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Chapter 18

Mycorrhizal community structure of Scots pine trees influenced by emissions from aluminum smelter

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Abstract

Ectomycorrhizas of Scots pine were studied in three forest stands in the vicinity of an aluminum smelter: one young stand (0.4 km from the smelter) and two mature stands (2.5–3.0 km from the smelter). The aim of the study was to evaluate a possible influence of pollutants emitted from the smelter on the abundance and diversity of mycorrhizas in the upper soil layer (0–5 cm). Seventeen mycorrhizal morphotypes were distinguished in the root samples from the three study sites (10–13 at one site). The same dominant morphotype was observed at each of the sites. Biomass of fine roots and total number of mycorrhizas were significantly higher at the young forest stand than at the two mature stands, however the fungal biomass in the fine roots, measured as ergosterol content, was lowest at the young stand. The results suggest a negative, indirect effect of pollution on fine root production, and thus fewer short roots available for mycorrhizal colonization in mature forest stands, rather than a direct effect through the soil.

1. Introduction

Aluminum smelters emit considerable amounts of air pollutants that are known to influence the growth of woody plants. The main chemicals that contaminate both the air and the soil in the vicinity of aluminum smelters are sulfur dioxide (SO_2), fluorides, and oxides of nitrogen (NO_x). Although injury from SO_2 and NO_x is more common, fluorides are the most phytotoxic air pollutants and may injure plants at much lower concentrations (10–1000 times lower) than the other pollutants (Weinstein and Alscher-Herman, 1982). Gaseous and particulate fluorides can be taken up and accumulated by leaves

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of coniferous and broad-leaved species and may reduce total photosynthesis, mainly because they may precipitate loss of leaves (Keller, 1977). Scots pine is considered one of the most sensitive tree species to these air pollutants.

Completed in 1966, the Konin aluminum smelter is located in a forested area in the middle of the Polish lowlands. Since 1966, it has emitted toxic pollutants into the atmosphere. In 1969, emission of fluorine exceeded 600 tons per year, but since then, it has slowly decreased due to technological modernization. Currently, emissions are 37 tons per year. Annual sulfur emissions decreased from more than 200 tons per year in the 1980s to 60 tons in 1999. In the late 1970s, a protective area with four sub-zones indicating hazardous concentrations of fluorine was established in the vicinity of the smelter. Mature and young forests in the protective zone were composed mostly of Scots pine (*Pinus sylvestris* L.).

Ectomycorrhizal symbiosis is an integral part of the root system of Scots pine and is widely regarded as a key component for water and nutrient uptake and healthy growth. Because ectomycorrhizas are sensitive to changes in the environment (Smith and Read, 1997; Cairney and Meharg, 1999), we investigated the mycorrhizal coenosis (i.e., composition and abundance of morphotypes) of Scots pine trees growing near the aluminum smelter. Roots were sampled from 50-year-old trees that had been exposed to emissions from the smelter for 30 years and from 20-year-old trees that were planted after emissions were reduced by technological modernization. The concentration of ergosterol, the main fungal sterol and an important component of membranes of the mycelia of mycorrhizal fungi (Weete, 1989), was analyzed in fine root samples. Ergosterol is considered to be a sensitive, rapid, and convenient measure of metabolically active fungal biomass (Nylund and Wallander, 1992).

2. Materials and methods

2.1. Study sites

Three sites represent Scots pine forests located in the vicinity of the Konin aluminum smelter (Fig. 1). Site 1 (Sulanki) was situated 0.4 km from the smelter and represents 20-year-old Scots pine trees planted after emission controls had been incorporated. Sites 2 and 3 (Anielew and Rudzica, respectively) are composed of mature Scots pine trees (50 years old) located 2.5–3.0 km from the smelter. These sites were exposed to emissions for more than 30 years. The levels of emission of fluorine and sulfur from 1969 to 1999 are shown in Fig. 2. Currently, the annual input of fluorine in throughfall is 5 kg ha^{-1} in Sulanki and about 2 kg ha^{-1} in Anielew and Rudzica. The mean concentration of fluorine in 1993, based on 24-hour measurement, was about 1.4 µg m^{-3} at a distance of

