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CHANGES IN THE SPORE NUMBERS OF AM FUNGI AND IN AM COLONISATION OF ROOTS OF CLOVERS AND GRASSES ON A PEAT-MUCK SOIL WITH RESPECT TO MINERAL FERTILISATION

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Abstract

A 4-year plot experiment was conducted to determine the dynamics of changes in the spore density of arbuscular mycorrhizal fungi (AMF) and of the degree of endomycorrhizal colonisation of roots of clovers and meadow grasses on an organic peat-muck soil in a post-marshy habitat, taking into account the effect of mineral fertilisation (NPK). The experimental object comprised four plots that represented the fertilisation treatments, sown with white clover (*Trifolium repens* L.), red clover (*Trifolium pratense* L.), smooth meadow-grass (*Poa pratensis* L.), and a mix of grasses composed of perennial ryegrass (*Lolium perenne* L.), meadow fescue (*Festuca pratensis* Huds.), smooth meadow-grass (*Poa pratensis* L.), and cocksfoot (*Dactylis glomerata* L.). Analogous sowing was performed on control (non-fertilised) plots. It was found that spores of AMF occurred in 100% of the samples of the soil studied, and the average total number of AMF spores isolated from soil under the particular plant combinations was high and amounted to 1858 spores (range from 1392 to 2443) in 100 g of air-dried soil. The percentage share of the clover and grass roots colonised by indigenous endomycorrhizal fungi was very low and varied from 0 to 46 (average from 4.1% to 12.2%). No correlation was found between the spore numbers of AMF in the soil and the degree of mycorrhized roots of the clovers and grasses. Mineral fertilisation stimulated the sporulation of AM fungi but had no effect on root colonisation by these fungi.

Key words: AM fungi, Spore density, Colonisation of roots, Clovers, Grasses, Peat-muck soil.

Introduction

The root zone of most terrestrial plants (ca. 90% of species) is colonised by mycorrhizal fungi, both ecto- and endomycorrhizal ones. Green plants produce mainly endomycorrhiza, called the arbuscular mycorrhiza (AM) (Martin *et al*., 2001; Smith & Read, 2008; Minz & Ofek, 2011; Bonfante & Desiro, 2015), formed by fungi from Glomeromycota (Schüβler *et al*., 2001). Numerous authors report that AM fungi play an important role in plant nutrition and health status, and that the colonisation of roots by those microorganisms causes changes in the composition of root exudates and a reduction of their amount. This results in an increase in the amount of nutrients taken up by the plant, mainly phosphorus, but also nitrogen and potassium. Arbuscular mycorrhiza can also protect plants against the action of certain soil pathogens, e.g. fungi from the genera *Fusarium, Pythium*, *Phyhophtora*, *Rhizoctonia*, *Verticillium,* or *Thielaviopsis* (Harrier & Watson, 2004). Endomycorrhizal fungi also enhance the resistance of plants to abiotic stress factors such as drought, salinity, presence of heavy metals, or soil acidification (Księżniak *et al.,* 2001; Johansson *et al.,* 2004; Hajiboland *et al*., 2010; Minz & Ofek, 2011; Datta & Kulkarni, 2014; Liu *et al*., 2015; Pedranzani *et al.,* 2015). Lee *et al.* (2012) demonstrated that under drought stress conditions both the water potential and the photosynthetic activity of perennial ryegrass were higher in mycorrhized plants than in non-mycorrhized ones.

Studies on the occurrence of arbuscular fungi in non-cultivated and in tilled soils conducted in various parts of the world (Europe, Asia, Africa, North and South America) indicate their non-uniform distribution, determined primarily by the soil (soil type) and vegetation factors (kind of plants). Studies by numerous authors show that the number of spores arbuscular fungi are generally lower in soils which are poor in organic matter and nutrients than in soils rich in those components (Mullahey & Sped, 1991; Błaszkowski *et al.,* 2002; Iwaniuk & Błaszkowski, 2004; Kowalczyk & Błaszkowski, 2005; Panwar *et al.,* 2011).

One of the factors that have a significant effect on the mycorrhization of crop plants by arbuscular fungi is mineral fertilisation, mainly with phosphorus and nitrogen. The effect of N and P fertilisation on AM fungi depends on the initial level of these elements in the soil (Karanika *et al.,* 2008). A stimulating effect of nitrogen on the mycorrhizal colonisation of meadow grasses was demonstrated only when organic phosphorus was present in the soil, while no such effect was noted when the soil was fertilised with inorganic phosphorus. The effect of phosphorus fertilisation on the level of mycorrhizal colonisation depends also on the host plant. In the case of plants with low phosphorus requirements, fertilisation with this element reduces mycorrhizal colonisation. Increased colonisation, on the other hand, is observed in the case of plants with high requirements for the element (Karanika *et al.,* 2008). A varied response to fungal infection of various host plants under conditions of phosphorus fertilisation was also observed by Šmilauer & Šmilaeurová (2000). They conducted a study on grasslands under conventional use and showed that an addition of phosphates decreased the number of arbusculae and corresponded with the number of root fragments without the infection.

There is a lack of information on the occurrence of AM fungi and on mycorrhizal colonisation of meadow vegetation in organic soils, such as peat-muck soils. Similarly, there is no information on the effect of mineral fertilisation on AMF spore density and on mycorrhization of meadow plants: clovers and meadow grasses grown on peat-muck soils in post-marshy habitats. The purpose of this study, was: (1) to assess changes in the spore density of AM fungi and the degree of endomycorrhizal colonisation roots of clover and meadow grasses on an organic peat-muck soil in post-marshy habitat, (2) to determine the effect of mineral fertilisation (NPK) on the spore density and on the level of mycorrhization of clovers and grasses by AM fungi, and (3) to analyse the correlation between the spore number of AMF in the soil and the mycorrhization of plant roots.

Materials and Methods

Study site: The study was located at the Didactic-Research Station in Sosnowica (Lublin Province) belonging to the Faculty of Grassland and Landscape Management, University of Life Sciences in Lublin (south-eastern Poland). The geographic position of Sosnowica is defined by the coordinates of 51°31' north latitude and 23°04' east longitude (51°31'N, 23°04'E).

The soil on which the experiment was established was identified as peat-muck soils (Histosols acc. to FAO) with a medium degree of mucking (Mt II) developed from sedge-reed peats. The soils are classified as the type of muck soils, order of post-marshy soils, and division of hydrogenic soils. The mean temperatures in the vegetation season (from April to October) in the successive years of the study (2002-2004) were 13.5°C, 14.0°C and 14.3°C, respectively, and were equal to the multi-year mean. The mean rainfall in the months from April to October was higher in the first year (by 78.9 mm) and second (by 25.6 mm) year, compared to the multi-year mean (386.2 mm). In the third year of the study, in turn, it was lower by 104.4 mm.

Experiment description: The experiment was set up in April 2002. The experimental object comprised four fertilised plots (1-4) with dimensions of 2x2 m and four non-fertilised (control) plots (5-8) with a size of 2x2 m. The plots (fertilised and non-fertilised) were sown as follows (Table 1): 1 - white clover (*Trifolium repens*), 2 – red clover (*Trifolium pratense*), 3 – smooth meadow-grass (*Poa pratensis*) and 4 – grass mix: perennial ryegrass (*Lolium perenne*), meadow fescue (*Festuca pratensis*) smooth meadow-grass (*Poa pratensis*), cocksfoot (*Dactylis glomerata*). On plots 1-4, fertilisation with Polifoska 8-24-24 was applied (prior to the sowing and in spring 2003) at the dose of 80g/4 m^2, i.e. the doses applied ($kg \cdot ha^{-1}$) were N-16, P_2O_5-48, K_2O-48. In the course of the vegetation season, three cuts were harvested. Characteristics of the chemical properties of the soil assayed in the particular experimental treatments are presented in Table 2.

