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Maria Rudawska, Barbara Kieliszewska-Rokicka, Tomasz Leski

Effect of aluminium on *Pinus sylvestris* seedlings mycorrhizal with aluminium-tolerant and aluminium-sensitive strains of *Suillus luteus*

Abstract: Mycorrhizal syntheses of pine seedlings were conducted with *Suillus luteus* (L.) S.F. Gray, a strain No 14 characterised by high tolerance to Al^{3+} ions and a strain No 62, sensitive to aluminium. The experiment was performed as a semi-sterile culture in a peat-perlite medium with Al^{3+} ion concentration of 11 mM. Abundant coralloid and cluster mycorrhizas of *S. luteus* were formed on roots of the inoculated plants at the beginning of the experiment. Aluminium treatment limited mycorrhizal morphotypes to single and dichotomous and significantly reduced the total number of mycorrhizal tips but had no effect on extramatrical mycelium development in the potting substrate. Al treatment did not affect growth of the above-ground part of the tested plants but significantly reduced root growth of mycorrhizal seedlings. The effect of Al on the internal nutrient status was variable and not very much pronounced. A considerable amount of Al was absorbed by the roots and translocated to the shoots. Mycorrhiza formation with both strains of *S. luteus* did not prevent Al^{3+} translocation to the upper parts of the tested seedlings. The results suggest that low pH and high Al availability may harmfully influence mycorrhizal symbiosis of Scots pine (*Pinus sylvestris* L.) by a negative effect on fine-root production and fewer short root tips available for colonisation rather than through a direct negative effect of Al^{3+} ion concentrations on extramatrical mycelium in the soil.

Additional key words: ectomycorrhizal fungi, extramatrical mycelium, ergosterol

Address: M. Rudawska, B. Kieliszewska-Rokicka, T. Leski, Institute of Dendrology, 62-035 Kórnik, Poland

Introduction

Acidification of forest soils due to environmental pollution leads to elevated aluminium (Al) concentrations that may damage tree roots, giving rise to a reduced vitality of trees (Ulrich 1981). Acidic, sandy and nutrient poor soils with a low buffering capacity cover the largest forest areas of the Polish Lowland. On such soils, high rates of atmospheric deposition of acidic compounds (SO_2, NO_X, NH_3) may lead to an extremely low soil pH and consequently, to an increased availability of Al^{3+} ions in the soil solution. This may negatively influence Scots pine which is a dominant forest tree species in these areas. As Scots pine trees occur naturally on acidic soils, they have presumably evolved some degree of tolerance to Al in the soil solution. However, the ability to withstand the acidity and aluminium toxicity is restricted and Al should be considered a possible growth-limiting factor for pines on sites with increasing anthropogenic acidification. Ectomycorrhizal fungi and ectomycorrhizas are obligatorily linked with roots of coniferous trees and can increase the ability of plants to resist environmental stresses (Godbold et al. 1998). Reports on the role of mycorrhizas in the response of trees to Al treatment have often been contradictory. Mycorrhizas formed by *Paxillus involutus* with Norway spruce (*Picea abies*) and by *Pisolithus tinctorius* with pitch pine (*Pinus rigida*) prevented Al toxicity of seedlings (Wilkins and Hodson 1989; Cumming and Weinstein 1990; Hentchel et al. 1993). Also infection of *Pinus massoniana* with the mycorrhizal fungus *Pisolithus*

tinctorius increased the ability of the plant to resist the toxicity of artificial acid rain and Al stress (Kong et al. 2000). Göransson and Eldhuset (1991) showed that nonmycorrhizal Scots pine seedlings and seedlings ectomycorrhizal with *Suillus bovinus* responded in a similar way to increasing aluminium concentrations. At the same time Jentchke et al. (1991) did not find any beneficial effect of mycorrhizas with *Lactarius rufus* and *L. theiogallus* in preventing Al toxicity in Norway spruce seedlings. The above data as well as other findings, reviewed by Godbold et al. (1988), clearly show different roles of ectomycorrhizal fungi and ectomycorrhizas in amelioration of metal stress in forest tress. A number of ambiguous results may reflect a great variability in response of different tree species to aluminium (Schaedle et al. 1989) and a high variation in Al response between species and even strains of ectomycorrhizal fungi (Hintikka 1988; Browning and Hutchinson 1991; Jongbloed and Borst-Pauwels 1992; Leski et al. 1995; Rudawska and Leski 1998).