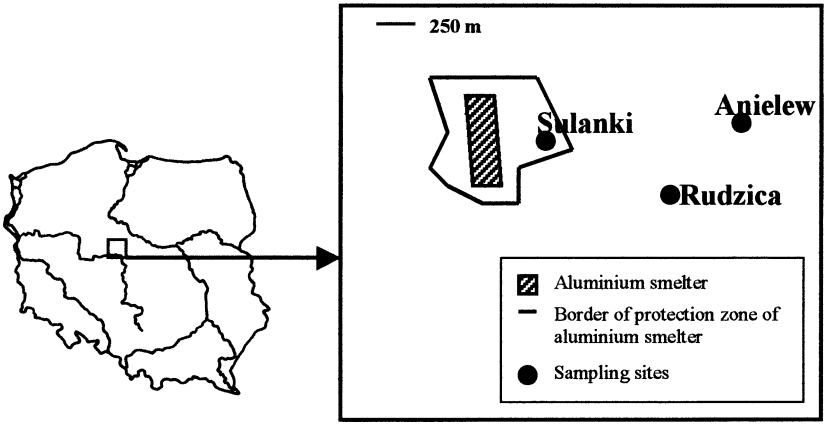

Figure 1. Monitoring sites.

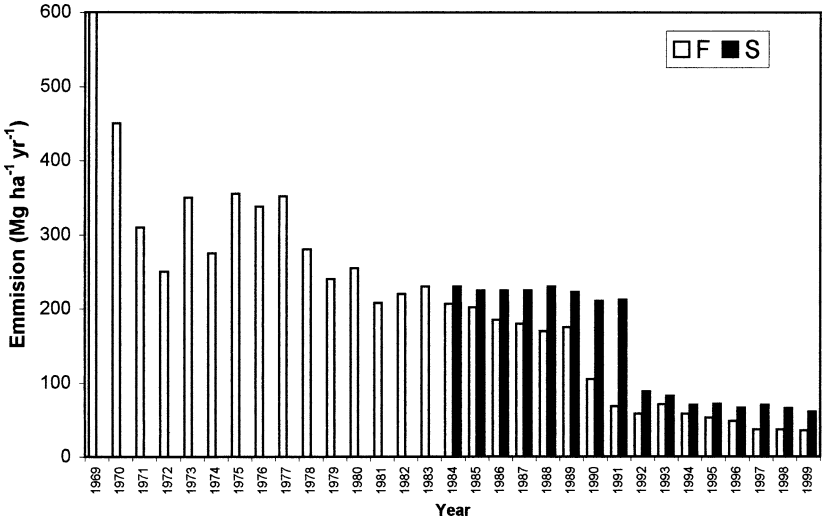

Figure 2. Emission of fluorine and SO_2 from the Konin aluminum smelter.

0.5 km from the smelter and below 1 $\mu g\,m^{-3}$ at 2.5–3.0 km from the emission source. The annual input of sulfur was similar at the three study sites, ranging from 22 to 27 $kg\,ha^{-1}$. Dust pollutants were also deposited in the area. In 1994, the annual dust deposit in Sulanki was 128 $tons\,km^{-2}$, in Rudzica 412 $tons\,km^{-2}$, and in Anielew 59 $tons\,km^{-2}$. Total concentrations of selected trace metals in soil in 1994 are shown in Table 1. The soils are dystric cam-

Table 1. Total concentration of selected trace metals in soil (mg kg^{-1}) in the vicinity of the study sites in 1994 (Pogodski, 1995)

Element	Study site		
	Sulanki	Rudzica	Anielew
Pb	8.9	13.7	53.5
Cd	14.6	2.2	5.3
Zn	3568	3712	352
Mn	194	798	6612
Ni	6.5	30.2	17.7
Cu	14.1	25.1	15.1

bisols at Sulanki, eutric cambisols at Anielew, and haplic podzols at Rudzica (FAO, 1988). Mean annual temperature in this region for the last decade was 9.1 °C and annual rainfall was 472 mm (Pogodski, 1995). Further characteristics of the investigation sites are summarized in Table 2 and are presented elsewhere (Staszewski et al., 1998).