Table 1. List of species and seed sowing standards (after Falkowski, 1982).

Species	Content in the mix (%)	Seed sowing norms ($kg \cdot ha^{-1}$)
White clover	-	13
Red clover	-	22
Smooth meadow-grass	20	20 (27)*
Perennial ryegrass	35	(62)*
Meadow fescue	35	(60)*
Cock's foot grass	10	(36)*

()* – Norms for a grass mix

Sampling methods: Samples were collected from June 2002 to October 2005, at three-week intervals throughout the vegetation season. From every plot, 10 soil samples were collected at a depth of 0-20 cm, together with the plant root systems. The averaged samples obtained were placed in plastic bags. The samples were carefully separated into soil and plant components. The plant roots with a small amount of soil (as a protection coating) were frozen (-18°C) and stored until the time of estimation of the degree of root colonisation by AMF. Fresh soil was crumbled and sieved with a 2 mm diameter mesh, dried to air-dry mass (ca. 20°C), and the spore numbers of endomycorrhizal fungi in the soil were assayed.

Table 2. Selected chemical properties of the peat-muck soil.

Treatment	Organic matter %	N total %	P total %	mg in 100g of soil			pH in KCl
				P_2O_5	K_2O	Available Mg	
B	72.61	2.76	0.20	82.30	15.00	18.90	3.67
C	73.83	2.83	0.25	220.20	9.40	7.00	3.39
W	73.10	2.83	0.23	162.10	10.60	7.50	3.67
M	76.55	2.85	0.22	142.80	10.00	8.70	3.69
Bn	64.29	2.35	0.24	102.60	11.70	7.30	3.74
Cn	57.34	1.94	0.21	100.20	10.90	6.30	3.90
Wn	76.12	2.95	0.23	133.10	9.00	4.60	3.77
Mn	72.47	2.80	0.21	88.00	11.60	5.30	3.71
	Mean for samples						
Non-fertilised soil	74.02	2.82	0.23	151.85	11.25	10.53	3.61
Fertilised soil	67.56	2.51	0.22	105.98	10.80	5.88	3.78

B – white clover, non-fertilised; **Bn –** white clover, fertilised; **C** – red clover, non-fertilised; **Cn** – red clover, fertilised; **W –** smooth meadow-grass, non-fertilised; **Wn** – smooth meadow-grass, fertilised; **M** – grass mix, non-fertilised; **Mn** – grass mix, fertilised

Spore assessment: Spores of AM fungi were assayed using the method of Allen *et al*. (1979) in the modification of Księżniak & Kobus (1998). 50 g weighed portions of air-dry soil were mixed with 50 g of sand with grain size above 400 µm and suspended in 100 ml of distilled water in Erlenmayer flasks (300 ml). The suspensions were shaken at 200 rpm for 4 hours and then centrifuged at 2000 rpm for 10 min. After rejecting the supernatant, a 2M solution of saccharose with 2 % sodium phosphate was added and the centrifugation (at 2000 rpm, for 10 min) was performed again. The supernatant obtained containing spores of AM fungi was passed though sieves of 400 µm, 150 µm, 100 µm, 75 µm, 51 µm, and 20 µm mesh size. Each of the fractions was transferred onto a separate acetate filter and next the count of spores obtained using a stereomicroscope and the spore numbers from the particular fractions were summed up. Each sample was analysed in two replicates and the numbers of AMF spores were arithmetic means from two assays. The number of AMF spores was given per 100 g of air-dry soil.

Mycorrhizal root colonisation assessment: The destaining of roots and staining of mycrorrhizas were performed according to Philips & Hayman (1970). Part of each root sample was carefully rinsed with tap water and cut into 0.5-1 cm fragments. Then the root fragments were heated at 90^{o}C for 30 min in 10% KOH and rinsed with distilled water. The samples were immersed in 10% HCl (neutralisation) for 1 hour. The roots were hot-stained (at 90^{o}C for 15 min.) with trypan blue in lactoglycerol (Kormanik & McGraw, 1982). For every root sample, double slides were performed using polyvinyl-lactoglycerol (PVLG) (Omar *et al.*, 1979; Koske & Tessier, 1983). Mycorrhizal colonisation of the roots was assayed with the use of a light microscope Nikon Labophot-2 at 100 x magnification. The presence of vesicles, arbuscules, or hyphae in the root tissue was observed in 50 separate fields of view. Percentage colonisation of the roots by AM fungi was calculated from the formula: number of colonised fragments /50 fragments of roots x 100. The observations of the slides were performed twice and the mean percentage degree of root colonisation by AM fungi was calculated.

Statistical analysis: The comparison of the mean values of the results was made with the use of ANOVA multi-criterion models with interactions and additive models. The significance of the differences among the mean values was estimated using Tukey's test at a significance level of $\alpha = 0.05$. The descriptive statics calculated included mean values and standard deviations. Correlation analyses of root colonisation and the number of spores were performed using the Pearson coefficient, estimating its significance at the level of $\alpha = 0.05$. Statistical analyses were performed using the program Statistica version 10 (StatSoft Inc., 2011).

Changes in the spore numbers of am fungi and in am colonisation of roots of clovers and grasses on a peat-muck soil taking into account mineral fertilisation

Results

Number of AM fungal spores: Spores of AMF were found in all samples (Figs. 1-4). The spore number in the rhizosphere of the two tested species of clovers: white clover (*Trifolium repens*) and red clover (*Trifolium pratense*) in 2002 ranged from 1509 to 2007 per 100 g d. m. of soil (Fig. 1a). Statistical analysis with Tukey's multiple tests based on HSD (Honest Significant Difference) showed a significantly higher mean number of spores only in rhizosphere soil of red clover treated with NPK, relative to the non-treated soil (Fig. 1a). Spore density in the second year (2003) varied from 1594 to 2245 per 100 g d. m. of soil. During the second year of analyses, the total average number of spores was somewhat higher than at the start of experiment. An increase was noted in spore density in the soil under both fertilised (NPK) clover species, in relation to the non-fertilised ones. However, the differences were not statistically significant (Fig. 1b).

The total mean number of AMF spores in the third year (2004) ranged from 1412 to 2296 per 100 g d. m. of soil. In the case of the red clover, fertilisation caused a significant increase in the numbers of AMF spores. In the case of the white clover, larger numbers of AMF spores were also noted in the fertilised version than in the non-fertilised soil, but the difference was not statistically significant (Fig. 1c). In the fourth year (2005), the average spore density in the rhizosphere of the clovers varied within the range from 1392 to 2443 in 100 g d. m. of soil (Fig. 1d). Analogously to the second and third year of the experiment, significantly highest numbers of mycorrhizal spores was isolated from the fertilised soil under the white clover. The lowest numbers of spores, with no statistically significant differences, were isolated from the non-fertilised soil under the red clover.

The spore numbers in the rhizosphere of the two tested clover species varied seasonally (Fig. a-d). The strongest growth of endomycorrhizal fungi took place in the summer months (June and August). The maximum value was noted for the fertilised red clover (year 2005), i.e. 3211 spores in 100 g of soil (term IV) – Fig. 1d. In September, a significant decrease in the numbers of spores was observed and on the final date (October) there was a significant increase in the numbers of AMF (Fig. 1d).

