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Effect of ammonium and nitrate nutrition on hydrolytic enzymes activity of Scots pine (*Pinus sylvestris* L.) roots and phosphorus content in shoots*

Abstract

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One-year-old pine seedlings transferred from nursery were cultivated in unsterilized forest soil in a greenhouse. Various forms and levels of nitrogen in nutrient solutions resulted after 10 months in various growth and mycorrhizal development of the seedlings. Nitrogen fertilization stimulated enzyme activity of acid phosphatase, α-galactosidase and β-glucosidase of roots and the cell-free acid phosphatase in soil. Ammonium nitrogen was more favourable for mycorrhizae development and the acid phosphatase activity in roots than nitrate supply. The phosphorus content was significantly higher in shoots of fertilized than of control seedlings.

Additional keywords: unsterilized soil, ectomycorrhizae, acid phosphatase, α-galactosidase, β-glucosidase.

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INTRODUCTION

Mycorrhizal symbiosis stimulates growth of mycorrhizal plants and improves their mineral nutrition, particularly phosphate nutrition. Mycorrhizal associations increase phosphatase activity of roots (Mousain and Salsac, 1984; Gianinazzi-Pearson and Gianinazzi, 1986) and phosphorus content in plant shoots (Bledsoe and Zasoski, 1983).

Some soluble acid phosphatase isoenzymes were found located on the outer face of plasmalemma making them readily accessible to substrate outside the cell (Zink and Veliky, 1979). This surface-localized activity appears to play an important role in utilization of soil phosphorus (Bowen, 1973).

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Various nitrogen forms (ammonium, nitrate) and levels influence mycorrhizal development of coniferous trees (Laiho, 1970; Bledsoe and Zasoski, 1983; Rudawska, 1986). Relations between nitrogen nutrition and activities of phosphatases in plant tissues have scarcely been investigated (Hewitt and Tatham, 1960; O'Connell and Grove, 1985; Kieliszewska-Rokicka, 1989).

In this paper the effects of various nitrogen sources and levels on the soluble acid phosphatase activity and phosphorus content of mycorrhizal Scots pine seedlings were studied.

MATERIAL AND METHODS

CULTIVATION OF PINE SEEDLINGS

One-year-old Scots pine seedlings with unidentified ectomycorrhizae were transferred from a nursery into the greenhouse and potted in forest soil. Seedlings were fertilized for 10 months twice a week with nutrient solutions in which the nitrogen sources and concentrations varied but the concentrations of other macro- and micronutrients were the same. The control seedlings received no nitrogen. The nitrogen sources and levels used in the experiment were presented in Table 1. Concentrations of nitrogen sources were chosen following Rudawska (unpublished data), who found that levels of nutrients higher than 1 mM in the case of urea and 2 mM in the case of ammonium tartrate caused an inhibition of seedlings growth after some months of fertilization.

Table 1

Concentration of nitrogen in nutrient solutions

Nitrogen source	Nirtogen concentration (mM)	Amount of nitrogen (mg seedling^{-1} week^{-1})
Control	0.00	0
Urea	0.36	2
Urea	0.90	5
Ammonium tartrate	0.36	2
Ammonium tartrate	1.79	10
Calcium nitrate	0.36	2
Calcium nitrate	1.79	10
Calcium nitrate	3.58	20

PREPARATION OF THE SOLUBLE ENZYME FRACTION

Pine roots were ground in liquid nitrogen and washed with 80% ice cold acetone followed by anhydrous ice cold acetone to obtain acetone powder. Soluble proteins were extracted with 100 mM acetate buffer pH 5.0 containing 0.01% Triton X100, 10 mM EDTA and 2.5% polyclar AT. After centrifugation the supernatant was used as the soluble enzyme fraction.

ENZYME ASSAYS

To assay accessible phosphatase activity, root tips were washed with distilled water and weighed. Samples of 20 mg fresh weight were placed in a medium consisting of 1.9 ml acetate buffer pH 4.8 and 100 μl of 40 mM p-nitrophenyl phosphate. Incubation took place at 35°C for 30 minutes. The reaction was stopped by adding 2.5 ml of 0.2 M Na_2CO_3 and the liberated p-nitrophenol determined at 400 nm. Enzyme activity was expressed as nkatal $(1\ g\ root\ tips)^{-1}$.

