Physiological activity of ectomycorrhizas in a moderately polluted forest (Ratanica catchment, southern Poland)

Abstract: Ectomycorrhizas of Scots pine (Pinus sylvestris L.) and beech (Fagus sylvatica L.) were sampled in a mature forest ecosystem exposed for more than 40 years to moderate levels of gaseous and dust pollutants. Soil of the forest site was characterised by low pH and accumulation of heavy metals (Pb, Mn, Zn, Cu, Cd, Fe). Mycorrhizal vitality and enzyme activity of the root-surface and soil acid phosphatase (AcPase) were studied at 17 measurement points (0–5 cm soil depth) in relation to the content of inorganic phosphate (Pi) and aluminium ions (Al^{3+}) in the soil. Anatomy of Scots pine and beech mycorrhizas taken from different measurement points was observed. The concentration of essential nutrients (C, N, P, Ca, Mg) and the ratios Ca/Al, Mg/Al and N/P were analysed in fine roots. High concentrations of Al^{3+} in the soil (40–118 meq kg^{-1}) and low levels of Pi (12–44 mg P_2O_5 kg^{-1}) were accompanied by high activity of the root-surface AcPase of pine and beech mycorrhizas (25–67 and 33–86 µmol pNP g^{-1} fresh weight h^{-1}, respectively) and soil AcPase (6.8–22.4 µmol pNP g^{-1} dry weight h^{-1}). The results indicate that fine tree roots are undoubtedly under stress as evidenced by a disturbance in P uptake and accumulation. However, the high vitality of mycorrhizas and the high Ca/Al ratio in fine roots suggest that the defence mechanisms of mycorrhizas and the rhizosphere are still able to ameliorate the influence of anthropogenic pollution.

Additional key words: acid phosphatase, fluorescein diacetate, soil, Fagus sylvatica, Pinus sylvestris, root anatomy

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Introduction

It is well established that anthropogenic stresses are important factors in forest decline (Schütt and Cowling 1985). Many forest ecosystems in Poland have been exposed to heavy industrial pollution, mainly sulphur dioxide (SO_2), nitrogen oxides (NO_x), hydrogen fluoride (HF), acid rain and heavy metals for about half a century (Białobok 1984; Godzik 1990). In recent years the phytotoxic effects of increased levels of tropospheric ozone (O_3) has been emphasised (Bytnerowicz et al. 1993; Godzik 1997). Although during the recent years the emissions of SO_2 to the atmosphere in Poland significantly decreased, toxic gases such as NO_x, HF, NH_3 and O_3 are important compounds influencing forest ecosystems and the area of injured forests is still increasing (Vancura et al. 2000).

In the early eighties attention has been paid to the response of tree root systems to anthropogenic pollutants. It has been suggested that forest decline is a consequence of damage to fine tree roots (Ulrich 1983; Meyer 1985) and that the sensitivity of roots to environmental pollution could be used as a marker for physiological damage to trees prior to visible symptoms of decline (Richards 1989). Forests of temperate regions usually grow on acidic soils, with a high accumulation of organic matter at the soil surface and limited availability of nutrients. An addi-
tional input of acid deposition may cause a decline in the decomposition rate of organic matter and chemical changes resulting in an increased availability of soluble aluminium, manganese and heavy metals and an accelerated leaching of base cations (Ca$^{2+}$, Mg$^{2+}$, K$^+$, Na$^+$) down the soil profile (Ulrich 1983; Grodziński et al. 1990).

