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Arbuscular mycorrhizal fungi (Glomeromycota) associated with roots of plants of the Lubuskie province

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The results of studies of the occurrence of arbuscular mycorrhizal fungi (AMF) and arbuscular mycorrhizae of the phylum Glomeromycota associated with roots of 31 cultivated, uncultivated and protected plant species growing at 103 sites of the Lubuskie province NW Poland are presented and discussed. The AMF most frequently found were members of the genus *Glomus*. Other relatively frequently revealed fungi were *Scutellospora* spp. Spore populations of AMF generally were more abundant and diverse in cultivated soils. Most protected plant species harboured AMF.

Key words: distribution, occurrence, cultivated, wild and protected plants, Poland

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) of the phylum Glomeromycota (Schüßler, Schwarzott and Walker 2001) belong to the most widely distributed soil micro-organisms in the world and are associated with ca. 70-90% of vascular land plants (Smith, Read 2008). At present, the phylum Glomeromycota consists of four orders, 10 families and 14 genera (Oehl, Sieverding 2004; Palenzuela et al. 2008; Schüßler et al. 2001; Sieverding, Oehl 2006; Walker et al. 2007). The most numerous group within the Glomeromycota is the genus *Glomus*, comprising ca. 53% of all species of the phylum known to date, i.e., ca. 220 (Błaszkowski 2003).

AMF are well known to increase productivity and vigour of plants, as well as their resistance to different abio- and biotic stresses (Schönbeck 1978; Koske et al. 2004). Additionally, AMF stabilize soils and improve their structure throughout binding sand grains and aggregate formation (Koske, Polson 1984). The effectiveness and stability of such influences are generally higher when AMF communities are more diverse (Bever et al. 1996; Klironomos et al. 2000).

Asexual spores of AMF are persistent propagules that remain infectious in the absence of host plants and under unfafourable conditions, e.g., because of influences of different agricultural practices. On the other hand, different plant species and farming practices may variously affect the spore production of different species of AMF (Bever et al. 1996; Jansa et al. 2002; Oehl et al. 2009). Recognition of the reasons that mainly affect AMF may be used in constructing of crop rotations and farming practices to retain high vitality of this important group of soil microorganisms.

Most studies of AMF diversity rely on morphological identification of spores extracted either from field-collected soil samples or/and trap cultures in which field soils with appropriate host plants are grown in a greenhouse (Oehl et al. 2009). However, each of these techniques has constrains. Examination of field soils frequently reveals AMF that do not sporulate in trap cultures (Błaszkowski, pers. observ.). On the other hand, cultivation of field soils in trap cultures may initiate sporulation of fungi forming spores seasonally, rarely or not at all in field conditions (Błaszkowski, Kovács and Balázs 2009; Błaszkowski, Tadych and Madej 2000; Błaszkowski et al. 2009; Stutz, Morton 1996). An ideal method of recognizing of AMF associated with plant roots seems to be that using molecular tools. However, its high costs and labor, as well as the lack of specific molecular markers for most existing AMF also restrict their wide application (Öpik et al. 2009).

One of the main aims of numerous plant protection projects are the preservation and protection of rare and endangered plant species (Zubek, Turnau and Błaszkowski 2005). Most of these plants probably co-occur with AMF, although the amount of literature data on this subject is very low. The recognition of AMF most frequently co-existing with such plant species may be used to protect them by introduction of these fungi into sites where the plants grow.

In soils of the Lubuskie province, only one species of AMF has been found to date, i.e., *Acaulospora thomii* Błaszk. (Błaszkowski 1988). Therefore, the aim of this study was to better know AMF associated with roots of cultivated, uncultivated (not protected) and protected plants growing in soils of this region.

MATERIALS AND METHODS

The study material and area. The study material were mixtures of rhizosphere soils and roots collected under 31 cultivated, wild (not protected), and protected plant species growing at 103 sites located in the Lubuskie province (Fig. 1).

Collection of rhizosphere soils and roots, establishment and growth of trap cultures, extraction of spores, staining of mycorrhizae, and identification of AMF. Rhizosphere soils and root fragments were collected from May to August of the years 2003-2006. They were taken from a depth of 5-30 cm using a small garden shovel and then placed in plastic bags. In the laboratory, they were air dried and then stored in a refrigerator at ca. 4°C for 1-4 months.

Trap cultures were established to obtain a large number of living spores and to initiate sporulation of species that were present but were not detected in the field collections (Stutz, Morton 1996). The method used to establish trap cultures, their