Our previous field and laboratory studies (Rudawska et al. 1996; Kieliszewska-Rokicka et al. 1998) indicated some negative impact of low pH and Al on mycorrhizal symbiosis of Scots pine and, on the other hand, a possibility of amelioration of aluminium stress by some selected *Suillus luteus* strains. *S. luteus*, a very common mycorrhizal symbiont of young Scots pine trees, proved to be very tolerant to aluminium when tested in pure culture condition (Leski et al. 1995). Strains of *S. luteus* originating from polluted areas (i.e. No 14) showed a considerable resistance to high Al concentration in pure culture conditions compared to strains from unpolluted stands at Puszcza Nadnotecka (i.e. No 62).

Many authors warn against extrapolating results from axenic culture to mycorrhizal symbiosis where at least two partners are involved. To date, there have been no reports considering whether the diverse responses of mycorrhizal fungi to Al in axenic culture could be reflected in similar responses in the symbiotic conditions. This is why this study was undertaken. Two strains of *Suillus luteus*: aluminium-tolerant (No 14) and aluminium-sensitive (No 62) were used for inoculation of Scots pine seedlings in semiaseptic conditions and the influence of low pH and aluminium on growth and mycorrhiza development was determined. Aluminium susceptibility was examined at Al concentration of 11 mM, representative of solution of highly polluted acidic forest soils (Eldhuset et al. 1987; Rudawska et al. 1996).

Material and methods

Fungal and plant material culture

Suillus luteus strains: No 14 and No 62, selected in a previous study (Leski et al. 1995) as tolerant and sensitive to aluminium respectively, were used for inoculation of Scots pine seedlings. Details of the origin, isolation and the criteria of selection were described in other papers (Leski et al. 1995; Rudawska et al. 1996). Mycelia for inoculation were cultivated in Petri dishes in a modified agar medium (Tomaszewski and Wojciechowska 1974) containing: 55 mM glucose, 14 mM maltose, 6.25 mM NH_4NO_3, 3.7 mM KH_2PO_4, 2 mM $MgSO_4$, 0.15 μM thiamine-HCl, 0.02 μM biotine, 0.1% (v/v) of a trace-element stock solution and agar (9 g l^{-1}). Fungal cultures had been cultivated in the darkness at 24°C for 3 weeks. Then the mycelia were transferred aseptically into distilled water, rinsed several times and homogenised for 5 seconds. Mycelial slurry of each strain was added to the potting substrate (mixture of 1 volume of peat and 4 volumes of sterilised perlite) in proportion 1:12 (v/v) in large plastic bags and shaken to distribute the inoculum evenly. The potting substrate with the inoculum was placed into plastic pots (volume 2,2 dm^3) and covered with a 2-cm layer of the peat-perlite containing no mycelium.

