the section plane, but binds weakly to the microfibrils appearing as arcs, since they lie oblique or transverse to the section plane (Fig. 5). The arcs are formed by many oligomers belonging to different fibrils, and few covalent linkages are available for binding (Fig. 6). WGA, on the other hand, links easily to the oligomer residues irrespective of their length. In conclusion, chitin in the VAM fungal spores examined consists of fibrils showing two different three-dimensional arrangements, parallel and helicoidal.

When the spores of VAM fungi germinate, the cytoplasm of the new hyphae is rich in organelles and nuclei in active division (Cooke et al., 1987; C. Marzachi, unpublished). The walls are thick, with a ply structure, and chitin can be localized by light and electron microscopy using WGA/FITC and WGA/gold complexes (Bonfante-Fasolo et al., 1986). However, the extraradical phase is not limited to this type of germinating mycelium, since in an established mycorrhiza, the root is surrounded by a mycelial weft of hyphae originating from internal mycelia. A large part of this mycelium appears empty and dead, as Schubert et al. (1987) showed by using fluorescein diacetate (FDA) as a probe of mycelium viability. Ultrastructural observations confirm that the hyphae are thick-walled and poor in cytoplasm, with irregularly distributed nuclei. We were able in this case also to demonstrate the presence of chitin fibrils in the wall, (Bonfante-Fasolo and Gianinazzi-Pearson, 1986) confirming biochemical analyses performed on the extramatrical mycelium. In order to quantify the development of the extra- and intraradical mycelia, Bethlenfalvay and his coworkers (1982) measured the amounts of chitin spectrophotometrically and observed that the extraradical mycelia attained a maximum weight at the onset of logarithmic growth of the colonized host and decreased thereafter.

In the presence of a compatible root, VAM fungi penetrate the host, giving rise to the intraradical phase, (see review by Bonfante-Fasolo, 1984). In the outer cell layers of many roots, the endophytes may form intracellular hyphal coils. These hyphae are usually very similar in wall structure and in cytoplasm to the extramatrical hyphae. Deeper in the root cortex, VAM fungi form intercellular hyphae positioned parallel to the long axis of the root, and spread the infection. So far there are few detailed time lapse studies on VAM development and these are limited to light microscope observations (Brundrett et al., 1985). By using a new procedure for observing the morphology of VAM fungi and a transplant method, these authors observed hyphal penetration about two days after initial contact, and arbuscule formation within 3 or 4 days. When VAM endophytes enter host cells, they stretch the host wall and cause the host plasmalemma to invaginate, forming a regularly ramified structure, the arbuscule (Fig. 7). Usually arbuscules as well as coils are regarded as being "intracellular" by workers involved in mycorrhizal studies. Conversely, in the legume-*Rhizobium* system, bacteroids surrounded by the peribacteroid membrane are regarded as being extracellular in a topological sense (Robertson et al., 1985). Regardless of the conflicting terminology, arbuscules are considered the key to plant-fungus

