

ACAULOSPORA NICOLSONII, A NEW ENDOGONACEOUS SPECIES FROM
GREAT BRITAIN

BY C. WALKER

Forestry Commission, Northern Research Station, Roslin, Midlothian, EH25 9SY

L. E. REED AND F. E. SANDERS

Department of Plant Sciences, Leeds University, Leeds LS2 9JT

A new species of endogonaceous fungus, *Acaulospora nicolsonii*, is described from Great Britain. Attempts to obtain the species in pure pot culture have failed, though it sporulated in a pot containing *Zea mays* and mycorrhizal *Glomus* species. Wound healing of the type previously thought to be confined to *Gigaspora* spp. was noted on a hypha attached to a sporiferous saccule of *A. nicolsonii*.

As part of a study of mycorrhizal fungi in Yorkshire, soil was sampled from a cropped field at the University of Leeds Experimental Field Station near Tadcaster, and open pot cultures (Gilmore, 1968) established with *Zea mays* L. After a year, spores were extracted from the pots by wet sieving and decanting (Gerdemann & Nicolson, 1963). Among several *Glomus* spp., an undescribed member of the genus *Acaulospora* Gerdem. & Trappe was found to be sporulating in one of the pots. Further samples from the same site confirmed its presence in the field, and it was later found at another location in Yorkshire and on a hill farm in northern Scotland.

To overcome the confusion caused by the use of the word 'vesicle' for several different structures found among members of the Endogonaceae, the terms 'vesicle' (Ames & Schneider, 1979; Gerdemann & Trappe, 1974; Walker & Trappe, 1981) and 'mother spore' or 'mother vesicle' (Hall, 1977; Mosse, 1970) have been replaced in this paper by 'sporiferous saccule', a phrase more accurately

describing the function of the swollen hyphal tip from which the spore is formed in *Acaulospora* and *Entrophospora* Ames & Schneider.

Descriptions of walls in this paper, including the use of the terms 'unit wall', 'laminated wall', 'membranous wall' and 'wall group', and the standardised lettering and numbering system, are based on the system proposed by Walker (1983), as is the presentation of the 'murograph' (Fig. 11), a graphic representation of the spore wall organization. Observations on spore colour were made with the aid of a stereomicroscope from fresh specimens, or spores preserved in 5% formaldehyde solution, immersed in a dish of water. There was little change of colour caused by the formaldehyde, though spores tended to yellow a little after fixation. Microscope observations were made on spores mounted in polyvinyl alcohol lactophenol (Walker, 1979a).

***Acaulospora nicolsonii* sp. nov.** (Figs 1-11)

(Etym.: In honour of Dr T. H. Nicolson, Uni-

versity of Dundee, in recognition of his important pioneering contributions to the study of endomycorrhizal Endogonaceae)

Sporae singulae in solo efformatae, hyalinae vel pallide avellaneae, globosae, subglobosae, vel pyriformes, 99–198 × 109–218 μm , lateraliter gestae per collare vel stipitem incrassatum usque ad 27 μm longum ad collum sacculi sporangiferi. Sporae tunica turba externa stratis tribus: exteriore hyalino, evanido, 0.5–1.5 μm crasso, adherenti, primo laevi, demum exasperato; mediano hyalino vel colorato, lamellato, 3–10 μm crasso, primo laevi, demum rimosulo; interiore pallide alutaceo, laxe adherenti, 0.5–1.5 μm crasso. Intra turmam externam membrana separata, hyalina, plerumque multo minus quae 0.5 μm crassa. Sacculus sporangifer hyalinus vel albus, globosus, 156–208 μm diam, collo 100–210 μm longo, ad maturitatem collabens.

Spores formed singly in the soil, laterally on the neck of a sporiferous saccule that collapses after the spore matures. Spores hyaline to pale yellow-brown, globose to subglobose to pyriform, 99–198 × 109–218 μm , attached to the saccule by a slightly raised collar 2–4 μm wide surrounding an ovoid hole 8–13 × 12–18 μm , or by a thickened stalk up to 27 μm long with walls approx 2 μm thick and a lumen 5–13 μm diam. Spore contents at maturity occluded by a septum formed by continuation of spore-wall growth, and plugged by a deposit of granular material between this septum and the inner wall. Spore wall an outer, brittle, wall group (Group A) (walls 1–3) enclosing an inner, membranous wall (Group B) (wall 4) (see micrograph, Fig. 11). Wall group A with an outer thin, hyaline, evanescent wall (wall 1), 0.5–1 μm thick, tightly adherent to a thick, brittle, hyaline to pale yellow-brown, laminated wall (wall 2), 3–10 μm thick, with up to 13 subequal laminae, enclosing a loosely adherent, pale yellow, brittle, unit wall, 0.5–1.5 μm thick (wall 3) that often separates when the spore is crushed. Inner wall (Group B, wall 4) very thin, hyaline, membranous, usually much less than 0.5 μm thick, but occasionally about 0.5 μm thick. Wall 1 at first smooth on outer surface, but later roughened as it breaks up and sloughs, leaving granular fragments attached to wall 2 which cracks in an irregular network with age. Spore contents at first appearing vacuolate, due to the presence of many oil droplets, but later becoming reticulate as the droplets apparently coalesce. Sporiferous saccule hyaline to white, globose, 156–208 μm diam; neck 100–200 μm long, tapering from about 50 μm diam at the saccule to 12–20 μm diam at its origin from a thin-walled, usually collapsed parent hypha, with a thickened wall (1–2.5(–7) μm) often continuous with, and not clearly differentiated from, the spore neck or collar, thus remaining attached to the spore after maturity. Saccule wall of a granular, evanes-

