

Observations on the mycorrhizal status of *Polygonum viviparum* in the Polish Tatra Mts. (Western Carpathians)

MICHAŁ RONIQUIER¹ and PIOTR MLECZKO²

¹W. Szafer Institute of Botany, Polish Academy of Sciences, Lubicz 46, PL 31 512 Kraków,
michal.ronikier@ib.pan.krakow.pl

²Institute of Botany, Jagiellonian University, Lubicz 46, PL 31 512 Kraków,
ubmleczk@cyf.kr.edu.pl

Ronikier M., Mleczko P.: *Observations on the mycorrhizal status of *Polygonum viviparum* in the Polish Tatra Mts. (Western Carpathians)*. Acta Mycol. 41 (2): 209-222, 2006.

Polygonum viviparum is one of very few herbaceous plants known to form ectomycorrhiza; in the Tatra Mts. it is one of dominants in the alpine zone, but also descends down to the feet of the massif. Specimens of this plant were collected from 5 sites at the altitude range 900-2150 m, from granite and limestone. It allowed an estimation of the ectomycorrhizal diversity as well as preliminary ecological observations. Roots were also stained in order to check potential presence of arbuscular mycorrhizal colonization. Ectomycorrhizae were present in all specimens (with 2-5 morphotypes observed on single plants). In total, 17 morphotypes were observed and briefly described. The most widespread were the mycorrhiza of *Cenococcum geophilum* and a brightly coloured morphotype resembling the ectomycorrhizae of *Russula* sp. No important differences in ectomycorrhizal colonization between low and high localities were found. Observed general differences in abundance and diversity of mycorrhiza in *P. viviparum* between sites could most probably be connected with plant community composition (presence/absence of ectomycorrhizal shrubs maintaining ectomycorrhizal fungi), although mycorrhizae were present also in sites devoid of other ectomycorrhizal plants. Structures associated to arbuscular colonization (vesicles, hyphal coils) were occasionally observed, but without formation of arbuscules.

Key words: *Polygonum viviparum*, ectomycorrhiza, arbuscular mycorrhiza, arctic alpine ecology, *Cenococcum geophilum*, rhizosphere

INTRODUCTION

Polygonum viviparum L. (*Polygonaceae*) is a widely distributed arctic-alpine species (Pawłowska 1972), commonly found in the arctic tundra and high mountain regions of the northern hemisphere. In the Tatra Mts. (the highest massif of the Carpathians, with maximum altitude of 2663 m) it is one of dominant species in the alpine and subnival zones (up to 2480 m a.s.l. in the Lodowy massif; Pawłowski

1956), but also descends down to the meadows at feet of the massif (900 m), which makes a very large altitudinal range. It is one of very few herbaceous plants known to form ectomycorrhiza (reviewed by Gardes and Dahlberg 1996). Hesselman (1900) first reported the presence of ectomycorrhizal tips with a Hartig net on the root system of *P. viviparum*. This observation was then confirmed for plants from different sites as Central Alps (Constantin and Magrou 1926; Peyronel 1930, 1937; Fontana 1977), Eastern Alps (Haselwandter and Read 1980; Read and Haselwandter 1981; Blaschke 1991a, 1991b), Tatra Mts. (Dominik et al. 1954), Rocky Mts. (Lesica, Antibus 1986; Massicotte et al. 1998), Alaska (Treu et al. 1996). However, some authors reported lack of ectomycorrhizae in their observations in both arctic (Bledsoe et al. 1990; Väre et al. 1992) and mountain (Nespiak 1953) sites, which suggests the existence of some constraints in the formation of symbiotic relationship of this type.

Most literature data are limited to the statement of the presence/absence of ectomycorrhizal structures, while there is only limited information on the diversity of *P. viviparum* ectomycorrhiza. Fontana (1977) gave account of the diversity of ectomycorrhizae of *P. viviparum* from Italian Alps, reporting presence of 16 morphotypes. Treu et al. (1996) mentioned different ectomycorrhizal tips morphology. Massicotte et al. (1998) described anatomical features of two herbaceous plants' ectomycorrhizae: *P. viviparum* and *Kobresia myosuroides*.

Some data suggest presence of two types of mycorrhizal colonization in *P. viviparum*. The parallel colonization of root system by arbuscular mycorrhizal fungi and ectomycorrhizal fungi was reported by Stahl (1900) from arctic site and by Blaschke (1991a, 1991b) from the Bavarian Alps. Nespiak (1953) also mentioned the AMF colonization of roots of *P. viviparum* in the Tatra Mts.

The aim of the present work was to describe the mycorrhizal status of *P. viviparum* in the Polish Tatra Mts. (Western Carpathians) and to contribute to the study of the diversity of mycorrhiza of this interesting arctic-alpine species. It is thought as a pilot study initiating more complex analyses of ectomycorrhizae in alpine habitats and their role in building the arctic-alpine macromycete diversity in the Tatra Mts.

MATERIALS AND METHODS

Description of the study sites and sampling of plant specimens. Five sampling sites were chosen on the stations of *Polygonum viviparum* growing on granitic and calcareous bedrock in Polish Tatra Mts., in different parts of the altitudinal range of the species (Tab. 1). Samples of plant root systems were collected twice during the vegetation period on three sites and once on two others (Tab. 1); 4–5 plants were collected from each site. Plants were taken together with the embedding soil (about 20×20×15 cm), transported in plastic bags and stored in a fridge (when quickly analyzed) or frozen at –20° C. Samples were soaked in water, then roots of *P. viviparum* were washed and carefully separated from roots of other plants. Only roots connected to rhizome were considered. Ectomycorrhizal colonization was analyzed and afterwards the roots were stained for checking the endophytic colonization.

