

## **ARBUSCULAR MYCORRHIZAL FUNGI ABUNDANCE, SPECIES RICHNESS AND COMPOSITION UNDER THE MONOCULTURES OF FIVE MEDICINAL PLANTS**

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**Abstract.** The presence of arbuscular mycorrhizal fungi (Glomeromycota, AMF) in soils may be crucial for sustainable agriculture. Although AMF impact on the performance and accumulation of therapeutic compounds of several medicinal plant species has been well documented, the investigations on the influence of medicinal plants being cultivated on AMF have been insufficiently studied. The effect of three-year monocultures of mycorrhizal (*Hypericum perforatum*, *Levisticum officinale*, *Mentha × citrata* subsp. *citrata* and *Thymus vulgaris*) and non-mycorrhizal (*Chelidonium majus*) medicinal plant species on AMF propagule abundance, species richness and composition was therefore examined. The AMF non-host plant species *C. majus* decreased the abundance of AMF propagules in the soil, whereas the mycorrhizal plants maintained the AMF propagule potential at the same level, however, they changed the composition of AMF species. The results showed that the choice of medicinal plant species, grown even for a relatively short period of time in a monoculture, can substantially alter the AMF potential of soils which in turn can influence the performance of other medicinal plants cultivated subsequently.

**Key words:** arable soil, arbuscular mycorrhiza (AM), cultivation, Glomeromycota

### **INTRODUCTION**

Due to the need of high quantity and quality of medicinal plants utilized in herbal industry, as well as the necessity of standardization of raw materials for pharmaceutical purposes, most medicinal plant species have been introduced into agriculture [van Wyk and Wink 2008]. The collection of medicinal plants from their wild habitats is presently

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scarce and it is presumed that in the nearest future the cultivation of medicinal plants would be the only way to obtain raw materials for herbal industry [Dachler and Pelzmann 1999, Jambor 2001]. Research emphasis is therefore on developing new agro-techniques to improve yield and quality of medicinal plant material. Such techniques may include the proper management of soil that favour soil microorganisms beneficial to plant performance, such as arbuscular mycorrhizal fungi (AMF) [Cameron 2010, Raviv 2010, Gianinazzi et al. 2010]. AMF have been found to stimulate growth, improve pathogen resistance, contribute to the formation of proper soil structure as well as influence the level of secondary metabolites in plants [reviewed in Smith and Read 2008]. The presence of these symbiotic soil microorganisms may therefore be crucial for sustainable agriculture [Gianinazzi et al. 2010]. Although AMF impact on the performance and accumulation of therapeutic compounds of several medicinal plant species has been well documented [Abu-Zeyad et al. 1999, Kapoor et al. 2002a, b, 2007, Copetta et al. 2006, Khaosaad et al. 2006, Toussaint 2007, Toussaint et al. 2007, Caccarelli et al. 2010, Zubek et al. 2010, 2012a], the investigations on the influence of medicinal plants being cultivated on AMF species richness and abundance have been insufficiently studied. To our best knowledge, only one paper concerning the impact of medicinal plants and contrasting fertilization regimes on AMF in arable soils has been published to date [Zubek et al. 2012b]. The aim of our present study was therefore to test whether three-year monocultures of mycorrhizal (*Hypericum perforatum*, *Levisticum officinale*, *Mentha × citrata* subsp. *citrata* and *Thymus vulgaris*) and non-mycorrhizal (*Chelidonium majus*) medicinal plant species influence the AMF community. We hypothesized that AMF species richness and propagule abundance in soil under monoculture of the non-mycorrhizal plant would be decreased in comparison to mycorrhizal ones. Additionally, as plants may vary in the degrees of their dependency on AMF [Smith and Read 2008], we expected differential impact of the mycorrhizal plant species cultivated on AMF community.

## MATERIAL AND METHODS

### Establishment of medicinal plant monocultures

The AMF abundance and species richness were studied in the monocultures of five perennial plant species of reputed medicinal value, included in the European Pharmacopoeia [2008], American Herbal Pharmacopoeia [1997–2005] and other pharmaceutical monographs [Barnes et al. 2007]: greater celandine (*Chelidonium majus* L.) cvar. ‘Cynaber’ (Papaveraceae), St. John’s wort (*Hypericum perforatum* L.) cvar. ‘Topaz’ (Hypericaceae), lovage (*Levisticum officinale* W.D.J. Koch.) cvar. ‘Amor’ (Apiaceae), peppermint (*Mentha × citrata* Ehrh. subsp. *citrata* (= *Mentha piperita* L.) population and thyme (*Thymus vulgaris* L.) cvar. ‘Słoneczko’ (Lamiaceae). The plants’ names follow Mirek et al. [2002], apart from *T. vulgaris*, which follows Anioł-Kwiatkowska [2003].

