The occurrence of arbuscular mycorrhizal fungi (Glomerales) in cultivated soils of Poland

JANUSZ BŁASZKOWSKI, ANNA IWANIUK, BEATA
CZERNIAWSKA

Department of Plant Pathology, University of Agriculture, Słowackiego 17, 71-434 Szczecin, Poland

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Summary

The occurrence of arbuscular mycorrhizal fungi (AMF) in cultivated soils of Poland was characterized based on 15-year investigations. Spores of AMF were isolated from both field-collected root-rhizosphere soil mixtures and trap cultures established with a part of these mixtures. The mixtures were collected from under 41 plant species. The plant species most frequently sampled were *Hordeum vulgare*, *Triticum aestivum*, and *Zea mays*. Spores of AMF were found in 97.5% of the field-collected root-soil samples and in 95.5% of trap cultures. The AMF predominating in populations of the spores revealed were members of the genus *Glomus*. The spores recovered belonged to 36 species. The AM fungal species most frequently occurring in cultivated soils of Poland were *Glomus caledonium*, *G. constrictum*, *G. deserticola*, and *G. mosseae*.

Key words: arbuscular mycorrhizal fungi, Glomerales, Glomeromycota, cultivated soils, Poland

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) of the order Glomerales, phylum Glomeromycota commonly occur in cultivated soils of different regions of the world (Błaszowski, 1991; Gianinazzi et al., 1989; Schenck and Kinloch, 1980). The fungi increase the growth and productivity of plants (Gerde mann, 1968), as well as their resistance to many disease causal agents and nematodes

(Schönbeck, 1978). Of the cultivated plants, only members of the families *Brassicaceae* and *Chenopodiaceae* have so far been considered to be non-mycorrhizal or to form infrequently mycorrhizae with these fungi (Gerde, 1968). However, results of recent investigations showed that a large part of AMF of different ecosystems is represented by non-sporulating species (Błaszowski et al., 2002; Stutz and Morton, 1996). Additionally, some species of AMF form mycorrhizae not reacting with the commonly used stains and, hence, may be omitted (Morton and Redecker, 2001).

According to Stutz and Morton (1996), an effective method initiating sporulation and, thereby, enabling to reveal the presence and species composition of the AMF associated is culturing of field-collected root-rhizosphere soil mixtures in trap cultures.

Therefore, beginning from 1996, the occurrence of AMF in cultivated sites of Poland was investigated based on both spores recovered from field-collected root-rhizosphere soils and trap cultures.

The aim of this paper is to present the occurrence of AMF associated with cultivated plants of Poland based on 15-year investigations.

MATERIALS AND METHODS

About 90% of the root-rhizosphere soil mixtures examined were collected during florescence or maturation of plants. The mixtures were excavated from a depth of 5-30 cm using a small trowel or a sampling stick. A 0.3-0.5-L mixture represented each plant. The mixtures were placed in plastic bags and then stored at 4°C for 2-6 months until processed. To 1996, spores of AMF were isolated only from field-collected root-soil samples. Later, 100 g of each of the root-soil mixture collected was used to determine the abundance of spores and the richness of species produced in the field. The other part was divided into three equal portions that were then mixed with an autoclaved coarse-grained sand (1:1 v/v). The mixtures were placed in 0.5-L plastic pots and separately seeded with *Plantago lanceolata*, *Sorghum vulgare*, and *Zea mays*. Hence, each plant growing in the field was represented by one root-soil sample collected from the field and three trap cultures.

The cultures were grown in a greenhouse for four months with supplemental 16-h lighting provided by sodic lamps placed 1 m above pots. Plants were watered 2-3 times a week. No fertilization was applied.

Spores of AMF were isolated from the root-soil mixture by wet sieving and decanting (Gerde and Nicolson, 1963). Both intact and spores crushed in polyvinyl/alcohol/ glycerin (PVLG) and a mixture of PVLG and Melzer's reagent were investigated.

