Ectomycorrhizae of Young and Mature Scots Pine Trees in Industrial Regions in Poland[1]

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Abstract

*Ectomycorrhizae of Scots pine (*Pinus sylvestris *L.) trees grown in forests influenced by different levels of air pollutants were investigated. Total numbers of mycorrhizal root tips in the soil horizons and the frequency of mycorrhizal morphotypes were compared as indicators of ectomycorrhizal status. The studies were conducted in two comparable young pine plantations in western Poland and in two mature forest ecosystems in southern Poland differing in pollution level. Soil at the polluted young pine plantation (Lubon) had lower pH and increased availability of aluminum ions than the control site (Kornik). During 3 years of observations a lower number (50 percent) of mycorrhizal root tips per soil volume were found at the polluted site compared to the control site. The percentage of particular mycorrhizal morphotypes in the two young pine plantations was similar. The mature pine stands represented heavily-polluted (Niepolomice Forest) and moderately-polluted (Ratanica forest catchment) forest ecosystems. The total number of mycorrhizae in the Niepolomice Forest was lower in sites located closer to the urban-industrial area than at more distant plots. In the Ratanica Forest catchment the total number of mycorrhizal root tips varied between the sampling sites; however, on an average it was considerably higher than in the Niepolomice Forest. The mycorrhizal diversity at the heavily-polluted stand was reduced (four mycorrhizal morphotypes) as compared to the moderately-polluted site (eight mycorrhizal morphotypes). The results indicated that ectomycorrhizae of mature Scots pine trees were more affected by environmental pollutants than mycorrhizae at young pine plantations.*

Introduction

Acidification of forest soils caused by acid deposition of sulfur and nitrogen compounds is considered the main factor of forest decline in Northern Europe (Ulrich 1983). At low pH (< 4.5) the rates of dissolution of minerals and solid complexes increase and the chemistry of soil changes (Ritchie 1995). Increased amounts of soluble aluminum, manganese, and other metals can reach concentrations that may be toxic to plants. At the same time, base cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) are also solubilized and easily leached from the upper rooting horizon of the soil. It is believed that aluminum toxicity is related to increased Ca/Al ratios in the forest soil rather than pure Al^{3+} concentration (Ulrich 1983).

Ectomycorrhizae are an integral part of the root system of Scots pine *(Pinus sylvestris* L.*)*, the dominant forest species in Poland. Mycorrhizae improve growth of plants by enhancing uptake of water and nutrients (particularly phosphorus) and are totally dependent on plant derived carbohydrates transported from shoot to root. Mycorrhizal Scots pine seedlings have been shown to be more tolerant to aluminum ions than non-mycorrhizal plants (Goransson and Eldhuset 1991). It is considered that ectomycorrhizae may protect tree roots from the effects of environmental pollution, acting as the physical and/or chemical barrier. On the other hand, ectomycorrhizae and ectomycorrhizal fungi may be negatively influenced by indirect factors, such as low photosynthetic rates after yellowing of needles or needle loss, or direct factors, such as low pH and high soluble aluminum concentrations, low availability of nutrients, elevated soil nitrogen, or drought. Soil texture, aeration, and humus compounds are also important factors affecting mycorrhizae (Kottke and others 1993).

Changes in the chemical composition of soil affect the production of fine roots, which potentially could develop mycorrhizae. A significant decrease of fine root biomass in soils degraded by environmental pollutants was documented for Scots pine (Jozefaciukowa and others 1995, Munzenberger and others 1995). Some authors indicated that environmental pollutants reduce the number of mycorrhizal root tips and the diversity of mycorrhizal morphotypes (Dighton and Skeffington 1987, Kowalski 1987, Kowalski and others 1989).

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Forest ecosystems in Poland have been under pressure of heavy industrial pollution, mainly SO_2, NO_x, and heavy metals, for about half a century (Godzik 1990). Although in recent years the levels of toxic pollutants emitted to the atmosphere have decreased, the southwestern part of Poland remains one of the most polluted areas in Europe (Tickle and others 1995). Toxic pollutants, such as heavy metals, accumulated in the contaminated soils and have affected the terrestrial and aquatic ecosystems.

This paper describes a study that compares the mycorrhizal status of Scots pine trees in forest ecosystems influenced by varying intensities of environmental pollution, using total number of ectomycorrhizal root tips in a soil volume and the diversity of mycorrhizal morphotypes as indicators.

