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ORIGINAL RESEARCH ARTICLE

Impact of aluminium sulphate fertiliser on selected soil properties and the efficiency and quality of pine seedlings in the forest ground tree nursery

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Abstract. The alkalisation of soil is a common phenomenon in forest ground nurseries. Liming, inadequate fertilisation and the use of hard water for irrigation are the main reasons for this alkalisation. The aim of this study was to investigate the effect of fertilisation with aluminium sulphate on soil pH, the activity of selected soil enzymes, efficiency as well as the growth parameters of pine seedlings.

The study was conducted in a forest nursery, on a plot with soil pH 6.4 in water and 5.9 in 1M KCl. Such a pH is not conducive to the production of conifer seedlings, particularly pines. Two different doses of aluminium sulphate fertiliser were applied: 740 kg ha^{-1} and 1110 kg ha^{-1}

Both doses significantly reduced the soil pH, whereas soil enzyme activity did not change. The lower dose had a positive impact on the growth parameters of pine seedlings, while the higher dose led to their deterioration. We observed statistically significant differences in average primary and lateral root lengths, number of short roots, and thickness of the neck root of seedlings. One- and 2-year-old seedlings did not show symptoms of nutrient deficiency and neither did concentrations of the investigated macronutrients and selected micronutrients in needles indicate such. After applying the higher fertiliser dose, we observed a favourable change in the composition of mycorrhizae. Out of the potential seedling pathogens we found *Cylindrocarpon* spp., *Fusarium* spp.,*Phytophthora* spp., *Pythium* spp. and *Rhizoctonia solani* of which the most frequent were *Fusarium oxysporom, Pythium* spp. and *R. solani*. Their occurrence frequency differed between the treatments used in this experiment.

This study confirms the positive effects of a low aluminium dose on the performance and growth parameters of pine seedlings. However, on the basis of the conducted experiments, it is difficult to say, whether this positive effect is due to a direct action of aluminium on the seedlings or rather an indirect effect caused by lowering the soil pH, which in tum impacts on mycorrhizae composition and hence pathogen development.

Key words: soil pH, aluminium sulphate, pine seedlings quality, ectomycorrhizae, damping-off

1. Introduction

Alkalisation of soil often occurs in forest ground nurseries. The reason can be both frequent liming and inadequate fertilisation as well as the use of hard water for irrigation. Maintaining optimal pH of the soil is important for the production of conifer seedlings (Januszek 1999). Between the number and size of annual pine seedlings and soil pH in H_2O, there is a strong, negative correlation, ranging from 4.0 to 6.5 (Januszek, Barczyk 2003). Pathogenic fungi that cause damping-off are known for their wide tolerance to soil pH.

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Furthermore, some degree of acidification of the soil is advantageous for most of forest trees due to symbiosis with ectomycorrhizal fungi (Mańka et al. 1987, Kowalski et al. 1996). Alkaline soil significantly reduces the rate of ectomycorrhizal contacts (Kowalski et al. 1996). It creates favourable conditions for the development of ectendomycorrhizae (Kowalski 1998).

Acidification of soil is a commonly recommended practice to tackle many root diseases, but the mechanism of suppression is not known (Fichtner 2003). For soil acidification, among others, aluminium sulphate is used, in the presence of which sulphuric acid is formed in the soil, and aluminium, entering the sorption complex, displaces hydrogen, making the pH even lower (Lityński, Jurkowska 1982).

The aim of the study was to: 1) examine the effectiveness of lowering the pH of the soil using aluminium sulphate, 2) assess the influence of the use of aluminium sulphate on soil properties and parameters of Scots pine seedlings and 3) assess the impact of this treatment on the efficiency of the emergence of Scots pine seedlings, spectrum of pathogens causing the symptoms of damping-off and state of mycorrhizae.

