

Arbuscular mycorrhiza of plants from the Mountain Botanical Garden in Zakopane

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The mycorrhizal status of 77 plant species collected from the Mountain Botanical Garden of the Polish Academy of Sciences in Zakopane (southern Poland) was surveyed. These plants include rare, endemic and threatened species in the Tatra Mts. (the Western Carpathians) and are maintained in the botanical garden in order to develop effective methods of protection and cultivation. Plants belonging to *Brassicaceae*, *Caryophyllaceae*, *Dryopteridaceae*, *Juncaceae*, *Polygonaceae*, *Rubiaceae* and *Woodsiaceae* families were nonmycorrhizal. 41 species formed AM symbiosis. Spores of nine AMF species (*Glomeromycota*), including *Archaeospora trappei*, *Glomus aggregatum*, *G. claroideum*, *G. constrictum*, *G. deserticola*, *G. geosporum*, *G. microcarpum*, *G. mosseae* and *G. rubiforme* were isolated for the first time from this region of Poland. In addition, the occurrence of the fine endophyte, *G. tenue* was detected in roots of 18 species from the study area, although formation of arbuscules by this fungus was observed rarely. AM fungi were sporadically accompanied by dark septate endophytes (DSE). 70% of nonmycorrhizal plant species were devoid of DSE.

Key words: arbuscular mycorrhiza (AM), Mountain Botanical Garden, Tatra Mts., rare and threatened plants, active plant protection

INTRODUCTION

A wide range of natural processes and diverse human activity have a strong impact on the stability of ecosystems, leading to the destruction of plants' habitats, plant endangerment or even extinction. The conservation of the most valuable taxa is the main goal of numerous plant protection projects and is considered as an obligation for a number of countries bound by international agreements (The Convention on the Conservation of European Wildlife and Natural Habitats 1979, World Conservation Strategy 1980, The Convention on Biological Diversity, Rio de Janeiro 1992).

Numerous scientific programmes, aiming at increasing the knowledge about the biology and ecology of rare and threatened species of the Tatras, have been carried out in the Mountain Botanical Garden of the Institute of Nature Conservation of the Polish Academy of Sciences in Zakopane. Certain especially valuable plant species have been investigated, both in the garden and in the Tatra Mts., in order to elaborate methods of their cultivation, propagation and protection (Piękoś-Mirkowa 1982, 1990, 2001; Mirek and Piękoś-Mirkowa 1990; Piękoś-Mirkowa and Łobarzewska 1990a, 1990b; Piękoś-Mirkowa and Kaczmarczyk 1990a, 1990b; Piękoś-Mirkowa, Mirek and Miechówka 1996). There is an urgent need for multidirectional studies on these species. The importance of the underground microbiota in ecosystem restoration of native vegetation becomes well recognised (Jasper 1994). Among microorganisms occurring in the rhizosphere, arbuscular mycorrhizal fungi (AMF) are known for providing important benefits to plants including enhanced nutrition, drought tolerance, biocontrol of pathogens and tolerance of pollutants (Smith and Read 1997; Leyval, Turnau and Haselwandter 1997; Kaldorf et al. 1999; Jeffries et al. 2003). In the mountains, the occurrence of AM seems to be especially important as with increasing altitude the rates of mineralisation of nutrients slow down and the availability of the major nutrients like nitrogen and phosphorus is restricted. In this situation, mycorrhizal association, if present, would be most likely to benefit both individual plants and whole plant communities (Read and Haselwandter 1981). The biodiversity of AMF has also been shown to influence the structure and species composition of plant communities (van der Heijden et al. 1998a, 1998b; Hartnett and Wilson 2002; Koide and Dickie 2002). They are also important in post *in vitro* acclimatisation of medical and agricultural plants (Hooker et al. 1994; Hamel 1996; Requena et al. 1997; Vosatka et al. 2000; Requena et al. 2001). So far, the application of AMF in active protection of rare and threatened species has not been adequately taken into consideration, however, the possibilities of the practical use of these microorganisms in plant conservation programmes are numerous (St. John 1999; Zubek 2001; Turnau and Haselwandter 2002). Moreover, during the processes of plant cultivation and propagation in botanical gardens, it is important to provide plants with the most suitable conditions (Puchalski 1995).

The main aim of the present work was to estimate the mycorrhizal status of selected plants from the Mountain Botanical Garden in Zakopane. Such information is considered a pre-requisite for making further active plant conservation projects successful (van der Heijden et al. 1998a; Turnau and Haselwandter 2002). Although the garden in Zakopane is the smallest botanical garden in Poland, it holds a rich collection of over 600 species (belonging to 72 families), including 90 rare, endemic and threatened plant species, which places the garden at the very top of all Polish botanical gardens as the regional genes bank of the Tatras' flora (Piękoś-Mirkowa 1991). The investigations were carried out only on plant species that were developed in sufficiently big populations to avoid the risk of destruction due to sampling. As so far no work had been carried out on the diversity of AMF in the region, the additional aim was to isolate, multiply and identify AMF from the soil of the Mountain Botanical Garden in Zakopane.

