Anales de Biología 26: 179-190, 2004

Ultrastructure of the mycorrhiza formed by *Tetraclinis articulata* (Vahl) Masters (*Cupressaceae*)

Asunción Morte & Mario Honrubia
Dpto. Biología Vegetal (Botánica), Facultad de Biología, Universidad de Murcia, Campus Universitario de Espinardo, Espinardo 30100, Murcia, Spain.

Correspondence
A. Morte
E-mail: amorte@um.es
Tel.: 34-968-367146
Fax: 968-363963
Received: 15 September 2004
Accepted: 15 October 2004

Abstract

The structural organisation of the endomycorrhiza in naturally infected seedlings and micropropagated and *Glomus* inoculated plants of *Tetraclinis articulata* was studied by means of light and electron microscopy. Hyphal spread from cell to cell in the host root was entirely intercellular. Intracellular hyphae crossing through the host walls were never observed. This could be favoured by the presence of numerous wall swellings at the contacting area among cell walls which are characteristic of *T. articulata* roots. These wall swellings could impede the crossing of the hyphae. The reduced interfacial region between both symbionts was observed with a fibrillar material in contact with the hyphal wall and, in some cases, in contact with the host plamalemma, although sometimes this region was occupied by small vesicles. The increase of the cytoplasmic organules both in the host cell and intracellular hyphae, during the arbuscular phase, indicated an increase of the metabolic activity of both symbionts. The membrane formations, generally referred to as plasmalemmasomes, appeared in the arbuscular interfacial zone and in the cytoplasm of the arbuscular hyphae. This is a typical arbuscular mycorrhiza.

Key words: Endomycorrhiza, *Tetraclinis articulata*, *Cupressaceae*, Ultrastructure, *Glomus*.

Resumen

Ultraestructura de la micorriza formada por Tetraclinis articulata *(Vahl) Masters (Cupressaceae).*

Se estudia la organización de la endomicorriza formada por *Tetraclinis articulata* tanto en plántulas de vivero colonizadas de forma natural como en plantas micropropagadas e inoculadas con *Glomus* sp. mediante miscroscopía óptica y electrónica. La infección se extendió de forma intercelular de una célula a otra en el interior de la raíz hospedante. Nunca se observaron hifas intracelulares atravesando las paredes de las células hospedantes. Esto pudo estar favorecido por la presencia de numerosos engrosamientos en las zonas de contacto entre las células, que son características de las raíces de *T. articulata*. Estos engrosamientos pudieron impedir el paso de las hifas. Se observó una reducida región interfacial entre ambos simbiontes con un material fibrilar en contacto con la pared de la hifa y, en algunos casos, en contacto con el plasmalema de la célula hospedante, aunque a veces esta región estuvo ocupada por pequeñas vesículas. El incremento de

los orgánulos citoplasmáticos tanto en la célula vegetal colonizada como en las hifas intracelulares, durante la fase arbuscular, indicó un aumento de la actividad metabólica de ambos simbiontes. Las formaciones de membrana, generalmente conocidas como plasmalemasomas, se observaron en las zona interfacial arbuscular y en el citoplasma de las hifas arbusculares. La organización corresponde a una micorriza arbuscular típica.

Palabras clave: Endomycorrhiza, *Tetraclinis articulata*, *Cupressaceae*, Ultraestructura, *Glomus*.

Introduction

There are numerous ultrastructural studies of the arbuscular mycorrhizae (AM) in various host families (Garriock et al. 1989, Bonfante-Fasolo & Grippiolo 1982, Jabaji-Hare et al. 1990, Kariya & Toth 1981, Kreutz-Jeanmaire & Dexheimer 1986, Kreutz-Jeanmaire et al. 1988, Gianinazzi-Pearson et al. 1981, Strullu 1978, Walker & Powell 1979, Yawney & Schultz 1990). However, there is no information about the ultrastructure of the AM in species of the *Cupressaceae* family. *Tetraclinis articulata* (Vahl) Masters is a cypress originating from North African and in Europe is only reported in Malta and the Southern Iberian Peninsula (Sierra de Cartagena) (Guerra et al. 1988). This species has been used in nurseries in southeaster Spain for revegetation programmes.

The presence of AM in *T. articulata* has been reported previously (Díaz & Honrubia 1993, Morte et al. 1996). The aim of the present work was to define the ultrastructural organisation of endomycorrhizas in naturally infected seedlings and micropropagated plants of *T. articulata post vitro* inoculated with *Glomus* sp., in order to determine whether they really differ from typical arbuscular mycorrhiza at the cellular level.

Material and methods

Material

Two types of samples have been studied:

1) Roots of micropropagated *T. articulata* plants inoculated with mycorrhizal roots of alfalfa with *Glomus* sp. at acclimatation stage, according to the method described by Morte et al. (1996) and Morte & Honrubia (1996). Roots of twenty plants were studied six months after inoculation.

2) Roots from six-month-old naturally infected seedlings of *T. articulata* obtained from El Valle tree nursery (La Alberca, Murcia) of the Servicio de Montes de la Consejería de Medio Ambiente de la Comunidad Autónoma de Murcia (Spain). Eighty plants were studied for one year.

Microscopy

One mm-long pieces of mycorrhizal root were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, for 3 h, at 4ºC. At the beginning of this process, a vacuum was created by means of a water vacuum pump (10-15 min) to facilitate the penetration of the fixative into the root tissues. The specimens were then rinsed three times in the phosphate buffer (30 min each time) and postfixed in 2% osmium tetroxide in the same buffer, for 2 h and 30 min at 4ºC. After postfixation, samples were soaked in the buffer overnight. Rinsed samples were poststained with uranyl acetate for 2 h, at 4 ºC. Samples were dehydrated through an alcohol series (30-100%) to propylene oxide and embedded in Spurr resin (Spurr 1969). Dehydratation and inclusion in resin were carried out at room temperature.

Samples were cut with a diamond knife ultramicrotome (Reichert Ultracut E). Semi thin sections (0.5 μm) were stained with toluidine blue for light microscope (Olympus BHT) observation.

Ultra thin sections (80 μm) were cut with the same ultramicrotome for electron transmission microscopy and stained with uranyl acetate for 5 min and lead citrate for 1 min (Reynolds 1963), and were examined using a Zeiss electron microscope 10 C at 75 Kv.

Results

Light microscopy study of the primary structure of the root of *T. articulata*

Roots of *T. articulata* were formed by an unistratified epidermis. The external cellular walls of the epidermal cells showed a thin cutine layer and the absence of root hairs.

Under the epidermis, there was a root cortex formed by four to six layers of parenchymal cells of greater size than epidermal cells. Their cellular walls presented swellings at the contact zone among cells. These parenchymal cells were disposed in concentric layers, leaving intercellular spaces among them.