Materials and Methods

Study Areas

The investigations were conducted at four Scots pine stands in Poland (*fig. 1*) influenced by different deposition rates of air pollution. At Lubon and Kornik young Scots pine trees planted in 1984 as 2-year-old seedlings were studied. The plot at Lubon is located about 2 km from a phosphate fertilizer plant established in 1917. The plant has emitted continual increasing levels of toxic air pollutants (Hernik 1987). In 1980 the emission of toxic gases were estimated: sulfur dioxide at 2,806 t yr^{-1}, fluorides at 162 t yr^{-1}, and nitric oxides at 850 t yr^{-1}. In recent years (since 1987) the amount of toxic pollutants emitted to the atmosphere has decreased considerably compared to previous years. In 1994 mean soil pH in the soil horizon 0-5 cm was: $pH_{water} = 4.2$, $pH_{salt} = 3.7$, and the Ca/Al ratio was 0.14 (Rudawska and others 1995). The Kornik stand is situated 12 km to the southeast of Lubon in the experimental forest of the Institute of Dendrology. This area, free of direct industrial pollution, was a control site. Soil at the control site was more than 10-fold less acidic than at the polluted site (mean $pH_{water} = 5.8$, $pH_{salt} = 4.9$). The Ca/Al ratio in the soil horizon 0-5 cm was 17 (Rudawska and others 1995). At the two young Scots pine plantations mycorrhizas were investigated, from autumn 1992 to autumn 1995, in the top 5 cm of the soil horizon. In 1995 additional samples from deeper soil layers were taken.

At the Niepolomice Forest and at Ratanica forest mature Scots pine trees (50-80 years old) were investigated. The Niepolomice Forest ecosystem has been affected for more than 40 years by gaseous and dust pollutants emitted by the adjacent urban-industrial area of Krakow (15-30 km west of the margin of the Forest) and by

Figure 1 — Experimental Scots pine plots in Poland.

the distant industrial area (Upper Silesian Industrial District — one of the most polluted areas in Central Europe, 70 km west of the Forest). The forest complex has been influenced by SO_2, NO_x, CO, CO_2, and heavy metals, including Fe, Zn, Pb, Cu, Cd, Ni (Manecki 1984). The average pH of soil in the upper 5 cm in 1994 was: $pH_{water} = 4.3$, $pH_{salt} = 3.3$. Scots pine is the major tree species of the forest stand, comprising 71 percent of the total area (Grodzinska 1984). Four sites (94, 124, 161, and 232) located on a west-east transect from Krakow were chosen as the experimental plots. Mycorrhizae were assayed in 1994, three times per year, at 0-5 cm and 5-10 cm soil depth.

The Ratanica experimental plot is located 40 km south of the Krakow urban area. It is a small forest catchment (88 ha) in the Carpatian foothills in the region of a drinking water reservoir, surrounded by intensively managed agricultural land. The catchment is influenced by gaseous and dust pollutants (containing SO_2, NO_2, Cl, Mn, Zn, Cu, Pb, Cd) transported there by winds from several distant industrial areas and by local agricultural emissions from surrounding villages (Niklinska and others 1995, Szarek 1995). Average pH in the organic horizon was reported as $pH_{water} = 3.9$ and in the mineral layer as $pH_{water} = 4.3$ (Grodzinska and Szarek 1995). The level of air pollution in this area was qualified as moderate but chronic (Grodzinska and Szarek 1995). The catchment is covered by a mixed forest with a predominance of Scots pine *(Pinus sylvestris* L.*)* and beech *(Fagus sylvatica* L.*).*

Mycorrhizal Frequency

Fine-root sampling was carried out with a 16 cm^3 soil corer from three places around a pine tree, at about 60-80 cm distance from the trunk. Samples were taken three times per year (May, August, October). At Lubon and Kornik 24 young trees at each plot were tested. At Niepolomice Forest six mature trees in each of the forest sites were investigated. At the Ratanica forest catchment three to seven mature Scots pine trees were studied in each of five areas of the entire catchment. Samples were taken to the laboratory and stored at 3 °C until analyzed. Roots were washed over a mesh, sorted, and analyzed under a stereomicroscope. The number of mycorrhizal root tips was counted in the root samples.