Most tree species in temperate forest ecosystems are ectomycorrhizal. Ectomycorrhizas (fungal mantle and extramatrical mycelium) are known to improve the uptake of water and nutrients (particularly nitrogen and phosphorus) by plants from the soil solution. The influx of Pi in mycorrhizal roots can be 3–5 times higher than in nonmycorrhizal roots (Smith and Read 1997). A major part of the phosphorus (up to 80% or more) contained in the upper horizons of forest soils, where most ectomycorrhizas are localised, is present in organic forms (e.g. Dalal 1977). Ectomycorrhizas are able to acquire large amounts of phosphorus from organic sources that are not available directly to the plant. One of the mechanisms by which mycorrhizal fungi and mycorrhizas may increase the uptake of phosphorus from the soil is the production of surface-accessible acid phosphatase (acid phosphomonoesterase $\equiv$ AcPase, EC 3.1.3.2) (Harley 1989; Smith and Read 1997). In ectomycorrhizal root tips the activity of AcPase has been detected mainly in the matrix of the fungal mantle and along the plasma membranes of the Hartig net (Dexheimer et al. 1986; Turnau and Dexheimer 1995). The activity of AcPase has been shown to be more active in ectomycorrhizal than in nonmycorrhizal roots (Smith and Read 1997). Activity of extracellular soil acid phosphatase may exist temporarily in complexes enzyme-substrate, adsorbed on clay minerals or associated with humic colloids (Burns 1982), takes part in the hydrolysis of polyphosphates and organic phosphates in the soil (Boutin and Roux 1974) and in Pi absorption by the plant (Bieleski 1973). Inverse relations have been shown between Pi supply and AcPase activity, both in soil (Spiers and McGill 1979) and in ectomycorrhizas of forest trees (Alexander and Hardy 1981; MacFall et al. 1992; Kieliszewska-Rokicka 1992; Pasqualini et al. 1992). Decreased activity of the root-surface AcPase has been reported from forest sites exposed to anthropogenic pollutants such as sulphur (Rejšek 1991) and the combined effects of toxic gases and heavy metals (Kieliszewska-Rokicka 1999). Activity of extracellular soil acid phosphatase can be depressed by increased concentrations of heavy metals in the soil (Tyler 1974; Juma and Tabatabai 1977; Kuperman and Carreiro 1997; Nowak et al. 1999).

It is considered that some parameters of fine roots and mycorrhizas, such as root biomass and turnover, the degree of mycorrhizal colonisation, anatomical changes of fine roots, nutrients and trace elements ratios and physiological activity, can be used as early indicators of environmental changes (Vogt et al. 1993). Extracellular soil AcPase can be a useful and sensitive estimate of metabolic response of soils to environmental stress (Tyler 1974; Nannipieri et al. 1990; Nannipieri 1994).

This study is a part of three-seasons of observation of the mycorrhizal status of Scots pine and beech in a small forest catchment of Ratanica stream located in southern Poland (49°51'N, 20°02'E) 40 km south of Cracow, in the Carpathian Foothills, in the vicinity of a big water reservoir supplying drinking water for the city of Cracow. The catchment has been exposed to moderate levels of gaseous and dust pollutants transported to this area by winds from distant industrial agglomerations (Upper Silesia, Cracow, Ostrava region in Czech Republic) (Tomaszewski and Walczewski 1992) and by local emissions from surrounding villages (Manecki and Tarkowski 1993). The Ratanica catchment belongs to a network of study plots of the International Co-operative Programme on Integrated Monitoring of Air Pollution Effects on Ecosystems in Europe. Numerous studies on air, soil, water and vegetation quality have been conducted in this forest ecosystem (see: Grodzińska and Weiner 1994; Grodzińska et al. 1995; Grodzińska and Szarek 1995; Grodzińska and Laskowski 1996; Kieliszewska-Rokicka et al. 1997).

The aim of the present study was to determine whether the long-term pressure of moderate anthropogenic pollution had an influence on the physiological mechanisms of phosphate mobilisation and uptake.

Forest soils contain extracellular acid phosphatase (AcPase) released by plant roots, mycorrhizas and soil microorganisms (fungi, bacteria, algae). The soil Acid phosphatase may exist temporarily in complexes enzyme-substrate, adsorbed on clay minerals or associated with humic colloids (Burns 1982), takes part in the hydrolysis of polyphosphates and organic phosphates in the soil (Boutin and Roux 1974) and in Pi absorption by the plant (Bieleski 1973). Inverse relations have been shown between Pi supply and AcPase activity, both in soil (Spiers and McGill 1979) and in ectomycorrhizas of forest trees (Alexander and Hardy 1981; MacFall et al. 1992; Kieliszewska-Rokicka 1992; Pasqualini et al. 1992). Decreased activity of the root-surface AcPase has been reported from forest sites exposed to anthropogenic pollutants such as sulphur (Rejšek 1991) and the combined effects of toxic gases and heavy metals (Kieliszewska-Rokicka 1999). Activity of extracellular soil acid phosphatase can be depressed by increased concentrations of heavy metals in the soil (Tyler 1974; Juma and Tabatabai 1977; Kuperman and Carreiro 1997; Nowak et al. 1999).