2.2. Root samples

The soil core samples were collected in May and August 1999, using a soil core sampler (diameter = 2 cm), from the organic layer (F/H horizon) to a depth of 5 cm after removal of the litter layer. Eighteen cores, randomly selected, were taken at each study site twice a year (May and August). All cores were taken 0.7–1 m from tree base. Intact soil cores were sealed in plastic bags and kept frozen for ergosterol analysis or stored for up to 3 months at 4 °C until used for morphotyping. Fine roots (< 2 mm) were separated from soil and organic matter using a sieve placed under a stream of cold water, and were then excised from the main root. Final separation and counting of roots were conducted under a stereomicroscope. Live and dead roots were determined by color and firmness. Dead roots were dark brown or black in color and shriveled. Ectomycorrhizal morphotypes were determined according to morphological traits (color, shape, and surface texture) and expressed as the total number of mycorrhizal root tips per 100 cm^3. Identifications were based on published descriptions (Ingleby et al., 1990; Agerer, 1987–1995; Danielson and Visser, 1989; Bradbury, 1998). Each Scots pine root tip colonized by an ectomycorrhizal fungus was counted. Dichotomous and coralloid ectomycorrhizas were counted as one morphotype.

2.3. Ergosterol analyses

Mycorrhizal roots for ergosterol analyses were separated from soil and organic matter within 2 days following sampling. They were excised from the

main root and cleaned with running water to remove adhering organic matter. Healthy-looking ectomycorrhizas (approximately 50–70 mg fresh weight) were chosen for subsequent ergosterol extraction, frozen in liquid nitrogen and kept at $-80\,^{\circ}$C until used. Freeze-dried root samples were ground with mortar and pestle. Ergosterol was extracted with 80% ethanol according to the procedure of Beguiristain and Lapeyrie (1997), separated by reverse phase HPLC on a Waters system, and detected using a UV detector at 280 nm, Waters Nova-Pak C18 column (150×4 mm) and 100% methanol (Baker) as a solvent, according to Nylund and Wallander (1992). Commercial ergosterol (5,7,22-Ergostarien-3ß-ol, Sigma) was used as a standard.

2.4. Chemical analyses

The total concentration of metals in the soil profile was extracted from replicate samples and quantified by atomic absorption spectrophotometry (Varian Spectra AA 300). Ions were determined by ion chromatograph Dionex X-100 with column AS4A. Organic C and total N were determined according to standard research methods (Ostrowska et al., 1991). Total fluorine concentration in soil, after combustion, was determined by the fluorine ion selective electrode (Reusmann and Westphalen, 1969). Soluble fluorine content in soil was determined according to Kaniewski and Kaniewski (1985).

2.5. Soil acidity

The pH of the soil layer from which roots were removed was measured using a glass electrode in soil-water and soil-salt (0.01 M KCl) suspensions (1 : 2 soil : solvent) immediately following sampling.

2.6. Statistical analysis

One-way ANOVA followed by the Tukey's *t*-test (StatSoft Inc. 1997, Statistica for Windows, 5.1, Tulsa, OK) was used to assess the effect of site and time of sampling on the different variables.

3. Results

Soil properties of all three stands are summarized in Table 2. The pH_{salt} of the soil at Sulanki was neutral (6.08) and medium acidic at Rudzica and Anielew (4.37 and 4.9, respectively). Concentrations of Ca, Mg, K, and Na in the soil and the value of cation exchange capacity (CEC) were relatively high at all three sites. The soils had medium concentrations of nitrogen, ranging from