MATERIALS AND METHODS

Site description. The material was collected from the Mountain Botanical Garden of the Polish Academy of Sciences located in Zakopane (834 m above sea level, 49°17'44"N, 19°57'04"E). The garden, founded in 1983, covers, in total, the area of 3000 m². Most of the plants in the garden have been cultivated from seeds collected in the field, and further propagated. The arrangement of the plants in the garden reflects the natural zones of vegetation in the Tatras. The substratum of the garden is either limestone or granite, which allows for the occurrence of both calcareous and granitic species.

Chemical analysis of soil samples. Soil samples were collected from five randomly chosen points on both main parts of the garden (calcareous and granitic) from the 0- to 15-cm-deep layer. The soil from the garden was classified as silty light loam in calcareous part and silty medium loam in granitic part (Tabs 1-2; Lityński, Jurkowska and Gorlach 1968; Kowalkowski et al. 1973).

Roots sampling and preparation. Plant material (1-3 specimens of each species) was collected during the flowering and early seed formation period (June and July in the 2002 and 2003). In total, roots of 77 plant species were collected and analysed. The shoots were deposited in the herbarium of the Institute of Botany of the Jagiellonian University (KRA).

Only roots attached to the main root of the plants were used for mycorrhizal assessment (to avoid the possibility of collecting roots from another species). The roots were prepared according to the modified Phillips and Hayman (1970) method. After careful washing in tap water, the roots were cleared in 10% KOH for 24 hours and subsequently rinsed in water. Dark, pigmented roots were bleached in a 10:1 mixture of 30% H₂O₂ and 25% NH₃ for 1-3 minutes and again rinsed in water. The material was acidified in 5% lactic acid in water for 24 h and stained with 0.01% aniline blue in 80% lactic acid for 24 h. The roots were stored in 80% lactic acid. The whole procedure was carried out at room temperature. Mycorrhizal frequency (F%), relative mycorrhizal root length (M%), intensity of colonisation within individual mycorrhizal roots (m%), relative arbuscular richness (A%) and arbuscule richness in root fragments where the arbuscules were present (a%) were assessed (Trouvelot, Kough and Gianinazzi-Pearson 1986; www.dijon.inra.fr/bbceipm/Mychintec/Mycocalc-prg/download.html). Additionally, attention was paid to the morphological characterisation of AM fungi, their percentage of roots colonisation and the occurrence of other root endophytes.

AMF spores isolation and identification. Soil samples collected from the rhizosphere of 25 selected plants were transported from the garden in plastic bags, placed in pots (200 ml) containing sterile substratum (sand : expanded clay 2:1, v/v). The cultures were kept in Sigma sunbags (B7026) in greenhouse conditions using *Plantago lanceolata* L. as a host plant (approximately 20 seedlings per pot). After six months spores were isolated using the wet filtering technique (mesh size 50 µm). Morphological properties of spores and their subcellular structures were determined in material mounted in a drop of polyvinyl alcohol/lactic acid/glycerol (PVLG) and in a mixture of PVLG/Melzer's reagent (4:1, v/v) on a slide (Omar, Bolland and Heaether 1979). Slides with isolated spores were deposited in the slide collection of the Department of Plant Pathology, University of Agriculture, Szczecin. Cultures of

Table 1
The chemical composition and properties (mean \pm SD) of soil from the Mountain Botanical Garden

	pH in H ₂ O	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			
						K ₂ O	P ₂ O ₅	MgO	CaO	K	Na	Mg	Ca
calcareous part	7.58 ± 0.05	0.53 ± 0.12	8.16 ± 0.12	14.07 ± 4.05	15.26 ± 1.32	17.68 ± 4.70	9.27 ± 4.33	189.00 ± 44.64	1310.40 ± 282.02	10.88 ± 2.20	8.16 ± 2.08	120.40 ± 27.00	936.00 ± 201.44
granitic part	7.46 ± 0.04	0.49 ± 0.04	7.32 ± 0.04	12.63 ± 1.47	15.34 ± 1.36	20.64 ± 7.71	4.20 ± 1.30	184.00 ± 50.42	817.60 ± 139.16	15.36 ± 7.78	5.36 ± 0.80	144.20 ± 22.35	584.00 ± 99.40

Table 2
The mechanical composition of soil (mean \pm SD) from the Mountain Botanical Garden [%]

particle size [mm]	1.0-0.1	0.1-0.05	0.05-0.02	0.02-0.005	0.005-0.002	<0.002
calcareous part	41.60 \pm 1.82	7.60 \pm 2.79	19.20 \pm 4.09	12.20 \pm 1.48	9.40 \pm 1.52	10.00 \pm 2.74
granitic part	36.20 \pm 8.35	12.60 \pm 3.91	16.20 \pm 4.82	14.80 \pm 2.59	9.60 \pm 2.07	10.60 \pm 0.55

selected AMF strains are kept under greenhouse conditions in the collections of the Jagiellonian University in Kraków and the University of Agriculture in Szczecin.