The monocultures were established in an experimental field of the Institute of Natural Fibres and Medicinal Plants, and located in Poznań-Plewiska, at 52°25’N, 16°58’E. Five fields, ca. 160 m<sup>2</sup> (4 × 40 m) each, were set up on the same soil type (luvisol) in

a complete block design. Before the monocultures were established in 2009, the fields had been subjected to identical mineral fertilization, tillage and crop rotation scheme. In order to maintain the high standards demand for medicinal plant raw materials, a moderate intensity of management, in line with good agricultural practice, was applied. Thus no herbicide and fungicide inputs were incorporated and manual weeding methods were used in each case before and throughout the medicinal plants cultivation. During the course of the cultivation (2009–2011), three universal fertilizers (N/CaO/MgO, 60:6:8 kg ha<sup>-1</sup>, respectively; P<sub>2</sub>O<sub>5</sub> – 70 kg ha<sup>-1</sup>; K<sub>2</sub>O – 100 kg ha<sup>-1</sup>) were applied, at the recommended rates [Kołodziej 2010]. Phosphorus and potassium fertilizers were incorporated in the spring of 2009, whereas nitrogen fertilizer was applied in mid-July 2009. Similarly, one application of universal fertilizer “Azofoska” (300 kg ha<sup>-1</sup>), containing N/P<sub>2</sub>O<sub>5</sub>/K<sub>2</sub>O, 1:0.5:1.4, respectively, was given in the spring of 2010 and 2011.

The seeds of medicinal plant cultivars originated from the maintenance breeding of the Institute of Natural Fibres and Medicinal Plants, Poznań. Earlier investigations have indicated that lovage, peppermint, St. John’s wort and thyme are colonized by AMF [Zubek and Błaszowski 2009, Zubek et al. 2011], whereas greater celandine is a non-mycorrhizal species [Zubek et al. 2012c]. Seedlings of a particular species, which had previously been grown in a greenhouse, were planted in the spring of 2009 in randomly chosen fields.

### Field sampling

The material for the analyses was collected in the third year of plant cultivation. The plants were harvested during the flowering and early seed formation period, in June 2011. Root systems with soil were excavated intact to the depth of ca. 20 cm and placed in plastic bags. Then the roots were cleaned mechanically of soil and placed in plastic jars containing 50% ethanol in water. Ten replicate samples of each plant species and soil from each field were collected, giving a total of 50 samples. The root and soil samples were transported to the laboratory. The roots were stained for the visualization of fungal endophytes as described below. The soil was used for chemical analyses and was also utilized in a laboratory experiment aimed at determining the colonization potential of AMF propagules (see below).

### Chemical analyses of the soil

The total phosphorus content was determined in an ammonium lactate extraction conducted in accordance with the Egner-Rim method, total nitrogen by means of the Kjeldahl method, and total carbon using the Tiurin method. Exchangeable cations were measured with a flame photometer and spectrophotometer in ammonium acetate [Moczek and Drzymała 2010].

### Estimation of the AMF colonization potential

In order to determine the colonization potential of AMF propagules, namely the spores, mycelium in roots and extraradical mycelium, which were present in the soils tested, a laboratory experiment was conducted using *Plantago lanceolata* L. as the host

plant. For this purpose, each soil sample collected from under a particular medicinal plant species was placed in 500 ml pot. Approximately 10 *P. lanceolata* seeds were sieved in each pot. After seed germination (5 days), the seedlings were thinned out to obtain 7 plants per pot. The pots were kept in sealed Sigma-Aldrich sunbags under plant growth chamber conditions at  $22 \pm 2^\circ\text{C}$ . The light regime was  $100\text{--}110 \mu\text{mol PAR photons} \times \text{m}^{-2} \times \text{s}^{-1}$ , 12/12 h. The pots were arranged in a random manner. The cultures were watered, using 50 ml of distilled water, once a week. After six weeks of growth, the plants were harvested and the roots were stained (see below) for the visualization of the AMF mycelium.

