

## A new fungal species forming arbuscular mycorrhizas: *Glomus viscosum*

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A species of *Glomus* forming arbuscular mycorrhizas was established in pot culture with several plants. The fungus forms abundant hyaline to white spores in loose clusters. In addition to the normal spore wall which is continuous with the wall of the subtending hypha, the spores of this species produce an apparently mucilaginous outer coating, resulting in the adherence of minute soil particles which were shown to be composed of soil particles by EDAX analysis. The fungus is named *Glomus viscosum* in reference to the sticky outer coating. The spores, germination characteristics, and mycorrhizas are described and illustrated, and the symbiotic behaviour is discussed.

An undescribed species of *Glomus* was found in the substrate of potted magnolia (*Magnolia soulangeana* (Soulang) Bodin var. *nigra*), from a nursery in Pescia, Tuscany, Italy. Spores of the fungus were used to inoculate seedlings of several different plants, including *Trifolium repens* L., *Medicago sativa* L., *Fragaria vesca* L., *Allium porrum* L., *Pisum sativum* L. and *Plantago lanceolata* L. Arbuscular mycorrhizas were successfully established with all these hosts. The species is described and named *Glomus viscosum* sp. nov.

The appearance of the spores was affected by an apparent outer covering of opaque material which at first was thought to be of fungal origin. This layer was studied histologically and with energy dispersive X-ray microanalysis (EDAX) to ascertain its true origins.

The terms 'wall' and 'wall layer' have been used interchangeably in species descriptions of members of the Glomales. The true nature of the spore wall structure in this species is readily observed, so we have employed a more accurate use of these terms in this paper.

### MATERIALS AND METHODS

The potted plants consisting of rooted apical cuttings of *M. soulangeana* were purchased from Sonnoli Nurseries, Uzzano, Pistoia, Italy as 30–40 cm tall plants. The potting substrate consisted of a mixture of peat imported from either Russia or Germany (the nursery owner could not be certain which particular batch had been used in this instance) and beech (*Fagus sylvatica* L.) leaf-mould of local origin. No greater detail of substrate origin could be ascertained.

