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Diversity of spruce ectomycorrhizal morphotypes in four mature forest stands in Poland

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Abstract: *Ectomycorrhizal communities structure of Norway spruce (Picea abies* L. (Karst.) was studied in four mature forest stands: Brenna, Salmopol, Zwierzyniec and Mirachowo. Morphological classification was used to distinguish the major mycorrhizal types associated with spruce in different forest types. Three of the forest stands were located within the natural geographical range of Norway spruce (Brenna, Salmopol, Zwierzyniec) and one (Mirachowo) was located in so-called "spruce-less zone". The sites differed in terms of environmental pollution. The mountain sites (Brenna, Salmopol) were characterized by relatively high levels of air pollution. The upland forest stand (Zwierzyniec), located in the southeastern part of Poland, was affected by a moderate pollution. The lowland stand in northern Poland (Mirachowo) was free from direct impact of anthropogenic pollution. The level of mycorrhizal colonization was 100% at all the study sites. Thirty-seven mycorrhizal morphotypes were distinguished in total. The number of ectomycorrhizal morphotypes varied between sites from 12 in Salmopol to 28 in Zwierzyniec. From one to three dominant morphotypes were found at the study site. Site-specific morhotypes were also observed. The frequency of mycorrhizal morphotypes differed between the forest stands.

Additional key words: air pollution, ectomycorrhizal diversity, *Picea abies,* soil

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Introduction

Norway spruce (*Picea abies* (L.) Karst.) is one of the main components of Polish forests. Under natural conditions spruce develops ectomycorrhizal symbiosis with a wide range of ectomycorrhizal fungi (Kårén and Nylund 1997; Erland et al. 1999). Morphological characteristics of ectomycorrhizas, such as colour and texture of the mantle surface, the abundance and structure of extramatrical mycelium, depend on the fungal species. Ectomycorrhizal morphotypes, which generally represent various fungal species, may differ in the physiological traits, such as carbon demand, efficiency in nutrients uptake, temperature and pH optimum, drought tolerance, response to different environmental pollutants etc. The development of the symbiosis (initiation, ageing and death of ectomycorrhizas) of forest trees in temperate regions is a dynamic process, dependent on the growth cycle of the phytobiont, weather conditions and the plant and the fungus species. Anthropogenic impacts have been observed to alter the abundance of ectomycorrhizal colonization and mycorrhizal community structure of spruce (e.g. Lehto 1994; Kårén and Nylund 1997; Qian et al. 1998; Cairney and Meharg 1999; Jonsson et al. 2000) and to decrease the number of ectomycorrhizal fungal species and the yield of fungal fruitbodies (e.g. Rühling et al. 1984; Wojewoda and

Ławrynowicz 1986; Jansen 1988; Lepšova and Mejstřik 1989). A link between mycorrhiza decrease and a decline of forest trees was observed by many authors (e.g. Kottke et al. 1993; Vogt et al. 1993; Markkola et al. 1995; Münzenberger et al. 1995).

Toxic gases such as NO_x, HF, NH_3, O_3 and dusts containing heavy metals are important pollutants that influence forest ecosystems in Poland. Although during the recent years the emission of SO_2 to the atmosphere in most of Poland has significantly decreased, the south-western part of Poland remains one of the most affected by anthropogenic pollution part of Europe and the area of injured forests is still increasing (Vančura et al. 2000).

Norway spruce is the second, after Scots pine, important forest tree in Europe, including Poland. On the other hand, a substantial decline in this tree species, due to air pollution, has been observed for more than 30 years in many forests in Europe (e.g. Ulrich et al. 1980; Rehfuess 1985; Cape et al. 1990) and in southern Poland (Godzik 1984; Godzik and Szdżuj 1994; Grodzińska and Szarek-Łukaszewska 1998; Staszewski et al. 1998).

