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Cattedra di Viticoltura dell'Università di Torino, Italia

Some ultrastructural features of the vesicular-arbuscular mycorrhiza in the grapevine

by

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Quelques caractéristiques ultrastructurelles des mycorrhizes du type vésiculaire arbusculaire de la vigne

Résumé. — On a étudié au moyen de microscopes electroniques à transmission et à balayage la morphologie de racines mycorrhizées prélevées dans les vignobles.

L'attention a été concentrée sur les formes les plus répandues de l'endophyte, c'est-à-dire les hyphes intercellulaires et les arbuscules. Les hyphes intercellulaires, qui transmettent l'infection, ont un protoplasme muni de noyaux, de mitochondries, de vacuoles avec des globules sombres aux électrons et des micro-organismes semblables à des bactéries.

A la suite de la pénétration dans la cellule de la plante-hôte, l'hyphe forme plusieures branches. L'arbuscule ainsi formé remplit complètement la cellule corticale. On a pu observer et décrire les différentes phases de dégradation de l'arbuscule.

Les cellules de l'hôte infectées montrent outre les organelles normales des plastides avec de l'amidon en contact étroit avec l'endophyte.

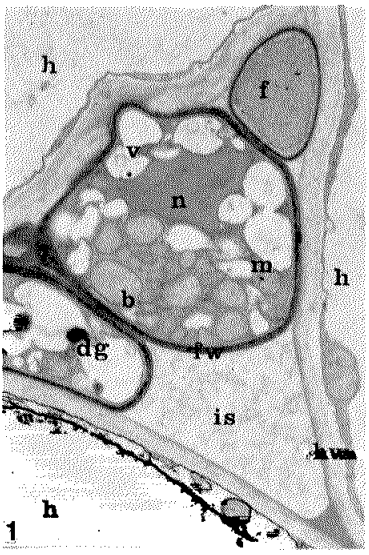
Introduction

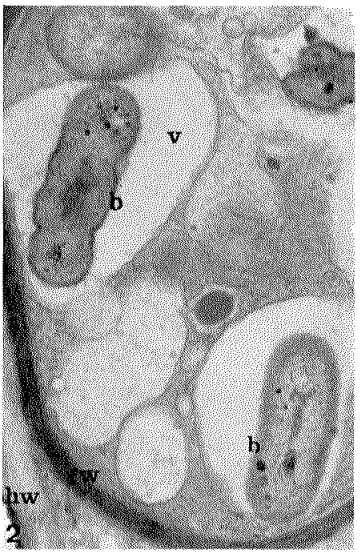
The interest towards the vesicular-arbuscular (v.a.) mycorrhizae was renewed during last years in consideration of the relevant role of such association for the plant nutrition.

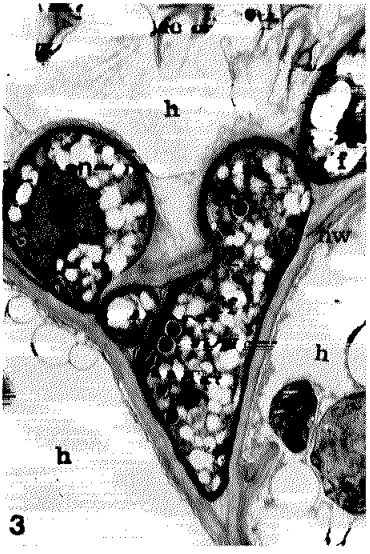
In fact, the passage of sugars from the plant to the fungus and viceversa the flow of phosphate towards the plant prompt many effects, the most prominent of which is the improved growth of the mycorrhizal plants (MOSSE 1973, HARLEY 1975, LEWIS 1975, GEBBING *et al.* 1977).

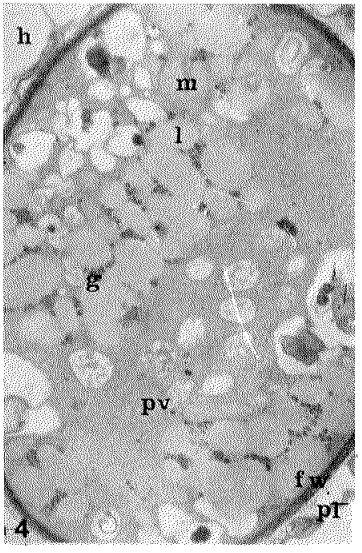
The occurrence of a v.a. mycorrhiza in grapevine roots was recognized long time ago (PETRI 1907, PEYRONEL 1923), but only recently this association got some sporadic evaluation. POSSINGHAM and GROOT OBBINK (1971) considered the grapevine mycorrhiza with particular reference to the plant growth and nutrition, while DEAL *et al.* (1972) studied the features of the mycorrhizal infection during the replanting of vineyards. Moreover, these authors described the morphological features of the infection, characterized by appressoria, inter-intracellular hyphae and arbuscules. Similar results were obtained also by GEBBING *et al.* (1977) and FONTANA *et al.* (In press).