RESULTS

Mycorrhizal status of plants. Arbuscular mycorrhiza, including formation of arbuscules, which is the structural criterion of AM symbiosis, was observed in 41 species constituting 53% of all species collected (Tab. 3). Strongly mycorrhizal ($M > 60\%$) were *Asarum europaeum* (Aristolochiaceae), *Bellidiastrum micheli*, *Homogyne alpina*, *Solidago alpestris* (Asteraceae), *Veratrum lobelianum* (Liliaceae), *Plantago atrata* (Plantaginaceae), *Potentilla aurea* (Rosaceae). The most abundant arbuscule formation was found in *Asarum europaeum*, *Homogyne alpina*, *Solidago alpestris* and *Veratrum lobelianum* (Tab. 3). In roots of these plants coarse fungal endophytes (mycelium diameter above $2\ \mu\text{m}$) dominated. The fine endophyte, usually assigned as belonging to *Glomus tenue* (Greenhall) Hall, characterised by mycelium ca. $1\ \mu\text{m}$ diameter, abundant small vesicles or swellings of diameter varying from $3\text{--}9\ \mu\text{m}$ and finger-like branches, was found sporadically and was never observed to form arbuscules in these plants.

Low mycorrhizal colonisation ($M < 10\%$) was found in *Matteucia struthiopteris* (Polypodiaceae), *Doronicum austriacum* (Asteraceae), *Campanula polymorpha* (Campanulaceae), *Allium ursinum* (Liliaceae), *Aquilegia vulgaris*, *Delphinium elatum* subsp. *elatum* (Ranunculaceae), *Saxifraga paniculata* (Saxifragaceae), *Valeriana simplicifolia* (Valerianaceae) and *Viola reichenbachiana* (Violaceae). Also in the case of these plants, the fine endophyte usually did not form arbuscules, although its frequency was higher than in the first group. The mycelium including hyphae and coils of appearance similar to AMF mycelium were found in roots of *Polygala amara* subsp. *brachyptera* (Polygalaceae) and *Viola biflora* (Violaceae), however, no arbuscules were developed. In the case of *Delphinium elatum* subsp. *elatum*, no coarse mycelium of AMF was found, nevertheless slight colonisation of the fine endophyte was present.

The most common were plant species of medium mycorrhizal colonization (M from 10 to 60%). Almost in half of these plant species, coarse fungi were accompanied by the fine endophyte, still arbuscules were scarce. The most common arbuscules of *G. tenue* were found in the case of *Leucanthemum waldsteinii* (Asteraceae).

In general, the presence of the fine endophyte, *Glomus tenue*, was detected in roots of 18 plant species. If present, the fungus occupied less than 8% of the roots analysed. The arbuscules were present only in roots of four species (Tab. 4).

The examined plants from the *Gentianaceae*, *Poaceae*, *Polypodiaceae* and *Primulaceae* families showed *Paris*-type of AM colonisation. Species of *Campanulaceae* and *Crassulaceae* showed both structural classes of AM symbiosis but the individual species were either *Arum*- or *Paris*-type. The intermediate AMF colonisation of the roots was found among representatives of the *Ranunculaceae* (Tab. 3). Mycorrhizal plants of remaining families were of the *Arum*-type.

No AMF structures were found in roots of 33 plant species including all examined species from the *Brassicaceae*, *Caryophyllaceae*, *Dryopteridaceae*, *Juncaceae*, *Polygonaceae*, *Rubiaceae* and *Woodsiaceae* families. AMF were not observed either in

Table 3
Mycorrhizal status and the occurrence of dark septate endophytes (DSE) in the roots of plants from the Mountain Botanical Garden

Family	Plant species (Category of threat and the legal status of the taxon in Poland)	Mycorrhizal status according to literature	AM type	Mycorrhizal parameters [%]					C	V	DSE
				F	M	m	A	a			
Dryopteridaceae	<i>Dryopteris filix-mas</i>	1+, 2+	-	0	0	0	0	0	-	-	-
	<i>Gymnocarpium robertianum</i>	NS	-	0	0	0	0	0	-	-	-
	<i>Asplenium viride</i>	1+/-, 2+	-	0	0	0	0	0	-	-	-
Polypodiaceae	<i>Cystopteris alpina</i>	NS	-	0	0	0	0	0	-	-	-
	<i>Manteucia struthiopteris</i> (prot.)	NS	P	46.7	5.4	11.6	0.5	9.9	-	-	-
	<i>Athyrium filix-femina</i>	1+/-	-	0	0	0	0	0	-	-	-
Woodsiaceae	<i>Astrantia major</i>	1+/-, 2+	A	90	29.4	32.6	2.5	8.4	+	+	-
	<i>Mutellina purpurea</i>	NS	-	0	0	0	0	0	-	-	+
	<i>Asarum europaeum</i> (p.prot.)	1+/-, 2+	A	100	91.7	91.7	68.2	74.4	-	+	+
Aristolochiaceae	<i>Artemisia eriantha</i> (LR)	NS	-	0	0	0	0	0	-	-	+
	<i>Belladonna michelii</i>	2+	A	86.7	65.7	75.8	31.8	48.3	+	+	+
	<i>Centaurea mollis</i> (P-se)	NS	A	84.6	48.1	56.8	11.1	23	-	-	-
Borraginaceae	<i>Cicerbita alpina</i>	1+/-, 2+	A	56.7	15.4	27.1	3.2	20.9	+	-	-
	<i>Doronicum austriacum</i> (prot.)	NS	A	10	0.5	5	0.3	66.7	-	-	-
	<i>Homogyne alpina</i>	1+, 2+	A	100	75.4	75.4	70.8	93.9	-	+	+
Borraginaceae	<i>Leucanthemum waldsteinii</i> (P-se)	2+	A	93.3	56.4	60.4	42.8	75.8	-	-	+
	<i>Solidago alpestris</i>	2+	A	93.7	60.9	65	60	98.5	-	+	+
	<i>Symphytum cordatum</i> (P-se)	NS	-	0	0	0	0	0	-	-	-
Borraginaceae	<i>Symphytum tuberosum</i>	1+/-	A	53.3	14	26.3	8.7	61.9	+	-	-