### Determination of fungal root colonization

The roots of field-collected medicinal plant species and *P. lanceolata* roots were stained according to the Phillips and Hayman [1970] protocol with minor modifications incorporated by Zubek et al. [2011]. After staining, the roots were cut into ca. 1 cm fragments. Thirty randomly chosen root fragments per replicate sample were mounted on slides in glycerol:lactic acid (1 : 1, v:v), and analysed using Nikon Eclipse 80i light microscope with differential interference contrast (DIC). Mycorrhizal colonization assessment was carried out according to the Trouvelot method [Trouvelot et al. 1986]. The parameters analysed were mycorrhizal frequency, relative mycorrhizal root length, and relative arbuscular richness. An estimate of mycorrhizal frequency (F%) is given as the ratio between root fragments colonized by AMF mycelium and the total number of root fragments analysed. The relative mycorrhizal root length (M%) is an estimate of the amount of root cortex that is colonized by AMF relative to the whole root system. Arbuscule abundance (A%) is an estimate of arbuscule richness across the entire root system [Trouvelot et al. 1986].

In the root material under investigation the mycelia of dark septate endophytes (DSE) were also observed. The presence of DSE was therefore additionally assessed. The estimated frequency of DSE ( $F_{\text{DSE}}\%$ ) mycelium occurrence in roots was calculated in the same way as that used for the presence of AMF.

### AMF species richness

**Establishment of the trap cultures.** Ten trap cultures were established on soil collected from under each plant species, giving a total of 50 cultures. For the establishment of the trap culture, 100 g of fresh soil was placed into  $9 \times 12.5$  cm, 500 ml, plastic pots and mixed with autoclaved, commercially available, coarse-grained sand, the proportions being grains 1.0–10.0 mm in diam. – 80.50%; grains 0.1–1.0 mm in diam. – 17.28%; and grains < 0.1 mm in diam. – 2.22%. *P. lanceolata* was used as the host plant. The pots were arranged in a random manner and kept under greenhouse conditions.

**Spore isolation and identification.** Six months after the establishment of the trap cultures, AMF spores were extracted using the wet sieving and decanting method [Gerdemann and Nicolson 1963]. The morphological properties of the isolated spores and their subcellular structures were determined in material mounted on a slide in a drop of polyvinyl alcohol/lactic acid/glycerol (PVLG) and in a mixture of PVLG/Melzer re-

agent (4 : 1, v:v) [Omar et al. 1979]. Observation of AMF spore characteristics was performed using an Olympus BX51 light microscope. The fungal species and family names are after Oehl et al. [2011a, b], apart from *Paraglomus majewskii* and *Scutellospora dipurpurescens* which follow Błaszczowski et al. [2011] and Schüßler and Walker [2010], respectively. The slides containing the isolated spores have been deposited in the slide collection of the Department of Plant Protection, West Pomeranian University of Technology, Szczecin.

### Statistical analysis

Analysis of the soil chemical properties and the data obtained from fungal root colonization assessments was conducted using one-way analysis of variance. Significance of differences between treatments was tested following Tukey ( $p < 0.05$ ). The analyses were carried out using STATISTICA ver. 10 (Statsoft).

## RESULTS

### Soil properties

The plant species differed in their effects on soil chemical properties, in particular on soil pH and potassium, phosphorus, calcium, sodium and magnesium levels. However, no consistent trends in the content of these elements were observed for a particular plant species. No statistically significant differences were found in the content of organic matter, nitrogen and carbon. The chemical properties of soils collected from under cultivated medicinal plant species are reported in Table 1a, b.

### Fungal root colonization of medicinal plants

**Arbuscular mycorrhizal fungi.** Arbuscular mycorrhizae were observed in lovage, peppermint, St. John's wort and thyme. The mean values of mycorrhizal frequency parameter (F) ranged from 44.9% in lovage to 52.5% in St. John's wort and did not differ statistically between the plant species (fig. 1). In the case of relative mycorrhizal root length (M), the mean values of M were lowest in thyme (22.5%) and highest in peppermint (26.1%), however, no statistically significant differences were observed. No statistically significant differences were also found in relative arbuscular richness (A). The mean values of this parameter varied from 14.9% in thyme to 25.4% in lovage (fig. 1). No AMF mycelium was found in the roots of greater celandine.