Characterization of Ectomycorrhizal Morphotypes

Each root tip was characterized according to morphological traits (shape, color, surface texture). The ectomycorrhizal morphotypes were distinguished and named according to the main types of ectomycorrhizal systems presented by Agerer (1987-1995) (*fig. 2*).

- **Type A:** Simple (unramified), elongate my corrhizal root tips, with a thin fungal mantle, their diameters were similar to non-mycorrhizal roots, yellow-brown in color, the surface was more or less smooth.
- **Type A:**$_1$ Simple, elongate, thick mycorrhizae, their diameters were mostly three times the diameter of the supporting roots, light yellow in color, abundant fungal mantle with extramatrical hyphae on the surface.
- **Type B:** Dichotomous, elongate, thin mycorrhizae, orange-brown in color. The fungal mantle thin with a very smooth surface, no evidence of extramatrical hyphae.
- **Type C:** Shortened, abundantly dichotomizing mycorrhizae, light yellow, with extramatrical mycelium.
- **Type D:** Stumpy coralloid mycorrhizae with thickened yellow-brown branches. The surface was more or less smooth, without extramatrical hyphae. Older forms were darker in color and had a wrinkled appearance.

- **Type E:** *Cenococcum sp.* characteristically jet-black mycorrhizae with abundant emanating black hyphae stiffly projecting around the root tip. Infection by the fungus was also overgrowing other types of mycorrhizae, as A and B. Type
- **Type F:** Tuberculate mycorrhizae with a thick mantle and a smooth surface.
- **Type G:** Monopodial-pinnate mycorrhizae with thick fungal mantle, yellow or yellow- brown, with a smooth surface.
- **Type H:** Monopodial-pyramidal mycorrhizae with thick mantle, white-yellow or yellow- brown, smooth or grainy surface, old forms brown, with wrinkled surface.

Statistics

Data were processed using Duncan's multiple range test (ANOVA) or Scheffe test (Statistix 4.1). Statistical significance was accepted at $P < 0.01$.

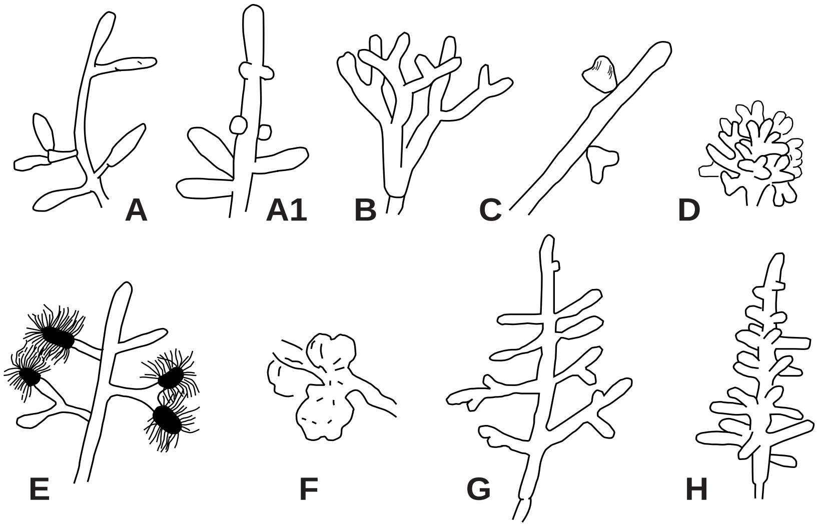

Figure 2 — Ectomycorrhizal morphotypes distinguished on Scots pine roots at the experimental plots:
A, A$_1$ - simple mycorrhizae;
B, C - dichotomous mycorrhizae;
D - coralloid mycorrhizae;
E - mycorrhizae with *Cenococcum* sp.;
F - tuberculate;
G - monopodial-pinnate;
H - monopodial- pyramidal.

Results

Young Plantations of Scots Pine (Lubon and Kornik)

At the polluted site (Lubon) the total number of mycorrhizal root tips in the upper soil horizon (0-5 cm), expressed per volume of soil sample, was significantly lower than at the control site in Kornik (*fig. 3*), although at both sites non-mycorrhizal roots were hardly found. The dry mass of fine roots (< 2 mm diameter) was slightly lower in soil samples from the polluted than from the control site; however, the differences were not significant (data not shown). In the 15-30 cm soil depth the average number of mycorrhizal root tips per soil volume was a little higher (not significantly) at the polluted than at the control plantation (*fig. 4*).