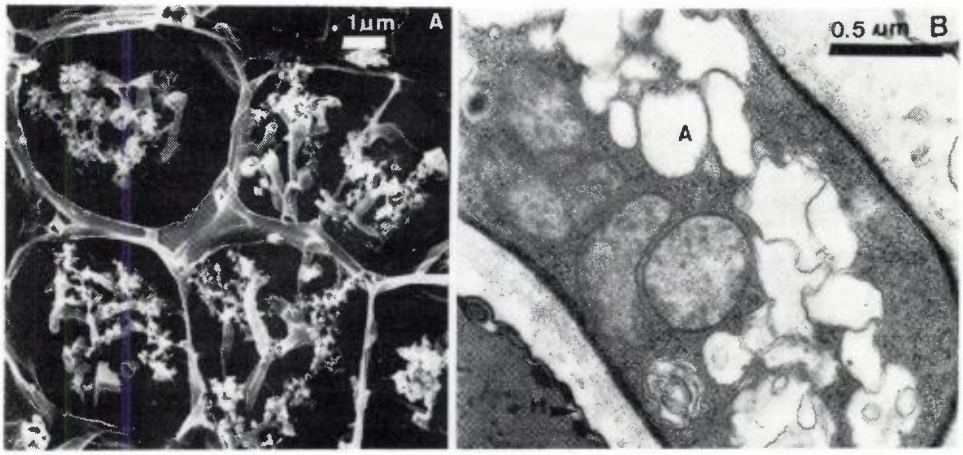

Figure 7. Plant mycorrhizal fungus interaction: the cellular level. (A) Hyphae of *Glomus caledonium* — seen by scanning electron microscopy — strongly colonize the cortical cells of *Glinkgo biloba*, forming arbuscules. (B) Thin-walled arbuscular hyphae of *G. fasciculatum* — seen by transmission electron microscopy — are surrounded by the host plasmalemma, A: arbuscule; H: host plasmamembrane.

interaction, since they are the site of nutritional exchange (Cox and Tinker, 1976). Morphologically, arbuscular branches represent the apical structures of the infection unit with a regular distribution of nuclei (Bonfante-Fasolo et al., 1987).

The arbuscular wall has a very distinct morphology; it is up to ten times thinner (e.g. 50 nm) than the extraradical hyphal wall, loses its fibrillar texture and becomes amorphous (Bonfante-Fasolo, 1982) Fig. 7). Chitin chains can no longer be detected in the wall of the arbuscule and chitinase gold-complexes no longer label it, while oligomers of N-acetylglucosamine can be discerned by the use of WGA linked to ferritin or gold (Bonfante-Fasolo, 1982; Bonfante-Fasolo et al., 1986) (Fig. 8).

Biochemical analyses of chitin content performed by measuring the quantity of glucosamine demonstrate the presence and quantity of chitin in the intraradical mycelium (Bethlenfalvay et al., 1982), while the affinity ultrastructural techniques give other information. The latter indicate that during the arbuscular phase, chitin fibrils disappear and chitin polymerization probably does not occur, since only sugar residues of N-acetylglucosamine are found. In VAM systems arbuscular hyphae are the apical portion of the