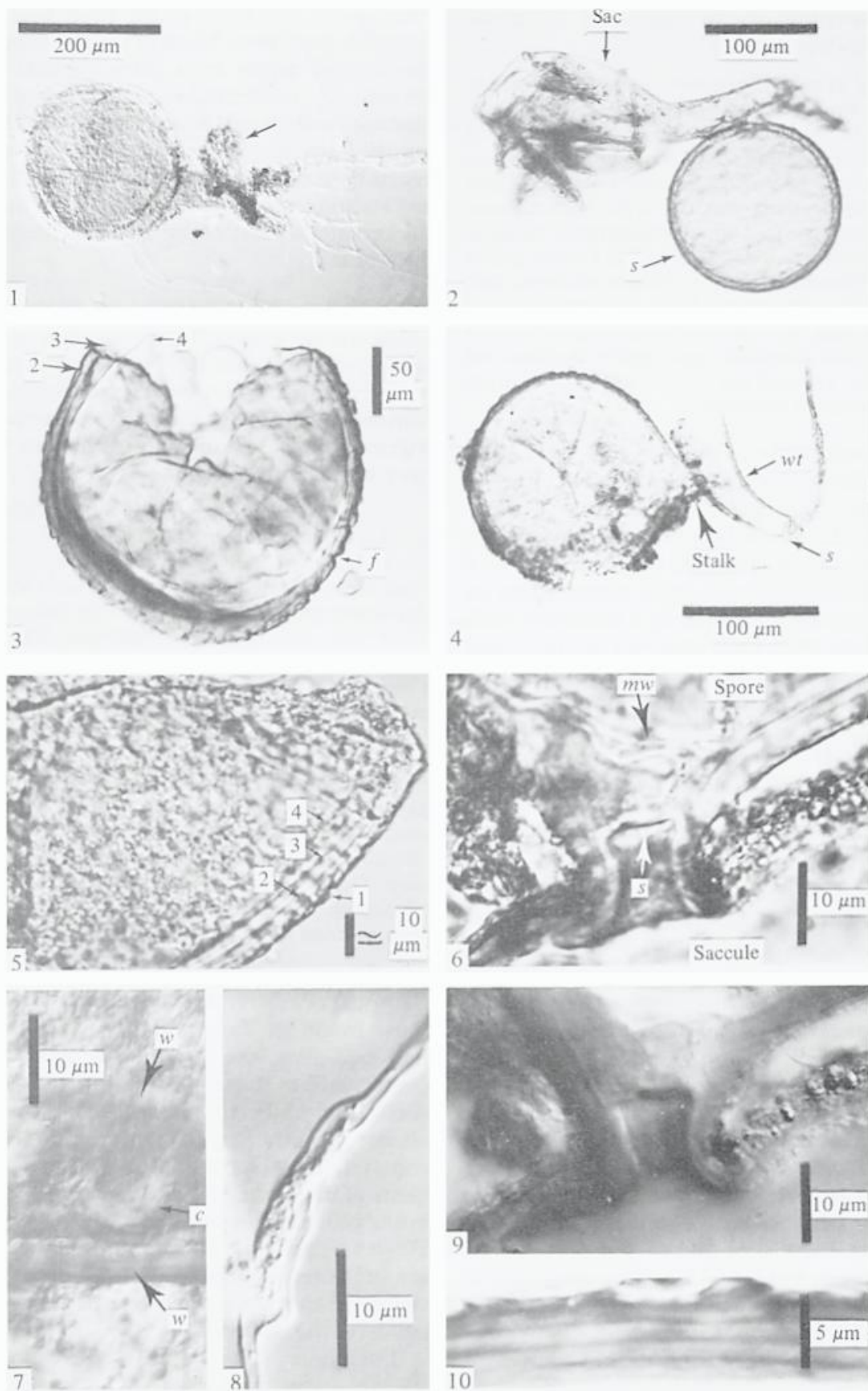
cent outer layer, 0.5–1.5 μm thick, overlaying an inner layer 0.5–1 (–5.5) μm thick that is thickest near the spore base. Neck of saccule separated from the hyphal origin by a thick septum (Fig. 4, s), formed by continuation of its inner wall-layer. Saccule collapsing at maturity, as the contents empty into the spore. Spores and saccule not reacting to Melzer's reagent. In cotton blue, walls 1–3 staining very pale blue, wall 4 not staining, and the reticulate parts of the spore contents becoming dark blue.