Mycorrhizal morphotypes distinguished on pine roots at the experimental plots (*fig. 2*), corresponded to the main types of ectomycorrhizal systems described by Agerer (1987-1995). At the control plantation at Kornik and the polluted site at Lubon, a maximum of seven morphotypes (A, A$_1$, B, C, D, E, F) was found (*table 1*). At both sites, the most frequent were the simple (unramified) mycorrhizae A and A$_1$. The frequency of the thin A type, considered as the less efficient, was similar at both plots. The dichotomous mycorrhizae with abundant fungal mantle (type C) and coralloid mycorrhizas (type D) were present at both polluted and unpolluted plantations at low frequencies.

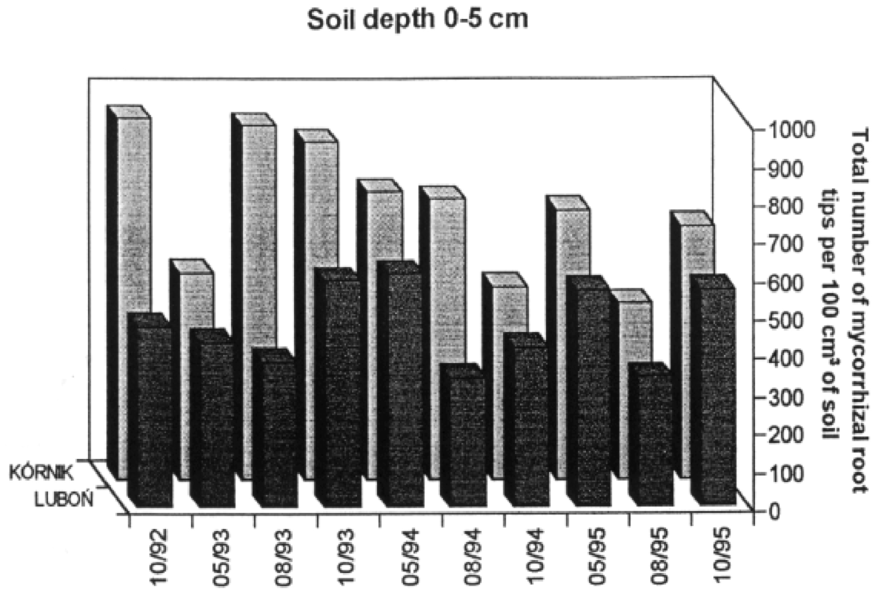

Figure 3 — Total number of ectomycorrhizal root tips in soil samples (100 cm^3) taken from a 0-5 cm depth at the control (Kornik) and the polluted (Lubon) Scots pine plantations, from 1992 to 1995, three times per year. Data are means of 24 replicates. The differences between the sites are statistically significant ($P < 0.01$, Scheffe test).

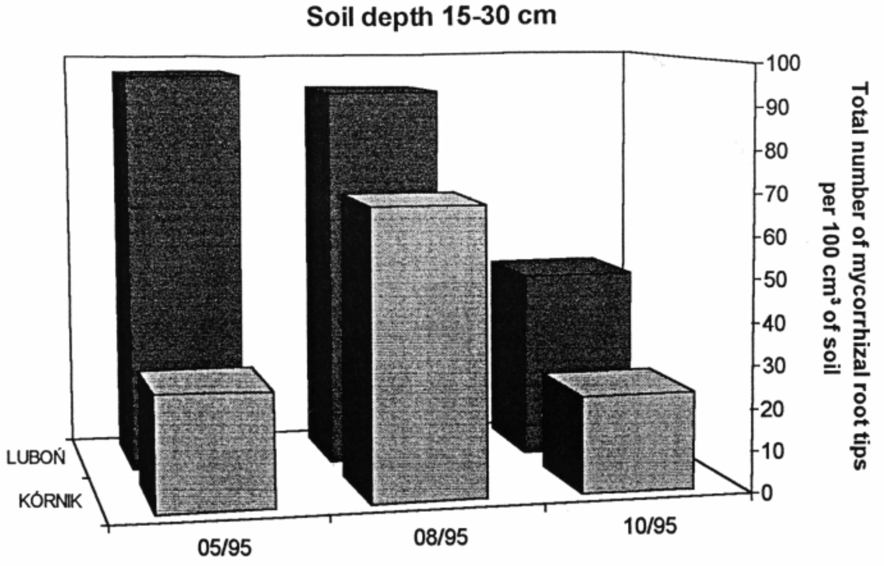

Figure 4 — Total number of mycorrhizal root tips in soil samples (100 cm^3) taken from a 15-30 cm depth at the control (Kornik) and polluted (Lubon) Scots pine plantations in 1995. Data are means of 24 replicates. The differences between the sites are not significant statistically, according to the Scheffe test.