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Materials and method

Study site

The entire Ratanica catchment covers 241.5 ha. The upper part of the catchment (88 ha) is covered by mixed forest with a predominance of *Pinus sylvestris* (L.) (63%) and *Fagus sylvatica* (L.) (21%), while the lower part is occupied by fields and meadows (Raimer et al. 1990). The highest point of the catchment is a hill of 427 m above sea level and the lowest one lies at 270 m a.s.l. More than half of the area is situated from 330 to 390 m a.s.l. The soil of the Ratanica catchment is an acidic podsol with a layer of mor-type humus. The soil solution is strongly acidic, especially in the upper part of the soil profile (Grodzińska and Laskowski 1996). The average concentration of SO$_2$ in the period 1991–1994 was 21.1 mg m$^{-3}$ with SO$_2$ as high as 100 mg m$^{-3}$ and NO$_2$ – 8.7 mg m$^{-3}$ with high values up to 30 mg m$^{-3}$ (Szarek 1995). The average concentration of tropospheric O$_3$ between June 1995 and October 1995 was 36.2 ppb (16.6–55.8) (Grodzińska and Laskowski 1996). The annual dust fall in the area of Ratanica forest was reported as 35–48 ton km$^{-2}$ (Manecki and Tarkowski 1993). The concentrations of heavy metals (Pb, Mn, Zn, Cu, Cd, Fe) in the upper soil layers (particularly in the organic layer) were higher than at unpolluted sites in Poland (Grodzińska and Laskowski 1996), however only the concentration of Pb exceeded the range accepted as normal (Kabata-Pendias and Pendias 1979). The deposition of nitrogen (mainly as NH$_4^+$) in the Ratanica forest ecosystem was estimated as 24 kg ha$^{-1}$ year$^{-1}$. The mean annual precipitation in the period 1991–1994 was 700 mm (Grodzińska and Laskowski 1996). The concentrations of sulphur in needles and Fe and Pb in bark of pines grown in Ratanica forest were significantly higher than in relatively unpolluted areas in Poland (Molski and Dmuchowski 1990; Szarek et al. 1993).

Most of the chemical analyses mentioned above were carried out at 29 points, located at three transects traced across the forested part of the catchment and on a permanent plot (PP), of 0.25 ha, located in the upper part of the catchment at an elevation of 370–380 m a.s.l. (Grodzińska and Laskowski 1996) (Fig. 1).

In the present studies soil cores were taken at every second transect point and at the permanent plot (17 measurement sites). The vitality of mycorrhizas was observed at 8 measurement sites.

Fig. 1. A – Location of the Ratanica catchment: a – watershed of Dobczyce Reservoir basin, b – Ratanica catchment; B – Location of the measurement points in the Ratanica catchment (according to: Grodzińska 1994)
Sampling and sample preparation

Soil core samples were collected in October 1997 with a 5 cm dia soil corer to a depth 5 cm after removal of the litter layer. All cores were taken at 0.7–1 m from tree bases. Intact soil cores were sealed in plastic bags and kept cool until used, within one week of collection. Soil samples for AcPase activity determination were sieved at 2 mm sieve to remove roots. Fine roots for vitality estimation were separated from soil and organic matter on a sieve under a stream of cold water, excised from the main root and cleaned with running water to remove adhering organic matter. Final separation of roots was conducted under a stereomicroscope. All visibly dead and injured roots, as well as roots of annual plants, were removed.

Microscopic studies

To estimate the development of the fungal mantle and the Hartig net, fresh ectomycorrhizas were cut into transverse sections using a razor blade. The sections were placed in a drop of glycerol and examined under a light microscope (Nikon, Optiphot-2) with a camera attachment (Nikon, Microflex UFX-DX). At each measurement point 50 transverse sections prepared from different mycorrhizal morphotypes were analysed.

FDA (fluorescein diacetate) vital staining of ectomycorrhizas

Fresh mycorrhizas (60–80 from one measurement point) were sectioned longitudinally using a razor blade. The fresh sections were stained immediately with the FDA-stain according to Ritter et al. (1986) for 5–15 minutes, washed with distilled water and observed under a fluorescence microscope (Olympus Provis). Vitality of mycorrhizas, characterised by the light green FDA-fluorescence was documented with a microscope camera using Fuji film, 400 ASA. Five stages of ectomycorrhizal vitality were distinguished according to Ritter et al. (1989): 1) entirely vital – all regions of mycorrhiza are vital (the fungal mantle, the cortex including the Hartig net, the central cylinder, the meristem), 2) largely vital – most of the mycorrhizal compartments are vital (the cortex with the Hartig net, the central cylinder, the meristem), 3) reduced vital – only the central cylinder and meristem are vital, 4) dying mycorrhiza – only the central cylinder is vital, 5) dead mycorrhiza – all root compartments are dead.