**Other fungal root endophytes.** Dark septate endophytes (DSE) were found in all the plant species; however, they were not present in all the root samples. The mean frequency of DSE occurrence in roots ( $F_{DSE}$ ) was low and ranged from 0.8 in greater celandine to 7.5 in peppermint and did not differ statistically between the plant species. The percentage of DSE root colonization was low in all the plant species (data not shown). Only single hyphae, accompanied sporadically by sclerotia, were found in the outer cortex and rhizodermis. No other fungal endophytes were observed in the roots under study.

Table 1a. The chemical properties of the soil (mean  $\pm$ SD; N = 10) collected from the experimental fields under cultivation of five medicinal plant species: *Chelidonium majus*, *Hypericum perforatum*, *Levisticum officinale*, *Mentha  $\times$  citrata* subsp. *citrata* and *Thymus vulgaris*

Treatment	pH (H <sub>2</sub> O)	pH (KCl)	N %	C %	Organic matter %	C/N
<i>C. majus</i>	7.1 $\pm$ 0.2 <sup>a</sup>	6.6 $\pm$ 0.3 <sup>a</sup>	0.09 $\pm$ 0.01 <sup>a</sup>	1.29 $\pm$ 0.18 <sup>a</sup>	2.22 $\pm$ 0.31 <sup>a</sup>	13.7 $\pm$ 1.9 <sup>a</sup>
<i>H. perforatum</i>	7.2 $\pm$ 0.2 <sup>b</sup>	7.0 $\pm$ 0.7 <sup>a</sup>	0.09 $\pm$ 0.01 <sup>a</sup>	1.14 $\pm$ 0.13 <sup>a</sup>	1.97 $\pm$ 0.23 <sup>a</sup>	13.6 $\pm$ 2.3 <sup>a</sup>
<i>L. officinale</i>	7.8 $\pm$ 0.2 <sup>c</sup>	7.2 $\pm$ 0.1 <sup>b</sup>	0.10 $\pm$ 0.02 <sup>a</sup>	1.37 $\pm$ 0.28 <sup>a</sup>	2.40 $\pm$ 0.45 <sup>a</sup>	13.9 $\pm$ 2.0 <sup>a</sup>
<i>M. <math>\times</math> citrata</i>	6.8 $\pm$ 0.2 <sup>d</sup>	6.3 $\pm$ 0.1 <sup>c</sup>	0.09 $\pm$ 0.01 <sup>a</sup>	1.28 $\pm$ 0.14 <sup>a</sup>	2.21 $\pm$ 0.24 <sup>a</sup>	15.0 $\pm$ 2.2 <sup>a</sup>
<i>T. vulgaris</i>	7.6 $\pm$ 0.2 <sup>c</sup>	7.2 $\pm$ 0.1 <sup>b</sup>	0.09 $\pm$ 0.02 <sup>a</sup>	1.22 $\pm$ 0.26 <sup>a</sup>	2.00 $\pm$ 0.46 <sup>a</sup>	14.5 $\pm$ 2.0 <sup>a</sup>

Table 1b. The chemical properties of the soil (mean  $\pm$ SD; N = 10) collected from the experimental fields under cultivation of five medicinal plant species: *Chelidonium majus*, *Hypericum perforatum*, *Levisticum officinale*, *Mentha  $\times$  citrata* subsp. *citrata* and *Thymus vulgaris*

