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A NEW SPOROCARPIC SPECIES OF ACAULOSPORA (ENDOGONACEAE)[1]

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ABSTRACT

A new species of Acaulospora, A. myriocarpa, a mycorrhizal-forming fungus in the Endogonaceae (Endogonales, Zygomycetes) is described. This hyaline, small-spored species forms spores singly in soil and roots but also produces them in sporocarps and sporulates in soil crevices, within empty seed teguments, cast insect exoskeletons, and empty spores of other Endogonaceae. Spores of A. myriocarpa are of similar size and appearance to A. trappei. Spores detached from the hyphal terminus can be confused possibly with Glomus occultum, G. diaphanum and G. microcarpum. The known distribution of A. myriocarpa is restricted to Colombia and Peru.

INTRODUCTION

A small-spored, hyaline, sporocarp-forming species of Acaulospora was observed first in Carimagua, Meta, Colombia in 1981. On first examination, this species was perplexing because spores associated with this fungus could be placed in the genera Glomus, Entrophospora and Acaulospora based on their configuration with or without the hyphal terminus. In addition, until recently (Berch, 1985), no species of Acaulospora was known to form sporocarps. The purposes of this paper are to describe this new fungus, compare it to species of similar morphology and size and discuss some of its unique features. Spore wall terminology follows that of Walker (1983).

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ACAULOSPORA MYRIOCARPA Spain, Sieverding et Schenck (Figs. 1,2 and 3)

Sporae singulatim in terra vel in sporocarpiis efformatae; sporocarpia irregularia, hyalina,parva,725 X 525 µm vel magna 22 X 8 mm, sine periodio; sporae penduculo gestae vel sporae sessiles, in subattenuata hypha cum terminatione inflata globosa. Sporae hyalinae, globosae vel subglobosae, 32-90 µm diam., vel irregulares, 28-95 X 28-80 µm. Tunicae sporarum 1.5-3.5 µm crassae; tunica exterior rigida, hyalina, 0.75-2 µm crassae, secundo interior rigida, hyalina, 0.3-1.5 µm crassae, tertio membranaceo (<0.3 µm crasso) inseparabili e tunica secundo. Sporae cum pedicellibus aliquando. Formans mycorrhizas sed sine vesiculis vel arbusculis.

Spores formed singly in the soil and adjacent to roots or in irregular-shaped small, 725(-1050) X 525(-906) µm, to occasionally large, upto 22 X 8mm, sporocarps without a peridium; spores frequently formed within soil crevices and inside roots, empty spores of other Endogonaceae, within insect carapaces, and old seed testa. Spores borne on a short pedicel, 2.8-12 long X 2.7-8 µm wide, or sessile on a slightly tapering hypha, 5-8 µm wide, terminating in a globose to ovoid terminus, 25-100 X 30-95 µm; hyphal terminus contents hyaline to subhyaline, granular to reticulate; terminus wall 0.5-0.75 µm thick; as the spore forms the terminus empties, collapses and the terminus hypha proximal to the pedicel may attenuate. Spores hyaline, mostly globose to subglobose, (22)32-90 µm, or cylindrical, ovoid, pyriform to irregular, (23)28-95(114) X (21)28-80(96) µm; spore contents hyaline and granular; composite spore wall hyaline, 1.5-3.5(7) µm thick, of 3 walls (1-3) in one group; wall 1 rigid, 0.75-2 µm, wall 2 rigid 0.3-1.5 µm; spore walls staining dull yellow in Melzer's reagent; wall 3 membranous (<0.3 µm thick) closely appressed to wall 2, seen best in stained preparations; hyphal pedicel occasionally (10-20% of spores) remaining attached to the spore after separation from the hypha and terminus. Hyphae within the root staining faintly with trypan blue and no typical vesicles or arbuscules observed.

TYPE: Obtained from a pot culture with Pueraria phaseoloides (Roxb.) Benth. (tropical kudzu), culture C-7 at Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia; originally collected by Joyce Spain from native grasses in an acidic loam (pH 4.5) at Reserva-Carimagua, Meta, Colombia. Holotype OSC; isotypes FLAS and FH.

SPECIMENS EXAMINED: Numerous specimens of A. myriocarpa were collected from several regions in Colombia. Spore sizes of pot-cultured isolates of these specimens varied considerably but because of the gradual overlap in spore