EM observations – light and scanning electron microscopy. Ectomycorrhizal tips were observed submerged in water, using dissecting microscope. Morphotypes were distinguished according to methods described by Agerer (1986, 1987–2002, 1991),

Table 1

Description of sampling sites (all within the Western Carpathians, the Tatra Mts.).
In the "sampling" column: 1 sampling in May 1997; 2 sampling in September 1997

No	Site location			Sampling	Other EM plants in the vicinity
	Description	Alt. [m]	Coordinates		
1	N slopes of the Nosal peak (1205 m); fresh meadow at forest edge	910	N 49°16'55" E 19°59'10"	12	<i>Picea abies</i>
2	SW slopes of the Przełęcz między Kopami pass (1550 m); clearings among dwarf pine shrubs	1545	N 49°15'07" E 20°00'13"	12	<i>Pinus mugo</i>
3	SW slopes of the Kasprowy Wierch peak (1985 m); alpine grassland on granite	1960	N 49°13'56" E 19°58'50"	12	[distant stands of <i>Salix herbacea</i>]
4	W crest of the Szpiglasowy Wierch peak (2172 m); alpine grassland on granite	2150	N 49°11'53" E 20°02'28"	2	
5	N slopes of the Małolączniak peak (2069 m); alpine grassland on limestone	2070	N 49°14'12" E 19°55'15"	2	<i>Dryas octopetala</i> , <i>Salix reticulata</i>

using mantle "scrapings" for plan views. In some cases (abundant morphotypes) basic macrochemical stainings (KOH, FeSO₄, sulphovanilin, Melzer's reagent) were also made. Non-identified morphotypes were named following rules proposed by Agerer (1996).

Additionally, ectomycorrhizal structure on longitudinal and cross sections were observed. Ectomycorrhizal tips for sections were embedded in the synthetic resin (Historesin embedding kit – Leica, Germany; prepared according to manufacturer's instructions) and cut on the microtome (Leica RM 2135, Germany; 6–7 µm). Slides were observed using microscope with differential interference contrast (DIC). Ectomycorrhizal tips were stored in FAA solution (Gerlach 1972).

For observations in scanning electron microscope, fresh ectomycorrhizal tips were fixed in 2% glutaraldehyde solution in cacodylate buffer and dehydrated in the increasing acetone and ethanol concentration series (Massicotte et al. 1987).

EM quantitative comparison. Quantitative comparison of ectomycorrhizal colonization was very difficult to estimate due to the tight mixture of roots in case of alpine grassland samples and thus only approximate data could be obtained. Ectomycorrhizal tips were counted and related to the length of the roots, as follows:

$$M = \frac{\text{number of mycorrhizal tips}}{\text{length of roots [cm]}}$$

Each site was characterized by a mean M value from all specimens (4–5). Additionally, the ratio of alive and dead mycorrhizal tips (M^{l/d}) was calculated.

AM colonization. For estimation of endophytic colonization, roots of *P. viviparum* were stained according to the modified method of Philips and Hayman (1970). Roots were softened using 7% KOH solution, washed with water and bleached with H₂O₂ containing NH₃ (10:1 v/v) for a few minutes. The material was then acidified in 5% lactic acid solution and stained with 0,01% cotton blue (anilin blue, methyl blue)

solution in lactic acid. All steps were conducted in room temperature and lasted (apart from bleaching) 24 h each. Stained material was stored in pure lactic acid. Prior to staining the roots were kept in 50% ethanol solution.

RESULTS

Diversity of ectomycorrhizae on *Polygonum viviparum*. Seventeen morphotypes of ectomycorrhizae were isolated from collected samples (cf. Fig. 1A–D). The highest number of morphotypes was found on the sites 1, 5 and 2 (10, 8 and 7 morphotypes, respectively) (Tab. 2). The highest morphotype number per sample (plant) was 5–6 on three mentioned sites, whereas on the sites 3 and 4 it did not exceed 2. One morphotype was identified as formed by *Cenococcum geophilum* Fr. Based on presence of clamps, six morphotypes were identified as members of Basidiomycota (“*P. lanata*”, “*P. vulpina*”, “*P. aurata*”, “*P. tuberoidea*”, “*P. aspera*”, “*P. tenua*”).

Ectomycorrhizae were formed mainly on delicate, secondary roots, the side branches of dark roots growing from the rhizome. All ectomycorrhizae were simple and did not form any ramifications. In some tips traces of renewed growth were visible in the form of slight segmentation (beaded mycorrhiza; Agerer 1991).

Morphotypes differed significantly in tips length. Some were short (ca. 1 mm), and often of big diameter (e.g. “*Polygonirhiza epidermoidea*”, “*P. lacteocinerea*”), while some others were elongated (up to 3 mm). A high diversity of fungal mantle structures was also observed. Several morphotypes had a primitive, plectenchymatous mantle (e.g. “*P. vulpina*”), or the mantle of an intermediate character between plectenchymatous and pseudoparenchymatous, with hyphae distinguishable yet considerably thickened (e.g. “*P. maculata*”, “*P. salebrosa*” and “*P. fusca*”). Pseudoparenchymatous mantles of different types were represented by “*Polygonirhiza epidermoidea*”, “*P. lacteocinerea*”, “*P. aurata*” and “*P. tuberoidea*”. Morphotypes varied also in respect of extramatrical structures. Some of them produced very abundant extramatrical mycelium, forming

Table 2
Synopsis table of the morphotype occurrence in sampling sites
(numbers of sites refer to Tab. 1)

Morphotype	Site	1	2	3	4	5
<i>Cenococcum geophilum</i>		■	■	■	■	■
“ <i>P. arenaria</i> ”		■				
“ <i>P. aspera</i> ”		■	■			
“ <i>P. aurata</i> ”						■
“ <i>P. epidermoidea</i> ”						■
“ <i>P. fusca</i> ”		■	■			■
“ <i>P. granulosa</i> ”						■
“ <i>P. lacteocinerea</i> ”		■	■	■	■	■
“ <i>P. lanata</i> ”			■		■	
“ <i>P. maculata</i> ”						■
“ <i>P. radiata</i> ”		■				
“ <i>P. rufocystidiata</i> ”		■				
“ <i>P. salebrosa</i> ”		■				
“ <i>P. tenua</i> ”		■				
“ <i>P. terrea</i> ”						■
“ <i>P. tuberoidea</i> ”			■			
“ <i>P. vulpina</i> ”		■	■			■

even woolly clusters, as in “*P. lanata*”, only few emanating hyphae were produced by e.g. “*P. terrea*” and “*P. vulpina*”, a smooth mantle surface was observed e.g. in “*P. epidermoidea*”. Abundant, very fine emanating hyphae of “*P. arenaria*” were covered with a secretion causing sticking of sand particles. Cystidia were present in three morphotypes: “*P. aurata*”, “*P. tuberoidea*” and “*P. rufocystidiata*”.