Mature Pine Trees (The Niepolomice Forest and Ratanica Forest Catchment)

In the Niepolomice Forest the total number of ectomycorrhizae was lower at the sites located closer to the urban-industrial centers than at the more distant sites, however, in August and October, in the soil depth 0-5 cm, the differences were not significant statistically (*fig. 5*). Four mycorrhizal morphotypes (A, A_1, B, D) were found on pine roots in the Niepolomice Forest (*table 2*). Generally, Scots pine roots developed at these forest sites mostly simple, thin mycorrhizae A (up to 84 percent). The dichotomous mycorrhizae (B) and coralloid morphotypes (D) were typically a low percentage of the mycorrhizae in the plots, with a few exceptions.

Considerable variations in the total number of mycorrhizae per soil volume (from 360 to 2,030) were found among 22 pine trees tested in the Ratanica forest catchment, however, the mean values count for various sites of the forest were similar or differed slightly (*fig. 6*). The average number of mycorrhizal root tips in the Ratanica forest catchment, in soil depth 0-5 cm, was more than 100 percent higher in relation to the Niepolomice Forest.

Table 1 — *Frequency of mycorrhizal morphotypes of Scots pine at two soil depths: 0-5 cm and 15-30 cm, at control (Kornik) and polluted (Lubon) plantations. Root samples were observed in May, August, and October 1995.*[1]

Time	Site	Percentage of mycorrhizal morphotypes						
		A	A_1	B	C	D	E	F
		Soil depth 0-5 cm						
05/95	Kornik	61 ± 16	21 ±11	7 ± 3	1 ± 1	5 ± 5	0	5 ± 5
	Lubon	53 ± 17	20 +14	13 + 8	1 + 1	10 + 3	0	3 + 3
08/95	Kornik	74 ± 7	5 ± 3	14 ± 6	2 ± 2	3 ± 2	2 ± 2	0
	Lubon	60 ± 13	8 ± 7	23 ± 10	1 ± 1	7 ± 7	1 ± 1	0
10/95	Kornik	73 ± 7	10 ± 5	6 ± 3	3 ± 2	5 ± 5	3 ± 3	0
	Lubon	59 ± 15	14 ± 7	9 ± 3	7 ± 7	8 ± 3	3 ± 3	0
Soil depth 15-30 cm								
05/95	Kornik	67 ± 22	10 ±10	9 ± 7	1 ± 1	9 ± 9	0	3 ± 3
	Lubon	60 ± 24	11 ± 11	12 ± 9	0	17 ± 12	1 ± 1	0
08/95	Kornik	79 ± 9	1 ± 1	13 ± 7	0	6 ± 4	1 ± 1	0
	Lubon	60 ± 13	1 ± 1	17 ± 4	0	20 ± 10	0	2 ± 2
10/95	Kornik	71 ± 17	14 ± 14	9 ± 9	1 ± 1	4 ± 4	1 ± 1	0
	Lubon	47 ± 27	12 ±10	10 ± 6	4 ± 4	15 ± 12	0	12 ± 12

[1] Data are means ± SD (n = 24).

