Mycorrhizal status of selected herbaceous plants in *Molinia* meadows of Folusz, near Szubin (Poland)

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Abstract. This preliminary study examined the arbuscular mycorrhizal (AM) status of native plant species in the *Molinia* meadows of Folusz in the Kujawy region, Poland. Root samples from ten plant species characteristic of *Molinia* meadows (*Betonica officinalis*, *Dianthus superbus*, *Galium boreale*, *Inula salicina*, *Ostericum palustre*, *Sanguisorba officinalis*, *Selinum carvifolia*, *Serratula tinctoria*, *Silaum silaus*, and *Succisa pratensis*) were collected in August 2014. Root colonization by AM fungi was considered an indicator of symbiosis development. AM was found in all plant species examined. The frequency of mycorrhizal structures ranged between 68% and 99%. The intensity of mycorrhizal colonization in roots varied with the plant species, ranging between 3.9% (*O. palustre*) and 40.1% (*B. officinalis*). To our knowledge, this is the first study to examine the mycorrhizal status of three species: *D. superbus*, *O. palustre*, *S. carvifolia*. Because AM colonization depends on plant genotype and environmental factors, further studies should examine seasonal variability in plant root colonization and the compositions of AM fungal communities, which may serve as indicators of site conditions.

Key words: arbuscular mycorrhiza, Glomeromycota, mycorrhizal colonization, Natura 2000 site, soil.

1. Introduction

Molinia meadows comprise a valuable, semi-natural grassland ecosystem. They are covered by a mixture of grasses, herbaceous plants, and other low-growing plant species. *Molinia* meadows are created and maintained by people that practice a specific type of low-intensity, traditional farming that includes grazing, mowing at least once a year, and no fertilization (Kącki & Załuski 2004; Michalska-Hejduk & Kopeć 2012). Cessation of farming practices can induce ecosystem transformations into herbaceous fields, scrubland, forests, or sedge rushes (Michalska-Hejduk & Kopeć 2012). In the European Union, this meadow region is protected by law (Habitat Directive), because it provides habitats for many rare and protected plants and birds (Kącki & Załuski 2004; Matuszkiewicz 2005; Michalska-Hejduk & Kopeć 2012; Kulik 2013).

Under natural conditions, 80% of all vascular plant species develop mycorrhizas, which are mutual symbiotic associations between plant roots and fungi specialized for growth in soils and plants. Mycorrhizas improve plant nutrition and water balance, increase plant growth, promote the establishment of plants in new areas, and play an important role in plant functions and in the function of entire ecosystems (Brundrett 2004; Smith & Read 2008). Arbuscular mycorrhizas (AMs) are produced by Glomeromycota fungi in roots of a wide range of plant species, particularly herbaceous plants and grasses. Arbuscular mycorrhizal fungi (AMF) also improve soil conservation by contributing to the soil aggregation process, controlling soil erosion, and maintaining nutrient reserves (Miller & Jastrov 1992).

AM is the most common mycorrhizal association in the world; it is widespread in all types of environments (Trappe 1987; Allen 1991). Also, AMF are the main components of soil microbiota (Smith & Read 2008). Nevertheless, only about 3% of known plant species have been examined to study associated mycorrhizal fungi (Trappe 1987; Wang & Qiu 2006). Little is known about AMF colonizations in plants in *Molinia* meadows and the relationships between site conditions, plant development, and mycorrhiza. Only a few studies have focused on the AM status of plants characteristic of this type of habitat, including Harley & Harley (1987a, b; 1990), Eriksen et al. (2002), Fuchs & Haselwandter (2004), and Wang & Qiu (2006).

Environmental factors, such as land use changes, soil fertilization, or contamination with xenobiotic substances, can affect the functions of above-ground and below-ground biota. Thus, mycorrhizal status might serve as an indicator of environmental changes. This study aimed to examine the presence of mycorrhizal symbiosis and to assess the frequency and abundance of mycorrhizal fungi in roots of ten selected herbaceous plant species, characteristic of *Molinia* meadows. These plants represented seven families (Table 1). The results broadened our knowledge of the ecology of this valuable, semi-natural grassland ecosystem.

