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Arbuscular Mycorrhizal and Dark Septate Endophyte Colonization along Altitudinal Gradients in the Tatra Mountains

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Abstract

The evaluation of fungal root endophytes of two multizonal mountain plant species (*Soldanella carpatica* and *Homogyne alpine*) in relation to altitude was conducted. The comparison of root colonization by coarse arbuscular mycorrhizal fungi (AMF) and the fine AMF endophyte (*Glomus tenue*), as well as the presence of dark septate endophytes (DSE) were assessed along altitudinal gradients (1000–2050 m a.s.l.) on calcareous and non-calcareous substrata in the Tatra Mts. (Western Carpathians). Additionally, AMF species composition in the rhizosphere of the investigated plants was determined. Coarse AMF dominated over the fine endophyte in roots of *S. carpatica* and *H. alpina*. In the case of *S. carpatica*, there was a tendency for coarse AMF colonization decline with increasing altitude, while the reverse trend was observed for the fine endophyte. In contrast, the altitudinal patterns of the two types were opposite in *H. alpina*. Fifteen AMF species associated with the rhizosphere of *S. carpatica* were identified at the sites located in the Western Tatra Mountains, whereas spores of only four species were isolated from the rhizosphere of *H. alpina* in the High Tatra Mountains. None of the identified AMF species was observed to occur both in the High and Western Tatra Mts. DSE accompanied AMF in the roots of *S. carpatica* and *H. alpina* at each site; however, the root colonization by this group of fungi was low. The DSE colonization did not have a consistent relationship with altitude in both plant species. The results suggest that at the investigated altitudes factors such as the type of substrata, host plants, and local plant species composition may play a more important role in determining root colonization as well as the establishment of a local AMF community than the climatic changes with increasing elevation above sea level.

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Introduction

In high mountain environments, vascular plants have developed numerous adaptations imposed by stress factors of the harsh conditions resulting from the increase in altitude (e.g. short growing season, low temperatures, and shallow soils). These adaptations are manifested in various growth forms, life strategies, and physiological processes (Mirek, 1996; Körner, 1999). Adaptive strategies can also involve symbiotic association with mycorrhizal fungi, as several types of mycorrhizas occur in mountain plant communities, e.g. ectomycorrhiza, ericoid, orchid, and arbuscular mycorrhizas (Haselwandter and Read, 1980; Read and Haselwandter, 1981; Körner, 1999). Among them only arbuscular mycorrhiza (AM) has been found even in the highest habitats (Haselwandter and Read, 1980; Read and Haselwandter, 1981).

The Tatra Mts. are located in the Western Carpathians and constitute the highest massif within the whole Carpathian Range. This massif shows all features characteristic of an alpine system with well developed altitudinal belts up to the alpine and subnival zones. It also represents the center of alpine plant biodiversity in this part of Europe (Mirek, 1996; Piękoś-Mirkowa et al., 1996). The occurrence of mycorrhizal fungi and other endophytes within roots of Tatra Mts. plants was investigated in the 1950s by a group of Polish mycologists (Nespiak, 1953; Dominik and Nespiak, 1954; Dominik et al., 1954a, 1954b; Dominik and Pachlewski, 1956). Recently, we have explored the arbuscular mycorrhiza development of a number of rare, endemic, and threatened species from this mountain range (Zubek et al., 2005, 2008). However, root endophyte (including AMF) colonization of the plant species occurring in the Tatras in relation to elevation has not been studied. Moreover, reports in the literature concerning the occurrence of arbuscular mycorrhiza in relation to elevation are inconsistent (Haselwandter and Read, 1980; Read and Haselwandter, 1981; Ruotsalainen et al., 2004). Hence, surveys of root endophyte colonization in a broad range of mountain plant species are important for understanding the mycorrhizal colonization patterns along altitudinal gradients.

