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ORIGINAL PAPER

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Occurrence of some Glomales in Finland

Abstract By using trap plants, 17 species of arbuscular mycorrhizal fungi (AMF) belonging to the order Glomales were identified in 266 soil samples collected in the period 1987–1989. Of the identified isolates, 87.1% belonged to the genus *Glomus* Tulasne & Tulasne, 8.5% to *Acaulospora* Gerdemann & Trappe, 4.1% to *Scutellospora* Walker & Sanders and 0.3% to *Entrophospora.* Of the individual species identified, *Glomus hoi* Berch & Trappe was the most frequently identified, followed by *G. fistulosum* Skou & Jakobsen and *G. mosseae* (Nicol. & Gerd.) Gerdemann & Trappe. Only small differences in AMF trapping ability were observed between the four trap plants used, *Trifolium pratense* L., *Zea mays* L., *Allium cepa* L. var. *cepa* and *Fragaria* × *ananassa* Duch. *T. pratense* was chosen for further study because it had the highest AMF sporulation index in trap cultures and it also performed better than the other plants when grown in soils with different physical and chemical properties. The proportion of soil samples where AMF were identified decreased from close to 100% in the southern and central parts of Finland to about 50% in northern Finland.

Key words Arbuscular mycorrhiza · Glomales · Trap plant · Geographical distribution

Introduction

Arbuscular mycorrhizal fungi (AMF) are widely distributed in nature. They are found in the tropics (Redhead 1977; Al-Garni and Daft 1990; Ganesan et al. 1991; Sieverding 1991) as well as in temperate (Gerdemann and Trappe 1974; Hall 1977; Walker et al. 1982) and arctic regions (Katenin 1972; Laursen and Schmielewski 1982). The distribution patterns of AMF genera vary between different climatological regions. Based on the manual of Schenck and Perez (1988), Allen (1991) surveyed the locations where species of the Glomales had been described.The genus *Glomus* Tul. & Tul. has been described in all climatic regions, but especially in temperate regions, while species of the genera *Acaulospora* Gerd. & Trappe and *Sclerocystis* Berch & Broome have been most frequently described in tropical regions. Only a few species of *Glomus* have been described in arctic and alpine locations.

Finland belongs to the temperate region but the most northern parts of the country are subarctic. No survey of the native Finnish AMF, or those of other Scandinavian countries, has been conducted, although *G. versiforme* (Karst.) Berch was originally described by Karsten (1884), according to Berch and Fortin (1983). However, Muromtsev et al. (1987) report that AMF are commonly found in the roots of agricultural crops in the soddy-podzolic soils of northwestern Russia, an area bordering on Finland.

In this present study, 266 soil samples from various parts of Finland were trapped for AMF with different mycorrhiza-forming plants. Special emphasis was placed on the distribution of AMF in different geographical regions of Finland.

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Materials and methods

Soil samples

A total of 266 soil samples were collected: in 1987 between 25 August and 22 October (52 samples), in 1988 between 13 September and 27 September (65 samples), and in 1989 between 5 September and 9 October (149 samples). Five subsamples from each location were pooled and mixed thoroughly. The majority of samples was collected from agricultural soil with grass as the standing crop (62%). The other main standing crops were cereals (16%) and wild strawberry (11%). Soil samples were taken from under each crop plant. The cultivation history of the collection sites was not recorded. The sampling area covered almost the whole of Finland, from approximately 61 to 68°N and from 22 to 30°E, and included different soil types.

Trapping of AMF

The original soil samples were diluted with steam-sterilized sand (1:1, v:v) and sown or planted with a trap plant. The soil mixture was fertilized with 0.5 g l^{-1} of Osmocote Plus (18N:11P:10K, Sierra UK Ltd.), a controlled-release (8–9 months) fertilizer. Soil samples from 1987 and 1988 were trapped for AMF by sowing seeds of onion (*Allium cepa* L. var. *cepa* 'Stuttgarter Riesen'), red clover (*T. pratense* L. 'Bjursele') and maize (*Z. mays* L. 'North Star'), or by transplanting micropropagated strawberry (*Fragaria* × *ananassa* Duch. 'Senga Sengana') in the soil-sand mixture. Soil samples from 1989 were trapped for AMF by sowing red clover in the soil mix. There were two replicate pots for each trap plant. After sowing or transplanting the trap plant, the pots were transferred to a greenhouse (22/18° C day/night, photoperiod 16/8 h day/night). When necessary, artificial light (Philips High Pressure Quicksilver HPI 400 W) was supplied. Trapping of AMF was performed during the autumn and winter of 1988, 1989 and 1990 from soil samples collected in the period 1987–1989. No extra fertilizer was given to the trap plants during trapping, which lasted 4–6 months. At the end of the trapping phase, the green parts of the plants were cut off and the pots left to dry in the greenhouse. The dry soil was stored at 8° C far a few weeks (up to 3 months) before examination of the fungi.