Treatment	Total content mg 100 g <sup>-1</sup> of dry soil							Exchangeable cationsmg 100 g <sup>-1</sup> of dry soil				
	K <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>	CaO	K	Na	Ca	Mg					
<i>C. majus</i>	17.7 $\pm$ 1.7 <sup>b</sup>	20.6 $\pm$ 2.9 <sup>bc</sup>	192.4 $\pm$ 43.1 <sup>c</sup>	20.9 $\pm$ 5.4 <sup>a</sup>	3.3 $\pm$ 0.7 <sup>b</sup>	139.5 $\pm$ 30.5 <sup>c</sup>	4.0 $\pm$ 1.9 <sup>bc</sup>					
<i>H. perforatum</i>	14.8 $\pm$ 3.6 <sup>c</sup>	29.1 $\pm$ 4.6 <sup>a</sup>	229.6 $\pm$ 49.2 <sup>b</sup>	11.5 $\pm$ 1.5 <sup>bc</sup>	3.4 $\pm$ 0.4 <sup>b</sup>	165.6 $\pm$ 30.4 <sup>b</sup>	6.6 $\pm$ 1.2 <sup>a</sup>					
<i>L. officinale</i>	24.0 $\pm$ 3.8 <sup>a</sup>	24.1 $\pm$ 5.7 <sup>b</sup>	445.2 $\pm$ 125.6 <sup>a</sup>	20.7 $\pm$ 4.9 <sup>a</sup>	4.8 $\pm$ 0.8 <sup>a</sup>	321.4 $\pm$ 93.3 <sup>a</sup>	5.1 $\pm$ 2.5 <sup>b</sup>					
<i>M. <math>\times</math> citrata</i>	16.7 $\pm$ 3.4 <sup>bc</sup>	18.0 $\pm$ 7.5 <sup>c</sup>	208.6 $\pm$ 23.6 <sup>bc</sup>	13.3 $\pm$ 2.8 <sup>b</sup>	3.3 $\pm$ 0.7 <sup>b</sup>	151.2 $\pm$ 12.1 <sup>bc</sup>	4.2 $\pm$ 0.5 <sup>bc</sup>					
<i>T. vulgaris</i>	12.5 $\pm$ 0.5 <sup>d</sup>	21.5 $\pm$ 4.1 <sup>bc</sup>	425.8 $\pm$ 155.7 <sup>a</sup>	10.7 $\pm$ 1.9 <sup>c</sup>	5.3 $\pm$ 1.2 <sup>a</sup>	285.8 $\pm$ 100.6 <sup>a</sup>	3.5 $\pm$ 3.5 <sup>c</sup>					

Within each parameter, levels not connected by the same letter indicate statistically significant differences; for each P < 0.05

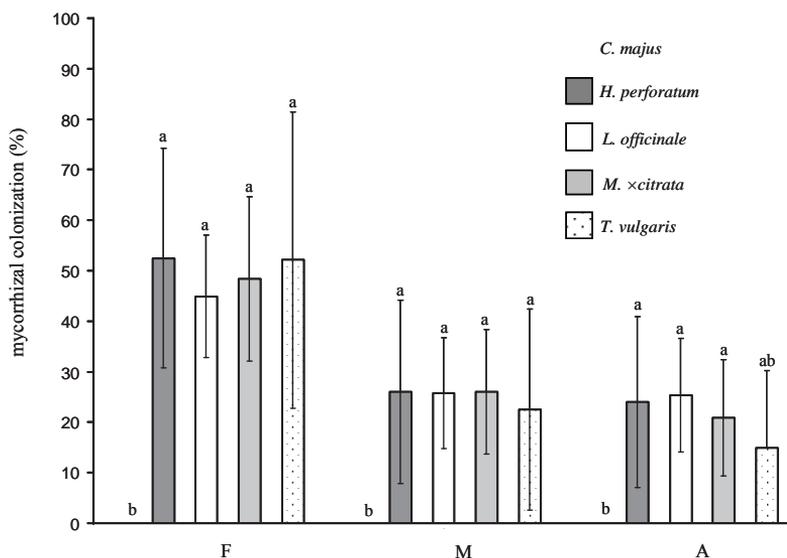


Fig. 1. The mycorrhizal parameters of the medicinal plant species collected from the experimental fields (mean  $\pm$ SD; N = 10): F – mycorrhizal frequency, M – relative mycorrhizal root length, A – relative arbuscular richness. Within each mycorrhizal parameter levels not connected by the same letter indicate statistically significant differences; for each P < 0.05.