The results concerning the AMF numbers in soil from the plots with the smooth meadow-grass (*Poa pratensis*) and the grass mix in the years 2002-2005 are presented in Fig. 2a-d. Statistical analysis with Tukey's multiple tests based on HSD did not reveal any significant differences between the spore density under the cultivars of the smooth meadow-grass and the grass mix (in both fertilisation versions) in the year 2002. In 2003, statistically significantly lower values were noted only in relation to the AMF numbers in the soil from the non-fertilised of the grass mix. In 2004 and 2005, the mineral fertilisation caused a significant increase in the numbers of endomycorrhizal fungi in the soil under the smooth meadow-grass and the grass mix (Fig. 2c-d).

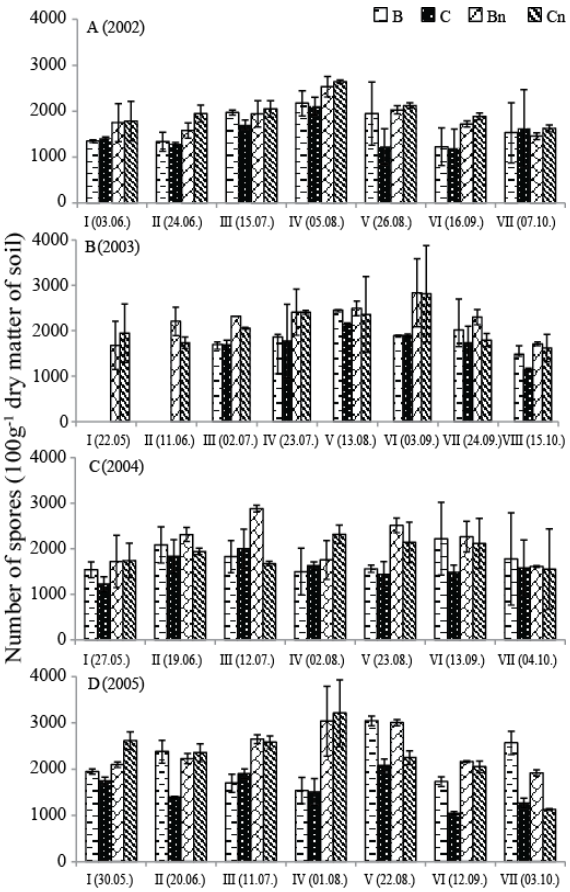

Fig. 1. Spore numbers of AM fungi under cultures of white and red clover in 2002-2005
Means ± standard deviations. Identical letters are not statistically different (Tukey, α=0.05): 2002 – B - ab; C – b; Bn – a; Cn – a. 2003 – B – ab; C – ab; Bn – a; Cn – ab. 2004 - B - a; C – b; Bn – a; Cn – a. 2005 – B – ab; C – c; Bn – a; Cn – ab. 2002-2005 (means) B – b; C – c; Bn – a; Cn – ab. Abbreviations: B – white clover, non-fertilised; Bn – white clover, fertilised; C – red clover, non-fertilised; Cn – red clover, fertilised

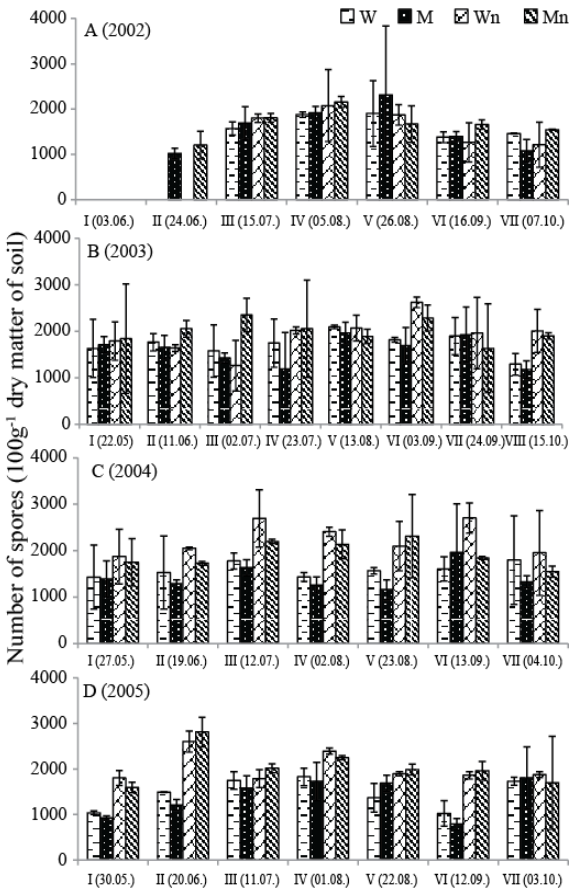

Fig. 2. Spore numbers of AM fungi under cultures of smooth meadow-grass and grass mix in 2002-2005
Means ± standard deviations. Identical letters are not statistically different (Tukey, α=0.05): 2002 – W - ab; M – ab; Wn – ab; Mn – ab. 2003 – W - ab; M – b; Wn – ab; Mn – ab. 2004 - W - b; M – b; Wn – a; Mn – a. 2005 – W - c; M – c; Wn – b; Mn – b. 2002-2005 (means) W – c; M – c; Wn – b; Mn – b. Abbreviations: W – smooth meadow-grass, non-fertilised; Wn – smooth meadow-grass, fertilised; M – grass mix, non-fertilised; Mn – grass mix, fertilised

Analyses of the vegetation period of the smooth meadow-grass and the grass mix demonstrated the strongest growth of AM fungi in the summer months (sampling terms IV - VI) - Fig. 2.

The mean values for four years ranged from 1477 to 2228 spores (mean of 1858) per 100 g d. m. of soil (Fig. 3). It was shown that in the years 2004 and 2005 fertilisation caused a significant increase in the density of AM fungal spores in soil under the smooth meadow-grass, the grass mix, and the red clover. In the first two years of the experiment (2002-2004), larger numbers of AMF spores were noted also in soil from the variants with the NPK fertilisation (Fig. 3), but the differences were statistically insignificant (except for the red clover – 2002) (Fig. 1, 2). Comparing the mean numbers of AMF spores (for all the plant variants during the four years of analyses), it was found that significantly lower number of spores were isolated in the first year of the experiment. In the subsequent years, the differences among the particular variants were not significant (Fig. 3). However, a seasonal character of the occurrence of AMF was noted. Significantly highest numbers were observed in the summer months, and the lowest at the start and towards the end of plant vegetation (Table 3).

Arbuscular mycorrhizal colonisation: All of the examined plants formed typical AM symbiosis because at least arbuscules or arbuscules and vesicles were found in the root tissues. However, during the years of the experiment, a very low percentage of roots of the studied plant species were colonised by endomycorrhizal fungi. The percentage share of the clover and grass roots colonised by AMF varied from 0 to 46% (average of 4.1% to 12.2%) – Fig. 4 and 5. In the first year of the experiment (2002), the mean level of root infection of the clovers and grasses by AM fungi did not exceed 4 % and 2 %, respectively (Fig. 4a and 5a). Only in July (sampling date III), it reached a value of 14 % in the case of the fertilised white clover. In 2002, absence of AM colonisation of roots of the fertilised smooth meadow-grass was noted as well (Fig. 5a). In the second year of the experiment (2003), the mean level of root infection of

the clover and grasses was still very low, from ca. 2 to 7% and 6%, respectively (Fig. 4b and 5b). Only in the case of the fertilised red clover, the degree of mycorrhization of the roots was significantly lower than in the other experimental variants, which did not differ significantly from one another.