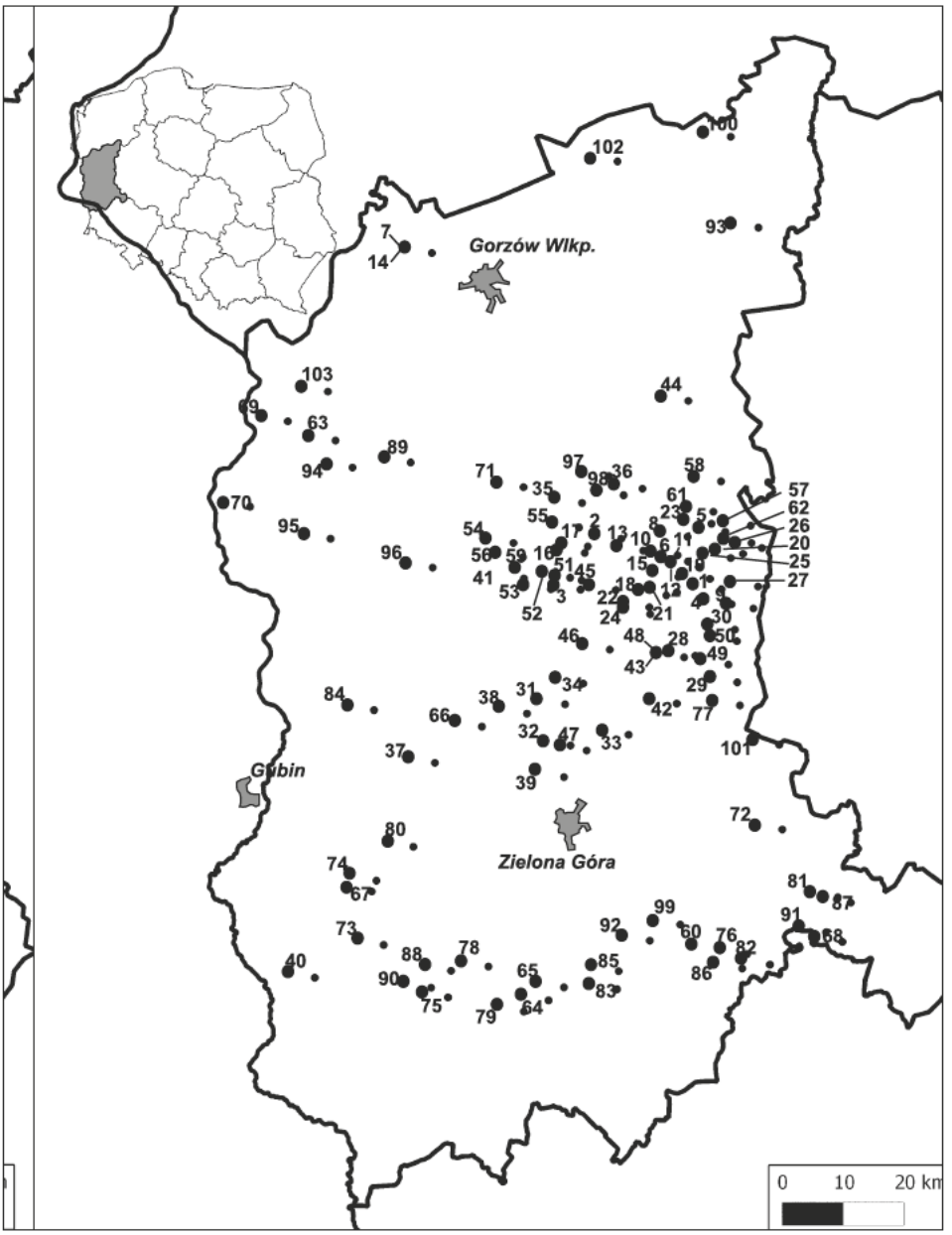

Fig. 1. Sites of collection of the rhizosphere soil and roots of cultivated, uncultivated (not protected), and protected plants in the Lubuskie province.

Cultivated plants. *Asparagus officinalis* L.:* 26; 27; Trzciel; Lutol Mokry; 25.04.2004; 25.05.2004; 43-45, 126-128; 46-48; *Avena sativa* L.; 4; 5; 6; 31; 32; 33; 72; 73; 74; Dąbrówka Wlkp.; Sierczynek; Łagowiec; Przetocznica; Brody; Mozów; Lipka; Jasień; Godno; 2.07.2003; 3.08.2003; 2.08.2003; 12.08.2004; -"-; -"-; 18.06.2005; -"-; -"-; 4; 5; 6; 57; 58; 59; 136; 137; 138; *Brassica napus* L.: 1; 6; 12; 15; 19; 64; 65; 66; 67; Rogoziniec; Łagowiec; Lutol Suchy; Brójce; Chociszewo; Jabłonów; Wrzesiny; Radnica; Górzyn; 25.04.2004; -"-; -"-; -"-; -"-; 18.06.2005; -"-; -"-; -"-53; 51; 50; 49; 52; 129; 130; 131;

132; *Beta vulgaris* L.: 1; 2; 3; 28; 29; 30; 68; 69; 71; Rogoziniec; Kaława; Nowa Wioska; Brudzewo; Babimost; Zbąszynek; Krzepielów; Żabice; Grochowo; 22.07.2003; 2.08.2003; -"-; 12.08.2004; -"-; -"-; 18.06.2005; 19.06.2005; -"-; 1; 2; 3; 54; 55; 56; 133; 134; 135; *Hordeum vulgare* L.: 1; 1; 7; 8; 29; 29; 34; 35; 75; 76; 77; Rogoziniec; -"-; Wysoka; Bukowiec; Babimost; -"-; Skąpe; Pieski; Lubomyśl; Różanówka; Leśniki; 22.07.2003; -"-; 2.08.2003; 3.08.2003; 12.08.2004; -"-; -"-; 12.08.2004; 18.06.2005; -"-; -"-; 1; 7; 8; 9; 55; 61; 60; 62; 139; 140; 141; *Secale cereale* L.: 9; 10; 11; 36; 37; 38; 78; 79; 80; Samsonki; Stary Dwór; Lutol Suchy (st. kolejowa); Międzyrzecz; Połupin; Sycowice; Gorzupia; Dzietrzychowice; Dęby; 22.07.2003; 2.08.2003; 3.08.2003; 12.08.2004; -"-; -"-; 18.06.2005; 18.06.2005; -"-; 10; 11; 12; 63; 64; 65; 142; 143; 144; *Solanum tuberosum* L.: 4; 40; 47; 48; 49; 50; 90; 91; 92; Dąbrówka Wlkp.: Łazy; Pomorsko; Smardzewo; Podmokle Małe; Kosieczyn; Surowa; Krążkowo; Wrociszów; 12.08.2004; -"-; -"-; -"-; -"-; -"-; 18.06.2005; -"-; -"-; 80; 75; 76; 77; 78; 79; 154; 155; 156; *Triticum aestivum* L.: 8; 10; 14; 15; 32; 32; 39; 81; 82; 83; Bukowiec; Stary Dwór; Wysoka; Brójce; Brody; -"-; Czerwieńsk; Jutrzenka; Kierzno; Styrułów; 12.08.2004; 2.08.2003; -"-; 3.08.2003; 12.08.2004; -"-; -"-; 18.06.2005; 18.06.2005; -"-; 66; 17; 16; 18; 58; 68; 67; 145; 146; 147; *XTriticose-cale* Wittmack: 12; 13; 41; 42; 43; 84; 85; 86; Lutol Suchy; Szumiąca; Łagów (k/Gronowa); Łęgowo; Smardzewo; Lubogoszcz; Podbrzeże; Dębianka; 22.07.2003; 2.08.2003; 12.08.2004; -"-; -"-; 18.06.2005; -"-; -"-; 13; 14; 69; 70; 71; 148; 149; 150; *Zea mays* L.: 16; 17; 18; 44; 45; 46; 87; 88; 89 Staropole; Boroszyn; Myszęcin; Przytoczna; Rubinów; Chociule; Stare Strącze; Bieniów; Drogomin; 2.08.2003; -"-; -"-; 11.08.2004; 12.08.2004; -"-; 18.06.2005; -"-; 19.06.2005; 19; 20; 21; 72; 73; 74; 151; 152; 153.