Activity of root surface acid phosphatase

AcPase activity associated with excised ectomycorrhizal root tips was measured using a modified method of Bartlett and Lewis (1973). The incubation medium contained 1.8 ml of 0.1 M Na-acetate buffer, pH 5.0, 0.2 ml of 0.05 M p-nitrophenyl phosphate (pNPP) and 10–20 mg fresh weight of root tips. In the reference samples roots were omitted. After 1h in a shaking bath at 30°C, the roots were removed and 1ml of 0.5 M NaOH. The liberated p-nitrophenol (pNP) was determined at 400 nm. Enzyme activity was expressed as μmol pNP g⁻¹ fresh weight h⁻¹.

Activity of soil acid phosphatase

Two ml of 0.2 M phosphate buffer (pH 6.5), 0.5 ml of 0.115 M p-nitrophenyl phosphate (pNPP) and 0.1 ml of toluene were added to 0.5 g of air-dried soil and incubated at 37°C for 1h. Adding 2 ml of 0.5 M NaOH and 0.5 ml of 0.5 M CaCl₂ stopped the reaction. After filtration the solution was diluted 10 times and the liberated p-nitrophenol determined at 400 nm (Tabatabai and Bremner 1969). Each sample was accompanied by a reference sample, where p-nitrophenol was omitted.

Chemical analyses of soil and fine roots

The pH of the soil layer from which roots were removed was measured with a glass electrode in soil-water and soil-salt (0.01 M CaCl₂) suspensions (Anonymous 1994). The concentration of P₂O₅ in soil was measured by the method of Egner-Riehm, the concentration of extractable aluminium and hydrogen (Al³⁺, H⁺) were estimated by the method of Sokolow (Ostrowska et al. 1991). The phosphorus concentration (PO₄-P) in fine roots was measured using a standard colorimetric method (Allen 1989). Carbon and nitrogen content were estimated by isotope ratio mass spectrometry (IRMS) Europa 20–20. The concentration of Ca in the plant material was measured by atomic emission spectrometry (Flapho 4) and concentrations of Mg and Al by atomic flame absorption spectrophotometry (Varian 20 BQ) (Grodzińska and Laskowski 1996).

Statistics

Relationships between AcPase activities and Pi content in soil were evaluated using correlation and regression analyses (p<0.05).

Results

Phosphate and aluminium content in soil

The concentrations of extractable Pi, measured as P₂O₅, aluminium ions (Al³⁺), hydrogen ions (H⁺) and pH values of the upper soil layer (0–5 cm) in the experimental forest are given in Table 1. All soil samples were generally strongly acidic, however variability among the 17 measurement sites was found. Available phosphorus concentration varied among the measurements sites within a range from 12 to 44 mg P₂O₅ kg⁻¹ of soil and the concentration of Al³⁺ from 40.6 to 118.4 meq kg⁻¹ of soil.
Anatomy of ectomycorrhizas

The microscope observations of the transverse and the longitudinal sections of mycorrhizas revealed the presence of different types of fungal mantle on the surface of the fine roots and the Hartig net in the intercellular spaces of the root cortex. In beech mycorrhizas the Hartig net penetrated the cortex to a depth of 1–3 cortical cell layers and in Scots pine mycorrhizas reached the endodermis (Fig. 2).

Vitality of ectomycorrhizas

Different stages of mycorrhizal vitality that reflect phases in the ageing process of ectomycorrhizas are presented in Fig. 3. The vitality of Scots pine and beech ectomycorrhizas was estimated at 8 measurement sites (1, 5, PP, 11, 14, 17, 22, 27). Frequency of mycorrhizas with vital fungal hyphae (stages 1 + 2) varied slightly between the measurement points and was from 40 to 53% for Scots pine and from 42 to 57% for beech. The frequency of dead mycorrhizas was from 10 to 19% for Scots pine and from 11 to 16% for beech (Fig. 4). The differences in mycorrhizal vitality did not differ significantly between the measurement sites.