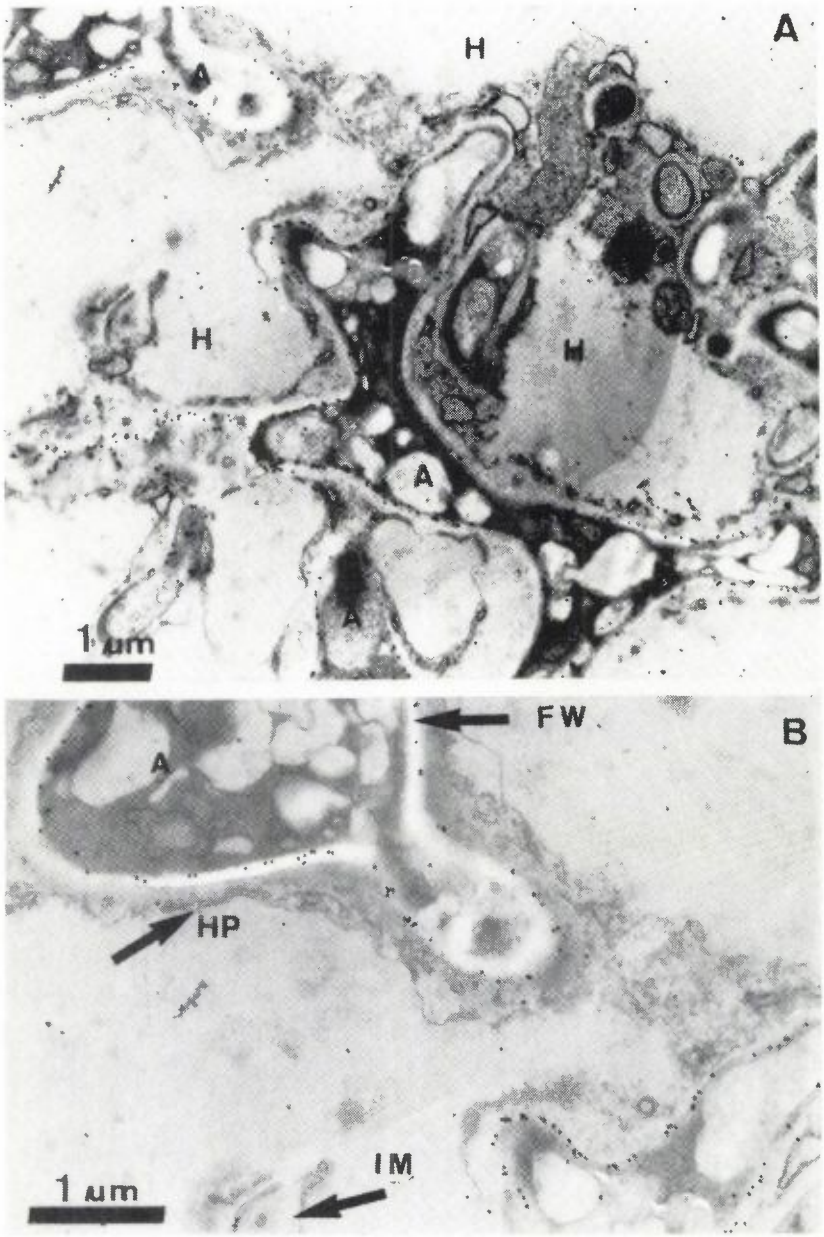

Figure 8. A. Ultrathin section of *Vitis vinifera* colonized by *Glomus fasciculatum*. Treatment with WGA/gold. Gold label occurs on the fungal wall. B. Magnification of an arbuscular branch showing that the interfacial space is not labelled. H: host cell; A: arbuscule; FW: arbuscule cell wall; HP: host plasmamembrane; IM: interfacial zone.

infecting unit, whereas the coils and the intercellular hyphae form the basal parts. Wessels (1986) claims that in the apical region of filamentous fungi the wall is plastic due to the presence of yet uncrystallized chitin at the growing apex. This is said to contrast "the conformational state of chitin in subapical parts and indicates a time gap between polymerization of the chitin chains and their final integration into the wall structure" (Wessels, 1986). The scheme suggested by this author for some ascomycetes and basidiomycetes may be applicable to VAM fungi, where the wall is also organized differently in basal and apical parts of the infection unit. However, VAM fungi are much more complex than fungi in pure culture, due to their growth inside the root. Host influence on fungal morphogenesis must therefore be considered (see below). The conclusion is that the walls of VAM fungi become simplified as they pass from the extraradical to the intraradical condition. Changes occur at different levels: there is a progressive thinning (from 12–15μm in the spore to 50 nm in the arbuscule) and the structure alters from fibrillar to amorphous, probably due to a different number of N-acetylglucosamine residues in the chitin chain.

Various reasons can explain wall thinning. A general mechanism of cell wall reduction in long standing symbioses has been suggested as a selective mechanism against redundancy (Margulis, 1981) and this could apply to VAM fungi. There may be active deposition of new wall material that remains thin, or one can speculate that a more complex regulation mechanism is directed by the host (Bonfante-Fasolo and Gianinazzi-Pearson, 1986). The host might control fungal development by modulating (among the other mechanisms) the production of hydrolytic enzymes, the role of which is very important during the host-pathogen interactions (Halbrock et al., 1986). Chitinase is of particular interest, since its role is well known both (1) in fungal growth and branching, (Gooday et al., 1986) when produced by the fungus, and (2) in the control of pathogenic fungi when produced by plants as a defense mechanism (Schlumbaum et al., 1986; Boller, 1986). Some observations show an increase in chitinase activity in mycorrhizal plants (Dehne and Schonbeck, 1978) or a peak of the same enzyme activity limited to the beginning of the VAM infection establishment (T. Boller and P. Spanu, personal communication). The presence of both plant and fungal chitinase in VAM suggests that the enzyme may play a role in fungal morphogenesis: it could be responsible for the amorphous wall and the apical branchings, characteristic of the arbuscule.