Table 2. Parameters of soil (0–5 cm depth) at the three study sites

Soil parameters	Study site		
	Sulanki	Rudzica	Anielew
pH_{water}	6.66	6.01	6.06
pH_{salt}	6.08	4.37	4.9
Ca ($cmol_c\,kg^{-1}$)	5.36	6.8	2.67
Mg ($cmol_c\,kg^{-1}$)	0.75	1.19	0.52
K ($cmol_c\,kg^{-1}$)	0.94	3.22	1.15
Na ($cmol_c\,kg^{-1}$)	0.12	0.68	0.49
CEC = $cmol_c\,kg^{-1}$) (Ca + Mg + K + Na)	7.2	11.9	4.8
N_{total} (%)	0.091	0.171	0.14
$C_{organic}$ (%)	0.91	3.16	0.89
C/N	10	18.5	6.35
F_{total} ($mg\,kg^{-1}$)	48.5	51.8	26.5
$F_{soluble}$ ($mg\,kg^{-1}$)	11.2	< 1	3.8

0.09% in Sulanki to 0.17% in Rudzica. The soil C : N ratio ranged from 6.3 in Anielew to 18.5 in Rudzica. Total concentrations of fluorine in soil were similar in Sulanki and Rudzica and lower in Anielew; however, the highest concentration of soluble fluorine was found in the stand situated closest to the smelter (Table 2).

An Anova with site as a single factor yields a significant effect on fine root biomass of tested trees ($F = 5.38,\ P = 0.010045$). Screening of the roots in May and August 1998 showed no difference in the fine root biomass between the time of sampling ($F = 0.32,\ P > 0.5$). Tukey's t-test revealed a significant difference between the young and the mature stands ($P < 0.05$) but there was no significant difference between the two mature stands ($P = 0.95$) (Fig. 3(A)). However, in both mature stands (Rudzica and Anielew), there was a tendency for a lower fine root biomass to occur in August than in May. Almost all Scots pine fine root tips were mycorrhizal at all three sites, but the total number of mycorrhizas per 100 cm^3 was significantly lower (Tukey's t-test, $P < 0.05$) in the two mature stands (Rudzica and Anielew) than in the young stand (Sulanki) (Fig. 3(B)). The fewest number of mycorrhizal tips was recorded for the mature Scots pine at Rudzica. The relationship between live and dead mycorrhizas was statistically not significant irrespective of site ($F = 0.23,\ P > 0.7$) and time of sampling ($F = 0.55,\ P > 0.4$); however, slightly more dead mycorrhizas were observed in May than in August (Fig. 3(C)).

Approximately 6 400 ectomycorrhizal tips were harvested and examined in the course of this study. Based on the gross morphology, 17 mycorrhizal morphotypes were distinguished (Table 3). Patterns of abundance of mycorrhizal morphotypes associated with young and mature pine demonstrated that each

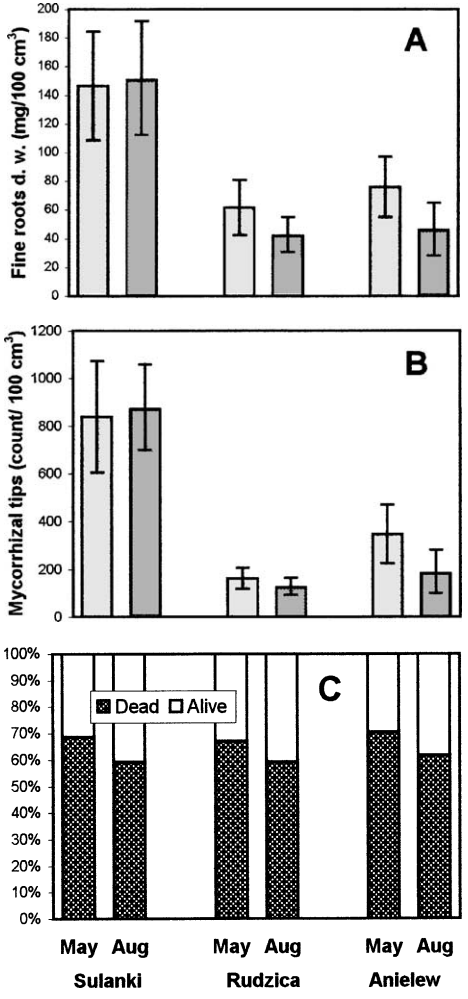

Figure 3. (A) Fine root biomass taken from 0–5 cm depth; (B) Total number of mycorrhizal tips in soil samples (per 100 cm^3); (C) Frequency of dead and alive mycorrhizas at three Scots pine sites (one young stand in Sulanki and two mature stands in Anielew and Rudzica) in the vicinity of the Konin aluminum smelter. Bars indicate standard deviation ($n = 18$).