diameter among the isolates on P. phaseoloides (Fig. 4) they were considered variants of the holotype rather than new taxa. Paratypes: (listed with CIAT pot culture numbers and followed by spore diameter measurements) C-4-10 associated with roots of Manihot esculenta Crantz in acid clay, Mondomito, Cauca; 27-57 µm. C-20-5 associated with roots of M. esculenta in acidic sand, Alegria-Carimagua, Meta; 40-90 µm. C-22-1 associated with roots of Allium porrum L. in red clay loam, farm of Mr. Baron, Bitaco, Valle; 51-82 µm. C-29-4 from soil for greenhouse experiments in Quilichao, Cauca; 30-61 µm. C-37-7 associated with roots of Stylosanthes sp. at the CIAT field station in Quilichao, Cauca; 45-75 µm. C-44-6 associated with roots of native grasses at Piragua Grande near Buenaventura, Valle; 33-65 µm. C-88-1 from soil on the CIAT station at Popayan, Cauca, collected by J. Haanschoten; 31-51 µm. C-94-3 associated with roots of M. esculenta at site D-4 on the CIAT station, Quilichao, Valle; 33-50 µm. C-98-2 from a pot culture (Car-45) of Acaulospora appendicula Spain, Sieverding & Schenck on P. phaseoloides at CIAT, Cali, Cauca; 41-64 µm. C-100-3 from a pot culture (Car-29) of A. appendicula on P. phaseoloides at CIAT, Cali, Cauca; 30-45 µm. Sporocarps were not found with all the above isolates but all spores had two walls. All the paratypes have been sent to the OSC herbarium. Also observed in Tarapoto, San Martin, Peru at two locations associated with roots of tropical grasses and legumes (pot culture C-46-7) and with roots of Brachyaria sp. (pot culture C-47-6).

ETYMOLOGY: Myriocarpa (Gr. Myrio = innumerable; L. carpa = fruit) referring to the abundance of spores and sporocarps produced by this species.

MYCORRHIZAL ASSOCIATIONS: Known to form mycorrhizal associations with Peuraria phaseoloides, Manihot esculenta, Coffea arabica L., Brachyaria sp., Stylosanthes sp., and Allium porrum L.

DISTRIBUTION: Known only from Colombia and Peru.

DISCUSSION

Some spores of A. myriocarpa, especially those which were stained with trypan blue or cotton blue, have a separation between the inner wall and a membrane retaining the spore contents. The thickness of this membrane was not possible to determine accurately with a light microscope (X1250) in unstained spores because of its thin nature and its adherence to the inner rigid wall. In stained preparations, this membrane separated from the inner rigid wall and measured less than 0.3 µm in thickness. This increased thickness of the membrane in stained spores may have resulted from stain particle

deposition. This membranous wall is included in the murograph (Fig. 2) but is labeled with an asterisk as being difficult to see.

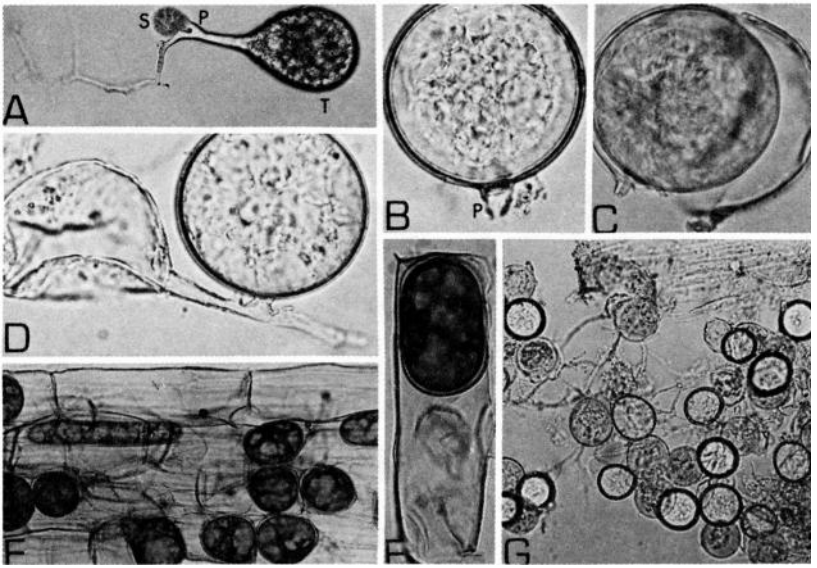

FIG. 1. Acaulospora myriocarpa. A) Initial stages of spore formation. (T=hyphal terminus; P=pedicel; S=initial spore) X250. B) Mature spore with attached pedicel (P) X400. C) Broken outer wall on mature spore, X400. D) Mature spore attached to pedicel, hypha, and collapsed terminus, X400. E) Spores (stained with trypan blue) in a root, X400. F) Mature spore (stained with trypan blue) and collapsed terminus in a single cortical cell, X400. G) Mature spores at the edge of a broken sporocarp, X250.

The only other sporocarp-forming species of Acaulospora, A. sporocarpia Berch, (Berch, 1985) can be readily separated from A. myriocarpa. Spores of A. sporocarpia are dark brown to black, are considerably larger (160-200 X 150-175 µm) than A. myriocarpa, and are formed in dark brown sporocarps. However, A. myriocarpa can be easily confused with A. trappei Ames & Linderman because both species produce spores that are hyaline, thin walled, of similar size and both form spores in roots. Spores of A. myriocarpa in sporocarps would readily be separable from A. trappei since A. trappei does not form sporocarps. In addition, spores of A. myriocarpa have two distinct walls and spores formed singly in soil frequently

have a pedicel attached, features absent in A. trappei. However, many spores of A. myriocarpa lack a pedicel or the pedicel can be lost by abrasion in soil.

