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Acaulospora mellea and *A. trappei*, fungi new for Poland

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Spores of *Acaulospora mellea* and *A. trappei* are described and illustrated. *Acaulospora mellea* occurs relatively infrequently in Poland. It was found in 30 of the more than 1300 soil samples examined representing different uncultivated and cultivated sites. Spores of *A. trappei* were only found in six field-collected soils. However, the occurrence of this fungus in many pot cultures with soils from various uncultivated and agricultural sites indicated that the species was common among arbuscular mycorrhizal fungi in Poland.

Key words: *Acaulospora mellea, A. trappei*, arbuscular fungi, *Glomales.*

INTRODUCTION

Investigations of arbuscular mycorrhizal fungi in Poland revealed two next species new in this country: *Acaulospora mellea* Spain et Schenck and *A. trappei* Ames et Linderman. Despite few reports on the occurrence of these fungi, they are probably widely distributed in the world.

The aim of the present paper was to characterize the morphological features of *A. mellea* and *A. trappei* spores and to show the distribution of these fungi in the world.

MATERIALS AND METHODS

Collection of soil samples, establishment of trap and single-species pot cultures, as well as growth conditions were described in a previous paper (Błaszkowski and Tadych 1997). The growth medium of single-species pot cultures was an autoclaved sand of maritime dunes adjacent to Świnoujście (pH 6.7; 12 and 26 mg L^{-1} P and K, respectively). The host species used in both trap and single-species cultures was *Plantago lanceolata*. Trap and single-species pot cultures were harvested at approximately 1-month intervals, beginning 2 months and ending 11 months after plant emergence. Spores were extracted by wet sieving and decanting (Gerdemann and Nicolson 1963). Mycorrhizae were revealed following clearing and staining root fragments according to Phillips and Hayman (1970).

Morphological properties of spores and their subcellular structures were determined based on at least 80 and 50 spores of each species mounted in polyvinyl alcohol/lactic acid/glycerol (PVLG; Koske and Tessier 1983) and a mixture of PVLG Melzer's reagent (1:1, v/v), respectively. Terminology of spore structure followed Franke and Morton (1994), Spain et al. (1989), Stürmer and Morton (1997) and Walker (1983). Spore colour was examined under a dissecting microscope on fresh specimens immersed in water. Colour names are from Kornerup and Wanscher (1983). The classification was based on Morton and Benny (1990). Nomenclature of plants was after Mirek, Piękoś-Mirkowa, Zając A. and Zając M. (1995). Specimens were mounted in PVLG on slides and deposited in the Department of Plant Pathology (DPP), Agricultural Academy in Szczecin, Poland.

Abbreviations: Bł. – Błaszkowski; DPP – Department of Plant Pathology; u.coll. – unnumbered collection.

DESCRIPTION AND DISCUSSION

Acaulospora mellea Spain et Schenck

Sporocarps unknown. Spores single in the soil, formed laterally on the neck of a sporiferous saccule (Fig. 1). *Spores* pale yellow (3A3) to orange (5B8); globose to subglobose; (98-) 116 (-140) in μm diam; rarely ovoid; 100 × 130 μm, attached to the saccule by a slightly raised collar 8.1–10.5 μm wide × 3.7–5.0 μm long surrounding a hole 7.5–9.8 μm diam. Spore contents at maturity occluded by a septum formed by continuation of spore-wall growth. *Subcellular structure of spores* consisting of three walls (spore wall and two inner flexible walls; Figs 2–4). *Spore wall* comprising three adherent layers (layers 1–3). Layer 1 evanescent, hyaline, (0.8-) 1.0 (-1.3) μm thick, usually completely sloughed in mature spores. Layer 2 laminated, pale yellow (3A3) to orange (5B8), (2.2-) 2.8 (-3.9) μm thick. Layer 3 semiflexible, hyaline, (0.5-) 0.7 (-0.8) μm thick, separable from layer 2. Inner wall 1 consisting of two tightly adherent semi-flexible, hyaline layers, each 0.4-0.6 μm thick (Figs 2 and 3).