Identification and characterization of AMF

The trap culture substrate was first decanted and sieved through 0.5-mm and 0.074-mm sieves. Material remaining on the sieves was washed with water into Petri dishes. Spores, either single ones (from the 0.074-mm sieve) or spores adhering to roots (from the 0.5-mm sieve), were picked out of the Petri dishes under a dissecting microscope and mounted in polyvinyl alcohol-lactoglycerol (PVLG) or PVLG/Melzer's reagent (4:1, v:v). Taxonomic features of the spores were studied under a dissecting microscope and a compound microscope with a Nomarski differential interference contrast attachment (NIC). Both bright-field microscopy and NIC were used to illustrate the fungi (Figs. 1–16). Original taxonomic papers and the INVAM identification manual (Schenck and Perez 1988) were used for identification of species.

AMF spore abundance in trap culture pots was roughly estimated on a scale of 0–3 (0 = no spores, 1 = few spores, 2 = moderate number of spores, 3 = abundant spores). For the evaluation of AMF colonization, the roots of trap plants were carefully washed and then bleached in KOH and stained with acid fuchsin according to the method of Kormanik et al. (1982). The colonization percentage was determined by the gridline intersect method (Giovannetti and Mosse 1980).

Pure culture of AMF strains

Red clover and strawberry were used to establish pure cultures of AMF strains from trap cultures. A multispore inoculum consisting of 20–30 similar spores from a trap culture were placed on a small piece of paper at the bottom opening of a small funnel made of filter paper. The funnel was filled with a sterile peat-sand mixture (1:3, v:v) and placed into a bigger pot filled with the same growth substrate. The substrate was fertilized with 2 g l^{-1} bone meal and limed with Dolomite lime to pH 6.0. Red clover seeds or micropropagated strawberry plantlets were placed to grow inside the funnel. When growth had been established, plants were given a phosphorus-free Hewitt solution (Hewitt 1952) once a week. After 3–4 weeks, roots had grown out of the funnel and this was then removed. The plants were allowed to grow for a further 4 months or more, after which the morphology of spores from the resulting pot culture was studied in detail. Whenever the pure cultures contained two or more AMF strains, the funnel procedure described above was repeated to separate the strains.

Herbarium collections

As a result of this study, a collection of approximately 100 live strains of Glomales was compiled in 1994 at the Laukaa Research and Elite Plant Unit. Some of these strains have been deposited in the Banque Européenne des Glomales at the Forestry Authority, Northern Research Station, Roslin, Scotland, UK.

Results

AMF

AMF spores were found in 49% of the decanted and sieved original soil samples. After trapping with a plant, sporulation was observed in 83% of the samples. When root colonization is also taken into account, 90% of the 266 soil samples yielded AMF spores and/or colonization after trapping. Of the identified isolates, 87.1% belonged to the genus *Glomus* Tulasne & Tulasne, 8.5% to *Acaulospora* Gerdemann & Trappe, 4.1% to *Scutellospora* Walker & Sanders and 0.3% (only two isolates) to *Entrophospora* Ames & Schneider. *Gigaspora* Gerdemann & Trappe, emend. Walker & Sanders and *Sclerocystis* Berch & Broome, emend. Almeida & Schenck were not found. Spores of *Glomus* spp. were usually more numerous in trap culture soils than those of *Acaulospora, Entrophospora* or *Scutellospora.* In 37.1% of the soil samples, only one AMF species was detected. The proportions of soil samples containing two, three, four or five species were 23.9%, 16.0%, 4.9% and 1.1%, respectively.