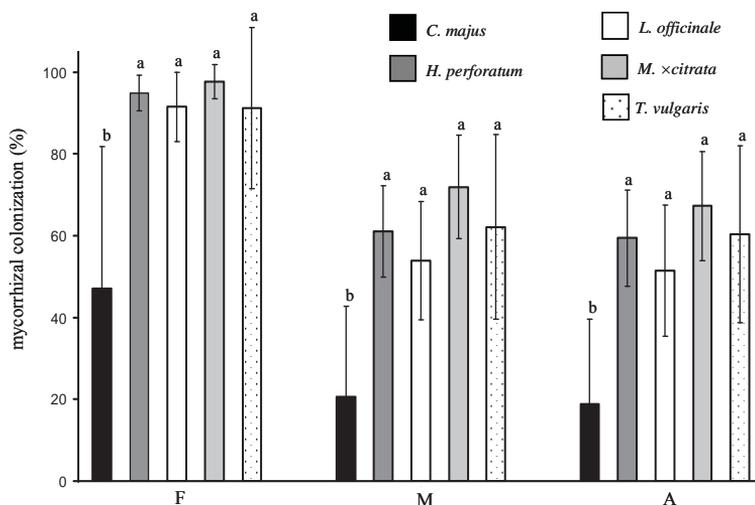


Fig. 2. The effect of medicinal plant species under cultivation on the mycorrhizal colonization of *Plantago lanceolata* grown in the soils collected from the experimental fields. Mycorrhizal parameters (mean  $\pm$ SD; N = 10): F – mycorrhizal frequency, M – relative mycorrhizal root length, A – relative arbuscular richness. Within each mycorrhizal parameter levels not connected by the same letter indicate statistically significant differences; for each P < 0.05.

### AMF colonization potential

Arbuscular mycorrhizae were observed in all the *P. lanceolata* root samples. No other fungal endophytes were found in any of the root materials analyzed. All mycorrhizal parameters (F, M, A) were significantly lower in *P. lanceolata* grown on the soil collected from under the non-mycorrhizal species greater celandine. The AMF colonization potential was similar in the case of the soils collected from under the mycorrhizal plants (fig. 2).

### AMF species richness

In total, the spores of 8 AMF species were isolated from the trap cultures established from the soils collected in the field; *Archaeospora trappei* (R.N. Ames & Linderman) J.B. Morton & D. Redecker (Archaeosporaceae), *Claroideoglossum claroideum* (N.C. Schenck & G.S.Sm.) C. Walker & A. Schüßler (Entrophosporaceae), *Funneliformis caledonius* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler, *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler, *Glomus irregulare* Błaszcz., Wubet, Renker & Buscot, *Septoglossum constrictum* (Trappe) Sieverd., G.A. Silva & Oehl (Glomeraceae), *Paraglossum majewskii* Błaszcz. & Kovács (Paraglomeraceae) and *Scutellospora dipurpureascens* J.B. Morton & Koske (Scutellosporaceae). In addition, one spore morphotype with glomoid spores similar to those of *Diversispora* spp. was found (tab. 2).

Table 2. The occurrence of arbuscular mycorrhizal fungi (Glomeromycota) species in the trap cultures established from soils collected from under monocultures of five medicinal plants

Fungal species	Plant species				
	<i>C. majus</i>	<i>H. perforatum</i>	<i>L. officinale</i>	<i>M. × citrata</i> subsp. <i>citrata</i>	<i>T. vulgaris</i>
<i>Archaeospora trappei</i>				+	
<i>Claroideoglossum claroideum</i>	+	+	+	+	+
<i>Funneliformis caledonius</i>	+				+
<i>Funneliformis mosseae</i>	+	+	+	+	+
<i>Glomus irregulare</i>	+			+	
<i>Paraglossum majewskii</i>	+				+
<i>Scutellospora dipurpureascens</i>		+	+		
<i>Septoglossum constrictum</i>			+		+
Morphotype with glomoid spores similar to those of <i>Diversispora</i> spp.	+	+	+	+	

The spores of *C. claroideum* were most frequently isolated and were present in 30 out of 50 cultures. *F. mosseae* and *Diversispora* sp. were found with the mid-frequency in the cultures, in 12 and 9, respectively. The other AMF species were detected in 2–4 cultures. Among the species that occurred rarely, *A. trappei* was exclusively found in

the cultures established from the soils of peppermint. *F. caledonius* and *P. majewski* were related to both greater celandine and thyme, *S. constrictum* to lovage and thyme, *G. irregulare* to greater celandine and peppermint, whereas *S. dipurpurens* to lovage and St. John's wort (tab. 2).