Seeds of *Pinus sylvestris* (L.), collected from the seed orchard of the Institute of Dendrology in Kórnik, Poland, were surface sterilised in 30% hydrogen peroxide for 20 min, rinsed with sterile water and germinated in the peat-perlite substrate (1:4) in a growth chamber at a high light intensity (7500 mW m^{-2}), a 16-h photoperiod, and a temperature of 25:20°C (light : dark). Five weeks old pine seedlings were transplanted into pots that were divided into six study groups: non-treated and Al treated controls (designated -Al, -S.lut. and +Al, -S.lut.), plants inoculated with the Al tolerant *S. luteus* strain No 14 (designated –Al, +S.lut. and +Al, +S.lut.) and plants inoculated with the Al sensitive *S. luteus* strain 62 (designated –Al, +S.lut.62 and +Al, + S.lut.62). Ten pots were used for each treatment with about 25 seedlings per pot. The seedlings had been cultivated for the next 6 weeks in the conditions described above and irrigated with 1/4 Laiho (1970) nutrient solution (150 ml/each time) in order to keep the peat-perlite moderately moist. Low fertilisation level was kept to obtain high frequency of mycorrhizas (Rudawska 1986). After six weeks, random observations of ectomycorrhizas on roots of inoculated seedlings were conducted in order to check if the inoculation was efficient. During the following weeks the plants were grown in greenhouse conditions at a 16-h photoperiod supplemented by additional irradiance from sodium lamps (7500 mW m^{-2}). Half of the pine seedlings from each study group were watered twice a week with 50 ml of the nutrient solution of Laiho (1970) adjusted to pH 3.8 and containing $Al_2(SO_4)_3$ at Al concentration 11 mM. Plants watered twice a week with 50 ml of the same nutrient solution without Al

served as a control. Once a week all the pots were fertilised with 1/4 Laiho nutrient solution of pH 3.8 (150 ml).

Analysis of seedling roots, shoots and needles

Six months after germination, all the seedlings were harvested, and their roots were washed free of potting substrate. External conditions of the seedlings were assessed and shoot height was recorded. The root system of the seedlings was then examined for ectomycorrhizas with a stereomicroscope (6.5 to 50× magnification). The degree of ectomycorrhizal colonisation was determined by counting ectomycorrhizas on the entire root system of 10 seedlings chosen at random from each treatment. Ectomycorrhizal formation was expressed as a percent of the total feeder roots that were ectomycorrhizal.

The seedlings were divided into needles, stem and roots, oven dried at 60°C for 48 h and weighed. For analyses of Ca, K and Mg in roots, stems and needles, perchloric acid-nitric acid (1:4, v/v) digestion of dried material was used. Calcium, magnesium and potassium analysis of the digest was carried out using Varian 20 BQ atomic absorption spectrophotometer. The aluminium content of the plant material was analysed by plasma emission spectrophotometry from ashed and HCl-dissolved material. For nitrogen analyses, the standard Kjeldahl method was applied, whereas phosphorus was analysed by the molybdenum blue method. The analyses were done by the Research Laboratory, Institute of Botany in Krakow.

Ergosterol analysis of extramatrical mycelium

The ergosterol assay was used to estimate metabolically active biomass of extramatrical mycelium in the peat-perlite substrate. Ergosterol was determined by HPLC as described by Nylund and Wallander (1992) and Kårén and Nylund (1996). Ten grams of the peat-perlite sample (each in 4 replications) was suspended in 50 ml of ethanol and extracted by shaking for 30 minutes. After filtering the extraction, the procedure was repeated. The combined extracts were evaporated to dryness at 40°C. The dry extracts were redissolved in 0.5 ml of methanol prior to quantification using a reverse-phase HPLC system (Waters) with UV detector 280 nm, column Waters Nova-Pak C18 (150 × 4 mm) and 100% methanol (Baker) as a solvent and 5,7,22-Ergostarien-3B-ol, (Sigma) as standard.

Statistical methods

Data were summarised and analysed by using statistical software procedures (Statistica PL 1997). The data were subjected to one-way analysis of variance (ANOVA). Comparison between means was carried out using Tukey's range test.

Results

Visual observations

Growth of the upper part of the plants was significantly stimulated by inoculation with the ectomycorrhizal fungus *S. luteus* No 14 and, to a lesser degree, by aluminium treatment (Fig. 1). Untill the end of the experiment, all the seedlings grew well, and the aluminium concentration (11 mM) did not cause any significant visual symptoms in seedling needles. Visual symptoms of Al toxicity were seen in roots of all the aluminium treated seedlings. The roots were shortened and thick, occasionally spotted brown; however, no discoloration or root necrosis was noticed.