Hartig net was of paraepidermal type in all examined ectomycorrhizae (Agerer 1991), fungal colonization was limited to anticlinal walls of rhizodermal cells (Fig. 1F). The hyphae of Hartig net were ramified and closely cohering together forming “palmetti” structures. Root cells of Hartig net zone were strongly elongated transversally (CCq 0.32, comp. Agerer 1987-2002).

Key for the determination of ectomycorrhizae. The dichotomous key is presented below in order to facilitate the identification of morphotypes described in this paper. Both macroscopical features (colour, surface structure) and microscopical characteristics of fungal mantle and extramatrical structures in “plan view” were included.

- 1. Mycorrhiza brownish-black or black 2
- 1*. Mycorrhiza of other colour 7
- 2. Surface rough and shiny, with long, rigid and dark emanating hyphae. Star-like mantle structure.
Mantle homogenously black (Fig. 1A), thick and rigid, densely plectenchymatous, outer layer formed by thick-walled, strongly pigmented cells, forming distinctive star-like arrangements (Fig. 1E) (in their centers the “cells” are small, centripetally elongated). Emanating hyphae thick-walled, with regularly distributed septa. Clamps lacking. Inner mantle layer formed by hyphae with weaker stained, thinner walls. Mycorrhiza rarely > 1 mm long, usually of a big diameter.
..... *Cenococcum geophilum*
- 2*. Mycorrhiza with other features 3
- 3. Abundant orange-brown cystidia present, well visible under dissecting microscope.
Mycorrhiza very dark brown, blackish, mantle surface slightly rough (Fig. 1B). Cystidia elongated, thick-walled with small diameter, narrowing to a sharp top, without clamps at base (Fig. 2G). Outer mantle layer pseudoparenchymatous, cells rounded, of different sizes. Deeper layer dense, intermediate between plectenchymatous and pseudoparenchymatous “*Polygonirhiza rufocystidiata*”
- 3*. Mycorrhiza without orange-brown cystidia 4
- 4. Mantle (plectenchymatous or pseudoparenchymatous) with visible star-like structure (but never with long, rigid emanating hyphae; cf. 2 – *Cenococcum geophilum*) 5
- 4*. Mantle pseudoparenchymatous, epidermoid or angular, without star-like pattern ... 6
- 5. Mantle pseudoparenchymatous, built by angular cells. Thick (> 5 µm in diameter), dark brown emanating hyphae with grainy surface and clamps.
Mycorrhiza very dark brown with a rigid, cloddish surface. Outer mantle layer built of big, strongly stained polygonal cells forming a characteristic „rosette” pattern (Fig. 2B) (cells dimensions diminish centripetally). Inner layer pseudoparenchymatous, cells thinner-walled and without pattern. Emanating hyphae frequently ramified and interconnected. Intrahyphal hyphae present “*Polygonirhiza aspera*”

- 5***. Mantle plectenchymatous. Extramatrical structures not observed. Mycorrhiza brownish-black. Mantle surface slightly rough. Outer mantle layer a dense plectenchyma built by long, irregular hyphae converging radially and forming a „rosette” pattern. Inner layer pseudoparenchymatous, formed by round cells. *“Polygonirhiza radiata”*
- 6**. Outer mantle pseudoparenchymatous with angular hyphal cells. Inner mantle layer intermediate between plectenchyma and pseudoparenchyma. Emanating hyphae fine, hyalin without clamps, with rare, fine septa and slightly incrustated cell walls. Mycorrhiza black, surface slightly cloddish. *“Polygonirhiza salebrosa”*
- 6***. Outer mantle pseudoparenchymatous with epidermoid hyphal cells. Inner mantle intermediate between plectenchymatous and pseudoparenchymatous. Towards the inside of the mantle hyphae become thinner and less stained. Extramatrical structures not observed
Mycorrhiza dark brown to blackish. Mantle surface very rough and slightly shiny. *“Polygonirhiza fusca”*
- 7**. Light or dark brown coloured mycorrhiza **8**
- 7***. Mycorrhiza golden-greenish, orange-brown or orange **11**
- 7****. Mycorrhiza whitish, grey, yellowish or pinkish **13**
- 8**. Light-brown coloured mycorrhiza with distinctly rough, cloddish mantle surface. Mantle thick and rigid, plectenchymatous, hyphae brown and thick-walled, often triangular in shape, usually arranged in a distinct „rosette-like” arrangement (Fig. 2C); responsible for the cloddish macroscopic mantle character. Extramatrical structures not observed *“Polygonirhiza granulosa”*
- 8***. Mycorrhiza with other features **9**
- 9**. Mycorrhiza brown. Mantle thin, intermediate between plectenchymatous and pseudoparenchymatous, formed by thin-walled hyphae without clamps. Emanating hyphae rare, hyaline, ramified, with clamps and opened anastomoses. *“Polygonirhiza tenua”*
- 9***. Mycorrhiza unhomogeneously brown (with irregular, darker spots). Mantle surface smooth and shiny **10**
- 10**. Mycorrhiza dark brown with greyish shade, with distinct darker spots. Mantle surface smooth and shiny. Mantle thin, plectenchymatous. In the top part of mycorrhizal tip fine, thin-walled emanating hyphae without clamps
..... *“Polygonirhiza terrea”*
- 10***. Light brown mycorrhiza with slight olivaceous shade and distinct small darker spots (Fig. 1D). Mantle surface smooth, shiny. Mantle intermediate between plectenchymatous and pseudoparenchymatous (Fig. 2D), hyphae immersed in matrix material. Extramatrical structures not observed *“Polygonirhiza maculata”*
- 11**. Mycorrhiza orange-brown. Outer mantle layer plectenchymatous, formed by a dense net of hyphae, with characteristic groups of parallel hyphae. Inner layer formed by larger hyphae, closer to irregular pseudoparenchyma. Emanating hyphae rare, fine, hyalin, thin-walled, with clamps. Cystidia lacking.
Mycelium without colour reaction to KOH, FeSO₄ and sulphovanilin
..... *“Polygonirhiza vulpina”*