## DISCUSSION

A better understanding of the factors influencing AMF diversity is clearly required both from an agronomic and ecosystem perspective and the effects of plant species is of considerable interest [Johnson et al. 2003]. It could be especially crucial in view of growing need of pharmaceutical industry for herbal materials that are presently obtained mostly from large-scale cultivation. In our study, the influence of five medicinal plants grown under monoculture on AMF species richness, composition and propagule abundance is reported. We found a negative effect of the non-mycorrhizal species greater celandine on AMF propagule abundance as well as the impact of the plants under study on AMF species composition in soils. We demonstrated that these influences may develop relatively fast, i.e. within three growing seasons.

It is well known that plant species can differ in their capacity to influence soil organic matter and soil nutrient availability [Bezemer et al. 2006, Pérez-Bejarano et al. 2010]. In turn, the differences in soil chemical properties may influence soil microbial communities. The levels of Ca and P as well as soil pH may have an impact on AMF. In Koomen's et al. [1987] studies, most AMF tested preferred a near-neutral pH. Although Zubek et al. [2009] did not find a close correlation between AMF species richness and soil pH, they found the highest diversity in calcareous substrata. Moreover, higher P contents have been found to have a negative impact on AMF colonization in some cases [Duan et al. 2010, Entz et al. 2004], though not in others [Vosátka 1995, Ryan and Ash 1999, Zubek et al. 2012b]. In our research, the medicinal plants had various effects on soil chemical properties, however, the changes were neither consistent nor unidirectional and thus it is difficult to draw any conclusion on the impact of soil chemical properties on AMF.

The lower abundance of AMF propagules in the soil under non-mycorrhizal greater celandine found in our study is in line with other observations where also non-host plant species under cultivation, particularly from the Brassicaceae family, have been found to reduce AMF abundance [Gavito and Miller 1998, Arihara and Karasawa 2000, Troeh and Loynachan 2003, Vestberg et al. 2005]. Here we demonstrate, to the best of our knowledge for the first time, such a negative impact of the representative of Papaveraceae. In the case of mycorrhizal plant species, we found no differences in the abundance of AMF propagules. This suggests that the cultivation of these plants maintain the amount of AMF propagules in the soil at about the same level. This is in accordance with earlier observations where mycorrhizal plants under cultivation had also no effect on AMF colonization potential in soils [Zubek et al. 2012b]. However, the incorporation of various mycorrhizal plant species in the crop rotation has clearly affected the amount and function of AMF in other studies [Arihara and Karasawa 2000, Troeh and Loynachan 2003, Vestberg et al. 2005], that was probably due to differences in the degrees of mycorrhizal dependency between the plant species tested [Vestberg et al. 2005].

The total number of AMF species found in our study is in accordance with previous investigations, where comparable low species richness was found in agroecosystems [Franke-Snyder et al. 2001, Jansa et al. 2002, Vestberg et al. 2005]. This is, however, much lower than the 35 species identified by Oehl et al. [2004], but using both direct isolation of spores from soils and the trap culture method. Surprisingly, the number of AMF species observed in our study is almost twice lower than the number found in our previous investigations on arable soils conducted in the neighboring area [Zubek et al. 2012b]. This might be due to differences in management history of these areas, including tillage, the use of fertilizers and plants in rotation.

The AMF species found in the present study commonly occur in Poland and have also wide distribution in the world [Błaszowski 2012]. Two species, *C. claroideum* and *F. mosseae* were the most frequently detected fungi. The results compare with previous studies, where either both species [Vestberg et al. 2005, Zubek et al. 2012b], or *F. mosseae* alone [Oehl et al. 2003, 2004], appeared to be generalists in arable soils in central Europe. In this study, six AMF species were detected exclusively in the trap cultures established from the soils collected from under only one or two medicinal plant species. Although most AMF associate with a wide range of hosts, a selectivity of some plant species for particular fungal symbionts exists [Helgason et al. 2002, Wubet et al. 2006, Smith and Read 2008]. Vestberg et al. [2005] reported AMF host specificity in several crops. In contrast, Franke-Snyder et al. [2001] found a homogeneity of AMF communities in plant/host combinations. Nevertheless, in the case of AMF species found rarely in this study, their occurrence might have been determined not by plant identity, but may be owing to their rarity.