Mycorrhizal development and seedling growth

At harvest, the peat-perlite substrate from the variants of the experiment inoculated with both, *S. luteus* No 14 and *S. luteus* No 62, as well as the one not treated with Al, was heavily colonised by fungal hyphae. Root systems of the seedlings inoculated with both *S. luteus* strains were intensively branched and heavily colonised (84–95%). Several mycorrhizal morphotypes were distinguished: 1) single, swollen mycorrhizas with an abundant mantle; 2) dichotomously branched, thick mycorrhizas; 3) coralloid mycorrhizas; 4) cluster mycorrhizas. The most abundant were the coralloid and cluster mycorrhizal morphotypes. In several cases, fruitbody primordia of *S. luteus* developed inside the substrate. Mycorrhizal plants were colonised mainly by the inoculated mycobiont. Occasionally, the non-mycorrhizal control contained an infection originating from air-borne spores of *Thelephora terrestris*. Aluminium treatment significantly reduced quality and quantity of mycorrhizas infection. Although mycorrhizal roots'developed in the Al treated pots, the coralloid and cluster mycorrhizas were not observed, and only a low percentage (12–25%) of dichotomous and single mycorrhizas were present (Table 1). Ergosterol concentration in extramatrical mycelium for inoculated pots was 138–270 $\mu g\ g^{-1}$ dry weight of the peat-perlite substrate, whereas for the non-inoculated pots there was 80–100 $\mu g\ g^{-1}$ dry weight of the peat-perlite substrate (Fig. 2). Ergosterol analysis of the potting substrate showed no limiting effect of aluminium on development of extramatrical mycelium. In the case of the Al-tolerant strain No 14, some increase (although not significant) in the amount of mycelium was even noticed. In the presence of Al, the extramatrical mycelium developed by the aluminium resistant strain

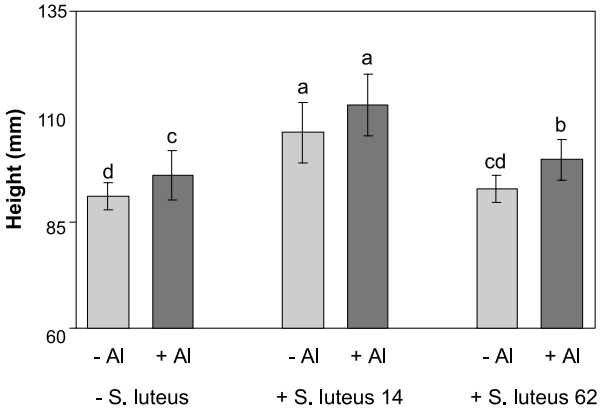

Fig. 1. Shoot height of *Pinus sylvestris* seedlings treated with aluminium [as $Al_2(SO_4)_3 \cdot 18\ H_2O$, 11 mM, pH 3.8] and mycorrhizal with *Suillus luteus* strains selected as tolerant (No 14) and sensitive (No 62) to aluminium. Bars indicate standard error (N=20–23). Means between treatment not sharing a common letter are significantly different; Tukey's test, p= 0.05

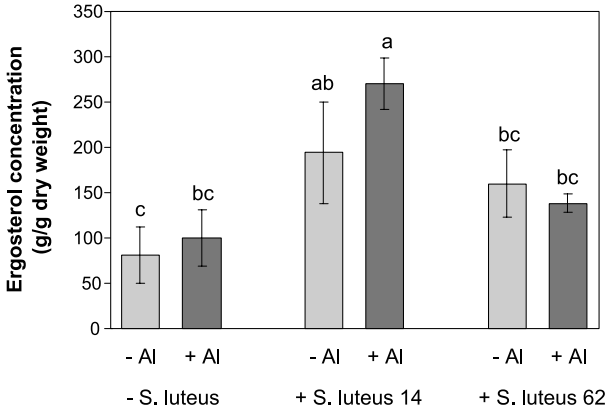

Fig. 2. Biomass of *extramatrical mycelium* measured as ergosterol content, in the peat-perlite substrate from the pots of *Pinus sylvestris* seedlings treated with aluminium [as $Al_2(SO_4)_3 \cdot 18\ H_2O$, 11 mM, pH 3.8] and mycorrhizal with *Suillus luteus* strains selected as tolerant (No 14) and sensitive (No 62) to aluminium. Bars indicate standard error (N=20–23). Means between treatment not sharing a common letter are significantly different; Tukey's test, p= 0.05