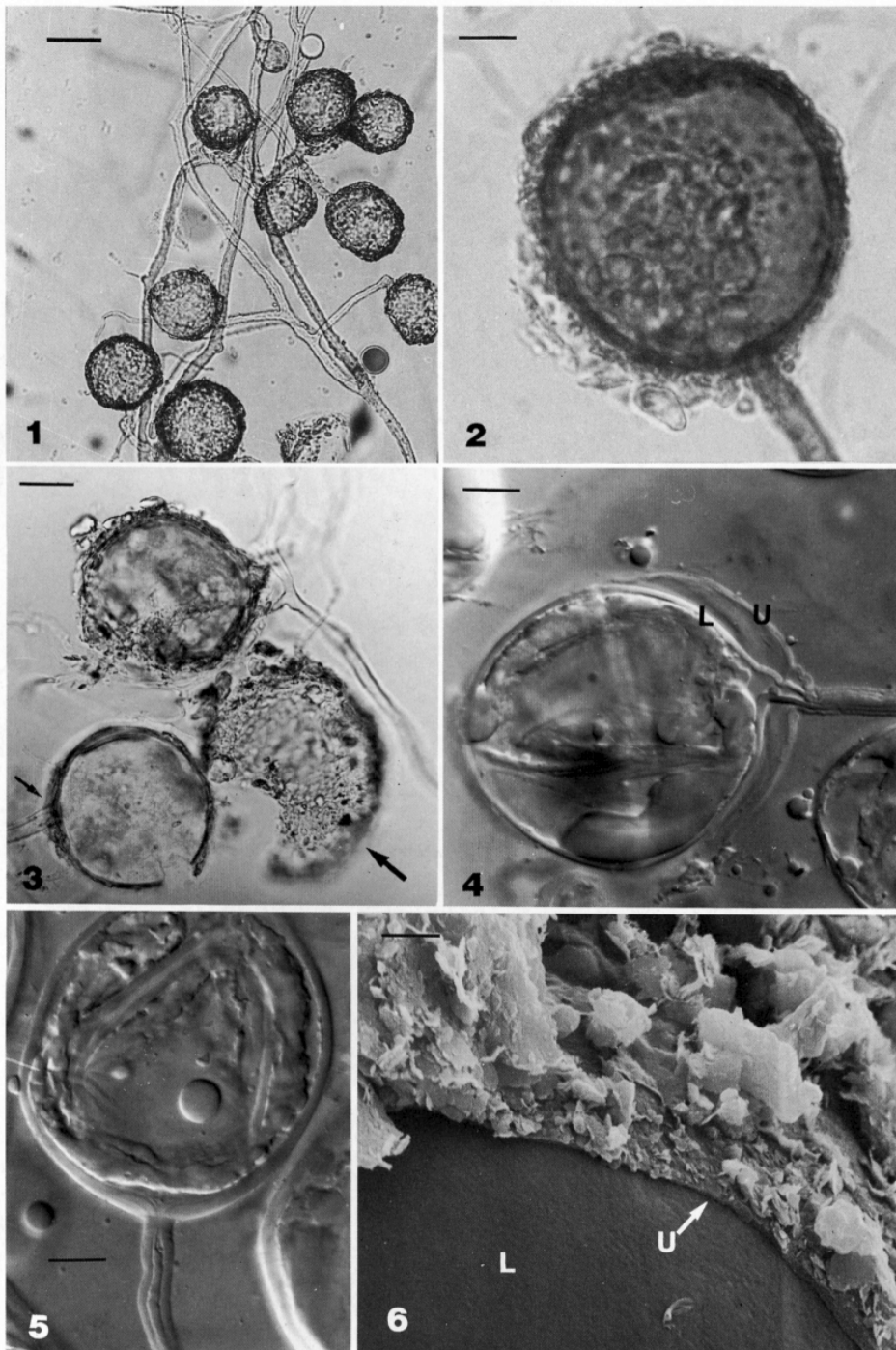
For isolation, clusters of spores extracted by wet-sieving were used to inoculate the roots of seedlings which were subsequently potted in autoclaved (1 h at 120 °C) coarse sand. These were then maintained in open pot culture (Gilmore, 1968) until mycorrhizas and spores were produced.

Spores for further study were produced in pot cultures made by germinating surface-sterilized seeds of *M. sativa* or *T. repens*, germinated in sterile sand mixed (1:4, v/v) with substrate from an existing stock culture. The plants were grown in a glasshouse (day/night temperature 21°/10°; max. light intensity 100  $\mu\text{E m}^{-2} \text{s}^{-1}$ ), watered daily with tap-water, and fertilized weekly with 50 ml of quarter-strength Hoagland's solution. Alternatively, pre-germinated seedlings of *P. lanceolata* were planted into a small quantity of pot culture substrate containing spores, mycelium and colonized roots and placed centrally in a 9 cm pot of sterile sand. The pots were then placed in plant growth bags and maintained in a growth chamber (Walker & Vestberg, 1994).

Germination was examined from spores placed between filter membranes buried in wet, sterilized sand and incubated in the dark at 20°. Germination was monitored under a dissecting microscope, and germinated spores were transferred to microscope slides for study at higher magnification under a compound microscope.

