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ENDOGONACEOUS MYCORRHIZAL ENDOPHYTES IN FLORIDA[1]

T. H. NICOLSON [2] AND N. C. SCHENCK

Plant Pathology Department, University of Florida, Gainesville, Florida 32611

SUMMARY

In Florida 21 species of Endogonaceae forming vesicular-arbuscular mycorrhizae are reported. Eight species of the genus *Glomus*, including a new species *G. clarus*, and three species of *Sclerocystis* make up the chlamydosporic species found in Florida. Seven species of *Gigaspora*, including four new species, *G. nigra*, *G. pellucida*, *G. rosea*, and *G. gregaria*, and three species of *Acaulospora*, including one new species, *A. gerdemannii*, make up the azygosporic species found. Ten species are reported from Florida for the first time.

Gerdemann and Trappe in their revision of the family Endogonaceae (Zygomycetes, Mucorales) recognized seven genera (7). Four of these genera, i.e., *Glomus, Gigaspora, Acaulospora* and *Sclerocystis*, contain species that formed vesicular-arbuscular (VA) mycorrhizae on the roots of plants. Several species within these four genera have been reported from Florida on soybean (14), citrus (10) and Everglades flora (9). The areas of Florida represented by these surveys encompass most of the state since soybeans are mainly grown in north Florida, citrus predominates in central Florida, and the Everglades are in south Florida. The purpose of this paper is to summarize the reports of VA-mycorrhizal fungi in Florida and to describe six new species of VA-mycorrhizal fungi.

MATERIALS AND METHODS

Rhizosphere-soil samples, 0–15 cm depth, were sieved for spores using the procedure of Gerdemann and Nicolson (6). Spores and detritus were collected on 40, 60, and 170-mesh sieves (375 μm, 250 μm, 90 μm sieve openings, respectively) and added to tap water in 9-cm

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Petri plates for examination under a dissecting microscope (× 20–70). Spores of mycorrhizal fungi were removed from the detritus by means of microspatulas or pipettes, transferred to Ringer's solution (**4**) in small vials and subsequently added to autoclaved field soil for establishing pot cultures. Plants used for mycorrhizal hosts in these pot cultures consisted of either sorghum (*Sorghum halepense* (L.) Pers.), bahia grass (*Paspalum notatum* Flugge) or soybean (*Glycine max* (L.) Merr.)

In addition, some rhizosphere-soil samples were placed in autoclaved 15-cm pots and planted with soybean seed. At plant maturity, the soil was sampled, wet sieved, and examined for spores of mycorrhizal fungi. Roots of pot-culture and field-grown plants were gently washed in tap water, cleared in hot 10% KOH, and stained in lactophenol containing trypan blue in a manner similar to that described by Phillips and Hayman (**13**). Stained roots were examined for internal structures of VA-mycorrhizal fungi under a microscope (× 40–150).

Holotypes of all new species described in this paper have been deposited at the Oregon State University Herbarium (OSC), Corvallis, Oregon. Isotypes have been deposited at the Farlow Herbarium (FH) of Harvard University, Cambridge, Massachusetts and the University of Florida Herbarium (FLAS), Gainesville.

CHLAMYDOSPORIC SPECIES

Chlamydospores of species in the genus *Glomus* and *Sclerocystis* are not recovered in sievings of rhizosphere soil in Florida as frequently as spores of azygosporic species. However, they may be more common in Florida than their recovery from rhizosphere soil would indicate. Cleared and stained roots containing vesicles typical of those formed by species of *Glomus* and *Sclerocystis* have frequently been observed in Florida yet few or no chlamydospores were recovered in soil sievings from around these same roots. When soybeans have been planted in such soil in pots in the greenhouse, chlamydospores are frequently recovered from the soil. In general, spores of *Glomus* species are recovered more frequently than spores of *Sclerocystis* species.

The genus Glomus.—Eight species of the genus *Glomus* have been found in Florida. They occur geographically throughout the state and have been observed to some degree in all areas in Florida examined for VA-mycorrhizal fungi. A listing of the species observed in Florida is given below.

GLOMUS MOSSEAE (Nicol. & Gerd.) Gerd. & Trappe. (7)

Glomus mosseae has been found rarely in Florida on both soybean and citrus (10). On soybean, spores of *G. mosseae* have never been recovered directly from the field. However, spores of *G. mosseae* have been recovered in the greenhouse from soybean grown in soil obtained from a field previously planted to soybean. Although both ectocarpic spores and spores within sporocarps have been recovered, ectocarpic spores predominate. Ectocarpic spores of *G. mosseae* are globose to irregular in shape, pale yellow in color, usually with typical funnel-shaped subtending hyphae. Pot cultures established on bahia grass from these spores have resulted in typical *G. mosseae* mycorrhizae and spores.

GLOMUS MACROCARPUS Tul. & Tul. var. MACROCARPUS (7)

Glomus macrocarpus var. *macrocarpus* is perhaps the most common and widely distributed species of *Glomus* in Florida. It has been found in soybean-rhizosphere soil in several locations in north and central Florida, in several citrus nurseries and groves in central Florida and in several sites in the Everglades (9). Spores are produced singly in soil and have never been observed in sporocarps. Spores vary exceedingly in size but most commonly measure 75–150 μm in diam. Spores are white when immature, becoming yellow to dark brown with maturity. Spore numbers in rhizosphere soil are usually low, 1–10 spores/50 ml of soil, compared to spore numbers of other VA-mycorrhizal fungi in Florida.

Two main forms of *G. macrocarpus* var. *macrocarpus* occur in Florida. The most common type forms thick, laminate walls (5.0–12.0 μm diam) which become dark brown in color at maturity. The other variant forms thinner walls (3.6–5.0 μm diam) which remain yellow to light brown at maturity. Both forms have been maintained in pot cultures of bahia grass for several years and each remains distinct. Because of the variability within *G. macrocarpus* as described by Nicolson and Gerdemann (11) and by Gerdemann and Trappe (7), there seems little justification or value in describing a new variety of *G. macrocarpus* at this time.

GLOMUS MACROCARPUS var. GEOSPORUS (Nicol. & Gerd.) Gerd. & Trappe (7)

This variety of *G. macrocarpus* has been found in north Florida from rhizosphere-soil samples of soybean, grape (*Vitis labrusca* L.) and Bahia grass and in south Florida from the Everglades. Spores are dark

brown to black with thick walls (10–12 μm) and occur singly or in clusters of two to three spores. Although it has been found at several locations, spore numbers have been few on each occasion and several attempts to establish pot cultures on bahia grass or soybean have thus far failed.

GLOMUS FASCICULATUS (Thaxter sensu Gerd.) Gerd. & Trappe. (7)

Glomus fasciculatus was collected from soybean (14), bahia grass and citrus (10) in Florida. Spores are formed free in the soil or in dense sporocarps several mm long around plant roots. Spores of this species are found only occasionally in rhizosphere-soil sievings, and it has been difficult to establish in pot cultures on soybean or bahia grass. *Glomus fasciculatus* is believed to be more common in Florida than sieved-spore numbers would indicate. Cleared and stained plant roots have been observed with chlamydospores within the cortex, similar in size and shape to those of *G. fasciculatus,* but no spores were recovered in rhizosphere-soil sievings from the same plant.