site was characterized by one or two dominant types and a greater number of rare morphotypes (Fig. 4). The richness of mycorrhizal morphotypes was quite similar among the three pine stands. The young, 20-year-old Scots pine at Sulanki averaged 13 morphotypes and the 50-year-old trees at Rudzica and Anielew were characterized by 12 and 10 morphotypes, respectively. Three of the 17 ectomycorrhizal morphotypes (Nos. 4, 5, 7) occurred at only one study

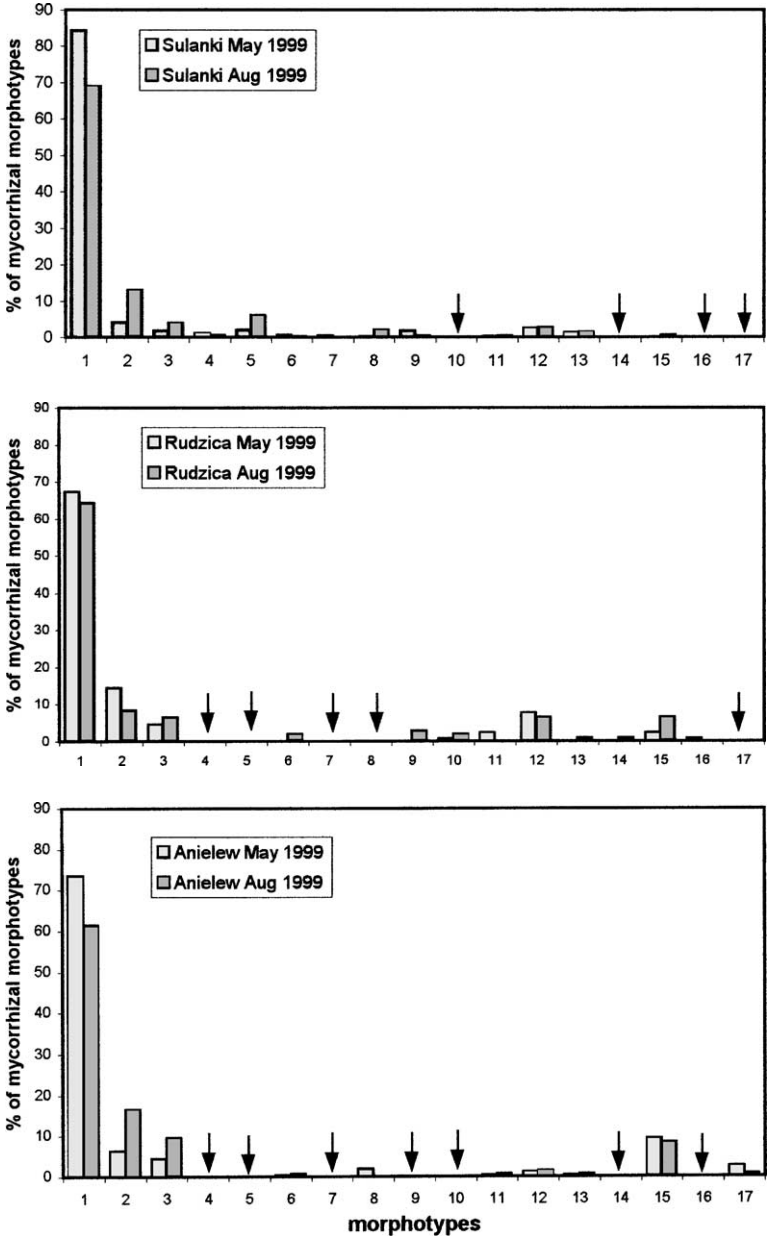

Figure 4. Patterns of abundance of the ectomycorrhizal morphotypes at three Scots pine sites, demonstrating that one mycorrhizal morphotype dominated on each site (↓ morphotype lacking at given site).