Seventeen fungal species were identified (Table 1). In addition, six unidentified species of *Acaulospora,* two species of *Glomus* and one species of *Scutellospora* were found. Most of the identified fungi are illustrated in Figures 1–16. *G. hoi* Berch & Trappe (Fig. 1) was the most frequently identified species. It was also the only relatively common *Glomus* species found in original soil samples without trapping. *G. fistulosum* (Figs. 3, 4) was also commonly found after trapping, while *G. mosseae* (Fig. 2), *G. caledonium* (Figs. 5, 6), *G. claroideum, G. pustulatum* (Fig. 7), *G. fasciculatum* (Fig. 8), *G. rubiforme* (Fig. 10) and a species resembling *G. clarum* (Fig. 9) occurred relatively infrequently. Within the genus *Glomus,* a large number of unidentified isolates (125) with hyaline or nearly hyaline spores were found. These are either unknown species or juvenile spores of species such as *G. clarum, G. claroideum, G. fistulosum* or *G. hoi.* From the genus *Acaulospora,* only *A. scrobiculata* (Fig. 13) and another species resembling *A. laevis* (Fig. 14) were identified.

The species *G. caledonium, G. clarum, G. claroideum, G. etunicatum, G. hoi, G. fasciculatum, G. fistulosum, G. mosseae* and *G. pustulatum* were successfully grown as pure cultures in the roots of strawberry and red clover. An unidentified *Glomus* species was successfully cultured in the roots of maize, but not in strawberry or clover. *G. rubiforme* was not obtained as