The internal limit of the parenchymal cortex was formed by the endodermis. The endodermis of the root of *T. articulata* was formed by a cellular layer whose transversal walls showed a thick Caspary band (Fig. 1).

The vascular primary tissue was surrounded by a region of cells which form the pericycle. This pericycle was formed by two layers of parenchymal cells with thin walls and it was in direct contact with the protoxylem and protophloem, forming the so-called central stele. The studied root samples had a protoxylem with three fascicles of xylematic vessels forming a triarc root system. The protophloem was disposed among the protoxylem fascicles (data not shown).

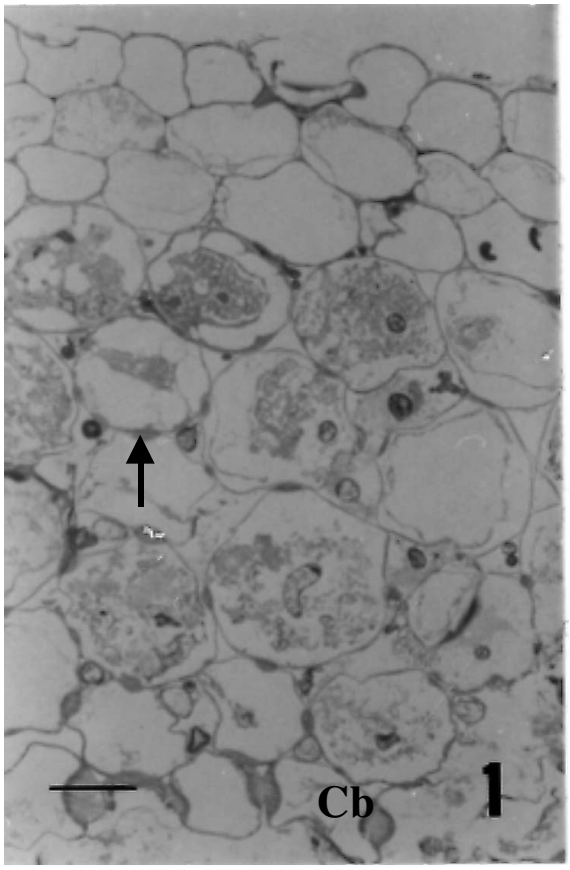


Figure 1. Semithin transverse section of an infected root of micropropagated *Tetraclinis articulata* plant with *Glomus sp.* Intracellular hyphae crossing through the host walls were not observed. Arrow: wall swellings. X800. Scale bar = 10 μm.

Structure of the mycorrhiza formed by *Glomus* sp. in roots of micropropagated plants of *T. articulata*

The semi-thin sections showed that fungal colonization mostly affected the parenchymal cells and the intercellular spaces between them. Endodermis and central stele were never colonized (Fig. 1).

At ultrastructural level, the intercellular hyphae of the outer parenchymal cellular layers showed a thick lamellar wall formed by several layers (Fig. 2). The cytoplasm contained numerous vacuoles, some of them with frequently eccentric dense granules and lipid globules (Fig. 2).

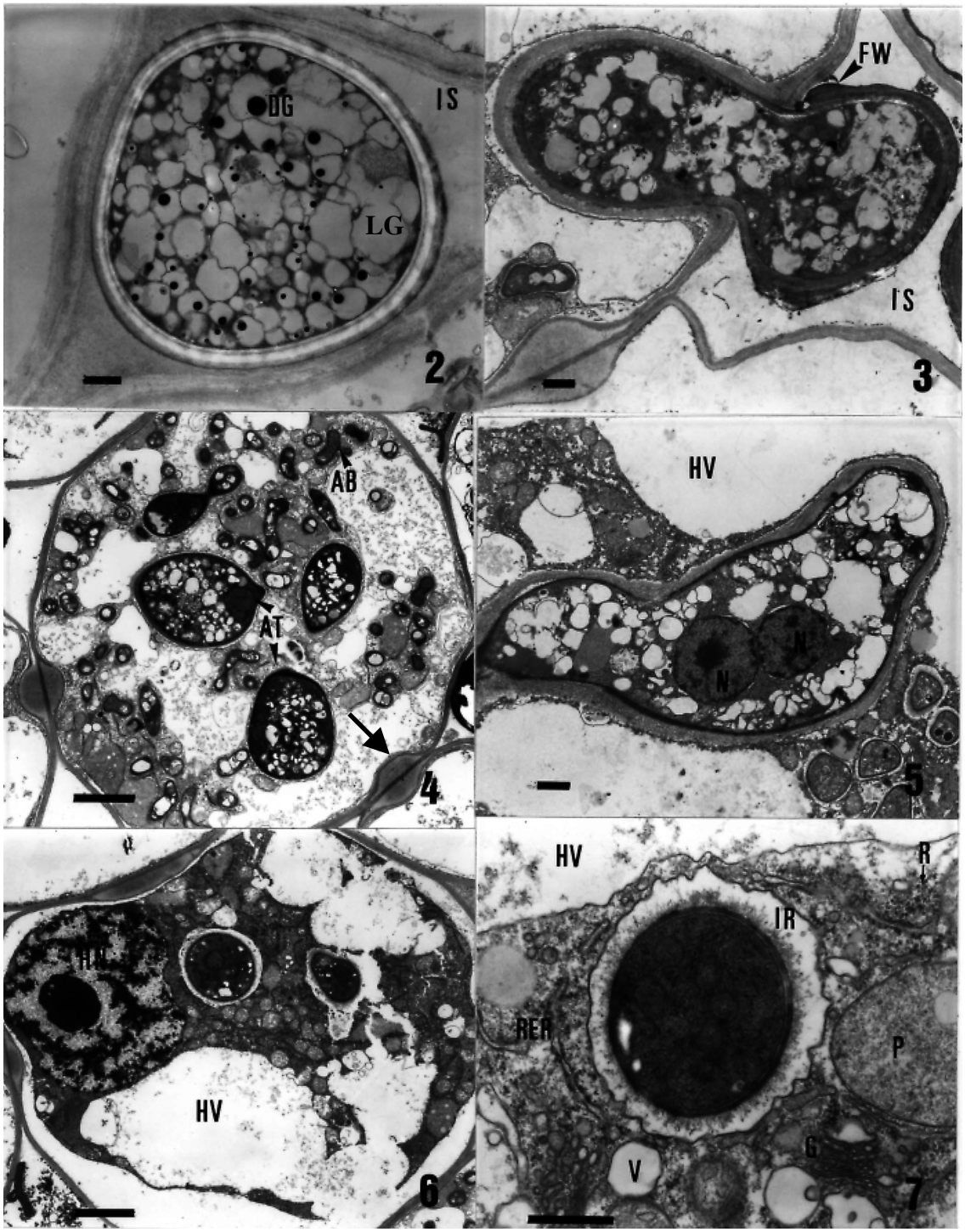
In the intercellular spaces, there were hyphae in contact with a distension of the host wall and invagination of the plasmalemma around the fungal branch. The fungal wall became thin at the zone into the host cell at the entry points (Fig. 3).