- 11***. Mycorrhiza orange or goldenish, with pseudoparenchymatous mantle. Numerous hyalin, elongated cystidia, often with clamps **12**
- 12**. Mycorrhiza short spiny, yellowish-brown with golden-green glaze (Fig. 1C). Mantle surface smooth and shiny, with hyalin cystidia. Outer mantle layer a very loose hyphal net, growing on intermediate, pseudoparenchymatous layer with angular, thin-walled cells. Inner mantle layer formed by small, irregular cells. Cystidia growing from angular cells, short and obtuse (top rounded), most with a clamp in 1/3 or 1/2 of their length. Cystidium wall much thicker in the proximal part and thinner above the clamp "*Polygonirhiza aurata*"
- 12***. Mycorrhiza short spiny, orange. Mantle surface smooth or slightly fibrous with numerous hyaline cystidia. Outer mantle layer a loose net of thick hyphae without clamps, with rare anastomoses. Intermediate layer pseudoparenchymatous with angular, thick-walled cells; the structure of inner layers less regular. Cystidia very numerous, obtuse, thick-walled, often with clamps at septa (Fig. 1F). This morphotype resembles macroscopically those formed by *Tuber* sp. (although such identity is excluded by presence of clamps). Although macroscopically very different from "*P. aurata*", microscopical structure and cystidia resemble that morphotype. It cannot be excluded that "*P. tuberoidea*" and "*P. aurata*" are formed by very close (or even the same) taxa of fungi "*Polygonirhiza tuberoidea*"
- 13**. Mycorrhiza with abundant emanating hyphae **14**
- 13***. Mycorrhiza with smooth surface, no emanating hyphae observed **15**
- 14**. Small, yellowish mycorrhiza with very fine emanating hyphae. Mantle surface covered with sand particles. Mantle thin, plectenchymatous "*Polygonirhiza arenaria*"
- 14***. Mycorrhiza whitish-grey (older parts often pinkish-brown), big – reaching length of 3 mm. Mantle plectenchymatous, formed by a dense hyphal net with matrix material. Hyphae becoming thicker towards the inner layers of the mantle, without clamps. Emanating hyphae abundant, often forming cottony concentrations, hyalin, thin-walled, with clamps, T-shaped branching, local inflations and frequent anastomoses. No colour reaction to KOH, FeSO₄ and Melzer's reagent "*Polygonirhiza lanata*"
- 15**. Mycorrhiza with whitish-creme colour. Mantle thin (cortical cells visible) with smooth and shiny surface. Outer mantle covered by a loose net formed by branched hyphae without clamps. Mantle beneath net pseudoparenchymatous, epidermoid (Fig. 2E). Inner layer plectenchymatous "*Polygonirhiza epidermoidea*"
- 15***. Mycorrhiza whitish-grey (Fig. 1A). Mantle surface smooth and shiny, in older mycorrhizae cortical root cells visible. Mantle thick, outer layer pseudoparenchymatous composed of roundish hyphal cells (Fig. 2E), in inner layer hyphal segments of smaller diameter, less regular, and elongated. Small mycorrhiza with a considerably big diameter and rounded top. Mycelium not stained by KOH, FeSO₄ and sulphovanilin "*Polygonirhiza lacteocinerea*"

Quantitative aspects of ectomycorrhizal colonization. Ectomycorrhiza was present in all analyzed samples. Number of mycorrhizal tips differed strongly between sites (Tab. 3). The richest specimens were characterized by M value near 0.2 (sites 1, 2, 5), whereas the quotient did not exceed 0.01 for plants from the site 3.

Table 3

Quantity of living and dead mycorrhizae (as M values with corresponding standard deviations in brackets) and average numbers of morphotypes per plant on study sites

Site	Sampling period	Living mycorrhizae (M)	Dead mycorrhizae (M)	Average number of morphotypes on plant
1	Spring	0.112 (0.068)	0.252 (0.102)	3.5
	Autumn	0.075 (0.034)	0.073 (0.023)	3.3
2	Spring	0.100 (0.081)	0.130 (0.084)	3.0
	Autumn	0.086 (0.020)	0.045 (0.017)	4.0
3	Spring	0.015 (0.008)	0.062 (0.032)	1.0
	Autumn	0.028 (0.025)	0.091 (0.068)	1.6
4	Autumn	0.071 (0.014)	0.032 (0.017)	1.7
5	Autumn	0.117 (0.063)	0.050 (0.027)	3.8

Average density of mycorrhizal tips for all analysed plants was almost equal in spring and autumn: 0.084 and 0.082 respectively. The ratio: alive vs dead mycorrhizae, was estimated for all sites. In all spring samples, number of living mycorrhizae was considerably lower than dead ($M^{ld} < 1$). This quotient was the lowest on the site 3 ($M^{ld} < 0.16$).