To assay total soluble phosphatase activity, 250 μl 0.1 M acetate buffer pH 4.8, 50 μl of 15 mM p-nitrophenyl phosphate and 50 μl of the soluble enzyme fraction were mixed and incubated for 30 minutes at 35°C. The reaction was stopped by addition of 2.5 ml of 0.2 M Na_2CO_3 and liberated p-nitrophenol determined at 400 nm. Total enzyme activity was expressed as nkatal $(100\ mg\ acetone\ powder)^{-1}$.

To assay α-galactosidase and β-glucosidase activities, 250 μl phosphate/citrate buffer (pH 5.0; 0.2/0.1 M), 50 μl of 10 mM p-nitrophenyl-α-D-galactopyranoside or p-nitrophenyl-β-D-glucopyranoside (Sigma Chemical Co, USA), respectively, and 100 μl of enzyme preparation were mixed and incubated for 1 hour at 35°C. The reaction was stopped by addition of 2.5 ml 0.2 M Na_2CO_3 and p-nitrophenol released was determined at 400 nm. Enzyme activities were expressed as $pkatal\ (100\ mg\ acetone\ powder)^{-1}$.

Acid phosphatase activity in soil was determined by using of p-nitrophenyl phosphate as a substrate as described by Tabatabai and Bremner (1969).

PHOSPHORUS ANALYSIS

Phosphorus content in pine needles was measured with the method of Kuttner and Lichtenstein (1932) as modified by Humphries (1956).

RESULTS

Table 2

Effect of various nitrogen sources and concentrations on growth of Scots pine seedlings and mycorrhizal development

Nitrogen source	Nitrogen concentr. [mM]	Fresh weight [g] shoot [1]	Fresh weight [g] root [1]	Mycorrhizal development [2]
Control	0.00	18.8a	17.1b	+++
Urea	0.36	24.5ab	14.7a	+++
Urea	0.90	32.3b	17.4b	+
Ammonium tartrate	0.36	30.4b	19.3bc	++
Ammonium tartrate	1.79	45.8d	19.9c	+
Calcium nitrate	0.36	28.8b	14.1a	–
Calcium nitrate	1.79	27.0b	17.4b	–
Calcium nitrate	3.58	35.4bc	17.1b	–

[1] Values in each row followed by the same letter do not differ significantly ($p=0.01$).

[2] +++ strong mycorrhizal infection, ++ moderate mycorrhizal infection, + slight mycorrhizal infection, – no infection.

ACCESSIBLE ACID PHOSPHATASE ACTIVITY

Acid phosphatase activity of excised root tips was stimulated by urea 0.36 mM, ammonium tartrate 1.79 mM and calcium nitrate 1.79 mM as compared to the non-fertilized control (Fig. 1). Low concentration of nitrogen (0.36 mM) both in ammonium and nitrate forms and the highest level of calcium nitrate (3.58 mM) did not increase the accessible acid phosphatase.

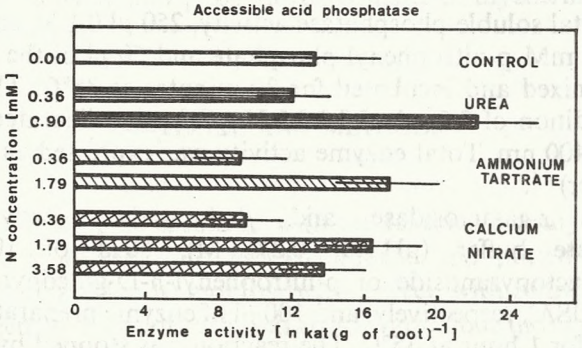

Fig. 1. Activity of accessible acid phosphatase of excised roots of Scots pine seedlings supplied with various forms and levels of nitrogen. Error bars indicate standard deviations

TOTAL SOLUBLE ENZYME ACTIVITY OF PINE ROOTS

Acid phosphatase activity was stimulated by all nitrogen treatments except of urea at higher concentration (0.90 mM) and calcium nitrate at the highest concentration (3.58 mM) (Fig. 2). The stimulation was most pronounced in roots of seedlings fertilized with higher level of ammonium tartrate (1.79 mM).