Large hyphae together with small arbuscular hyphal branches were observed in the same parenchymal cell (Fig. 4). These arbuscular hyphae filled almost all the cytoplasm of the host cell. The largest hyphae corresponded to the arbuscule trunk; these hyphae had a uniformly thin wall, numerous nuclei with nucleoli, mitochondria and some vacuoles (Fig. 5).

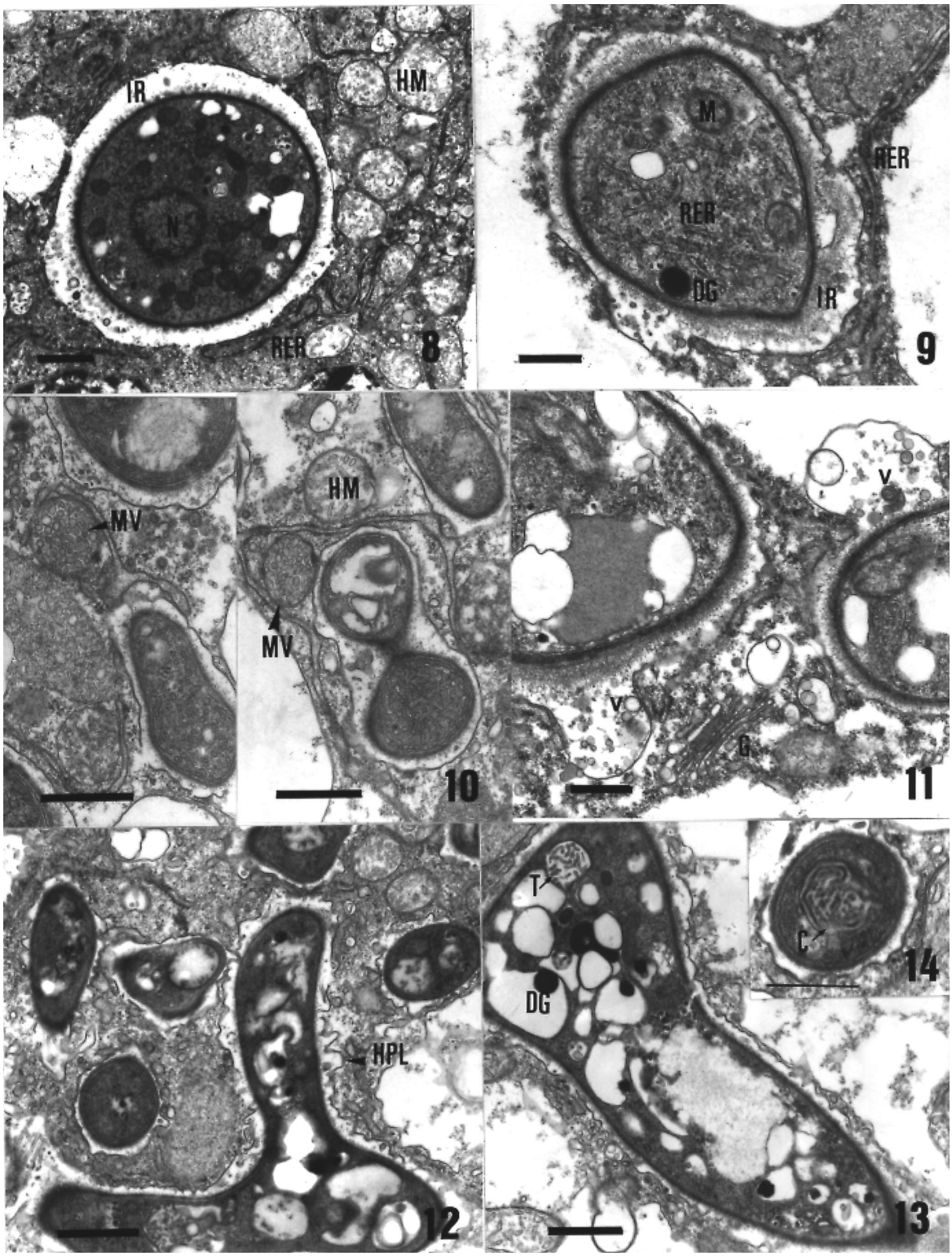
The host cells presented an increased cytoplasmic content compared to uninfected cells, especially mitochondria, endoplasmic reticulum and some plastids, although the characteristic amyloplasts of the uninfected parenchymal cells were not observed. The nucleus of the host cell, with an eccentric nucleolus, increased its size lightly, although it continued in a typical peripheral position, and the host vacuole was smaller than the vacuole of the uninfected cells (Fig. 6).

The arbuscular branches and the host plasmalemma were not observed to be in direct contact but were always separated by an interfacial region. This reduced interfacial region between both symbionts was observed with a fibrillar material in contact with the hyphal wall and, in some cases, in contact with the host plamalemma, although sometimes this region was occupied by small vesicles (Figs. 7, 8 and 9).