Inner wall 2 composed of two adherent hyaline layers (layers 1 and 2). Layer 1 flexible, beaded, (0.5-) 0.9 (-1.0) μm thick. Layer 2 amorphous, 10.0–12.5 μm thick in PVLG (Fig. 4), (0.8-) 1.1 (-1.2) μm thick and beetroot purple (13D8) in Melzer's reagent (Fig. 3). Spore contents of hyaline oil droplets. *Sporiferous saccule* hyaline; globose to subglobose (Fig. 1); 90–130 μm in diam; neck 50–80 μm long, tapering from 17–24 μm in diam at the saccule to 15–20 μm in diam at the point of spore attachment. Saccule wall of a hyaline, smooth, 0.8–1.2 μm thick layer. Saccule collapsing at maturity and usually detached among mature spores.

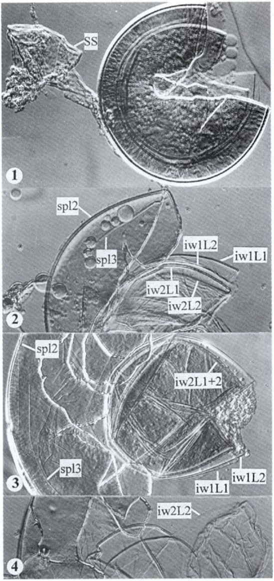
Distribution and habitat: *Acaulospora mellea* was found in 30 of the over than 1300 soil samples examined (Fig. 9). The species occurred among roots of wild and cultivated plants growing in forest nurseries, uncultivated and cultivated soils, as well as in maritime sand dunes. It is probably commonly associated with roots of different plants of the Hel Peninsula (reported as *Acaulospora* 61 and *A. polylamina*; Błaszkowski 1993, 1994). The spore abundance of this species per 100 g dry soil ranged from 1 to 157 (mean 20.2). The proportion of spores of *A. mellea* in the spore populations of arbuscular fungi recovered ranged from 0.8 to 81.3% (mean 12.1%). The species was isolated together with 2–11 other species of arbuscular fungi, including *A. koskei* Błaszk., *A. lacunosa* Morton, *A. morrowiae* Schenck et Smith, an undescribed *Acaulospora* sp., *Glomus aggregatum* Schenck et Smith emend. Koske, *G. caledonium* (Nicol. et Gerd.) Trappe et Gerd., *G. clarum* Nicol. et Schenck, *G. constrictum* Trappe, *G. deserticola* Trappe et al., *G. dominikii* Błaszk., *G. etunicatum* Becker et Gerd., *G. fasciculatum* (Thaxter) Gerd. et Trappe emend. Walker et Koske, *G. mosseae* (Nicol. et Gerd.) Gerd. et Trappe, an undescribed *Glomus* sp., *Scutellospora armeniaca* Błaszk. and *Sc. dipurpurescens* Morton et Koske. It was also found with zygospores of *Endogone flammicorona* Trappe et Gerd. (*Endogonaceae*) and chlamydospores of *Complexipes moniliformis* Walker emend. Yang et Korf (*Ascomycota*), probably associated with mycorrhizae of neighbouring *Pinus sylvestris*. The chemical properties of soil samples from which *A. mellea* was isolated ranged: pH 3.8–6.2 (in 1 N KOH), NO_3 19–190, P 8–37, K 8–80, Mg 2–270, Cl 13–120 (mg/kg).