Table 3. Classsification of ectomycorrhizal (ECM) morphotypes found on the roots of Scots pine trees in the vicinity of the Konin aluminum smelter

ECM morphotype	Description
Type 1	Dark brick to brown, with a pale tip, fairly straight and infrequently dichotomously branched, discontinuous mantle reticulate and shiny, ectendomycorrhizal character
Type 2	Orange to light brown, mostly unbranched, extended, often segmented with thick, smooth, and shiny mantle
Type 3	Light brown, fairly long, simple to branched with dense, abundant brown hyphae surrounding the mantle
Type 4	Brown, fairly long, simple to branched with loose gray hyphae surrounding the mantle
Type 5	Light brown, simple and dichotomously branched, tips smooth and shiny, lower part covered by white-silver hyphae, compact strands emerging from the mantle
Type 6	Light brown, thick, mostly unbranched and or dichotomizing at the tip, smooth and matte
Type 7	Black to dark maroon, unbranched or dichotomously branched, smooth and shiny
Type 8	Light brown, unbranched or infrequently dichotomously branched, dense silver-white hyphae surrounding the mantle, *Hebeloma*-like
Type 9	Beige, dichotomous to coralloid with a thick mantle overgrowing branching short roots, abundant rhizomorphs with beige and pinkish hues
Type 10	White, dichotomously branched with thick, smooth greyish-white mycelium, thin white strands
Type 11	Whitish to buff, dichotomous, thick, short and stubby, smooth mantle
Type 12	Beige to brown, coralloid, smooth mantle
Type 13	Beige to brown, coralloid with abundant light brown hyphae surrounding the mantle
Type 14	Beige to brown, coralloid with abundant greyish brown hyphae surrounding the mantle
Type 15	Black, mantle angular stellate arrangement, with smooth, stiff hyphae radiating from mantle, *Cenococum*-like
Type 16	Brown, coralloid to subtuberculate and clusters, loose brown strands, *Suillus*-like
Type 17	Pallid to black, subfloccose, simple and very infrequently branched, abundant black hyphae loosely surround the base of the mycorrhiza, *Mycelium radicis atrovirens*-like

site (Site 1 in Sulanki, the young stand of Scots pine situated closest to the aluminum smelter). Several morphotypes were found on only a very few ectomycorrhizal root tips, sometimes only in one survey, May or August. At all three sites, the dominant ectomycorrhizal morphotype was Type 1, accounting for 85% of the morphotypes at Sulanki (young stand) and about 70% at Rudzica and Anielew. This dominant morphotype was brown, smooth, and rarely branched, showing evidence of ectendomycorrhizal penetration. Other mor-

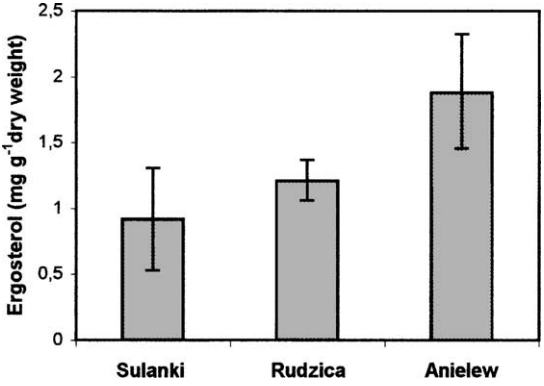

Figure 5. Average ergosterol concentration in mycorrhizas of Scots pine, collected in August 1999 at one young (Sulanki) and two mature (Anielew, Rudzica) forest sites in the vicinity of the Konin aluminum smelter. Bars indicate standard deviation ($n = 18$).

photypes accounted for only a small percentage of the mycorrhizas colonizing the roots of Scots pine.