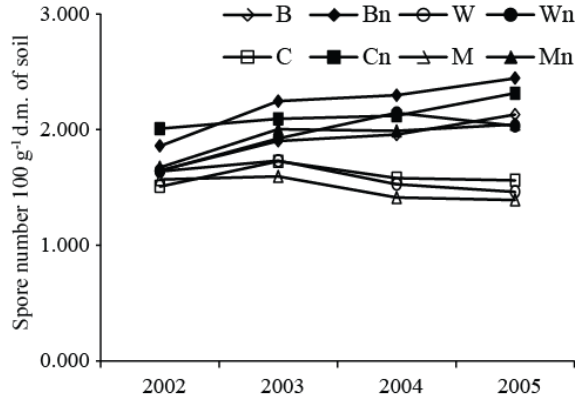

Fig. 3. Comparison of the mean AMF spore number in the particular years of the experiment
Identical letters are not statistically different (Tukey, α=0.05): 2002 – b; 2003 – a; 2004 – a; 2005 – a. Abbreviations as for Fig. 1-2.

In the subsequent year (2004), the mean level of mycorrhizal colonisation of the roots of the clovers and grasses reached a value of 11.7% and persisted within the range from 5.2% to 18% (Fig. 4c and 5c). The highest mean percentage of colonised roots was noted in the plot with the fertilised grass mix and it was statistically significant. In turn, analysis of variance did not reveal any significant differences between the root colonisation levels of the clovers studied. In the fourth year of the experiment (2005), the average levels of root colonisation of both clover species oscillated between 1.6 and 13.9%, which was slightly lower than in the preceding year (Fig. 4d). In the case of the red clover and grass mix, the mineral fertilisation caused a significant increase in the root colonisation levels (Fig. 4d and 5d).

The study showed significant growth of arbuscular mycorrhizas in the roots of the clovers and grasses in the first three years of the experiment. In the fourth year (2005), the intensity of endomycorrhizal colonisation decreased significantly relative to the year 2004 and stabilised at the level from 2003 (Fig. 6).

Both indicators of the growth of arbuscular fungi, i.e. root colonisation and sporulation, indicate stronger growth of these fungi in perennial communities of meadow vegetation in a post-marshy habitat than in young habitats (non-stabilised).

Correlation analysis: No significant correlation was found between spore density and AMF colonisation of the roots of the clovers and grasses (2002: r - 0.099, p - 0.33; 2003: r - 0.034, p - 0.27; 2004: r - 0.103, p - 0.12; 2005: r - 0.052, p - 0.58; 2002-2005: r - 0.077, p - 0.07).

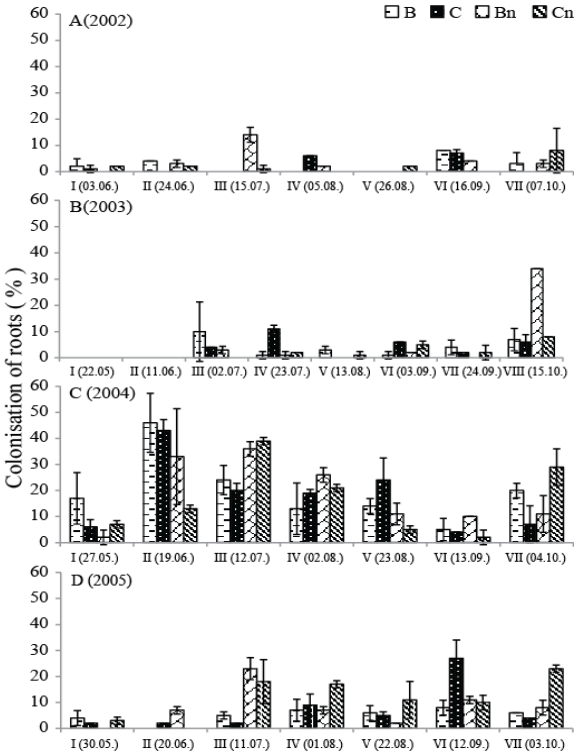

Fig. 4. Colonisation of the roots of the white and red clover by AMF in 2002-2005
Means ± standard deviations. Identical letters are not statistically different (Tukey, α=0.05): 2002 – B - ab; C – ab; Bn – a; Cn – ab. 2003 – B – ab; C – a; Bn – ab; Cn – b. 2004 - B - abc; C – abc; Bn – ab; Cn – ab. 2005 – B – bc; C – b; Bn – b; Cn – a. 2002-2005 (means) B – b; C – c; Bn – a; Cn – ab. Abbreviations as for Fig. 1.

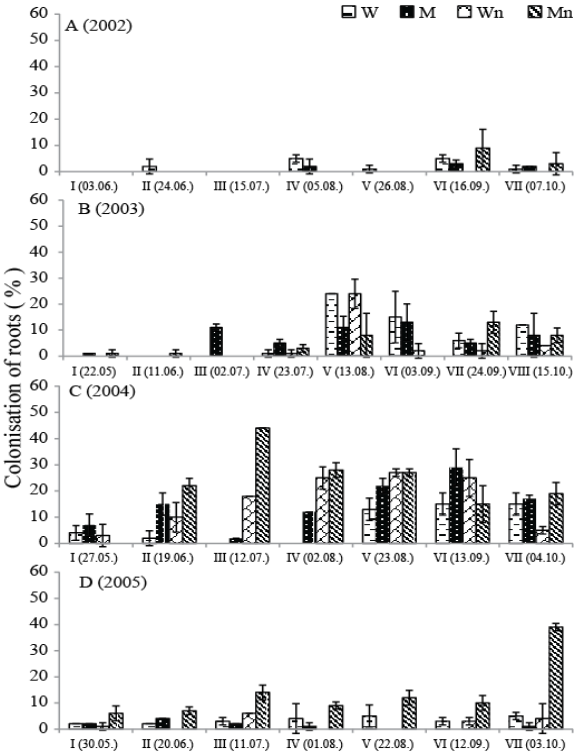

Fig. 5. Colonisation of the roots of the smooth meadow-grass and the grass mix by AMF in 2002-2005
Means ± standard deviations. Identical letters are not statistically different (Tukey, α=0.05): 2002 – W - ab; M – ab; Wn – b; Mn – ab. 2003 – W - ab; M – a; Wn – ab; Mn – a. 2004 - W - c; M – bc; Wn – bc; Mn – a. 2005 – W - cd; M – d; Wn – cd; Mn – a. Abbreviations as for Fig. 2.

Table 3. The mean of the AMF spore number (for four years) in soil (100 g^{-1} d. m. of soil) under the cultures of clovers and grasses.