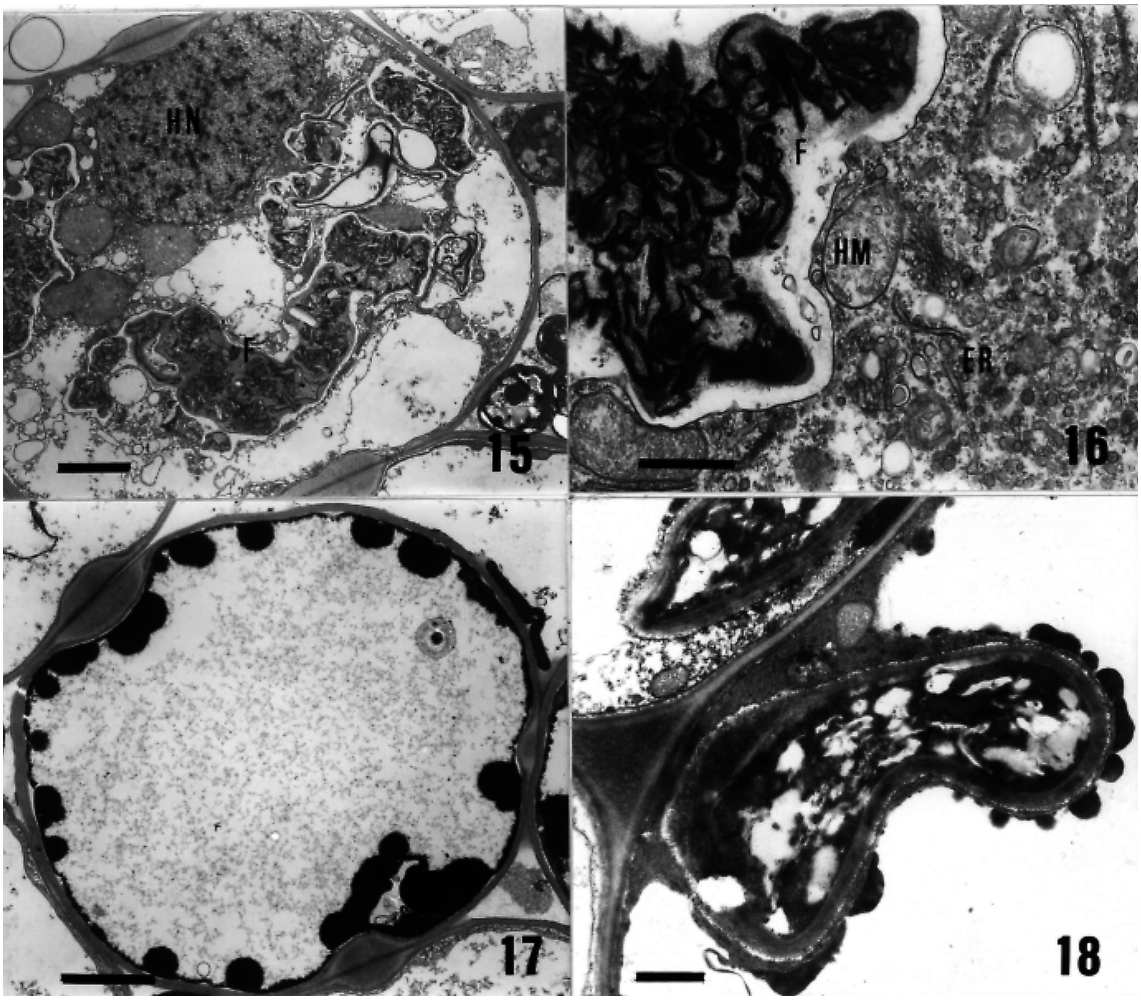
The host cytoplasm close to the interfacial zone presented Golgi systems with dilated cisternae at its extremes (Figs. 7 and 11), numerous large mitochondria with transversal crests disposed in an irregular way, and abundant smooth cisternae of rough endoplasmic reticulum (RER) (Figs. 8 and 9). Some of these RER cisterns had numerous and long ramifications and they were very close to the interfacial region (Fig. 8). There were also many free ribosomes in the host cytoplasm as well as plastids, some of them



Figures 2-7. Mycorrhization of micropropagated *Tetraclinis articulata* plant with *Glomus* sp. Fig. 2. Intercellular hyphae with a lamellar wall. The cytoplasm shows numerous vacuoles with eccentric dense granules and lipid globules. X6000. Scale bar = 1 µm. Fig. 3. Hyphae of *Glomus* sp. penetrating into the cell from an intercellular space. The fungal wall became thin at the zone into the host cell at the entry point. X6000. Scale bar = 1 µm. Fig. 4. Parenchymal cell infected by large hyphae together with small arbuscular branches. The central vacuole is fragmented. Wall swellings (arrow) at the contact zone among cells were observed. X2750. Scale bar = 4 µm. Fig. 5. Large hyphae corresponding to the arbucule trunk. The dense cytoplasm contained numerous nuclei, mitochondria and some vacuoles. X5500. Scale bar = 1 µm. Fig. 6. Detail of a parenchymal cell during the first stage of the fungal colonization. The cell presented an increased cytoplasmic content and the nucleus increased its size lightly although it continued in a peripheral position. X2750. Scale bar = 4 µm. Fig. 7. Detail of the interfacial region between an intracellular hypha and the host plasmalemma. The area of the fungal side of the region is occupied by a fibrillar electrodense matrix and, on the plasmalemmal side, there is a translucent space sometimes occupied by the same fibrillar material of the fibrillar matrix. The host cytoplasm showed an hyperactive Golgi system with dilated 'trans' cisternae at its extremes, abundant cisternae of RER with clustered ribosomes, plastids and vacuoles. X24000. Scale bar = 0.5 µm.



Figures 8-14. Mycorrhization of micropropagated *Tetraclinis articulata* plant with *Glomus* sp. Fig. 8. RER cisternae of the host cytoplasm close to the interfacial region. Fungal cytoplasm with eccentric nucleus, numerous mitochondria and small vesicles were observed. X10000. Scale bar = 1 µm. Fig. 9. RER cisternae were also present in the fungal cytoplasm. X24000. Scale bar = 0.5 µm. Fig. 10. Multivesicular bodies in the host cytoplasm close to the interfacial region. X16000. Scale bar = 1 µm. Fig. 11. Host cytoplasm showed some vesicles in small vacuoles and host plasmalemmasomes. X20000. Scale bar = 0.5 µm. Fig. 12. Detail of the arbucular colonization. Host plasmalemma was invaginated around the small arbuscular branches increasing the contact area between both symbionts. X13750. Scale bar = 1 µm. Figs. 13 and 14. Details of fungal plasmalemmasomes containing tubules and concentric layers of membranes. X13750. Scale bar = 1 µm (Fig. 13). X32000. Scale bar = 0.5 µm (Fig. 14).



Figures 15-18. Mycorrhization of micropropagated *Tetraclinis articulata* plant with *Glomus* sp. Fig. 15. Cortical root cell with highly vacuolated arbuscular tips and degenerated arbuscular tips. X2750. Scale bar = 4 µm. Fig. 16. Detail of an hyphal mass that has degenerated to a stage where individual hyphal tips, which have collased, are no longer in contact with the host cytoplasm. The host cytoplasm still contained numerous mitochondria, plastids, dictyosomes, vesicles and endoplasmic reticulum. X13750. Scale bar = 1 µm. Fig. 17. Some uninfected root cells contained osmiophilic droplets (arrow) of round shape and joined to the tonoplast. X3465. Scale bar = 4 µm. Fig. 18. Some hyphae were observed pressing against the central vacuole with osmiophilic droplets. X8800. Scale bar = 1 µm.

containing electron dense globules but free of starch (Figs. 7 and 9).