Ergosterol, as a marker of living fungal mycelium, was analyzed in mycorrhizas collected in August. Ergosterol concentration ranged between 0.9 and 1.85 mg g^{-1} dry weight and was significantly influenced by site ($F = 21.39$, $P = 1 \times 10^{-6}$). Mycorrhizas from the mature forest site at Anielew contained significantly more ergosterol than mycorrhizal samples from Sulanki or Rudzica (Tukey's t-test, $P < 0.005$) (Fig. 5).

4. Discussion

Soil acidity (medium acidic at Rudzica and Anielew and neutral at Sulanki) was satisfactory for Scots pine growth (Puchalski and Prusinkiewicz, 1975) and mycorrhizal development (Smith and Read, 1997). All study sites were characterized by a high saturation of the soil sorption complex by base cations (Ca, Mg, K, and Na) and by rather high effective cation exchange capacity. However, soils in the vicinity of the Konin aluminum smelter were polluted with phytotoxic trace metals (Cd, Zn) that exceeded "normal" levels (Cd: 0.01–7 mg kg^{-1}; Zn: 10–300 mg kg^{-1} (Kabata-Pendias and Pendias, 1993)). Availability of the metals was probably low because of the rather high soil pH (Tables 1, 2). In the present study, the C : N ratio indicated a high nitrogen soil content, especially in Sulanki and Anielew (Uggla and Uggla, 1979).

All three Scots pine stands had similar numbers of ectomycorrhizal morphotypes, which may be considered an index of richness of fungal species. The sites were characterized by one very common morphotype and greater numbers

of several rare morphotypes. This pattern of mycorrhizal diversity has been observed in other studies using sporocarp counting (Ohtonen and Markkola, 1989; Bradbury et al., 1998; Jonsson et al., 1999) and direct mycorrhizal morphotyping using morphological and molecular techniques (Visser, 1995; Gardens and Bruns, 1996; Kåren and Nylund, 1997; Gehring et al., 1998; Jonsson et al., 1999). The dominant morphotype at all three sites was brown, smooth, thin-mantle ectendomycorrhiza (Type 1), which occupied 60–85% of the root tips, depending on the site and time of the sampling (Fig. 4). Ectendomycorrhizas are noticed by other authors as typical for highly fertilized nurseries and disturbed sites (Mikola, 1965, 1988; Danielson, 1991). A similar morphotype was also found in several studies at highly contaminated sites in Poland (Kowalski, 1987; Kowalski et al., 1989; Kieliszewska-Rokicka et al., 1997). Ectendomycorrhizas can develop under conditions of very low irradiance and presumably low photosynthate production (Mikola, 1965), which may suggest higher competitiveness of this kind of symbiosis over other ectomycorrhizal fungi (Yu et al., 2001).

Ectomycorrhizal richness observed in this study was slightly lower than that found by other authors (Gehring et al., 1998; Jonsson et al., 1999; Saikkonen et al., 1999). Some authors determined that ectomycorrhizal morphotypes were not highly correlated with attributes of ecosystem productivity (i.e., nutrients and moisture) (Gehring et al., 1998) or environmental stress (Rygiewicz et al., 2000), suggesting a degree of stability in the overall structure of the community (Kåren and Nylund, 1997; Goodman and Trofymow, 1998). Similarly, others have found declining changes in the frequency of individual morphotypes due to environmental stresses (Jones et al., 1997; Wöllecke et al., 1999; Peter et al., 2001). In our study, environmental pollution seems to influence ectomycorrhizal richness and total number of mycorrhizal tips, especially in the case of mature stands of Scots pine. The total number of fine root biomass and mycorrhizal tips produced by young Scots pine trees at the forest site (Sulanki) located 0.5 km from the aluminum smelter was similar to that found in earlier studies in Scots pine stands of comparable age (Rudawska et al., 1995, 1996; Kieliszewska-Rokicka et al., 1997). Mature Scots pine trees located 2.5–3 km from the smelter (Rudzica and Anielew) developed significantly fewer mycorrhizas and fine root biomass than Scots pine trees of comparable age at a moderately polluted forest stand in our previous study (Kieliszewska-Rokicka et al., 1997). The number of mycorrhizas was similar to that occurring on mature Scots pine trees growing in a heavily polluted forest stand exposed for more than 40 years to gaseous and dust pollutants emitted by the adjacent urban-industrial area of Kraków and by the distant industrial area of the Upper Silesian Industrial District, one of the most polluted areas of central Europe (Kieliszewska-Rokicka et al., 1997). This indicated that the old stands growing in the vicinity of the aluminum smelter are significantly affected by the