Terms	Combination								Mean for terms	Tukey's test
	B	C	Bn	Cn	W	M	Wn	Mn		
I	1695	1530	1868	2140	1287	1239	1819	1695	**1679**	cd
II	2046	1478	2111	2068	1571	1279	2223	2122	**1866**	abc
III	1778	1839	2489	2187	1686	1583	1867	2077	**1938**	ab
IV	1721	1705	2556	2757	1744	1565	2259	2169	**2059**	a
V	2409	1792	2609	2224	1662	1764	1965	1971	**2049**	a
VI	1761	1332	2230	2184	1371	1328	2063	1943	**1776**	bcd
VII	2092	1493	1840	1444	1725	1590	1777	1624	**1698**	bcd
VIII	1483	1136	1711	1619	1295	1179	2008	1902	**1542**	d
Mean of combination	**1919**	**1587**	**2228**	**2129**	**1578**	**1477**	**1995**	**1949**		

values in the same column followed by different letters are significantly different $\mathbf{HSD_{comb.}=211.46}$; $\mathbf{HSD_{term.}=244.20}$
Explanations as for Table 2

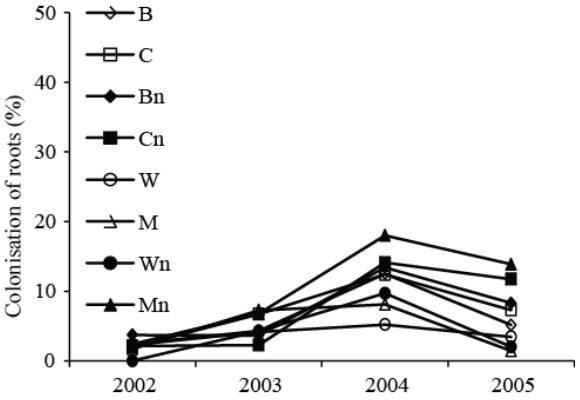

Fig. 6. Comparison of the mean levels of AMF root colonisation in the particular years of the experiment
Identical letters are not statistically different (Tukey, α=0.05): 2002 – c; 2003 – b; 2004 – a; 2005 – b. Abbreviations as for Fig. 1-2.

Discussion

Spore density: The results of this study showed that the total count of AMF spores (mean for 4 years) in the studied peat-muck soil under the clovers and grasses varied from 1477 to 2228 spores in 100 g of d. m. of the soil (mean of 1858). The values obtained were generally higher than those noted for clovers and grasses grown on soils conventionally referred to as mineral soils, and on sandy soils in particular. In turn, they were similar to values obtained for soils rich in organic matter. Mullahey & Speed (1991), who studied the abundance of AMF in a meadow culture of 4 species of indigenous grasses: *Schizochyrium soloniferum* Nash; *Andropogon copillipes* Nash; *Andropogon virginicus* L. and *Aristida stricta* Mchx (Florida, USA) on a podzolic soil found only from 23 to 265 spores per 100 g of d. m. of soil. Higher numbers of AMF than in light soils have been found under grasses on soils richer in organic matter, mineral colloids, and nutrients (Lugo & Cabello, 2002). An even greater variation in the number of spores of endomycorrhizal fungi, even up to 4083 in 100 g of soil, with a minimum of 8 spores/100 g, was observed by Lugo & Cabello (2002) in studies on natural grass communities of Argentina situated on soils classified as Humic Cambisol and Haplic Phaeozem.

Although the literature on the subject does not provide information on the occurrence of AMF under meadow vegetation in post-marshy habitats, data on the cultivation of other plants on organic soils indicate that these habitats are characterised by high abundance of AM fungi. This is indicated in a study of Księżniak & Kobus (1998), who, isolated 1720 spores /100 g of soil under a culture of barley and oat on a silty-peat soil.

Our study shows that the kind of plant had a significant effect on the density of spores of arbuscular fungi in the peat-muck soil studied. This was manifested in a significantly greater number of spores in the soil under the clovers than grasses (with the exception of the non-fertilised red clover). The overall mean number of spores isolated from the soil under the clovers was 1916, and in the case of the grasses, it was 1730 spores in 100 g of soil. As opposed to the grasses (smooth meadow-grass and grass mix), for which no significant differences were observed in the numbers of spores between the treatments, a significantly larger number of spores was found under the white clover (1919) compared to the red clover (1587), but only in the non-fertilised treatment. Similar tendencies were indicated in studies concerning these clover species growing on mineral soils. Data compiled by Błaszkowski (1993a, b) and Błaszkowski *et al*. (2002) (Poland) indicate that the abundance of AM fungi in the rhizosphere soil of the white clover is several-fold higher than in that of the red clover. Comparatively, however, those values were 7-fold (white cover) and 32-fold (red clover), respectively, lower than the values obtained in this study.

The differences observed in this study in the numbers of AMF spores under various plants (clovers, grasses) growing on the same soil should be attributed to the

diversified composition of root secretions of these plants, and in consequence to the differences of their effect on endomycorrhizal fungi. This explanation is in agreement with the thesis of Nasrullah *et al.* (2010), who attribute the greater number of spores of AM fungi as well as the degree of colonisation roots of various plants (wheat and maize) cultivated on the same soils to root secretions, which stimulate spore germination and the level of their infectiousness. Analogous conclusions concerning the mycotrophicity of plants inhabiting the same soils were formulated earlier by Sharif and Moawad (2006).

Our study indicates that NPK fertilisation caused significant stimulation of AM fungal sporulation in all 4 plant variations. The mean numbers of AMF spores in soils fertilised with NPK were significantly higher than in the non-fertilised soil (control). The explanation of this fact poses considerable interpretation difficulties. As demonstrated by various authors (Sharif *et al.*, 2006; Nasrullah *et al*., 2010), greater numbers of spores of endomycorrhizal fungi are noted in non-fertilised soils than in fertilised ones. On the other hand, Panwar *et al.* (2011) observed a reverse effect while analysing over a short period of time (8 months) the influence of the content of available phosphorus on the density of AMF spores in wheat rhizosphere on a grey-brown podzolic soil. A similar reaction with relation to nitrogen is indicated in the studies by Anderson *et al.* (1984) and Kowalczyk (2008). According to the authors cited, the concentration of spores of AM fungi is correlated with the content of nitrogen in the soil. Given the low levels of phosphorus in hydrogenic soils (among which the studied peat-muck soils are classified), it can be assumed that the fertilisation of the clovers and grasses with mineral phosphorus in these habitats may have contributed to increased sporulation of AM fungi. This may have been achieved through improvement of the condition of the plants due to more effective photosynthesis, and thus to the influx of greater amounts of assimilates to the roots – source of C and energy for the fungi. This suggestion appears to be supported by the observations of Escudero and Mendoza (2005), who suggest that fertilisation with phosphorus at a deficit of the element in soil may lead to an increase in the number of spores. Moreover, the authors point out that the AMF spore density is a result of interactions between soil, plant, and climate factors, and may be specific in individual cases.

The analysis of the effect of the vegetation season on the AMF spore density in the successive years of the study (2002-2005) demonstrated that it was the highest in the summer months and the lowest in spring and in late autumn. Significantly lower numbers of spores at the start and towards the end of vegetation were observed in all the combinations of the test plants. Absence of any effect of plant species on the seasonal dynamics of the abundance of spores of arbuscular fungi was also observed by Muthukumar and Udaiyan (2002) in a study on two species of sedges (*Cyperus iria* and *C. rotundus*) from the family Cyperaceae growing on semi-barren tropical grasslands in India.

In the light of own observation, it appears that the higher AMF sporulation under the clovers and grasses in the summer months was caused by higher moisture content of the peat-muck soil and higher temperature in that period compared to the spring and autumn months. Meteorological data for the study area (data not shown) indicate that the values of mean monthly sums of precipitations for a multi-year period and air temperatures were higher in the months from June to August than from April to May and from September to October. Such conditions were also favourable for the growth of the clover and grass species studied, which have high or moderate water requirements and are generally (with the exception of the red fescue) sensitive to low temperatures (Warda, 2005). Indirectly, through their effect on the plants, the moisture- temperature conditions affected also the process of AMF sporulation, which could intensify in optimum conditions for the physiological processes and *vice versa.*

Colonisation of roots: Cade-Menum *et al*. (1991) report an effective impact of AM fungi on plants at root colonisation above 50%. In turn, Sanders *et al*. (1977) conclude that already 10% colonisation of roots by AM fungi contributes to a significant increase in the amount of phosphorus absorbed from the soil, and Volkmar & Woodbury (1989) observed a 25% increase in the mass of barley shoots even at 2-7% colonisation of roots.