To study the colonization characteristics of the fungus, seedlings of *Helianthus annuus* L. were placed between Millipore membrane filters embedded in sterile sand and gently removed after 2 wk (Giovannetti & Citeresi, 1993). At sequential harvests, mycelial growth and appressorium formation could be observed by opening the sandwich and staining with 0.05% trypan blue in lactic acid. After 1 month, the characteristics of intraradical colonization were observed following clearing and staining the roots (Phillips & Hayman,

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**Figs 1–6.** Photomicrographs (brightfield illumination unless otherwise stated) of *Glomus viscosum*. **Fig. 1.** A loose cluster of mature spores (bar, 50  $\mu\text{m}$ ). The spores, with their roughened appearance, are firmly attached to a coarse coenocytic mycelium. **Fig. 2.** A specimen in the early stage of development of the viscous mucigel-like outer coating. Fine soil particles are attached and incorporated (bar, 25  $\mu\text{m}$ ). **Fig. 3.** Two spores, one of which has been manipulated to remove the outer wall layer and its attached particles (large arrow), leaving the smooth main structural spore wall layer still attached to the subtending hypha (small arrow) (bar, 25  $\mu\text{m}$ ). **Fig. 4.** A spore, photographed with Nomarski differential interference contrast (DIC) enhancement, treated with bleach to remove the mucigel-like covering and its soil particles. The smooth, unit wall layer (U) and the laminated main structural wall layer (L) are indicated (bar, 25  $\mu\text{m}$ ). **Fig. 5.** DIC image illustrating the wall thickening beginning to occlude the pore between the persistent subtending hypha and the spore contents (bar, 25  $\mu\text{m}$ ). **Fig. 6.** SEM illustrating the outer surface of the main structural wall layer (L), the outer unit wall layer (arrowed, U), and the mucigel-like layer with adherent and incorporated soil particles (bar, 5  $\mu\text{m}$ ).

≤ 1 µm crassum; stratum interius e laminis compositum, 1–3 µm crassum, concolor cum exteriore. Hyphae subtendentes 8–11 µm diametro, tunicae 1·8–2·3 µm crassae. In maturis chlamydo-sporis exterius stratum mucilaginem exsudans, cui solum multum adherat. Hypha sustinens alba ad sporae basim 8–10 µm diametro. Formans arbusculares mycorrhizae.

Holotypus PI-HMZ 10: Isotypus E.

Spores borne in loose clusters in the soil, on abundant coarse, thick walled hyphae: hyaline to white, often covered by an adherent layer of fine soil particles: globose to subglobose, 44–97 × 46–94 µm (mean 60 × 58 µm,  $n = 100$ , s.d. 3·25).

Spore wall structure of a single wall (wall group *sensu* Walker, 1983) of two layers (walls *sensu* Walker) continuous with the wall layers of subtending hypha. Outermost wall layer up to 1 µm thick, lacking evident laminations (a unit wall *sensu* Walker), often difficult to discern under the light microscope. Innermost layer laminated, 1–3 µm thick, constituting the main structural spore wall layer (a laminated wall *sensu* Walker). With age, the outermost layer exuding a mucigel-like substance with attached and incorporated minute soil particles, resulting in an apparent third wall layer, up to 10 µm thick.

Subtending hypha single, straight, concolorous with spore wall, remaining attached to spores even after wet sieving, 8–11 µm diam., thickening internally with age, and exuding a sticky substance to which soil particles may be attached. Not morphologically distinguishable from the extra-matrical hyphae which form a coarse, coenocytic or very sparsely septate mycelium.

Spores mostly lacking occlusion but sometimes occluded by spore wall thickening at the spore base. Germination by development of a germ-tube from the broken end of a subtending hypha after production of a balloon-like swelling. Multiple germ-tubes and germination directly through the spore wall not observed.

Forming mycorrhizas with coarse arbuscules and hyphal coils. Neither vesicles nor spores observed within roots.

*Type:* consisting of spores on microscope slides, and spores and mycorrhizas in 5% formaldehyde solution and in 0·025% sodium azide solution, from an arbuscular mycorrhizal pot culture with *M. sativa*. *Holotype:* Herbarium Horti Botanici Pisani (PI). *Isotype:* Royal Botanic Garden, Edinburgh (E). *Type culture* registered with the Banque Européenne des Glomales as BEG 27, maintained in a pot culture of *M. sativa* in the greenhouse of the Institute of Agricultural Microbiology, University of Pisa, Italy (culture number IMA 4), and with *P. lanceolata* at the Forestry Authority's Northern Research Station, Roslin, U.K.