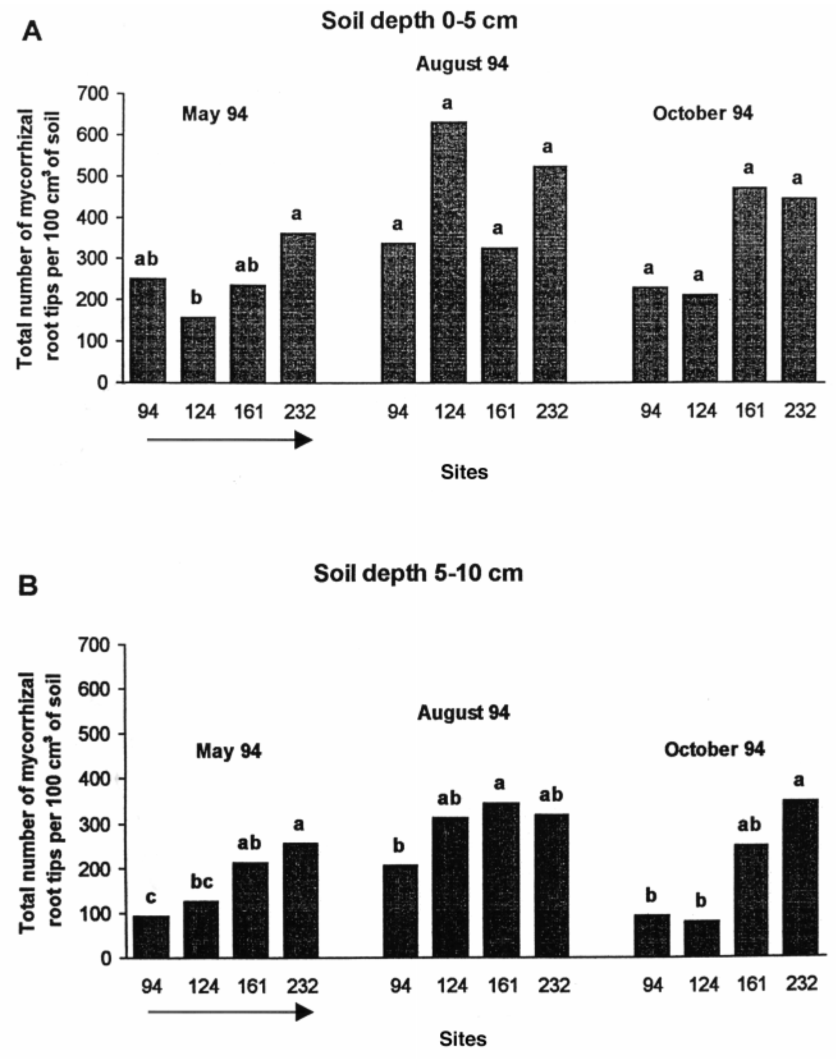

Figure 5 — Total number of mycorrhizal root tips in soil samples (100 cm^3) taken from 0-5 cm and 5-10 cm depths at four sites (94, 124, 161, 232) in the Niepolomice Forest, located along a transect extending from the Krakow area, the source of air pollutants. Data are means of six replicates. Means followed by the same letter do not differ significantly ($P < 0.01$, ANOVA, Duncan's test).

Eight morphotypes of pine ectomycorrhizae were distinguished in the Ratanica forest catchment (*table 3*). The average frequency of the simple, thin mycorrhizas (type A), considered as the less beneficial for a tree, was lower than 50 percent. Occurrence of new morphotypes, compared to the Niepolomice Forest was observed: abundantly dichotomizing (C), monopodial-pinnate (G), monopodial-pyramidal (H), and Cenococcum sp. mycorrhizae (E).

Discussion

Mycorrhizal status of forest trees provides accurate information about changes in the forest soil affected by environmental pollution. In this study reduced numbers of Scots pine mycorrhizae were found at the severely polluted forest stands in Poland (Lubon, the Niepolomice Forest) compared to the slightly or moderately polluted sites (Kornik, Ratanica forest catchment) (*figs. 3, 5, 6*). Significant reduction in mycorrhizae was found at 11-18 year-old Scots pine plantations (Kowalski and others 1989) and in mature Scots pine stands (Ohtonen and others 1990) influenced by industrial pollutants. A decrease in numbers of mycorrhizae was also caused by low pH and available aluminum in perlite / peat cultures (Metzler and Oberwinkler 1987). The influence of other important factors on mycorrhizae, like soil type and soil moisture must also be considered (Kottke and others 1993).

Frequencies of ectomycorrhizal morphotypes are considered accurate indicators of the mycorrhizal status of forest trees. Dighton and Skeffington (1987) observed that artificial acidic precipitation and an increase of Al^{3+} concentration in

Table 2 *— Frequency of mycorrhizal morphotypes of Scots pine at soil depths: 0-5 cm (I) and 5-10 cm (II) at four forest sites in order of decreasing pollution exposure (94, 124, 161, 232) in the Niepolomice Forest.* [1]