Our study indicates that endomycorrhizal colonisation of meadow plants on peat-muck soil was not significant: the average level of mycorrhization of the clovers and grasses did not exceed 12.2%. The highest colonisation rates (up to 18%) were exhibited by the roots of grasses grown in the grass mix fertilised with NPK, and the lowest – by the roots of the non-fertilised smooth meadow-grass (maximum 5.2%). The data obtained are similar to the lower range of values concerning the degree of mycorrhization of crop plants and wild-growing plants on the soils in Poland given by Błaszkowski (1993a). On average, it amounted to 13.4% - 27.9%. In the case of the individual species of grasses and clovers studied in our experiment, the values obtained were close to those recorded by Błaszkowski (1993a). The author reported that the level of colonisation of the smooth meadow-grass (*Poa pratensis*) was 10.1% and the value for red clover (*Trifolium pratense*) reached 17.7%. Low mycorrhization rates (from 6 to 28%) of grass roots from meadow areas (podzolic soils) in Florida were observed also by Mullahey & Speed (1991).

According to Błaszkowski (1993a), the relatively low level of endomycorrhizal colonisation suggests that the plants studied may have been growing under conditions that were unfavourable for arbuscular fungi. In post-marshy habitats, one of the factors can be the excessive level of soil moisture. For example, no colonisation of roots by AM fungi has been observed in the rush family (Juncaceae) inhabiting wetland habitats (Tadych & Błaszkowski, 1999). On the other hand, it is known that in grass communities the spread of mycorrhizal infection is facilitated due to the close neighbourhood of roots (Lugo *et al.,* 2003). However, unlike in natural grass communities, where due to the strong competition for nutrients the plants grow under conditions of nutrient stress, in meadow communities on cultivated soils the content of nutrients is higher, and thus

the level of AMF colonisation can be low. It is known that mycorrhizal colonisation of cultivated plants is lower than that of wild-growing plants, mainly due to lower competition of the plants for nutrients (Read *et al.,* 1976). An additional problem may result from the poor or non-existent pigmentation of mycorrhizal structures of many species of Glomeromycota, which makes them undetectable in microscopic observations (Morton & Redecker, 2001).

Although in this study no statistically significant differences were noted between the mycorrhization of the clovers and the grasses (in both fertilisation treatments), in percentage values the mycorrhization was lower for the grasses (with the exception of the fertilised grass mix) than for the clovers. Similar trends were noted in two greenhouse experiments by Zhu *et al.* (2000), who studied the preferences of the perennial ryegrass (*Lolium perenne*) and the white clover (*Trifolium repenes*) to mycorrhizal associations with AM fungi. In both cases, higher mycotrophicity was shown for the white clover than for the perennial ryegrass. A higher level of colonisation of roots of legumes than grasses was also noted by Karanika *et al.* (2008) in pot experiments with such grass species as cocksfoot (*Dactylis glomerata*), sheep fescue (*Festuca ovina*), perennial ryegrass (*Lolium perenne*) and smooth meadow-grass (*Poa pratensis*) and legumes: bird's-foot trefoil (*Lotus cornitulatus*), alfalfa (*Medicago sativa*) and white clover (*Trifolium repenes*). This was attributed to the stronger branching of the root system of grasses with more abundant root hairs, which reduce the dependence of grasses on AMF in the uptake of nutrients. Additionally, grasses have generally lower requirements for phosphorus, which ensures lower dependence on AMF. In contrast, the high level of colonisation of legumes is attributed to their greater demand for phosphorus in the process of binding of atmospheric nitrogen (Mamolos *et al.,* 2005; Karanika *et al.,* 2007).

Our study did not show any effect of mineral fertilisation on the degree of colonisation of plant roots by AM fungi. The exception was the grass mix for which a significantly higher level of colonisation was demonstrated in the combination with NPK fertilisation. The fact of NPK fertilisation contributed to increased mycorrhization of grass roots (*Schizochyrium scoparium* Michx., *Muhlenbergia capillaries* Lam., *Andropogon glomeratus* Walt) was reported earlier by Nijjer *et al.* (2010). Different results were obtained by Titus & Lepš (2000), who studied the effect of NPK fertilisation on the vegetation of an oligotrophic meadow (Czech Republic), including grasses (*Malcus lanatus* - tufted grass and *Molinia caerulea* – purple moor grass Moench).

Studies conducted by Thomson *et al.* (1992) indicate that increased levels of colonisation of plant roots (underground clover *Trifolium subterraneum* L., Italian ryegrass *Lolium rigidum*, barley *Hordeum leporium* Link., and *Arctotheca calendula* Levyns) by AM fungi after the application of phosphorus to the soil is observed at lower doses, which supply no more than 60-65% of the plant requirements and thus maintain a serious deficit of the element. Our study indicates that the level of phosphorus in the combination with the fertilised grass mix was lower by 32% than in the combination without phosphorus fertilisation. Therefore, the deficit of phosphorus observed could have contributed to increased mycorrhization of the plant roots compared to the non-fertilised combination. In the other plant combinations, the level of phosphorus in both fertilisation treatments was similar, which could explain the lack of an effect of mineral fertilisation on the degree of endomycorrhizal colonisation of those plants.

Studies conducted by various authors (Muthukumar & Udaiyan, 2002; Escudero & Mendoza, 2005; Rodríguez-Echeverría *et al.,* 2008, Xin *et al*., 2012) concerning endomycorrhizas of grasses, sedges, and legumes clearly indicate a seasonal nature of AMF root colonisation. Analysing the seasonal changes in the intensity of endomycorrhizal colonisation of the grasses and clovers in our study, we noted the significantly weakest growth of arbuscular mycorrhizas only at the start of plant vegetation (term I). The numerical values obtained for the other dates of analyses were higher and did not differ significantly from one another. Higher levels of colonisation of roots of meadow plants by AMF in the later months of the vegetation season (summer months) were noted earlier by Lugo *et al*. (2003), Mandyam & Jumpponen (2008) Titus & Lepš (2000). The main causes of the intensification of endomycorrhizal colonisation during the vegetation season include primarily increased requirements for phosphorus caused by blooming and/or fruition at a simultaneous slowdown of root growth (Titus & Lepš, 2000). The weaker endomycorrhizal colonisation of meadow plants at the beginning of the vegetation season may be an effect of slower root growth of the plants relative to the rate of formation of AM structures (Titus & Lepš, 2000).