The aim of the present study was to evaluate fungal root endophytes of two multizonal plant species (*Soldanella carpatica* and *Homogyne alpina*) growing in the Tatra Mts. along altitudinal gradients. The first species was investigated on calcareous and the second on non-calcareous substrata. Three types of root endophytes were included in the study: (1) coarse AMF; (2) the fine AMF endophyte *Glomus tenue*, which was found to dominate in some alpine habitats (Haselwandter and Read, 1980); and (3) dark septate endophytes (DSE), considered to be frequent root colonizers of plants in alpine and arctic ecosystems (Jumpponen, 2001). The AMF species composition in the rhizosphere of the investigated plants at different sites along altitudinal gradients was also determined. This research may contribute towards understanding the role of fungal root endophytes in the functioning of mountain plant species.

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Materials and Methods

SITE DESCRIPTION AND FIELD SAMPLING

Two plant species were selected for this study, based on their wide altitudinal distribution: *Soldanella carpatica* Vierh. (Primulaceae) and *Homogyne alpina* (L.) Cass. (Asteraceae). Moreover, in our previous research these species were found to be colonized by both AMF and DSE (Zubek et al., 2005, 2008).

For each species, the material was collected from three selected locations in the Polish Tatra Mts. along an altitudinal gradient ranging from montane to alpine. Numbers and location of the sampling sites were as follows: *Soldanella carpatica* in the Western Tatra Mts. (calcareous substrata): I—Nosal Mt., 1000 m a.s.l. (49°16′47″N, 19°58′59″E), II—Piec rock in Czerwone Wierchy massif, 1510 m a.s.l. (49°14′49″N, 19°53′11″E), III—Kozi Grzbiet ridge, 2050 m a.s.l. (49°13′59″N, 19°54′40″E); *Homogyne alpina* in the High Tatra Mts. (non-calcareous substrata): IV—Niżna Polana glade below Wołoszyn Mt., 1040 m a.s.l. (49°14′51″N, 20°05′44″E), V—Szeroki Piarg above Morskie Oko lake, 1550 m a.s.l. (49°11′28″N, 20°04′22″E), VI—Marusarzowa Turnia Mt., 2050 m a.s.l. (49°11′41″N, 20°05′05″E).

The plants were collected during the period of early seed formation in September 2004 (*S. carpatica*) and September 2005 (*H. alpina*). At every sampling site, 10 replicate samples containing 1–3 individuals growing together were collected from the area (5 × 5 m) which had similar vegetation. Root systems with soil were excavated intact and transported in plastic bags to the laboratory.

Phytocoenotic status of the investigated species at each site was characterized by recording associated plant taxa using the Braun-Blanquet method (Braun-Blanquet, 1964) (Table 1). Plant species names were given after Mirek et al. (2002).

Chemical analyses of soil from the sampling sites were also conducted (Table 2). Rhizosphere soils excavated with the roots of each plant species at each site were mixed and analyzed as bulk samples. The total phosphorus content was determined in ammonium lactate extraction according to the Egner-Rim method, total nitrogen by the Kjeldahl method, and total carbon by the Tiurin method (Lityński et al., 1968; Kowalkowski et al., 1973). Cation exchanges capacity (CEC) was calculated as the sum of different cations measured with a flame photometer and spectrophotometer in ammonium acetate (Lityński et al., 1968; Kowalkowski et al., 1973).

ASSESSMENT OF FUNGAL ROOT COLONIZATION

Roots were prepared according to Phillips and Hayman (1970) with modifications. After washing in running tap water, roots were cleared in 10% KOH for 24 h and subsequently rinsed in water. The material was acidified in 5% lactic acid in water for 24 h, then stained with 0.01% aniline blue in 80% lactic acid for 24 h, and finally stored in 80% lactic acid. Both clearing and staining were carried out at room temperature.