<i>Brassicaceae</i>	<i>Arabis alpina subsp. alpina</i>	-	0	0	0	0	0	0	0	0	-	-	-	-
	<i>Cardamine trifolia</i>	-	0	0	0	0	0	0	0	0	-	-	-	-
	<i>Cochlearia latrae</i> (VU, T-se)	NS	-	0	0	0	0	0	0	0	-	-	-	-
	<i>Dentaria enneaphyllos</i>	NS	-	0	0	0	0	0	0	0	-	-	-	-
	<i>Dentaria glandulosa</i> (P-sc)	2-	-	0	0	0	0	0	0	0	-	-	-	-
	<i>Lanaria rediviva</i>	NS	-	0	0	0	0	0	0	0	-	-	-	-
	<i>Thlaspi caerulescens</i>	1-	-	0	0	0	0	0	0	0	-	-	-	-
<i>Campulidaceae</i>	<i>Campulula polytrichophila</i> (P-sc)	NS	A	30	0.6	1.9	0.02	2.9	0.02	2.9	-	-	-	+
	<i>Phyteuma orbiculare</i> (prot.)	1+, 2+	P	87.1	21	24.1	1.5	7.3	1.5	7.3	+	+	-	-
	<i>Phyteuma spicatum</i>	1+/-, 2-	P	63.3	10.1	15.9	1.3	13.1	1.3	13.1	+	+	-	-
<i>Caryophyllaceae</i>	<i>Cerastium latrae</i> (W-c)	NS	-	0	0	0	0	0	0	0	-	-	-	-
	<i>Dianthus plumarius subsp. proteox</i> (prot., W-c)	2-	-	0	0	0	0	0	0	0	-	-	-	-
	<i>Silene acaulis</i>	1+/-, 2+/-	-	0	0	0	0	0	0	0	-	-	-	+
<i>Corvalliaceae</i>	<i>Maianthemum bifolium</i>	1+/-, 2+	A	96.7	48.9	50.6	24.5	49.5	24.5	49.5	-	-	+	+
<i>Cruciferae</i>	<i>Rhodiola rosea</i>	2+/-	P	93.3	32.7	35	11.2	34.4	11.2	34.4	-	-	+	-
	<i>Sedum alpestre</i>	2-	A	70	27.7	39.6	0.5	1.7	0.5	1.7	-	-	+	+
<i>Euphorbiaceae</i>	<i>Euphorbia amygdaloides</i>	1+	A	90	46.3	51.5	31	67	31	67	-	-	-	+
	<i>Mercurialis perennis</i>	1+/-, 2-	A	100	41.2	41.2	23.6	57.4	23.6	57.4	-	-	-	-
<i>Gentianaceae</i>	<i>Gentiana asclepiadea</i> (p. prot.)	2+	P	68.7	51.7	59.6	50.5	97.7	50.5	97.7	+	+	-	-
	<i>Gentiana clausi</i> (prot.)	2+	P	100	39.5	39.5	34.5	87.5	34.5	87.5	+	+	-	-
<i>Hypericaceae</i>	<i>Hypericum maculatum</i>	1+, 2+/-	A	50	24.2	48.4	10.3	42.7	10.3	42.7	-	-	-	-
<i>Juncaceae</i>	<i>Luzula sylvatica</i>	1-, 2-	-	0	0	0	0	0	0	0	-	-	-	+
<i>Lamiaceae</i>	<i>Thymus pulcherrimus</i> (P-c)	NS	A	84.4	42.6	50.4	31.2	73.3	31.2	73.3	-	-	-	+

Tab. 3 cont.