Uncultivated (not protected) plants. *Achillea millefolium* L.: 2; 19; 20; 51; 52; 53; Kaława; Chociszewo; Jasieniec; Lubrza; Romanówek; Bucze; 2.08.2003; 22.07.2003; 2.08.2003; 13.08.2004; -"-; -"-; 23; 22; 24; 81; 82; 83; *Cirsium arvense* L.: 21; 22; 23; 51; 54; 55; Wilenko; Wityń; Żydowo; Lubrza; Jemiołów; Zarzyń; 3.08.2003; -"-; 3.08.2003; 13.08.2004; -"-; -"-; 25; 26; 27; 84; 85; 86; *Melandrium album* L.: 51; 52; 56; Lubrza; Romanówek; Łagów; 13.08.2004; -"-; -"-; 87; 88; 89; *Sedum maximum* (L.) Hoffm.: 26; 26; 51; 59; 95; 96; 97; Trzciel; -"-; Lubrza; Żelechów; Rzepin; Torzym; Górzyce; 3.08.2003; 15.08.2004; -"-; -"-; 19.06.2005; -"-; -"-; 32; 95; 81; 94; 161; 160; 162; *Carex sylvatica* Huds: 57; 58; 93; 94; 95; Rybojady; Borowy Młyn; Drezdenko; Ośno Lubuskie; Rzepin; 15.08.2004; -"-; 18.06.2005; 19.06.2005; -"-; 90-91; 92; 157; 158; 159; *Equisetum arvense* L.: 5; 25; 26; 55; 56; 57; Sierczynek; Bieleń; Trzciel; Łagów; Rybojady; 3.08.2003; -"-; 3.08.2003; 13.08.2004; -"-; -"-; 33; 31; 32; 96; 97; 98; *Trifolium arvense* L.: 2; 53; 54; 64; 70; 98; Kaława; Bucze; Jemiołów; Jabłonów; Drzecin; Jagielniki; 12.08.2004; 13.08.2004; -"-; 18.06.2005; 19.06.2005; 18.06.2005; 99; 100; 101; 164; 163; 165; *Hypericum perforatum* L.: 51; 52; 54; 96; 99; 100; Lubrza; Romanówek; Jemiołów; Torzym; Nowa Sól; Dobiegniew; 13.08.2004; -"-; -"-; 19.06.2005; 18.06.2005; 19.06.2005; 102; 103; 104; 168; 166; 167; *Juncus effusus* L.: 26; 56; Trzciel; Łagów; 15.08.2004; 21.06.2005; 105-107; 169-171; *Plantago arenaria* Waldst. et Kit.: 26; 26; Trzciel; -"-; 15.08.2004; 22.06.2005; 108-110; 172-174; *Plantago lanceolata* L.: 16; 20; 23; 54; 57; 60; Staropole; Jasieniec; Żydowo; Jemiołów; Rybojady; Siedlisko; 2.08.2003; 3.08.2003; -"-; 13.08.2004; -"-; -"-; 34; 35; 36; 113; 111; 112; *Corynephorus canescens* (L.) P. Beauv.: 26; 94; 101; 102; Trzciel; Ośno Lubuskie; Stary Jaromierz; Skólsko; 15.08.2004; 19.06.2005; 18.06.2005; -"-; 114-116; 176; 175; 177; *Polygonum persicaria* L.: 5; 13; 23; 58; 61; 62; Sierczynek; Szumiąca; Żydowo; Borowy Młyn; Siercz; Świdwowiec; 3.08.2003; 2.08.2003; 3.08.2003; 15.08.2004; -"-; -"-; 39; 37; 38; 117; 118; 119; *Rumex acetosella* L.: 4; 6; 17; 37; 52; 63; Dąbrówka Wlkp.; Łagowiec; Boroszyn; Połupin; Romanówek; Gronów; 22.07.2003; 2.08.2003; -"-; 12.08.2004; 13.08.2004; 12.08.2004; 40; 41; 42; 120; 122; 121; *Potentilla anserina* L.: 26; 103; Trzciel; Słońsk; 15.08.2004; 19.06.2005; 123-125; 178-180.

Protected plants. *Vinca minor* L.: 56; 56; Łagów; -"-; 21.06.2005; 24.06.2006; 217-218; 226-228; *Hedera helix* L.: 56; 56; Łagów; -"-; 13.08.2008; 21.06.2005; 211-213; 219-223; *Helichrysum arenarium* (L.) Moench: 26; 26; Trzciel; -"-; 3.08.2003; 15.08.2004; 181-185; 196-198; *Jovibarba sobolifera* (Sims.) Opiz: 26; 26; Trzciel; -"-; 3.08.2003; 15.08.2004; 186-190; 199-201; *Convallaria majalis* L.: 56; 56; 56; Łagów; -"-; -"-; 17.04.2004; 9.04.2005; 8.04.2006; 205-207; 214-216; 224-225; *Lycopodium clavatum* L.: 26; 26; Trzciel; -"-; 3.08.2003; 15.08.2004; 191-195; 202-204.