Time	Site	Percentage of mycorrhizal morphotypes							
		A		A_1		B		D	
		I	II	I	II	I	II	I	II
05/94	94	82 ± 11	75 ±19	10 ± 7	7 ± 7	4 ± 3	16 ±14	3 ± 3	1 ± 1
	124	74 ± 24	59 ±25	17 ± 23	8 ± 7	5 ± 3	6 ± 5	4 + 4	27 ±25
	161	50 ± 6	35 ± 25	45 ± 19	64 ±32	3 ± 3	1 ± 1	2 ± 2	0
	232	65 ± 16	68 ±17	26 ± 13	24 ± 12	2 ± 2	4 ± 4	6 ± 5	4 ± 4
08/94	94	84 ±13	77 ±17	6 ± 6	14 ± 14	6 ± 6	5 ± 5	4 ± 2	4 ± 4
	124	58 ± 13	76 ± 7	37 ± 17	18 + 9	2 ± 2	2 ± 2	3 ± 2	4 ± 2
	161	70 ± 18	70 ± 22	23 ± 16	25 ± 24	2 + 2	2 ± 2	6 ± 5	3 ± 3
	232	59 ±30	80 ± 21	32 ± 12	17 ± 17	4 ± 3	1 ± 1	5 ± 4	2 ± 2
10/94	94	60 ± 10	73 ± 9	25 ±12	19 ± 13	6 ± 3	3 ± 3	8 ± 4	4 ± 4
	124	80 ± 19	77 ±127	7 ±6	8 ±8	5 ± 5	8 ± 7	9 ± 8	6 ± 5
	161	70 ±19	56 ±27	28 ±17	44 ± 31	2 ± 2	0	1 ± 1	0
	232	62 ±19	81±13	25 ±16	15 ±10	8 ± 5	1 ± 1	5 ± 4	2 ± 2

[1] Data are means ± SD (n = 6)

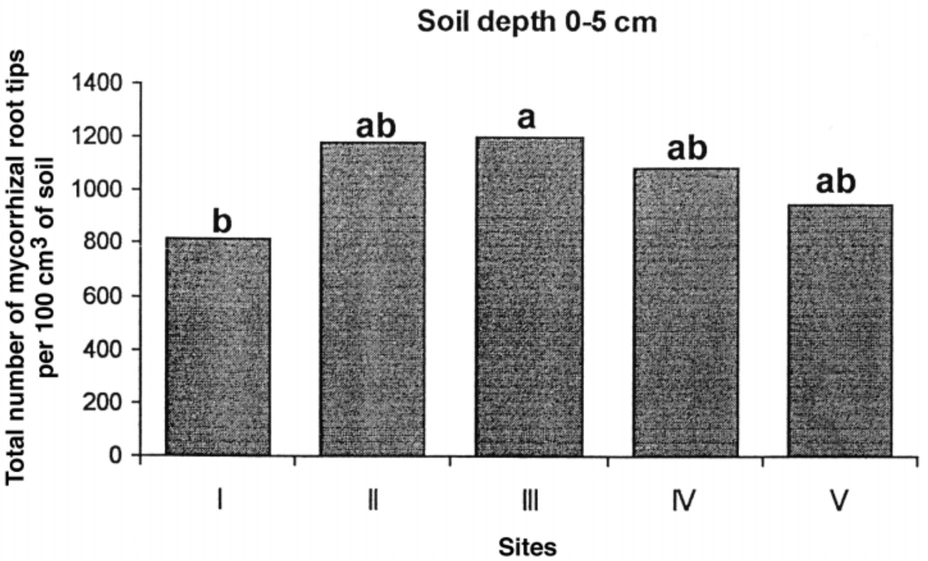

Figure 6 — Total number of mycorrhizal root tips in soil samples (100 cm^3) taken from a 0-5 cm depth at five sites in the Ratanica forest catchment. Data are means of three to seven replicates. Means followed by the same letter do not differ statistically ($P < 0.01$, ANOVA, Duncan's test).

soil solution significantly reduced the branching of fine roots of Scots pine and decreased the occurrence of two coralloid mycorrhizal morphotypes. Mycorrhizal roots of Scots pine at highly contaminated areas in Poland occur mainly as simple mycorrhizae with a scant fungal mantle, whereas in forests with low-level industrial pollution, mycorrhizae are more commonly of the dichotomous morphotypes (Kowalski 1987, Kowalski and others 1989).