<i>Liliaceae</i>	<i>Allium ursinum</i>	1+	A	40	9.6	24	4.7	48.6	-	-	-
	<i>Lilium martagon</i> (prot.)	1+	-	0	0	0	0	0	-	-	-
	<i>Paris quadrifolia</i>	1+/-, 2+	-	0	0	0	0	0	-	-	-
	<i>Polygonatum odoratum</i>	1+/-	A	80.7	24.4	30.2	3.8	15.5	-	+	-
	<i>Polygonatum verticillatum</i>	1+/-, 2+/-	-	0	0	0	0	0	-	-	-
	<i>Vernum lobelianum</i> (prot.)	2+	A	100	69.9	69.9	68	97.3	+	+	+
	<i>Toxicaria calycularis</i> (prot.)	2-	-	0	0	0	0	0	-	-	+
<i>Plantaginaceae</i>	<i>Plantago atrata</i> (VU, IUCN)	NS	A	100	76.7	76.7	50.1	65.4	+	+	+
<i>Poaceae</i>	<i>Avenula planiculmis</i> (VU)	NS	P	79.3	33.6	42.4	22.4	66.5	+	+	-
	<i>Deschampsia caespitosa</i>	1+	-	0	0	0	0	0	-	-	-
	<i>Sesleria latrae</i> (W-se)	NS	P	75	16.2	21.7	4.5	27.7	+	+	+
<i>Polygonaceae</i>	<i>Polygala amara</i> subsp. <i>brachyptera</i>	2+	A	11.1	3.3	30	0	0	-	+	-
	<i>Polygonum bistorta</i>	1+, 2+	-	0	0	0	0	0	-	-	+
	<i>Polygonum viviparum</i>	1+/-ecto, 2+/-ecto	-	0	0	0	0	0	-	-	+
	<i>Rumex scutatus</i>	1-	-	0	0	0	0	0	-	-	+
<i>Primulaceae</i>	<i>Primula auricula</i>	2+	-	0	0	0	0	0	-	-	-
	<i>Primula elatior</i> (p. prot.)	1+, 2+	-	0	0	0	0	0	-	-	-
	<i>Soldanella carpatica</i> (W-e)	2+	P	96.7	29	30	28.4	98	-	+	+

		1+/-	A/P	80	31	38.7	5.7	18.5	-	-	+
<i>Ranunculaceae</i>	<i>Anemone nemorosa</i>	1+/-	A/P	80	31	38.7	5.7	18.5	-	-	+
	<i>Aquilegia vulgaris</i> (prot.)	1+, 2+	A	16.7	1.7	10	0.5	30	-	-	-
	<i>Callianthemum corniculatum</i> (LR)	NS	-	0	0	0	0	0	-	-	+
	<i>Delphinium elatum</i> subsp. <i>elatatum</i>	NS	A	13.3	0.4	3	0	0	-	-	+
	<i>Ranunculus alpestris</i>	1+	A	93.3	30.5	32.7	5.3	17.3	-	+	-
	<i>Ranunculus platentifolius</i>	NS	A/P	93.3	49.3	52.9	42.4	85.9	-	-	-
	<i>Thalictrum aquilegifolium</i>	1+	A	98	47.8	48.8	17.9	37.6	+	-	-
	<i>Thalictrum minus</i> subsp. <i>minus</i>	1+	-	0	0	0	0	0	-	-	+
	<i>Trollius europaeus</i> (prot.)	1+	A/P	83.3	34	40.8	4.5	13.4	-	+	+
<i>Rosaceae</i>	<i>Dryas octopetala</i>	1+/-ecto, 2+/-ecto	-	0	0	0	0	0	-	-	-
	<i>Potentilla aurea</i>	2+	A	91.1	68.9	75.6	58.9	85.5	-	-	-
<i>Rubiaceae</i>	<i>Galium odoratum</i> (p.prot.)	NS	-	0	0	0	0	0	-	-	-
<i>Saxifragaceae</i>	<i>Saxifraga paniculata</i> (prot.)	2+	A	44.4	6.7	15	2.8	41.5	-	+	+
<i>Scrophulariaceae</i>	<i>Digitalis grandiflora</i> (p.prot.)	NS	A	73.3	35.8	48.9	14.5	40.6	+	+	+
<i>Valerianaceae</i>	<i>Valeriana simplicifolia</i>	NS	A	53.3	7.4	13.9	2.5	34.5	-	-	+
<i>Violaceae</i>	<i>Viola biflora</i>	2+/-	A	33.3	0.6	1.8	0	0	-	+	+
	<i>Viola reichenbachiana</i>	1+/-	A	40	9.8	24.6	4.1	41.6	-	+	-

Explanations: Names of plants after Mirek et al. (2002). Category of threat to the taxon in Poland and on a global scale after Kaźmierczakowa and Zarzycki (2001); LR – lower risk, VU – vulnerable, IUCN – species included in the world list of threatened plant species. The legal status of the taxon in Poland after Piękoś-Mirkowa and Mirek (2003): prot. – protected plant species, p.prot. – partly protected plant species, Endemism of the species after Piękoś-Mirkowa et al. (1996): Pe – Pan-Carpathian endemic species, We – West-Carpathian endemic species, P-se – Pan-Carpathian subendemic species, W-se – West-Carpathian subendemic species, T-se – Tatra subendemic species. Mycorrhizal status according to literature: 1 – Harley and Harley (1987); 2 – Nespiak (1953), Dominik and Nespiak (1954), Dominik et al. (1954a, 1954b), Dominik and Pachlewski (1956); (+) AM present, (-) AM absent, (+/-) AM in some stands present and in other absent, ecto – ectomycorrhiza present, NS – not surveyed according to the available literature. AM type: A – *Arum* type, P – *Paris* type, A/P – intermediate type. Mycorrhizal parameters: F – mycorrhizal frequency, M – relative mycorrhizal root length, m – intensity of colonisation within individual mycorrhizal roots, A – relative arbuscular richness, a – arbuscule richness in root fragments where the arbuscules were present (Troville et al. 1986). AM structures: C – coils, V – vesicles, DSE – dark septate endophytes; (+) DSE present, (-) DSE absent.