*After the plant species name, the number of location(s), the name of location(s), the date(s) of collection, and the number of samples are listed, respectively.

growing conditions, and the methods of spore extraction and staining of mycorrhizae were as those described previously (Błaszkowski, Renker and Buscot 2006). The host plants were *Plantago lanceolata* and *Zea mays*.

Morphological properties of spores and their subcellular structure were determined based on examination of at least 50 spores mounted in water, lactic acid, polyvinyl alcohol/lactic acid/glycerol (PVLG; Omar, Bollan and Heather 1979), and a mixture of PVLG and Melzer's reagent (1:1, v/v). Spores at all developmental stages were crushed to varying degrees by applying pressure to the cover slip and then stored at 65° C for 24 h to clear their contents from oil droplets. They were then examined under an Olympus BX 50 compound microscope equipped with Nomarski differential interference contrast optics. Microphotographs were recorded on a Sony 3CDD color video camera coupled to the microscope.

AMF were identified according to original descriptions, specimens collected by J. Błaszkowski, and descriptions and illustrations presented in Błaszkowski (2003) and Morton (2002).

Chemical and statistical analyses. For chemical analyses, 81 soil samples were selected, in which pH (in 1N KCl), the contents of N, P, K, organic C (in $g \cdot kg^{-1}$ of dry matter), and available forms of P, K, and Mg (in $mg \cdot kg^{-1}$ of dry matter) were determined. Each cultivated and uncultivated (not protected) plant species was represented by three randomly selected soil samples, and each protected plant species by one.

Differences in the structure of AMF communities were investigated by determining the frequency of occurrence of species, spore abundance and species richness, and by calculating dominance coefficients (Górny, Gruma 1981). Spore abundance and coefficients of dominance were determined based on spores isolated only from field-collected samples. Frequency of occurrence and species richness were calculated based on spores isolated from both field-collected samples and trap cultures. 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 50 or 100 g dry soil. Dominance coefficient expresses the proportion of the number of spores of a particular species in all spores of AMF recovered.

Coefficients of correlation were used to determine relationships between the spore abundance and soil chemical properties.

RESULTS AND DISCUSSION

Arbuscular mycorrhizal fungi and arbuscular mycorrhizae associated with cultivated and uncultivated (not protected) plants

Arbuscular mycorrhizal fungi. The occurrence of AMF in cultivated and uncultivated soils was determined based on spores isolated from field-collected rhizosphere soil-root samples and trap cultures established from a part of each field sample. The soil-root samples came from under 25 plant species belonging to 16 families (Fig. 1).

Of them 10 were cultivated plant species, and the others uncultivated once, including medicine plants. Each cultivated plant species was represented by 9 soil-root samples, and uncultivated one by 6.

A total of 40287 spores of AMF were isolated, including 11517 spores from field samples (of which 39.6% come from under cultivated plants) and 28770 spores from trap cultures. The spores represented 9 of the 14 exiting genera of the Glomeromycota (Tab. 1). Most species (18) were from the genus *Glomus*. The genus *Acaulospora* was represented by 5 species, and the genera *Entrophospora*, *Gigaspora*, *Pacispora*, and *Scutellospora* by 5 species each. One species each came from the genera *Ambispora* and *Paraglomus*. Additionally, not numerous spores of unrecognized species of the genera *Gigaspora*, *Glomus*, and *Scutellospora* were found.

Occurrence of AMF. Spores of AMF occurred in 92.8% of field-collected soil samples. They represented 13 species of *Glomus*, 5 of *Acaulospora*, 3 of *Scutellospora*, 2 each of *Entrophospora*, *Gigaspora* and *Pacispora*, and 1 of *Ambispora* (Tab. 1). In the root zone of cultivated plants, 15 species in three genera occurred. Roots of wild plants harboured 28 species in 7 genera and not numerous spores of unrecognized species of *Glomus* and *Scutellospora*. Of the AM fungal species identified in field soils, 21 sporulated in trap cultures (Tab. 1). Spores of *Acaulospora capsicula*, *Ac. lacunosa*, *Ac. koskei*, *Ac. mellea*, *Entrophospora baltica*, *Gigaspora gigantea*, and *Glomus fuegianum* were revealed only in field soils.

In trap cultures, 27 species and not numerous spores of unrecognized morphotypes were revealed.

The AMF most frequently occurring in the Lubuskie province soils were members of the genus *Glomus* (Tab. 1); they occurred in 87.2% of soils, of which 47.0% represented cultivated plants. *Glomus* spp. were associated with all plant species of the families *Asteraceae*, *Caryophyllaceae*, *Cyperaceae*, *Equisetaceae*, *Hyperiaceae*, and *Polygonaceae*. Other relatively frequently revealed AMF were *Scutellospora* spp. (more frequently co-occurred with most families of uncultivated plants) and *Pacispora* spp. (were more frequently harboured by families of cultivated plants).

Members of the genera *Acaulospora*, *Ambispora*, *Gigaspora*, and *Paraglomus* were recorded only in soils from under uncultivated plants (Tab. 1).

The disclosure of 10 species and one undescribed morphotype in trap cultures that were not found in field soils confirms conclusions of, e. g., Błaszkowski, Tadych and Madej (2002), Stütz & Morton (1996) and Jansa et al. (2002) that a large part of AMF may not sporulate in the field at all or their sporulation is seasonal.