2. Study area

This study was conducted in *Molinia* meadows (subtype *Selino carvifoliae-Molinietum*) located in Folusz (17°49' E, 52°47' N), near Szubin, in Gąsawka Valley (Gąsawka River – left tributary of Noteć River), ca. 30 km southwest of Bydgoszcz city (Kuyavian-Pomeranian Province). The study area was about 50 ha (Krasicka-Korczyńska & Rutkowski 2005), which formed part of the Szubin-Łabiszyn Plain Microregion (315.353), located in the Mesoregion, Toruń Basin (Kondracki 2000). According to Matuszkiewicz (2008), Folusz belongs to the Szubin Subdistrict, which is part of the Chodzież District. The *Molinia* meadows are surrounded on the south and east by moraine hills. The central part of this area includes a complex of fishing ponds; the northwestern area harbors numerous post-excavation peat pits. The *Molinia* meadows are composed of muck soils. Selected soil properties are shown in Table 2. The meadows are mowed, usually twice a year. The *Molinia* meadows community was classified as *Selino-Molinietum brometosum erecti* (Matuszkiewicz 2005). This community represents a typical *Molinia* meadow, with many characteristic species, including *Betonica officinalis*, *Dianthus superbus*, *Galium boreale*, *Inula salicina*, *Molinia caerulea*, *Ostericum palustre*, *Selinum carvifolia*, *Succisa pratensis*, *Serratula tinctoria*, *Gentiana pneumonanthe*, *Laserpitium prutenicum*, *Silaum silaus*. This area harbors 27 endangered or protected plant species (Krasicka-Korczyńska & Rutkowski 2005). In 2010, this area was classified as a Natura 2000 site, called "*Molinia* meadows in Folusz" (code PLH040027).

3. Materials and methods

Among the plant species typical of *Molinia* meadows (Matuszkiewicz 2005; Michalska-Hajduk & Kopeć 2012), we selected the ten most frequently observed species at the study site for AM evaluation. We employed the nomenclature of plant species described by Mirek et al. (2002), the phytocenotic character of species, and the names of plant communities described by Matuszkiewicz (2005).

We collected random specimens of the following herbaceous plants: *Betonica officinalis*, *Dianthus superbus*, *Galium boreale*, *Inula salicina*, *Ostericum palustre*, *Sanguisorba officinalis*, *Selinum carvifolia*, *Serratula tinctoria*, *Silaum silaus* and *Succisa pratensis*. Triplicate samples of each plant were collected with a spade in August 2014. Root systems were cut off, placed in plastic bags, and transported to the laboratory. Fungal structures in roots were visualized according to the method described by Kormanik and McGraw (1982), with modifications. Briefly, fresh fine roots (about 0.1 g) were cut into 1-cm segments, cleared for 20 min in 10% KOH, and stained in 0.05% trypan blue solution for 8 min. The entire procedure was carried out at 90°C in a water bath. The stained roots were stored in lactoglycerol. The mycorrhizal colonization in each root system was estimated with the method described by Trouvelot et al. (1986). Thirty root segments (30 cm) from each sample were examined with a light microscope under 100x magnification (Zeiss Axiostar Plus). We recorded the frequency of mycorrhiza in the root system (F%), the intensity of the mycorrhizal colonization in the root system (M%), and the arbuscule abundance in the root system (A%). Mycorrhizal colonization was quantified with the software program, 'Mycocalc' (http://www2.dijon.inra.fr). Soil analyses were performed in the laboratory of the Department of Agricultural Chemistry of the University of Science and Technology, in Bydgoszcz.

4. Results

The ten plant species selected for AM evaluation at the *Molinia* meadows in Folusz near Szubin represented 10 plant genera and 7 families (Table 1). All plants investigated were colonized by AM fungi. The presence of arbuscules, vesicles, hyphal coils, and intraradical hyphae were observed in roots of all plant species, with the exception of *G. boreale, S. silaus*, and *S. pratensis*, which had roots without hyphal coils (Fig. 1). The degree of AM colonization varied among plant species (Fig. 2). *B. officinalis* (Lamiaceae) had the highest and *O. palustre* (Apiaceae) had the lowest AM parameter values (F%, M%, and A%). The average AM frequency (F%) was relatively high in all the plant species investigated; it ranged from 62.2% (*O. palustre*) to 98.9% (*B. officinalis*). The mycorrhizal

Table 1. Characteristics of growth and flowering period of plant species investigated in this study (Rutkowski 2007)