The share of morphotypes in the total number of mycorrhizal tips was very differentiated. *Cenococcum geophilum* and “*Polygonirhiza lacteocinerea*” were clearly dominant, constituting appr. 23 % of all mycorrhizal tips each. “*Polygonirhiza vulpina*”, “*P. aspera*”, “*P. arenaria*” and “*P. fusca*” represented 8–9 % of total number of ectomycorrhizae each. Other morphotypes were less numerous, and sometimes limited only to few tips (“*P. granulosa*”, “*P. aurata*”, “*P. terrea*”, “*P. maculata*”). Domination of *Cenococcum geophilum*, “*Polygonirhiza lacteocinerea*” and “*P. vulpina*” was correlated with their presence in all (in the case of two first) or most (3 – in case of the third) sites; to the contrary, “*P. tuberoidea*” and “*P. arenaria*” were limited to single sites only. Some differences were observed in presence of morphotypes in samples collected in spring and autumn (sites 1, 2 and 3). Five morphotypes on the site 1 (“*P. aspera*”, “*P. rufocystidiata*”, “*P. radiata*”, “*P. salebrosa*”, “*P. tenua*”) and two on the site 2 (“*P. tuberoidea*” and “*P. aspera*”) were present only in spring (in the case of “*P. tuberoidea*” it was connected with a clear domination of this morphotype in the samples). On the other hand, *Cenococcum geophilum* was almost absent in spring, while in autumn it was very frequent in all sites. This was also true for “*P. arenaria*” on the site 1 (only 4 tips observed in spring and very abundant occurrence in autumn). These single observations are too scarce, however, to formulate any general conclusions.

There were no clear differences between sites in the average number of mycorrhizae in relation to altitude. Although the M value for the high mountain site 3 was low, the data for two other high-altitude stands (4 and 5) were higher and comparable with data for the lower located sites (1 and 2).

Endomycorrhiza in *Polygonum viviparum*. Prevailing part of roots did not manifest any traces of AMF colonization, however, several roots taken from site 2 in spring contained intraradical structures resembling those formed by endomycorrhizal fungi (members of Glomeromycota) – massive hyphae, coils and vesicles. No arbuscules, however, were observed.

DISCUSSION

Presence and diversity of ectomycorrhizae on *Polygonum viviparum* in the Tatra Mts. Seventeen ectomycorrhizal morphotypes were described in the samples from the Tatra Mts. Comparable number was reported in the observations from Italian Alps (Fontana 1977). The diversity of morphotypes on single specimens of *P. viviparum* reported from the Alps also corresponds well with the situation in the Tatra Mts. Fontana (1977) observed 2–3 morphotypes on average on a single plant, with maximum of 5 different mycorrhizae. The plants from Denali National Park (Alaska) had at least 3 morphotypes each (Treu et al. 1996). Also in the material from Rocky Mts. “several morphotypes” were mentioned (Massicotte et al. 1998). The universal presence of ectomycorrhizal colonization in *P. viviparum* on sites in whole altitudinal range of Tatra Mts. is in agreement with majority of observations. However, it does not correspond with some data, especially that of Nespíak (1953), who found specimens without any ectomycorrhizal colonization in two alpine sites in High Tatra. However, comparison of data (from literature and present observations) for plants growing on different sites reveal rather small direct role of the position above sea level in the detected number of ectomycorrhizae, even though mycorrhizal colonization generally decreases with altitude (reviewed in Körner 1999). More probably, it could be suspected that the composition of the plant communities might have a strong influence on the ectomycorrhizal population of *P. viviparum*. A distinct, positive relationship was observed between the diversity of ectomycorrhizae of *P. viviparum*, and the presence of other ectomycorrhizal plants in its vicinity (cf. Tab. 1). On plants growing near *Picea abies* (L.) H. Karst. (site 1), *Pinus mugo* Turra (site 2) or *Salix reticulata* L. and *Dryas octopetala* L. (site 5), the mycorrhizal colonization and/or the number of morphotypes was considerably higher than on specimens originating from the sites where *P. viviparum* was the only ectomycorrhizal plant (sites 3 and 4). Similarly, in the paper by Dominik et al. (1954) an abundant ectomycorrhizal colonization was reported for specimens from Kominiarski Wierch (1829 m) in the Tatra Mts., where *P. viviparum* grew together with *Pinus mugo*, while lack of ectomycorrhiza in the study by Nespíak (1953) from the Przełęcz Białczańska Pass (2080 m) in a patch of alpine grassland *Oreochloa distichae*-*Juncetum trifidi* could have resulted from lack of ectomycorrhizal plants. The presence of perennial, obligatorily mycorrhizal dwarf shrubs could play an important role in the creation and maintenance of the bank of ectomycorrhizal fungal inoculum (Väre et al. 1992). Importance of this factor increases with the environmental stress at high altitude locations, diminishing the capability of fungi to grow and form fruitbodies. Nevertheless, a quite high level of mycorrhizal colonization on the site 4 (although with only 3 morphotypes, two of them common for all the investigated sites), also devoid of ectomycorrhizal shrubs, suggests a notable autonomy of *P. viviparum* in the formation and maintenance of ectomycorrhiza.

The presence of alive ectomycorrhizae in samples collected in spring and in autumn, together with a similar average number of mycorrhizal tips, suggest that the colonization is generally stable throughout the year. The absence of several morphotypes in spring could be the result of weaker ability to recolonize the roots after winter dormancy, however other reasons (eg. patchy distribution of mycelium) cannot be excluded.