Root-surface and soil acid phosphatase

The activity of the surface AcPase of Scots pine mycorrhizas ranged between 25.3 and 66.7 µmol pNP g⁻¹ fresh weight h⁻¹ and the enzyme activity of beech mycorrhizas between 33 to 86.4 µmol pNP g⁻¹ fresh weight h⁻¹, depending on the measurement site. The highest AcPase activities were found at measurement sites No 5, 9 and 25 (Table 2).

The activity of extracellular AcPase in the soil horizon from which the mycorrhizas were taken varied between 6.8 at measurement site No 17 and 22.4 at measurement site No 5 (Table 2). An inverse significant correlation (p<0.05) between the concentration of extractable inorganic phosphorus in the soil layer 0–5 cm and the extracellular soil AcPase activity and the root-surface AcPase of ectomycorrhizas of Scots pine and beech was found (Fig. 5).

Nutrient content in fine roots

The concentrations of C, N, Pi, Ca, Mg and Al analysed in fine roots (0<1 mm) of Scots pine taken from the upper soil layer (0–5 cm) are presented in Table 3. The percentage of C in pine fine roots was similar at all sites (38–45,%) and the percentage of N varied between 1,20 and 1,91%. The concentration of Pi in...
Fig. 2. Microscope observations of ectomycorrhizas. A – crosssection of Scots pine mycorrhiza (scale mark=50 μm); B – crosssection of beech mycorrhiza (scale mark=50 μm); C – longitudinal section of Scots pine mycorrhiza (scale mark=200 μm); D – longitudinal section of beech mycorrhiza (scale mark=100 μm); m = fungal mantle, Hn = Hartig net, e = endodermis; M = meristem, Vc – vascular cylinder
pine fine roots was generally low (0.05–0.15%). The proportions of nitrogen in relation to C and Pi in fine roots are presented in Table 3.

Fine roots of Scots pine and beech accumulated high concentrations of extractable aluminium (Al\(^{3+}\)) and also high amounts of Ca and Mg. The Ca/Al and Mg/Al (mol/mol) ratios were calculated (Table 3).

**Discussion**

This study demonstrated the relations between soil parameters in a moderately polluted forest and some aspects of physiological activity of ectomycorrhizas of Scots pine and beech. We have focused on the concentration of available inorganic phosphorus in the soil and the efficiency of mycorrhizas in phosphorus mobilisation and accumulation of Pi in fine roots. The average concentration of Pi in the Ratanica catchment was relatively low (30.3 mg P\(_2\)O\(_5\) kg\(^{-1}\) of soil), as compared with other forest sites in Poland, however some differences in Pi content among the 17 measurement sites in the Ratanica forest were found (Table 1). Similar or lower levels of Pi in the upper soil layer have been found at severely polluted sites, such as Świerklaniec in Upper Silesia (5–8 mg P\(_2\)O\(_5\) kg\(^{-1}\) of soil), Szklarska Poręba in the Sudeten Mountains (9–12 mg P\(_2\)O\(_5\) kg\(^{-1}\) of soil) (Walendzik et al. 1995a,b) and in the vicinity of the Warsaw Steelworks (12–44 mg P\(_2\)O\(_5\) kg\(^{-1}\) of soil) (Kieliszewska-Rokitka 1999), whereas soil in relatively unpolluted areas (Biłowieża National Park and Kampinos National Park) contained higher concentrations of Pi (30–50 and 83 mg P\(_2\)O\(_5\) kg\(^{-1}\) of soil, respectively) (Walendzik et al. 1995a; Kieliszewska-Rokitka 1999). Soil in the Ratanica forest was further characterised by low soil pH, high concentrations of Al\(^{3+}\) (Table 1) and accumulation of heavy metals in the upper soil layers (Niklińska et al. 1995; Grodzińska and Laskowski 1996).

The average concentration of aluminium ions (Al\(^{3+}\)) in the upper soil layer in the Ratanica forest was relatively high (69.6 meq kg\(^{-1}\) of soil) as compared with forest sites characterised by strongly acidic soils, such as Szklarska Poręba (55 meq kg\(^{-1}\) of soil) and Świerklaniec and Biłowieża National Park (10–20 meq kg\(^{-1}\) of soil) (Walendzik et al. 1995a,b). High levels of Al\(^{3+}\) resulted from the dissolution of inorganic Al at low soil pH is considered as an important stress factor for tree roots in forest ecosystems,