Table 4
Mycorrhizal colonisation of roots by *Glomus tenue* and other AMF species [%]

Family	Plant species	<i>Glomus tenue</i>					other AMF species				
		F	M	m	A	a	F	M	m	A	a
Polypodaceae	<i>Mattesia striatoptera</i>	6.7	0.1	1	0	0	46.7	5.4	11.6	0.5	9.9
	<i>Astrantia major</i>	-	-	-	-	-	90	29.4	32.6	2.5	8.4
Aristolochiaceae	<i>Asarum europaeum</i>	-	-	-	-	-	100	91.7	91.7	68.2	74.4
	<i>Belladonna micheli</i>	-	-	-	-	-	86.7	65.7	75.8	31.8	48.3
Asteraceae	<i>Centaurea mollis</i>	-	-	-	-	-	84.6	48.1	56.8	11.1	23
	<i>Cicorbia alpina</i>	6.7	0.1	1	0	0	56.7	15.4	27.1	3.2	20.9
Doronicaceae	<i>Doronicum austriacum</i>	-	-	-	-	-	10	0.5	5	0.3	66.7
	<i>Homogone alpina</i>	4.4	0.8	17.5	0	0	100	75.4	75.4	70.8	93.9
Leucanthemaceae	<i>Leucanthemum waldsteinii</i>	6.7	0.3	5	10	0.03	93.3	54.7	58.6	76.6	41.9
	<i>Solidago alpestris</i>	-	-	-	-	-	93.7	60	65	60	98.5
Borraginaceae	<i>Symphytum tuberosum</i>	6.7	0.3	5	0	0	50	13.9	27.7	8.7	62.6
	<i>Camparula polymorpha</i>	10	0.2	2.3	0.02	7.1	22.6	0.5	2.1	0.02	3.3
Campanulaceae	<i>Phyteuma orbiculare</i>	76.7	7.9	10.3	0	0	66.7	8.1	12.1	1	12
	<i>Phyteuma spicatum</i>	20	4	20	0	0	50	5.4	10.9	1.3	24.2
Convallariaceae	<i>Maianthemum bifolium</i>	-	-	-	-	-	96.7	48.9	50.6	24.2	49.5
	<i>Rhodola rosea</i>	6.7	0.1	1	0.01	10	93.3	32.7	35	11.2	34.4
Crassulaceae	<i>Sedum alpestris</i>	-	-	-	-	-	70	22.7	39.6	0.5	1.7
	<i>Euphorbia amygdaloides</i>	17.6	2.3	13.3	0	0	90	43.3	47.9	29.6	68.3
Euphorbiaceae	<i>Mercurialis perennis</i>	-	-	-	-	-	100	41.2	41.2	23.6	57.4
	<i>Geniana asclepiadea</i>	-	-	-	-	-	86.7	51.7	59.6	50.5	97.7
Gentianaceae	<i>Geniana clusii</i>	-	-	-	-	-	100	39.5	39.5	34.5	87.5
	<i>Hypericum maculatum</i>	3.3	0.03	1	0	0	46.7	24.2	51.8	10.3	42.7
Labiatae	<i>Thymus pulcherrimus</i>	-	-	-	-	-	84.4	42.6	50.4	31.2	73.3

<i>Liliaceae</i>	<i>Allium ursinum</i>	-	-	-	-	-	-	-	40	9.6	24	4.7	48.6
	<i>Polygonatum odoratum</i>	-	-	-	-	-	-	-	80.7	24.4	30.2	3.8	15.5
	<i>Véronique lobelianum</i>	-	-	-	-	-	-	-	100	69.9	69.9	68	97.3
<i>Plantaginaceae</i>	<i>Plantago atrata</i>	-	-	-	-	-	-	-	100	76.7	76.7	50.1	65.4
<i>Ponceae</i>	<i>Avenula planiculmis</i>	20.7	3.9	18.7	0.4	9.8	-	-	78.3	32.3	41.4	21.2	65.5
	<i>Sesleria latiae</i>	-	-	-	-	-	-	-	75	16.2	21.7	4.5	27.7
<i>Polygonaceae</i>	<i>Polygonum amara subsp. brachyptera</i>	-	-	-	-	-	-	-	11.1	3.3	30	0	0
<i>Primulaceae</i>	<i>Soldanella carpatica</i>	-	-	-	-	-	-	-	96.7	29	30	28.4	98
<i>Ranunculaceae</i>	<i>Anemone nemorosa</i>	-	-	-	-	-	-	-	80	31	38.7	5.7	18.5
	<i>Aquilegia vulgaris</i>	-	-	-	-	-	-	-	16.7	1.7	10	0.5	30
	<i>Delphinium elatum subsp. elatum</i>	13.3	0.4	3	0	0	-	-	-	-	-	-	-
	<i>Ranunculus alpestris</i>	13.3	0.4	3	0	0	-	-	93.3	29.7	31.8	5.3	17.5
	<i>Ranunculus plantaginifolius</i>	-	-	-	-	-	-	-	93.3	49.3	52.9	42.4	85.9
	<i>Thalictrum aquilegifolium</i>	-	-	-	-	-	-	-	98	47.8	48.8	17.9	37.6
	<i>Trollius europaeus</i>	3.3	0.03	1	0	0	-	-	83.3	34	40.8	4.5	13.4
<i>Rosaceae</i>	<i>Potentilla aurea</i>	-	-	-	-	-	-	-	91.1	68.9	75.6	58.9	85.5
<i>Saxifragaceae</i>	<i>Saxifraga paniculata</i>	2.2	0.02	1	0	0	-	-	44.4	6.7	15	2.8	41.5
<i>Scrophulariaceae</i>	<i>Digitalis grandiflora</i>	-	-	-	-	-	-	-	73.3	35.8	48.9	14.5	40.6
<i>Valerianaceae</i>	<i>Valeriana simplicifolia</i>	3.3	0.03	1	0	0	-	-	53.3	7.4	13.9	2.5	34.5
<i>Violaceae</i>	<i>Viola biflora</i>	6.7	0.1	1	0	0	-	-	33.3	0.3	1	0	0
	<i>Viola reichembachiana</i>	-	-	-	-	-	-	-	40	9.8	24.6	4.1	41.6