The mycorrhizal associations of this species are unknown. It was found sporulating in a pot containing *Z. mays*, but the pot also contained spores of the known endomycorrhizal species, *Glomus caledonicum* (Nicol. & Gerd.) Trappe & Gerd. Attempts to isolate the species in pure pot culture have so far failed.

Specimens examined: North Yorkshire, Tadcaster, Headley Hall Farm (University of Leeds Agricultural Field Station), 21 Apr. 1981, Walker 281, OSC (Holotype), K. From a pot culture with *Z. mays*, in soil (Aberford series clay-loam) taken from beneath oats (*Avena sativa* L.), (Grid Ref. SE 445414). Sutherland, Rhian (north of Lairg), 18 May 1981, Walker 303, OSC (Paratype). From a very wet, sandy soil, beneath moorland grasses (principally *Festuca ovina* L.) and butterwort (*Pinguicula vulgaris* L.), on the banks of the small river, Abhainn Sgeamhaidh (Grid Ref. NC 576164). North Yorkshire, north of Bellerby Military Camp, near Leyburn (Grid Ref. SE 057965) 11 July 1981, Walker 338, from around roots of *F. ovina*, *Poa pratensis* L., and spring sandwort (*Minuartia verna* (L.) Hiern.), on an old lead-mining spoil heap. There was insufficient material in this last collection for depositing in an herbarium, but the specimens have been retained in the senior author's collection.

The spores from Sutherland, and from Bellerby Camp, Yorkshire, are somewhat different from those found in Tadcaster. In specimens from the first two sites, the sporiferous saccule usually is detached from mature spores, and the fissures in the surface of older spores are generally very deep, almost penetrating the spore wall (Fig. 3). In contrast, most spores from Tadcaster have remnants of the saccule attached, and the fissures in the outer wall are not usually so deep (Figs 2, 4, 10). However, this last collection contains spores that are indistinguishable from those in the others, and the differences are considered to be examples of intraspecific variation.

The outer layer (wall 1) of young spores is usually distinct, but gradually is lost or almost lost, as it breaks down. Wall 2 is always distinct, and usually laminated, but often the laminae are difficult to observe. On a few specimens no lamination can be seen, and the wall appears to be an amorphous unit wall. Wall 3 is often difficult to observe



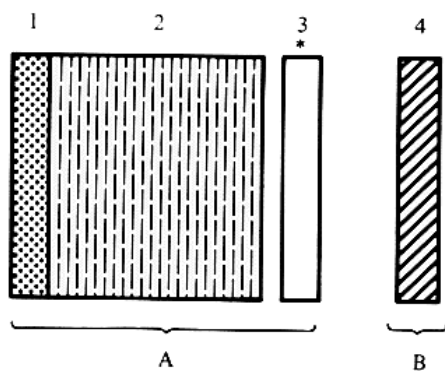


Fig. 11. Murograph of *A. nicolsonii*. The evanescent wall, the laminated wall, and the membranous wall are shaded respectively with dots, dashes and 45° lines. The unit wall is unshaded (after Walker, 1983).

and is missing on some specimens. Such specimens have vacuolate contents, and it is possible that they are at an early stage of development, before this wall is formed. Occasionally, the inner lamina of wall 2 separates, to give the appearance of another unit wall. The inner, membranous wall (wall 4) seems always to be present in healthy spores, and is separated completely from the other walls to form an endospore. However, wall 4 is usually missing from parasitized spores.

Wound healing as described for *Gigaspora gigantea* (Nicol. & Gerd.) Gerd. & Trappe by Gerdemann (1955) was observed on a hypha attached to an *A. nicolsonii* sporiferous saccule (Fig. 8). This phenomenon appears not to have been reported

before for an *Acaulospora* sp., and can be considered as evidence for a close phylogenetic relationship between *Acaulospora* and *Gigaspora*, reinforcing other similarities, such as germination characteristics, wall structure and wall ornamentation (Koske, Miller & Walker, 1983; Mosse, 1970; Walker, 1979b).