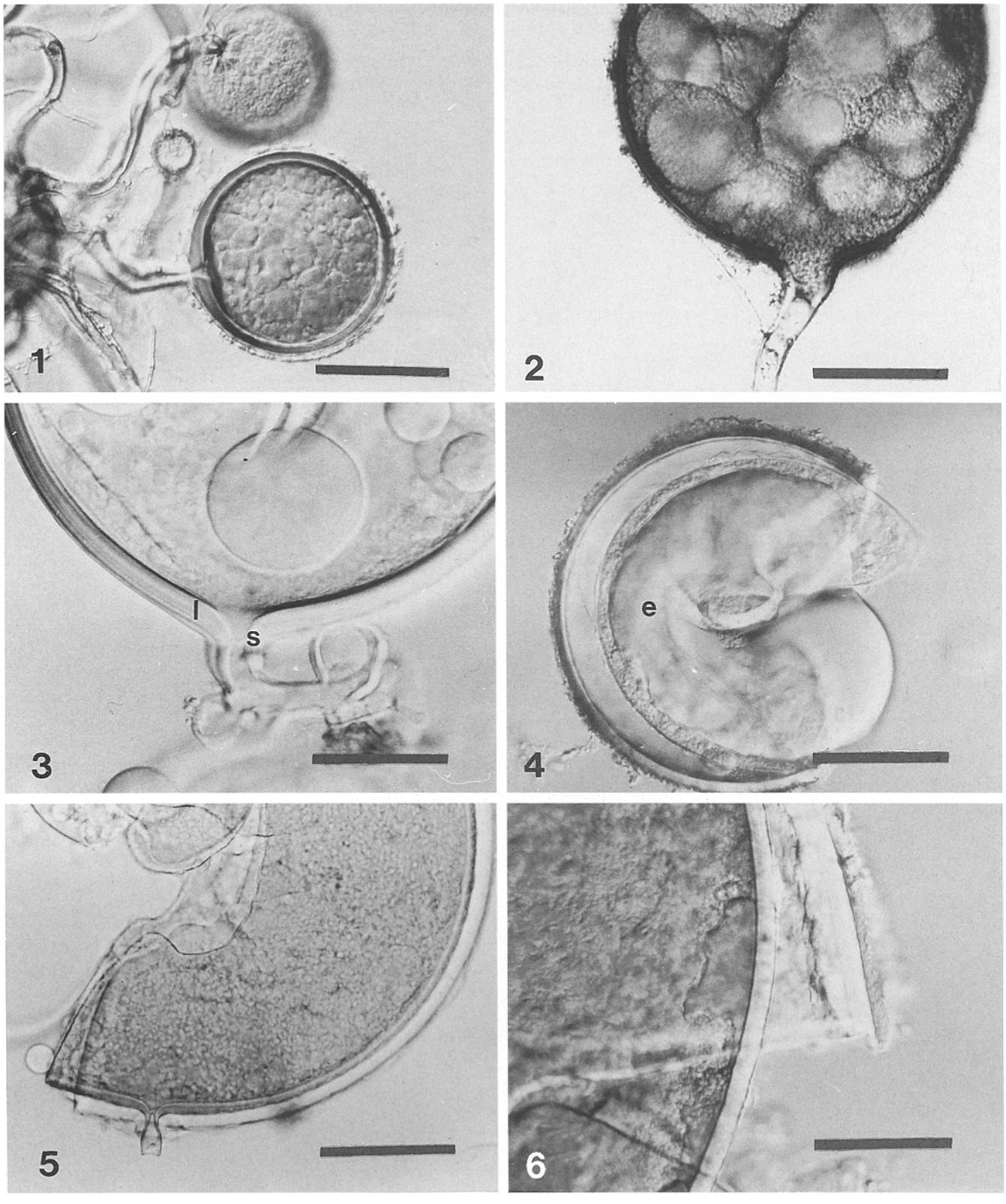

Fig. 1 Intact spores of *Glomus hoi* V98. Nomarski interference contrast (NIC); *bar* 50 μm

Fig. 2 Intact spore of *Glomus mosseae* V81 b. Bright field microscopy (BFM); *bar* 50 μm

Fig 3 Crushed young spore of *Glomus fistulosum* V128 (BEG31) showing occlusion of the spore by a septum (*s*) and laminar wall layer (*l*). NIC; *bar* 25 μm

Fig. 4 Crushed old spore of *Glomus fistulosum* V127 showing a so-called endospore (*e*). NIC; *bar* 50 μm

Fig. 5 Crushed spore of *Glomus caledonium* V111a. BFM; *bar* 50 μm

Fig. 6 Crushed spore of *Glomus caledonium* V111a showing a complex wall structure with layers in two groups. NIC; *bar* 25 μm

Table 1 Glomales species identified in trap cultures and pure cultures from 266 soil samples collected in the period 1987–1989 in Finland

Fungus	Number of times trapped
Acaulospora	
Acaulospora scrobiculata Gerd. & Trappe	5
A species resembling *A. laevis* Trappe	8
A species resembling *A. nicolsonii* Walker, Reed & Sanders	6
Acaulospora spp.	16
Entrophospora	
Entrophospora infrequens (Hall) Ames & Schneider	2
Glomus	
G. caledonium (Nicol. & Gerd.) Trappe & Gerdemann	3
G. claroideum Schenck & Smith	4
G. constrictum Trappe	1
G. etunicatum Becker & Gerdemann	1
G. fasciculatum Thaxt. emend. Walker & Koske	5
G. fistulosum Skou & Jakobsen	63
G. hoi Berch & Trappe	108
G. macrocarpum Tul. & Tul.	1
G. mosseae (Nicol. & Gerd.) Gerd. & Trappe	23
G. pustulatum Koske, Friese, Walker & Dalpe	5
G. rubiforme (Gerd & Trappe) Almeida & Schenck	9
A species resembling *G. clarum* Nicol. & Schenck	9
Glomus species 3	4
Glomus spp.	125
Scutellospora	
Scutellospora calospora (Nicol. & Gerd.) Walker & Sanders	14
Scutellospora spp.	3

a pure culture. Species of *Acaulospora* were less successfully grown as pure cultures. Only one species and one isolate of this genus, i.e. a species resembling *A. nicolsonii,* grew in the roots of red clover. Attempts to grow *Entrophospora infrequens* (Figs. 11, 12) and species of *Scutellospora* (Figs. 15, 16) failed.

Effect of trap plant

Of the trap plants used, red clover grew in all 117 soil samples collected in the period 1987–1988 (Table 2). Of the other trap plants, a small proportion died, i.e. 1.7%, 5% and 2.5% of onion, strawberry and maize, respectively. With regard to AMF sporulation in trap plant soil, onion showed the highest trapping ability by producing AMF spores in 72.5% of the trap cultures, while strawberry was the poorest (51.7%). When both root colonization and sporulation are considered, maize showed the highest trapping ability with AMF infection and/or sporulation in 82.5% of the samples studied (Table 2). In 1989, only red clover was used as trap plant and a very high proportion of trap cultures (96%) were mycorrhizal that year.