No 14 was significantly more abundant than the mycelium developed by the aluminium sensitive strain No 62.

The strain of *S. luteus* No 14, tolerant to Al, positively affected the plant growth, expressed as a height of shoots and biomass production (dry weight) (Fig. 1, Table 1). The strain of *S. luteus* No 62, selected as sensitive to aluminium, even though it formed abundant mycorrhizas in the non-treated pots, was less effective in growth stimulation of the pine seedlings. Aluminium affected growth parameters of the seedlings by increasing in all cases shoot height and shoot and needles biomass. Root dry weight was significantly lower in mycorrhizal plants treated with aluminium; however, no significant influence of Al was found in the non-inoculated plants.

Chemical composition of roots, shoots and needles

The concentrations of N, P, K, Ca, Mg, Al, and Ca/Al ratio in different parts of the seedlings from all of the treatments are given in Table 2. Aluminium had little influence on the nitrogen concentration in roots, stems and needles. In roots of the seedlings mycorrhizal with the *S. luteus* strain No 14 and treated with Al, a sudden increase in nitrogen content was noticed. Phosphorus concentration in different parts of the seedlings was influenced by aluminium and mycorrhizal fungi. Treatment with Al caused a reduction in the concentration of P in root, shoot and needles of the pine seedlings. All the mycorrhizal seedlings treated with Al had a significantly lower P concentration in root and needles than the uninoculated seedlings. Aluminium treatment did not consistently

Table 1. Growth parameters and mycorrhizal infection of *Pinus sylvestris* seedlings treated with aluminium [as $Al_2(SO_4)_3 \cdot 18\ H_2O$, 11 mM, pH 3.8] and mycorrhizal with *Suillus luteus* strains selected as tolerant (No 14) and sensitive (No 62) to aluminium. Mean value ± SD. Means between treatment not sharing a common letter are significantly different; Tukey's test, p= 0.05

Treatment	Dry weight (mg)			Mycorrhiza (%)
	Root	Shoots	Needles	
- Al, - S. lut.	119.2 ± 9.5 ab	55.9 ± 1.9 c	129.5 ± 7.3 c	10
+ Al, - S. lut.	125.3 ± 18 ab	77.2 ± 9.1 ab	176.4 ± 25 ab	8
- Al, + S. lut. 14	157.4 ± 22 a	81.1 ± 8.7 ab	165.3 ± 30 abc	95
+ Al, + S. lut. 14	110.9 ± 17 b	93.7 ± 10 a	197.9 ± 31 a	12
- Al, + S. lut. 62	123.2 ± 40 ab	60.1 ± 7.4 c	125.6 ± 13 c	84
+ Al, + S. lut. 62	91.2 ± 1.8 c	69.1 ± 5.9 bc	141.4 ±14 bc	25

Table 2. Element concentrations in different parts of *Pinus sylvestris* seedlings treated with aluminium [Al as $Al_2(SO_4)_3 \cdot 18 H_2O$, 11 mM, pH 3.8] and mycorrhizal with *Suillus luteus* strains selected as tolerant (No 14) and sensitive (No 62) to aluminium. Means between treatment not sharing a common letter are significantly different; Tukey's test, p= 0.05