Arbuscular mycorrhizal and DSE colonization was assessed using a Nikon Eclipse 800 light microscope with Nomarski interference contrast optics. Fine endophyte [usually considered *Glomus tenue* (Greenall) I.R. Hall; Thippayarugs et al., 1999; Dodd et al., 2000] AM-type colonization was counted separately from coarse AM-type colonization. The fine endophyte was identified on the basis of the following characteristics: mycelium ca. 1 μm diameter, deep blue stained hyphae, small vesicles or swellings of a diameter varying from 3 to 9 μm and fan-shaped branches. Mycorrhizal development was assessed according to the Trouvelot et al. method (1986). The following parameters were evaluated: mycorrhizal frequency (F), relative mycorrhizal root length (M), and relative arbuscular richness (A). 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 analyzed. The relative mycorrhizal root length (M%) is an estimate of the amount of root cortex that became mycorrhizal relative to the whole root system. Arbuscule abundance (A%) is an estimate of arbuscule richness in the whole root system. In the case of DSE colonization, the frequency of DSE mycelium (hyphae and sclerotia) occurrence in roots (F_{DSE}%) was estimated similarly as it was calculated for the presence of AMF. The obtained data were analyzed with the Kruskal-Wallis test ($p < 0.05$) using STATISTICA ver. 7.1 (Statsoft).

ISOLATION AND IDENTIFICATION OF AMF SPORES

Soil samples were collected in the six studied locations in the Tatra Mts., from which the plant material was obtained. Spores of AMF were isolated by centrifuging the soil in 50% sucrose solution and filtering (mesh size 50 μm) (Brundrett et al., 1996). Morphological properties of 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's reagent (4:1, v/v) (Omar et al., 1979). Slides with isolated spores were deposited in the slide collection of the Department of Plant Protection, West Pomeranian University of Technology, Szczecin. Fungal species nomenclature followed Walker and Trappe (1993).

Results

ARBUSCULAR MYCORRHIZA

Arbuscular mycorrhizas with arbuscules, which are the structural and functional criterion of the symbiosis, were observed in roots of *Soldanella carpatica* and *Homogyne alpina* examined at each sampling site. In roots of both plant species, coarse AMF (mycelium diameter above 2 μm) were more abundant than the fine endophyte along altitudinal gradients (Table 3). The fine endophyte accompanied coarse AMF and was rarely observed to form arbuscules. *S. carpatica* showed *Paris*-type colonization while the mycorrhiza of *H. alpina* was of the *Arum*-type morphology.

In the case of *S. carpatica*, there was a tendency of coarse AMF colonization to decline with increasing altitude; however, statistically significant differences were detected only in relative arbuscular richness (A) between the highest site (III) and the other two sites (I, II). In the case of the fine endophyte, an opposite trend was observed and significant differences were found between all parameters characterizing mycorrhizal colonization of roots, but only for the highest site (III) compared to the lowest (I) site (Table 3).

In contrast, in the root systems of *H. alpina*, the opposite patterns were observed. The coarse AM shift and fine endophyte decline in mycorrhizal colonization were found with increasing altitude. Nevertheless, statistically significant differences were observed between the highest (VI) and the lowest (IV) site in the case of relative mycorrhizal root length (M) and relative arbuscular abundance (A) for coarse AMF, and mycorrhizal frequency (F) for fine endophyte (Table 3).

AMF SPECIES DIVERSITY

In total, spores of 19 AMF species were extracted from the rhizosphere soil samples collected in the Tatra Mts. (Tables 4 and

TABLE 1

Plant species composition on sites of *Soldanella carpatica* along an altitudinal gradient in the Western Tatra Mts. (calcareous substrata; I = 1000 m a.s.l., II = 1510 m a.s.l., III = 2050 m a.s.l.) and *Homogyne alpina* in the High Tatra Mts. (non-calcareous substrata; IV = 1040 m a.s.l., V = 1550 m a.s.l., VI = 2050 m a.s.l.); abundance of plant species according to Braun-Blanquet method: species absent (−), few with small cover (+), 1 = numerous but <5% cover, 2 = 5–25% cover, 3 = 25–50%, 4 = 50–75%, 5 = 75–100%.