Composition of mycorrhizal morphotypes of Scots pine in various polluted and unpolluted forest ecosystems in Poland was investigated recently (e.g. Kowalski 1987; Kowalski et al. 1989; Rudawska et al. 1995, 2003; Kieliszewska-Rokicka et al. 1997), however, the information available on the mycorrhizas of Norway spruce stands located in Poland date fifties and early sixties (Dominik and Niespiak 1953; Dominik et al. 1954; Dominik and Pachlewski 1956; Dominik 1961; Wojciechowska 1960). Therefore this study was undertaken, as a part of a larger investigation of the biodiversity and biomass of mycorrhiza at Norway spruce in diverse forest types.

The aims of the present study were: 1) to compare the variability of ecomycorrhizal morphotypes of Norway spruce at four mature forest stands in different regions of Poland (mountains, upland, lowland) and influenced by diverse levels of air pollutants, 2) to find the dominating and specific morphotypes of spruce mycorrhiza at each of the study sites. To show ectomycorrhizal diversity of Norway spruce from different sites the morphological classification was applied as a useful method in primary studies, prior to molecular analysis, the next step of the investigations (Sakakibara et al. 2002).

Material and methods

The study sites represented mature forests (Fig. 1). Two of the sites, Salmopol and Brenna, were located in the Beskid Śląski Mountains, Zwierzyniec – in the Roztocze National Park (upland) and Mirachowo – in the Kaszuby Protected Landscape Area (lowland). Only Mirachowo was located out of the natural geographical range of *Picea abies* (Boratyński 1998). The sites differed in climatic conditions, soil and forest type (Table 1) and in the levels of anthropogenic pollutants in the air. The study sites Brenna and Salmopol are located in the area which were influenced for many years by elevated levels of SO_2 and NO_x, and heavy metals, particularly in the heating period (autumn-spring) (Godzik 1984; Godzik and Sienkiewicz 1990). Annual mean concentration of SO_2 reported by Staszewski et al. (1998) (Brenna 30.1 μg m^{-3}, Salmopol – 34.5 μg m^{-3}) was higher than proposed by ICP Forests (International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests) as critical (20 μg m^{-3}). Although the level of industrial emissions in this region have been declining since 1989, and the monthly concentrations of SO_2 during vegetation seasons 1998 –1999 was lower than 10 μg m^{-3} (Bytnerowicz et al. 2002), a high level of defoliation of forest trees were observed (Wawrzoniak et al. 1996, Staszewski et al. 1998). The Zwierzyniec area was influenced by a moderate level of SO_2 and low level of NO_x and Mirachowo study site was located in a region free from a direct air pollution (Dmuchowski and Wawrzoniak 1994).

The size of the study plots was 50 by 50 m at each of the site. Mycorrhizas from the top 5 cm of the soil horizon were investigated. At least 45 cores (Ø=5 cm) were taken in July 2001. Mycorrhizal root tips were observed under a stereomicroscope (Zeiss) with a photo-camera. Only healthy mycorrhizas with a firm stele and an intact fungal mantle were chosen for morphotyping. Ectomycorrhizal morphotypes were determined according to the morphological traits (colour, shape, surface texture). In some cases

Fig. 1. Localization of the experimental plots in Poland

Table 1. The main characteristics of the four study sites

Site characteristics	Brenna	Salmopol	Mirachowo	Zwierzyniec
Latitude (N)	49°43'	49°41'	54°24'	50°36'
Longitude (E)	18°56'	18°58'	18°02'	22°58'
Altitude (m a.s.l.)	679	1000	150	240
Mean annual precipitation (mm)	1150	1200	600	650
Mean annual temperature (°C)	7.2	7.0	7.0	7.5
Soil type	brown podzolized soil	podzolic soil	brown sandy clay and peat transitional soil	marsh soil developed on river sands
Soil pH_{water}	3.6–3.9	3.5–3.9	3.8–4.2	3.7–4.1
Forest stand type	spruce forest: *Picea abies*, practically homogenous (planted)	spruce forest: *Picea abies* practically homogenous (planted)	coniferous forest: Picea abies–Pinus sylvestris (planted, naturally regenerated)	moist mixed forest: *Picea abies-Pinus sylvestris-Fagus sylvatica-Alnus glutinosa* (natural)
Age of spruce (yr)	115	75	66–100	70

cross-sections of mycorrhizas were examined under a light microscope. The morphotypes were compared to published descriptions (Agerer, 1987–2000). The quantity of mycorrhizal morphotypes was determined as 1) the average percentage of the morphotype in all samples examined at a study site, 2) the frequency of mycorrhizal morphotypes – as a ratio of the number of samples containing the morphotype to the total number of soil samples studied.