In the present studies, no evident differences in the proportion of ectomycorrhizal morphotypes were found between the polluted and the control young Scots pine plantations (*table 1*); however, considerable differences in the occurrence of mycorrhizal morphotypes were observed between the severe-polluted and moderately-polluted mature pine trees (*tables 2, 3*). Simultaneously, at the polluted young pine plantation we recorded a more abundant occurrence of species and carpophores of ectomycorrhizal fungi (Rudawska and others [In press]). Termorshuizen and Schaffers (1987) observed that mycorrhizal fruitbodies were not depressed by air pollution in young stands of Scots pine (5-13 years), but they were reduced in mature stands (50 years). This phenomenon could be explained by relatively low susceptibility of mycorrhizal fungi species living in symbiosis with young Scots pine trees (Shaw and others 1992). Ectomycorrhizal fungi show differential responses to Al in pure cultures (Kieliszewska-Rokicka and others [In press], Thompson and Medve 1984). Since Scots pine tolerates a wide range of ecological conditions and it is also classified as relatively tolerant to soluble Al, (Schaedle and others 1989) the mycorrhizae formation could be limited by the sensitivity of fungi rather than of the plant (Metzler and Oberwinkler 1987).

Reduced mycorrhizal diversity in the Niepolomice Forest compared to the Ratanica forest catchment suggests a lower biodiversity of mycorrhizal species at the severely-polluted mature pine stand than in the moderately-polluted forest. Kowalski and others (1989) reported that the reduction in diversity of mycorrhizal morphotypes at a contaminated Scots pine plot was accompanied by significant changes in the composition of mycorrhizal fungi species and a decrease in the production of fruitbodies. Termorshuizen and Schaffers (1991) have found a negative correlation between SO_2 concentration in the air and the number of mycorrhizal species and carpophores in mature Scots pine forests in The Netherlands. Carpophores of mycorrhizal fungi and fungal species have declined during this century in Europe (Arnolds 1991). The disappearance of various species of mycorrhizal fungi has been observed also in many areas in Poland (Wojewoda and Lawrynowicz 1986).

Environmental pollutants undoubtedly affect mycorrhizae directly, inhibiting the uptake of nutrients and causing nutrient imbalance in root and shoot (Janhunen and others 1994) or limiting growth of the mycorrhizal root tips (Metzler and

***Table 3** — Frequency of mycorrhizal morphotypes of Scots pine in the top 5 cm of soil at five sites in the Ratanica forest catchment. The fine-root samples were collected in August 1995.*[1]

Site	**Percentage of mycorrhizal morphotypes**							
	A	A_1	B	C	D	E	G	H
I	54 ± 12	8 ± 6	10 ± 5	7 ± 1	5 ± 3	10 ± 1	4 ± 4	2 ± 2
II	37 ± 12	33 ± 7	8 ± 4	10 ± 5	4 ± 3	5 ± 5	1 ± 1	2 ± 2
III	52 ± 8	31 ± 9	4 ± 2	7 ± 1	3 ± 1	1 ± 1	1 ± 1	1 ± 1
IV	50 ± 9	26 ± 11	12 ± 5	4 ± 3	3 ± 1	3 ± 2	1 ± 1	1 ± 1
V	42 ± 17	26 ± 16	15 ± 8	7 ± 2	5 ± 1	2 ± 1	1 ± 1	2 ± 2
MEAN	47 ± 7	25 ± 10	10 ± 4	7 ± 2	4 ± 1	4 ± 3	2 ± 1	2 ± 1

[1] Data are means ± SD (n= 3-7).

Oberwinkler 1987). At young Scots pine plantation contaminated by soluble Al, an increased Al/Ca ratio in needles was correlated with a decrease in the net photosynthesis, biomass production, and allocation to roots (Reich and others 1994). Simultaneously, a high, negative correlation between aluminum content in pine needles and the number of mycorrhizal root tips in the upper 5 cm of soil has been recorded for the young pine trees (Rudawska and others 1995). Other external factors, like drought, frost, and pathogens could intensify the effects of environmental pollutants.

Conclusions

The decrease in the number and diversity of mycorrhizae at the heavily polluted sites is presumably related to a direct negative effect of aluminum and other metals on fungal hyphae as well as the influence of pollutants on photosynthesis and transport of carbohydrates to roots. Mycorrhizae of mature Scots pine trees seem to be more sensitive to the stress factors than mycorrhizae of young trees. As Scots pine changes ectomycorrhizal partners during its ontogeny, a modification of mycorrhizal status with the age of the plantation could be possible.

Acknowledgments

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