2. Materials and methods

The study was conducted in the forest ground nursery in the Krzeszowice Forest District (Regional Directorate of State Forests in Cracow), in which difficulties in the production of Scots pine seedlings were occurring due to the high intensity of damping-off. On the chosen research plot, brown rusty pseudogley soil – Stagni Cambic Arenosol (Classification of forest soils 2000) – occurred, which developed from slightly loamy water-glacial sands cross-layered with loose sands (49–125 cm) resting on loamy sand and underlain (150 cm) by sandy loam. In October 2002, before the start of the study, soil samples collected from the cultivation level determined the pH in H_2O in the range of 6.09–6.19, the pH in 1M KCl in the range 5.08–5.17, hydrolytic acidity within 0.31–2.65 cmol (+) kg^{-1} of soil, decreasing with the soil profile, and the degree of base saturation (V%) in the range of 59.3–97.5%.

Commercial aluminium sulphate [$Al_2(SO_4)_3 \times 14H_2O$], comprising 57.8% of aluminium sulphate [$Al_2(SO_4)_3$], was applied to lower the pH of the soil. The experiment was performed using randomised blocks, in five repetitions, on 15 plots with dimensions of 3×4.5 m each. Aluminium sulphate was spread on the experimental plot on 07.04.2003. The dosages of aluminium sulphate were determined by laboratory incubation. Two doses of aluminium sulphate were used that drew the pH in 1M KCl of studied soil close to 4.2 (Januszek, Barczyk 2003): variant Al1 – dose of 740 kg ha^{-1}, and variant Al2 – 1110 kg ha^{-1}. After spreading aluminium sulphate on soil, it was mixed by cultivating and harrowing. In all plots, including the control plots (without aluminium sulphate), such fertilizers were used: potassium sulphate in a dose of 180 kg ha^{-1} (75 kg ha^{-1} K) and magnesium sulphate in a dose of 210 kg ha^{-1} (20 kg ha^{-1} Mg). Scots pine seeds were sown on 24.04.2003 in a dose of 0.3 kg ar^{-1} using row seeding. Before sowing, the seeds were dressed with Funaben T (3 g kg^{-1} of seeds).

The study of soil properties

In 2003 and 2004, at 4–6-week intervals, five soil samples per plot were taken from cultivated level (0–25 cm) using Egner's stick. Subsequently, the samples from each plot were thoroughly mixed in a bulk sample. After drying them to air-dry state and sieving through a sieve with an aperture of 2 mm, the soil pH was measured in H_2O and 1M KCl using the potentiometric method maintaining the weight of the soil to the volume of solution as 1:2.5. For samples collected in July 2004, additionally were determined: hydrolytic acidity and base exchange capacity by Kappen method, with the calculation of sorption capacity (T) and the degree of base saturation (V%), organic carbon content with Tiurin's oxidometric method and total nitrogen by the Kjeldahl method of calculation of the C:N ratio. In samples with a natural moisture, taken in August 2003 and July 2004, were also determined: phosphatase activity by Kramer and Erdei (Haziev 1976) and invertase activity by Ščerbakova (Haziev 1976).

The study of acid phosphatase activity area: the ends of the roots and growth parameters of 1- and 2-year-old seedlings

On 6–8 November 2003, from each plot 30 1-year-old pine seedlings were collected at random. Seedlings along with a small amount of soil were placed in plastic bags, transported to the laboratory and placed in a refrigerator at 4–5°C. All the seedlings were measured with an accuracy of 0.1: the height of the aboveground part [cm], the main root length [cm] and the root neck diameter [mm].

For further study, 10 plants were selected randomly from each batch of 30 seedlings per plot. Roots were rinsed with distilled water and dried on filter paper. Then, in each seedling, the endings (10 mm long) of lat-

eral roots were cut off to become a sample for analysis of approximately 50 mg. Analyses were performed according to the methodology described by Januszek and Januszek (2000). In October 2004, from each plot, 30 2-year-old seedlings of pine were collected and the activity of acid phosphatase and growth parameters were determined again in the manner described above.

The study of damping-off severity and spectrum of seedling pathogens

This part of the study included only two variants of experiment: control (0) and a higher dose of aluminium sulphate (Al2). As a measure of the severity of damping-off (pre-emergence and post-emergence), seedling efficiency was adopted, which was estimated in September 2003. Pine seedlings were counted in randomly marked out sections of seed rows. Within each plot, there were six such sections with a length of 1 m each.

