THE ULTRASTRUCTURE OF WALLS IN SOME SPOROCARPIC SPECIES OF *DENSOSPORA, GLOMUS* AND *ENDOGONE*

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Abstract

The ultrastructure of walls of spores and hyphae from *Densospora solicarpa*, *Densospora tubaeformis*, *Glomus cuneatum*, *Glomus fuegianum* and *Youngiomyces aggregatus* were examined in the transmission electron microscope. Particular attention was paid to the surface of the spores and surrounding hyphae. Both spores and hyphae of the two *Densospora* species have a single laminate wall, and three layers or zones based on differences in electron density are apparent. Spores of *Glomus cuneatum* have a single wall similar to *Densospora* but with an electron dense zone in the outer part of the wall. Spores and hyphae of *Glomus fuegianum* have three walls in contrast to *Densospora* and *Glomus cuneatum*. *Endogone aggregata* spores have two walls with the wall of hyphae having a similar appearance to the outer wall of spores. In addition, all five fungi have an extra layer of fibrillar material outside the wall which may function in attachment. In *Densospora solicarpa* a Hartig net and sheath, typical of ectomycorrhiza, were observed in colonised roots from pot cultures.

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Introduction

Densospora is a genus of aseptate fungi with four species, some of which form ectomycorrhizal associations (McGee 1996). Sporocarps from these species have been found in and on soil under undisturbed vegetation at a number of sites in Australia. The taxonomic position of this genus is unclear (McGee 1996).

The number of layers or components in the wall has been used as a taxonomic characteristic in the Glomales and Endogonales (Bonfante & Vian 1984, McGee 1996, McGee & Trappe 2002, Morton & Redecker 2001, Morton *et al.* 1995, Walker 1983, Walker & Vestberg 1998). The wall is usually examined under the light microscope, and this information included in the species description. However, the details of wall structure in the light microscope are not always clear and, on occasions, better detail can be gathered from ultrastructural studies.

The walls of some spores of *Densospora* are comparatively thick. These spores can form trumpet-like extensions (Tandy 1975) though we have only observed them when using an alkaline mordant. The thickened walls and their dramatic expansion are not seen in the walls of spores of *Glomus*, and it indicates a unique attribute of walls differentiating *Densospora* from *Glomus*. Otherwise, the spore walls in *Densospora* appeared in the light microscope to be similar to those in some species of *Glomus* that have a single cell wall (McGee 1996).

Observations of structures seen in the light microscope less than 2 μm thick are unreliable. Ultrastructural studies can be used to verify the wall structure seen in the light microscope and can provide additional information on the composition of walls. Indeed, some of the small hyphae surrounding spores in sporocarps (McGee 1996, McGee & Trappe 2002, Tandy 1975) cannot be easily seen in the light microscope. Use of the electron microscope enables clarification of their structure.

The primary purpose of the present study was to examine the ultrastructure of the walls of spores and hyphae of *Densospora solicarpa* McGee and *D. tubaeformis* (Tandy) McGee in order to augment our understanding of this genus. In particular, we examined the number of layers or laminations, thickness, electron density and outer surface of the cell walls of spores and surrounding hyphae.