Frequency of occurrence of species. The species most frequently occurring in cultivated soils of the Lubuskie province were *Gl. mosseae* (present in 80.0% of soils) and *Gl. claroideum* (62.2%; Tab. 1). Fungi relatively frequently recorded (present in 15-30% of soils) also were *Gl. caledonium* and *P. franciscana*. In uncultivated soils, the most frequently found fungi included *Gl. constrictum* (51.1%) and *Gl. claroideum* (44.0%), followed by *Ac. lacunosa*, *Gl. macrocarpum*, and *S. dipurpurescens*.

Dominance. The eudominants (of a coefficient of dominance of D>20%) of cultivated sites were *Gl. claroideum* and *Gl. deserticola* (Tab. 2). The group of dominants (D=10-20%) formed only *Gl. mosseae*. The subdominants (D=5-10%) were *Gl. caledonium*, *P. scintillans*, and *S. dipurpurescens*. In uncultivated soils, the eudominants were *Gl. constrictum* and *Gl. claroideum*. *Glomus badium*, *Gl. deserticola*, *Gl. lamel-*

Table 1
Frequency of occurrence of AMF isolated from under cultivated (a) and uncultivated (not protected) plants (b) of the Lubuskie province

Fungus	Frequency of occurrence (%)					
			Trap cultures with			
	Field soils		*P. lanceolata*		*Z. mays*	
	a	b	a	b	a	b
Acaulospora capsicula		5.56				
Acaulospora lacunosa		16.67				
Acaulospora koskei		1.11				
Acaulospora mellea		4.44				
Acaulospora paulinae		1.11		1.11		
Ambispora gerdemannii		13.33				2.22
Archaeospora trappei				2.22	2.22	4.44
Entrophospora baltica		1.11				
Entrophospora infrequens		1.11	5.56	3.33	4.44	2.22
Gigaspora gigantea		1.11				
Gigaspora margarita		2.22		4.44		2.22
Gigaspora sp.						1.11
Glomus aggregatum	5.56	10.00	3.33	11.11	2.22	2.22
Glomus badium	1.11	8.89		3.33		1.11
Glomus caledonium	17.78	5.56	37.8	10.00	30.00	17.78
Glomus claroideum	27.78	20.00	56.7	44.44	62.22	40.00
Glomus clarum				5.56		
Glomus constrictum	28.89	51.11	11.1	26.7	1.11	8.89
Glomus deserticola	24.44	25.56	15.6	10.00	1.11	
Glomus fasciculatum	4.44	12.22	2.22	4.44	2.22	
Glomus fuegianum		1.11				
Glomus geosporum	3.33	5.56			2.22	
Glomus lamellosum			1.11	7.78	1.11	6.67
Glomus macrocarpum	8.89	16.67	1.11			
Glomus microcarpum	1.11	6.67		1.11		1.11
Glomus mosseae	80.00	26.67	61.1	31.11	60.00	25.56
Glomus pansihalos				1.11		
Glomus pustulatum				1.11		1.11
Glomus rubiforme		1.11				1.11
Glomus verruculosum			1.11		2.22	
Glomus 178			3.33	4.44	2.22	7.78
Glomus sp.		1.11	1.11	2.22	1.11	2.22
Pacispora franciscana	16.67	8.89	11.1	3.33	10.00	3..33
Pacispora scintillans	12.22	7.78	8.89	3.33	6.67	2.22
Paraglomus laccatum				1.11		4.44
Scutellospora armeniaca	1.11	1.11				1.11
Scutellospora dipurpurescens	8.89	23.33	8.89	17.78	5.56	14.44
Scutellospora pellucida		6.67				5.56
Scutellospora sp.		1.11				

losum and *S. dipurpurescens* were dominants, and *E. infrequens*, *Gl. caledonium*, and *Gl. mosseae* - subdominats.

The data presented above confirm Błaszkowski's (1993) and Gerdemann's (1968) conclusions that AMF are common soil fungi and coexist with most vascular cultivated and uncultivated plant species of the world.

The abundant and diverse spore populations of *Glomus* spp. revealed in the study discussed here indicate a good adaptation of these fungi to a wide range of soil conditions (Anderson, Liberta and Dickman 1984; Grey 1991; Jansa et al. 2002; Porter, Robson and Abbott 1987). Species of *Gigaspora* and *Scutellospora* prefer warmer

Table 2
Dominance of AMF associated with cultivated (a) and uncultivated (b; not protected) plants of the Lubuskie province

	Dominance (%)					
Fungus			Trap cultures with			
	Field soils		*P. lanceolata*		*Z. mays*	
	a	b	a	b	a	b
	1	2	3	4	5	6
Acaulospora capsicula		0.07				
Acaulospora lacunosa		0.22				
Acaulospora koskei		0.01				
Acaulospora mellea		0.06				
Acaulospora paulinae		0.01		0.02		
Ambispora gerdemannii		0.59				0.09
Archaeospora trappei				2.33	0.42	1.50
Entrophospora baltica		0.01				
Entrophospora infrequens		0.01	0.46	7.00	0.07	4.13
Gigaspora gigantea		0.03				
Gigaspora margarita		0.06		0.09		0.09
Gigaspora sp.						0.06
Glomus aggregatum	1.78	2.54	0.08	1.90	0.08	0.06
Glomus badium	0.24	12.84		1.90		0.26
Glomus caledonium	2.17	0.27	2.47	6.04	8.74	2.34
Glomus claroideum	4.10	3.10	67.44	27.37	66.00	56.72
Glomus clarum				3.29		
Glomus constrictum	4.43	32.91	0.17	4.01	0.12	2.17
Glomus deserticola	53.47	17.04	6.07	5.53	0.01	
Glomus fasciculatum	0.20	1.23	0.02	0.22	0.03	
Glomus fuegianum		2.81				
Glomus geosporum	0.59	0.25			0.42	
Glomus lamellosum			0.07	10.43	0.04	13.15
Glomus macrocarpum	0.35	4.23	0.02			
Glomus microcarpum	0.11	4.27		2.86		0.03
Glomus mosseae	17.64	3.10	10.59	4.60	15.00	8.49
Glomus pansihalos				0.07		
Glomus pustulatum				0.16		0.49
Glomus rubiforme		3.16				1.10
Glomus verruculosum			0.01		0.15	
Glomus 178			0.46	0.75	2.45	0.84
Glomus sp.		0.03	0.15	2.11	0.03	0.06
Pacispora franciscana	4.67	0.79	3.27	0.84	4.01	0.32
Pacispora scintillans	5.90	0.46	2.40	1.24	0.60	0.17
Paraglomus laccatum				0.02		4.33
Scutellospora armeniaca	0.07	0.04				0.75
Scutellospora dipurpurescens	4.30	7.36	6.30	17.23	1.83	2.40
Scutellospora pellucida		2.25				0.46
Scutellospora sp.		0.22				