α-galactosidase activity was stimulated by the lower concentration of urea (0.36 mM), both concentrations of ammonium tartrate (0.36 mM and 1.79 mM) and higher concentrations of calcium nitrate (1.79 mM and 3.58 mM). At the higher level of urea (0.90 mM) and the lowest concentration of calcium nitrate no stimulation of soluble α-galactosidase was found (Fig. 3).

β-glucosidase activity was stimulated markedly by lower urea level (0.36 mM), higher concentration of ammonium tartrate (1.79 mM) and higher levels of calcium nitrate (1.79 mM and 3.58 mM). Stimulation by 3.58 mM calcium nitrate was most pronounced (Fig. 4). In roots treated with 0.90 mM urea and 0.36 mM calcium nitrate the activity of β-glucosidase was at the same level as in the control.

ACID PHOSPHATASE ACTIVITY IN SOIL

Figure 5 shows that nitrogen sources and levels influenced activity of cell-free acid phosphatase. Lower concentration of urea (0.36 mM) did not influence significantly this activity but higher level (0.90 mM) decreased it.

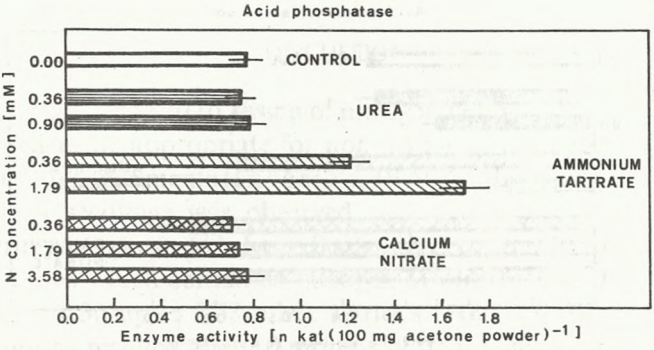

Fig. 2. Activity of total soluble acid phosphatase of roots of Scots pine seedlings supplied with various forms and levels of nitrogen

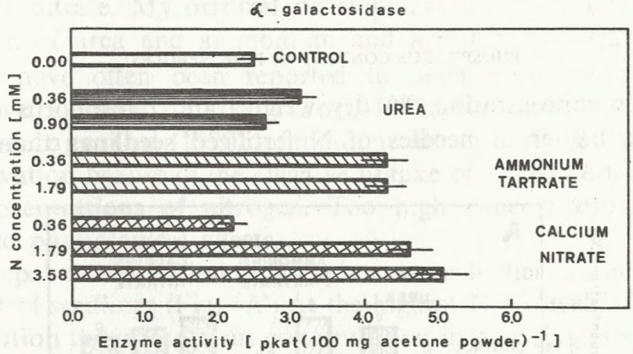

Fig. 3. Activity of total soluble α-galactosidase of roots of Scots pine seedlings supplied with various forms and levels of nitrogen

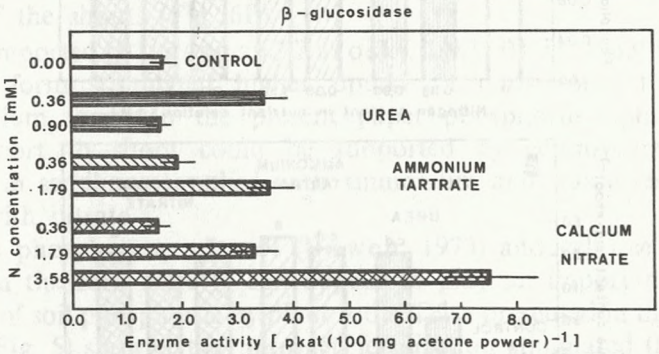

Fig. 4. Activity of total soluble β-glucosidase of roots of Scots pine seedlings supplied with various forms and levels of nitrogen

Ammonium tartrate at lower concentration (0.36 mM) stimulated the activity but at a higher level (1.79 mM) decreased it very markedly. Calcium nitrate stimulated acid phosphatase in the soil at all three concentrations used in the experiment (0.36 mM, 1.79 mM, 3.58 mM).