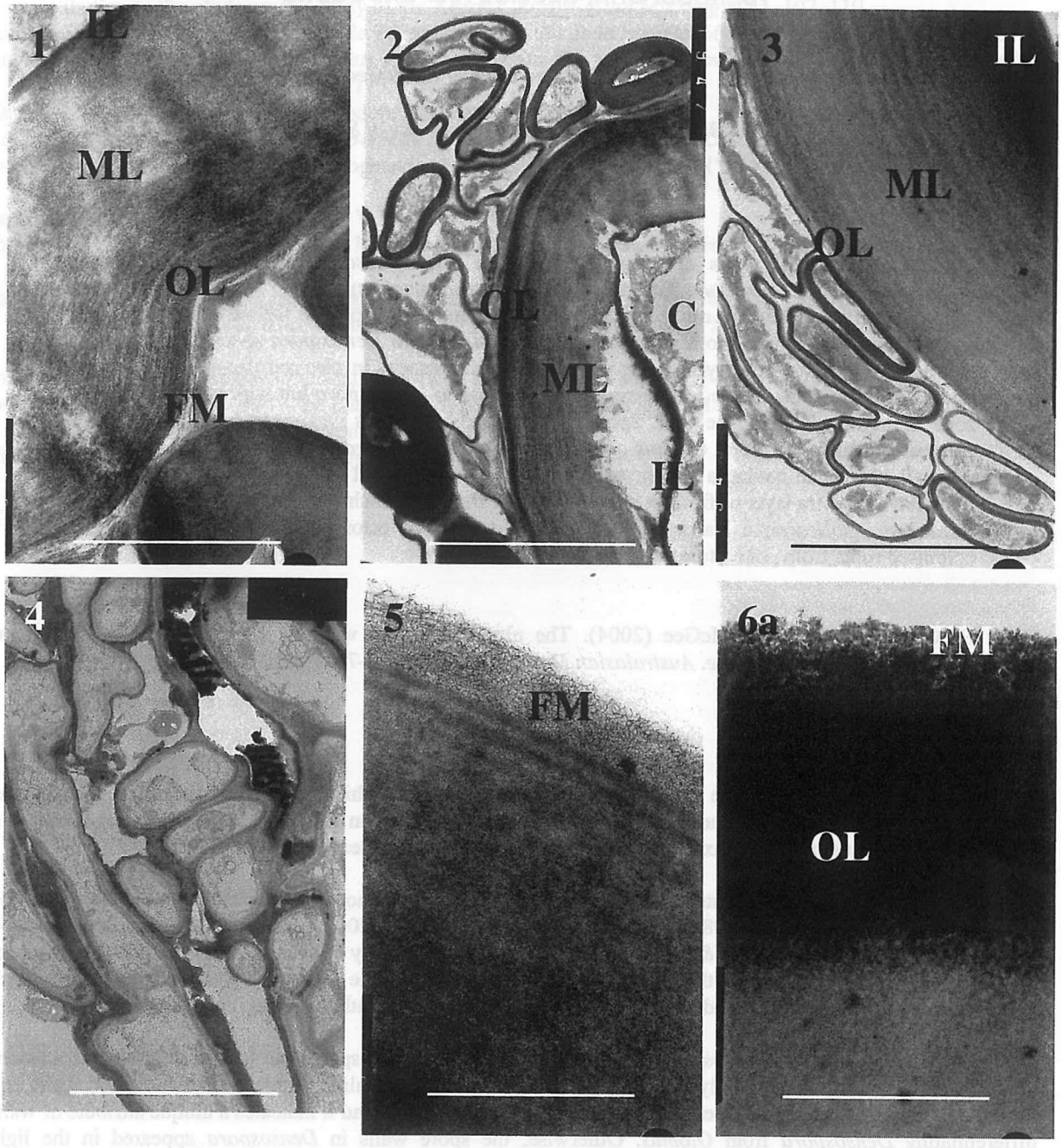
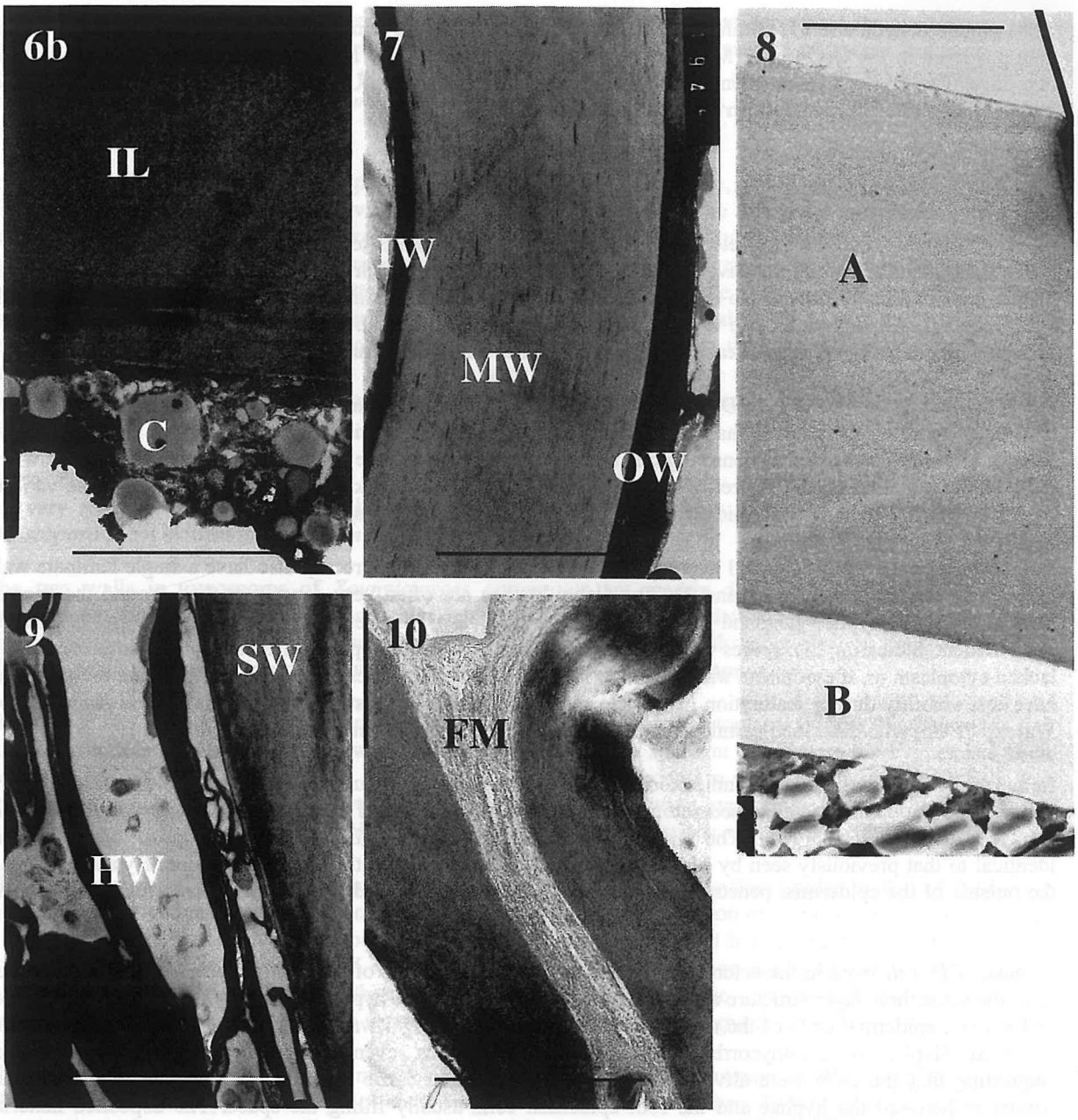