(Koske 1981; Schenck, Graham and Green 1975) and more sandy soils (Błaszkowski 1993b).

Spore density. The overall mean spore density of AMF in field soils collected under cultivated plants was 50.7 and ranged from 0 to 925 spores in 100 g dry soil. In the rhizosphere of uncultivated plants, the values were 77.3 and 0 to 865 in 100 g dry soil, respectively.

Most spores were isolated from under *Beta vulgaris*, *Hypericum perforatum*, *Polygonum persicaria*, and *Trifolium arvense* (Tabs 3 and 4).

Table 3
Spore abundance and species richness of AMF associated with roots of 10 cultivated plant species (means)

Plant species	Spore abundance	Species richness		
	Field soils*	Field soils*	Trap cultures with**	
			P. lanceolata	*Z. mays*
Asparagus officinalis	11.00	2.00	1.78	1.78
Avena sativa	46.89	2.56	2.33	2.22
Beta vulgaris	119.00	2.56	2.89	2.00
Brassica napus	32.25	2.56	1.69	2.22
Hordeum vulgare	35.00	2.78	2.56	1.78
Secale cereale	54.67	3.22	1.89	2.58
Solanum tuberosum	15.11	1.78	2.56	2.20
Triticum aestivum	102.78	2.44	2.33	1.56
XTriticum secalum	19.00	1.89	2.50	1.86
Zea mays	46.67	2.67	2.78	1.67

* in 100 g dry soil ** in 50 g dry soil

Table 4
Spore abundance and species richness of AMF associated with roots of 15 uncultivated (not protected) plant species (means)

Plant species	Spore abundance	Species richness		
	Field soils*	Field soils*	Trap cultures with**	
			P. lanceolata	*Z. mays*
Achillea millefolium	121.33	3.00	2.33	1.67
Carex sylvatica	10.00	2.17	1.67	1.60
Cirsium arvense	87.33	4.00	3.50	2.33
Corynephorus canescens	6.00	1.17	1.33	0.17
Equisetum arvense	67.17	3.67	0.67	2.00
Hypericum perforatum	149.00	3.17	1.33	1.83
Juncus effusus	10.33	1.33	1.17	1.67
Melandrium album	144.50	3.83	2.50	1.67
Plantago arenaria	30.00	2.00	1.83	2.50
Plantago lanceolata	55.83	2.83	2.67	1.80
Polygonum persicaria	181.17	2.33	2.17	1.50
Potentilla anserina	12.40	2.17	1.50	0.00
Rumex acetosella	34.83	3.00	1.50	1.17
Sedum maximum	35.25	2.33	2.00	2.00
Trifolium arvense	277.00	2.50	2.50	2.17

* in 100 g dry soil ** in 50 g dry soil

Species density. The overall mean species density of AMF in field soils from under cultivated plants was 2.44 and ranged from 0 to 7 in 100 g dry soil. The mean species density of AMF associated with uncultivated plant was 2.63 within the range 0-6 in 100 g dry soil. In trap cultures with soils and roots of cultivated plants and the host plants *P. lanceolata* and *Z. mays*, the overall mean species density was higher by 12.4% and 19.1%, respectively, than in those with soils and roots of uncultivated plants (Tabs 3 and 4).

In the field, of the cultivated plants, most species were harboured by *Secale cereale*, and of uncultivated plants – *Cirsium arvense*, *Equisetum arvense* and *Melandrium*

album (Tabs 3 and 4). In trap cultures representing cultivated plants, most species were found when the growing media were soil-root samples from under *B. vulgaris* and *Z. mays* (Tab. 3). When trap cultures contained soils and roots from under uncultivated plants, most species came from those representing *Ci. arvense* (Tab. 4).

The species most frequently revealed in the spore populations of AMF associated with roots of cultivated and uncultivated plants of the Lubuskie province, i.e., *Gl. claroideum*, *Gl. constrictum*, *Gl. deserticola*, *Gl. mosseae* and *S. dipurpurescens*, have many times been found in cultivated and uncultivated sites of different regions of the world (Błaszkowski 1993a; Jansa et al. 2002).

Literature data on the sporulation of AMF in cultivated *versus* uncultivated soils are contradictory. As found in this study and that of Błaszkowski (1993a), AMF produced more spores in uncultivated soils, probably because of the lack of inhibitory influences of agricultural practices. According to Oehl et al. (2005), intensive agricultural farming decreases spore production and the numbers of species, especially those from the genus *Glomus*. In contrast, Jansa et al. (2002) concluded that some taxa of AMF are activated in conditions of agricultural soils. The over 2-fold higher frequency of occurrence of *S. dipurpurescens* in uncultivated soils probably resulted from high sensitivity of this species to agricultural practices. Spores of *Scutellospora* spp. generally are much larger than those of other AMF and easier undergo destructions (Błaszkowski 2003).

Arbuscular mycorrhizae. The occurrence of arbuscular mycorrhizae (AM) in this group of plants was determined based on 75 root samples. Each plant species was represented by three root samples.