Response of the host to fungal colonization

Results with different host plants clearly show that the response to fungal colonization differs in the different layers of the root (Bonfante-Fasolo, 1984). Coils usually occur in the epidermis or outer cortex, while arbuscules mainly develop in the inner cortex.

Cytological changes have been observed only in the cells infected by arbuscules; the other cell types do not appear to be modified in the presence of coils. The cytoplasm remains as a thin peripheral layer, the vacuole is central, and amyloplasts are intact. The interfacial material surrounding the coils and continuous with the host wall is thick. In the presence of arbuscules, the host protoplasm increases in volume, and the host plasmalemma proliferates up to ten times around the hyphal branches (Toth and Miller, 1984). Compared with the peripheral membrane of a cell from the same tissue in an uninfected root, the host plasmalemma surrounding the hyphal branches shows some modifications of certain enzyme activities. ATPase activity has been localized on the plasmalemma around the arbuscule (Marx et al., 1982) as well as neutral phosphatase activity, typical of the plasmalemma in differentiating root cells and absent in differentiated cortical cells (Jeanmaire et al., 1985). Plastids are often modified and may be arrested at the protoplastid phase. The large central vacuole divides into many small vacuoles. The host nucleus becomes lobed, but does not change its ploidy, in contrast to the behaviour suggested for nuclei of orchids when infected by *Rhizoctonia* (Berta et al., 1986). Nuclei from mycorrhizal and non-mycorrhizal roots show different degrees of condensation of the chromatin, which appears more dispersed in the mycorrhizal cells, with the nucleolus showing interpenetrated fibrillar and granular parts. All these features suggest that the nuclei in cells containing arbuscules are involved in intense transcriptional activity (Berta et al., 1986).

In contrast to the striking changes shown by the fungal wall, no clear modification can be discerned in the host cell wall. Cortex cells have a typical primary wall with a fibrillar structure and cellulose can be located using a cellulase-gold probe. Only slight textural changes observable in the cortical cell walls of mycorrhizal roots: they appear swollen after weak chemical extractions in comparison with the non-mycorrhizal ones (Vian and Bonfante-Fasolo, in preparation).

As with some biotrophic pathogenic interactions, at the moment of fungal penetration the host plasmalemma invaginates and proliferates around the fungus (Fig. 9). New wall material is laid down by the host between the host

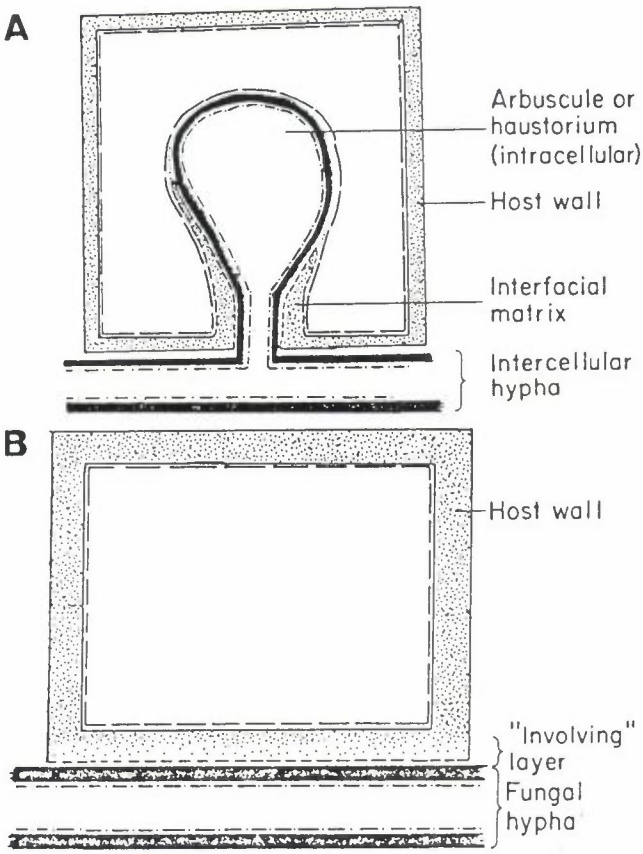

Figure 9. Scheme showing the contact between fungal and host walls during the intercellular phase (B) and the contact between host and fungus during the intracellular phase (A), when the fungus penetrates the host cell, by invaginating the host plasmalemma (from Harley and Smith, 1983).

plasmalemma and the intracellular fungal branches. This material remains thick around the fungal coil, but becomes thin around the arbuscule branches (Bonfante-Fasolo, 1984). It is formed of polysaccharides, strongly reactive to the Thiery test and is continuous with the primary wall (Scannerini and Bonfante-Fasolo, 1979). Neither chitin nor callose could be detected.