On a quantitative AMF sporulation scale from 0 to 3 (Table 2), red clover had the highest average index (0.96), followed by maize (0.75), onion (0.56) and strawberry (0.48). In the trap cultures of 1987 and 1988, red clover trapped the genera *Acaulospora, Glomus* and *Scutellospora,* onion and strawberry trapped *Acaulospora* and *Glomus,* and maize trapped only *Glomus.*

Geographical distribution of AMF

To evaluate the distribution of AMF in different parts of Finland, the country was roughly divided into four areas: (1) southern Finland, (2) the Lake District including eastern Finland, (3) western Finland and (4) northern Finland. The numbers of soil samples collected from different areas varied considerably. With red clover as the trap plant, the proportion of samples in which AMF fungi was obtained was highest in the Lake District (92%), southern and western Finland (90%) and lowest in northern Finland (about 50%). When all four trap plants are taken into consideration, the corresponding values for the four areas 1–4 were 100%, 96%, 92% and 50%, respectively.

Effect of standing crop on AMF distribution

Soil samples were collected from three main standing crops. Two crops were agricultural, viz. grass mix and cereal, and one was wild strawberry. Of the samples collected from soil under wild strawberry, 96% yielded AMF, while the soils under agricultural crops yielded less AMF, 78% and 72% under grass and cereal, re-

Table 2 Effect of trap plant on the occurrence of arbuscular mycorrhizal fungi spores and on the sporulation index according to a scale from 0 to 3

Parameter	Trap plant			
	Onion	Red clover	Maize	Strawberry
Trap plant survival (%)	98.3	100.0	97.5	95.0
Sporulating trap cultures (%)	72.5	54.2	68.3	51.7
Trap cultures sporulating and/or with root colonization (%)	77.5	80.8	82.5	66.7
Sporulation index	0.56	0.96	0.75	0.48