Family	Plant species	Height [cm]	Flowering period
Apiaceae	*Ostericum palustre* Besser	50-125	VI-IX
	Selinum carvifolia (L.) L.	30-100	VII-IX
	Silaum silaus (L.) Sch. et Thell.	50-100	VI-IX
Asteraceae	*Inula salicina* L.	30-60	VI-VIII
	Serratula tinctoria L.	30-100	VII-IX
Caryophyllaceae	*Dianthus superbus* L.	20-50	VI-IX
Dipsacaceae	*Succisa pratensis* Moench	15-80	VII-IX
Lamiaceae	*Betonica officinalis* L.	30-75	VI-IX
Rosaceae	*Sanguisorba officinalis* L.	30-90	VI-IX
Rubiaceae	*Galium boreale* L.	20-60	V-VIII

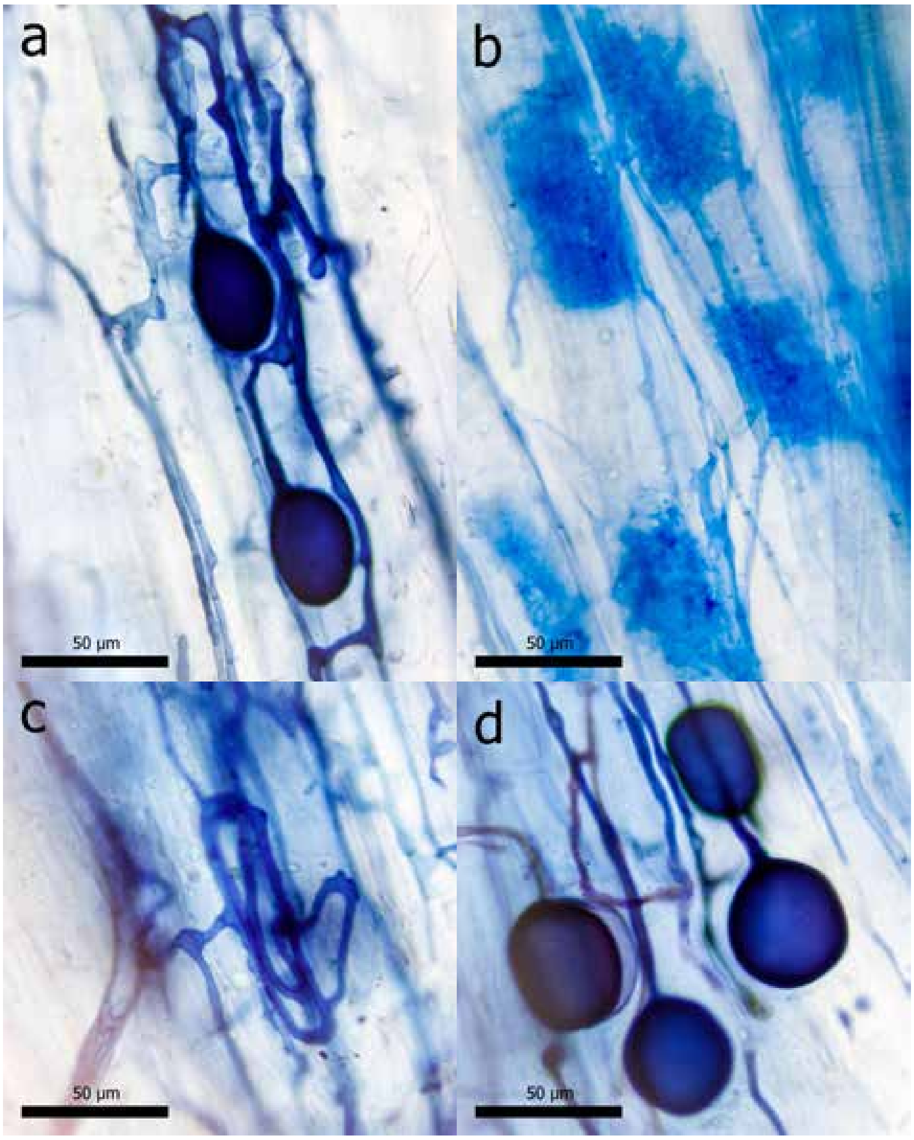

Figure 1. Arbuscular mycorrhiza in plant roots: (a) vesicles and intraradical hyphae within a root of *Ostericum palustre*; (b) arbuscules and intraradical hyphae within cortical cells of a root of *Selinum carvifolia*; (c) hyphal coil within a root of *Dianthus superbus*; (d) vesicles and intraradical hyphae within a root of *D. superbus*