The aim of the present work is to supplement the morphological analysis, studying at the ultrastructural level the intercellular hyphae and the arbuscules, which are the most common features of the v.a. mycorrhizae in the grapevine grown in field.









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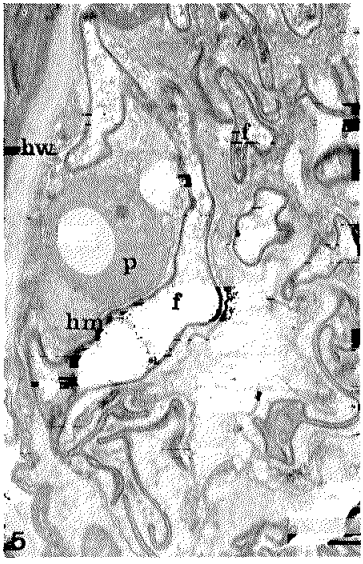

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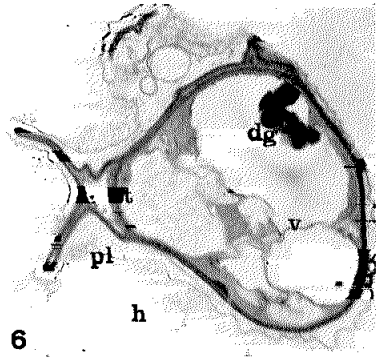

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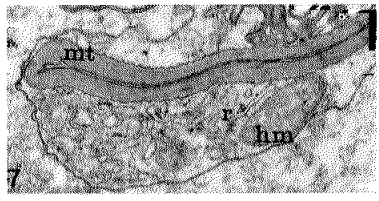

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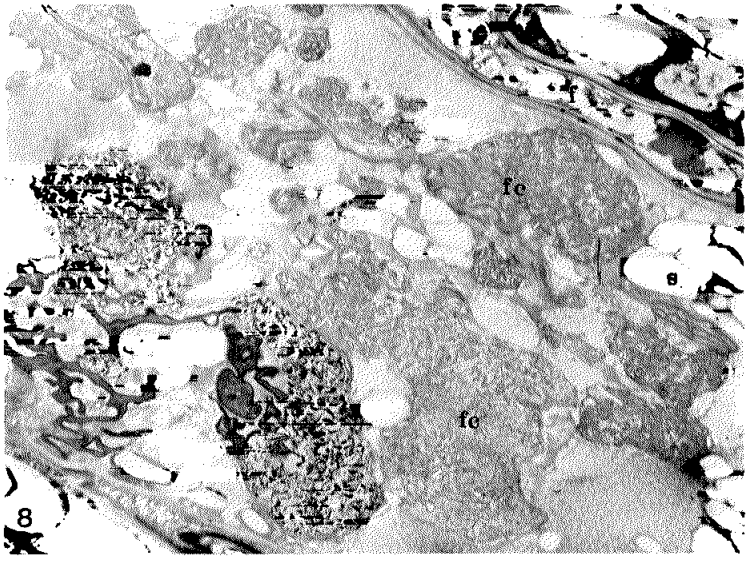

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Material and methods

Roots of Kober 5 BB grafted with *Vitis vinifera* cultivar Barbera grown in an experimental vineyard were collected once a month for a year. Infection was detected with hand cut sections stained with Lugol and lactic blue solutions and observed by a light microscope.

Fig. 1: Hyphae spreading the infection in the intercellular space. Their protoplasm shows small bacteria-like organisms, a nucleus and vacuoles with electron dense globules. × 15,000.

Fig. 2: Bacteria inside a fungal vacuole. They are endowed with a thin stratified wall and minute electron dense granules. × 38,400.

Fig. 3: Hyphal penetration from the intercellular space towards the host cell. The host plasma membrane surrounds the fungal branch. × 8,200.