Single spores of A. myriocarpa with a pedicel can sometimes be confused with Glomus species (Fig. 1B), especially spores of G. occultum Walker, G. diaphanum Morton & Walker and G. microcarpum Tul. & Tul. Spores of A. myriocarpa and both G. diaphanum and G. occultum have two separable walls. Glomus diaphanum has the inner wall inserted into the hyphal attachment and this arrangement can be seen when spores are broken and the two walls disassociate. This does not occur with A. myriocarpa. Glomus occultum has a thin outer wall that is frequently associated with debris and a thicker inner wall. In A. myriocarpa the outer wall is slightly thicker than the inner wall (Fig. 2) and no debris is associated with the outer wall (Fig. 1 B,C and D). With G. microcarpum the spore wall is single, frequently has laminations, and is some what thicker (4-6 µm) than that of A. myriocarpa (1.5-3.5 µm).

As mentioned earlier, our first observations of A. myriocarpa revealed spore types that resembled three genera. Typical Acaulospora-like attachments to the spore were observed but in addition Entrophospora-like attachments were noted. These resulted from the attenuation or loss of the proximal hypha associated with the tapering hypha at the pedicel (Fig. 3). This spore-terminus conformation much resembled that of Entrophospora. The Glomus-like appearance of the pedicel attached to the spore resulted when the separation of the spore from the hypha occurred at the point where the pedicel was attached to the tapering hypha (Fig. 1B; Fig. 3).

A word of caution is appropriate regarding research with and identification of small, hyaline-spored species of Endogonaceae. These species can be easily overlooked in soils, especially if very fine sieves (25 µm diam.) are not used routinely in surveys for mycorrhizal fungi. These hyaline, small-spored species can be a problem also when inoculating "control" pots with pot culture sievings to establish similar microflora to that which occurs in mycorrhizal inoculated treatments. Many of these spores are small enough in diameter, especially without their attached hyphae, to pass through most sieves routinely used to exclude spores of mycorrhizal fungi from these sievings. This can result in "control" treatments with mycorrhizae.

Many of these hyaline, small-spored species cannot be distinguished readily from each other with a dissecting microscope (X70-X100). A species determination may be impossible even if examined with a compound microscope,

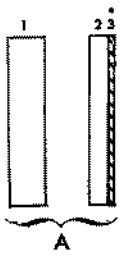

Fig. 2. Murograph of the wall structure of Acaulospora myriocarpa which consists of two unit walls and a thin membranous wall. Diagram after Walker, 1983.

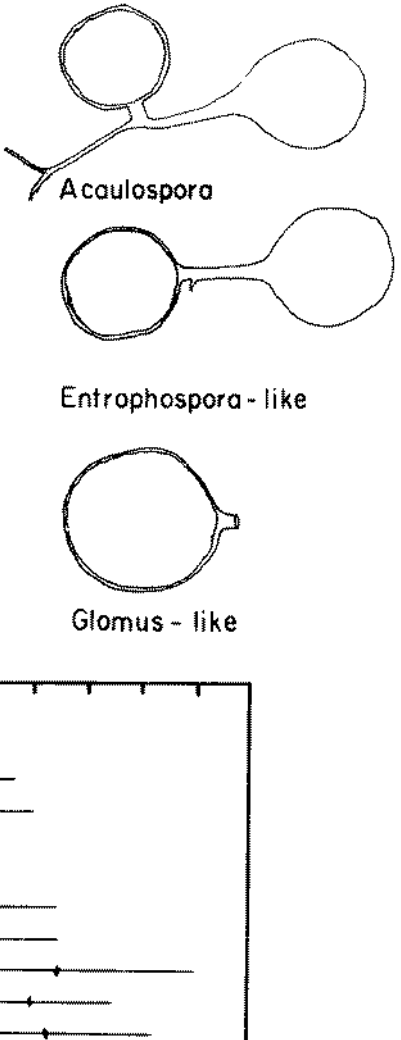

Fig. 3. Diagram of the spores of Acaulospora myriocarpa with and without the attached terminus showing the three configurations observed.

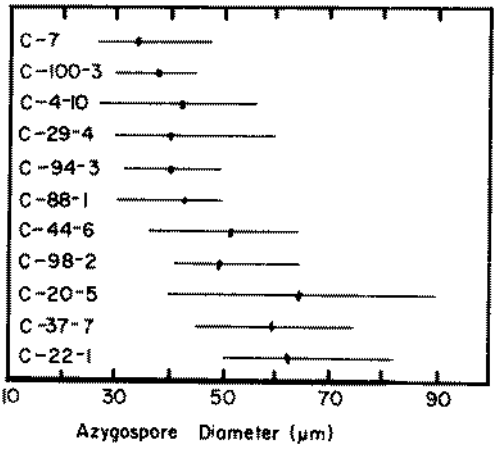

Fig. 4. Range of spore diameters of eleven isolates of Acaulospora myriocarpa. Mean spore diameter indicated by the mark on each line.

especially if hyphal attachments are missing. Therefore, we recommend establishment of pot cultures from single spores so that a positive determination can be made later on pot cultured spores with intact hyphal attachments and spores in different stages of maturity and development.

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