GLOMUS ETUNICATUS Becker & Gerd. (2)

This species was first observed in Florida by Nemec on citrus and was referred to as *G. caledonius* (Nicol. & Gerd.) Trappe & Gerd. (10). Its occurrence on citrus is common but it has not been recorded on other crop plants in Florida. Chlamydospores of this species have two walls. The outer wall is ephemeral and readily sloughs off; the species can be distinguished from other *Glomus* species occurring in Florida by this characteristic. However, mature spores of *G. etunicatus* without the outer wall could be confused with other *Glomus* species, especially *G. macrocarpus.*

GLOMUS MICROCARPUS Tul. & Tul. (7)

This small-spored species has been recovered rarely from rhizosphere soil in Florida. Chlamydospores are formed in loose clusters in pot culture on tobacco (*Nicotiana tabacum* L. 'Hicks') and no sporocarps have been observed. Spore diam ranged from 20 to 35 μm; spore walls were thin and light yellow in color.

GLOMUS TENUIS (Greenall) Hall (8)

The mycelium of this fungus was noted on one occasion from roots of bahia grass from Okeechobee, Florida. The mycelium of *G. tenuis*

stains deeply with trypan blue on cleared and stained roots. Hyphae are narrow (1.5–3.0 μm diam), irregularly shaped, with hyphal swellings (5.0–10.0 μm). Hyphal coils are common within root cortical cells and arbuscules are abundant. Although no spores were observed, *G. tenuis* can be identified by its typical narrow hyphae with short lateral projections and the stranding of hyphae in the cells into "ropes."

GLOMUS FULVUS (Berk & Broome) Gerd. & Trappe (7)

Glomus fulvus was collected in south Florida (Dade County) by Rolf Singer at several locations. Specimens deposited in the Farlow Herbarium (Numbers 1150, 1312, 1357, 1420) by Singer and by Thaxter (No. 5055) were examined. Sporocarps are light brown to ochraceous, variable in size and shape, ovate to pulvinate, 3 × 3 mm to 12 × 20 mm; chlamydospores are globose to obovate, 55–115 × 43–75 μm; spore walls are 2.0–4.5 μm thick; the hyphal attachment at the spore is 4.8–12.0 μm thick.

Glomus clarus Nicolson & Schenck, sp. nov. FIG. 2d-g

Chlamydosporae singulatim vel in fasciculis parvis hypogaeis, et plerumque intra radicem formatae, rumpentes aetate corticem; sporae hyalinae, globosae vel subglobosae 68–290 μm diametro, pro maxima parte ± 190 μm diametro; materia sporae hyalina, constans e globulis magnitudinis variabilis; tunicae sporae complexae, hyalinae, eatate lutescentes; tunicae 7–31 μm diametro constantes e tunica interiore (2–9 μm) et exteriore (5–20 μm) haud facilitier separabilibus; tunica interior aliquot (2–5) stratorum 0.5–2.5 μm latorum; nonnullae sporae tunica hyalina et mucilagina (0.5–2.0 μm) quae eatate verrucosa vel rugosa plicis usque ad 5 μm altitudine fit; hyphae subtendentes 15–80 μm latae tunicis crassis (7–39 μm) extensis usque ad 400 μm infra sporam, leviter tenuioribus distantia a spora crescente. Orificium pori ad chlamydosporam 3–5 μm latum septo protuberante in poro secendente materiam sporae ab hyphis subtendentibus. Formans vesicular-arbusculares mycorrhizas.

Chlamydospores formed singly or in small clusters in the soil, also commonly formed within the root, rupturing the cortex with age; spores hyaline, globose to subglobose, 68–290 μm in diam, mostly ± 190 μm; spore contents hyaline, consisting of globules of variable size; spore walls complex, hyaline, becoming yellow with age; walls 7–31 μm in diam consisting of an inner (2–9 μm) and outer wall (5–20 μm) that do not separate readily; inner wall of several (two to five) layers of 0.5–2.0 μm in width; some spores with a hyaline outer mucilaginous coat (0.5–2.0 μm) which with age becomes verrucose or rugose with folds up to 5 μm in height; subtending hypha 15–80 μm wide with thick walls (7–39 μm) extending up to 400 μm below the spore, becoming slightly thinner with increasing distance from the spore. Pore opening to the chlamydospore 3–5 μm in width with a bulging septum in the pore

separating the spore contents from the subtending hyphae. Forming vesicular-arbuscular mycorrhizae.

Distribution and mycorrhizal associations: from roots of peanut (*Arachis hypogea* L.) near Marianna, Florida, soybean roots at Gainesville, Florida and bahia-grass roots at Ona and Okeechobee, Florida. Maintained in pot culture on soybean and bahia grass.

Etymology: Latin, *clarus* (clear, transparent) referring to the hyaline spore wall.

Type: from soil on the Agronomy Farm, University of Florida collected July, 1975 (Schenck 05) and maintained in pot culture on bahia grass (OSC; isotypes, FH and FLAS).

Glomus clarus can be separated readily from other described species of *Glomus* by its thick, hyaline spore walls and large spore size.

The genus Sclerocystis.—Three species have been observed in Florida. Species of this genus are not commonly recovered in sievings of rhizosphere soil in Florida but they may be more common than their recovery from soil would indicate. Chlamydospores of *Sclerocystis* species have been most frequently recovered from rhizosphere soil in north and central Florida.

SCLEROCYSTIS COREMIOIDES Berk & Broome. (7)

This species has been found most frequently in north Florida. In some locations (Gainesville), numerous sporocarps have been sieved regularly from soil on a coarse sieve (375-μm openings). The older sporocarps found in rhizosphere soil are dark brown to black in appearance, with soil impregnating the outer layers of the peridium. These sporocarps have the appearance of round sclerotia but reveal the compact, ellipsoid chlamydospores when crushed.

SCLEROCYSTIS SINUOSA Gerd. & Bakshi (**5**)

Sporocarps of this species have been found around, or on, roots of citrus, soybean and bahia grass in central Florida on several occasions. In addition, sporocarps have been observed forming within the roots of citrus and bahia grass. Spores have been seen in cleared roots forming around a central plexus of hyphae without a peridium. As the sporocarp develops it crushes the cortical cells eventually filling the cortex on one side of the root. The sporocarp sometimes distends the root but more frequently attains a maximum diam equal to the diam of the root

where it is formed. Later a peridium of sinuous hyphae becomes apparent. This is believed to be the first report of this phenomenon.

This species is readily identified in microscope-slide mounts (× 100–450) by the thick sinuous hyphae that enclose the sporocarp. The sporocarps are similar in size and appearance to *S. coremioides* and can be confused with this species except for the sinuous hyphae of *S. sinuosa* which cover the sporocarp. Pot cultures of *S. sinuosa* have been maintained on bahia grass for several years.