Fig. 4: Ultrastructural organization of a large intracellular hypha. A light osmiophilic wall surrounds a protoplasm with mitochondria, polyvesicular bodies, lipid droplets and glycogen particles. × 17,500.

Fig. 5: Fungal branches of different size in cortical cell. The cytoplasm appears highly vacuolated and degenerated. Host mitochondria and amyloplasts are evident among the hyphae. × 18,500.

Fig. 6: Apical fungal branch. A septum divides the basal part from the collapsed apical one. A plasma membrane surrounds all the branches giving rise to a space containing an almost electron transparent material. × 11,500.

Fig. 7: Thin collapsed hypha surrounded by electron dense material. Host rough reticulum is evident. × 16,000.

Fig. 8: A cortical cell completely filled up with large fungal clumps. × 5,400.

Key to the lettering see Figs. 13 and 14.

Fig. 1: Hyphes intercellulaires qui transmettent l'infection dans la racine de l'hôte. Les protoplasmes des hyphes renferment des organismes ressemblant à des bactéries de petite taille, un noyau et des vacuoles avec des globules sombres aux électrons. × 15.000.

Fig. 2: Deux bactéries à l'intérieur des vacuoles du champignon symbiotique. Les bactéries sont entourés par leurs parois cellulaires et renferment dans leur protoplasmes de petits grains sombres aux électrons. × 38.400.

Fig. 3: Hyphes pénètrant dans une cellule de l'hôte. Les branches intracellulaires des hyphes sont entourées de la membrane cytoplasmatique de l'hôte. × 8.200.

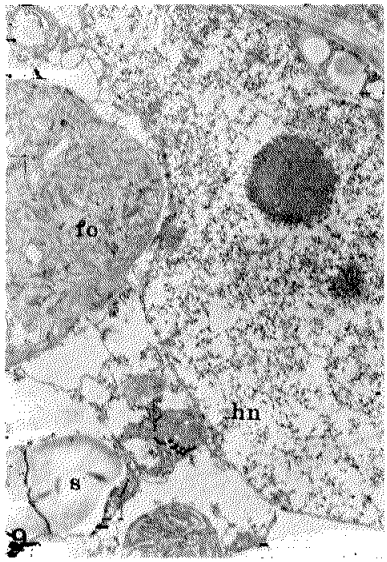
Fig. 4: Ultrastructure d'une grande hyphe à contour circulaire qui vit à l'intérieur de la cellule hôte. Le protoplasme du champignon symbiotique possède des mitochondries, des corps polyvésiculaires, des globules lipidiques et du glycogène. L'hyphe est entourée par une paroi sombre aux électrons. × 17.500.

Fig. 5: Groupement d'hyphes de taille différente dans une cellule corticale de la mycorrhize. Leur contenu cellulaire est sortout formé par des vacuoles et le cytoplasme fongique disparaît progressivement. Parmi les hyphes les mitochondries et les plastides amylifères de l'hôte sont visibles. × 18.500.

Fig. 6. Partie terminale de l'arbuscule. Une cloison transversale sépare la partie basale et la partie apicale de l'hyphe qui est aplatie et possède un contenu cellulaire en cours de lyse. Une couche presque transparente aux électrons se trouve entre le plasmalemme de l'hôte et la parois du champignon symbiotique. × 11.500.

Fig. 7: Petite hyphe aplatie entourée d'une couche sombre. Dans la cellule hôte le réticulum endoplasmique avec ses ribosomes est évident. × 16.000.

Fig. 8: Cellule corticale remplie de grands amas résiduels d'hyphes. × 5.400.
Liste des abréviations voir Fig. 13 et 14.



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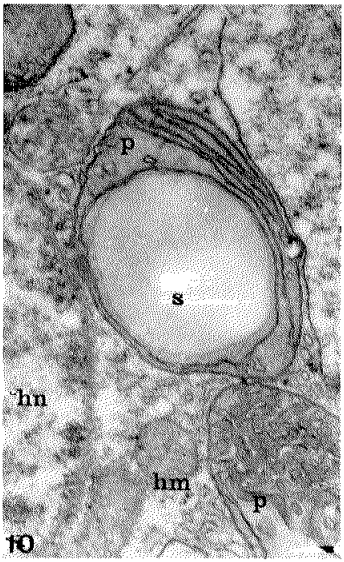