The spectrum of pathogens was studied in seedlings with symptoms of damping-off collected in May and June 2003. From each plot of control variant and the variant of Al2, 30 seedlings were randomly collected, i.e. a total of 150 seedlings per each variant of the experiment. Isolation of pathogenic fungi was performed on selective media: P_5ARP and P_5ARPH (Jeffers and Martin 1986) and standard potato dextrose agar in each case a sample of 50 seedlings (N=50) taken from the control and Al2 variant according to the methodology described earlier (Stępniewska 2003). The resulting colonies were identified on the basis of morphological criteria.

The study of the root system architecture and mycorrhizae of 1- and 2-year old pine seedlings

This part of the study also included only two variants of experiment: control (0) and a higher dose of aluminium sulphate (Al2). On 6 and 8 November 2003, from each plot, six 1-year-old seedlings were collected randomly, i.e. 30 of each variant of the experiment. In the laboratory, they were rinsed from the remaining soil, placed in Erlenmeyer flasks and covered with formalin acetic alcohol (Russell 1974). For each seedling was determined: the sum of lengths of lateral roots of each order, number of short roots and frequency of ectomycorrhizae and ectendomycorrhizae estimated according to their morphological and anatomical features. On 5 and 19 November 2004, 2-year-old seedlings were collected for analysis, in the same way as 1-year-olds. The analysis was performed similarly, but the presence of short roots and mycorrhizae were checked on lateral roots of each seedling on the basis of 20 randomly taken sections, each of about 5 cm (total of 1 m).

The content of macro-and micronutrients in the needles of Scots pine seedlings

The contents of nutrients (N, P, K, Ca, Mg, S, Cu, Fe, Mn, Zn) were determined for the 1-year needles collect-ed from seedlings in November 2003 and for the needles of 1- and 2-year-olds, mixed in equal proportions, taken from the seedlings in October 2004. The sulphur content was determined by nephelometry after mineralisation of needles in concentrated HNO_3. Total nitrogen content was determined by Kjeldahl method. The remaining elements studied were determined after mineralisation of needles in concentrated HNO_3 and $HClO_4$ mixed in a 3:1 ratio. The phosphorus content was determined by colorimetry, and the remaining metals were determined spectrophoto-metrically using an atomic absorption spectrophotometer of Varian Spectr AA-20 type (Ostrowska et al. 1991).

Statistical analysis of results

Statistica 9 was applied for statistical analysis of data; Kruskal–Wallis test and Mann–Whitney U test for differences between means. For the tests, the following significance levels were adopted: $p = 0.05$, $p = 0.01$ and $p = 0.001$.

3. Results

Physical and chemical properties of the soil

The pH of the soil in the cultivated level of plots determined in 2002, before the experiment started, did not vary significantly and ranged from 5.81 to 6.52 in H_2O and from 4.61 to 5.95 in KCl (Table 1).

In 2003 and 2004, soil pH measured at cultivated level in control variant (O), variant with a smaller (Al1) and higher (Al2) dose of aluminium sulphate ranged respectively: 4.43–6.59, 4.69–6.22 and 4.66–6.41 in H_2O and 3.97–5.86, 4.25–5.29 and 4.15–5.57 in KCl (Table 1). In 2003, the average pH values of the soil in variants Al1 and Al2 were lower than in the control, respectively, by 0.17 and 0.33 pH units in H_2O, and 0.11 and 0.20 pH units in KCl (Table 1). In 2004, a similar relationship was observed, and the differences were respectively: 0.19 and 0.28 pH units in H_2O, and 0.17 and 0.24 pH units in KCl (Table 1). In 2003, the differences between the control

variant and variants of Al1 and Al2 were statistically significant only in the case of soil pH in H_2O in August (Fig. 1) and were, respectively, 0.28 and 0.63 pH units. In 2004, a statistically significant difference between the control variant and variants Al1 and Al2 was found in June and July. They were, respectively, 0.21 and 0.53 pH in June and 0.25 and 0.45 pH in July (Fig. 1). The biggest differences between the soil of the control (O) and fertilised plots (Al1 and Al2) in terms of pH in H_2O were recorded in July and August 2003, respectively, 0.34 and 0.63 pH units (Fig. 1), and in terms of pH in KCl in May 2004 and August 2003, respectively, 0.20 and 0.42 pH units (Fig. 2). Taking all soil pH values recorded in 2003 and 2004 together (50 repetitions per each variant of the experiment), there were significantly lower pH values, both in H_2O and in KCl (Table 1), in the variant Al2 than in the control. The average pH values of the soil in the Al1 and Al2 variants were lower than in the control, respectively, by 0.18 and 0.27 pH units in H_2O and 0.15 and 0.23 pH units in KCl (Table 1). Soil pH values in H_2O in 2004 were significantly higher than in 2003, both for the control variant, as well as variants Al1 and Al2 by, respectively, 0.39, 0.37 and 0.44 pH units on average (Table 1). The values of soil pH in KCl in the years 2003 and 2004 in different variants were similar and statistically undifferentiated (Table 1).