roots of *Dryas octopetala* (*Rosaceae*), that, on the contrary, formed ectomycorrhizal association.

AMF spores isolated from the trap cultures. Spores of nine species were obtained from 25 trap cultures that were established from the material collected from the Mountain Botanical Garden. They were identified as *Archaeospora trappei* (R.N. Ames & Linderman) J.B. Morton & D. Redecker, *Glomus aggregatum* N.C. Schenck & S.M. Sm. emend. Koske, *G. claroideum* N.C. Schenck & S.M. Sm., *G. constrictum* Trappe, *G. deserticola* Trappe, Bloss & J.A. Menge, *G. geosporum* (Nicol. & Gerd.) C. Walker, *G. microcarpum* Tul. & C. Tul., *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe and *G. rubiforme* (Gerd. & Trappe) R.T. Almeida & N.C. Schenck. The same fungal species were found in the trap cultures from the calcereous and granitic part of the garden. Isolates of *G. mosseae* and *G. constrictum* were the most commonly obtained in pot cultures.

Presence of other root endophytes. Dark septate endophytes (DSE) were found in the root cortex of 31 species (Tab. 3). The regularly septated mycelium was accompanied sporadically by sclerotia. The observed mycelium stained with anilin blue or remained brownish. DSE were found together with AMF in the case of 21 species, however, they were not abundantly developed. They were even less common in nonmycorrhizal plant species.

DISCUSSION

First studies concerning arbuscular mycorrhizas of the Tatras' plant communities had been carried out in the fifties of the 20th century by the group of Polish mycologists (Nespiak 1953; Dominik and Nespiak 1954; Dominik, Nespiak and Pachlewski 1954a, 1954b; Dominik and Pachlewski 1956). These studies, however, did not include rare, endemic and threatened plant species. Moreover, no mycorrhizal investigations have been carried out in the Mountain Botanical Garden. The access to the garden enabled investigations of a number of rare species in a relatively short time and without collecting them from their natural habitats, where the material sampling could create an additional threat.

No AM colonisation was found in the investigated representatives of the *Brassicaceae*, *Caryophyllaceae*, *Dryopteridaceae*, *Juncaceae*, *Polygonaceae*, *Rubiaceae* and *Woodsiaceae* families. The *Brassicaceae* and *Caryophyllaceae* are constantly considered as nonmycorrhizal (Gerdemann 1968, Trappe 1987). The absence of AM in species belonging to these families was usually explained by the presence of chemical barriers, low root exudation, lack of signaling compounds or cell wall structure and physiology (DeMars and Boerner 1996). Nevertheless, according to literature, AM structures were observed among some representatives of the *Brassicaceae* family (Ross and Harper 1973; Tommerup 1984; DeMars and Boerner 1996; Orłowska et al. 2002; Regvar et al. 2003). However, so far there is no evidence of AM formation under laboratory conditions, what suggests that the mycorrhiza under field conditions might be stimulated by unknown stress factors. In the present study, not only AM structures were lacking but also no other root endophytes were found in all species examined from the *Brassicaceae*.

53% of the investigated plants were colonised by AMF, although the level of mycorrhizal colonisation differed among plant species. The differences in mycorrhizal parameters can be expected not only because of the preferences of the in-

dividual plant species to allow for abundant or sparse AM fungal colonisation, but also can depend on factors such as season, stage of plant development, susceptibility to inoculation and plant nutritional status (Friese and Allen 1991; Sanders and Fitter 1992). Nevertheless, mycorrhizal colonisation level can not serve as an indicator of the effectiveness of the symbiosis and the dependency of a plant on mycorrhiza (McGonigle 1988; Lieberei and Feldmann 1990; Hetrick and Wilson 1991). The present results concerning mycorrhizal development can be treated only as a survey of plant mycorrhizal status under the conditions of the Mountain Botanical Garden and can be used to select plant species that could be of interest for further investigations concerning application of AMF in plant protection. As repeated sampling could threaten the existing populations, material collection was limited to the flowering/seed production stage, as it is recognised that high percentage of AM colonisation usually correlates with the most active growth of the plant host (Nicolson and Johnston 1979; Van Duin, Rozema and Ernst 1990; Ietswaart, Griffioen and Ernst 1992).