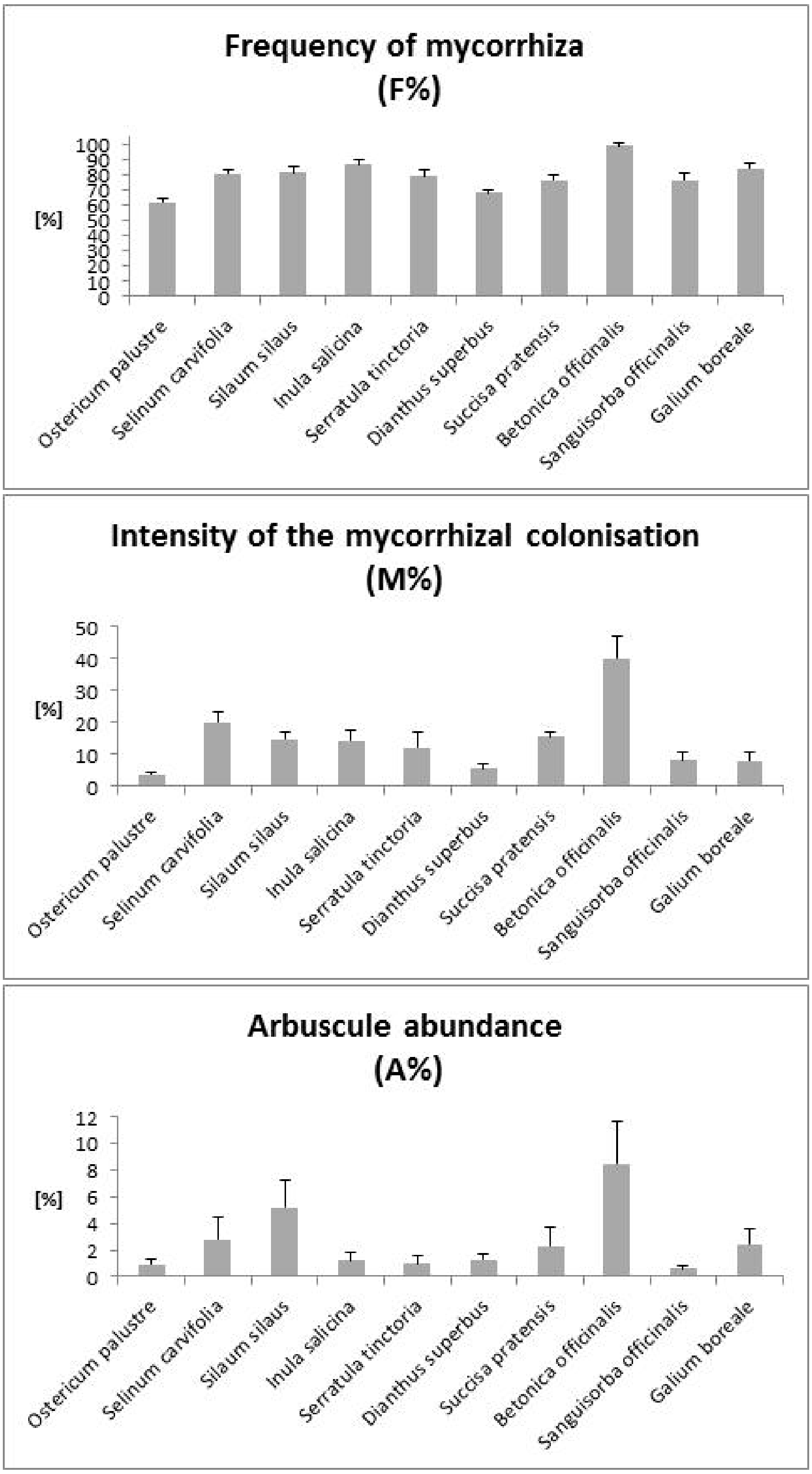

Figure 2. Mycorrhizal colonization of selected herbaceous plants characteristic of *Molinia* meadows, located in Folusz, near Szubin. (a) Frequency of mycorrhiza in the root system (F%); (b) intensity of mycorrhizal colonization in the root system (M%); (c) arbuscule abundance in the root system (A%). Data are means (n=3); bars indicate standard deviations