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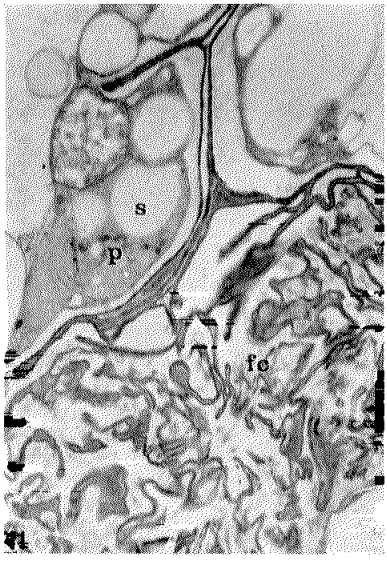

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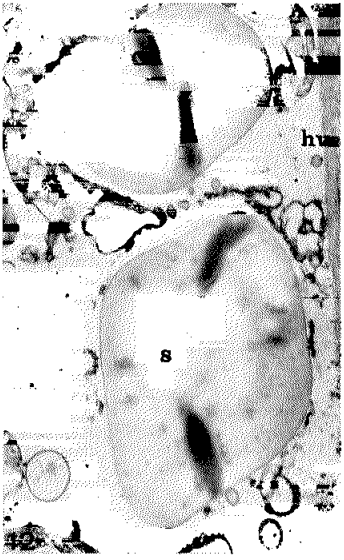

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For transmission electron microscopy the roots were fixed in 2.5 % glutaraldehyde in 0.1 M cacodylate buffer pH 7.2 for 1½ h, postfixed in 1 % OsO_4 in the same buffer for 3 h, dehydrated in the graded ethanol series and embedded in Durcupan ACM. Thick sections (1 μm) were stained with toluidine blue for light microscope observation, while thin sections (70 nm) after contrasting with uranyl acetate and lead citrate were examined with a Philips 300 electron microscope.

For scanning electron microscopy the material was fixed and dehydrated as described above till absolute ethanol, followed by a graded ethanol/amyl acetate series. Then it was dried with a critical point dryer (Denton), fractured in liquid nitrogen, coated with gold in a vacuum evaporator (Leyboldt-Heraeus EPA 30) and finally observed in a Siemens Autoscan electron microscope, operating at an accelerating voltage of 20 kV.

Results

A massive infection caused by a phycomicetous fungus, tentatively identified as a *Glomus* by the chlamydospores collected from the soil taken from around the roots (Fontana *et al.*, in press), was always detectable in the roots. The fungus with intercellular hyphae and intracellular arbuscules is found in the central and inner layers of the cortex. It never proceeds beyond the endodermis.

At the ultrastructural level, in the swollen intercellular spaces, the hyphae (3—5 μm in diameter) spreading the infection are found (Fig. 1). They show an electron opaque wall with a fibrillar texture. Their protoplasm sometimes shows usual organelles (nuclei, mitochondria) but more often highly vacuolated areas with dense globules. Glycogen particles and bacteria-like microorganisms are also observed. These last are found inside a vacuole and display different shapes. Rod-shape bacteria (0.8 μm long and 0.2 μm large) have a finely stratified wall and a cytoplasm endowed with minute electron dense granules (Fig. 2).

The intercellular hypha displays a direct contact between its wall and the host wall. Only in the intercellular spaces traces of fibrillar material are sometimes observed. From the intercellular spaces the endophyte passes into the host cell (Fig. 3); the distension of the host wall and the invagination of the plasma membrane around the fungal branch are observed. Inside the host cell the fungus gives

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Fig. 9: Large nucleus near a fungal clump in an infected cell. × 10,200.

Fig. 10: Plastid with starch in a poorly infected cell. × 22,000.

Fig. 11: Amyloplast and mitochondrion in close contact with a fungal clump. × 18,500.

Fig. 12: Large starch granules in an uninfected cell. × 24,000.

Key to the lettering see Figs. 13 and 14.