	Western Tatra Mts.			High Tatra Mts.		
Plant species	I	II	III	IV	V	VI
Aconitum firmum Rchb.	−	−	1	−	−	−
Androsace obtusifolia All.	−	−	+	−	−	−
Anemone narcissifolia L.	−	−	+	−	−	−
Asplenium viride Huds.	−	+	−	−	−	−
Anthoxanthum alpinum Á. Löve & D. Löve	−	−	1	−	−	1
Athyrium distentifolium Tausch ex Opiz	−	−	−	−	2	−
Athyrium filix-femina (L.) Roth	−	−	−	+	−	−
Bartsia alpina L.	−	−	1	−	−	−
Botrychium lunaria (L.) Sw.	−	+	−	−	−	−
Calamagrostis arundinacea (L.) Roth	2	−	−	−	−	1
Calamagrostis villosa (Chaix) J. F. Gmel.	−	−	−	−	5	−
Campanula alpina Jacq.	−	−	−	−	−	+
Campanula polymorpha Witasek	−	2	−	−	−	−
Cardaminopsis halleri (L.) Hayek	−	−	+	−	−	−
Carex capillaris L.	−	1	−	−	−	−
Carex firma Host	2	2	1	−	−	−
Carex parviflora Host	−	−	1	−	−	−
Carex sempervirens Vill.	1	−	+	−	−	2
Carlina acaulis L.	2	−	−	−	−	−
Clematis alpina (L.) Mill.	+	−	−	−	−	−
Cicerbita alpina (L.) Wallr.	−	−	−	+	−	+
Cystopteris fragilis (L.) Bernh.	−	−	−	−	+	−
Deschampsia caespitosa (L.) P. Beauv.	−	−	−	+	−	−
Doronicum austriacum Jacq.	−	−	−	2	−	−
Dryopteris expansa (C. Presl) Fraser-Jenk. & Jermy	−	−	−	−	+	−
Erigeron uniflorus L.	−	−	1	−	−	−
Euphrasia salisburgensis Hoppe	2	−	−	−	−	−
Festuca picta Kit.	−	−	−	−	1	2
Festuca versicolor Tausch	−	1	−	−	−	−
Galeobdolon luteum Huds.	−	−	−	+	−	−
Galium anisophyllon Vill.	−	+	−	−	−	−
Gentiana asclepiadea L.	−	−	−	2	+	−
Gentiana punctata L.	−	−	−	−	1	2
Gentianella lutescens (Velen.) Holub subsp. *lutescens*	1	−	−	−	−	−
Geum montanum L.	−	−	2	−	+	1
Gymnadenia conopsea (L.) R. Br.	+	−	−	−	−	−
Gymnocarpium dryopteris (L.) Newman	−	−	−	1	−	−
Gymnocarpium robertianum (Hoff.) Newman	−	−	−	1	−	−
Hieracium alpinum L. s.l.	−	−	−	−	−	2
Hieracium villosum Jacq.	−	2	−	−	−	−
Homogyne alpina (L.) Cass.	1	−	2	4	3	4
Huperzia selago (L.) Bernh. ex Schrank & Mart.	−	−	−	−	+	−
Hutchinsia alpina (L.) R. Br.	−	−	+	−	−	−
Hypericum maculatum Crantz	−	−	−	1	−	−
Juncus trifidus L.	−	−	−	−	+	−
Juniperus communis L. subsp. *alpina* (Sm.) Čelak.	+	−	−	−	−	−
Leontodon hispidus L.	1	−	−	−	−	−
Leucanthemum waldsteinii (Sch. Bip.) Pouzar	−	−	−	+	−	−
Luzula alpino-pilosa (Chaix) Breistr.	−	−	−	−	+	−
Luzula luzuloides (Lam.) Dandy & Wilmott	−	−	−	+	−	−
Luzula sylvatica (Huds.) Gaudin	−	−	−	+	−	−
Maianthemum bifolium (L.) F. W. Schmidt	2	−	−	−	−	−
Mercurialis perennis L.	2	−	−	−	−	−
Minaurtia verna (L.) Hiern	−	1	−	−	−	−
Mutellina purpurea (Poir.) Thell.	−	−	2	−	2	3
Myosotis sylvatica Ehrh. ex Hoffm.	−	−	−	+	−	−
Oxalis acetosella L.	−	−	−	+	−	−
Parnassia palustris L.	−	1	−	−	−	−
Pedicularis verticillata L.	−	1	+	−	−	−
Phleum commutatum Gaudin	−	−	1	−	−	−
Phyteuma orbiculare L.	+	2	+	−	−	−

TABLE 1

Continued.