SCLEROCYSTIS RUBIFORMIS Gerd. & Trappe. (7)

This species has been observed only once from Florida on a sample of pangola digitgrass (*Digitaria decumbens* Stent.) from the Agricultural Research Center at Ona, Florida. Sporocarps of *S. rubiformis* are readily recognizable by their "blackberry"-like appearance. Chlamydospore measurements are within the range listed by Gerdemann and Trappe (7).

AZYGOSPORIC SPECIES

Spores of the genera *Gigaspora* and *Acaulospora* are the most frequently recovered of the mycorrhizal fungi in rhizosphere soil in Florida. Most species occur in north Florida in greatest abundance but some occur throughout the state. Spores of azygosporic species from soil were generally collected on a medium sieve (250-μm sieve openings) since they were larger in diam than the chlamydosporic species which are usually recovered in a fine sieve (90-μm sieve opening).

The genus Gigaspora.—The species in this genus can be separated into two groups, those with dark-colored spores and those with light-colored spores. All species of *Gigaspora* have been observed to form only arbuscles in roots (no vesicles) in pot culture on soybean, sorghum or bahia grass. They all have external accessory vesicles that are formed on hyphae outside the root either in clusters or singly. Seven species of *Gigaspora* have been found in Florida and are listed below.

Dark-spored species:

In Florida, isolates of a *Gigaspora* species that resembled *Gigaspora coralloidea* Trappe, Gerd. & Ho in external appearance of the azygospores were found to differ in several characteristics originally described for this species (7). These difference were considered sufficient to justify the description of a new species.

Gigaspora gregaria Schenck & Nicolson, sp. nov. FIG. 1b

Azygosporae hypogaeae singulatim formatae, globosae, 250–448 μm diametro, rubri-brunneae, eatate fuscantes; projecturae irregulariter formatae 1–7 × 3–12 μm continae super superficiem sporae; tunica sporae 11–15 μm crassa includens membranam 1–2 μm; tunica composita e duabus partibus haud faciliter separabilibus sed aspectabilibus in partibus tunicarum fractarum; tunica exterior arto-

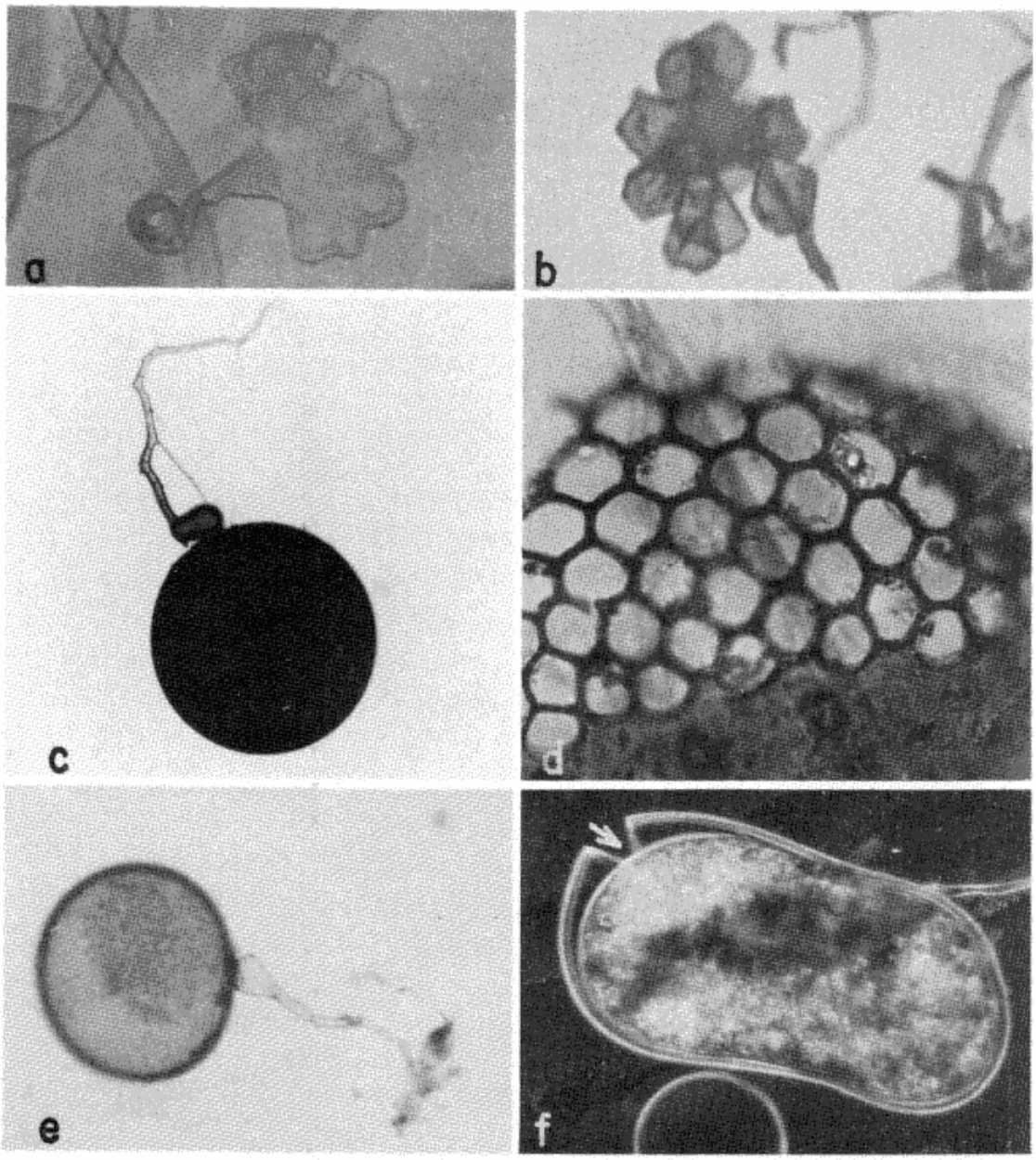

FIG. 1. Species of *Gigaspora*. a. *G. coralloidea*, typical soil-borne vesicle from a type specimen obtained from J. M. Trappe, × 400; b. *G. gregaria*, typical clustered vesicles, × 250; c, d. *G. nigra:* c. azygospore with attached suspensor and hyphae, × 70; d. reticulation of the outer wall, × 400; e, f. *G. pellucida:* e. azygospore with suspensor and attached hyphae, × 150; f. broken azygospore showing inner and outer wall separated (arrow), dark field microscopy photograph, × 150.

brunnea, 6–9 μm crassa projecturis inclusis; tunica interior 5–7 μm crassa, subbrunnea, hyalina. Colligatio hyphae suspensori similis 39–80 μm lata, bulbosa, tunicis subbrunneis usque ad 4 μm crassis. Vesiculae terrestres, globosae vel subglobosae, fasciculis trium ad sedecim, 14–31 × 22–48 μm, plerumque tuberculatae, projecturis variantibus ab 0.5–2.4 × 1.2–4.8(–9.6) μm sed haud ramosis vel coralloideis; vesiculae exorientes ex hyphis circinatis vel tortis, subbrunneis, tenuiter tunicatis, affixis ad hyphas atrobrunneas; tunicae vesicularum ± 1.0 μm crassae.