**Distribution, habitat and mycorrhizal associations.** Precise origins and distribution unknown, because the substrate, from which it was isolated was a mixture from Italy and either Russia or Germany. Recovered from a potted plant of *M. soulangeana* and producing arbuscular mycorrhizas in pot culture with *A. porrum*, *F. vesca*, *H. annuus*, *Malus communis* L., *M. sativa*, *Prunus domesticus* L., *Prunus persica* (L.) Batsch, *P. sativum*, *T. repens*, and *P. lanceolata*. The species has been in continuous pot culture since 1984.

The species differs from most other members of the genus by the possession of hyaline to white spores. *Glomus albidum* C. Walker & L. H. Rhodes, *Glomus claroideum* N. C. Schenck &

G. S. Sm., *Glomus clarum* T. H. Nicolson & N. C. Schenck, *Glomus laccatum* Blaszk, *Glomus diaphanum* J. B. Morton & C. Walker, *Glomus leptotichum* N. C. Schenck & G. S. Sm., and *Glomus occultum* C. Walker also have spores which may be hyaline or white. However, *G. albidum*, *G. diaphanum* and *G. claroideum* each have spores with a separable, flexible inner wall formed after initial spore development (Walker & Rhodes, 1981; Schenck & Smith, 1982; Morton & Walker, 1984). This wall is not continuous with a layer of the subtending hypha in these species. No such wall exists in spores of *G. viscosum*. The spores of *G. clarum* (Nicolson & Schenck, 1979) have, at maturity, a coloured, laminated inner wall layer. *G. occultum* and *G. viscosum* have spores with a similar wall structure and with overlapping size ranges (Walker, 1982), but the subtending hypha of the former is much less prominent, being thinner, shorter, and less persistent than that of the latter, resulting in spores of *G. occultum* usually becoming detached, rather than in clusters as in *G. viscosum*. In addition, the outer wall layer of *G. occultum*, while being of a similar mucilaginous nature to that of *G. viscosum*, is much less prominently developed, and its spores are more angular in shape. *G. leptotichum* spores are much larger than those of *G. viscosum*, and they become yellow with age (Schenck & Smith, 1982), and the chlamydo-sporis of *G. laccatum* do not possess a mucilaginous outer layer and have a much more distinctly laminated structural wall layer than those of *G. viscosum*.

## RESULTS AND DISCUSSION

**Spore development.** The use of the terms 'wall' and 'wall layer' have been discussed by several authors (Berch, 1986; Walker, 1992; Franke & Morton, 1994; Rosendahl, Dodd & Walker, 1994). In many fungi, the true nature of the spore wall structure is not known. In such circumstances, there is as yet no reason to abandon the arbitrary definitions of Walker (1983) and subsequent workers (see Morton, 1988), but in *G. viscosum* the wall development is readily observable, and we have followed the lead of Franke & Morton (1994), who introduced an ontogenetic analysis of wall structure development.

Chlamydo-sporis of *G. viscosum* develop by the blastic production of a globose to subglobose transparent spore with a 2-layered wall, continuous with that of both the somatic and subtending (sporogenous) hypha, and an open pore. A mucilage-like substance, presumably a polysaccharide, is then produced. Soil particles then become attached to, and embedded within, this substance, resulting in a rough, opaque, outer coating. As the spore ages, thin, additional layers are deposited internally, considerably thickening the laminated wall layer. The extra-matrical hyphae also become thickened in this way and can be extremely coarse and thick-walled. The majority of spores appear to lack any form of occlusion, but where closure occurs, it is by a simple differential thickening of the wall ('spore wall thickening' *sensu* Gerdemann & Trappe, 1974) (Fig. 5). Although these spores may be termed chlamydo-sporis, they are not separated from their sporogenous hyphae except by thickening of the wall and they perhaps should be given another name to distinguish them

from chlamydospores *sensu strictu* which are formed from a cell and which possess a secondary wall (Hawksworth, Sutton & Ainsworth, 1983).