Plant species	Western Tatra Mts.			High Tatra Mts.		
	I	II	III	IV	V	VI
Phyteuma spicatum L.	−	−	−	+	−	−
Pimpinella major (L.) Huds.	+	−	−	−	−	−
Poa alpina L.	−	−	+	−	−	−
Polygala amara L. subsp. *brachyptera* (Chodat) Hayek	1	−	−	−	−	−
Polygonum bistorta L.	−	−	−	−	−	+
Polygonum viviparum L.	−	1	−	−	−	−
Potentilla aurea L.	−	−	2	−	−	−
Potentilla crantzii (Crantz) Beck ex Fritsch	−	2	−	−	−	−
Potentilla erecta (L.) Raeusch.	+	−	−	−	−	−
Prenanthes purpurea L.	−	−	−	+	−	−
Primula elatior (L.) Hill	−	1	+	−	−	−
Ranunculus alpestris L.	−	−	+	−	−	−
Ranunculus repens L.	−	−	−	+	−	−
Rhinanthus minor L.	1	2	−	−	−	−
Rhodiola rosea L.	−	−	+	−	−	−
Rubus idaeus L.	−	−	−	1	−	−
Rumex alpestris Jacq.	−	−	−	+	−	−
Salix reticulata L.	−	−	5	−	−	−
Salix silesiaca Willd.	+	1	−	−	−	−
Saxifraga aizoides L.	−	−	2	−	−	−
Saxifraga moschata Wulfen	−	−	1	−	−	−
Saxifraga wahlenbergii Ball	−	+	−	−	−	−
Scabiosa lucida Vill.	1	+	−	−	−	−
Sedum fabaria W. D. J. Koch	−	−	−	+	−	−
Silene acaulis (L.) Jacq.	−	−	2	−	−	−
Soldanella carpatica Vierh.	2	3	2	−	1	+
Solidago alpestris Waldst. & Kit.	−	−	−	−	+	+
Sorbus aria (L.) Crantz	+	−	−	−	−	−
Thymus alpestris Tausch ex A. Kern.	−	2	−	−	−	−
Thymus pulcherrimus Schur	+	−	−	−	−	−
Tofieldia calyculata (L.) Wahlenb.	1	−	−	−	−	−
Trifolium badium Schreb.	−	1	−	−	−	−
Trifolium pratense L.	−	1	−	−	−	−
Urtica dioica L.	−	−	−	+	−	−
Veronica aphylla L.	−	+	+	−	−	−
Veronica fruticans Jacq.	−	+	−	−	−	−
Vaccinium gaultherioides Bigelow	−	−	−	−	−	+
Vaccinium myrtillus L.	+	−	−	+	4	4
Veratrum lobelianum Bernh.	−	−	−	−	+	2

5). In the case of *S. carpatica* sites (calcareous substrata), 15 species were identified. Spores of *Acaulospora paulinae*, *Glomus claroideum* and *Glomus constrictum* were isolated from each site along the gradient (Table 4). In the case of *H. alpina* sites (non-calcareous substrata), four species were isolated from the plant rhizosphere. All of them represented the genus *Acaulospora*. Among them, only *Acaulospora cavernata* occurred at all altitudes (Table 5). Interestingly, none of the identified AMF species were detected both in the Western and High Tatra Mts. (calcareous and non-calcareous sites, respectively).

DARK SEPTATE ENDOPHYTES

Dark septate endophytes (DSE) accompanied AMF in the roots of *S. carpatica* and *H. alpina* at each site, but root colonization by this group of fungi was low. Single, regularly septated DSE hyphae with rarely observed sclerotia were detected in the outer cortex. The mycelium stained with aniline blue or remained brownish (unstained). DSE root colonization did not have a consistent relationship with altitude in the case of both plant species (Table 3).