Results

The level of mycorrhizal colonization was 100%. Thirty seven mycorrhizal morphotypes were distinguished in total. The morphological features of all the morphotypes are described in Table 2. The most frequent mycorrhizal morphotypes are presented in the Fig. 2. Thirteen morphotypes were found in Brenna, 12 in Salmopol, 15 in Mirachowo, and 28 morphotypes Zwierzyniec. The frequency of the all morphotypes in the upper soil horizon (0–5 cm) at the forest plots studied are shown in the Table 3 and the average percentage of the morphotypes in the soil samples is shown in the Fig. 3. Five morphotypes (T1, T2, T3, T6, T23) were common for all the study sites (Table 3, Fig. 3).

The mycorrhizal communities at the mountain sites (Salmopol, Brenna) were dominated by three morphotypes (T1 – light brown mycorrhiza with thin, transparent mantle and colour-less extramatrical hyphae, T2 – light brown to grey brown smooth mycorrhiza, similar to *Lactarius* sp. and T3 – green-yellow mycorrhiza similar to *Russula ochroleuca* (Agerer 1987–2000) (Fig. 2). The three morphotypes comprised about 70% of the total number of mycorrhizas (Fig. 3). At the upland site Zwierzyniec and the lowland site Mirachowo a high frequency and abundance of the morphotype T6 (the black *Cenococcum geophilum*-like mycorrhizas) was found (22% and 29%, respectively), whereas at the both mountain sites this morphotype was present only in a low percentage (1.2% in Salmopol, 2.7% in Brenna) (Table 2, Fig. 3).

At each of the forest plot some site-specific mycorrhizal morphotypes were distinguished: one morphotype in Mirachowo (T38), two in Salmopol (T11, T12), three in Brenna (T17, T19, T20) and thirteen specific morphotypes in the Zwierzyniec forest (Table 2, Fig. 3).

Discussion

In this study the diversity of spruce ectomycorrhizas was determined by morphological classification. This method, based on macroscopic features of the fungal mantle and extramatrical mycelium, let us to analyse a large number of ectomycorrhizas, to distinguish variable mycorrhizal morphotypes, to evaluate their abundance and frequency in the forests studied. Although only a low number of species-specific morphotypes may be distinguished by morphotyping, this method is considered as a useful and necessary preliminary approach in studies of ectomycorrhizal communities, prior to DNA analyses (Hagerman et al. 1999; Sakakibara et al. 2002).

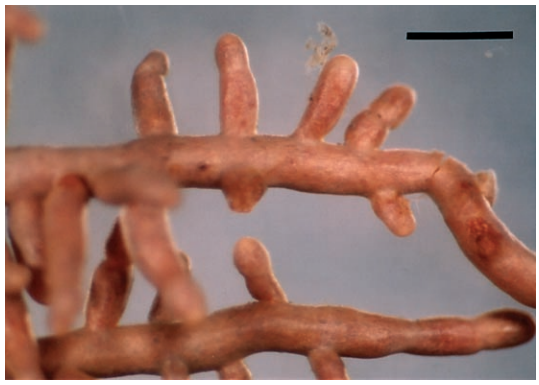
The number of ectomycorrhizal morphotypes distinguished in this study (from 12 in the mountain site in Salmopol up to 28 at the upland site in Zwierzyniec) is comparable to the range of fungal species (12–32) found by other authors in spruce forest in Europe using morphotyping (Trošt et al. 1999) and molecular methods (Dahlberg et al. 1997; Kårén and Nylund 1997; Jonsson et al. 2000; Fransson et al. 2000; Mahmood et al 2002). Dominik (1961) in recapitulation of his investigation of ectomycorrhizas of Norway spruce in forests located both, in lowland and in high mountains in Poland, distinguished in total, by morphotyping and anatomical studies, 39 mycorrhizal types. The author described for instance in Tatra Mountans 14 morphotypes, in Karkonosze Mountains – 8 morphotypes, 25 – in northern part of