Azygospores formed singly in soil, globose, 250–448 μm diam, red brown becoming dark brown with maturity; irregular shaped projections 1–7 × 3–12 μm continuous over the spore surface; spore wall 11–15 μm thick enclosing a membrane 1–2 μm; wall consisting of two parts not readily separable but apparent on portions of fractured walls; outer wall dark brown, 6–9 μm thick including projections; inner wall 5–7 μm thick, light brown, and transparent. Suspensor-like hyphal attachment 39–80 μm wide, bulbous, walls light brown, up to 4 μm thick. Soil-borne vesicles globose to subglobose, in clusters of 3–16, 14–31 × 22–48 μm, usually tuberculate, with projections ranging from 0.5–2.4 × 1.2–4.8(–9.6) μm but never becoming branched or coralloid; vesicles arising from coiled or twisted light-brown, thin-walled hyphae attached to dark-brown hyphae; vesicle walls ± 1.0 μm thick.

Distribution and mycorrhizal associations: *Gigaspora gregaria* has been collected from soybean-rhizosphere soil regularly in northwest Florida and occasionally in central Florida. Azygospores are found regularly in northwest Florida associated with soybeans on newly cleared land. This species has been maintained in pot culture for several years on sorghum, bahia grass, soybean, or pepper (*Capsicum annuum* L.).

Etymology: Latin, *gregaria* (in clusters) referring to the soil-borne vesicles which occur in clusters.

Type: from soybean-rhizosphere soil at the Agricultral Research Center near Jay, Florida. September, 1972 (Schenck 04); (OSC, isotypes, FH, FLAS) Maintained in pot culture on bahia grass.

Gigaspora gregaria can be separated from *G. coralloidea*, which it resembles most closely, by its soil-borne vesicles which occur in clusters (not singly as in *G. coralloidea*, FIG. 1a) with the vesicles having a knobby surface with mostly short projections which do not become branched or coralloid, and by its tightly fused, two-layered azygospore wall; the outer wall with projections and dark brown and the inner wall light brown in color and transparent.

GIGASPORA HETEROGAMA (Nicol. & Gerd.) Gerd. & Trappe (7)

This species is widespread in Florida and is the most common dark-spored *Gigaspora* species in the state. Although it has been recovered

from most areas, it is most common in north and central Florida. Azygospores are tan to light brown, globose, 160–260 μm diam, usually with a laterally attached suspensor-like cell 29 × 29 μm to 39 × 58 μm. Spore walls are 5–10 μm thick, consisting of two fused walls, surrounding a membrane (1–2 μm thick) that is quite durable and persists after spore breakage. The inner and outer walls are apparent on broken spores; the inner wall is light brown and transparent, 3–5 μm thick; the outer wall is dark brown, (3–5 μm thick), with minute projections 0.5–2.0 μm high. Laminations within the walls, 0.5–1.0 μm, are apparent on some spores. Vesicles are formed in clusters of four to nine on coiled dark-brown hyphae exterior to the root, are smooth to knobby, 17 × 19 to 29 × 43 μm. Spores of *G. heterogama* resemble those of *G. coralloidea* but are generally smaller, lighter brown, have a lateral attachment to the suspensor-like cell and frequently have a white outer "coat" on the spore wall visible with reflected light under a dissecting microscope. The apparent white outer "coat" was not observed in water or lactophenol-slide preparations when examining the wall for details. The apparent "coat" must be the result of light reflections from the numerous small projections on the outer spore wall. Without the suspensor-like cell attachment on the azygospore, spores of *G. heterogama* can be confused with spores of *Acaulospora laevis*.

Gigaspora nigra J. F. Redhead sp. nov. FIG. 1c, d

Azygosporae singulatim in terra formatae, atrobrunneae velatrae sphaericae, 297–555(–1050) μm diametro in medis 402 μm, tunica interiore et exteriore. Tunica exterior atra vel atrobrunnea alveolata poris maioribus, 7–10 μm diametro, subjectis poris minoribus compositis e seriebus spirarum; tunica interior subbrunnea, hyalina, aliquot sed continuarum laminarum. Colligatio hyphae suspensorii similis, brunnea, lateraliter affixa, 40–60 × 80–120 μm saepe producens hypham brevem extensam ad tunicam sporae. Accessoriae vesiculae terrestres, atrobrunneae, globosae (21–36 μm diametro) vel subglobosae, laeves vel nodosae projecturis 2.5–7.0 μm altis, fasciculis plerumque arctis trium ad duodecim in hyphis circinatis vel tortis exorientibus ex hyphis rectis 4.8–9.6 μm latis. Germinatio sporae tubis germationis uno ad multos iuxta partem colligationis sporae; tube germationis exorientes e cellulis peripheralibus et preformatis intra sporam.

Azygospores formed singly in the soil, dark brown to black, spherical, 297–500(–1050) μm diam (mean 402 μm) with an inner and outer wall. Outer wall black to dark brown, pitted with larger pores, 7–10 μm diam, overlaying smaller pores consisting of a series of coils; inner wall light brown, transparent, of several laminae but continuous. Suspensor-like hyphal attachment brown, attached laterally, 40–60 × 80–120 μm, often producing a peg-like hypha extending to the spore wall. Accessory soil-borne vesicles dark brown, globose (21–36 μm diam) to subglobose, smooth to knobby with projections 2.5–7.0 μm in height, in usually

tight clusters of 3 to 12 on coiled or twisted hyphae arising from straight hyphae 4.8–9.6 μm in width. Spore germination with one to many germ tubes near the attachment region of the spore; germ tubes arising from preformed peripheral compartments within the spore. Forming arbuscular mycorrhizae.

Distribution: sieved from soil samples from three different areas in Nigeria (12) and associated with diverse flora. In Florida, collected from rhizosphere samples of soybean (*Glycine max*) in Escambia County and at Live Oak. Mycorrhizal associations: established in pot culture on *Nauclea diderrichii* (Nigerian isolates) and on soybean and bahia grass (Florida isolates). Arbuscules only noted in the roots of mycorrhizal plants.

Type: Florida isolate from Escambia County, September, 1970 (Schenck 01); established on bahia-grass pot culture (OSC; isotypes FH, FLAS).

Etymology: Latin, *nigra* (black, shiny), referring to the outward appearance of the azygospores.

Gigaspora nigra can be readily separated from other dark-spored species of *Gigaspora* by its large black spores with pores in the outer wall. White spores occasionally appear in pot cultures of *G. nigra* intermixed with characteristic black spores. White spores reach the same dimensions as black spores and have the same wall characteristics. For these reasons they are not considered immature forms of *G. nigra*; but all attempts to establish mycorrhizae with these "mutant" spores have failed. For a detailed account of spore characteristics of the Nigerian isolate see the paper of Old et al. (12).