Morphotypes recorded on *Polygonum viviparum*. A comparison of ectomycorrhizae found in the Tatra Mts. with those from other sites is difficult, as very few descriptions are available. Dominik et al. (1954) described an „ectotrophic mycorrhiza of the A type“; this type comprises a simple mycorrhiza without important ramifications nor growth modifications, with a primitive (plectenchymatous), loose mantle and Hartig net of different depth (Dominik 1961). The paper includes some general remarks on the mycorrhiza of *P. viviparum*, but does not allow comparison of morphotypes. One of dominant morphotypes in Tatra Mts. was formed by an ascomycete *Cenococcum geophilum*. As a result of its commonness and a very characteristic appearance, the presence of this mycorrhiza is reported in most investigations from the whole distribution area of *P. viviparum* (e.g. Fontana 1977; Read and Haselwandter 1981; Treu et al. 1996; Massicotte et al. 1998). It is not surprising, as the mycorrhiza of this fungus was described from many plant hosts (Maia et al. 1996), and it was found on several other arctic-alpine species, as *Dryas octopetala*, *D. integrifolia*, *Salix* spp., *Kobresia belliardii* (Fontana 1963; Trappe 1964; Read, Haselwandter 1981; Massicotte 1998). In lowlands *Cenococcum* often dominates in dry environments (Trappe 1964). It is not the case in the mountains, characterized by relatively high falls and long snow depositions. As suggested by Read & Haselwandter (1981), the commonness of this fungus in such areas could be connected with an efficient spread strategy, that is the production of very resistant and abundant sclerotia, rather than any special symbiotic features. The high resistance of *Cenococcum* to frost, experimentally demonstrated by Corbery and Le Tacon (1997) can also be important factor. The species from the genera *Amanita*, *Inocybe* and *Russula* were also reported to form mycorrhiza with *P. viviparum* (reviewed in Gardes and Dahlberg 1996); an ectomycorrhiza of *Alnicola cholea* and *P. viviparum* was also recently described (Moreau et al. 2006). A morphotype called “*Polygonirhiza lacteocinerea*” strongly resembles an alpine mycorrhiza formed by *Russula nana* (*Russula emetica* Fr. var. *alpestris* Boud.), described by Fontana (1977). The presence of this mycorrhiza in Tatra Mts. is probable since this fungus is very common in the alpine belt there (Nespiak 1960; M. Ronikier, pers. obs.). In the case of some morphotypes, many characteristics link them to the mycorrhizae described on trees. “*Polygonirhiza epidermoidea*” share mantle features with e.g. the mycorrhizae of some *Russula* spp. (epidermoid mantle structure with inner plectenchymatous layer, scarce extramatrical hyphae). A similar mycorrhiza is formed by *Russula firmula* on *Pinus mugho* (Treu 1990). The morphotype “*Polygonirhiza aurata*” seems to be close to the ectomycorrhiza of *Tomentella galzini* described on *Quercus* (Jakucs et al. 1997 as „*Quercirhiza fibulocystidiata*”; Kõljalg et al. 2001). Both have an olive-green colour, pseudoparenchymatous, angular mantle structure and very characteristic cystidia with single clamps and thick walls below them. The only difference between these two morphotypes seems to be the lack of ramified emanating hyphae in the mycorrhiza of *P. viviparum*. Also the features of the group of „black” mycorrhizae found on *P. viviparum* lead to suppose their possible relationships with several tree mycorrhizae, e.g. “*Piceirhiza nigra*” (Gronbach 1988), identified as formed by a member of *Thelephoraceae* (Agerer et al. 1995). These morphotypes, having a pseudoparenchymatous mantle structure (“*Polygonirhiza aspera*”, “*P. rufocystidiata*”, “*P. salebrosa*”, “*P. fusca*”), could be formed by this group of fungi. Representatives of *Thelephoraceae* forming dark mycorrhizae are mostly species

producing resupinate fruit-bodies on wood; interestingly, such morphotypes clearly dominated in sites in the vicinity of *Picea abies* or *Pinus mugo* (cf. Tab. 1, 2), so they could be formed not by alpine fungi but species related with trees – *Tomentella* spp. It is not possible, however, to refer such an assumption to available mycological data from the Tatra Mts. as this group of fungi has not been studied in this area so far. A very characteristic star-like hyphae arrangement and the presence of dark, incrustated emanating hyphae in “*Polygonirhiza aspera*” resembles strongly “*Fagirhiza setifera*” (Brand 1991), however the *Fagus* mycorrhiza has abundant cystidia, lacking in the *P. viviparum* mycorrhiza.

The general morphological aspects of ectomycorrhiza formed by *P. viviparum* in the Tatra Mts. fit well descriptions by Blaschke (1991a), Treu et al. (1996) and Massicotte et al. (1998). It may be concluded that this species forms exclusively simple, cylindrical or club-shaped mycorrhizae. The anatomical features of these mycorrhizae, particularly the characteristic growth modification of epidermal cells (Massicotte et al. 1998), are similar to mycorrhizae of other Angiosperms, including trees, e.g. beech (Agerer 1991; Smith, Read 1997).

Presence of arbuscular mycorrhiza in *Polygonum viviparum*. Traces of probable arbuscular mycorrhizal colonization were sporadically observed. The roots contained some characteristic structures typically associated with the arbuscular mycorrhiza, as hyphae, coils and vesicles, but they were not accompanied by arbuscules. Most observations of *P. viviparum* reported lack of endomycorrhizal colonization, although it was incidentally found in arctic sites (Stahl 1900) as well as in the mountains (Nespiak 1953; Blaschke 1991a, 1991b). Nespiak (1953) noticed exclusively endomycorrhizal colonization without ectomycorrhizae in the same root system, while Blaschke (1991a, 1991b) found both kinds of symbiosis occurring together. None of these authors, however, mentioned the formation of arbuscules in the roots. The infection of non-host roots by AM fungi, including formation of vesicles, was reported in some cases; the main signal which controls the development of functional symbiosis probably acts by triggering fungal genes responsible for change of hyphal growth and physiology during arbuscule formation (review in Giovannetti and Sbrana 1998). Considering the presence of some AM structures in *P. viviparum* roots, the capacity of this plant to form this kind of symbiosis seems to be probable even if not important ecologically. Lack of arbuscules could possibly be also due to their short-lived appearance during the vegetation period, as it was reported in the study of the high-mountain *Ranunculus adoneus* colonization by *Glomus tenuis* (Mullen, Schmidt 1993). Regular phenological study or controlled cultures would be necessary to verify potential factors responsible for establishment of arbuscular mycorrhiza in *P. viviparum*.