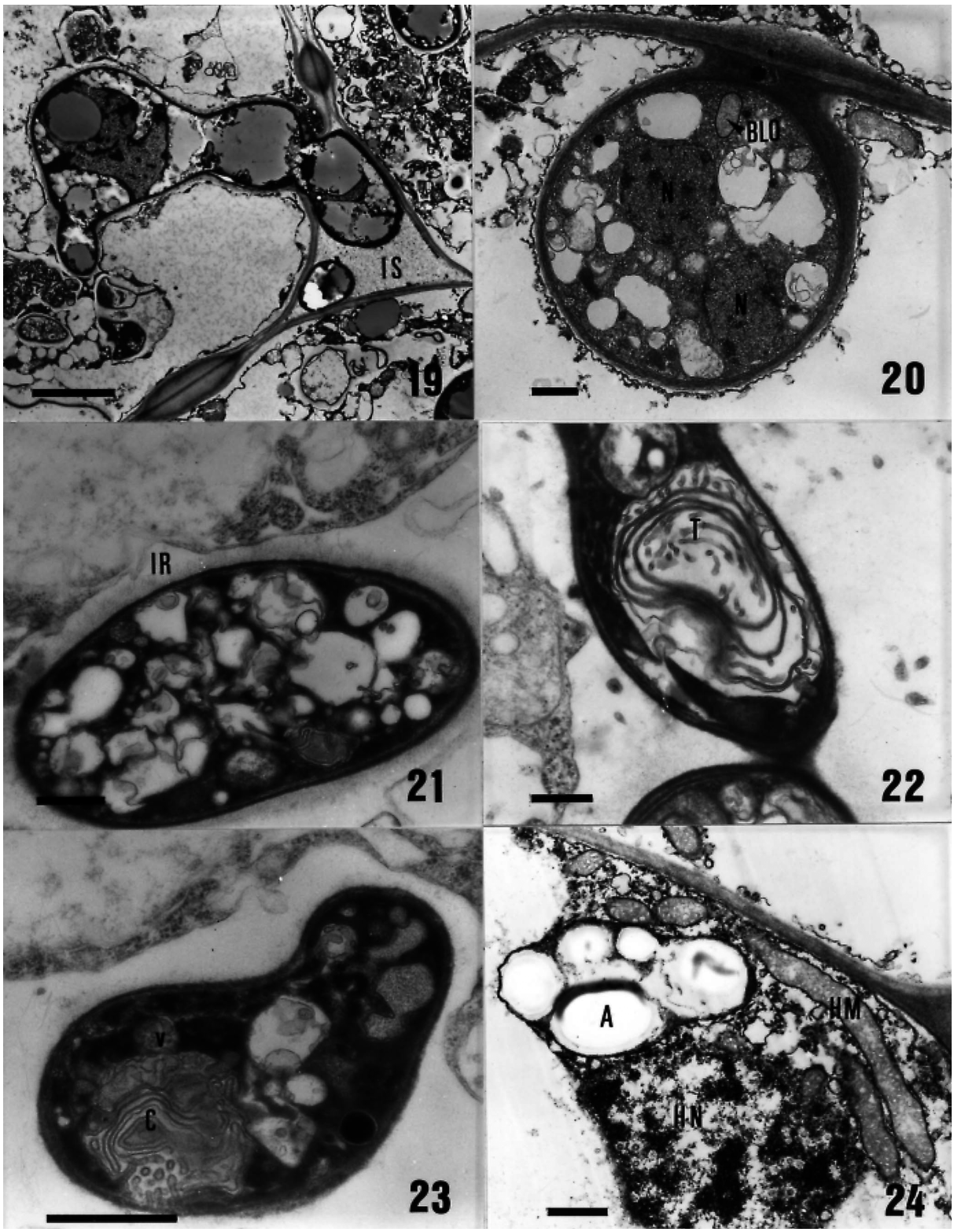
The presence of membranous vesicles is a general characteristic of arbuscular interface. They were observed near young and vacuolates hyphae. These membrane formations or host plasmalemmasomes vary in form and size, the most frequent being like round vesicles distributed in the interfacial space (Fig. 11). Multivesicular bodies in the host cytoplasm close to the interfacial regions were also observed (Fig. 10).

Host plasmalemma was invaginated round the small arbuscular branches (Fig. 12).

The fungal cytoplasm was characterized by the presence of a rounded, slightly eccentric and electrodense nucleus, abundant round mitochondria with transversal crests, RER cisternae and free ribosomes (Figs. 8 and 9), electrondense globules in vacuoles or closely ensheathed by a membrane and large lipidic areas (Figs. 9 and 13). The membrane formations of the fungus or fungal plasmalemmasomes forms fine tubules (Fig. 13) and a concentric layered configurations or complex «finger-print»-like formations (Fig. 14). They were most developed in the fine arbuscular hyphae where they could occupy nearly all the hypha lumen (Fig. 14).

All parts of the arbuscule (trunk, smaller branches and degenerated and collapsed hyphae in clumps) were evident among the cytoplasm of the same host cell (Fig. 14).

When the fungal deterioration was observed, the cytoplasm of the host cell still contained numerous



Figures 19-24. Structure of the mycorrhiza formed in roots of *T. articulata* seedlings in natural conditions. Fig. 19. Hypha penetrating, from the intercellular space towards the host cell, and its accompanying restriction. X3750. Scale bar = 4 µm. Fig. 20. The intracellular hypha of the trunk of the arbuscule was multinucleated and contained bacteria-like organisms. X8000. Scale bar = 1 µm. Fig. 21. Interfacial region with both fibrillar and translucent areas. X25000. Scale bar = 0.5 µm. Figs. 22 and 23. Intercellular hyphae with membrane formations or fungal plasmalemmasomes: vesicles, tubules and concentric layered formations. X20000. Scale bar = 0.5 µm (Fig. 22). X37500. Scale bar = 0.5 µm (Fig. 23). Fig. 24. Peripheral cytoplasm of an uninfected cell with amyloplasts, mitochondria and free ribosomes. X10500. Scale bar = 1 µm.

mitochondria, plastids, dictyosomes, vesicles and endoplasmic reticulum (Fig. 16). Empty hyphae and collapsed fungal wall forming clumps were observed in the host cytoplasm. The trunk arbuscular hyphae were very vacuolated. However, in this arbuscular senescent stage the host cell had not yet the appearance of an infected cell, since it looked more like an uninfected cell with a large vacuole, a peripheral cytoplasm with a lower number of organelles and an eccentric, slightly oval nucleus (Fig. 15). A greater number of intercellular hyphae were observed in this stage. The central vacuole of the uninfected root cell presented osmiophilic droplets, most probably of polyphenolic nature (Fusconi and Bonfante-Fasolo 1984), of round shape and joined to the tonoplast (Fig. 17). Some hyphae were observed pressing against the central vacuole (Fig. 18). However, arbuscular fungal development was not observed in this type of cells.

Structure of the mycorrhiza formed in roots of *T. articulata* seedlings in natural conditions

Mycorrhizal colonization affected only the cortical cells and the intercellular spaces among these cells. No intracellular hyphae were observed in the epidermal cells.

There were abundant intercellular hyphae in the outer parenchymal cortex some of them appearing in the same intercellular space. These hyphae were independent of each other; there was no common matrix among them (Fig. 19). In the swollen intercellular spaces, hyphae which spread the infection were found. Hyphae constriction was observed at the cell-wall entry points (Fig. 19).