**Spore germination.** No spore dormancy was observed in *G. viscosum*. Germination of more than 90% of spores occurred within 9 d. Germination took place directly through the subtending hypha (Fig. 7), a mode of germination found in some other *Glomus* species. The germ-tube forms a balloon-shaped swelling (8–10 µm diam.) at the germination point, before continuing growth. Germination characteristics may have some utility in separating groupings of species currently placed within *Glomus* (Walker, 1982).

**Mycorrhizas.** Some authors have proposed consideration of mycorrhizal morphology, as well as intraradical fungal development, for the taxonomic differentiation of AM endophytes (Abbott & Robson, 1979; Abbott, 1982). However, such criteria have not been widely applied, although recently, Walker *et al.* (1993) included a description of mycorrhizas in the protologue of an arbuscular mycorrhizal fungus, *Scutellospora castanea* C. Walker. In the plants tested to date, no vesicle has been found, the colonization consisting only of hyphal coils and coarse arbuscules (Figs 8–10). Several species of *Glomus* have been reported to form such 'atypical' mycorrhizas (Schenck & Smith, 1982; Miller & Walker, 1986). The presence or absence of intraradical vesicles is one of the characters used to separate the suborder Glomineae from the suborder Gigasporineae (Morton & Benny, 1990), and some doubts as to the value of this as a supra-generic characteristic must be expressed if this feature is inconsistent.

**Histochemical investigations.** At first it was thought that the fine particles attached to the outer surface of the spores were of fungal origin, but the histochemical investigations indicated otherwise. The PARS test indicated the presence of polysaccharides in the spore wall layers, and probably in the outer mucigel-like coating, but the fine plate-like particles produced no reaction. After staining with Coomassie brilliant blue, the spore stained blue, indicating the presence of proteins, but neither the matrix nor the particles stained. The Uvitex was absorbed by all wall layers, shown by a blue fluorescence under uv light, but again the particles did not fluoresce. Similarly, a violet stain, indicating the presence of chitin, appeared in the wall layers after treatment with KOH and potassium iodide, but was lacking in the particles.

Coomassie brilliant blue stain and PARS test demonstrated that the particles in the outer mucilagnous layer did not consist of proteins or polysaccharides. The use of optical brighteners has proved to be a valuable aid in understanding the structures of higher fungi (Romero & Minter, 1988). The use of such fluorochromes has been proposed recently in the study of AM and other fungi (Giovannetti *et al.*, 1991; Nicholas, Williams & Hunter, 1994). In this work Uvitex stain, which binds to polysaccharides with  $\beta$ -linkages (Hughes & McCully, 1975), was absorbed to some extent by the outer mucigel-like layer, but not by the embedded and attached particles, showing that these were not constructed of polysaccharides such as chitin.

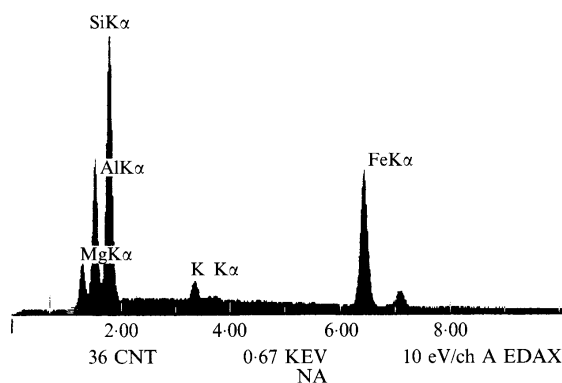


Fig. 11. X-ray energy spectrum from the surface of a *Glomus viscosum* spore.