Discussion

The positive effects of arbuscular mycorrhizal fungi on their hosts, e.g. improved acquisition of water and soil nutrients, increased pathogen resistance, better growth and reproduction, have been well documented (Smith and Read, 1997). The benefits plants derive from the symbiosis might be especially important for high mountain species growing in harsh environments. However, in ecosystems such as alpine and arctic habitats, it may be too costly for a plant to maintain a fungal partner under stressful conditions (Ruotsalainen et al., 2004). It has been proposed that the advantage of the mycorrhizal symbiosis for the host plant decreases with increased elevation above sea level. Hence, it could be beneficial for plants to maintain different fungal colonization levels at different altitudes (Ruotsalainen et al., 2004). Nevertheless, different patterns have been observed in mycorrhizal colonization in relation to altitude. The coarse AMF declined with increasing elevation (Haselwandter and Read, 1980; Read and Haselwandter, 1981), but also the lack of a consistent relationship with altitude was observed (Väre et al., 1997; Ruotsalainen et al., 2004). The fine endophyte was found to be

TABLE 2

The chemical properties of soil from the sites along altitudinal gradients in the Tatra Mts.; W.T. = Western Tatra Mts. (calcareous substrata), H.T. = High Tatra Mts. (non-calcareous substrata).

Site	pH (H_2O)	N (%)	C (%)	Organic matter (%)	C/N	Contents total in mg 100 g^{-1} of dry soil		Exchangeable cations in mg 100 g^{-1} of dry soil			
						P_2O_5	CaO	K	Na	Mg	Ca
Soldanella carpatica											
W.T. I—1000 m	7.24	0.82	13.29	22.91	12.90	5.00	665.00	8.00	5.50	23.25	475.00
W.T. II—1510 m	7.38	0.27	3.43	5.92	16.30	0.20	252.00	4.25	1.87	8.25	180.00
W.T. III—2050 m	7.40	0.32	4.47	7.71	13.50	1.40	350.00	8.00	2.75	10.25	250.00
Homogyne alpina											
H.T. IV—1040 m	4.35	0.97	12.06	29.79	12.50	2.60	504.00	20.00	1.80	360.00	6.00
H.T. V—1550 m	4.20	1.19	20.64	35.58	17.40	7.00	560.00	24.80	1.60	400.00	17.00
H.T. VI—2050 m	4.15	1.20	17.24	29.77	14.30	11.40	283.00	25.60	2.00	202.00	9.00

more common at high than at low altitudes (Haselwandter and Read, 1980; Ruotsalainen et al., 2004). In our research, we found different patterns of AMF colonization in the two studied plant species, *S. carpatica* and *H. alpina*. This suggests that at the investigated altitudes factors like substrata and host plant species play a more important role in determining root colonization than climatic changes with increasing altitude above sea level. Ruotsalainen et al. (2004) found that *Sibbaldia procumbens* had high coarse AM colonization levels at high altitudes in comparison to other plants. It was concluded that this may be due to the species dependency on the symbiosis (Ruotsalainen et al., 2004). Moreover, vegetation coverage may also affect mycorrhizal colonization patterns along altitudinal gradients as mycorrhizal colonization percentage has been found to be positively related to host plant density (Hartnett et al., 1993; Genney et al., 2001; Ruotsalainen et al., 2004). Nevertheless, in our studies, the sampling of *S. carpatica* was only on the calcareous substratum and *H. alpina* on non-calcareous. Both species occurred at each substrata, however, they were not present at each altitude/site. Moreover, when the species was present at the particular site, not enough replicates were possible to collect due to small number of individuals at the site. Therefore, the species were not collected on both soils, but only on the substratum where they were abundant. In this case, when the investigations were limited to one species on each of the substrata, the obtained results might be from host plant, soil type, or community vegetation type as well as elevation.

The mycelium of the fine endophyte was detected in roots of the two investigated plant species. However, the colonization was not abundant in comparison to coarse AMF. In the previous study (Zubek et al., 2008), we found that this endophyte was occurring abundantly in roots which were devoid of coarse AMF. The fine endophyte was also observed to form arbuscules only in roots where there were no other Glomeromycota species (Turnau et al., 2005). This could suggest that the fungus may be the main root colonizer only in the absence of competition with other AMF (Turnau et al., 2005; Zubek et al., 2008). Hence its role might be important at higher altitudes, where coarse AMF occur rarely or are not present. The fine endophyte was observed to become dominant above 3000 m a.s.l. in the Alps (Read and Haselwandter, 1981).