Figure 1. Cross section of the wall of a blastospore of *Densospora solicarpa* showing three layers. Scale bar = 1.2 µm. **Figure 2.** Cross section of a blastospore and glebal hyphae adjacent to a blastospore of *Densospora solicarpa* showing variation in thickness of glebal hypha. Note that the wall layers may separate on sectioning. Scale bar = 2.9 µm. **Figure 3.** Cross section of a blastospore and glebal hyphae of *Densospora solicarpa*. The wall shows variation in electron density. Scale bar = 2.9 µm. **Figure 4.** Ectomycorrhizal hyphae of *Densospora solicarpa*. There is little variation in wall thickness. Scale bar = 5.3 µm. **Figure 5.** Cross section of fibrillar outer layer blastospore of *Densospora tubaeformis*. Scale bar = 1.2 µm. **Figure 6a & b.** Cross section of a blastospore of *Glomus cuneatum*. The outer layer (a) tends to be more electron dense than the inner layer (b). Scale bar = 2.9 µm. **Figure 7.** Cross section of a blastospore of *Glomus fuegianum* showing three distinct walls. Scale bar = 3.5 µm. **Figure 8.** Cross section of a zygospore of *Youngiomyces aggregatus* showing two distinct walls. Scale bar = 4.6 µm. **Figure 9.** Cross section of hyphae tightly packed around the zygospore of *Youngiomyces aggregatus*. Scale bar = 7 µm. **Figure 10.** Fibrillar matrix surrounding the hyphae of *Youngiomyces aggregatus*. Scale bar = 1.2 µm. (Figures 1–6a, p. 74, figures 6b–10, p. 75).

Abbreviations: A. Zygosporangium wall; B. Zygospore wall; C. Cytoplasm; FM. Fibrillar material; IL. Inner layer; ML. Middle layer; OL. Outer layer; IW. Inner wall; MW. Middle wall; OW. Outer wall; HW. Hyphal wall; SW. Spore wall.



Further, we compared the wall ultrastructure of the two species of *Densospora* with other species of sporocarpic aseptate fungi. Since we had sporocarps of *Glomus cuneatum* McGee & Cooper, *Glomus fuegianum* (Spegazzini) Trappe & Gerdemann and *Youngiomyces aggregatus* Y.J. Yao (formerly *Endogone aggregata* Tandy) available, we examined their spores and adhering hyphae.