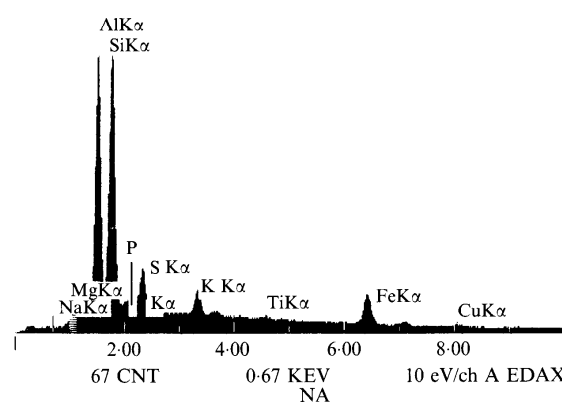


Fig. 12. X-ray energy spectrum from a *Glomus viscosum* hyphal surface.

**EDAX analysis.** The EDAX analysis of several points on the surface of both spores and hyphae gave similar spectra characterized by the constant detection of silicon. Other elements were also detected such as Al, Fe, Mg and K. Two typical spectra are shown in Figs 11 and 12, taken from spores and from hyphal surfaces. The analysis thus revealed the composition of the attached particles. The occurrence of Si, Al, Mg, Fe and K indicated that they are composed mainly of minerals of the mica and chlorite groups. The percentage of Fe observed in our results was higher than that expected for chlorites; this value may be explained by the occurrence of Fe-oxides and Fe-hydroxides, commonly associated with silicates in the soil (Guillet & Souchier, 1982). The data revealed an elemental composition of the particles similar to that of soil clay minerals. When treated with strong sodium hypochlorite solution (domestic bleach) (approx. 3.5% NaOCl), the wall layers remained intact, but the mucigel-like layer dissolved to leave a deposit of mineral particles. The conclusion drawn from this and both the histochemical and EDAX analysis is that the particles are not of fungal origin, but come directly from the soil. The same conclusion was drawn from a similar investigation of the outer surface layer of spores of *Glomus dimorphicum* Boyetchko & J. P. Tewari (1986).

All the data discussed up until now indicate that particles of the clay fraction of the soil may be adsorbed to the external wall of *G. viscosum*, although the mechanism by which this adhesion occurs is not known. We can only speculate that polysaccharides with adhesive qualities are involved. The adhesion of clay particles to fungal surfaces has been reported for some soilborne fungi. Electron microscope studies showed that the hyphae of *Gaeumannomyces graminis* (Sacc.) Arx & Olivier were surrounded by clay particles and the potential importance of clays in the interaction between *G. graminis* and its antagonists has been hypothesized (Campbell, 1983). Reisinger *et al.* (1977) gave direct experimental evidence confirming that the clay particles covering the hyphae and spores of *Beauveria bassiana* (Bals.-Criv.) Vuill. could exert a protective action on the fungal structures by reducing the rate of lysis caused by bacteria. We can hypothesize that, also for *G. viscosum*, the clay flakes surrounding the hyphae and the spores might play a role in the protection of the organism against antagonistic micro-organisms. Moreover, the great surface of intimate contact between the mineral soil particles and the extramatrical hyphae of the endophyte might account for the high efficiency of this isolate (M. Giovannetti, unpublished data). Such a beneficial effect of mucigel has been discussed in relation to plant roots by Uren (1993). The more extensive exploration of soil by hyphae than by roots would also suggest that adhesive material of this sort may have an important role in soil aggregation.

**Symbiotic behaviour.** When germinated spores of *G. viscosum* encountered the roots of the sunflower, appressoria formed within 42 h. Intraradical penetration occurred within 50 h, resulting in the formation of hyphal coils penetrating the cortical cell walls. Whereas the diameter of intraradical hyphae ranged from 3.4 to 4.5  $\mu\text{m}$ , the penetrating hyphae had a notably larger diameter, ranging from 9.2 to 10.3  $\mu\text{m}$ . The number of appressoria formed on the roots of the host plant 3 wk after germination was 4  $\text{mm}^{-1}$  of root length. Different forms of appressoria were observed, from irregular to lenticular (18.4–23  $\times$  6.9–13.8  $\mu\text{m}$ ) to papillate (32.2–85.1  $\times$  13.8–16.1  $\mu\text{m}$ ).