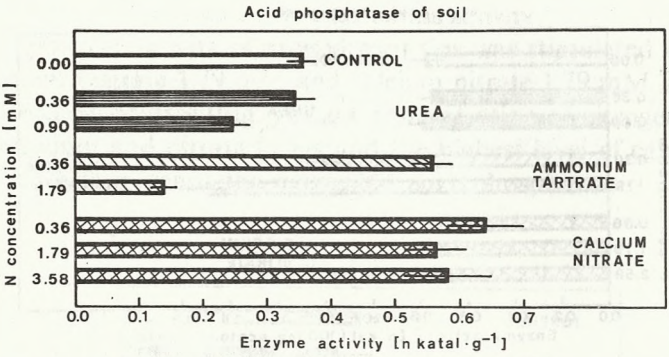

Fig. 5. Activity of acid phosphatase of soil after 10 months of fertilization with various forms and levels of nitrogen

PHOSPHORUS CONTENT IN PINE NEEDLES

Phosphorus concentration (% dry weight) and phosphorus content (mg shoot^{-1}) were higher in needles of N fertilized seedlings than in control seedlings (Fig. 6A, B).

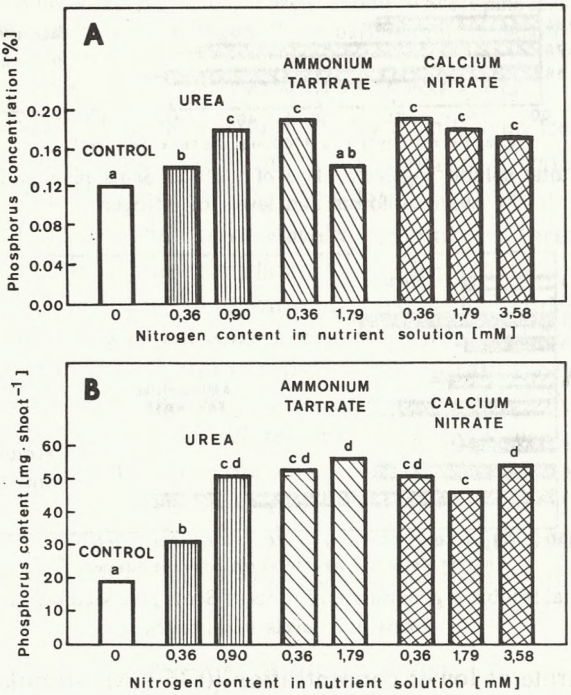

Fig. 6. Phosphorus concentration (A) and phosphorus content (B) in shoots of Scots pine seedlings supplied with various forms and levels of nitrogen. Columns with the same letters do not differ significantly ($p = 0.05$)

DISCUSSION

Concentration range 20 to 50 mg of nitrogen per liter (about 1.5 to 3.5 mM) was considered to be appropriate for non-mycorrhizal Scots pine seedlings in nutrient solution experiments (Ingestad, 1979). In this range optimum uptake of nutrients by seedlings was observed.

In experiments presented here Scots pine seedlings were treated with nutrient solutions which contained nitrogen at optimal or lower than optimal concentrations (0.36 up to 3.58 mM). Various forms and levels of nitrogen in nutrient solutions resulted after 10 months of treatment in various growth and mycorrhizal development of seedlings. Total biomass of seedlings treated with ammonium tartrate was significantly higher than biomass of seedlings supplied with urea or nitrate. Mycorrhizal development was reduced by the higher concentration of urea and ammonium and arrested by nitrate (Table 2).

Conifers have often been reported to react positively to ammonium nitrogen (Evers, 1963; Christersson, 1972; Nelson and Selby, 1974). According to Ingestad (1979) ammonium nitrogen should be preferred in conifer cultivation beause of the effective uptake of ammonium. This concerns optimal concentrations of nitrogen. Too high concentrations could exert unfavourable physiological effects.