The fungi were identified according to their original descriptions (Schönbeck and Pérez, 1990), revisions (Morton, 1995; Frank and Morton, 1994; Stürmer and Morton, 1997), information and specimens obtained from Prof. R. E. Koske (Rhode Island University, U.S.A.), Prof. J. M. Morton (West

Virginia University, U.S.A.), Prof. J. M. T r a p p e (Oregon State University, U.S.A.), and Dr. C. W a l k e r (U.K.). Vouchers of all the fungal species recovered are preserved in the authors' collections. Nomenclature of the fungi mentioned in this paper follows W a l k e r and T r a p p e (1993). The classification is that of S c h ü s s l e r et al. (2001).

Differences in the structure of arbuscular fungal communities were investigated by determining the frequency of occurrence of species, spore abundance and species richness and by calculating dominance coefficients (G ó r n y and G r u m a, 1981). Frequency of occurrence was calculated by determining the percentage of field-collected samples and trap cultures from which spores of a particular species were recovered. Spore abundance and species richness were defined by determining the number of spores and species, respectively, occurring in 100 g dry soil. Dominance coefficient expresses the proportion of the number of spores of a particular species in all spores of AMF recovered. Spore abundance, species richness, and coefficients of dominance were determined based on spores isolated only from field-collected samples.

RESULTS

In the years 1985-2000, a total of 335 mixtures of roots and rhizosphere soils were collected from different cultivated sites of the former 11 voivodeships of Poland. About 90% of the mixtures came from the former Szczecin and Gdańsk voivodeships. The mixtures were collected from under 41 plant species. The plant species most frequently sampled were *Hordeum vulgare*, *Triticum aestivum*, and *Zea mays*.

Spores of AMF were found in 97.5% of the field-collected root-soil samples and in 95.5% of trap cultures established from the root-soil mixtures collected in 1996-2000. The spores represented all of the existing genera of the order Glomerales (Table 1). The AMF predominating in populations of spores revealed in both the field-collected root-soil samples and trap cultures were members of the genus *Glomus*. Fungi of the genus *Acaulospora* ranked second.

The spores recovered belonged to 36 species of AMF (Table 1), i. e., ca. 21.2% of all the taxa described in the phylum Glomeromycota. The species most frequently found in the field samples were *Glomus deserticola* (present in 76.5% samples), followed by *Gl. mosseae* (59.3%), *Gl. constrictum* (43.9%), *Gl. caledonium* (34.1%), and *Gl. geosporum* (31.8%). Six species, i. e., *Archaeospora trappei*, *Gl. clarum*, *Gl. intraradices*, *Gl. laccatum*, *Gl. spurcum* and *Gl. verruculosum*, sporulated only in trap cultures.

Taking into account all the spores of AMF recovered from field samples, the eudominants (coefficient of dominance >10.0%) were *Gl. claroideum*, *Gl. deserticola*, *Gl. dominikii*, *Gl. fasciculatum*, and *Gl. mosseae*. *Glomus caledonium*, *Gl. constrictum*, and *Gl. geosporum* classified as dominants (D=5.1-10.0%). The group of subdominants (D=2.1-5.0%) was formed by *Gl. etunicatum*, *Gl. macrocarpum*, and *Gl. microcarpum*.

Table 1.

The occurrence of arbuscular mycorrhizal fungi in field-collected root-soil samples and trap cultures