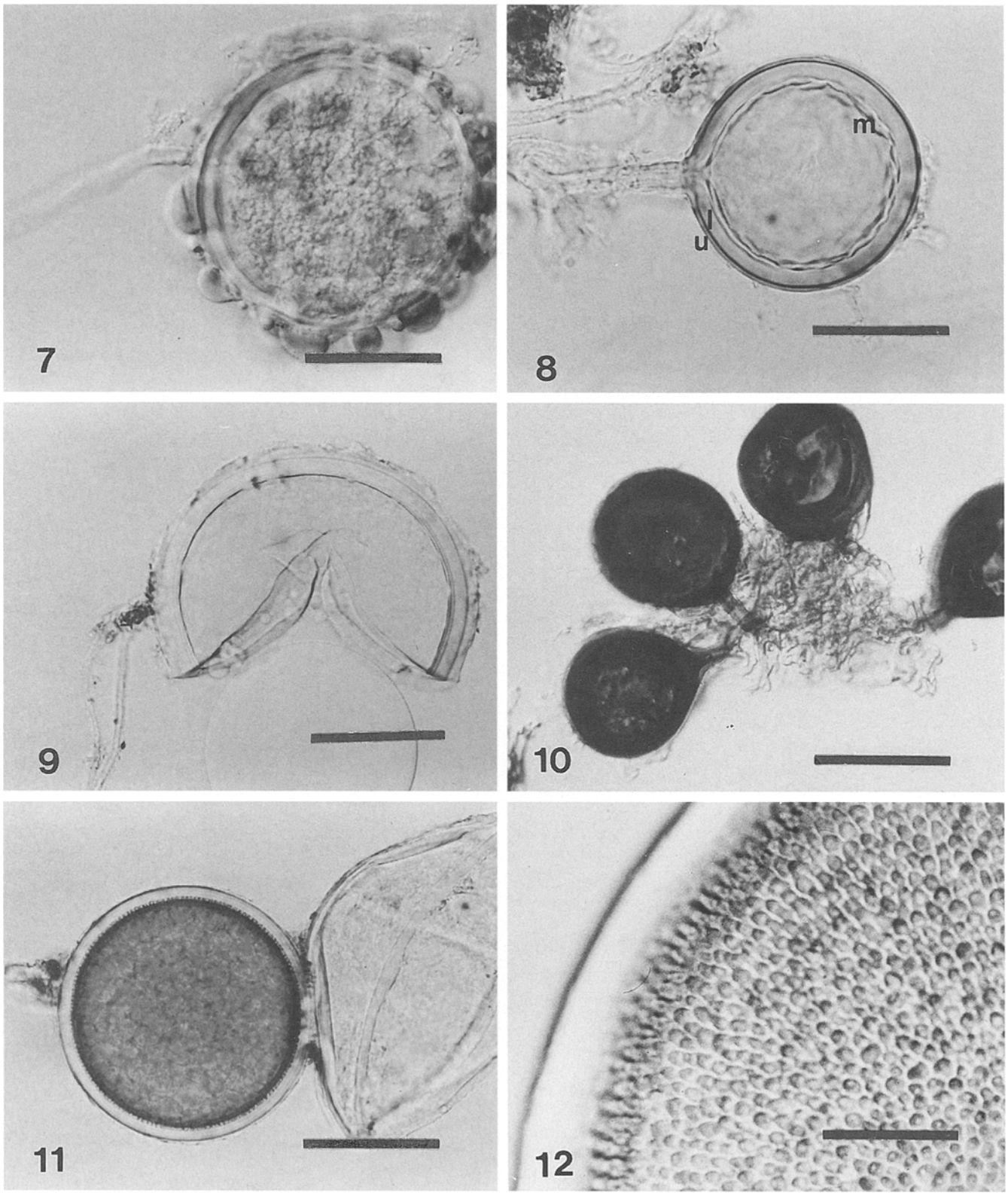

Fig. 7 Intact spore of *Glomus pustulatum* V122a showing blister-like thickenings at the spore surface. BFM; *bar* 25 μm

Fig. 8 Intact spore of *Glomus fasciculatum* V122c showing a thick wall of three layers, a unit wall layer (*u*), a laminar wall layer (*l*) and an innermost membranous layer (*m*). BFM; *bar* 50 μm

Fig. 9 Crushed spore of a *Glomus clarum*-like fungus (V106) showing a thick and complex wall of several layers. BFM; *bar* 50 μm

Fig. 10 Sporocarpic spores of *Glomus rubiforme* V38b. BFM; *bar* 50 μm

Fig. 11 *Entrophosphora infrequens* V122e showing a mature spore and a collapsed sporiferous saccule. BFM; *bar* 50 μm

Fig. 12 Ornamentations of the spore wall surface of *Entrophospora infrequens* V122e. NIC; *bar* 10 μm