The symbiotic behaviour of an AM fungal isolate depends on its ability to colonize the roots of a host plant rapidly and extensively. The first factor influencing fungal infectivity is the dormancy of spores, which occurs in some species of AM fungi and which can negatively influence infection, delaying the formation of germinative hyphae and appressoria (Tommerup, 1983). Unlike other *Glomus* species *G. viscosum* spores germinated promptly in wet filter membranes, exhibiting no spore dormancy. In this study we measured the time required by *G. viscosum* to form appressoria on the roots of a host plant, together with their number and shape. This characteristic, which is a key factor in the symbiotic behaviour of each mycobiont, might represent another important feature to consider when characterizing species and isolates of arbuscular mycorrhizal fungi.

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## REFERENCES

- Abbott, L. K. (1982). Comparative anatomy of vesicular–arbuscular mycorrhizas formed on subterranean clover. *Australian Journal of Botany* **30**, 485–499.
- Abbott, L. K. & Robson, A. D. (1979). A quantitative study of spores and anatomy of mycorrhizas formed by a species of *Glomus*, with reference to its anatomy. *Australian Journal of Botany* **27**, 363–375.
- Berch, S. M. (1986). Endogonaceae: taxonomy, specificity, fossil record, phylogeny. *Frontiers in Applied Mycology* **2**, 161–188.
- Boyetchko, S. M. & Tewari, J. P. (1986). A new species of *Glomus* (Endogonaceae, Zygomycotina) mycorrhizal with barley from Alberta. *Canadian Journal of Botany* **64**, 90–95.
- Campbell, R. (1983). Ultrastructural studies of *Gaeumannomyces graminis* in the water films on wheat roots and the effect of clay on the interaction between this fungus and antagonistic bacteria. *Canadian Journal of Microbiology* **29**, 39–45.
- Franke, M. & Morton, J. B. (1994). Ontogenetic comparisons of arbuscular mycorrhizal fungi *Scutellospora heterogama* and *Scutellospora pellucida*: revision of taxonomic character concepts, species descriptions, and phylogenetic hypotheses. *Canadian Journal of Botany* **72**, 122–134.
- Gahan, P. B. (1984). *Plant Histochemistry and Cytochemistry*. Academic Press: London, U.K.
- Gerdemann, J. W. & Trappe, J. M. (1974). The Endogonaceae in the Pacific Northwest. *Mycologia Memoir* **5**, 1–76.
- Gilmore, A. E. (1968). Phycomycetous mycorrhizal organisms collected by open-pot culture methods. *Hilgardia* **39**, 87–105.
- Giovannetti, M., Avio, L. & Salutini, L. (1991). Morphological, cytochemical, and ontogenetic characteristics of a new species of vesicular–arbuscular mycorrhizal fungus. *Canadian Journal of Botany* **69**, 161–167.
- Giovannetti, M. & Citernes, A. S. (1993). Time-course of appressorium formation on host plants by arbuscular mycorrhizal fungi. *Mycological Research* **97**, 1140–1142.
- Gillet, B. & Souchier, B. (1982). Amorphous and crystalline oxyhydroxides and oxides in soils (iron, aluminium, manganese, silicon). In *Constituents and Properties of Soils* (ed. M. Bonneau & B. Souchier), pp. 21–42. Academic Press: London, U.K.
- Hawksworth, D. L., Sutton, B. C. & Ainsworth, G. C. (1983). *Ainsworth & Bisby's Dictionary of the Fungi (Including the Lichens)*, 7th ed. Commonwealth Mycological Institute: Kew, U.K.
- Hughes, J. & McCully, M. E. (1975). The use of optical brighteners in the study of plant structure. *Stain Technology* **50**, 319–329.
- Miller, D. D. & Walker, C. (1986). *Glomus maculosum* sp. nov. (Endogonaceae): an endomycorrhizal fungus. *Mycotaxon* **25**, 217–227.
- Morton, J. B. (1988). Taxonomy of VA mycorrhizal fungi: classification, nomenclature, and identification. *Mycotaxon* **32**, 267–324.
- Morton, J. B. & Benny, G. L. (1990). Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): a new order, Glomales, two new suborders, Glomineae and Gigasporineae, and two new families, Acaulosporaceae and Gigasporaceae, with an emendation of Glomaceae. *Mycotaxon* **37**, 471–491.
- Morton, J. B. & Walker, C. (1984). *Glomus diaphanum*: a new species in the Endogonaceae common in West Virginia. *Mycotaxon* **21**, 431–440.
- Nicholas, R. O., Williams, D. W. & Hunter, P. A. (1994). Investigations of the value of  $\beta$ -glucan-specific fluorochromes for predicting the  $\beta$ -glucan content of the cell walls of zoopathogenic fungi. *Mycological Research* **98**, 694–698.
- Nicolson, T. H. & Schenck, N. C. (1979). Endogonaceous mycorrhizal endophytes in Florida. *Mycologia* **71**, 178–198.
- Omar, M. B., Bolland, L. & Heather, W. A. (1979). A permanent mounting medium for fungi. *Bulletin of the British Mycological Society* **13**, 13–32.
- Phillips, J. M. & Hayman, D. S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55**, 158–161.