The present study is the first contribution focused on the mycorrhiza of *Polygonum viviparum* in the Tatra Mts. and the Carpathians. The results showing that ectomycorrhizal colonization is a regular situation in this species, but affected by several factors, should be the starting point for future studies employing rigorous morphological/anatomical descriptions of morphotypes, regular survey of carpophores on permanent plots and employing DNA comparisons of mycorrhizae and carpophores. Including comparative analysis of diversity of mycorrhizae in neighbouring ectomycorrhizal plants in plant communities with *P. viviparum* would al-

low a direct estimation of share/independence of the ectomycorrhizal diversity of *P. viviparum*.

Acknowledgments: This paper is based on an MSc project carried out by M. Ronikier under supervision of Prof. Katarzyna Turnau (Jagiellonian University, Kraków). The authors thank Prof. K Turnau for helpful discussions and comments, and Dr. Anna Ronikier for help in gathering plant samples and comments on the manuscript.

REFERENCES

- Agerer R. 1986. Studies on ectomycorrhizae II. Introducing remarks on characterization and identification. *Mycotaxon* 26: 473–492.
- Agerer R. (ed.) 1987–2002. Colour atlas of ectomycorrhizae. Einhorn Verlag, Schwäbisch Gmünd, p. 31–33i.
- Agerer R. 1991. Characterization of ectomycorrhizae. *Methods Microbiol.* 23: 25–73.
- Agerer R. 1996. Characterization of ectomycorrhizae: a historical overview. *Descr. Ectomyc.* 1: 1–22.
- Agerer R., Klostermeyer D., Steglich W. 1995. *Piceirhiza nigra*, an ectomycorrhiza on *Picea abies* formed by a species of *Thelephoraceae*. *New Phytol.* 131: 377–380.
- Blaschke H. 1991a. Multiple mycorrhizal association of individual calcicole host plants in the alpine grass heath zone. *Mycorrhiza* 1: 31–34.
- Blaschke H. 1991b. Distribution, mycorrhizal infection and structure of roots of calcicole floral elements at treeline, Bavarian Alps, Germany. *Arct. Alp. Res.* 23: 444–450.
- Bledsoe C., Klein P., Bliss L. C. 1990. A survey of mycorrhizal plants on Truelove Lowland, Devon Island, N.W.T., Canada. *Can. J. Bot.* 68: 1848–1856.
- Brand F. 1991. Ektomykorrhizen an *Fagus sylvatica*. Charakterisierung und Identifizierung, ökologische Kennzeichnung und unsterile Kultivierung. *Libri Bot.* 2: 3–229.
- Constantin J., Magrou J. 1926. Contribution à l'étude des racines des plantes alpines et de leurs mycorrhizes. *C. R. Acad. Sci.* 181: 26–29.
- Corbery Y., LeTacon F. 1997. Storage of ectomycorrhizal fungi by freezing. *Ann. Sci. Forest.* 54: 211–217.
- Dominik T. 1961. Klucze do oznaczania mikoryz. *Zesz. Nauk. Wyższej Szkoły Rolniczej w Szczecinie* 5: 63–81.
- Dominik T., Nespiak A., Pachlewski R. 1954. Badanie mykotrofizmu roślinności zespołów na skałkach wapiennych w Tatrach. *Acta Soc. Bot. Pol.* 23: 471–485.
- Fontana A. 1963. Micorrize ectotrofiche in una Ciperacea: *Kobresia bellardi* Degl. *Giorn. Bot. Ital.* 70: 639–641.
- Fontana A. 1977. Ectomycorrhizae of *Polygonum viviparum* L. In: Abstracts of the 3rd North American Conference on Mycorrhizae, Athens, Georgia, p. 53.
- Gardes M., Dahlberg A. 1996. Mycorrhizal diversity in arctic and alpine tundra: an open question. *New Phytol.* 133: 147–157.
- Gerlach D. 1972. Zarys mikrotechniki botanicznej. PWRiL, Warszawa, p. 48.
- Giovannetti M., Sbrana C. 1998. Meeting a non host: the behaviour of AM fungi. *Mycorrhiza* 8: 123–130.
- Gronbach E. 1988. Charakterisierung und Identifizierung von Ektomykorrhizen in einem Fichtenbestand mit Untersuchungen zur Merkmalsvariabilität in sauer beregneten Flächen. *Bibl. Mycol.* 125: 1–216.
- Haselwandter K., Read D. J. 1980. Fungal associations of roots of dominant and sub dominant plants in high alpine vegetation systems with special reference to mycorrhiza. *Oecologia* 45: 57–62.
- Hesselman H. 1900. Om mykorrhizabildningar hos arktiska växter. *Bih. Svens. Vetensk. Akad. Handl.* 26: 1–46.
- Jakucs E., Agerer R., Bratek Z. 1997. „*Quercirhiza fibulocystidiata*” + *Quercus* sp. (In:) R. Agerer, R. M. Danielson, S. Egli, K. Ingleby, R. Treu (eds). *Descr. Ectomyc.* 2: 67–71.
- Körner C. 1999. Alpine plant life. Functional plant ecology in high mountains ecosystems. Springer Verlag, Berlin Heidelberg, pp. 338.
- Kõljalg U., Jakucs E., Bóka K., Agerer R. 2001. Three ectomycorrhizae with cystidia formed by different *Tomentella* species as revealed by rDNA ITS sequences and anatomical characteristics. *Folia Cryptog. Estonica* 38: 27–39.