Table 2. The morphological features of ectomycorrhizal (ECM) morphotypes of Norway spruce collected in the upper soil horizon (0–5 cm at the four study sites (Salmopol, Brenna, Zwierzyniec, Mirachowo)

ECM type	Description
T1	Light brown, monopodial pinnate and pyramidal, tips straight to bent; very thin, transparent mantle with colour-less extramatrical hyphae;
T2	light brown to grey brown, monopodial pinnate, tips bent; smooth, compact mantle, no extramatrical hyphae; mycorrhizas similar to *Lactarius* sp.*;
T3	Yellow or green-yellow, monopodial pinnate, tips straight to bent; thick mantle with yellow spots and few extramatrical hyphae; the mantle pseudoparenchymatous with angular cells – mycorrhizas similar to *Russula ochroleuca**;
T4	Silver-white, irregular pinnate, tips bent; thick, smooth and shiny mantle with white rhizomorphs; mycorrhizas similar to *Boletus* sp.*;
T5	Silver-gold, unbranched, tips straight to bent; thick mantle with few extramatrical hyphae, soil particles attached to the mantle surface;
T6	Coal black, mostly unbranched, tips straight; mantle surface grainy and shiny, black, spiny extramatrical hyphae; mycorrhizas similar to *Cenococcum geophilum**;
T7	Light brown, monopodial pinnate, tips bent; mantle thin, abundant white extramatrical mycelium; mycorrhizas similar to *Cortinarius* sp.*;
T8	Light brown, irregular pyramidal, tips straight to bent; mantle smooth and matt, soil particles attached to the mantle surface;
T9	Light brown to pink, monopodial pinnate, tips straight to bent; mantle thin, smooth and matt with yellow grains and few emanating hyphae;
T10	Dark brown, unbranched, tips straight; abundant yellow, woolly, extramatrical hyphae;
T11	Dark brown with yellow, bent tips, unbranched; mantle thin, matt with few emanating hyphae;
T12	Brown, monopodial pyramidal, tips bent; abundant white extramatrical hyphae, rhizomorphs;
T13	Grey-green with light brown tips, monopodial pyramidal; mantle smooth, metallic shiny, with white extramatrical hyphae;
T14	Olive to dark brown with light tips, branching irregular, tips bent; yellow-green extramatrical hyphae and rhizomorphs;
T15	dark brown, monopodial pyramidal, tips straight to bent; mantle surface grainy with brown, woolly extramatrical hyphae;
T16	Yellow to light brown, monopodial pyramidal, tips bent; mantle surface matt with few extramatrical hyphae;
T17	Brown, unbranched, tips straight to bent; white, cotton extramatrical mycelium, white rhizomorphs;
T18	Olive –green, irregular pyramidal, tips bent; mantle thick, smooth, shiny with grey extramatrical mycelium and orange grains;
T19	Brown, monopodial pinnate, tips bent; mantle thin; partly white deposit on the surface of the mantle, abundant, cottony, white to beige extramatrical mycelium;
T20	Brown, monopodial pinnate, tips straight to bent; thin matt mantle, partly white-silver deposit at the surface of the mantle;
T21	Light brown, irregularly pinnate, tips bent; mantle reticulate with white rhizomorphs;
T22	Green-olive, unramified, tips straight and thick; mantle with white to light yellow rhizomorphs;
T23	Bright yellow and white, irregularly pinnate, tips straight to bent; mantle cottony with emanating extramatrical mycelium, abundant bright yellow rhizomorphs; mycorrhizas similar to *Piloderma croceum**;
T24	Brown, irregularly pinnate and unramified, tips bent; mantle reticulate with emanating, white, extramatrical hyphae, partly white deposit at the surface of the mantle;
T25	Brown, unramified, tips straight and short; smooth, thin and transparent mantle;
T26	Dark brown, unramified, monopodial pinnate; thick, matt mantle with light brown emanating hyphae;
T27	Brown, monopodial pinnate, tips straight to bent, thin, smooth, transparent mantle, partly white deposit at the surface of the mantle;
T38	Grey-metallic, monopodial pinnate; tips thin, straight to bent; smooth mantle;
T29	Brown-orange, unramified; tips straight, short; smooth, matt mantle;
T30	Yellow-orange, unramified, tips straight;
T31	Light brown, monopodial pinnate and monopodial pyramidal; smooth mantle with abundant white, cottony extramatrical hyphae;
T32	Light brown to beige, unramified, tips straight; matt, reticulate mantle with white extramatrical hyphae and rhizomorphs;
T33	Light brown to beige, irregularly pinnate, thick mycorrhizas; reticulate surface of the mantle with white deposit, smooth, rhizomorphs;
T334	Beige, monopodial pinnate, tips straight to bent; mantle reticulate;
T335	Brown to dark brown, monopodial pinnate, tips straight to bent; matt, smooth mantle with extramatrical hyphae, soil particles attached to the mantle surface;
T336	Light brown, irregularly pinnate, pyramidal, tips straight to bent; smooth, thick mantle with white deposit, mantle with extramatrical hyphae and thick, beige rhizomorphs, soil particles attached to the surface of the mantle;
T37	Light brown to yellow, unramified and monopodial pinnate, tips straight and bent, smooth and matt mantle;