Fungal species	Frequency of occurrence	
	field samples	trap cultures
	(%)	
<i>Acaulospora bireticulata</i> Rothwell et Trappe	0.6	-
<i>Acaulospora capsicula</i> Błasz.	2.9	0.6
<i>Acaulospora cavernata</i> Błasz.	2.9	-
<i>Acaulospora lacunosa</i> Morton	1.7	-
<i>Acaulospora paulinae</i> Błasz.	3.5	0.6
<i>Acaulospora polonica</i> Błasz.	1.7	-
<i>Acaulospora mellea</i> Spain et Schenck	1.7	-
<i>Acaulospora thomii</i> Błasz.	0.6	0.6
<i>Archaeospora trappei</i> (Ames et Linderman) Morton et Redecker	-	3.7
<i>Entrophospora infrequens</i> (Hall) Ames et Schneider	2.3	0.6
<i>Gigaspora gigantea</i> (Nicol. et Gerd.) Gerd. et Trappe	2.3	-
<i>Glomus aggregatum</i> Schenck et Smith emend. Koske	8.7	0.6
<i>Glomus caledonium</i> (Nicol. et Gerd.) Trappe et Gerd.	34.1	40.1
<i>Glomus clarum</i> Nicolson et Schenck	-	0.6
<i>Glomus claroideum</i> Schenck et Smith	11.7	48.8
<i>Glomus constrictum</i> Trappe	43.9	21.6
<i>Glomus deserticola</i> Trappe, Bloss et Menge	76.5	48.2
<i>Glomus dominikii</i> Błasz.	30.3	21.6
<i>Glomus etunicatum</i> Becker et Gerd.	17.9	1.2
<i>Glomus fasciculatum</i> (Thaxter) Gerd. et Trappe emend. Walker et Koske	30.1	2.5
<i>Glomus fuegianum</i> (Spegazzini) Trappe et Gerd.	2.9	-
<i>Glomus geosporum</i> (Nicol. et Gerd.) Walker	31.8	4.3
<i>Glomus heterosporum</i> Smith et Schenck	6.9	-
<i>Glomus intraradices</i> Schenck et Smith	-	0.62
<i>Glomus laccatum</i> Błasz.	-	6.2
<i>Glomus macrocarpum</i> Tul. et Tul.	24.9	-
<i>Glomus microcarpum</i> Tul. et Tul.	6.4	-
<i>Glomus mosseae</i> (Nicol. et Gerd.) Gerd. et Trappe	59.5	66.7
<i>Glomus occultum</i> Walker	5.8	-
<i>Glomus pansihalos</i> Berch et Koske	2.9	1.2
<i>Glomus przewliscense</i> Błasz.	0.6	-
<i>Glomus spurcum</i> Pfeiffer, Walker et Bloss emend. Kennedy, Stutz et Morton	-	1.2
<i>Glomus verruculosum</i> Błasz.	-	0.6
<i>Scutellospora dipurpurea</i> Morton et Koske	9.3	4.3
<i>Scutellospora nodosa</i> Błasz.	0.6	-
<i>Scutellospora pellucida</i> (Nicol. et Schenck) Walker et Sanders	2.9	-

Of the plant species most frequently sampled in the field (Table 2), most spores of AMF were harboured by *Fragaria vesca* (av. 115.7 spores in 100 g dry soil), followed by *Triticum aestivum* (97.4), *Beta vulgaris* (89.9), and *Zea mays* (57.4). Most species of AMF occurred among roots of *T. aestivum* (av. 3.7 in 100 g dry soil), then *Z. mays* (3.4), *T. secalum* (3.3), and *Vicia faba* (3.0).

Considering the results of isolations of AMF from both the field samples and trap cultures, the AM fungal species most frequently occurring in cultivated soils of Poland are *Gl. caledonium*, *Gl. claroideum*, *Gl. constrictum*, *Gl. deserticola*, and *Gl. mosseae*.

Table 2.

Spore abundance* and species richness* of arbuscular mycorrhizal fungi among 12 most frequently examined plant species

Plant species	n	Spore abundance	Species richness
<i>Avena sativa</i>	20	54.0	5.3
<i>Beta vulgaris</i>	21	89.9	2.3
<i>Festuca ovina</i>	7	101.4	3.3
<i>Fragaria vesca</i>	7	115.7	2.0
<i>Hordeum vulgare</i>	23	40.7	2.8
<i>Lupinus luteus</i>	4	87.3	4.8
<i>Medicago sativa</i>	4	46.8	3.8
<i>Trifolium pratense</i>	9	69.6	4.1
<i>Triticum aestivum</i>	101	97.4	3.7
<i>Triticum secalum</i>	18	43.6	3.3
<i>Vicia faba</i>	6	43.4	3.0
<i>Zea mays</i>	35	57.4	3.4

*in 100 g dry soil

n – number of root-soil mixtures investigated

DISCUSSION

The results of the 15-year investigations of the occurrence of AMF in cultivated soils of Poland characterized here support Błażkowski's (1991) and Gerde m a n n's (1968) conclusions that members of the order Glomales belong to the most common soil fungi of the world and are associated with most vascular plants.