Acaulospora mellea is probably a widely distributed arbuscular mycorrhizal fungus in the world. It was previously recorded in cultivated and uncultivated soils of Florida, Massachusetts, North Carolina, Rhode Island, U.S.A. (Bever, Morton, Antonovics and Schultz 1996; Douds and Schenck 1990; Koske and Gemma 1997; Schenck, Spain and Howeler 1984; Sylvia 1986), Brazil (Grandi, Grandi and Trufem 1991; Schenck, Spain and Howeler 1984), Mexico (Estrada-Torres, Varela, Hernandez-Cuevas and Cavito 1992), Colombia (Dodd, Arias, Koomen and Hayman 1990; Saif 1987; Sieverding 1989; Sieverding and Toro 1988; Sieverding, El-Sharkawy, Hermandez and Toro 1986), Cameroon (Musoko, Last and Mason 1994) and China (Mei-ging and You-shan 1992).

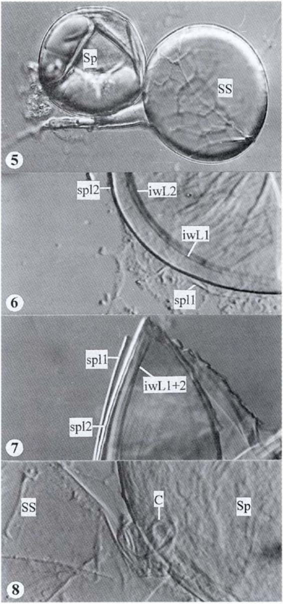
Mycorrhizal associations: In the field, *Acaulospora mellea* was associated with roots of *Ammophila arenaria, Corynephorus canescens, Crataegus monogyna, Festuca arundinacea, F. rubra, Hieracium umbellatum, Holcus mollis, Hordeum vulgare, Juncus balticus, Juniperus communis, Leymus arenarius, Lupinus angustifolius, Poa pratensis, Rosa canina, Triticum aestivum, T. secalum* and *Vicia sativa.* In pot cultures, this fungus formed arbuscular mycorrhizae with *S. sudanense* and *T. repens.*

Polish collections examined: Wybrzeże Trzebiatowskie: Mrzeżyno, 09.1987, Bł., 1471, DPP; Równina Nowogardzka: Nowogard, 09.1987, Bł., 1268–1270, DPP, and Równina Goleniowska: Glewice, all under *R. canina,* 09.1987, Bł., 1518–1522, DPP. Równina Pyrzycko-Stargardzka: Lipnik, under *T. secalum,* 07.1989, Bł., 1452–1464, DPP; under *T. aestivum,* 07.1992, Bł., u. coll., DPP; under *Hordeum vulgare, Lupinus angustifolius, Triticum secalum, Vicia sativa,* 07.1996, Bł., u. coll., DPP. Wybrzeże Słowińskie: Słowiński National Park, under *Corynephorus canescens,* 09.1994, Bł., u. coll., DPP; under *Juncus balticus,* 07.1995, Bł., u. coll., DPP; under *Hieracium umbellatum,* 08.1996, Bł., u. coll., DPP. Pobrzeże Kaszubskie: Jastrzębia Góra, under *Poa pratensis,* 11.1986, Bł., 1220–1225, DPP; under *P. pratensis,* 09.1986, Bł., u. coll., DPP; under *Crataegus monogyna,* 10.1987, Bł., u. coll., DPP. Mierzeja Helska: Chałupy, under *Rosa canina,* 07.1986, Bł., 798–797, DPP; under *Ammophila arenaria,* 09.1988, Bł., u. coll., DPP; under *Festuca arundinacea,* 09.1988, Bł., u. coll., DPP; 07.1989, Bł., u. coll., and 08.1989, under *Rosa canina,* Bł., u. coll., DPP; Kuźnica, under an unknown grass, 08.1985, Bł., u. coll., DPP; under *Ammophila arenaria,* 08.1988, Bł., u. coll., DPP; under *Crataegus monogyna,* 07.1989, Bł., u. coll., DPP. Garb Tarnogórski: Pustynia Błędowska, under *Juniperus communis,* 08.1995, Bł., u. coll., DPP; under *Leymus arenarius,* 08.1995, Bł., u. coll., DPP; under *Corynephorus canescens,* 08.1995, Bł., u. coll., DPP; under *Holcus mollis, Festuca rubra,* 06.1997, Bł., u. coll., DPP.