Light-spored species:

The light-colored spores of *Gigaspora* species are the most numerous mycorrhizal spores collected in rhizosphere soil in Florida. They occur in samples from extreme north Florida to extreme south Florida Everglades and the Keys. Spores of these species are most frequently collected from rhizosphere-soil samples in the 250-μm sieve using the wet-sieving technique. Four species of light-spored *Gigaspora* have been found in Florida.

GIGASPORA MARGARITA Becker & Hall (3)

This most common species in Florida is particularly numerous in cultivated sandy soil around the roots of agronomic crops and citrus.

Azygospores are formed singly in the soil, 255–385 μm in diam, white turning brown with age, and borne on swollen hyphal tips (a suspensor-like structure) 24–58 μm in diam. Vesicles with spines are formed in the soil on coiled hyphae in clusters of 4 to 12.

Two variants of *Gigaspora margarita* have been found in Florida. Both produce white-colored azygospores singly in the soil and have echinulate, soil-borne vesicles in clusters on coiled hyphae. One variant produces chalk-white azygospores (325–405 μm diam) with a white suspensor-like cell (40–58 μm diam) and white subtending hyphae. Another variant produces a translucent-white azygospore, 212–285 μm diam with a clear suspensor-like cell (28–39 μm diam). Both fall within the range of characteristics described for *G. margarita* and intergrade with it in appearance. Both have been maintained intermittently in pot culture on bahia grass and soybean and appear to maintain their characteristic color and shape. Because of their similarity to *G. margarita* it seems undesirable to describe them as separate variants or strains of *G. margarita* until further distinguishing characteristics can be determined.

GIGASPORA GIGANTEA (Nicol. & Gerd.) Gerd. & Trappe (7)

This species is found primarily in north Florida, particularly in the rhizosphere of soybean on newly cleared land. It does not seem to persist over 2 or 3 yr in cultivated soils. It is readily recognizable by its large (290–460 μm diam) greenish-yellow spores and clusters of echinulate vesicles produced in rhizosphere soil. A form of this species found on pasture grasses in north-central Florida has been maintained in pot culture for several years and has cream-yellow-colored azygospores. It resembles in appearance a cross between *G. margarita* and *G. gigantea* but has never given rise to spores completely resembling either *G. margarita* or *G. gigantea*. Azygospores of this "form" are produced singly in the soil, 220–350 μm in diam (285 μm average). Soil-borne vesicles are echinulate on coiled hyphae in clusters of 5 to 11. Additional studies will be necessary to determine if this "form" is sufficently different from either *G. margarita* or *G. gigantea* to describe as a new taxon.

Gigaspora pellucida Nicolson & Schenck, sp. nov. FIG. 1e, f, 2c

Azygosporae singulatim hypogaeae formatae, globosae vel irregulares. Sporae globosae 58–212 μm diametro; sporae difformes 107–183 × 145–241(–328) μm. Tunica sporae laevis, 6–12 μm crassa, composita e duobus tunicis separabilibus, exteriore fragili, 3–8 μm crassa, duarum vel trium laminarum; interiore flexili

globulis aetate provecta coalescentibus. Suspensorium hyalinum, 10–29 μm diametro, hyphis subtendentibus et hyalinis, 7–12 μm latis; tunicae hypharum 0.5–1.0 μm crassae, septus plurivus in hyphis sub suspensorio. Vesiculae terrestres, brunneae, singulatim vel in fasciculis ad quatuordecim repertae, in hyphis fuscis et circinatis; vesiculae obpyriformes vel irregulares, 19–38 μm latae, nodosae projecturis pro parte maxime 5 × 10 μm sed variantibus ab 2–9 × 5–15 μm. Formans mycorrhizas arbusculares.

Azygospores formed singly in the soil, globose or irregular. Globose spores 58–212 μm diam; irregularly shaped spores 107–183 × 145–241(–328) μm. Spore wall smooth, 6–12 μm thick, composed of two separable walls, the outer wall brittle, 3–8 μm thick, of two to three laminae; the inner wall pliable 1.5–5.0 μm thick. Walls and spore contents hyaline. Spore contents of small globules that coalesce with age. Suspensor-like cell hyaline, 10–29 diam, subtending hyphae hyaline, 7–12 μm wide, hyphal walls 0.5–1.0 μm thick, with several septa in the hyphae below the suspensor cell. Soil-borne vesicles brown, occurring singly or in clusters up to 14, on brown coiled hyphae; vesicles obpyriform to irregular in shape, 19–38 μm wide, knobby with projections mostly 5 × 10 μm but varying from 2–9 × 5–15 μm. Forming arbuscular mycorrhizae.

Distribution: Found in cultivated soils in north and central Florida. Found throughout the year from rhizosphere soil. Mycorrhizal association: forming mycorrhizae on soybean and bahia grass in pot culture.

Etymology: Latin, *pellucida* (clear or transparent), referring to the hyaline azygospore walls, contents and the subtending hyphae.

Gigaspora pellucida can be separated from other light-spored *Gigaspora* species by its hyaline spore walls and contents, and its double spore wall, the outer one of which is brittle and easily separable from a more pliable inner wall.

Type: from soybean-rhizosphere soil in Jay, Florida, October, 1972 (Schenck 02); maintained on bahia-grass pot culture in a greenhouse (OSC; isotypes FH, FLAS).

Gigaspora rosea Nicolson & Schenck, sp. nov. FIG. 2a, b

Azygosporae singulatim hypogaeae formatae, plerumque globosae, 230–305 μm diametro, interdum subglobosae, albae vel cremeae, colore roseolo in tunica azygosporae iuxta colligationem hyphae tegente usque ad dimidiam sporam. Color roseolus varians a roseo-roseolus ad minime aspectabile. Crassities tunicate azygosporae 2.4–7.5 μm, duobus ad quinque stratis inseparabilibus 1–2 μm crassis. Stratum tunicae exterioris laeve. Colligatio ad azygosporam similis suspensorii aliquando sphaerica 28–40 μm diametro, interdum subglobosa; hyphis subtendentibus 7–14 μm latis, tunicis hypharum 1–2 μm crassis, septatis. Vesiculae terrestres fasciculis quinque ad duodecim in hyphis circinatis; vesiculae singulae 19–32 μm latae, echinatae spinis 5.0 μm longis et 2.5 μm latis. Formans mycorrhizas arbusculares.