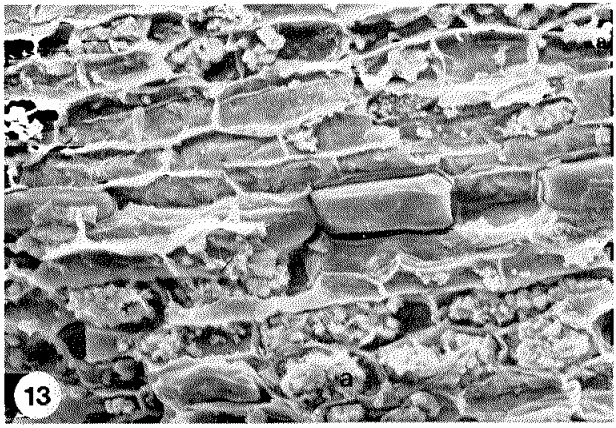
Fig.: 9: Noyau hypertrophié proche d'un amas résiduel du champignon dans une cellule infectée par le symbiote. × 10.200.

Fig.: 10: Plastide avec de l'amidon dans une cellule hébergeant peu de champignon. × 22.000.

Fig. 11: Un plastide avec de l'amidon et une mitochondrie voisins d'un amas résiduel du champignon. × 18.500.

Fig. 12: Grands grains d'amidon dans une cellule dépourvue de champignon symbiotique. × 24.000.

Liste des abréviations voir Fig. 13 et 14.



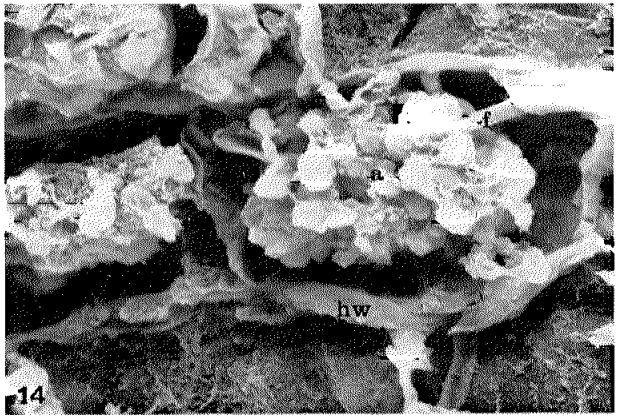


Fig. 13: Scanning electron micrography of an inner surface of mycorrhizal root. Endophytic arbuscules are detectable in most cortical cells. × 450.

Fig. 14: A higher magnification of an arbuscule in a late stage of deterioration. Round-shaped clumps are recognized. x 1,800.

Fig. 13: Micrographie électronique à balayage d'une mycorrhize fracturée à l'aide d'azote liquide. Les arbuscules du champignon symbiotique sont très répandus dans les cellules corticales de la mycorrhize. × 450.

Fig. 14: Détail d'un arbuscule en cours de destruction. Les amas résiduels sphériques du champignon sont évidents à l'intérieur de la cellule hôte. × 1.800.

Key to the lettering
a: arbuscule; b: bacterium; dg: dense globule; f: fungus cell; fc: fungal clump; fw: fungal wall; g: glycogen; h: host cell; hw: host wall; hm: host mitochondrion; hn: host nucleus; is: intercellular space; l: fungal lipids; m: fungal mitochondrion; mt: material

rise to the arbuscule; fungal branches of different size (from 4 μm to 0.3 μm) are found. While their wall is always osmiophilic, the cytological organization varies according to the size of such branches. In fact, the larger branches display all the usual organelles, i.e. nucleus (Fig. 3), mitochondria, polyvesicular bodies, lipid droplets surrounded by glycogen particles (Fig. 4). Also little vacuoles with dense globules and bacteria-like microorganisms in a degenerated stage are detectable. On the contrary, the smallest hyphae display large vacuoles with electron dense globules and a highly degenerated cytoplasm (Fig. 5). In these hyphae a transverse septum separates the still active part by the degenerated one; this last collapses and the two joined walls form a little clump (Figs. 5, 6, 7). A lot of these little clumps aggregate to form large amorphous masses. At the end of the process the infected cell is completely filled up with the fungal remnants (Fig. 8). The intracellular hyphae show another common feature: The host plasma membrane always separates them from the host cytoplasm. In this way an interfacial space (0.1 μm thick) filled with almost electron transparent material (Fig. 6) or strongly electron dense one (Fig. 7) is formed. Around the collapsed fungal branches host mitochondria and rough endoplasmic reticulum are evident (Fig. 7).