Arbuscules. Of cultivated plants, the highest levels of root colonization by arbuscules were found in *S. cereale* and *Z. mays* (Tab. 5). In wild plants, most arbuscules occurred in roots of *Hy. perforatum*, *Plantago arenaria*, and *P. lanceolata*. No arbuscules were found in roots of the cultivated *Brassica napus*, *B. vulgaris* and *Solanum tuberosum* and the uncultivated *Carex sylvatica*, *E. arvense*, and *Pol. persicaria*.

Vesicles. Of the 10 cultivated plant species, only roots of *Ho. vulgare* and *Z. mays* contained a high number of vesicles (Tab. 5). Of the wild plants, most vesicles were found in roots of *P. arenaria*, *P. lanceolata*, and *Sedum maximum*.

Intraradical hyphae. Most intraradical hyphae had roots of the cultivated *Hordeum vulgare* and *Z. mays* and the wild *Hy. perforatum* and *Sed. maximum* (Tab. 5). No intraradical hyphae were revealed in roots of *B. vulgare*, *Br. napus*, *C. sylvatica*, *E. arvense*, and *So. tuberosum*.

According to Sanders et al. (1977), already a 10% level of root colonization by AMF significantly increases the absorption of P from the soil. Volkmar and Woodbury (1989) found that 2-7% colonization of roots by AMF increased up to 25% the shot weight of *Ho. vulgare*.

The presence of arbuscules indicates a functional AM (Smith, Read 2008). However, arbuscules were not found in roots of *B. vulgaris* sampled in this study, which, on the other hand, harboured abundant spore populations of AMF. Intraradical components of AM of many species of the Glomeromycota either stain faintly or not at all and, thereby, may be omitted (Stutz, Morton 1996). In the literature, the amount of data on the active functioning of AM in roots of members of the family *Chenopodiaceae* increases (Landwehr et al. 2002).

Table 5
Mean percent of root length of cultivated and uncultivated (not protected) plants of the Lubuskie province with arbuscules, vesicles, and intraradical hyphae of AMF

Plant species	Arbuscules	Vesicles	Hyphae
	Cultivated plants		
Asparagus officinalis	5.00	8.00	25.00
Avena sativa	3.00	5.00	46.0
Beta vulgaris	0.00	0.00	0.00
Brassica napus	0.00	0.00	0.00
Hordeum vulgare	18.00	22.00	59.00
Secale cereale	32.00	1.00	53.00
Solanum tuberosum	0.00	0.00	0.00
Triticum aestivum	5.00	9.00	53.00
XTriticum aestivum	3.00	17.00	51.00
Zea mays	38.00	20.00	66.00
	Uncultivated plants		
Achillea millefolium	7.00	20.00	50.00
Carex sylvatica	0.00	0.00	0.00
Cirsium arvense	19.00	9.00	15.00
Corynephorus canescens	5.00	10.00	40.00
Equisetum arvense	0.00	0.00	0.00
Hypericum perforatum	27.00	21.00	65.00
Juncus effusus	5.00	15.00	42.00
Melandrium album	1.00	12.00	49.00
Sedum maximum	1.00	24.00	66.00
Plantago arenaria	28.00	40.00	42.00
Plantago lanceolata	31.00	31.00	22.00
Polygonum persicaria	0.00	1.00	49.00
Potentilla anserina	2.00	18.00	51.00
Rumex acetosella	4.00	4.00	22.00
Trifolium arvense	1.00	8.00	53.00

Arbuscular mycorrhizal fungi and arbuscular mycorrhizae associated with protected plants

Arbuscular mycorrhizal fungi. The occurrence of AMF associated with protected plants was determined based 48 rhizosphere soil-root samples collected under 6 plant species being fully or partly protected (Fig. 1). Each plant species was represented by 8 soil-root mixtures sampled at four sites of the Łagowo Landscape Park located in Łagowo and at two sites of the Pszczewo Landscape Park positioned in Trzciel (Fig. 1).

Spores of AMF occurred in 31.2% of soil-root samples. They belonged to 11 species of *Glomus*, two species each of *Acaulospora* and *Scutellospora*, one species each of *Ambispora*, *Archaeospora* and *Pacispora*, two undescribed morphotypes (one each of *Glomus* and *Scutellospora*), and not numerous spores of unrecognized fungi forming glomoid spores (Tab. 6).

AMF sporulated in 29 trap cultures with *P. lanceolata* as the host plant, i.e., 60.4% of all trap cultures established. The spores represented 12 species and two undescribed morphotypes (one each of *Glomus* and *Scutellospora*; Tab. 6).

Frequency of occurrence of species. The species of AMF most frequently co-occurring with roots of protected plants (present in >18% of samples and trap cultures) were *Gl. claroideum*, *Gl. constrictum*, and *S. dipurpurescens* (Tab. 6).

Table 6
Frequency of occurrence and dominance of AMF associated with roots of protected plant species

	Frequency of occurrence (%)		Dominance	
Fungus	Field soils	Trap cultures with *P. lanceolata*	Field soils	Trap cultures with *P. lanceolata*
Acaulospora lacunosa	6.25		17.86	
Acaulospora mellea		2.08		0.03
Ambispora gerdemannii	2.08		0.30	
Archaeospora trappei		6.25		0.62
Glomus aggregatum		2.08		0.03
Glomus badium	2.08		29.76	
Glomus claroideum	4.17	20.83	2.38	79.29
Glomus constrictum	18.75	2.08	24.11	0.03
Glomus geosporum	2.08		0.30	
Glomus lamellosum		12.50		5.27
Glomus macrocarpum		2.08		0.03
Glomus microaggregatum	2.08		2.98	
Glomus microcarpum	4.17		16.67	
Glomus mosseae	6.25	6.25	1.49	1.46
Glomus verruculosum		2.08		0.03
Glomus 178		2.08		0.35
Glomus sp.		6.25		6.24
Paraglomus laccatum		8.33		2.29
Scutellospora dipurpurescens	4.17	20.83	2.68	3.78
Scutellospora pellucida	2.08	4.17	1.49	0.49
Scutellospora 179		2.08		0.03

Dominance. The eudominants (D>20%) were *Gl. badium* and *Gl. constrictum* (Tab. 6). The group of dominants (D=10-20%) formed *A. lacunosa* and *Gl. microcarpum*. None of the other species found was a subdominant (D=5-10%).