*according to Agerer (1987–2000)

Morphotype T1

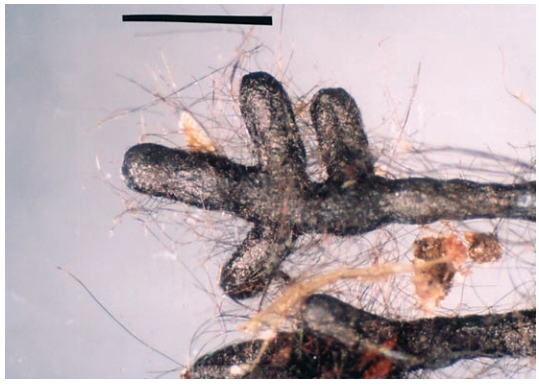


Morphotype T2 (similar to *Lactarius* sp.)

Morphotype T3 (similar to *Russula ochroleuca*)

Morphotype T4 (similar to *Boletus* sp.)

Morphotype T5

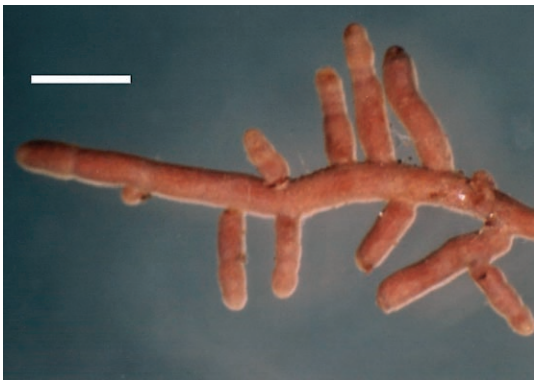


Morphotype T6 (similar to *Cenococcum geophilum*)

Morphotype T7 (similar to *Cortinarius* sp.)

Morphotype T8

Fig. 2. Dominating ectomycorrhizal morphotypes of *Picea abies* isolated from the upper soil layer (0–5 cm) in four mature forest sites (Brenna, Salmopol, Mirachowo, Zwierzyniec); (scale=1 mm)