Treatment	Element concentration						Ca/Al
	N	P	K	Ca	Mg	Al	
	mg/g						
root							
- Al, - S. lut.	13.2 bc	5.9 c	5.9 ab	5.0 a	3.2 a	0.64 f	5.20
+ Al, - S. lut.	12.7 c	5.6 c	6.3 a	1.3 d	1.5 b	5.45 b	0.16
- Al, + S. lut. 14	12.9 c	6.6 b	5.6 bc	4.2 b	2.9 a	0.83 e	3.40
+ Al, + S. lut. 14	17.1 b	4.6 d	4.2 cd	2.2 c	1.4 b	4.96 c	0.30
- Al, + S. lut. 62	13.7 b	7.7 a	3.7 d	4.5 b	2.9 a	1.11 d	2.80
+ Al, + S. lut. 62	13.7 b	3.9 e	5.1 c	1.0 d	1.4 b	7.80 a	0.09
shoot							
- Al, - S. lut.	8.5 a	3.4 a	7.5 a	3.3 b	2.1 ab	0.16 f	13.7
+ Al, - S. lut.	8.2 ab	2.2 b	7.2 a	1.7 cd	1.8 abc	1.72 c	0.66
- Al, + S. lut. 14	8.4 a	3.4 a	6.1 b	3.1 b	2.1 ab	0.29 d	7.02
+ Al, + S. lut. 14	7.8 b	2.0 b	5.3 c	2.1 c	1.6 bc	1.88 b	0.74
- Al, + S. lut. 62	8.5 a	3.4 a	4.3 d	5.6 a	2.3 a	0.20 e	18.7
+ Al, + S. lut. 62	8.2 ab	2.0 b	5.3 c	1.4 d	1.4 c	2.38 a	0.40
needles							
- Al, - S. lut.	1.25 a	3.8 b	6.4 c	3.8 a	2.8 c	0.16 f	16.3
+ Al, - S. lut.	1.23 ab	3.5 b	7.1 b	3.5 a	3.8 a	1.61 c	1.46
- Al, + S. lut. 14	1.36 a	5.2 a	5.7 d	3.7 a	3.1 bc	0.32 e	7.86
+ Al, + S. lut. 14	1.19 b	2.8 c	7.8 a	3.1 b	3.5 ab	3.50 a	0.59
- Al, + S. lut. 62	1.22 ab	3.8 b	5.0 e	3.8 a	3.0 bc	0.38 d	6.70
+ Al, + S. lut. 62	1.13 c	2.6 c	5.8 d	2.6 b	2.6 c	3.19 b	0.54

affect potassium content in roots and stems of the uninoculated plants. However, a lower K concentration was noticed in roots, stems and needles of plants mycorrhizal with the strain 62, sensitive to aluminium compared to the plants mycorrhizal with the strain No 14. In the needles, the internal potassium concentration was increased by aluminium (as a result of a decreased efflux from the roots). Aluminium treatment decreased the Ca concentration in roots and shoots of all the seedlings, whereas in the needles it was significantly lower only in the mycorrhizal plants. With respect to Mg, a consistent effect of aluminium was only shown in roots, where a decrease in this element was observed.

The aluminium concentrations in the roots and in the stem and needles are shown in Table 2. The small amount of Al in the non-treated control plants originated probably from the peat used as a compound of the substrate. All parts of the plants treated with solution containing Al^{3+} ions, accumulated a considerable amount of aluminium. The highest amount of Al was found in the roots, and the smallest amount in the shoots. Plants inoculated with the aluminium-sensitive strain No 62, accumulated the highest amount of Al in roots and shoots. Aluminium concentration in needles of the plants mycorrhizal with both strains of *S. luteus,* was considerably higher compared with the uninoculated control; however, the highest amount of aluminium in the needles was rfound in the plants mycorrhizal with the strain No 14. The molar Ca/Al ratio in roots was drastically reduced in the aluminium treated plants. The mycorrhizal plants had a lower Ca/Al ratio than the nonmycorrhizal control, which was a result of high aluminium absorption by mycelium of the mycorrhizal fungi.