Spores of 15 AMF species were found in the rhizosphere of *S. carpatica* collected from the sites in the Western Tatra Mts. (calcareous substrata), whereas only 4 species from a single genus were associated with *H. alpina* in the High Tatra Mts. (non-calcareous substrata). None of the identified AMF species was observed to occur both in the High and Western Tatra Mts. This suggests that bedrock and soil properties would play an important role in establishment of the AMF community. Literature data confirm the importance of soil pH in the symbiosis of plants with AMF. In Koomen's et al. (1987) studies, most AMF tested preferred a near neutral pH. Although Oehl et al. (2003, 2004) did not find a close correlation between AMF diversity and soil pH, they found the highest diversity in a calcareous grassland. Root colonization by *Glomus macrocarpum* has also increased with increasing rates of lime (Lambais and Cardoso, 1988). In our studies, however, host plants as well as the local plant species composition could also play an

TABLE 3

Fungal root colonization of *Soldanella carpatica* and *Homogyne alpina* at the sites along altitudinal gradients in the Tatra Mts.; W.T. = Western Tatra Mts. (calcareous substrata), H.T. = High Tatra Mts. (non-calcareous substrata); mycorrhizal parameters [%] (mean): mycorrhizal frequency (F), relative mycorrhizal root length (M), and relative arbuscular richness (A); F_{DSE} = frequency of dark septate endophytes (DSE) occurrence in roots [%] (mean); different letters after values indicate statistically significant differences ($p < 0.05$). AMF = arbuscular mycorrhizal fungi.

Site	Coarse AMF			Fine endophyte			F_{DSE}
	F	M	A	F	M	A	
Soldanella carpatica							
W.T. I—1000 m	65.88 a	15.52 a	3.24 b	8.12 b	1.18 b	0.07 b	14.95 ab
W.T. II—1510 m	64.07 a	9.73 a	1.96 b	17.59 ab	2.80 ab	1.53 ab	5.05 b
W.T. III—2050 m	43.42 a	6.69 a	1.03 a	33.18 a	6.74 a	2.56 a	19.02 a
Homogyne alpina							
H.T. IV—1040 m	75.14 b	32.39 b	10.89 b	37.94 b	7.26 a	0.79 a	1.71 a
H.T. V—1550 m	92.70 a	46.44 ab	16.81 ab	17.08 ab	2.51 a	0.81 a	7.02 a
H.T. VI—2050 m	90.34 ab	57.70 a	26.23 a	14.08 a	4.27 a	1.32 a	1.49 a

TABLE 4

AMF species (Glomeromycota) associated with *Soldanella carpatica* rhizosphere along an altitudinal gradient in the Western Tatra Mts. (calcareous substrata); I = 1000 m a.s.l., II = 1510 m a.s.l., III = 2050 m a.s.l.; AMF species present (+)/absent (−).

Family	Fungal species	Site		
		I	II	III
Ambisporaceae	*Ambispora gerdemannii* (S.L. Rose, B.A. Daniels & Trappe) C. Walker, Vestberg & Schuessler	+	−	−
Acaulosporaceae	*Acaulospora bireticulata* F. M. Rothwell & Trappe	+	+	−
	Ac. paulinae Blaszk.	+	+	+
	Ac. thomii Blaszk.	−	−	+
Pacisporaceae	*Pacispora franciscana* Sieverd. & Oehl	+	+	−
	Pac. robigina Sieverd. & Oehl	−	+	−
	Pac. scintillans (S. L. Rose & Trappe) Sieverd. & Oehl	−	+	−
Glomeraceae	*Glomus claroideum* N. C. Schenck & S. M. Sm.	+	+	+
	G. constrictum Trappe	+	+	+
	G. deserticola Trappe, Bloss & J. A. Menge	−	+	−
	G. fasciculatum (Thaxt.) Gerd. & Trappe emend. C. Walker & Koske	+	+	−
	G. geosporum (Nicol. & Gerd.) C. Walker	+	−	−
	G. macrocarpum Tul. & C. Tul.	+	−	+
	G. microcarpum Tul. & C. Tul.	−	+	−
	G. rubiforme (Gerd. & Trappe) R. T. Almeida & N. C. Schenck	+	+	−