emitted pollutants which negatively influence the mycorrhizal status of tested Scots pine. Observations from polluted forests across Europe (Jansen, 1988) suggest that the mycorrhizal fungi occurring in late succession, characteristic of older stands, are particularly sensitive to atmospheric pollutants and that the decreasing abundance of fungal species may be a factor contributing to forest decline.

The range of ergosterol concentrations in mycorrhizas of Scots pine found in this study (Fig. 5) was similar to that reported by Wallander et al. (1997) in different morphotypes of a mature Scots pine forest in Sweden. Variation in ergosterol concentrations between the mycorrhizal samples may reflect differences in fungal biomass (Sung et al., 1995) or in the extent of fungal membranes (Ruzicka et al., 2000) in the mycorrhizal root tips. Van Praag et al. (1994) reported lower ergosterol contents in rootlets of trees in a spruce stand showing early symptoms of decline. Acid rain treatment resulted in lower ergosterol content in the *Lactarius* mycorrhiza of *Betula pubescens* (Zobel et al., 1994); however, no significant differences in ergosterol concentrations were found in fine roots of Scots pine influenced by various SO_2 and NO_x emissions (Markkola et al., 1995). Ergosterol concentrations can vary between morphotypes of Scots pine ectomycorrhizas (Wallander et al., 1997; Rudawska et al., 2000). Low ergosterol content was reported for ectendomycorrhizas and simple ectomycorrhizal morphotypes with smooth, thin fungal mantles, and high ergosterol levels were reported for the coralloid morphotypes with thick mantles.

Surface characteristics of needles collected at all study sites (Sulanki, Anielew, Rudzica) shown by Staszewski et al. (1998) using SEM analysis were typical of this area, which has been influenced by industrial activities: high fluorine concentrations in the needles, low wettability (determined by the contact angle values), and wax structure degradation. These symptoms may be related to lower mycorrhizal colonization of roots in the mature stands. It has been also suggested that mature Scots pine forests have a greater sensitivity to environmental pollution than young stands (Shaw et al., 1992; Termorshuizen, 1993; Kieliszewska-Rokicka et al., 1997).

Air pollution is often only considered injurious to forest ecosystems if trees are visibly damaged. However, many physiological reactions may be affected even if the trees do not show any clear symptoms of injury. Negative effects of some environmental pollutants are often visible first in roots and their mycorrhizas. As far as we are aware, there is little or no information about the direct effect of fluorides on mycorrhizal fungi and mycorrhizas. However, it is unlikely that roots are exposed to high concentrations of fluorine. Except for a few mycorrhizal roots or hyphae occurring in the upper litter layers, it is unlikely that gaseous pollutants will have a direct effect on the physiology of mycorrhiza.

Trees and their mycorrhizas may be indirectly affected by pollutants. Scots pine is a fluoride-sensitive species and is characterized by a low fluoride accumulation coefficient (0.24 $m^3\,g^{-1}$ dry weight day^{-1}), compared with the median value (0.8 $m^3\,g^{-1}$ dry weight day^{-1}) estimated for more than 50 other species (Horntvedt, 1997). At background levels of fluoride, Scots pine may show slight damage (indicated by leaf necroses), leaving the cause of the damage in doubt. But indirect effects, due to reduced photosynthesis and belowground carbon allocation, may significantly influence the ectomycorrhizal community of tested trees.

In conclusion, this study has demonstrated the toxicity of the environment surrounding the aluminum smelter to ectomycorrhizas and plant symbionts, which has manifested itself in reductions in root biomass and ectomycorrhizal colonization. A molecular study is planned that will enable us to investigate in greater detail the effect of emissions from the aluminum smelter on the fungal community.

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