frequency was lower than 70% in only two plant species (*D. superbus* and *O. palustre*) (Fig. 2a). The intensity of mycorrhizal colonization in the root systems (M%) varied between 3.9% (*O. palustre*) and 40.1% (*B. officinalis*) (Fig. 2b). Arbuscule abundance in the root systems (A%) was relatively low; it comprised between 0.6% (*S. officinalis*) and 8.5% (*B. officinalis*) of the root systems (Fig. 2c).

5. Discussion

Although AM is the predominant type of mycorrhiza in land plants, AM colonization has been confirmed in only about 3% of plant species. The first list of mycorrhizal status in vascular plants included 1,101 species and 144 families of plants in British flora (Harley & Harley 1987a, b; 1990). Nineteen years later, Wang & Qiu (2006) developed a list of mycorrhizas in land plants, based on 659 papers, including those by Harley & Harley (1987a, b; 1990). That list comprised 3,617 species (angiosperms, gymnosperms, pteridophytes, and bryophytes), which belonged to 263 families.

Our research confirmed the AM status of seven species that were previously described by other authors as AM plants: *G. boreale*, *I. salicina*, *S. silaus*, *S. officinalis* (Harley & Harley 1987a, b; 1990), *B. officinalis* (Fuchs & Haselwandter 2004), *S. tinctoria* (Harley & Harley 1987a, b; 1990; Fuchs & Haselwandter 2004), and *S. pratensis* (Harley & Harley 1987a, b; 1990; Eriksen et al. 2002). To our knowledge, this study was the first to describe the mycorrhizal status of three plant species: *D. superbus*, *O. palustre*, and *S. carvifolia*. One of the species, *D. superbus*, belongs to the family Caryophyllaceae, previously considered 'nonmycorrhizal', because they comprise relatively large numbers of non-host plant species, which can adversely influence mycorrhizal fungi (Brundrett 2009). In our study, roots of *D. superbus* contained low levels of AM colonizations (M% 5.7, A% 1.3; Fig. 2); however, these levels were comparable to those observed in other plants that co-existed in the *Molinia* meadows of Folusz, such as *O. palustre* (Asteraceae), *G. boreale* (Rubiaceae), and *S. officinalis* (Rosaceae; Fig. 2). In the literature, reports have been contradictory concerning the mycorrhizal status of species that belong to the genus *Dianthus*. For example, *D. caryophyllus*, *D. gratianopolitanus*, and *D. plumarius* were listed as nonmycorrhizal plants (Harley & Harley 1987a, b, 1990; St-Arnaud et al. 1997; Zubek et al. 2008); however, *D. caryophyllus* was reported to develop AM associations with a variety of AM fungi in greenhouse conditions (Kerur & Lakshman 2009). According to Harley & Harley (1987a, b, 1990), *D. deltoides* is nonmycorrhizal. However, Lekberg et al. (2015) found low AM colonization in the roots of this plant grown in Danish coastal grasslands. Similarly, Pawłowska et al. (1996) and Gucwa-Przepióra & Błaszkowski (2007) reported the presence of AM structures in the roots of *D. carthusianorum*, a species listed as nonmycorrhizal by Harley and Harley (1987a, b, 1990). Moreover, Gucwa-Przepióra & Błaszkowski (2007) found relatively high AM parameter values (F%= 80.0; M%= 45.5; A%= 23.2) in roots of this plant species. It is noteworthy that many plant families and genera can contain both mycorrhizal and nonmycorrhizal species (Wang & Qiu 2006). Some authors suggested that, in some cases, AM structures may be present in plant roots, but they are not observed, because they do not react with commonly used dyes (Morton & Redecker 2001; Kowalczyk & Błaszkowski 2005). Lekberg et al. (2015) examined AM fungi in the roots of *D. deltoides* with microscopy, signature fatty acid (NLFA 16:1ω5) analysis, and molecular identification. They demonstrated that this species exhibited substantially lower AM colonization than that observed in neighboring plants. The authors suggested that some non-host plants may be colonized by AM fungi, when grown together with host plants. According to Lekberg et al. (2015), the distinction between host and nonhost plants is very subtle, and more research is needed to improve our understanding of plant-AM fungal interactions.