![Fig. 3. Stages of ectomycorrhizal vitality, determined by FDA vital staining of hand cut medial longitudinal sections: 1. entirely vital – all regions of mycorrhiza are vital (the fungal mantle, the cortex including the Hartig net, the central cylinder, the meristem, 2. largely vital – most of the mycorrhizal compartments are vital (the cortex with the Hartig net, the central cylinder, the meristem), 3. reduced vital – only the central cylinder and meristem are vital, 4. dying mycorrhiza – only the central cylinder is vital, 5. dead mycorrhiza – all root compartments are dead (according to: Ritter et al. 1989)](image1)

![Fig. 4. Frequency of vitality stages of ectomycorrhizas of Scots pine (Pinus sylvestris L.) and beech (Fagus sylvatica L.) in 8 measurement sites of the Ratanica catchment (see: Fig. 1). Sixty to eighty mycorrhizas taken from 0–5 cm soil depth were classified according to 5 vitality stages (see: Fig. 3)](image2)
Table 2. Enzyme activity of surface acid phosphatase (AcPase) of excised ectomycorrhizas of Scots pine (*Pinus sylvestris* L.) and beech (*Fagus sylvatica* L.) and of soil at 17 measurement sites in Ratanica catchment (see: Fig. 1). Soil and mycorrhizal root tips were taken from a 0–5 cm depth. AcPase activities of pine and beech ectomycorrhizas are the means of 7–11 analyses ± SD and AcPase activities of soil are the mean of 6 analyses ± SD.

<table>
<thead>
<tr>
<th>Measurement sites</th>
<th>AcPase activity of ectomycorrhizas (mol pNP·g⁻¹ fresh weight·h⁻¹)</th>
<th>AcPase activity of soil (mol pNP·g⁻¹ dry weight·h⁻¹)</th>
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<td>pine mycorrhizas</td>
<td>beech mycorrhizas</td>
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<td>1</td>
<td>45.5 ± 3.1</td>
<td>44.7 ± 6.2</td>
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<td>5</td>
<td>66.7 ± 7.1</td>
<td>86.4 ± 16.6</td>
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<td>7</td>
<td>59.5 ± 18.0</td>
<td>66.6 ± 7.7</td>
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<td>9</td>
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<td>Mean</td>
<td>45.8 ± 14.4</td>
<td>53.0 ± 15.8</td>
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Fig. 5. The relationships between the concentration of extractable phosphorus measured as P₂O₅ in soil and enzyme activity of surface acid phosphatase (AcPase) of ectomycorrhizas of Scots pine (*Pinus sylvestris* L.) (A) and beech (*Fagus sylvatica* L.) (B) and soil AcPase (C). Soil samples and mycorrhizal root tips were taken from 0–5 cm depth at 17 measurement sites in the Ratanica forest catchment (see: Fig. 1). AcPase activities of pine and beech ectomycorrhizas were means of 7–11 analyses at each site, and AcPase activities of soil samples were means of 6 analyses.
which inhibits the uptake and transport of essential cations (Ca$^{2+}$, Mg$^{2+}$) and important anions (PO$_4$$^{3-}$, SO$_4$$^{2-}$) (Foy et al. 1978). It is accepted that aluminium toxicity is related to a decreased Ca/Al ratio (<1.0) than to Al$^{3+}$ concentration alone (Ulrich 1983; Rost-Siebert 1983). The mobile Al$^{3+}$ and heavy metal ions in the soil may reduce P concentration by immobilisation in metal-polyphosphate complexes as confirmed by Turnau et al. (1993) who found a high content of Al, Pb, Cd, Zn in polyphosphate granules localised in the fungal vacuoles of Paxillus involutus/Pinus sylvestris mycorrhizas taken from a heavily polluted forest. Scots pine seedlings grown in the presence of increased levels of Al$^{3+}$ in the soil substrate had decreased concentrations of Pi in the root and shoot of mycorrhizal seedlings, as compared with the control (Kieliszewska-Rokicka et al. 1998; Rudawska et al. 2000). Moreover, acid deposition and high concentrations of heavy metals may be toxic to soil microorganisms involved in the decomposition of organic matter and nutrient cycling (Bååth 1989; Zwoliński 1995) and can inhibit enzymatic systems including acid phosphatase (Tyler 1974; Tyler et al. 1989; Kuperman and Carreiro 1997). One of mechanisms by which mycorrhizal fungi and mycorrhizas may increase the uptake of phosphorus from soils under Pi-limiting conditions is the production of a root-surface AcPase (Harley 1989; Smith and Read 1997).