In the host cells, there were large hyphae corresponding to the trunk of the arbuscule. The cytoplasm of these hyphae was less vacuolized than the cytoplasm of the intercellular hyphae, was multinucleated and contained bacteria-like organisms (Fig. 20). The interface with the host cell was formed by the fungal wall and the host plasmalemma which surrounds the hypha. The interfacial area also presented both fibrillar and translucent regions. The host cytoplasm close to the interfacial area presented numerous free ribosomes, vesicles enclosed in small vacuoles and some plastids (Fig. 21).

The intracellular hyphae also presented membrane formations or fungal pasmalemmasomes, such as vesicles (Fig. 21), tubules (Fig. 22) and concentric layered formations (Fig. 23).

Amyloplasts were only found in the peripheral cytoplasm of the uninfected cells (Fig. 24). Some cortical cells contained osmiophilic material, probably of polyphenolic nature. These cells were not colonized.

Discussion

No differences were observed in mycorrhizal ultrastructure of naturally or control-infected mycorrhizal roots of *T. articulata*. The small differences observed could be due to the different stage of mycorrhization of the samples at the time of collecting.

The light and electron microscope observations showed the constant presence of a typical arbuscular mycorrhiza similar to those already described by other authors in different herbaceous and woody plants (Scannerini & Bellando 1967, 1968, Scannerini 1972, Cox & Sander 1974, Kaspari 1975, Old & Nicolson 1975, Kinden & Brown 1975a, b, c, 1976, Scannerini & Bonfante-Fasolo 1975, 1977, 1979, 1983, Strullu 1978, Bonfante-Fasolo & Scannerini 1977, Bonfante-Fasolo 1978, Dexheimer et al. 1979, Holley & Peterson 1979, Gianinazzi-Pearson et al. 1981, Kariya & Toth 1981, Dexheimer et al. 1986, Kreuzt-Jeanmaire & Dexheimer 1986, Kreuzt-Jeanmaire et al. 1988, Yawney & Schultz 1990).

The mycorrhizal colonization only developed intensely in the parenchymal cortical cells and fungal spread from cell to cell was entirely intercellular. Intracellular hyphae crossing through the host walls were never observed. This could be favoured by the presence of numerous wall swellings at the contacting area among cell walls which are characteristic of *T. articulata* roots. These wall swellings could impede the crossing of the hyphae. In contrast, the intercellular hyphae were very rare in the gymnosperm *Taxus bacatta* (Strullu 1985) and *Acer sacharum* (Yawney & Schultz 1990) or inexistent as in the angyosperm *Gentiana lutea* (Jacquelinet-Jeanmougin et al. 1987).

The arbuscular development coincides with that described by other authors in other VA associations (Scannerini & Bonfante-Fasolo 1983). Large intracellular hyphae, corresponding to the trunk of the arbuscule, together with smaller hyphae, corresponding to the arbuscular branches, have been observed in the same host cell. This means that the smallest arbuscular hyphae were originated by repeated divisions of the trunk hyphae.

The bacteria-like organisms (BLOs) that appeared in the naturally infected roots of *T. articulata* were quite similar to those found in *Ornitogalum umbellatum* roots naturally infected with *G. fasciculatum* (Scannerini et al. 1975) and grapevine roots also na-

turally infected (Bonfante-Fasolo 1978). These BLOs have been observed in many AM fungi (Scannerini & Bonfante 1991) but they have never been identified. Biancotto et al. (1996) have shown that the endosymbiont of *G. margarita* was an rRNA group II pseudomonad (genus *Burkholderia*). They used PCR assays to demonstrate that the sequence came from the BLOs. They suggested that these bacteria are stable component of the fungal cytoplasm and that they must be taken into account when considering the extent of microbial biodiversity in ecosystems (Biancotto et al. 1996).

In the senescence stage of the fungus, the fact that clumps of collapsed fungal walls appear together with the large hyphae of the arbucular trunk suggests that arbuscular deterioration starts in the smallest branches and progresses to the trunk.

In the present study, no septa were observed in the fine endophyte, whilst they have been observed in the coarse arbuscular fungi at certain stages of mycorrhiza development, and particularly during arbuscular senescence (Cox & Sanders 1974, Scannerini et al. 1975, Kinden & Brown 1975c). Our results were similar with those obtained by Gianinazzi-Pearson et al. (1981) in the mycorrhiza formed by *G. tenuis* with *Rubus idaeus*.

The increase of the cytoplasmic organules both in the host cell and intracellular hyphae during the arbuscular phase indicated an increase in the metabolic activity of both symbionts. The cytoplasm of the colonized host cell presented the same characteristics which have been found in all AM: numerous mitochondria, active dictyosomes, RER and numerous isolated ribosomes. These characteristics indicated the existence of important interactions between both symbionts (Kreutz-Jeanmarie et al. 1988). The meaning of the numerous dictyosomes is not yet understood. They could play a role in the increased synthesis of plasma membranes (Münzenberger et al. 1992).

The plastids of the host cytoplasm contained some moderately electrodense globules inside. However, starch accumulations were never observed in infected cells of *T. articulata* roots. This absence or reduction in starch in the host cells has also been observed in some VAM (Kinden & Brown 1975c, Bonfante-Fasolo 1978, Gianinazzi-Pearson et al. 1981) and it has been interpreted as a manifestation, at structural level, of the change in carbon metabolism in the host cell, where an important part of carbohydrate would be solubilized and transferred to the mycorrhizal fungus (Kreutz-Jeanmaire et al. 1988).

The membrane formations associated with the plasmalemma and generally referred to as plasmalemmasomes (Marchant & Robards 1968, Marchant & Moore 1973) appeared in the arbuscular interfacial zone. These membrane formations represent an important proliferation of both the plant and fungal plasmalemmas, so that the surface where the two symbionts contact is greatly increased (Dexheimer et al. 1985) and is even more important than that estimated by Cox & Tinker (1976) on the basis of the invaginated host plasmalemma above. Moreover, these structures could be involved in exchange processes between the symbionts. These plasmalemmasomes have also been observed in other mycorrhizal associations (Dexheimer et al. 1982, Gianinazzi-Pearson et al. 1984, Dexheimer et al. 1985, Yawney & Schultz 1990). One of the functions that have been attributed to plant plasmalemmasomes is an involvement in cell wall synthesis (Marchant & Robards 1968, Mesquita 1970) and as a place of chain polymerization of polysaccharides (Roland 1973, Roland & Pilet, 1974). The plasmalemmasomes of the arbuscular fungus have been associated as much with hyphal wall synthesis (Marchant et al. 1967) as with hyphal vacuolation or autolysis (Coulomb 1973, Eyme & Angeli-Papa 1978) and the active secretion of substances (Setandreu et al. 1981). However, there are a very few studies which demonstrate each of these functions.