This study revealed significant differences in mycorrhizal frequency (F%), the intensity of colonization (M%), and the abundance of arbuscules in plant roots (A%) among the ten plant species examined (Fig. 2). However, these plants were collected only once, in August. Moreover, the levels of AM colonization observed in the plants examined in this study were lower than the levels observed in other studies. For example, in the roots of *S. officinalis* grown in Folusz, the parameters were: F% 81.3, M% 8.3, and A% 0.6 (Fig. 2). However, roots of the same species, grown in the Botanical Garden of Jagiellonian University in Kraków, and collected in June, showed parameters of: F% 91.3, M% 36.3, and A% 18.4 (Zubek et al. 2011).

The degree of mycorrhizal colonization can be determined by many factors, including the availability of nutrients and water in soil, the season, the density of inoculum, the vegetation development, and the genotypes of phytobionts and mycobionts (Friese & Allen 1991; Smith & Read 2008). According to several published papers, the colonization of plant roots by AM is variable throughout the year; it is associated with host phenology and climatic variations. Therefore, each plant species may have different seasonal patterns (Allen 1996; Garcia & Mendoza 2008; Lingfei et al. 2005). However, some studies have indicated that mycorrhizal colonization is more closely related to plant physiology than to phenology.

Arbuscules, the major sites of nutrient exchange between the fungus and host, are ephemeral structures, often absent in field-collected roots (Brundrett 2004). However, it has been reported that arbuscules are more abundant at the beginning of the growing season, during periods of ac-

Table 2. Selected properties of the soil in *Molinia* meadows in Folusz

Variables	Values
$pH(H_2O)$	6.55
pH (KCl)	6.38
N total [$g \cdot kg^{-1}$ air d.w.]	10.37
C organic [$g \cdot kg^{-1}$ air d.w.]	154.80
P available [$mg \cdot kg^{-1}$ air d.w.]	26.20
K available [$mg \cdot kg^{-1}$ air d.w.]	14.00

tive nutrient uptake (Allen 1983, Garcia & Mendoza 2008; Sawilska et al. 2010). Several authors observed increasing arbuscular abundance between spring and mid-summer and decreasing abundance from mid-summer to late autumn (Sawilska et al. 2010; Tyburska et al. 2013). Therefore, in the present study, the sampling date (August) may have influenced our findings of low arbuscule abundance in all the plants examined (A% = 0.6-8.5). Furthermore, several factors, like soil moisture, aeration, and temperature, might also influence arbuscule development (Khan 1995).

It is known that AM symbiosis improves phosphorus and nitrogen uptake under limiting conditions. However, high availability of exogenous phosphate and nitrate was shown to inhibit root colonization by the AM fungus, *Rhizophagus irregularis* (Nouri et al. 2014). That observation suggested that a feedback mechanism may cause plants to promote or limit AM colonization, according to nutrient needs. Our finding that the roots of plants from the *Molinia* meadows in Folusz had relatively low mycorrhizal AM colonization may have been linked to the relatively high N, C, and P concentrations in the soil (Table 2). Conversely, the average AM colonization was elevated in herbaceous plants grown at a soda-contaminated site; this might be linked to the relatively low concentration of available essential nutrients in the soil (Gucwa-Przepióra & Błaszkowski 2007).

In this study, the water status may have had an impact on the degree of mycorrhizal colonization in plant roots. *Molinia* meadows are characterized by variable levels of ground water. Ground water can be very high at the beginning of the growing season, and the meadows may be flooded. However, at the end of summer, groundwater is often very low, out of reach for the root systems of many plants (Michalska-Hejduk & Kopeć 2012).

To date, the "*Molinia* meadows in Folusz", Nature 2000 site (PLH040027), has mainly been investigated to examine floristic and phytosociological aspects (Krasicka-Korczyńska & Rutkowski 2005; Nienartowicz et al. 2014). The present study provided basic information on the mycorrhizal status of selected plant species characteristic of *Molinia* meadows. However, further research is needed to determine the seasonality of mycorrhizal colonization and the composition of the associated AM fungal community. That information might facilitate the design of future conservation and restoration programs that target semi-natural *Molinia* meadows.

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