Spore density. The overall mean spore density of AMF in field soils was 7.00 and ranged from 0 to 100 spores in 100 g dry soil. Most spores were associated with roots of *Jovibarba sobolifera*, and least with those of *Lycopodium clavatum* (Tab. 7).

Species density. The plant species harbouring most species was *J. sobolifera* (Tab. 7). Relatively high numbers of species were also associated with roots of *Convalaria majalis* and *L. clavatum*.

In Zubek's et al. (2005) investigations, the overall mean species richness of AMF co-existing with protected plants of the Mountain Botanical Garden in Zakopane

Table 7
Spore abundance and species richness of AMF associated with roots of six protected plant species in field soils (a) and trap cultures with *P. lanceolata* as the host plant (b; means)

	Spore abundance		Species richness	
Plant species	a	b	a	b
Convallaria majalis	12.5	207.9	0.13	1.1
Hedera helix	1.0	19.9	0.50	0.7
Helichrysum arenarium	7.2	16.4	0.25	0.7
Jovibarba sobolifera	17.5	16.2	1.13	1.2
Lycopodium clavatum	0.6	30.2	0.38	1.0
Vinca minor	3.1	69.7	0.87	0.6

Table 8
Mean percent of root length of protected plants of the Lubuskie province with arbuscules, vesicles, and intraradical hyphae of AMF

Plant species	Arbuscules	Vesicles	Hyphae
Convallaria majalis	29.00	8.00	60.00
Hedera helix	0.00	0.00	0.00
Helichrysum arenarium	29.00	31.00	50.00
Jovibarba sobolifera	19.00	23.00	52.00
Lycopodium clavatum	0.00	0.00	0.00
Vinca minor	1.00	22.00	54.00

was higher than that found in this study. However, in both investigations the species most frequently identified were *Gl. claroideum* and *Gl. constrictum*.

Arbuscular mycorrhizae. The occurrence of AM of protected plants of the Lubuskie province was determined based on 18 roots samples from six plant species. Each plant species was represented by three samples.

Arbuscules. The highest levels of root colonization by arbuscules were found in *Con. majalis* and *Helichrysum arenarium* (Tab. 8). No arbuscules were found in roots of *Hedera helix* and *L. clavatum*.

Vesicles. Most vesicles were found in roots of *Hel. arenarium*, then in those of *J. sobolifera* and *Vinca minor* (Tab. 8).

Intraradical hyphae. The plant species harbouring most intraradical hyphae was *Con. majlis* (Tab. 8). No intraradical hyphae were observed in roots of *H. helix* and *L. clavatum*.

The occurrence of spores of AMF vs. AM vs. in connection with soil chemical properties

Soil pH ranged from 3.67 to 8.28. The ranges of the total contents ($g \cdot kg^{-1}$ of dry matter) of organic C, N, P, and K were 0.42-2.0, 0.30-22.5, 0.13-2.02, and 0.10-4.63, respectively. The values of available forms of P, K, and Mg ($g \cdot kg^{-1}$ of dry matter) ranged 9.4-361, 4.90-327, and 13.4-98.8, respectively.

Rectilinear correlation analyses showed that the spore numbers of AMF associated with roots of wild plants significantly correlated with the contents of organic C ($r=0.38$; P,0.005) and available Mg ($r=0.55$; $P<0.05$). The contents of total ($r=0.50$; $P<0.005$) and available K ($r=0.43$; $P<0.05$) in soils from under cultivated plants influenced the abundance of *Gl. mosseae* spores. The contents of available Mg in soils of cultivated sites was linked with the numbers of *Gl. claroideum* spores ($r=0.90$; $P<0.05$). In uncultivated soils, the contents of organic C and total N and Mg correlated with numbers of *Gl. constrictum* spores: $r=0.66$, $P<0.05$; $r=0.59$, $P<0.05$; and $r=0.62$, $P<0.05$, respectively.

The levels of mycorrhizal colonization did not correlate with any of the soil chemical properties considered. Insignificant also were correlations between numbers of spores and species and levels of mycorrhizal colonization.

The results of our study contradict those of many workers who proved that higher contents of soil P generally decrease the production of spores by AMF and the composition and distribution of structures of AM inside roots (Smith, Read 1990). Alternatively, the activity of AMF did not correlate with soil P because of its changing availability at different levels of pH (Hayman 1970).

The lack of relationships between the level of mycorrhizal colonization and the spore abundance and the species richness may have resulted from that the stains used did not reveal structures of all AMF in reality existing inside roots (Morton, Redecker 2001).

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Arbuskularne grzyby mikoryzowe (Glomeromycota) związane z korzeniami roślin województwa lubuskiego

Streszczenie

Przedstawiono i przedyskutowano wyniki badań występowania arbuskularnych grzybów mikoryzowych (AGM) i mikoryz arbuskularnych z gromady Glomeromycota związanych z korzeniami 31 gatunków roślin uprawnych, nieuprawnych i chronionych rosnących w 103 stanowiskach województwa lubuskiego. Najczęściej znajdywanymi AGM byli przedstawiciele rodzaju *Glomus*. Często ujawniano również gatunki z rodzaju *Scutellospora*. Populacje zarodników AGM na ogół były bardziej obfite i różnorodne w glebach uprawnych. Większość gatunków roślin chronionych utrzymywała AGM.