important role in determining AMF species diversity at both investigated substrata, as the rhizosphere soil samples were collected from two different plant species. It has been generally believed that AMF have low host specificity (Smith and Read, 1997). Nevertheless, the few studies to date have consistently observed some physical and functional specificity in the symbiosis (McGonile and Fitter, 1990; Bever et al., 1996; Helgason et al., 2002; Aldrich-Wolfe, 2007). Consequently, there cannot be a clear separation between the possible influences of soil and plant factors on fungal species diversity in these studies.

Dark septate endophytes co-appear with mycorrhizal fungi in different ecosystems, especially in arctic and alpine habitats, over broad host and geographical ranges (Jumpponen, 2001). They were also found to accompany AMF in the roots of *S. carpatica* and *H. alpina* in the present study, but the level of colonization was low in both cases. There were no altitude-related shifts in the DSE root colonization, which is in agreement with the observations carried out by Ruotsalainen et al. (2004). However, more abundant DSE colonization at high than at low altitudes was found by Haselwandter and Read (1980) and Read and Haselwandter (1981). Different results obtained in studies on DSE infection patterns may be due to the fact that these endophytes represent a taxonomically and ecologically diverse group of fungi that probably includes saprobes, pathogens, and symbionts (Jumpponen, 2001; Schadt et al., 2001; Ruotsalainen et al., 2004). On the basis of our investigations, it is not possible to conclude the effect these fungi have on the plants that they inhabit. However, since some DSE strains have been shown to have positive effect on plant growth and nutrition (Haselwandter and Read, 1982; Wu and Guo, 2008), more attention should be paid to these endophytes in ecological studies. Similarly to AMF, they may improve plant performance in the case of taxa which are rarely colonized by Glomeromycota fungi, e.g. species from the Cyperaceae family (Miller et al., 1999). Haselwandter and Read (1982) found that two *Carex* species were colonized by DSE in the alpine habitats. They isolated DSE strains and reinoculated the seedlings of two host species in the laboratory conditions. The isolates were shown to stimulate plant growth or increase phosphorus concentration of these alpine species (Haselwandter and Read, 1982). In a similar experiment, Wu and Guo (2008) also observed that the mountain species *Saussurea involucrata* (Asteraceae) displayed enhanced growth when the plants were colonized by dark septate fungi.

In conclusion, our investigations revealed that (1) coarse AMF dominated over the fine AMF endophyte in roots of both plant species; (2) different patterns of these two types of AM colonization were observed in *S. carpatica* and *H. alpina*; (3) none of the identified AMF species was found to occur both in the High (non-calcareous substrata) and Western Tatra Mts. (calcareous substrata); and (4) DSE root colonization did not have a consistent relationship with altitude. The results suggest that at the investigated altitudes factors such as soil properties, host plants, and local plant species composition could play a more important role in determining root colonization as well as the establishment of a local AMF community than the climatic changes with increasing elevation above sea level. Further observations concerning mycorrhizal colonization of other alpine species as well as the assessment of *S. carpatica* and *H. alpina* response to inoculation with AMF and DSE in laboratory conditions are planned.

TABLE 5

AMF species (Glomeromycota) associated with *Homogyne alpina* rhizosphere along an altitudinal gradient in the High Tatra Mts. (non-calcareous substrata); IV = 1040 m a.s.l., V = 1550 m a.s.l., VI = 2050 m a.s.l.; AMF species present (+)/absent (−).

Family	Fungal species	Site		
		IV	V	VI
Acaulosporaceae	*Acaulospora alpina* Oehl, Sykorova & Sieverd.	−	−	+
	Ac. cavernata Blaszk.	+	+	+
	Ac. excavata Ingleby & C. Walker	+	+	−
	Ac. koskei Blaszk.	+	−	−

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