In this paper concentration of phosphorus was higher in shoots of fertilized than of control seedlings (Fig. 6A). At the highest NH_4^+ level (1.79 mM) in the nutrient solution the phosphorus concentration in pine shoots was lower than in shoots of seedlings treated with nitrate (Fig. 6A), however the phosphorus content of ammonium in supplied seedlings was higher because of high biomass of the shoots (Fig. 6B).

It was reported (Bledsoe and Zasoski, 1983; Bhat, 1983) that nitrogen in nitrate form stimulated higher uptake of phosphorus than nitrogen in ammonium form. In the present paper phosphorus uptake by roots and transport to shoot could be supported by ectomycorrhizae which developed in seddlings supplied with ammonium and was arrested in those supplied with nitrate.

Soluble phosphatases of root (Bowen, 1973) and cell-free phosphatase localized in the root zone of soil appear to play an important role in the utilization of soil phosphorus by plant roots. Data presented in this paper (Fig. 1, Fig. 2, Fig. 5) showed that nitrogen fertilization stimulated the activity of acid phosphatases which when released by pine roots and microorganisms to the soil could increase the pool of available phosphorus and enhance its content in pine shoots. However ammonium was more favourable for acid phosphatase activity of root than nitrate. Simultaneously higher levels of nitrate stimulated enzyme activities of other hydrolytic enzymes of pine root (Fig. 3, Fig. 4). Stimulation by ammonium nitrogen of soluble acid phosphatase

activity of Scots pine seedlings grown under axenic conditions was presented elsewhere (Kieliszewska-Rokicka, in the press).

Phosphorus uptake and transport from root to shoot appears to be an active process which is controlled by a negative feedback signal between shoot and root (Cram, 1976; Pitman and Cram, 1977). Schjørring and Jensén (1987) showed that the amount of phosphorus utilized from reserves was strongly reduced or almost eliminated in the presence of inhibitors of protein synthesis. It is thus possible that nitrogen supply and nitrogen status of plants could be important factors influencing biosynthesis of proteins involved in the processes of phosphorus release and transport.

LITERATURE

Bhat K. K. S., 1983. Nutrient inflows into apple roots. Plant and Soil, 71: 371–380.

Bledsoe C., Zasoski R. J., 1983. Effect of ammonium and nitrate on growth and nitrogen uptake by mycorrhizal Douglas-fir seedlings. Plant and Soil, 71: 445–454.

Bowen G. D., 1973. Mineral nutrition of ectomycorrhizae. In: Ectomycorrhizae. Their Ecology and Physiology. G. C. Marks and T. T. Kozlowski (eds.), Academic Press, New York, pp. 151–205.

Christersson L., 1972. The influence of urea and other nitrogen sources on growth rate of Scots pine seedlings. Physiol. Plant., 27: 83–88.

Cram W., 1976. Negative feedback regulation of transport in cells. The maintenance of turgor, volume and nutrient supply. In: Encyclopedia of Plant Physiology, New Series U. Lüttge and M. G. Pitman (eds.), Vol. IIA, Springer-Verlag, Berlin, pp. 284–316.

Evers F. H., 1963. Die Wirkung von Ammonium- and Nitratstickstoff auf Wachstum und Mineralstoffhaushalt von *Picea* und *Populus*. III. Einfluss auf den Mineralstoffhaushalt und Auswirkungen auf das Wachstum. Z. Bot., 51: 91–111.

Gianinazzi-Pearson V., Gianinazzi S., 1986. The physiology of improved phosphate nutrition in mycorrhizal plants. In: Physiological and Genetical Aspects of Mycorrhizae. V. Gianinazzi-Pearson and S. Gianinazzi (eds.), I. N. R. A., Paris, pp. 101–109.

Hewitt E. J., Tatham P., 1960. Interaction of mineral deficiency and nitrogen source on acid phosphatase activity in leaf extracts. J. Exp. Bot., 11: 367–376.