The composition of the cytoplasm of *Glomus* sp. is also similar to that described in other mycorrhizal associations with *Glomus* species previously mentioned. Intercellular and coiled hyphae of *Glomus* sp. have very little but uniform protoplasm, while the protoplasm of the arbuscules (large trunk and small branches) contains a normal complement of organelles, indicative of a very active mycelium. On the contrary, electrodense globules, corresponding to the polyphosphate granules described by Cox et al. (1975), are found in all the endophyte mycelium.

ABBREVIATIONS: A, amyloplast; AB, arbuscule branch; AT, arbuscule trunk; BLO, bacterium-like organism; C, concentric formations; Cb: Caspary band; DG, dense granule; ER, endoplasmic reticulum; F, fungal clump; FW, fungus wall; G, Golgi; HC, host cytoplasm; HM, host mitochondrion; HN, host nucleus; HPL, host plasmalemma; HV, host vacuole; IS, intercellular space; IR, interfacial region; M, fungus mitochondrion; MV, multivesicular bodies; N, fungus nucleus; P, plastid; R, ribosome; RER, rough endoplasmic reticulum; T, tubule; V, vacuole; v, vesicle.

References

Biancotto V, Bandi C, Minerdi D, Sirono M, Volker Tichy H & Bonfante P. 1996. An obligately endosymbiotic mycorrhizal fungus itself harbors obligately intracellular bacteria. Applied and Environmental Microbiology 62 (8): 3005-3010.

Bonfante-Fasolo P. 1978. Some ultrastructural features of vesicular-arbuscular mycorrhiza in the grapevine. Vitis 17: 386-395.

Bonfante-Fasolo P & Grippiolo R. 1982. Ultrastructural and cyrochemical changes in the wall of a vesicular-arbuscular mycorrhizal fungus during symbiosis. Canadian Journal of Botany 60: 2303-2312.

Bonfante-Fasolo P & Scannerini S. 1977. A cytological study of the vesicular-arbuscular mycorrhiza in Ornithogallum umbellatum L. Alliona 22: 5-12.

Coulomb C. 1973. Diversité des corps multivésiculaires et notion d'hétérophagie dans le méristème radiculaire de Scorzonère (Scorzonera hispanica). Journal of Microbiology 16: 345-360.

Cox G & Sanders F. 1974. Ultrastructure of the host-fungus interface in a vesicular-arbuscular mycorrhiza. New Phytologist 73: 901-912.

Cox G & Tinker PB. 1976. Translocation and transfer of nutrients in vesicular-arbuscular mycorrhizas. I. The arbuscule and phosphorus transfer: a quantitative ultrastructural Study. New Phytologist 77: 371-378.

Cox G, Sanders F, Tinker PB & Wild JA. 1975. Ultrastructural evidence relating to host-endophyte transfer in a vesicular-arbuscular mycorrhiza. In Endomycorrhizas. London, New York, San Francisco: Academic Press, pp. 297-312.

Dexheimer J, Gianinazzi S & Gianinazzi-Pearson V. 1979. Ultrastructural cytochemistry of the host-fungus interfaces in the endomycorrhizal association Glomus mosseae/Allium cepa. Zeitschrift für Pflazenphysiologie 92: 191-206.

Dexheimer J, Gianinazzi-Pearson V & Gianinazzi S. 1982. Acquisitions récentes sur la phisiologie des mycorrhizes VA au niveau cellulaire. Les Mycorrhizes: biologie et utilisation. Les Colloques de l'INRA, Dijon. INRA Publ.

Dexheimer J, Kreutz-Jeanmaire C, Gerard J, Gianinazzi-Pearson V, Gianinazzi, S. 1986. Approche cellulaire du fonctionnement des endomycorhizes à vésicules et à arbuscules: les plasmalemmes de l'interface. In Aspects physiologiques et génétiques des mycorrhizes (Gianinazzi-Pearson V & Gianinazzi S, eds.). Paris: INRA, pp. 277-283.

Dexheimer J, Marx M, Gianinazzi-Pearson V & Gianinazzi S. 1985. Ultracytological studies of plasmalemma formations produced by host and fungus in vesicular-arbuscular mycorrhizae. Cytologia 50: 461-471.

Díaz G & Honrubia M. 1993. Arbuscular mycorrhizae on Tetraclinis articulata (Cupressaceae): development of mycorrhizal colonization and effect of fertilization and inoculation. Agronomie 13: 267-274.

Eyme J & Angeli-Papa J. 1978. Evolution des hyphes et vacuolisation chez Agaricus bisporus et A. sylvicola. Mush. Sci. Proc. 10th Int. Congress on the Science and Cultivation of Edible Fungi 10: 505-527.

Fusconi A & Bonfante-Fasolo P. 1984. Ultrastructural aspects of host endophyte relationships in Arbutus unedo L. mycorrhizas. New Phytologist 96: 397-410.

Garriock ML, Peterson RL & Ackerley CA. 1989. Early stages in colonization of Allium porrum (leek) roots by the vesicular-arbuscular mycorrhizal fungus, Glomus versiforme. New Phytologist 112: 85-92.

Gianinazzi-Pearson V, Dexheimer J, Gianinazzi S & Jeanmaire C. 1984. Plasmalemma structure and function in endomycorrhizal symbiosis. Zeitschrift für Pflanzenphysiology 114: 201-205.

Gianinazzi-Pearson V, Morandi D, Dexheimer J & Gianinazzi S. 1981. Ultrastructural and ultracytochemical features of a Glomus tenuis mycorrhiza. New Phytologist 88: 633-639.

Guerra J, Alcaraz F, Egea JM, Hernández J, Carrión JS, Martínez-Sánchez JJ, Sánchez P. 1988. Densidad, Estructura Poblacional y Areas Potenciales para la Expansión de la Sabina Mora (Tetraclinis articulata) en el Litoral de la Región de Murcia. Murcia: ARMAN.

Holley JD & Peterson RL. 1979. Development of a vesicular-arbuscular mycorrhiza in bean root. Canadian Journal of Botany 57: 1960-1978.

Jabaji-Hare S, Therien J & Charest PM. 1990. High resolution cytochemical study of the vesicular-arbuscular mycorrhizal association, Glomus clarum x Allium porrum. New Phytologist 114: 481-496.

Jaquelinet-Jeanmougin S, Gianinazzi-Paerson V & Gianinazzi S. 1987. Endomycorrhizas in the Gentinaceae. II Ultrastructural aspects of symbiont relatonship in Gentiana lutea L. Symbiosis 3: 269-286.