Morphotype T9

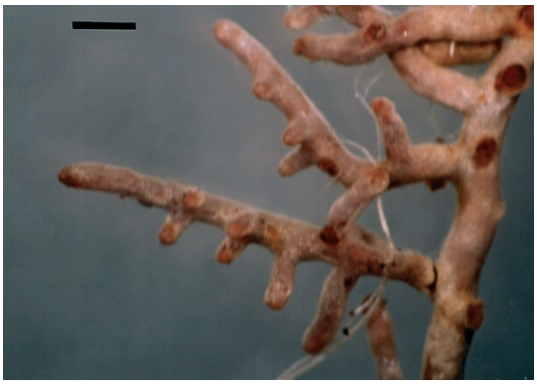
Morphotype T10

Morphotype T11

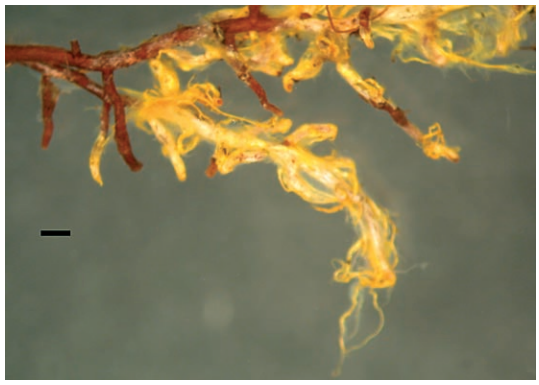
Morphotype T13

Morphotype T16

Morphotype T20



Morphotype T23



Morphotype T25 (similar to *Piloderma fallax*)

Fig. 2. Dominating ectomycorrhizal morphotypes of *Picea abies* isolated from the upper soil layer (0–5 cm) in four mature forest sites (Brenna, Salmopol, Mirachowo, Zwierzyniec); (scale=1 mm)