Our results revealed an inverse correlation between the concentration of Pi in the soil and the activities of the root-surface and soil extracellular AcPase. This indicates that the functioning of a feedback mechanism which induces the AcPase activity in response to a deficiency of plant and soil Pi as shown for ectomycorrhizal fungi and ectomycorrhizas (i.e. Alexander and Hardy 1981; MacFall et al. 1991; Kieliszewska-Rokicka 1992; Pasqualini et al. 1992) and for forest soil (i.e. Spiers and McGill 1979). Increased activities of the root-surface AcPase in Ratanica forest (Table 2, Fig. 5) suggest a high physiological activity of ectomycorrhizas as confirmed by FDA vital staining (Figs. 2, 4). The frequency of vital mycorrhizas, containing vital fungal hyphae (stages 1, 2) ranged between 20 and 40%, whereas the percentage of dead mycorrhizas was relatively low (between 10 and 18%). Similar results have been reported for unpolluted and moderately polluted forest sites in Poland (Józefaciukowa et al. 1995) and in Germany (Kottke et al. 1993; Münzenberger et al. 1995).

In trees, the concentration of elements in the fine roots is regarded as a better indicator of the nutritional conditions in the forest soil than the foliar concentration (Joslin et al. 1988; Person and Ahlström 1991). In spite of the increased phosphatase activity, the concentrations of Pi and the weight proportions

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Scots pine</th>
<th>Beech</th>
</tr>
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<tbody>
<tr>
<td>C (%)</td>
<td>42.3 ± 2.4</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>(39.3–44.8)</td>
<td></td>
</tr>
<tr>
<td>N (%)</td>
<td>1.58 ± 0.22</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>(1.20–1.91)</td>
<td></td>
</tr>
<tr>
<td>PO$_4$ – P (%)</td>
<td>0.093 ± 0.029</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>(0.05–0.15)</td>
<td></td>
</tr>
<tr>
<td>C/N (weight ratio)</td>
<td>26.8 ± 3.95</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>(21.2–34.7)</td>
<td></td>
</tr>
<tr>
<td>N : P (weight ratio)</td>
<td>100 : 5.8</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>(100 : 4.1–100 : 7.9)</td>
<td></td>
</tr>
<tr>
<td>Ca (mg g$^{-1}$ dry weight)</td>
<td>4.04 ± 0.75</td>
<td>3.73 ± 0.59</td>
</tr>
<tr>
<td></td>
<td>(3.26–4.75)</td>
<td>(3.15–4.33)</td>
</tr>
<tr>
<td>Mg (mg g$^{-1}$ dry weight)</td>
<td>1.77 ± 0.41</td>
<td>1.55 ± 0.28</td>
</tr>
<tr>
<td></td>
<td>(1.40–2.2)</td>
<td>(1.27–1.83)</td>
</tr>
<tr>
<td>Al (mg g$^{-1}$ dry weight)</td>
<td>1.69 ± 0.58</td>
<td>1.36 ± 0.62</td>
</tr>
<tr>
<td></td>
<td>(1.14–2.30)</td>
<td>(0.67–1.90)</td>
</tr>
<tr>
<td>Ca/Al (mol/mol)</td>
<td>1.79 ± 0.75</td>
<td>2.39 ± 1.74</td>
</tr>
<tr>
<td></td>
<td>(0.96–2.44)</td>
<td>(1.13–4.36)</td>
</tr>
<tr>
<td>Mg/Al (mol/mol)</td>
<td>1.28 ± 0.53</td>
<td>1.89 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>(0.67–1.67)</td>
<td>(0.75–3.03)</td>
</tr>
</tbody>
</table>

Table 3. Concentrations of nutrients in fine roots (Ø<2 mm) of Scots pine and beech in the Ratanica forest catchment. Data represent means and ranges of the nutrient concentrations and proportions in root samples taken from 0–5 cm depth at 12 measurement sites.
N/P in pine fine roots were below the range considered as optimal. According to Ingestad (1979), the optimum weight proportion of nitrogen to phosphorus for Scots pine is 100 N : 15 P. In this study Pi concentration was 2–3 times below the optimum resulting in a 100 N : 5.8 P average (Table 3).