In conclusion, there is new wall building by the host, comparable to that in elongating cells. However, this point deserves further investigation.

It is not clear whether the host wall plays a specific role by acting as a barrier to fungal penetration, as it does in plant-pathogen interactions. Pathogens penetrate the walls of their host by secreting a mixture of cell-wall degrading enzymes, while walls of plant cells can release molecules from microbial walls which activate a defense response in the plant (McNeil et al., 1984). This aspect of plant-fungus interaction in mycorrhizae has not been investigated so far. There are only assumptions based on morphological features. According to Bonfante-Fasolo (1984) and Gianinazzi-Pearson (1985), penetration and colonization patterns of VAM fungi are tissue and host dependent. For example, *Glomus fasciculatum* develops coils in *Ginkgo biloba* but does not in *Allium cepa*; it forms intercellular hyphae in *A. cepa* but not or very rarely in *G. biloba* (Bonfante-Fasolo and Fontana, 1985). We suggest that the wall components of the host are responsible for these changes and act as regulators. In *Ginkgo*, VAM fungi can cross the primary walls of the rhizodermic cells, but are blocked by the dark material, probably phenolics, in the intercellular spaces. Preliminary data obtained on purified cell wall fractions of *G. biloba* and *A. cepa* show that different types of phenols can be identified (syringic, vanillic, ferulic and caffeic acid). However, there are not obvious quantitative or qualitative differences between mycorrhizal and non-mycorrhizal plants (Maffei et al., 1986). The presence of cell wall phenols is therefore not a response to the fungal colonization, differently from what happens in some plant-pathogen interactions (Halbrock et al., 1986).

All these observations suggest that the host cell wall could modulate the pattern of colonization in VAM fungi, the enzymatic processes of which are still unknown. This is due to the strong microheterogenity shown by cell wall components in higher plants, and is not a response to symbiotic association. As McNeil et al. (1984) claim "... the complex carbohydrates of the walls of the different cells in individual organs and tissues of a single plant are different".

3. Conclusion and Speculations

Two points emerge from the discussion. First, the fungal wall changes its texture during symbiosis, particularly in the fine arbuscular branches. Second, the host cell wall apparently remains extensible, while the cytoplasm is greatly activated, mostly at the nuclear level, but only in the arbuscule-containing cells.

These two points observable morphologically, suggest a situation where the fungus — at least in part — grows under the control of the host plas-

mamembrane, and in which we believe there is a complex regulation. We can imagine that signal molecules produced by the fungus reach the host nucleus and stimulate some inducible genes, expression of which produces enzymes able to synthesize new membranes and cell wall material, which can be seen at the ultrastructural level. We speculate that chitin may act as one of these signal molecules, as has already been suggested both for chitin and chitosan in some pathogen/plant interactions (Pearce and Ride, 1982; Hadwiger et al., 1986). Chitin or N-acetyl-glucosamine oligomers may stimulate the production of enzymes such as chitinase, which is known to be an inducible enzyme (Hadwiger et al., 1986). The result of events of this kind would be (1) highly ramified hyphae and (2) a wall that remains thin and amorphous during the arbuscular phase.

In conclusion, the short-range interactions between the host and the fungus in VAM change throughout the root reflecting fungal morphology and the host cellular types. Cellular interactions are apparently determined by the surface properties of the partners and involve molecules that are typical components of the cell wall, such as chitin. These molecules would therefore play not only a structural role, but also an informational one.

Acknowledgements

This material is based on work supported by CNR, Italy, Special Grant I.P.R.A., Subproject 1, Paper No. 1651.

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