According to Gerde m a n n (1968), of cultivated plants, only species of the families *Brassicaceae* and *Chenopodiaceae* infrequently or do not at all form mycorrhizae with fungi of the order Glomerales. Błażkowski (1991) supported this opinion following the investigations of the occurrence of AMF in 173 root-rhizosphere soil mixtures collected from under 39 plant species cultivated in the former 11 voivodeships of Poland. These findings were based on investigations of the occurrence of mycorrhizal structures in roots treated with different stains and the isolation of spores of AMF from field-collected root-rhizosphere soil mixtures. However, mycorrhizae formed by some species of AMF do not react with commonly used stains (Morton and Redecker, 2001) and a large part of AMF does not

sporulate in the field or their sporulation is seasonal (Błaszowski et al., 2002; Gemma et al., 1989; Stützel and Morton, 1996). One of the methods enabling to reveal the existence of mycorrhizal association is culturing of field-collected root-rhizosphere soil mixtures in trap cultures (Stützel and Morton, 1996). This method used in this study showed that, e. g., *Brassica napus* and *B. oleracea* (*Brassicaceae*; data not presented here) yielded abundant and diverse populations of AMF in trap cultures, despite they did not harbour any spores of these fungi in the field. Additionally, in trap cultures, six species of AMF were revealed that did not produce spores in the field. Among them, *Archaeospora trappei* and *Glomus laccatum* occurred, i. e., fungi forming mycorrhizae not reacting in trypan blue, the most frequently used stains. Thus, the results of this study are in line with the motto of the COST Action 838 meeting recently held at Faro (Portugal) saying that „plants do not have roots, but have mycorrhizae”.

The dominance of members of the genus *Glomus* in cultivated soils of Poland supports earlier reports of a good adaptation of these fungi to a wide range physical and chemical conditions (Anderson et al., 1984; Haas and Menge, 1990; Porter et al., 1987). Daniels and Trappe (1980) found that the optimal temperature for germination of spores of *Glomus* spp. was 14-22°C, i. e., a temperature range of a vegetative period of Poland. Pirozynski (1968) hypothesized that temperature is the major factor determining the distribution and occurrence of fungi in general.

Considering the results of isolations of AMF from both the field samples and trap cultures, the AM fungal species most frequently occurring in cultivated soils of Poland are *Glomus claroideum*, *Gl. constrictum*, *Gl. deserticola*, and *Gl. mosseae*. These fungal species have also many times been found in cultivated soils of other regions of the world (Błaszowski, 1991; Vestberg, 1995).

Stahl and Christensen (1991) suggested that a broad distribution of species of AMF results from their genetic adaptability which leads to genetic differentiation between populations. Hence, the apparent tolerance to agrochemical practices of the fungal species found in this study is probably relatively stable. Thus, they should theoretically be the most appropriate AMF to use in inoculations of agricultural soils of Poland.

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Występowanie arbuskularnych grzybów mikoryzowych (Glomerales) w glebach uprawnych Polski

Streszczenie

Scharakteryzowano występowanie arbuskularnych grzybów mikoryzowych (AGM) w glebach uprawnych Polski na podstawie 15-letnich badań. Zarodniki AGM izolowano z mieszanin korzeni i gleby ryzosferowej zebranych z pola i kultur pułapkowych utworzonych z częścią tych mieszanin. Mieszaniny korzeni i gleby pobrano spod 41 gatunków roślin. Roślinami najczęściej badanymi były *Hordeum vulgare*, *Triticum aestivum* i *Zea mays*. Zarodniki AGM znaleziono w 97,5% prób zebranych z pola i 95,5% kultur pułapkowych. Grzybami dominującymi w populacjach wyodrębnionych zarodników byli przedstawiciele rodzaju *Glomus*. Wyizolowane zarodniki należały do 36 gatunków. Gatunkami AGM najczęściej występującymi w glebach uprawnych Polski były *Glomus caledonium*, *G. claroideum*, *G. constrictum*, *G. deserticola* i *G. mosseae*.