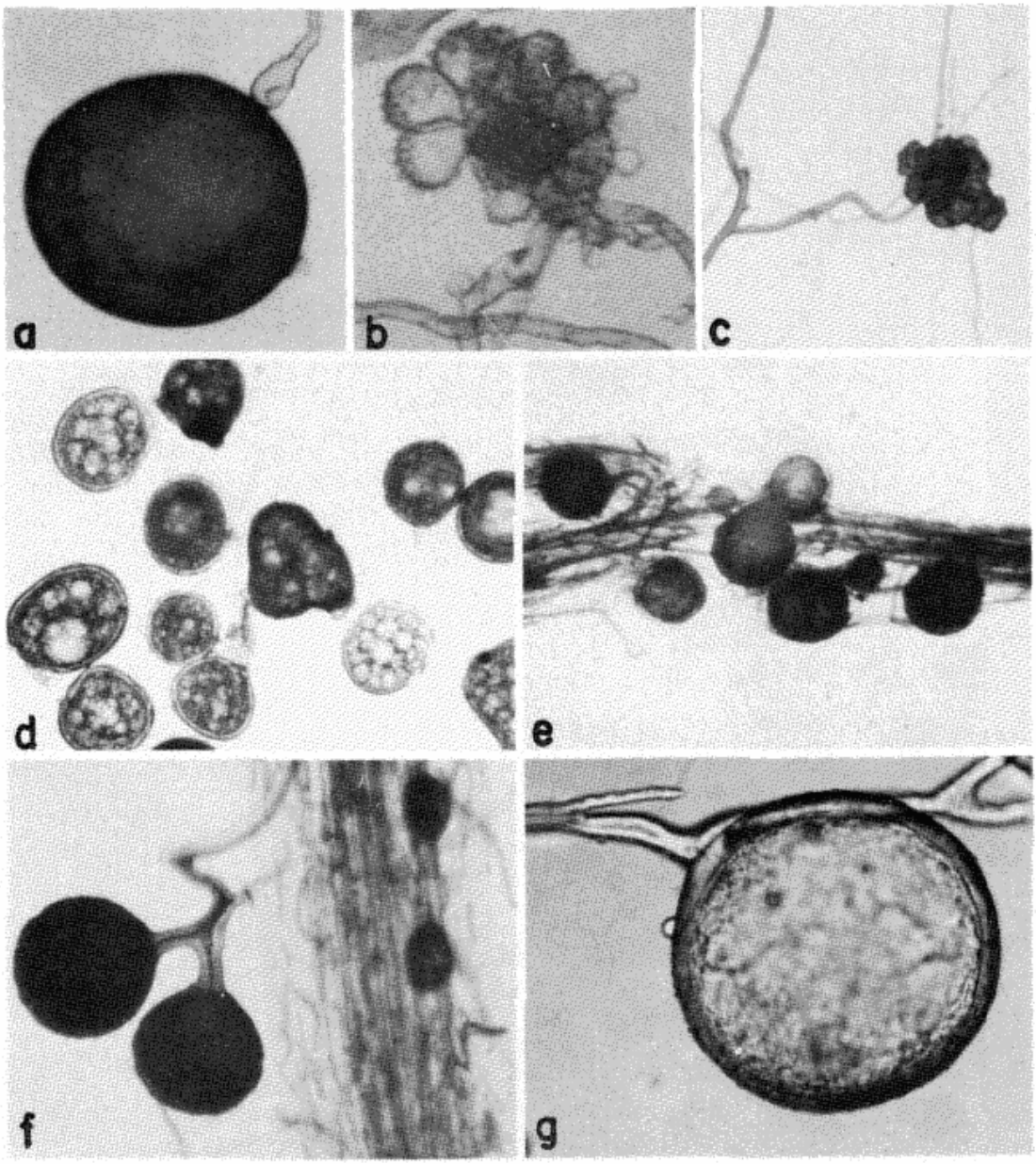

FIG. 2 a-c. Species of *Gigaspora*. a, b. *G. rosea:* a. azygospore and attached hyphae, × 100; b, soil-borne-vesicle cluster, × 250; c. *G. pellucida:* vesicle cluster, × 100. FIG. 2 d-g. *Glomus clarus:* d. chlamydospores, × 100; e. chlamydospores formed in the root cortex; spores and root stained with trypan blue, × 50; f. stained root containing two vesicles within the root and two chlamydospores, outer wall on the chlamydospores deeply stained, × 250; g. chlamydospore showing the thick wall, × 400.

Azygospores produced singly in soil, predominantly globose, 230–305 μm diam, occasionally subglobose, white to cream in color with a rose-pink tint on the azygospore wall near the hyphal attachment encompassing up to half the spore. Pink coloration variable from distinctly rose pink to barely detectable. Azygospore wall thickness 2.4–

7.5 μm, with two to five inseparable layers 1–2 μm thick. Outer wall layer smooth. Suspensor-like cell attachment to azygospore usually spherical 28–40 μm diam, occasionally subglobose; subtending hyphae 7–14 μm wide, hyphal walls 1–2 μm thick, septate. Soil-borne vesicles in clusters of 5 to 12 on coiled hyphae, individual vesicles 19–32 μm wide, echinulate with spines up to 5.0 μm long and 2.5 μm wide. Forming mycorrhizae with arbuscules.

Distribution: found occasionally in soybean-rhizosphere soil from north Florida. Observed only in the summer months but probably occurs year round. Mycorrhizal association: forming mycorrhizae on soybean and bahia grass in pot cultures.

Etymology: Latin, *rosea* (pink, rose colored), referring to the pink tint associated with azygospores of this species.

Type: originally from soybean-rhizosphere soil in Escambia County, Florida, September, 1972 (Schenck 03); maintained on bahia-grass pot culture in a greenhouse (OSC, isotypes FH, FLAS).

Gigaspora rosea can be distinguished from other light-spored species of *Gigaspora* by its rose-pink tint associated with the wall of the azygospore near the hyphal attachment. Its thin azygospore wall and generally smaller spore diam separate it from *G. margarita* which it resembles most closely. Its clusters of echinulate soil-borne vesicles distinguish it from *G. calospora* (Nicol. & Gerd.) Gerd. & Trappe, its white to cream appearance distinguish it from *G. pellucida,* and its nonseparable wall and smaller spore size separate it from *G. gilmorei* Trappe & Gerd.

The genus Acaulospora.—Three species of *Acaulospora* have been observed in Florida. Species of *Acaulospora* are found from north Florida to the south Florida Everglades. Their occurrence is common and in some soils they are the only mycorrhizal species found. The spores are produced as azygospores, as in *Gigaspora,* but the azygospores are formed laterally on swollen vesicles.

ACAULOSPORA LAEVIS Gerdemann & Trappe (7)

This species is by far the most common *Acaulospora* species found in Florida. It has been recovered from soybean-rhizosphere soil in numerous locations in north Florida, from grasses and citrus-rhizosphere soil in central Florida, and from a 17-yr-old woodland in the Everglades (9). Azygospores are borne laterally and singly in the soil on swollen hyphae ending in a vesicle. These spores become detached easily from the vesicles on which they are borne and are usually sieved from soil devoid of any hyphal attachments. They can be recognized by their

honey-brown color, globular contents and uniform size. Isolates of *A. laevis* from Florida have been maintained in pot culture on bahia grass and soybean where they form typical vesicular-arbuscular mycorrhizae.

ACAULOSPORA TRAPPEI Ames & Linderman (1)

This species has been recovered twice from grasses in central Florida, from rhizosphere soil of soybean, peanut, corn (*Zea mays* L.) and cotton (*Gossypium hirsutum* L.) in northwest Florida, and occurs commonly in tobacco (*Nicotiana tabacum* L.) rhizosphere soil in north-central Florida. Azygospores of *A. trappei* are small, colorless, and are usually recovered as spores without hyphal attachments. Pot cultures of *A. trappei* have been established on bahia grass where the fungus forms vesicular-arbuscular mycorrhizae.