Kariya N & Toth R. 1981. Ultrastructure of the mycorrhizal association formed between Zea diploperennis and Glomus fasciculatus. Mycologia 73: 1027-1039.

Kaspari H. 1975. Fine structure of the host-parasite interface in endotrophic mycorrhiza of tobacco. In Endomycorrhizas. London, New York, San Francisco: Academic Press, pp. 325-351.

Kinden DA & Brown MF. 1975a. Electron microscopy of vesicular-arbuscular mycorrhizae of yellow poplar. I. Characterization of endophytic structures by scanning electron stereoscopy. Canadian Journal of Microbiology 21: 989-993.

Kinden DA & Brown MF. 1975b. Electron microscopy of vesicular-arbuscular mycorrhizae of yellow poplar. II. Intracellular hyphae and vesicles. Canadian Journal of Microbiology 21: 1768-1780.

Kinden DA & Brown MF. 1975c. Electron microscopy of vesicular-arbuscular mycorrhizae of yellow poplar. III. Host-endophyte interactions during arbuscular development. Canadian Journal of Microbiology 21: 1930-1939.

Kinden DA & Brown MF. 1976. Electron microscopy of vesicular-arbuscular mycorrizae of yellow poplar. IV. Horst-endophyte interactions during arbuscular deterioration. Canadian Journal of Microbiology 22: 64-75.

Kreutz-Jeanmaire C & Dexheimer J. 1986. Etude ultrastructurale comparative des endomycorrhizes naturelles et artificielles de merisier. In Mycorrhizae: physiology and genetics. Dijon: Proceedings of the Ist ESM, INRA.

Kreutz-Jeanmaire C, Dexheimer J & Gerard J. 1988. Etude de l'organisation ultrastructurale des mycorhizes a vesicules et arbuscules dans deux Rosacees ligneuses (Prunus avium L. et Pyrus malus L.). Phytomorphology 38 (2, 3): 205-217.

Marchant R & Moore RT. 1973. Lomasomes and plasmalemmasomes in fungi. Protoplasma 76: 623-629.

Marchant R & Robards AW. 1968. Membrane system associated with the plasmalemma of plant cells. American Journal of Botany 32: 457-471.

Marchant R, Peat A & Bambury GH. 1967. The ultrastructural basis of hyphal growth. New Phytoloogist 66: 623-629.

Mesquita JF. 1970. Etude ultrastructurale de vésicules associées aux parois cellulaires dans les racines de l'Allium cepa L. et de Lupinus albus L. (Vésicules plasmalemmiques et plasmalemmasomes). Révision Cytologie et Biologie Végetale 33: 235-264.

Morte A & Honrubia M. 1996. Biotechnology in Tetraclinis articulata. In Biotechnology in Agriculture and Forestry. Trees IV, Vol. 35. (Bajaj YPS, ed.). Berlin, Heidelberg: Springer-Verlag, pp. 407-423.

Morte A, Diaz G & Honrubia M. 1996. Effect of arbuscular mycorrhizal inoculation on micropropagated Tetraclinis articulata growth and survival. Agronomie 16: 563-571.

Münzenberger B, Kottke I & Oberwinkler F. 1992. Ultrastructural investigations of Arbutus unedo - Laccaria amethystea mycorrhiza synthesized in vitro. Trees 7: 40-47.

Old KM & Nicolson TH. 1975. Electron microscopic studies of the mycorrhizal roots of sand dune grasses. New Phytologist 74: 51-58.

Reynolds ES. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. Journal of Cell Biology 17: 208-212.

Roland JC. 1973. The relationship between the plasmalemma and cell wall. International Review of Cytology. 36: 45-92.

Roland JC & Pilet PE. 1974. Implications du plasmalemme et de la paroi dans la croissance des cellules végétales. Experientia 30: 441-451.

Scannerini S. 1972. Ultrastruttura delle endomicorrize di Ornithogalum umbellatum all'inizio dell'attività vegetativa. Alliona 18: 129-150.

Scannerini S & Bellando M. 1967. Some ultrastructural features of endotrophic mycorrhiza in Ornithogalum umbellatum. Giorna Botanico Italiano 101: 313-314.

Scannerini S & Bellando M. 1968. Sull'ultrastruttura delle micorrize endotrofiche de Ornithogalum umbellatum in attività vegetativa. Atti dell'Accademia delle Scienze di Torino 102: 795-809.

Scannerini S & Bonfante-Fasolo P. 1975. Dati preleminari sull'ultrastruttura di vesicole intracellulari nell'endomicorrhiza di Ornithogalum umbellatum L. Atti dell'Accademia delle Scienze di Torino 109: 619-621.

Scannerini S & Bonfante-Fasolo P. 1977. Unusual pastids in an endomycorrhizal root. Canadian Journal of Botany 55: 2471-2479.

Scannerini S & Bonfante-Fasolo P. 1979. Ultrastructural cytochemical demonstration of polysaccharides and proteins within the host-arbuscules interfacial matrix in an endomycorrhiza. New Phytologist 83: 87-94.

Scannerini S & Bonfante-Fasolo P. 1983. Comparative ultrastructural analysis of mycorrhizal associations. Canadian Journal of Botany 61: 917-943.

Scannerini S & Bonfante P. 1991. Bacteria and bacteria-like objects in endomycorrhizal fungi (Glomaceae). In Symbiosis as a source of evolutionary innovation: speciation and morphogenesis. (Margulis L & Fester R, eds.). Cambridge, Mass.: MIT Press, pp. 273-287.

Scannerini S, Bonfante-Fasolo P & Fontana A. 1975. An ultrastructural model for the hostsymbiont interaction in the endotrophic mycorrhizae of Ornithogallum umbellatum L. In Endomycorrhizas. (Sanders FE, Mosse B & Tinker BP, eds.). London, New York, San Francisco: Academic Press, pp. 313-324.

Sentandreu R, Larriba G & Elorza MV. 1981. Secretion processes-general considerations and secretion in fungi. Encyclopedy of Plant Physiology N.S. 13B: 487-512.

Spurr AR. 1969. A low viscosity epoxy resin embedding medium for electron microcopy. Journal of Ultrastructural Research 26: 31-43.

Strullu DG. 1978. Histologie et citologie des endomycorrhizes. Physiologie Végétale 16: 657-669.

Strullu DG. 1985. Les mycorrhizes. Gebrüder Borntraeger, Berlin, Stuttgart.

Walker GP & Powell CL. 1979. Vesicular-arbuscular mycorrhizae in white clover: a scanning electron microscope and X-ray microanalytical study. New Zealand Journal of Botany 17: 55-59.

Yawney WJ & Schultz RC. 1990. Anatomy of a VA mycorrhizal symbiosis between sugar maple (Acer saccharum Marsh) and Glomus etunicatum Becker & Gerdemann. New Phytologist 144: 47-57.