Two structural classes of AM symbiosis, the *Arum*- and the *Paris*-type, were observed in plant roots from the Mountain Botanical Garden. The morphology of AM depends on plant and fungal identity (Smith and Smith 1997; Cavagnaro et al. 2001). Smith and Smith (1997), basing on the literature data, concluded that *Paris*-type occurs more frequently in the plant kingdom and predominates in ferns, gymnosperms and wild angiosperms while *Arum*-type dominates in cultivated herbs. In the present study, the *Arum*-type was, however, the dominant AM morphology.

Both coarse and fine endophytes were observed in roots collected from the Mountain Botanical Garden in Zakopane. The presence of the fine endophyte, *G. tenue*, was detected in roots of 18 plant species, however, the root length occupied by the fine endophyte was low and arbuscules, functionally the most important mycorrhizal structures, occurred only sporadically. This may suggest that *G. tenue* does not play an important role, compared to other AMF colonisers. Its role might be more important at higher altitudes. The fungus has commonly been observed in the Alps and has been shown to become dominant above 3000 m above sea level (Read and Haselwandter 1981).

Spores of nine AMF species were isolated from trap cultures established using soil and root samples collected from the field. As most of the plants cultivated in the garden have been propagated from seeds, the AMF of the garden soil are rather indigenous for the place and might have little in common with the AMF diversity in the natural stands of the plants investigated. In addition, the isolated strains are those that are easily obtained under greenhouse conditions; the most effective root colonizers in the garden might not be necessarily obtained. Nevertheless, the preparation of inoculum can be done only in the case of strains that sporulate well under greenhouse conditions. From this material, the most effective fungal strains assuring the successful plant growth could be selected.

Dark septate endophytes (DSE) occurred in the roots of several species collected from the Mountain Botanical Garden. Interestingly, most non-AM plants were also devoid of DSE which suggests similar defense mechanisms towards the both groups of fungi. According to literature, DSE association with plants can vary from negative to positive when measured by host performance or tissue nutrient concentrations, and in some cases this type of association has been classified as mycorrhizal

(Bartholdy, Berreck and Haselwandter 2001, Jumponen 2001). They are frequent colonisers of roots co-appearing with other mycorrhizal fungi in a variety of ecosystems, especially in arctic and alpine habitats (Haselwandter and Read 1980, Read and Haselwandter 1981, Bartholdy et al. 2001, Jumponen 2001). They should not be neglected as an additional tool in plant protection, although, their isolation and identification was not included within the scope of the present paper. Nevertheless, similarly to AM fungi that are already practically used in cultivation systems of agricultural and medical plants, they may strongly improve active protection of rare and threatened species.

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Mikoryza arbuskularna roślin z Górskiego Ogródu Botanicznego w Zakopanem

Streszczenie

Na terenie Górskiego Ogródu Botanicznego Instytutu Ochrony Przyrody PAN w Zakopanem znajduje się szereg gatunków roślin cennych dla naszej flory ze względu na ich rzadkość. Prowadzone są tu prace mające na celu poznanie biologii i ekologii gatunków rzadkich i zagrożonych, służące dalszym badaniom nad ich uprawą *ex situ*, zwiększeniem liczebności populacji oraz reintrodukcją w siedliska naturalne. Poznanie zależności interakcji z grzybami arbuskularnymi (*Glomeromycota*) stanowi istotny czynnik, który może w przyszłości przyczynić się do zwiększenia przeżywalności tych roślin zarówno na etapie uprawy, namnażania materiału, a także po reintrodukcji.

W badaniach uwzględniono 77 gatunków roślin tatrzańskich (w tym gatunki rzadkie, zagrożone, chronione oraz endemiczne), zebranych z Górskiego Ogródu Botanicznego. Określono stopień kolonizacji mikoryzowej z wyróżnieniem morfotypów grzybów arbuskularnych oraz identyfikowano zarodniki grzybów arbuskularnych izolowane z kultur pułapkowych, założonych na glebie pobranej z ogrodu. Stwierdzono następujące gatunki grzybów z grupy *Glomeromycota*: *Archaeospora trappei*, *Glomus aggregatum*, *G. claroideum*, *G. constrictum*, *G. deserticola*, *G. geosporum*, *G. microcarpum*, *G. mosseae* oraz *G. rubiforme*. Mikoryzę arbuskularną obserwowano u 41 gatunków roślin. Wysoki stopień kolonizacji mikoryzowej stwierdzono u: *Asarum europaeum* (*Aristolochiaceae*), *Bellidiastrum micheli*, *Homogyne alpina*, *Solidago alpestris* (*Asteraceae*), *Veratrum lobelianum* (*Liliaceae*), *Plantago atrata* (*Plantaginaceae*), *Potentilla aurea* (*Rosaceae*). Mikoryzy arbuskularnej nie stwierdzono u badanych przedstawicieli rodzin: *Brassicaceae*, *Caryophyllaceae*, *Dryopteridaceae*, *Juncaceae*, *Polygonaceae*, *Rubiaceae* oraz *Woodsiaceae*. U 31 gatunków obserwowano występowanie endofitycznych grzybów o ciemnych, septowanych strzępkach (DSE).