Spores of *Acaulospora mellea* may easily be confused with those of *A. dilatata* Morton and *A. morrowiae* Spain et Schenck due to the similarity in spore size and the structure of the spore wall and the two inner flexible walls. The presence of small pits in the laminate spore wall layer of *A. dilatata* spores (Morton 1986) seems to be the only property distinguishing this species from *A. mellea* having a smooth laminate layer. The spores of *A. mellea* compared with those of *A. morrowiae* are usually somewhat larger [average 116 in μm diam (pers. observ.), 120 μm in diam (Morton 1998)



Figs 1–4. *Acaulospora mellea.* 1. Slightly crushed spore with sporiferous saccule (SS). 2. Spore wall layers 2 and 3 (spl 2, spl 3) and two layers of inner wall 1 (iw1L1, iw1L2) and of inner wall 2 (iw2L1, iw2L2) of a spore crushed in PVLG are visible. 3. Spore wall and inner walls of a spore crushed in a mixture of PVLG and Melzer's reagent. 4. Amorphous layer (iw2L2) of the innermost wall 2 in PVLG. Fig. 1, × 1200, differential interference contrast (DIC); Figs 2–4, all × 1428, all DIC



Figs 5–8. *Acaulospora trappei.* 5. Spore (Sp) with sporiferous saccule (SS), DIC. 6–7. Spore wall layers 1 and 2 and layers 1 and 2 of inner wall. 8. Spore (Sp), cicatrix and sporiferous saccule (SS) are visible. Fig. 5, DIC, ×680; Fig. 6–8, all DIC, all ×1428

vs. 60–100 μm diam (M o r t o n 1998)] and darker-coloured [pale yellow to orange (pers. observ.), pale orange-brown to dark orange brown (M o r t o n 1998) vs. subhyaline to pale yellow-brown (M o r t o n 1998)].

Another arbuscular mycorrhizal fungus somewhat resembling *A. mellea* is *A. gedanensis* Błaszk. However, the latter species forms markedly smaller spores (55–88 μm in diam) of different properties of the inner wall layers (B ł a s z k o w s k i 1988b, 1994). The spore wall of *A. gedanensis* consists of an evanescent layer adherent to a yellow to pale brown laminate layer. According to M o r t o n (1998), the laminate layer of probably all *Acaulospora* spp. is associated with a tightly adherent, thin, flexible layer. This frequently makes impossible to recognize the layer in many species of this genus, likely including *A. gedanensis*. Compared with the two adherent semi-rigid layers of the inner wall 1 of *A. mellea* spores, the inner wall 1 of *A. gedanensis* spores is represented by a single, rigid, easily cracking layer in crushed spores. Additionally, in contrast to the beaded, flexible layer adherent to a highly plastic amorphous layer of the inner wall 2 of *A. mellea* spores, the inner wall 2 of *A. gedanensis* spores consists of two smooth, somewhat rigid layers. None of these layers stain in Melzer's reagent (vs. dark staining reaction in *A. mellea*).

Acaulospora trappei Ames et Linderman

Sporocarps unknown. *Spores* formed singly in the soil; laterally on the neck of a sporiferous saccule (Fig. 5); hyaline; globose to subglobose; (37.5-) 55.2 (-78.0) μm in diam, attached to the saccule by a small elevation (cicatrix; Fig. 8), 0.5–0.8 μm high, surrounding a hole, 5.0–6.9 μm in diam. *Subcellular structure of spores* of two walls (a spore wall and an inner wall), each comprising two hyaline layers (Fig. 6 and 7). None of these layers stain in Melzer's reagent. Spore wall layer 1 (0.5-) 0.6 (-0.7) μm thick, frequently folding in uncrushed spores mounted in lactic acid. Spore wall layer 2 (0.7-) 0.9 (-1.2) μm thick, usually adherent to layer 1 or slightly separated from this layer in crushed spores. Inner wall layers 1 and 2 ca 0.5 μm and (1.0-) 1.6 (-2.2) μm thick, respectively. *Sporiferous saccule* (Fig. 5 and 8) hyaline; globose to subglobose; 55–70 μm in diam or oblong, 50–60 × 70–80 μm; neck 80–120 μm long, tapering from 12.5–17.5 μm in diam at the saccule to 5.0–6.5 μm in diam at the point of spore attachment. Saccule wall of a hyaline, smooth, 1.0–2.5 μm thick layer (Fig. 5 and 8). Saccule collapsing at maturity and usually becoming detached in mature spores.