Acaulospora gerdemannii Schenck & Nicolson, sp. nov. FIG. 3a-d

Sporae singulatim hypogeae formatae, sessiles, in hypha 35–45 μm diametro lateraliter portatae, terminantes in vesicula sphaerica vel ellipsoidea. Vesiculae 290–365 μm diametro tunicis brunneis 10–12 μm crassis; vesiculae collabentes et post maturitatem sporae discedentes a spora. Sporae sphaericae 200–250 μm diametro, materia globulari et tunicis duplicibus. Tunica exterior 1.0–1.5 μm crassa, brunnea, cerebriformibus plicis 10–12 μm altis; tunica exterior aetate fragilescens; tunica interior 1.0–1.5 μm crassa, flexilis, hyalina, superficie ornata reticulo alveolato. Alveoli 7.5–10 μm lati. In veterioribus sporis tunica exterior faciliter discedens a tunica interiore. Hyphae infra sporae colligationem 10–12 μm crassae, saepe producentes multas hyphae subtiliter ramosas. Formans vesiculas nonlobatas in vesicular-arbuscularibus mycorrhizis.

Spores formed singly in the soil, sessile, borne laterally on a hypha 35–45 μm diam terminating in a spherical to ellipsoid vesicle. Vesicles 290–365 μm diam with brown walls 10–12 μm thick; vesicles collapsing and detaching from the spore after spore maturity. Spores spherical, 200–250 μm diam, with globular contents and double walls. Outer wall 1.0–1.5 μm thick, brown, and with cerebriform folds up to 10–12 μm tall; outer wall becoming brittle with age; inner wall 1.0–1.5 μm thick, pliable, hyaline with its surface ornamented with an alveolate reticulum; alveoli 7.5–10 μm in width; outer wall readily separating from the inner wall on older spores. Hyphae below the spore attachment 10–12 μm wide, frequently giving rise to numerous finely branched hyphae. Forming nonlobed vesicles in vesicular-arbuscular mycorrhizae.

Distribution and mycorrhizal associations: known from soil on the Agricultural Research Centers at Ona and Jay, Florida, and from tobacco and corn-rhizosphere soils near Live Oak, Florida. Forming mycorrhizae on bahia-grass pot culture in a greenhouse.

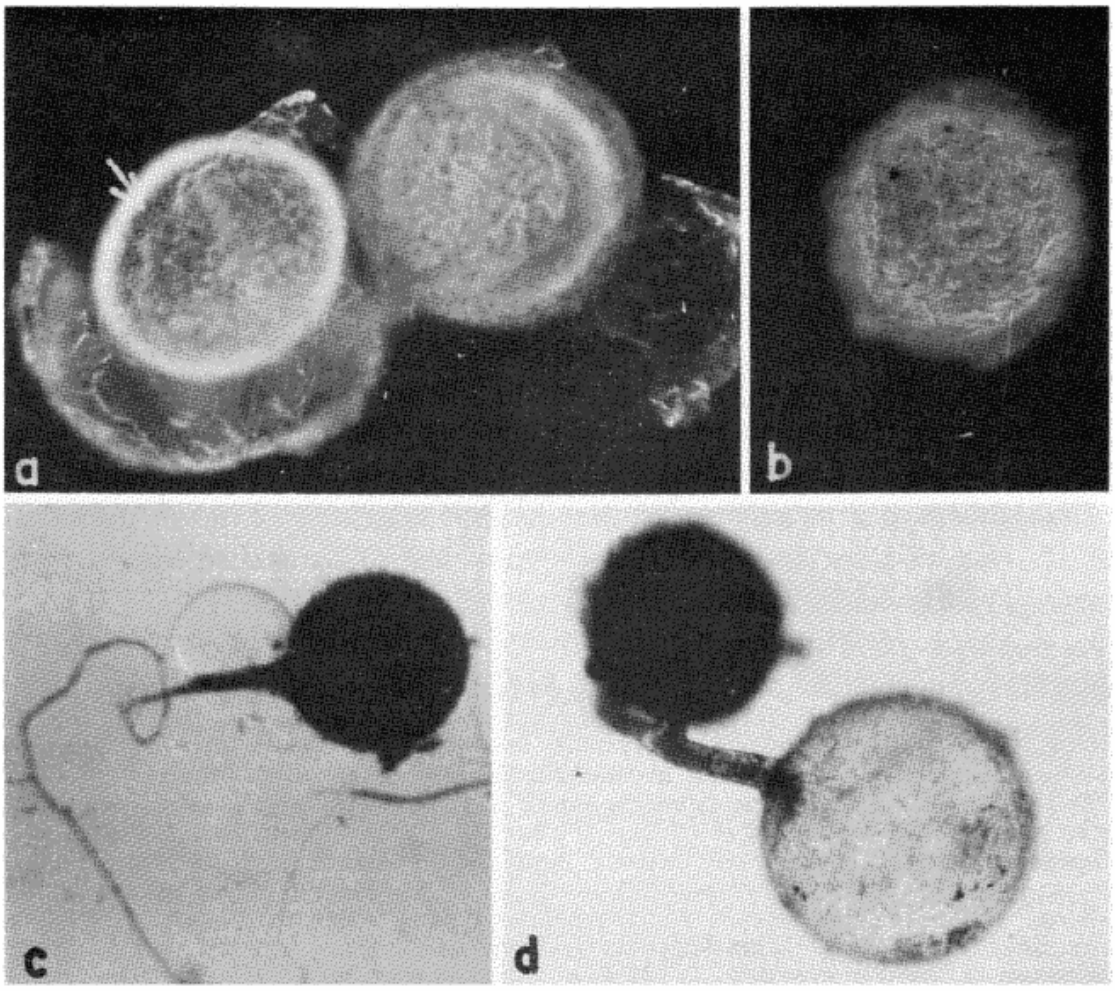

FIG. 3. *Acaulospora gerdemannii*. a. Two azygospores showing the outer (broken) and inner spore walls; note the reticulate pattern on the inner wall (arrow), dark field microscopy, × 100; b. Azygospore showing the cerebriform convolutions on the outer wall, dark field microscopy, × 100; c. Young vesicle before spore formation, × 40; d. Vesicle and newly formed azygospore, × 100.

Etymology: named in honor of Dr. James W. Gerdemann for his pioneer work with VA-mycorrhizal fungi.

Type: pot culture of bahia grass inoculated with spores obtained from Ona, Florida, October, 1977 (Schenck 07) (OSC; isotypes, FH & FLAS).

Acaulospora gerdemannii can be distinguished from other described species of *Acaulospora* by its readily separable walls and the cerebriform convolutions on the outer wall.

DISCUSSION

Species of all four genera in the Endogonaceae that form endomycorrhizae have been recovered in Florida. Species of *Gigaspora* predomi-

nate in north Florida with one species, *G. margarita,* being found in rhizosphere soil in all parts of the state thus far sampled. *Acaulospora laevis* appears to be the only species in the genus that has widespread distribution in Florida. The chlamydosporic species of *Glomus* and *Sclerocystis* are recovered generally less frequently than azygosporic species but may be more widely distributed and abundant than the recovery of their spores in rhizosphere soil would indicate.