Discussion

The Scots pine seedlings from our experiment proved to be extremely Al-tolerant. Treatment with soluble Al, at the concentration representative for a soil solution of an acidic and highly polluted forest soil, where root growth of Scots pine start to be reduced (Eldhuset et al. 1987), did not cause any visual symptoms of Al toxicity of the above-ground parts of the plants. Moreover, Al treatment even slightly stimulated shoot growth of the pine seedlings. Growth

stimulation by Al has also been observed before (Foy et al 1978). Keltjens and van Loenen (1989) explain that such a phenomenon occurs at low levels of Ca^{2+} supply (as in our experiment) and is connected with substitution of Ca^{2+} at root cation exchange capacity sites by Al^{3+} instead of H^+, which is more harmful. Also Wagatsuma and Kaneko (1987) found some positive effect of Al at an extremely low pH. Under high concentrations of H^+, aluminium seems to reduce destruction of the plasmalemma of root cells and consequently prevents root cells from K^+ leakage. In our experiment, aluminium significantly decreased root dry weight in the mycorrhizal seedlings compared with the mycorrhizal seedlings not treated with Al. It may mostly be attributed to a strong negative effect of aluminium on the mycorrhizas of both *S. luteus* strains used in this study. Al reduced root length and biomass and caused swollen root tips, similar to those described by other authors (Göransson and Eldhuset 1991). This may be a result of disturbed meristematic growth pattern (Schier and McQuattie 1995).

Scots pine is obligatorily equipped with mycorrhizas, and the uptake of mineral nutrients by this species highly depends on the mycorrhizal symbiosis. Therefore the intact mycorrhizal root association is of great importance to the Scots pine seedlings. These results indicate the negative impact of Al on the mycorrhizal symbiosis of the Scots pine seedlings grown at the high Al concentration, colonised with two strains of *S. luteus,* which were selected in previous *in vitro* studies (Leski et al. 1995) as tolerant and sensitive to aluminium. Al treatment caused the abundant mycorrhizas, which had formed on pine roots inoculated with both strains of *S. luteus,* to decline by the end of the experiment. Lower percentages of ectomycorrhizas resulted rather from fewer root tips available for colonisation than from the reduced growth of the ectomycorrhizal fungi in the potting substrate. Ergosterol analysis of the potting substrate showed no limiting effect of aluminium on development of the ectomycorrhizal extramatrical mycelium. In case of the Al-tolerant strain No 14, some increase in the amount of mycelium was even noticed. This is in agreement with our previous results which revealed a high tolerance of some *S. luteus* strains to an increased aluminium concentration and very abundant growth of the mycelia at concentrations of Al up to 27 mM (Leski et al. 1995). Thus, our findings suggest a possibility of extrapolation from the results obtained in *in vitro* culture to a situation when phytobiont and mycobiont grow in the mycorrhizal symbiosis. Untill now, the effects of acidification and Al ions on mycorrhizal seedlings of Scots pine were equivocal. What is lacking is a quantitative information on the degree to which the functioning of the mycorrhizal association may be impaired without severe consequences for tree growth. These results clearly indicate the negative impact of Al on the mycorrhiza of the Scots pine seedlings when the aluminium concentration was 11 mM. The Al content in roots of the tested plants increased with Al treatment and was independent of the presence of the mycorrhiza. In our previous study (Kieliszewska-Rokicka et al. 1998), when Scots pine seedlings were mycorrhizal with the same Al-tolerant strain of *S. luteus* (No 14) but grown at a lower aluminium concentration (4 mM), the content of Al in needles was 3 times lower. This suggests that the presence of the vigorous mycobiont inhibits the passage of aluminium to the stele for transport in the transpiration stream. However, when Al concentration in the growth medium and in the plant material exceeds some limits (as in our experiment), even the selected Al-tolerant mycorrhizal fungus is not capable of preventing the negative impact of Al on the root growth and, as a result, on the mycorrhizal symbiosis.