Materials and Methods

Hypogeous sporocarps of *Densospora solicarpa* McGee, *Densospora tubaeformis* (Tandy) McGee, *Glomus cuneatum* McGee & Cooper, *Glomus fuegianum* (Spegazzini) Trappe & Gerdemann and *Youngiomyces aggregatus* Y.T. Yao (previously called *Endogone aggregata* Tandy) were collected in the field and later fixed with 2.5% glutaraldehyde in 0.2M cacodylate buffer at pH 7.2 overnight at 4°C. After washing with 0.2M cacodylate buffer, the hyphae were postfixed in 1% OsO4 in 0.1M cacodylate buffer for two hours at room temperature. The samples were then washed with distilled water, dehydrated in a series of graded ethanol and finally acetone solutions, and infiltrated and embedded in Spurr's resin. The same procedure was used for fresh roots from a pot culture of *Melaleuca uncinata* R. Br. inoculated with *D. solicarpa* except that they were fixed in

2.5% glutaraldehyde and 2% paraformaldehyde for two days at room temperature and for four days at 4°C and then postfixed in 1% OsO4 in 0.1M cacodylate buffer overnight at 4°C. The resin was polymerized at 60°C for two days. The blocks were sectioned with a diamond knife (Diatome). Ultrathin sections were then examined with a Philips EM400 transmission electron microscope.

Results and Discussion

In the specimens of sporocarps selected for this study, the spore walls tended to be very hard, and there were many sand grains and other abrasive objects within the sporocarps which could not be removed. Therefore, the blocks were extremely difficult to cut into good sections. During sectioning the centre of the spores often fell out. Frequently the diamond knife would jump over hard objects in the blocks causing chatter. It was therefore necessary to cut many sections of each specimen in order to clarify the structure of the cell walls.

All of the spores sectioned from *Densospora solicarpa* have a single laminate wall. The outer and inner layers tend to be more electron dense than the middle layer, but there is considerable variability in electron density (Figure 1). Sometimes electron dense regions were apparent in the middle layer. These layers can separate when cut with the diamond knife (Figure 2). The walls of spores are very thick in comparison to those in the various types of hyphae. However, wall thickness was extremely variable in both spores and hyphae (Figure 3).

In *D. solicarpa* the walls of glebal hyphae appressed to spores in the sporocarp also have a single laminate wall of variable thickness (Figures 2 and 3). The glebal hyphae are often very densely packed around the spores. Several layers of hyphae surround the spores. Spores of *Densospora* retained their cytoplasm and organelles were evident, indicating that spores were potentially viable. The glebal hyphae surrounding the spores commonly lacked cytoplasm or, if cytoplasm was present, it lacked obvious organelles. Thus, it is possible that these hyphae have lost viability during maturation of the spore or storage. The suggestion of spore viability is supported by Warcup (1985) who described germination of single spores of *D. tubiforme* on agar.

In this study we also examined synthesised ectomycorhizae between *Melaleuca uncinata* R. Br. and *Densospora solicarpa* in pot culture. Synthesised ectomycorrhizae have been reported previously in *Densospora tubaeformis* (McGee 1996, Warcup 1985). The structure of the ectomycorrhiza formed by *Densospora solicarpa* was identical to that previously seen by McGee (1996) in *D. tubaeformis* in the light microscope. Hyphae surround the outside of the epidermis, penetrate between the epidermal cells but do not normally enter the cortex of the root.

Hyphae of *D. solicarpa* in the ectomycorrhizal sheath around the roots of *Melaleuca uncinata* from pot cultures have the same three layer structure as seen in spores (Figure 4). These hyphae are mostly on the outer surface of, or between, epidermal cells of the root. Very little variation in fungal wall thickness within the Hartig net was observed. Hyphae of ectomycorrhizae retained their organelles, even though they were densely packed, suggesting that the cells were alive when fixed. Much electron dense material, possibly phenolic, has been deposited between the hyphae and the root epidermal cells usually filling the space. The deposited material appears to seal the outside of the root and may ensure symplastic movement of water and nutrients (Vesk *et al.* 2000).

The pattern of three layers in the single walls of hyphae and spores of *D. solicarpa* was also found seen in *D. tubaeformis* (data not shown). In both species of *Densospora*, most hyphae and spores within the sporocarp had a thin layer of fibrillar material on the outside surface of the wall that appears particularly deep in the junctions between structures. This layer may enable fungal cells to attach to surfaces (Figure 5). Our observations show that spores and all hyphae of both *D. solicarpa* and *D. tubaeformis* have a single laminate wall, consisting of three layers based on differences in electron density. In addition, the enormous variation in thickness of spore walls was evident in hyphae. The similarity of structure of walls of spores and attached hyphae supports the hypothesis that spores form blastically.