The infected cells display usual organelles with nuclei of different size (from 4 to 8 μm in diameter) (Figs. 9 and 10). Moreover, many plastids containing a dense stroma, lamellae and/or tubules and starch are observed (Fig. 10). Amyloplasts are evident also in close association with the fungal branches, both vacuolated or collapsed (Figs. 5 and 11). Large starch granules are mostly found in the uninfected cells (Fig. 12).

The massive mycorrhizal infection is also detectable on the scanning electron microscope. Most of cortical cells are filled up with fungal arbuscules (Fig. 13). At higher magnification the late stage of the arbuscular deterioration becomes evident owing to the clumped morphological structures. The hyphal trunk giving rise to the whole arbuscule appears still in contact with the host wall (Fig. 14).

Discussion

The results here described, together with the light microscope observations of Fontana *et al.* (in press), show the constant presence in natural conditions of a v.a. mycorrhizal association between the roots of Kober 5 BB grafted with *Vitis vinifera* and a phycomycetous fungus.

At the ultrastructural level, the cytological organization of the endophyte (both in the intercellular hyphae and arbuscules) is quite similar to that already observed

in the interface space; n: fungal nucleus; p: plastid; pl: host plasma membrane; pv: fungal polyvesicular body; r: host endoplasmic reticulum; s: starch; st: septum; v: fungus vacuole.

Liste des abréviations

a: arbuscule; b: bactérie; dg: globule sombre aux électrons; f: hyphe; fc: amas résiduel du champignon; fw: parois du champignon; g: glycogène; h: hôte; hw: parois de l'hôte; hm: mitochondrie de l'hôte; hn: noyau de l'hôte; is: espace intercellulaire; l: globule lipidique du champignon; m: mitochondrie du champignon; mt: couche autour de l'hyphe intracellulaire; n: noyau du champignon; p: plastide; pl: plasmalemme de l'hôte; pv: corps polyvesiculaire du champignon; r: réticulum endoplasmique de l'hôte; s: amidon; st: cloison transversale; v: vacuole du champignon.

in other plants: onion (COX and SANDERS 1974), tobacco (KASPARI 1975), star of Bethlehem (BONFANTE-FASOLO and SCANNERINI, in press), trees as yellow poplar (KINDEN and BROWN 1975 a and b, 1976). Some features of the aseptate fungus are in fact common in all these types of associations; few areas endowed with usual organelles alternate with highly vacuolated areas, often containing dense globules, possibly of polyphosphate nature (COX *et al.* 1975). Moreover, the formation and the deterioration of the arbuscule in the grapevine mycorrhiza, as well as the presence of a space between the fungus wall and the plasma membrane, closely correspond to the characters found in the other associations above quoted. Also the observed bacteria are quite similar to those found in *Ornithogalum umbellatum* roots (SCANNERINI *et al.* 1975). On the contrary, some structural features of the grapevine mycorrhiza are peculiar, and particularly the mass of the fungus is so heavy to fill up the whole host cell. Accordingly, in the infected cell there is no evidence of a massive synthesis of cytoplasm, which is a major host response to the mycorrhizal infection (COX and TINKER 1976). Most of the arbuscules examined are found in late stage of deterioration, but surrounded by active intercellular hyphae, while in the other mycorrhizae studied the most typical picture shows arbuscule branches of different size. This fact could suggest a very short life-span of the arbuscule. Another feature is quite important: Large amyloplasts are found also in those infected cells containing arbuscule branches and clumps. Generally starch is completely absent in the infected cells (KINDEN and BROWN 1975 a and b, GERDEMANN 1975) or plastids are of unusual structure (SCANNERINI and BONFANTE-FASOLO 1977). Only STRULLU (1976) found amyloplasts in the endomycorrhiza of *Taxus baccata*. Thus, in the infected cells of the grapevine roots the presence of a large quantity of starch suggests that only a part of the infected cell sugars is freely used by the endophyte, while another part is stored in the starch granules.

Summary

A morphological analysis using transmission and scanning electron microscopy was carried out about the mycorrhizal roots of grapevine, grown in the field. Only the most common features of the endophyte, i.e. intercellular hyphae and arbuscules, were studied. The intercellular hyphae spreading the infection showed a protoplasm endowed with nuclei, mitochondria, vacuoles with dense globules and bacteria-like microorganisms. After the penetration inside the host cell, many fungal branches were found. The so formed arbuscule filled up the whole cortical cell. The different stages of the arbuscular deterioration were observed and described. The infected host cell showed beyond the usual organelles plastids with starch in close contact with the endophyte.

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