Humphries E. C., 1956. Mineral components and ash analysis. In: Moderne Methoden der Pflanzenanalyse. K. Peach and M. V. Tracey (eds.), Springer Verlag, V. I, p. 487.

Ingestad T., 1979. Mineral nutrient requirements of *Pinus sylvestris* and *Picea abies* seedlings. Physiol. Plant., 45: 373–380.

Kieliszewska-Rokicka B., 1989. Acid phosphatase activity in roots of Scots pine (*Pinus sylvestris* L.) seedlings fertilized with different nitrogen sources. Agric. Ecosystems Environ., 28: 229–234.

Kieliszewska-Rokicka B., 1991. Acid phosphatase activity in mycorrhizal and non-mycorrhizal Scots pine seedlings in relation to nitrogen and phosphorus nutrition. Acta Soc. Bot. Pol., in the press.

Laiho O., 1970. *Paxillus involutus* as a mycorrhizal symbiont of forest trees. Acta Forest. Fenn., 106: 1–72.

Mousain D., Salsac L., 1984. Effet du phosphate et du calcium sur la croissance et l'accumulation ionique chez les champignons ectomycorhiziens en culture *in vitro*, Can. J. Bot., 62: 2600–2609.

Nelson L. E., Selby R., 1974. The effect of nitrogen sources and iron levels on the growth and composition of Sitka spruce and Scots pine. Plant and Soil, 41: 573–588.

O'Connell A. M., Grove T. S., 1985. Acid phosphatase activity in karri (*Eucalyptus diversicolor*) in relation to soil phosphate and nitrogen supply. J. Exp. Bot., 36: 1359–1372.

Pitman M. G., Cram W. J., 1977. Regulation of ion content in whole plants. Symp. Soc. Exp. Biol., 31: 391–424.

Rudawska M., 1986. Sugar metabolism of ectomycorrhizal Scots pine seedlings as influenced by different nitrogen forms and levels. In: Physiological and Genetical Aspects of Mycorrhizae. V. Gianinazzi-Pearson and S. Gianinazzi (eds.), I. N. R. A., Paris, 389–394.

Schjørring J. K. and Jensén P., 1987. Phosphorus export from roots to shoots of barley, buckwheat and rape seedlings with different P status. Physiol. Plant., 70: 58–64.

Tabatabai M. A., Bremner J. M., 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil. Biol. Biochem., 1: 301–307.

Zink M. W., Veliky I. A., 1979. Acid phosphatases of *Ipomea* sp. cultured *in vitro*. I. Influence of pH and inorganic phosphate on the formation of phosphatases. Can. J. Bot., 57: 739–753.

Wpływ nawożenia amonowego i azotanowego na aktywność enzymów hydrolitycznych korzeni i na zawartość fosforu w pędach sosny zwyczajnej (*Pinus sylvestris* L.).

Streszczenie

Jednoroczne siewki sosny przeniesiono ze szkółki leśnej do szklarni, posadzono w niesterylizowanej glebie leśnej i podlewano pożywkami zawierającymi azot w różnych formach chemicznych (mocznik, winian amonowy, azotan wapniowy) i stężeniach (w zakresie od 0.36 mM do 3.58 mM).

Mocznik i winian amonowy w niższych stężeniach wpływały korzystnie na rozwój mikoryz. Wyższe stężenia azotu w formie mocznika (0.9 mM) i w formie winianu amonowego (1.79 mM) ograniczyły znacznie, a azotan (we wszystkich stężeniach) zahamował powstawanie mikoryz.

Zawartość fosforu w pędach siewek nawożonych była istotnie wyższa niż w pędach siewek kontrolnych. Nawożenie silnie stymulowało aktywność enzymatyczną kwaśnej fosfatazy, α-galaktozydazy i β-glukozydazy w korzeniach siewek oraz kwaśnej fosfatazy zawartej w glebie. Aktywność kwaśnej fosfatazy korzeni była wyższa w siewkach hodowanych w obecności azotu amonowego, natomiast aktywność kwaśnej fosfatazy gleby była silnie stymulowana w obecności azotu w formie azotanu.

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