According to Rost-Siebert (1983), the Ca/Al ratio in plants tissue is more important than the absolute concentration of Ca and Al. In our experiment the Al concentration used reduced the Ca/Al ratio to values below the critical level (less than 1). Because of a considerably higher Al accumulation in mycorrhizal plants, Ca/Al ratio was consistently lower in the seedlings mycorrhizal with both *S. luteus* strains than in the nonmycorrhizal control. A drop in Ca/Al from 5.2 to 0.30 and 0.09 in roots mycorrhizal with the Al-tolerant (No 14) and the Al-sensitive (No 62) strains of *S. luteus* respectively, significantly affected root growth of the plants and, in consequence, the mycorrhiza formation. In some previous experiments conducted with the same Al-tolerant strain of *S. luteus* but at a lower aluminium concentration (4 mM), the Ca/Al ratio exceeded 1, and the mycorrhizal symbiosis remained nearly untouched (Kieliszewska-Rokicka et al. 1998). Thus it may be concluded that in soil solution with a low mineral nutrient content, the aluminium limit for the ectomycorrhizal Scots pine seedlings exceeds 4 mM but is significantly lower than 11 mM.

Although laboratory experiments must be interpreted with caution, our studies showed that the mycorrhizal strains selected in the axenic culture reflected their ability to resist high Al concentration under controlled conditions. Because mycorrhizal fungi are not equally effective in stimulating and protecting the plants, laboratory experiments could help to determine their potential to ameliorate Al toxicity and select the most efficient strains for inoculating different trees in reforestation programs.

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Wpływ glinu na siewki sosny zwyczajnej inokulowane dwoma szczepami grzyba *Suillus luteus*: tolerancyjnym i wrażliwym na glin

Streszczenie

Syntezę mikoryzową siewek sosny (*Pinus sylvestris*) z odpornym na glin szczepem *Suillus luteus* nr 14 i wrażliwym na glin szczepem nr 62 przeprowadzono w warunkach półsterylnych w szklarni. Po nawiązaniu mikoryzy siewki przez 6 miesięcy traktowano roztworem glinu o stężeniu 11 mM przy pH = 3,8. W warunkach kontrolnych (bez glinu) oba szczepy *S. luteus* tworzyły obfite mikoryzy pojedyncze oraz dichotomicznie i koralowato rozgałęzione. Traktowanie glinem zredukowało morfotypy mikoryzowe do pojedynczych i dichotomicznych oraz znacząco zmniejszyło całkowitą liczbę wierzchołków mikoryzowych na korzeniach siewek, ale nie ograniczyło rozwoju grzybni ekstramatrykalnej. W warunkach kontrolnych (bez glinu) rozwój grzybni ekstramatrykalnej, mierzony zawartością ergosterolu w podłożu był podobny w przypadku obu testowanych szczepów. Traktowanie glinem spowodowało pewien wzrost rozwoju grzybni ekstramatrykalnej szczepu tolerancyjnego i nieznaczne zahamowanie wzrostu szczepu wrażliwego. Glin ograniczył znacząco wzrost korzeni, natomiast nie stwierdzono negatywnego wpływu glinu na wzrost części nadziemnych siewek. Siewki traktowane glinem absorbowały w korzeniach znaczne ilości tego metalu, który był także przemieszczany do pędów. Stwierdzono niewielki wpływ glinu na zawartość pierwiastków mineralnych w siewkach. Badania wykazały, że przy zastosowanym wysokim stężeniu glinu szczep Nr 14 wyselekcjonowany w warunkach *in vitro* jako odporny na glin, nie zahamował przemieszczania jonów Al^{3+} do części nadziemnych siewek sosny. Wyniki sugerują, że szkodliwe działanie jonów glinu na symbiozę mikoryzową w warunkach niskiego pH polega raczej na ograniczeniu liczby wierzchołków korzeni drobnych, które potencjalnie mogą tworzyć mikoryzy, niż na bezpośrednim wpływie na grzybnię mikoryzową.