Acaulospora nicolsonii can be distinguished from other members of the genus (Walker & Trappe, 1981; Trappe, 1982) by the size, light colour, and multiple wall structure of its spores, which in older specimens have deep surface fissures. The retention of part of the sporiferous saccule frequently occurs in the species as found in the holotype collection from a pot culture, but not in the specimens found in field collections. The long stalk formed on some spores can give them the appearance of chlamydospores from the genus *Glomus* if the remnants of the sporiferous saccule are detached completely, but collections usually contain other specimens with which to make comparisons.

We wish to thank Dr J. M. Trappe, USDA, Forest Service, for his helpful review of the manuscript, and for preparing the Latin diagnosis.

REFERENCES

- AMES, R. N. & SCHNEIDER, R. W. (1979). *Entrophospora*, a new genus in the Endogonaceae. *Mycotaxon* 8, 347-352.
- GERDEMANN, J. W. (1955). Wound-healing of hyphae in a phycocomycetous mycorrhizal fungus. *Mycologia* 47, 916-918.

Figs. 1-10. *Acaulospora nicolsonii* sp. nov. Photographs taken with a Zeiss Photomicroscope III fitted with Nomarski differential interference contrast equipment.

Fig. 1. Sporiferous saccule showing beginnings of spore formation (arrowed) (photographed between crossed polarizing filters with substage Wollaston prism).

Fig. 2. Saccule (sac) collapsed, with spore (s) still attached. Brightfield microscopy.

Fig. 3. Mature spore without attached saccule remnants. Wall 1 is sloughed, and the laminated wall (2) and attached unit wall (3) can be seen, with the inner, membranous wall (4) broken in this crushed specimen. The deep fissures are clearly evident (f). Differential interference contrast microscopy.

Fig. 4. A mature spore showing a distinct stalk and wall-thickening (wt) of the attached saccule neck. The septum separating the saccule from the parent hypha can be seen on the left (s). Brightfield microscopy.

Fig. 5. Detail of wall-structure on a mature spore. The remnants of the evanescent wall (1), the laminated wall (2), the brittle, unit wall (3) and the inner, membranous wall (4) are arrowed. In this spore, as in the spore in Fig. 3, the membranous wall has fractured upon crushing along with the other wall layers. Brightfield microscopy.

Fig. 6. Details of the stalk and occlusion of spore contents, from the spore in Fig. 4. The membranous wall (mw), granular plug (p) and septum (s) are arrowed. Brightfield microscopy.

Fig. 7. Details of the collar (c) of a mature spore. The outer walls (w) of the sporiferous saccule neck can be seen. Brightfield microscopy.

Fig. 8. Wound-healing in a hypha found attached to a saccule. Differential interference microscopy.

Fig. 9. Photograph by differential interference microscopy of the neck and occlusion seen in Fig. 6.

Fig. 10. Detail of the outer wall group of a mature spore. The granular material at the top of the picture is wall 1, the evanescent wall, in the process of sloughing. Four laminations can be seen in wall 2. Walls 3 and 4 are not present in the field of view. Brightfield microscopy.

- GERDEMANN, J. W. & NICOLSON, T. H. (1963). Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society* **46**, 235-244.
- 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.
- HALL, I. R. (1977). Species and mycorrhizal infections of New Zealand Endogonaceae. *Transactions of the British Mycological Society* **68**, 341-356.
- KOSKE, R. E., MILLER, D. D. & WALKER, C. (1983). *Gigaspora reticulata*: a newly described endomycorrhizal fungus from New England. *Mycotaxon* **16**, 429-435.
- MOSSE, B. (1970). Honey-coloured, sessile *Endogone* spores. I. Life history. *Archiv für Mikrobiologie* **70**, 167-175.
- ROTHWELL, F. M. & TRAPPE, J. M. (1979). *Acaulospora bireticulata* sp.nov. *Mycotaxon* **8**, 471-475.
- TRAPPE, J. M. (1982). Synoptic keys to the genera and species of zygomycetous mycorrhizal fungi. *Phytopathology* **72**, 1102-1108.
- WALKER, C. (1979a). The mycorrhizist and the herbarium: the preservation of specimens from VA mycorrhizal studies. In *Program and Abstracts, 4th North American Conference on Mycorrhiza*. Fort Collins, Colorado.
- WALKER, C. (1979b). Hybrid poplar mycorrhizae and endogonaceous spores in Iowa. Ph.D. Thesis, Iowa State University, Ames, Iowa.
- WALKER, C. (1983). Taxonomic concepts in the Endogonaceae: spore wall characteristics in species descriptions. *Mycotaxon* **18**, 443-455.
- WALKER, C. & TRAPPE, J. M. (1981). *Acaulospora spinosa* sp.nov. with a key to the species of *Acaulospora*. *Mycotaxon* **12**, 515-521.