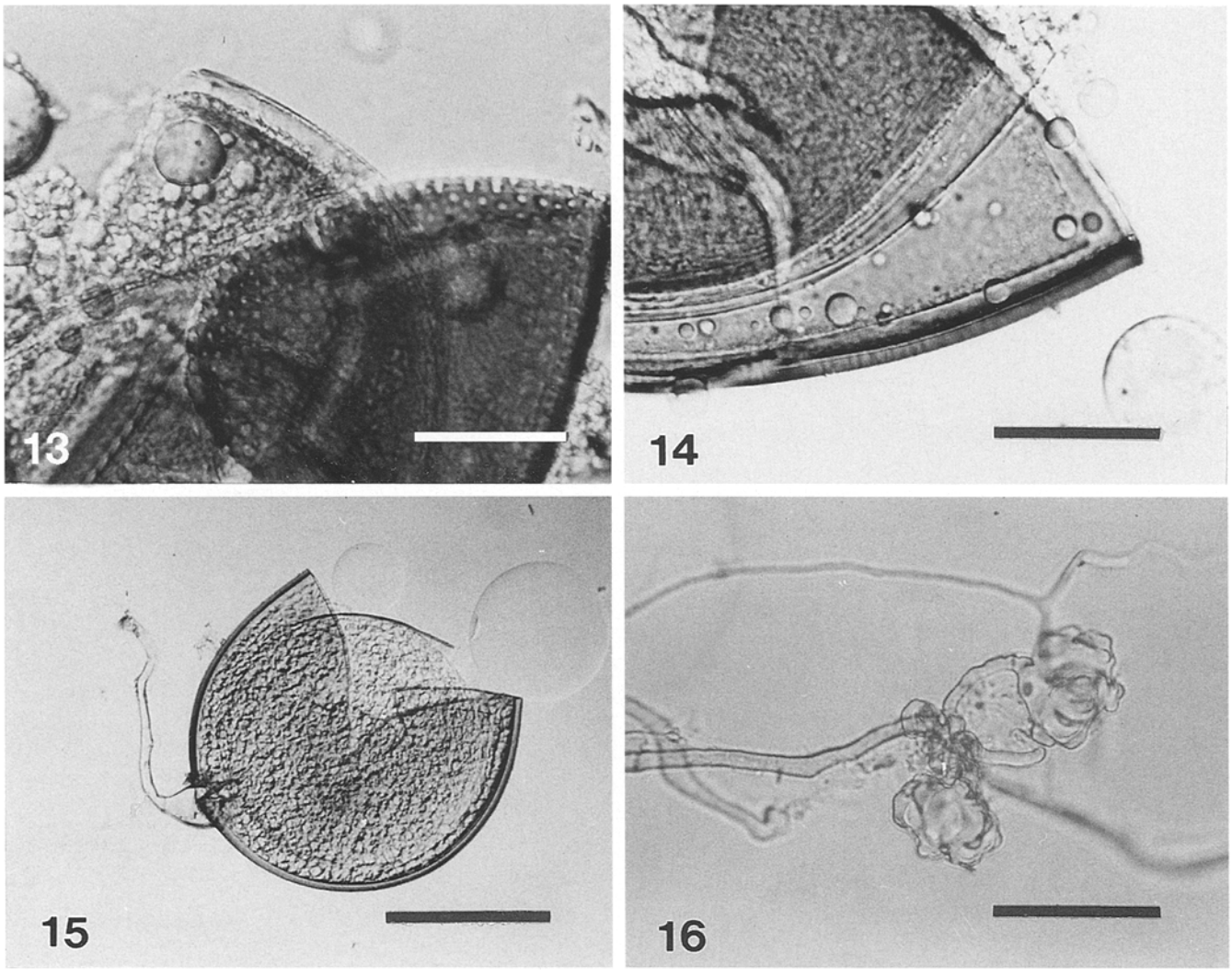

Fig. 13 Crushed spore of *Acaulospora scrobiculata* V55b showing the complex wall structure of this fungus. NIC; *bar* 25 μm

Fig. 14 Crushed spore of an *Acaulospora laevis*-like fungus (V69) showing the complex wall structure of this fungus. NIC; *bar* 25 μm

Fig. 15 Crushed spore of *Scutellospora* sp. V39/87. NIC; *bar* 100 μm

Fig. 16 Knobby auxiliary cells of a *Scutellospora* sp. BFM; *bar* 25 μm

spectively. Of the individual fungal species, *G. hoi* was associated with wild strawberry more than grass and cereal (Fig. 17). However, *G. mosseae* was trapped more frequently in grassland and cereal soils than in wild strawberry soil. *Acaulospora* was not found in the strawberry soil, but in approximately 10–15% of the grass and cereal soils (Fig. 17). The genus *Scutellospora* was found more frequently in grassland soil (8.6%) than in cereal (2.3%) and wild strawberry (3.6%) soils.