Plant species differ in their association with AMF and the degree of dependency on the symbiosis, and the cropping sequence and pattern can modify the AMF status in the soil. Preceding crops, therefore, can affect AM formation and the growth of succeeding crops. It was shown that maize and mandarin orange growth after cultivation of mycorrhizal plants was higher than that after cultivation of a non-mycorrhizal ones in different soil types [Karasawa et al. 2001, Panja and Chaudhuri 2004]. Moreover, some mycorrhizal plants gave highest growth benefit to mandarin orange plants apparently by increasing the soil AMF potential more than other mycorrhizal pre-crops [Panja and Chaudhuri 2004]. In our study, the use of non-mycorrhizal plant had a negative impact on the abundance of fungal propagules. Consequently, the growth rate and accumulation of secondary metabolites, that have been found to be enhanced by AMF for several plant species [reviewed in Gianinazzi et al. 2010], of mycorrhiza-dependent medicinal plants cultivated in this area in the future may be affected by the previously cultivated plant, especially non-mycorrhizal one. Similarly, AMF species specificity was found in the impact on the production of therapeutic compounds in several medicinal plant species [e.g. Copetta et al. 2006, Toussaint et al. 2007, Ceccarelli et al. 2010]. In view of this fact, the changes in AMF species composition in soils due to long-term plant monoculture may consequently influence the quality and quantity of medicinal plant species cultivated in the future on these soils if succeeding plant species rely on a symbiosis with particular fungi. It seems an important factor to consider when designing medicinal plant rotation.

## CONCLUSIONS

It can be concluded that the three-year cultivation of the non-host plant species greater celandine decreases the abundance of AMF propagules in the soil, whereas the mycorrhizal plants under study keep the number of indigenous AMF propagules at the same level, however, may change the composition of AMF species. The results show that choice of a medicinal plant species by farmers, grown even for a relatively short period in a monoculture, can substantially alter the AMF potential of soils that can influence the performance of succeeding medicinal plants cultivated. The effects of these changes on medicinal plants grown in the soil under study need, however, to be further investigated. Nevertheless, it seems important to select medicinal plant species in rotation not only focusing on the improvement of nutrition, soil structure, and pathogen stress reduction but also to facilitate AMF maintenance and development. Among the countries of Europe, Poland is one of the leaders in the production of medicinal plant raw materials for the herbal industry [Seidler-Łożykowska et al. 2005, Pisulewska and Janeczko 2008]. In this context, our studies may well contribute to the development of effective methods of sustainable agricultural production of medicinal plants.

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## **BOGACTWO I ZRÓŻNICOWANIE GATUNKOWE ORAZ DOSTĘPNOŚĆ PROPAGUL GRZYBÓW ARBUSKULARNYCH W MONOKULTURACH PIĘCIU ROŚLIN LECZNICZYCH**

**Streszczenie.** Obecność symbiotycznych grzybów arbuskularnych (Glomeromycota) w glebach może być istotna dla zrównoważonego rolnictwa. Choć wpływ tych mikroorganizmów na witalność i produkcję metabolitów wtórnych przez rośliny lecznicze był badany w ostatnich latach, niewiele wiadomo na temat oddziaływania uprawianych roślin leczniczych na te grzyby. Celem pracy było więc określenie wpływu trzyletniej uprawy mikoryzowych (*Hypericum perforatum*, *Levisticum officinale*, *Mentha × citrata* subsp. *citrata* i *Thymus vulgaris*) i niemikoryzowych (*Chelidonium majus*) gatunków roślin leczniczych na liczbę propagul, bogactwo oraz zróżnicowanie gatunkowe grzybów arbusku-

larnych. Uprawa *C. majus* spowodowała spadek liczby propagul tych mikroorganizmów w glebie. W przypadku roślin mikoryzowych dostępność propagul utrzymywała się na podobnym poziomie. Uprawiane rośliny miały jednak wpływ na skład gatunkowy grzybów arbuskularnych. Uprawa badanych roślin leczniczych na danym terenie, nawet przez stosunkowo krótki czas, wpływa na zmianę składu gatunkowego i dostępność propagul grzybów arbuskularnych w glebie, co w konsekwencji może mieć wpływ na witalność roślin leczniczych, zależnych od symbiozy mikoryzowej, uprawianych w przyszłości na tym terenie.

**Słowa kluczowe:** gleba rolnicza, mikoryza arbuskularna, Glomeromycota, uprawy

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