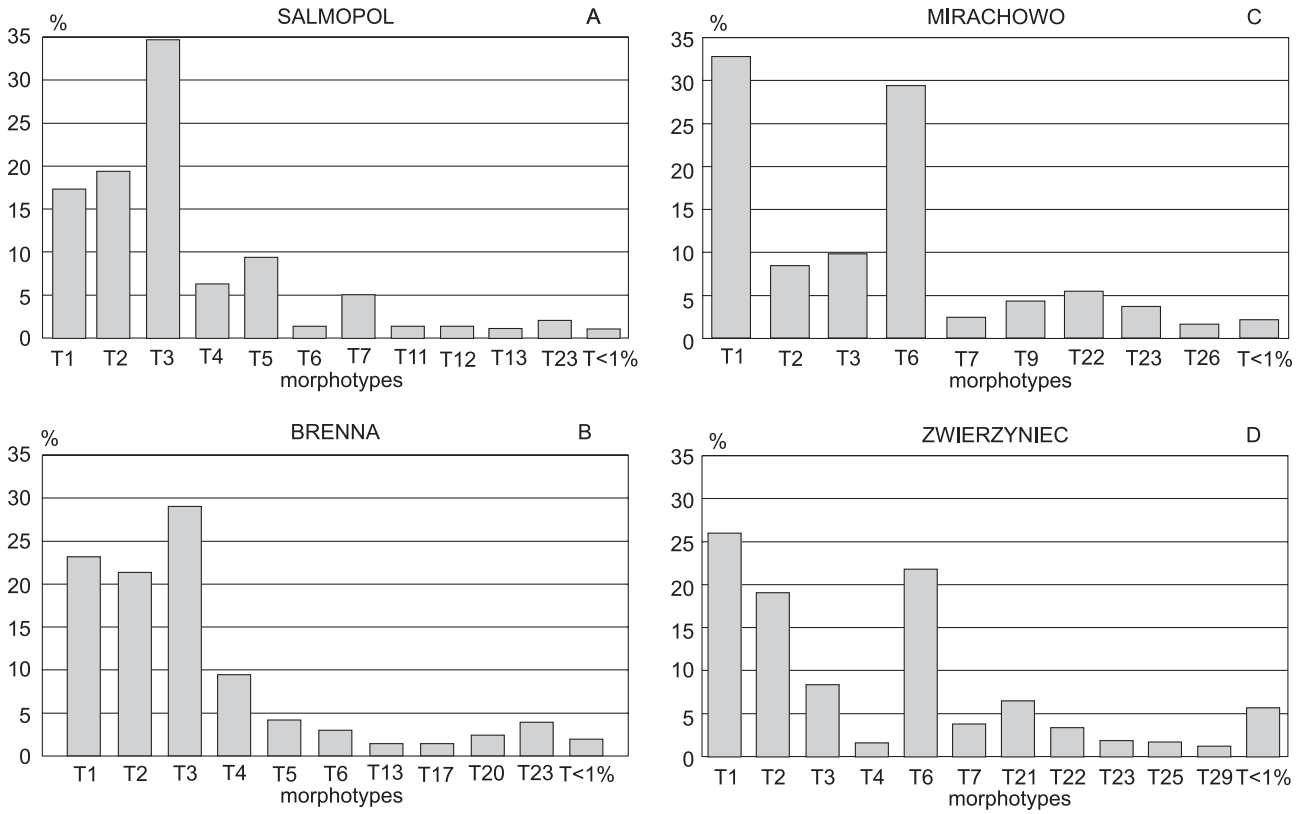


Fig. 3. Relative abundance (mean percent) of ectomycorrhizal morphotypes of *Picea abies* isolated from the upper soil layer (0–5 cm) in four mature forest sites (Brenna, Salmopol, Mirachowo, Zwierzyniec)

Poland and 16 – in the dysjunction area (Boratyński 1998).

The numbers of morphotypes described in this study at Brenna and Salmopol were relatively low (13 and 12, respectively) (Table 2, Fig. 3). The reduced mycorrhizal variability could be affected by the character of the forest stands, practically homogenous Norway spruce, with only few young specimens of other trees species (*Abies alba, Fagus silvatica*), by severe climatic conditions, the drought recorded in Poland in the study year and the year before and also by air pollution. Similarly, Dominik et al. (1954) and Dominik and Pachlewski (1956) observed lower variability of ectomycorrhizas of Norway spruce in Tatry Mountains in the upper montana zone, than in the lower montana zone.

The forests in the Beskid Mountains were classified as strongly damaged (Grodzińska and Szarek-Łukaszewska 1997). One of the aboveground symptoms of forest injury was a high defoliation in spruce stands in Salmopol (40%) and Brenna (30%) (Staszewski et al. 1998). Some authors reported a strong reduction in ectomycorrhizal morphotypes at heavily polluted coniferous and broad-leaved forest stands (e.g. Kieliszewska-Rokicka et al. 1997; Vinceti et al. 1998; Baxter et al. 1999; Wöllecke et al. 1999; Erland et al. 1999). Moderate levels of pollution may reduce the biomass of fine roots and change the ectomycorrhizal community structure, but not decrease the number of mycorrhizal species in coniferous forests (e.g. Rudawska et al. 1995, 2003; Kårén and Nylund 1997). A shift in frequency and abundance of some fungal species was observed at study plots heavily polluted by nitrogen (e.g. Kårén and Nylund 1997; Fransson et al. 2000).

In the present study a relatively high mycorrhizal diversity (28 morphotypes) was found at the upland stand Zwierzyniec, a mixed forest, where the dominating species *Picea abies* was accompanied by *Pinus sylvestris, Fagus sylvatica* and *Alnus glutinosa,* contrary to the mountain plots – practically homogenous Norway spruce stands. (Table 2, Fig. 3). Moreover, soil substrate at the site in Zwierzyniec represented more fertile and more moist type than the soils at the mountain sites. The moisture availability in soil is known to control biomass of soil microorganisms, including mycorrhizal fungi (e.g. Insam et al. 1989). Both, the diversity of the plant species and the soil conditions could influence the richness mycorrhizal symbionts (Johnson et al. 1993; Smith and Read 1997).

Spruce at all the study sites were dominated by 1–3 ectomycorrhizal morphotypes (Fig. 3), in accordance with a general ecological feature of domination of species in communities and biocoenoses (e.g. Krebs 1996). At the mountain sites, Brenna and Salmopol, mycorrhizal communities were dominated by the morphotype T3 (the frequency: 35 and 28%, respectively), however the morphotypes T1 and T3 also