Like the two species of *Densospora* examined in this study, *Glomus cuneatum* has a single laminated wall around the spores (Figure 6). In mature spores this wall has two layers. The outer portion appears more electron dense while the inner layer is more electron transparent. The electron density of the outer layer appears to increase in more mature spores, consistent with the dark reddish brown pigment seen in the light microscope (McGee & Trappe 2002). In some spores, a lighter portion appears at the spore surface suggesting that the

increased electron dense portion is due to deposition of material and that the deposition may be uneven. The electron dense outer layer is not seen in hyphal walls. Also, the outside surface of spores has a very thin amorphous surface layer with densely packed subtending hyphae attached to it. This very thin layer of fibrillar material on the outside surface of the wall is found in both hyphae and spores, and is similar to the surface layer of *Densospora*.

In contrast to the previous fungi, the spores of *Glomus fuegianum* have three, clearly differentiated, wall layers (Figure 7) consistent with light observations (McGee & Trappe 2002). The thin outer and inner layers were relatively electron dense, and the thicker middle layer was relatively electron transparent. The structure of the walls also differed from that of *Densospora* and *G. cuneatum*. The outer wall was strongly laminated with laminations tending to separate in a pattern similar to *Densospora*. The middle wall appeared much more diffuse and homogenous. Laminations were thicker and graded one into the next. Laminations of the inner wall appeared to be tightly packed. Glebal hyphae also had three walls. However, in hyphae, the middle layer was much thinner in comparison to the inner and outer layers. The very thin electron dense outer layer in spores of *Glomus fuegianum* was often fused with the outer layer of adjacent spores or the many glebal hyphae that surround spores.

Unlike the two species of *Densospora* and *G. cuneatum*, the electron density in sections of *G. fuegianum* does not vary across a single wall. Also the wide variation in thickness of the wall seen in the two species of *Densospora* is not evident in *G. fuegianum*.

The two walls in zygospores of *Youngiomyces aggregatus* are readily differentiated (Figure 8). In our preparations, the wall of the zygosporangium (outer wall) was thicker, relatively electron dense with broad diffuse laminations. The outer wall also appeared to have a very thin, slightly more electron dense inner edge. The wall of the zygospore (inner wall) was electron transparent with diffuse laminations.

The hyphae that surround the spores were tightly packed and often lacked cytoplasm (Figure 9). All hyphae had one electron dense wall. Laminations were difficult to see and were apparent only where the hyphae had been torn from adjacent structures. Hyphae were almost completely embedded in a less electron dense matrix that appeared to completely fill the gaps between adjacent hyphae and between hyphae and spores. The difficulty of separating spores from surrounding hyphae indicates that the matrix functions as an adhesive (Figure 10).

The ultrastructure of the walls of zygospores of *Youngiomyces aggregatus* was similar to that of *Endogone flammicorona* (Bonfante-Fasolo & Scannerini 1976). Apart from the separation of gametangia in *Y. aggregatus*, a major difference between the two species is the presence of an organized hyphal mantle over the spores of *E. flammicorona* (Yao *et al.* 1996). In *Y. aggregatus*, spores were arranged in an aggregate that was held together by tightly appressed glebal hyphae that lacked any pattern on the spore surface. Further, the walls are undifferentiated, and lack the enormous variation in thickness found in *Densospora*.

The two species of *Densospora* and some species of *Glomus*, such as *G. cuneatum*, have a single laminate wall (Bonfante-Fasolo & Grippiolo 1982, McGee & Trappe 2002, Walker & Vestberg 1998). Apart from the extreme variation in thickness, the walls of hyphae and spores of *Densospora* cannot be easily distinguished from species of *Glomus* that have a single wall. Further comparison of the walls of *Densospora* and other zygosporic aseptate fungi is warranted.

A new fungal phylum, the Glomeromycota (Schussler *et al.* 2001) includes all of the arbuscular mycorrhizal fungi. Although *Densospora* has morphological characteristics similar to *Glomus* and *Endogone*, the phylogenetic relationship of *Densospora* to *Glomus* and *Endogone* is not known at present. This investigation indicates that *Densospora* has a cell wall ultrastructure resembling that of *Glomus* more closely than *Endogone*. Determination of the placement of the genus awaits molecular analysis.

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