P o l i s h c o l l e c t i o n e x a m i n e d: Uznam and Wolin: Świnoujście – present in trap pot cultures established based on soils collected under many different dune colonizing plant species, Bł., u. coll., DPP. Wybrzeże Słowińskie: Słowiński National Park, associated with roots of

A. arenaria, 09.1993, Bł., 2224 – 2228, DPP, among roots of *Juncus articulatus*, 09.1994, Bł. 2229 – 2234, DPP, in the root zone of *J. balticus*, 08.1996, Bł. 2235 – 2240, DPP. Garb Tarnogórski: Pustynia Błędowska, among roots of *Festuca rubra*, 08.1995, Bł. 2241 – 2245, DPP, in the root zone of *J. communis*, 09.1996, Bł., u. coll., DPP.

Distribution and habitat: Of the more than 1300 field-collected soil samples, spores of *A. trappei* were only found in six (Fig. 9). Four of these samples represented dune soils of the Baltic Sea and two came from inland dunes of the Błędowska Desert located in the south of Poland (50°22'N, 19°34'E). The plant species associated with *A. trappei* were *Ammophila arenaria*, *Festuca rubra*, *Juncus articulatus*, *J. balticus*, *Juniperus communis* and *Linaria odora*.

The spore abundance of *A. trappei* in the field-collected samples ranged from 1 – 39 (average 11.7) in 100 g dry soil. The proportion of spores of the

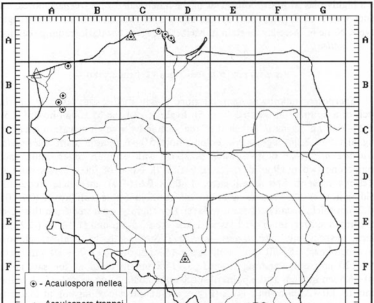


Fig. 9. Distribution of *Acaulospora mellea* and *A. trappei* in Poland.

species in the spore populations of all arbuscular mycorrhizal fungi isolated ranged from 2.2–100% (average 39.5%). The arbuscular mycorrhizal fungal species richness in the samples containing *A. trappei* ranged from 2–5 (mean 3.3) in 100 g dry soil. The fungi co-occurring with *A. trappei* in the field were *Acaulospora dilatata*, *A. koskei*, *A. lacunosa*, *Endogone maritima* Błaszk. et al., *Glomus pustulatum* Koske et al., an undescribed *Glomus* 107, *Scutellospora armeniaca* and *S. dipurpurescens*. Additionally, spores of *A. trappei* occurred in many pot cultures with *Plantago lanceolata* as the host plant established based on soils collected from dunes adjacent to Świnoujście (53°55'N, 14°14'E) and in some recently examined pot cultures representing cultivated soils of the former Szczecin voivodeship.

Despite the infrequent presence of *A. trappei* spores in the field-collected soil samples, their very frequent occurrence in pot cultures established based on soils of different uncultivated and cultivated sites indicates that the fungus is a common species of arbuscular mycorrhizal fungal communities in Poland. *Acaulospora trappei* produces spores with very delicate layers probably undergoing decomposition easily due to the activity of soil microorganisms. Hence, the soils collected during maturation of plants lacked *A. trappei* spores, although the fungus was present in plant roots subsequently used to establish pot cultures. Mycorrhizal root fragments are an important source of inoculum of arbuscular mycorrhizal fungi (Hepper 1981).