were present in a high percentage at the both sites. The morphotype T3, very likely *Russula ochroleuca* mycorrhiza (Agerer 1987–2000) was present also at the sites in Mirachowo and Zwierzyniec, however in lower percentage (9 and 8%, respectively). This ectomycorhizal morphotype was found in a very low frequency (<3%) by Kårén and Nylund (1997) in a Norway spruce stand in southwestern Sweden. Clearly expressed domination of two ectomycorrhizal morphotypes (T1 and T6) was found in Mirachowo. At the Zwierzyniec study site three frequent morphotypes were observed (T1, T2, T6) (Fig. 3). The morphotype T6 (the black *Cenococcum*-like mycorrhiza) was present also at the mountain stands, however in rather low amounts. This morphotype was frequently found connected with *Picea* sp., both mature trees and young seedlings (e.g. Hagerman et al. 1999; Mah et al. 2001). Classification of the black *Cenococcum*-like morphotype based only on morphological features may be sometimes incorrect and detailed anatomical and/or molecular studies should be performed. Kårén and Nylund (1997), using DNA analysis identified in the black morphotypes two separate mycorrhizas: *Cenococcum geophilum* and Piceirhiza bicolorata.

Table 3. Frequency of ectomycorrhizal morphotypes at the four mature forest stands (Salmopol, Brenna, Mirachowo, Zwierzyniec) in 2001

ECM type	Salmopol	Brenna	Mirachowo	Zwierzyniec
T1	1.00	0.94	1.00	0.71
T2	0.75	0.67	0.37	0.60
T3	1.00	0.8	0.53	0.43
T4	0.83	0.67	–	0.20
T5	0.67	0.33	0.05	–
T6	0.50	0.40	0.89	0.74
T7	–	–	0.16	0.28
T8	0.17	0.07	–	–
T9	0.08	–	0.26	0.03
T10	0.25	–	–	–
T11	0.25	–	–	–
T12	0.17	0.13	–	–
T13	–	0.07	–	0.11
T14	–	–	–	0.06
T15	–	–	–	0.03
T16	–	0.07	–	–
T17	–	0.13	–	–
T18	–	0.27	–	–
T19	–	–	–	0.23
T20	–	–	0.53	0.31
T21	0.17	0.27	0.32	0.20
T22	–	–	–	0.03
T23	–	–	–	0.03
T24	–	–	0.05	0.03
T25	–	–	–	0.03
T26	–	–	–	0.03
T27	–	–	–	0.03
T38	–	–	0.10	0.03
T39	–	–	0.05	0.08
T30	–	–	0.05	0.03
T31	–	–	0.05	0.08
T32	–	–	–	0.03
T33	–	–	–	0.03
T34	–	–	–	0.06
T35	–	–	–	0.06
T36	–	–	0.05	–
T37	–	–	–	0.03

The diversity of mycorrhizal morphotypes distinguished in this study could be underestimated or overestimated. Comparison studies conducted by other researchers who used both, morphotyping and DNA analyses indicated that some morphotypes may represent more than one RFLP pattern and that the same RFLP pattern could be found in more than one mycorrhizal morphotype. For example, based on the morphological features, Erland et al. (1999) have distinguished only 4 to 6 ectomycorrhizal morphotypes in two 60-year-old Norway spruce forests in Sweden. Further RLFP analysis of ribosomal DNA showed, however, that the morphotypes included, respectively, 7 and 13 ectomycorrhizal species., at least 5 fungal taxa were found among "brown smooth" mycorrhizas of spruce. Sakakibara et al. (2002) identified six different species of *Lactarius* in an ectomycorrhizal morphotype called "*Lactarius*". Kårén and Nylund (1997) have distinguished 16 mycorrhizal morphotypes of Norway spruce, using the morphological classification, and 21 different RLFP patterns, using the fungal ribosomal DNA analysis. The most abundant morphotype decribed by these authors was the morphotype A – "brown, thin, transparent mantle, usually with extramatrical hyphae (EH), but in some cases only with few EH" which comprised mycorrhizal fungi of 13 different RFLP patterns. On the other hand, the overestimation of mycorrhizal species by morphotyping is also possible (M. Rudawska and T. Leski, personal communication).

The results of our study give basic comparative information on the numbers, frequency and abundance of mycorrhizal morphotypes of Norway spruce in different mature forests in Poland. Verification of the morphological classification by molecular techniques is the subject of our further investigations.

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