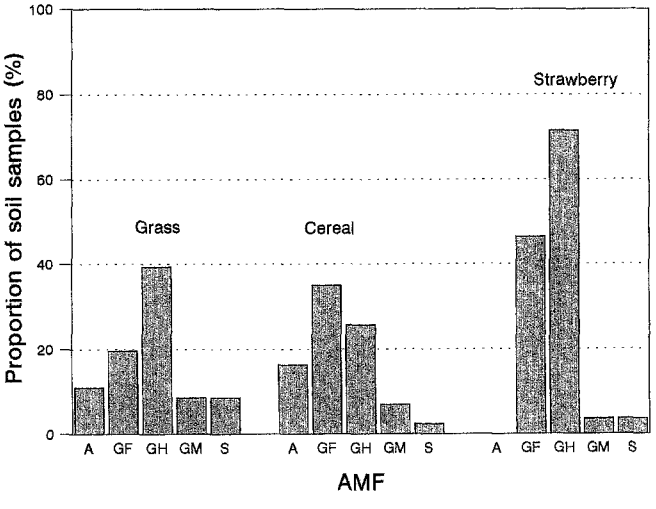

Fig. 17 Effect of standing crop (grass, cereal and wild strawberry) on the proportion of soil samples where different arbuscular mycorrhizal fungi (*AMF*) were trapped. (*A Acaulospora* spp., *GF Glomus fistulosum, GH Glomus hoi, GM Glomus mosseae, S Scutellospora* spp.)

Discussion

This survey shows that AMF (Glomales) are extensively distributed in Finland. Of the identified isolates, almost 90% belonged to the genus *Glomus*. This is not surprising as *Glomus* has been reported to be the most common AMF genus globally (Stahl and Christensen 1982) and predominant in temperate regions (Gerdemann and Trappe 1974; Miller et al. 1985; Błaszkowski 1989; Kühn 1992).

The AMF species identified here all originated from pot cultures of original soils with four different trap plants. Therefore, the results are not directly comparable with those obtained in surveys done by sieving or centrifugation of in situ soil samples, or by collection of sporocarps from the soil surface (Tandy 1975). As pointed out by Miller et al. (1985), pot cultures with trap plants may reveal species not recovered by sieving and vice versa.

Most of the AMF species identified in this study, for example *G. mosseae, G. clarum, G. fasciculatum, G. etunicatum, A. laevis* and *A. scrobiculata* occur all over the world. However, surprisingly, the species most commonly found in this study, *G. hoi,* has been reported only from America (Berch and Trappe 1985). *G. hoi* closely resembles *G. aggregatum* and *G. intraradices,* and this may explain the few findings of this fungus. *G. fistulosum* is another rarely reported but, in this study, commonly occurring fungus. This species was first found in arable land in Denmark by Skou and Jakobsen (1989), but has been found also in the Czech Republic (M. Gryndler, personal communication) and the UK (C. Walker, personal communication). *G. fistulosum* resembles closely both *G. maculosum* and *G. claroideum. Entrophospora infrequens,* a controversial AMF species which has never been grown as a pure culture, was found under red clover. In a Canadian study, this species was also observed in connection with red clover (Boyetechko and Tewari 1987).

The plants chosen for trapping AMF in this study (red clover, maize, onion and strawberry) are all well-known for their good trapping ability. Therefore, it is not surprising that their ability to trap AMF was rather similar. However, red clover was overall the best plant to use because it performed well in soils with different physical and chemical properties and, moreover, produced the highest amounts of spores in the pot cultures. The reasons for this are not known.

The proportion of soil samples from which AMF could be trapped decreased considerably when going from southern to northern Finland. Apart from the considerable differences in temperature and in lengths of growing season, there are also edaphic differences and differences in host plant composition between these areas; for example, no soil was collected under wild strawberry in northern Finland. Therefore, no single factor can be distinguished as the most important one. Temperature may be important, as reported by Koske (1987), who found that the mean AMF species abundance was positively correlated with increasing temperature in a sand dune study in the USA. In a survey of AMF on Nigerian vegetation types, Redhead (1977) found that AMF were more frequent in savannas than in lowland rain forests. However, Miller et al. (1985) found no evident patterns in the geographical distribution of AMF associated with 18 different apple rootstocks in the USA.

In this present study, the effect of the standing crop is indirect as the trap plants were not the same as the standing crop, with the exception of strawberry. Despite the small number of standing crops, some conclusions can be made. Strawberry was the only wild plant used, and a higher proportion of AMF-positive soil samples were collected from under wild strawberry than from under the cultivated crops. This agrees with other studies where spores were found in greater numbers under wild plants than under cultivated plants (Hetrick and Bloom 1983; Miller et al. 1985; Błaszkowski 1989), but the converse has also been reported (Mosse and Bowen 1968; Schenck and Kinloch 1980). Furthermore, Schenck and Segueira (1987) found more spores per unit of soil in agroecosystems than in native ecosystems, but a greater diversity of spores in the native ecosystems than in the agroecosystems.

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