Although records of *A. trappei* are sparse, the fungus probably has a worldwide distribution. It was previously found in cultivated and uncultivated soils of California (Ames and Linderman 1976), Florida (Schenck and Kinloch 1980; Schenck and Smith 1981), Kansas (Hetrick and Bloom 1983), North Carolina (Bever et al. 1996), Oregon (Ames and Linderman 1976), U.S.A., Israel (Haas and Menge 1990) and Australia (Abbott 1982; Gazey et al. 1993; Scheltema et al. 1987; Tommerup 1988). However, none of these soils represented a dune site. Our observations suggest *A. trappei* to be a frequent inhabitant of dune soils.

Mycorrhizal associations: In the field *Acaulospora trappei* was associated with vesicular-arbuscular mycorrhizae of *Ammophila arenaria*, *Festuca rubra*, *Juncus balticus*, *J. articulatus*, *Juniperus communis* and *Linaria odora*. *Acaulospora trappei* formed vesicular-arbuscular mycorrhizae in pot cultures with *Plantago lanceolata*.

Compared with many other *Acaulospora* spp., *A. trappei* is unique due to the properties of its spore wall and flexible inner wall. We were not able to follow the ontogenesis of *A. trappei* spores. However, the position of the four layers relative to each other in crushed specimens suggests that spores of this fungus contain two walls: a spore wall and a flexible inner wall, each of which comprises two layers. Layer 1 of the spore wall is very thin and continuous with a sporiferous saccule layer. However, compared with other species of the genus *Acaulospora*, this layer is relatively permanent. It is present in most spores coming from pot cultures even more than a year old (vs. this layer is usually highly sloughed or absent in mature spores of many other *Acaulospora* spp.). Layer 2 is somewhat thicker and either adheres to layer 1 or slightly separates from it in crushed spores. This layer corresponds to a laminate layer of other *Acaulospora* spp.

Layer 1 of the flexible inner wall is very thin and usually adheres to the much thicker and more rigid innermost layer 2. Hence, it is very difficult to see. Forcibly crushing revealed this layer in some spores. This layer corresponds to a beaded layer of many other species of the genus *Acaulospora*.

Under a stereomicroscope, spores of *A. trappei* are almost indistinguishable from those of *G. laccatum* Błaszk. and *G. occultum* Walker. The latter two species form hyaline spores of similar size, whose subtending hypha frequently breaks at the spore base. Hence, the spores resemble those of *Acaulospora* spp. Examination of crushed spores under a light microscope readily separates the three fungal species. Although difficult to see, only *A. trappei* has a thin layer associated with a relatively thick innermost layer. The two layers correspond with the two-layered flexible innermost wall of most of the other species of the genus *Acaulospora*.

The spore wall of *G. laccatum* consists of a thin, sloughing outermost layer associated with a laminate layer composed of some easily separating laminae (Błaszkowski 1988a; Błaszkowski pers. observ.). *Glomus occultum* produces spores with a wall comprising three layers: a sloughing outermost layer adherent to two permanent layers similar in thickness (Walker 1982).

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Acaulospora mellea i *A. trappei*, nowe gatunki w Polsce

Streszczenie

Opisano zarodniki *Aaulospora mellea* i *A. trappei*, dwóch nowych gatunków grzybów w Polsce. Pierwszy w Polsce występuje stosunkowo rzadko; został znaleziony w 30 z ponad 1300 zbadanych prób glebowych reprezentujących różne stanowiska na ziemiach nieuprawnych i uprawnych. Zarodniki *A. trappei* wykryto tylko w sześciu próbach glebowych. Jednak występowanie tego grzyba w wielu kulturach wazonowych zawierających próby glebowe pochodzące z różnych stanowisk nieuprawnych i uprawnych wskazuje, że grzyb ten jest powszechnie obecny wśród arbuskularnych grzybów mikoryzowych Polski.