- Lacey J. 1996. Spore dispersal its role in ecology and disease: the British contribution to fungal aerobiology. *Mycol. Res.* 100: 641–660.
- Lesica P., Antibus R. K. 1986. Mycorrhizae of alpine fell field communities on soils derived from crystal line and calcareous materials. *Can. J. Bot.* 64: 1691–1697.
- Massicotte H. B., Melville L. H., Peterson R. L., Luoma D. L. 1998. Anatomical aspects of field mycorrhizas on *Polygonum viviparum* (Polygonaceae) and *Kobresia bellardi* (Cyperaceae). *Mycorrhiza* 7: 287–292.
- Massicotte H. B., Peterson R. L., Ashford A. E. 1987. Ontogeny of *Eucalyptus pillularis*-*Pisolithus tinctorius* ectomycorrhizae. I. Light and scanning electron microscopy. *Can. J. Bot.* 65: 1927–1939.
- Maia L.C., Yano A.M., Kimbrough J.W. 1996. Species of *Ascomycota* forming ectomycorrhizae. *Mycotaxon* 43: 371–390.
- Moreau P. M., Mleczko P., Ronikier M., Ronikier A. 2006. Rediscovery of *Alnicola cholea* (Cortinariaceae): taxonomic revision and description of its mycorrhiza with *Polygonum viviparum* (Polygonaceae). *Mycologia* 98 (3): 468–478.
- Mullen R. B., Schmidt S. K. 1993. Mycorrhizal infection, phosphorus uptake and phenology in *Ranunculus adoneus*: implications for the functioning of mycorrhizae in alpine systems. *Oecologia* 94: 229–234.
- Nespiak A. 1953. Badanie mykotrofizmu roślinności alpejskiej ponad granicą kosodrzewiny w granitowych Tatrach. *Acta Soc. Bot. Pol.* 22: 97–125.
- Nespiak A. 1960. Notatki mykologiczne z Tatr. *Fragm. Flor. Geobot.* 4: 709–724.
- Pawłowska S. 1972. Charakterystyka statystyczna i elementy flory polskiej. (In:) W. Szafer, K. Zarzycki (eds). *Szata roślinna Polski*. 1. PWN, Warszawa: 129–206.
- Pawłowski B. 1956. *Flora Tatr. Rośliny naczyniowe*. 1. PWN, Warszawa: 204–205.
- Peyronel B. 1930. Simbiosi micorrizica tra piante alpine e Basidiomiceti. *Nuovo Giorn. Bot. Ital.* 37: 655–663.
- Peyronel B. 1937. Osservazioni e considerazioni sul fenomeno della micorrizza al Piccolo San Bernardo. *Nuovo Giorn. Bot. Ital.* 44: 587–594.
- Phillips J., Hayman D. S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* 55: 158–161.
- Read D. J., Haselwandter K. 1981. Observations on the mycorrhizal status of some alpine plant communities. *New Phytol.* 88: 341–352.
- Smith S. E., Read D. J. 1997. *Mycorrhizal symbiosis*. Academic Press.
- Stahl E. 1900. Der Sinn der Mycorrhizenbildung. Eine vergleichend biologische Studie. *Jahrb. Wissenschaft. Bot.* 34: 539–668.
- Trappe J. M. 1964. Mycorrhizal hosts and distribution of *Cenococcum graniforme*. *Lloydia* 27: 100–106.
- Treu R. 1990. Charakterisierung und Identifizierung von Ectomycorrhizen aus dem Nationalpark Berchtesgaden. *Bibl. Mycol.* 134: 80–84.
- Treu R., Laursen G. A., Stephenson S. L., Landolt J. C., Densmore R. 1996. Mycorrhizae from Denali National Park and Preserve, Alaska. *Mycorrhiza* 6: 21–29.
- Väre H., Vestberg M., Eurola S. 1992. Mycorrhiza and root associated fungi in Spitsbergen. *Mycorrhiza* 1: 93–104.

Obserwacje statusu mikoryzowego *Polygonum viviparum* w polskich Tatrach (Karpaty Zachodnie)

Streszczenie

Polygonum viviparum jest jednym z nielicznych gatunków roślin zielnych, które tworzą ektomikoryzę. W Tatrach rdest żyworodny należy do gatunków dominujących w piętrze alpejskim, występuje również niżej sięgając do podnóża masywu. Celem badań była wstępna analiza różnorodności ektomikoryz tworzonych przez ten gatunek w Tatrach oraz ogólna analiza jej zależności od warunków ekologicznych takich jak wysokość nad poziom morza oraz skład zbiorowisk roślinnych. Korzenie *P. viviparum* były również dodatkowo badane pod kątem obecności kolonizacji endomikoryzowej.

Próby korzeni zebrano z 5 stanowisk na podłożu granitowym i wapiennym, rozmieszczonych w przedziale wysokości 900–2150 m n.p.m. Ektomikoryzy były obecne na wszystkich badanych okazach *Polygonum viviparum*; na pojedynczych roślinach obserwowano 2–5 morfotypów. W sumie zaobserwowano i krótko scharakteryzowano 17 morfotypów ektomikoryz. Najbardziej rozpowszechnione we wszystkich próbach były mikoryza *Cenococcum geophilum* oraz niezidentyfikowany, jasno zabarwiony morfotyp przypominający mikoryzy *Russula* sp. Nie stwierdzono znaczących różnic w poziomie kolonizacji ektomikoryzowej pomiędzy stanowiskami różniącymi się położeniem nad poziomem morza. Zaobserwowane różnice w liczebności i różnorodności mikoryz *P. viviparum* na poszczególnych stanowiskach wiązać można najprawdopodobniej ze składem gatunkowym zbiorowisk roślinnych – obecnością krzewinek ektomikoryzowych spełniających zasadniczą rolę w utrzymywaniu populacji grzybów ektomikoryzowych. Należy jednak podkreślić, że ektomikoryzy obserwowano również na stanowiskach, gdzie *P. viviparum* było jedynym potencjalnym symbiontem ektomikoryzowym.

Regularnie obserwowano kolonizację korzeni *Polygonum* przez grzyby endofityczne. W kilku korzeniach odnotowano obecność struktur charakterystycznych dla mikoryzy arbuskularnej (pęcherzyki, peletony), jednak nie towarzyszyły im wykształcone arbuskule.