Correlations between the numbers of AMF and the degree of mycorrhization: In this study, no correlation was noted between the AMF spore numbers and the degree of mycorrhization of roots of clovers and meadow grasses. Analogous results for a variety of plants were obtained by number of workers (Błaszkowski, 1993a, b; Jacobson, 1997; Escuardo & Mendoza, 2005; Li *et al.,* 2007; Rodríguez-Echeverría *et al.,* 2008; Kowalczyk & Błaszkowski, 2011). A different tendency, i.e. towards increased mycorrhization with increasing abundance of AMF spores in soil, was noted by Sharif *et al.* (2006) and by Nasrullah *et al.* (2010). In turn, Lugo & Cabello (2002) and Lugo *et al.* (2003) observed an opposite relation: a high level of AMF root colonisation was coupled with a low spore numbers in the soil, and *vice versa*. The authors attributed this effect to seasonal changes in the spore diversity and to low specificity of the host plant. According to Rodríguez-Echeverría *et al.* (2008), who demonstrated absence of a correlation between the AMF spore density and the AMF root colonisation of European beachgrass (*Ammophila arenaria* L. – *Poaceae*), the production of spores and the level of root colonisation are not mutually correlated. Although sporulation is affected by climatic conditions and by the phenology of the plant, the level of colonisation does not depend on the climate or on the phenology of the plant. Also, the results of our study seem to support the absence of a correlation between the AMF

spore numbers and root colonisation. In addition, the lack of a correlation between the level of mycorrhization and the spore numbers in the soil may be the result of the dyeing applied, which does not reveal all structures of AM fungi that actually exist within the roots (Morton & Redecker, 2001).

Conclusions

The abundance of arbuscular mycorrhizal fungi in peat-muck soil under cultures of clovers and meadow grasses was high and increased after the application of mineral fertilisation. In contrast to the numbers of AM fungi in the soil, the degree of endomycorrhizal colonisation of roots of the grasses and clovers was low and did not depend on the spore density of the fungi in the soil or on NPK fertilisation. The study indicated stronger growth of the fungi and arbuscular mycorrhiza of meadow vegetation on peat-muck soil in the perennial communities than in the young ones (non-stabilised).

References

Allen, M.F., T.S. Moore, Jr.M. Christensen and N. Stanton. 1979. Growth of vesicular-arbuscular-mycorrhizal and nonmycorrhizal *Bouteloua gracilis* in a defined medium. *Mycologia,* 71: 666-669.

Anderson, R.C., A.E. Liberta and L.A. Dickman. 1984. Interaction of vascular plant and vesicular-arbuscular mycorrhizal fungi across a soil moisture-nutrient gradient. *Oceologia*, 64: 111-117.

Błaszkowski, J. 1993a. Comparative studies of the occurrence of arbuscular fungi and mycorrhizae (Glomales) in cultivated and uncultivated soils of Poland. *Acta Mycol.,* 28: 93-140.

Błaszkowski, J. 1993b. The occurrence of arbuscular mycorrhizal fungi and mycorrhizae (Glomales) in cultivated and uncultivated soils of Poland. *Phytopath. Pol*., 5: 9-15.

Błaszkowski, J., A. Iwaniuk and B. Czerniawska. 2002. The occurence of arbuscular mycorrhizal fungi (Glomeromycota) in cultivated soils of Poland. *Acta Agrobot*., 55: 41-48.

Bonfante, P. and A. Desiro. 2015. Arbuscular Mycorrhizas: The lives of beneficial fungi and their plant hosts. In: (Ed.): Lugtenberg, B. *Principles of Plant-Microbe Interactions*. Springer Inter. Publ., Switzerland, pp. 235-245.

Cade-Menun, B.J., S.M. Berch and A.A. Bomke. 1991. Seasonal colonization of winter wheat in South Coastal British Columbia by vesicular-arbuscular mycorrhizal fungi. *Can. J. Bot.,* 69: 78-86.

Datta, P. and M. Kulkarni. 2014. Influence of two, AM'' fungi in improvement of mineral profile in *Arachis hypogaea* L. under salinity stress. *Lagume Res.*, 37: 327-328.

Escudero, V. and R. Mendoza. 2005. Seasonal variation of arbuscular mycorrhizal fungi in temperate grasslands along a wide hydrologic gradient. *Mycorrhiza*, 15: 291-299.

Falkowski, M. 1982. *Polish grasses*. PWRiL, Warszawa, (in Polish).

Hajiboland, R., N. Aliasgharzadeh, S. Laiegh and C. Poschenrieder. 2010. Colonization with arbuscular mycorrhizal fungi improves salinity tolerance of tomato (*Solanum lycopersicum* L.) plants. *Plant Soil*, 331: 313-327.

Harrier, L.A. and C.A. Watson. 2004. The potential role of arbuscular mycorrhizal (AM) fungi in the bioprotection of plants against soil-borne pathogenes in organic and/or other sustainable farming systems. *Pest. Manag. Sci.,* 60: 149-157.

Iwaniuk, A. and J. Błaszkowski. 2004. Arbuscular fungi and mycorrhizae of agricultural soils of the Western Pomerania. Part I. Occurrence of arbuscular fungi and mycorrhizae. *Acta Mycol*., 39: 65-91.

Jacobson, K.M. 1997. Moisture and substrate stability determine VA-mycorrhizal fungal community distribution and structure in an arid grassland. *J. Arid. Environ.,* 35: 59-75.

Johansson, J.F., L.R. Paul and R.D. Finlay. 2004. Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. *FEMS Microbiol. Ecol.,* 48: 1-13.

Karanika, E.D., D.A. Alifragis, A.P. Mamolos and D.S. Veresoglou. 2007. Differentiation between responses of primary productivity and phosphorus exploitation to species richness. *Plant Soil*, 297: 69-81.

Karanika, E.D., O.K. Voulgari, A.P. Mamolos, D.A. Alifragis and D.S. Veresoglou. 2008. Arbuscular mycorrhizal fungi in northern Greece and influence of soil resources on their colonization. *Pedobiologia*, 51: 409-418.

Kormanik, P.P. and McGraw A.C. 1982. Quantification of vesicular-arbuscular mycorrhizae in plant roots. In: (Ed.): Schenk, N.C. *Methods and Principles of Mycorrhizal Research*, Amer. Phypathol. Soc. St. Paul, Minn, USA, pp. 37-45.

Koske, R.E. and B. Tessier. 1983. A convenient permanent slide mounting medium. *Mycol. Soc. Am. Newslett.*, 34: 59.

Kowalczyk, S. 2008. Fungi and arbuscular mycorrhizas (Glomeromycota) of soils of the Lubuskie Province. *Doctoral dissertation*, AR Szczecin, (in Polish).

Kowalczyk, S. and J. Błaszkowski. 2005. Arbuscular mycorrhizal fungi of soils of the Lubuskie Province. *Acta Agrobot.,* 58: 453-474, (in Polish).

Kowalczyk, S. and J. Błaszkowski. 2011. Arbuscular mycorrhizal fungi (Glomeromycota) associated with roots of plants of the Lubuskie Province. *Acta Mycol*., 46: 3-18.

Księżniak, A. and J. Kobus. 1998. Occurrence of spores of VAM fungi and colonisation of roots of crop plants in various soils of Poland. In: (Eds.): Sawicka, A. and G. Durska. *Ecological aspects microbiology of soil*. Poznań, (in Polish), pp. 149-154.

Księżniak, A., J. Kobus and A. Perzyński. 2001. An attempt to use of bacteria and AM fungi in protection of cereal plants against *Gaeumannomyces graminis* var. *tritici*. Bull. Pol. Acad. Scien, *Biol. Sci.,* 49: 353-355.

Lee, B.R., S. Muneer, J.C. Avice, W.J. Jung and T.H. Kim. 2012. Mycorrhizal colonisation and P-supplement effects on N uptake and N assimilation in perennial ryegrass under well-watered and drought-stressed conditions. *Mycorrhiza*, 22: 525-534.

Li, L., Y. Zhang and Z.W. Zhao. 2007. Arbuscular mycorrhizal colonization and spore density across different land-use types in a hot and arid ecosystem, Southwest China. *J. Plant. Nutr. Soil Sci*., 170: 419-425.