Although accurate notations of soil type, *p*H and nutrients levels on all samples have not been kept, observations would indicate that species occurrence in Florida is influenced by these factors. As examples, *A. laevis* has been recovered from soils (*p*H 4.0–4.5) with native grass and palmetto vegetation in central Florida to the exclusion of all other species of mycorrhizal fungi. However, species of two other genera have been successfully established in this same soil while *Acaulospora* did not reestablish well when the soil was limed to *p*H 5.5. Several *Gigaspora* species are recovered from north-Florida soils when land is newly cleared but they are not found in fields after several years of soil cultivation and fertilization.

The climate and soil types in Florida are varied but appear to encourage development of azygosporic species of Endogonaceae. More species of *Gigaspora* have been reported from Florida than elsewhere in the world. Only four of the 11 named species, *G. gilmorei, G. calospora, G. coralloidea* and *G. aurigloba* Hall, have not been found in the state. The occurrence of chlamydosporic species of Endogonaceae may be more prevalent than indicated since vesicle development in roots is common and species of *Glomus* and *Sclerocystis* can be recovered on soybean and bahia grass when these are planted in rhizosphere soils.

KEY TO FLORIDA ENDOGONACEAE

1. Forming chlamydospores free in the soil, in sporocarps or within roots 2
1. Forming azygospores on terminally swollen hyphae 3
   2. Chlamydospores only in sporocarps containing a single tightly packed layer of spores around a central plexus of hyphae ..... *Sclerocystis*
   2. Chlamydospores usually free in soil or within roots; if in a sporocarp, not as in 2 above ..... *Glomus*
3. Azygospores terminally attached to a swollen hyphal tip (suspensor-like cell) that frequently bears a smaller hypha projecting towards the spore; spores usually free in soil with a bulbous (suspensor-like cell) attachment ..... *Gigaspora*
3. Azygospores laterally attached to a swollen hypha tapering to a large terminal vesicle which collapses with age; spores usually free in the soil with no hyphal attachment ..... *Acaulospora*

GLOMUS

1. Chlamydospore walls on mature spores predominantly hyaline, white or yellow occasionally becoming dark yellow to brown with age 2
1. Chlamydospore walls on mature spores predominantly brown to black, occasionally yellow brown when young 5
   2. Chlamydospore walls hyaline (becoming yellow with age), walls 7–31 μm thick, frequently formed within the roots ..... *G. clarus*
   2. Chlamydospore walls yellow to yellow brown, not formed within the roots .. 3
3. Hyphal attachment to spore frequently funnel shaped, spores 100–300 μm in diam, spores formed ectocarpically or within sporocarps of 1 to 10 spores ..... *G. mosseae*
3. Hyphal attachment not funnel shaped, spores usually less than 100 μm 4
   4. Spores greater than 50 μm in diam, usually formed in sporocarps above the soil surface ..... *G. fulvus*
   4. Spores 50 μm in diam or less, hypogeous ..... *G. microcarpus*
   4. No spores usually formed, when present 10–12 μm in diam; hyphae on roots thin (0.5–3.0 μm wide), with irregular swellings ..... *G. tenuis*
5. Spores formed ectocarpically or in old roots, spore diam not exceeding 150 μm 6
5. Spores not formed in old roots, spore diam frequently exceeding 150 μm 7
   6. Young spores with an ephemeral outer wall up to 5 μm thick and a laminate inner wall 2–8 μm thick ..... *G. etunicatus*
   6. Spores without an ephemeral outer wall; spore wall yellow brown, 3–17 μm in diam; spores frequently tightly packed in sporocarps ..... *G. fasciculatus*
7. Chlamydospores dark brown to black; not formed in sporocarps ..... *G. macrocarpus* v. *geosporus*
7. Chlamydospores yellow brown; spores formed ectocarpically or in sporocarps ..... *G. macrocarpus* v. *macrocarpus*

SCLEROCYSTIS

1. Sporocarps enclosed in a peridium 2
1. Sporocarps not in a peridium; resembling a miniature blackberry .. *S. rubiformis*
   2. Sporocarp peridium composed of thick walled, sinuous hyphae tightly enclosing the spores ..... *S. sinuosa*
   2. Sporocarp peridium without sinuous hyphae ..... *S. coremioides*

ACAULOSPORA

1. Azygospores over 100 μm in diam 2
1. Azygospores less than 100 μm in diam ..... *A. trappei*
   2. Azygospores with two readily separable walls; outer spore wall with cerebriform folds up to 12 μm tall ..... *A. gerdemannii*
   2. Azygospores without readily separable walls; walls smooth ..... *A. laevis*

GIGASPORA

(Including species not found in Florida)

1. Azygospores light brown to dark brown or black 2
1. Azygospores hyaline, white, yellow, or greenish yellow 5
   2. Azygospore wall black, pitted with pores; spores over 250 μm diam; accessory vesicles in clusters ..... *G. nigra*
   2. Azygospores light to dark brown 3
3. Azygospores 250 μm or larger 4
3. Azygospores usually less than 250 μm; outer wall with minute spines; accessory vesicles in clusters ..... *G. heterogama*
   4. Accessory vesicles in clusters; outer wall continuous with irregular shaped projections 1–7 × 3–12 μm ..... *G. gregaria*
   4. Accessory vesicles borne singly, coralloid; wall surface with openly spaced hyaline ridges 2.0 μm tall by 0.5–6.0 μm broad (not known to occur in Florida) ..... *G. coralloidea*
5. Azygospores with an outer wall readily separating under pressure from the inner wall; azygospores hyaline 6
5. Azygospore without separable walls 7
   6. Azygospores under 250 μm in diam; suspensor cell hyaline; accessory vesicles brown, knobby, borne singly or in clusters ..... *G. pellucida*
   6. Azygospores over 250 μm in diam; suspensor cell light brown; accessory vesicles pale brown, knobby, borne in clusters (not known to occur in Florida) ..... *G. gilmorei*
7. Azgospore walls consisting of one layer, usually less than 5 μm thick; spores hyaline, white or shades of yellow; accessory vesicles knobby, borne singly (not known to occur in Florida) ..... *G. calospora*
7. Azygospore walls consisting of more than one layer; accessory vesicles borne in clusters 8
   8. Azygospores predominantly some shade of yellow 9
   8. Azygospores predominantly some shade of white 10
9. Azygospores yellow to greenish yellow; accessory vesicles spiny, borne in clusters; suspensor-like cell 41–51 μm in diam ..... *G. gigantea*
9. Azygospores pale yellow to dull yellow, never turning greenish yellow; accessory vesicles in clusters, spiny to knobby; suspensor-like cell 40–70 μm diam (not known to occur in Florida) ..... *G. aurigloba*
   10. Azygospores white to cream with a pink tint in the area of the suspensor-like cell; spores usually less than 300 μm in diam ..... *G. rosea*
   10. Azygospores white to cream; usually greater than 300 μm in diam ..... *G. margarita*

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