A low Ca/Al (<0.1 mol/mol) in fine roots is considered as an indicator of the toxic effect of aluminium (Meiwes et al. 1986). Although the Ca/Al ratio in the soil of the Ratanica forest was much below the level believed as safe for plants (Table 2), the Ca/Al in fine tree roots was relatively high (0.96–2.44 in Scots pine and 1.13–4.34 in beech) (Table 3). The average Ca/Al ratio in fine roots in the Ratanica forest was higher than that found by Józefaciukowa et al. (1995) in fine roots (<2 mm) of Scots pine from 11 forest sites in Poland (Ca/Al=0.1–0.7) and by Persson and Majdi (1995) in fine roots (<2 mm) of mature Scots pine (Ca/Al=0.11–0.22) and Norway spruce (0.05–0.57) in various Swedish forest stands. Similarly, no deficiency of Mg was found in the fine roots of pine and beech from the Ratanica catchment (Table 3). Keltjens and van Loenen (1989), in hydroponic experiments, have found a higher accumulation of Al in roots of coniferous evergreens (Scots pine and Douglas-fir) than in deciduous broad-leaved species (oak and birch) and intermediate Al levels in larch. The differences in Al concentrations in fine roots of Scots pine and beech from Ratanica forest were not significant.

These results led us to the conclusion that the long-term influence of air pollution that induced significant changes in soil chemistry in the Ratanica forest catchment caused a moderately disadvantageous effect on the functioning of ectomycorrhizas of Scots pine and beech, as demonstrated by some nutrient imbalance in the fine roots of these trees. The anatomical structure of ectomycorrhizas, the relatively high frequency of vital ectomycorrhizas and the high activity of the enzyme involved in the nutritive metabolism (AcPase) showed that the physiological activity was not inhibited by the anthropogenic stress factors, however it was insufficient with respect to phosphate mobilisation and uptake. The high buffer capacity of the soil in Ratanica forest, especially in the organic soil layer, reported by Grodzińska and Laskowski (1996), could alleviate the potentially harmful effects of increased levels of aluminium and heavy metals ions in the soil.

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Aktywność fizjologiczna ektomikoryz w średnio zanieczyszczonym lesie (zlewnia potoku Ratanica, południowa Polska)

Streszczenie

Badania ektomikoryz sosny (*Pinus sylvestris* L.) i buka (*Fagus sylvatica* L.) prowadzono na Pogórzu Karpackim w dorosłym drzewostanie, który przez ponad 40 lat znajdował się pod wpływem gazowych i pyłowych zanieczyszczeń powietrza o średnich stężeniach. Gleby badanego ekosystemu leśnego czechowało niskie pH oraz znaczna akumulacja metali ciężkich (Pb, Mn, Zn, Cu, Cd, Fe). Badano zależność między stężeniem nieorganicznego fosforanu w glebie, a aktywnością enzymatyczną kwaśnej fosfatazy mikoryz i gleby. Analizowano także stężenia pierwiastków odżywczych (C, N, P, Ca, Mg) oraz jonów glin (Al$^{3+}$) w korzeniach drobnych. Mikoryzy sosny i buka były analizowane pod względem budowy anatomicznej oraz żywotności. Stwierdzono stosunkowo wysokość stężenie jonów Al$^{3+}$ w glebie (40–118 meq kg$^{-1}$) i niski poziom nieorganicznego fosforanu (Pi) (12–44 mg P$_2$O$_5$ kg$^{-1}$). Jednocześnie zarejestrowano wysoką aktywność powierzchniowej kwaśnej fosfatazy mikoryz sosny i buka (odpowiednio, 25–67 i 33–86 µmoli pNP g$^{-1}$ świeżej masy h$^{-1}$) oraz kwaśnej fosfatazy gleby (6,8–22,4 µmoli pNP g$^{-1}$ suchej masy h$^{-1}$). Stężenia fosforu w korzeniach drobnych (poniżej stężeń uważanych za optymalne) oraz wysoki stosunek N/P pokazują, że pobieranie i akumulację fosforu przez systemy korzeni drobnych sosny i buka jest niedostateczne, mimo wysokiej aktywności kwaśnej fosfatazy. Budowa anatomiczna mikoryz, stosunkowo duży udział żywych mikoryz w systemie korzeni drobnych oraz wysoki stosunek Ca/Al w korzeniach sosny i buka (odpowiednio, 1,79 i 2,38) sugerują, że mechanizmy obronne mikoryz i mikroorganizmów ryzosfery mają zdolność łagodzenia wpływu zanieczyszczeń antropogenicznych.