- Reisinger, O., Fargues, J., Robert, P. & Arnold, M.-F. (1977). Effet de l'argile sur la conservation des microorganismes. I. Etude ultrastructurale de la biodégradation dans le sol de l'hyphomycète entomopathogène *Beauveria bassiana* (Bals.) Vuill. *Annales de Microbiologie (Institut Pasteur)* **128 B**, 271–287.
- Romero, A. I. & Minter, D. W. (1988). Fluorescence microscopy: an aid to the elucidation of ascomycete structures. *Transactions of the British Mycological Society* **90**, 457–470.
- Rosendahl, S., Dodd, J. C. & Walker, C. (1994). Taxonomy and phylogeny of the Glomales. In *The Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems* (ed. S. Gianinazzi & H. Schüepp), pp. 1–12. Birkhäuser Press: Basel, Switzerland.
- Schenck, N. C. & Smith, G. S. (1982). Additional new and unreported species of mycorrhizal fungi (Endogonaceae) from Florida. *Mycologia* **74**, 77–92.
- Spain, J. L. (1990). Arguments for diagnoses based on unaltered wall structures. *Mycotaxon* **38**, 71–76.
- Tommerup, I. C. (1983). Spore dormancy in vesicular–arbuscular mycorrhizal fungi. *Transactions of the British Mycological Society* **81**, 37–45.
- Uren, N. C. (1993). Mucilage secretion and its interaction with soil, and contact reduction. *Plant and Soil* **155/156**, 79–82.
- Walker, C. (1982). Species in the Endogonaceae: a new species (*Glomus occultum*) and a new combination (*Glomus geosporum*). *Mycotaxon* **15**, 49–61.
- Walker, C. (1983). Taxonomic concepts in the Endogonaceae: spore wall characteristics in species descriptions. *Mycotaxon* **18**, 443–455.
- Walker, C. (1992). Systematics and taxonomy of the arbuscular endomycorrhizal fungi (Glomales) – a possible way forward. *Agronomie* **12**, 887–897.
- Walker, C., Gianinazzi-Pearson, V. & Marion-Espinasse, H. (1993). *Scutellospora castanea*, a newly described arbuscular mycorrhizal species. *Cryptogamie, Mycologie* **14**, 279–286.
- Walker, C. & Rhodes, L. H. (1981). *Glomus albidus*: a new species in the Endogonaceae. *Mycotaxon* **12**, 509–514.
- Walker, C. & Vestberg, M. (1994). A simple and inexpensive method for producing and maintaining closed pot cultures of arbuscular mycorrhizal fungi. *Agricultural Science in Finland* **3**, 233–240.

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