Liu T., M. Sheng, C.Y. Wang, H. Chen, Z. Li and M. Tang. 2015. Impact of arbuscular mycorrhizal fungi on the growth, water status, and photosynthesis of hybrid poplar under drought stress and recovery. *Photosynthetica*, 53: 250-258.

Lugo, M. and M. Cabello. 2002. Native arbuscular mycorrhizal fungi (AMF) from mountain grassland (Córdoba, Argentina) I. Seasonal variation of fungal spore diversity. *Mycologia*, 91: 579-586.

Lugo, M.A., M.E. González Maza and M.N. Cabello. 2003. Arbuscular mycorrhizal fungi in a mountain grassland II. Seasonal variation of colonization studied, along with its relation to grazing and metabolic host type. *Mycologia*, 95: 407-415.

Mamolos, A.P., C.V. Vasilokos and D.S. Veresoglou. 2005. Vegetation in contrasting soil water sites of upland herbaceous grasslands and N:P ratios as indicators of nutrient limitation. *Plant Soil*, 270: 355-369.

Mandyam, K. and A. Jumpponen. 2008. Seasonal and temporal dynamics of arbuscular mycorrhizal and dark septate endophytic fungi in a tallgrass prairie ecosystem are minimally affected by nitrogen enrichment. *Mycorrhiza*, 18: 145-155.

Martin, F.M., S. Perotto and P. Bonfante. 2001. Mycorrhizal fungi: a fungal community at the interface between soil and roots. In: (Eds.): Pinton, R., Z. Varanini and P. Nannipieri. *The Rhizosphere: Biochemistry and organic substances at the soill-plant interface*. Marcel Dekker, New York, pp. 263-296.

Minz, D. and M. Ofek. 2011. Rhizosphere microorganisms. In: (Eds.): Rosenberg, E. and U. Gophna. *Beneficial microorganisms in multicellular life forms*. Springer-Verlag, Berlin Heidelberg, pp. 105-122.

Morton, J.B. and D. Redecker. 2001. Two new families of Glomales, Archaeosporaceae and Paraglomaceae, with two new genera *Archaeospora* and *Paraglomus* based on concordant molecular and morphological characters. *Mycologia*, 93: 181-195.

Mullahey, J.J. and C.S. Speed. 1991. The occurrence of vesicular-arbuscular mycorrhizae on Florida range grasses. Soil and Crop. Sci. Soc. Flo., *Proc.*, 50: 44-47.

Muthukumar, T. and K. Udaiyan. 2002. Seasonality of vesicular-arbuscular mycorrhizae in sedges in a semi-arid tropical grassland. *Acta Oecol*., 23: 337-347.

Nasrullah, M. Sharif, K. Rubina and T. Burni. 2010. Occurrence and distribution of arbuscular mycorrhizal fungi in wheat and maize crops of Malakand division of North West Frontier Province. *Pak. J. Bot*., 42: 1301-1312.

Nijjer, S., W.E. Rogers and E. Siemann. 2010. The impacts of fertilization on mycorrhizal production and investment in Western Gulf Coastal grasslands. *Am. Midl. Nat*., 163: 124-133.

Omar, M.B., L. Bolland and W.A. Heather. 1979. A permanent mounting medium for fungi. *Bull. Brit. Mycol. Soc.,* 13: 31-32.

Panwar, V., M.K. Meghvansi and S. Siddiqui. 2011. Short-term temporal variation in sporulation dynamics of arbuscular mycorrhizal (AM) fungi and physico-chemical edaphic properties of wheat rhizosphere. *Saudi. J. Biol. Sci*., 18: 247-254.

Pendranzani H., M. Rodríguez-Rivera, M. Gutiérrez, R. Porcel, B. Hause and J.M. Ruiz-Lozano. 2015. Arbuscular mycorrhizal symbiosis regulates physiology and performance of *Digitaria eriantha* plants subjected to abiotic stresses by modulating antioxidant and jasmonate levels. *Mycorrhiza*, 1: 1-12.

Philips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc*., 55: 158-161.

Read, D.J., H.K. Koucheki and J. Hodgson. 1976. Vesicular-arbuscular mycorrhiza in natural vegetation systems. I. The occurrence of infection. *New Phytol.,* 77: 641-653.

Rodríguez-Echeverría, S., W.H.G. Hol, H. Freitas, W.R. Eason and R. Cook. 2008. Arbuscular mycorrhizal fungi of *Ammophila arenaria* (L.) Link: Spore abundance and root colonisation in six locations of the European coast. *Eur. J. Soil Biol*., 44: 30-36.

Sanders, F.E., P.B. Tinker, R.L.B. Black and S.M. Palmersley. 1977. The development of endomycorrhizal root systems. I. Spread of infection and growth-promoting effects with four species of vesicular-arbuscular endophytes. *New Phytol.,* 78: 257-268.

Schüβler, A., D. Schwarzott and C. Walker. 2001. A new fungal phylum, the *Glomeromycota*: phylogeny and evolution. *Mycol. Res.,* 105: 1413-1421.

Sharif, M. and A.M. Moawad. 2006. Arbuscular mycorrhizal incidence and infectivity of crops in North West Frontirer Province of Pakistan. *World J. Agric. Sci*., 2: 123-132.

Sharif, M., M.S. Sarir and Nasrullah. 2006. Field evaluation of arbuscular mycorrhizal fungi in wheat-maize cropping system in Hazara Division of North West Frontier Province. *Pak. J. Biol. Sci.,* 9: 487-492.

Šmilauer, P. and M. Šmilauerová. 2000. Effect of AM symbiosis exclusion on grassland community composition. *Folia Geobot*., 35: 13-25.

Smith, S.E. and D. Read. 2008. Mycorrhizal Symbiosis. (3rd Ed), Academic Press, Elsevier Ltd.

StatSoft, Inc. 2011. STATISTICA (data analysis software system), version 10. www.statsoft.com.

Tadych, M. and J. Błaszkowski. 1999. Growth responses of maritime sand dune plant species to arbuscular mycorrhizal fungi. *Acta Mycol.,* 34: 115-123.

Thomson, B.D., A.D. Robson and L.K. Abbott. 1992. The effect of long-term applications of phosphorus fertilizer on populations of vesicular-arbuscular mycorrhizal fungi in pastures. *Aust. J. Agric. Res*., 43: 1131-1142.

Titus, J.H. and J. Lepš. 2000. The response of arbuscular mycorrhizae to fertilization, mowing, and removal of dominant species in a diverse oligotrophic wet meadow. *Am. J. Bot*., 87: 392-401.

Volkmar, K.M. and W. Woodbury. 1989. Effects of soil temperature and depth on colonization and root and shoot growth of barley inoculated with vesicular-arbuscular mycorrhizae indigenous to Canadian prairie soil. *Can. J. Bot.,* 67: 1702-1707.

Warda, M. 2005. Persistency of *Trifolium repens* and sward productivity in low-input pasture on peat-muck soil. *Grassland Science in Europe*, 10: 372-375.

Xin, G., Y.E. Shaoping, E.N. Wu, Y. Wang and K. Sugawara. 2012. Seasonal dynamics in arbuscular mycorrhizal fungal colonization and spore numbers in the rhizosphere of *Dactylis glomerata* L. and *Trifolium repens* L. *Pak. J. Bot*., 44: 2087-2092.

Zhu, Y.G., A.S. Laidlaw, P. Christie and M.E.R Hammond. 2000. The specificity of arbuscular mycorrhizal fungi in perennial ryegrass-white clover pasture. *Agric. Ecosyst. Environ*., 77: 211-218.

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