The higher pH of soils in 2004 compared with 2003 may be associated with higher rainfall (in May 2003 and 2004, respectively, 117.8 and 42.6 mm) and higher temperatures in 2003 than in 2004 (monthly average temperatures between May and August of 2003 and 2004 were, respectively, 18.2 and 16.2°C), which could increase microbial activity in the soils (more CO_2) as well as intensify leaching of bases from the soils in 2003 compared with 2004 (data from Center for Poland's Climate Monitoring, Institute of Meteorology and Water Management in Warsaw, 2013).

Among the soil samples collected from variants of experiment in July 2004, there was no statistically significant difference in hydrolytic acidity, the sum of exchangeable bases, sorption capacity, the degree of base saturation, the concentration of organic carbon and total nitrogen, and the C:N ratio. In the soil of Al2 variant compared with the control, there was higher hydrolytic

Table 1. Mean (śr), minimal (min), maximal (max) values and standard deviation (sd) of soil pH in the cultivated horizon before (2002) and after (2003 i 2004) application of aluminium sulphate.

Term	Value	pH H_2O			pH KCl		
		Variant of the experiment					
		O	Al1	Al2	O	Al1	Al2
15.10.2002	śr (N = 5)	6.14^{a}	6.09^{a}	6.19^{a}	5.17^{a}	5.08^{a}	5.16^{a}
	min–max	5.87–6.34	5.81–6.35	5.89–6.52	4.65–5.95	4.61–5.44	4.89–5.51
	sd	0.219	0.222	0.248	0.517	0.318	0.226
	N	5	5	5	5	5	5
2003	śr (N = 20)	5.52^{aA}	5.35^{aA}	5.19^{aA}	4.86^{aA}	4.75^{aA}	4.66^{aA}
	min–max	4.43–6.44	4.69–5.94	4.66–5.99	3.97–5.97	4.25–5.60	4.15–5.32
	sd	0.487	0.292	0.410	0.557	0.326	0.361
	N	20	20	20	20	20	20
2004	śr (N = 30)	5.91^{aB***}	5.72^{abB***}	$5.63^{b***B***}$	4.89^{aA}	4.72^{aA}	4.65^{aA}
	min–max	5.00–6.59	5.28–6.22	5.22–6.41	4.10–5.86	4.23–5.29	4.24–5.57
	sd	0.337	0.255	0.274	0.479	0.297	0.309
	N	30	30	30	30	30	30
2003–2004	śr (N = 50)	5.75^{a}	5.57^{ab}	5.48^{b**}	4.88^{a}	4.73^{ab}	4.65^{b*}
	min–max	4.43–6.59	4.69–6.22	4.66–6.41	3.97–5.97	4.23–5.60	4.15–5.57
	sd	0.442	0.326	0.418	0.506	0.306	0.327
	N	50	50	50	50	50	50

Explanation: O – control variant without aluminium sulphate; Al1 – 740 kg $Al_2(SO_4)_3x18H_2O$ ha^{-1} (Commercial Product); Al2 – 1110 kg $Al_2(SO_4)_3x18H_2O$ ha^{-1} (Commercial Product); N – number of replications; little different letters of the alphabet mean differences in pH values between experience variants with probability: * <0.05, ** <0.01 and *** <0.001; different big letters of the alphabet mean differences in pH

Figure 1. The average values (n = 5) pH in H_2O of soil in the cultivated horizon of control variant (O), with a smaller (Al1) and higher (Al2) dose of aluminium sulphate

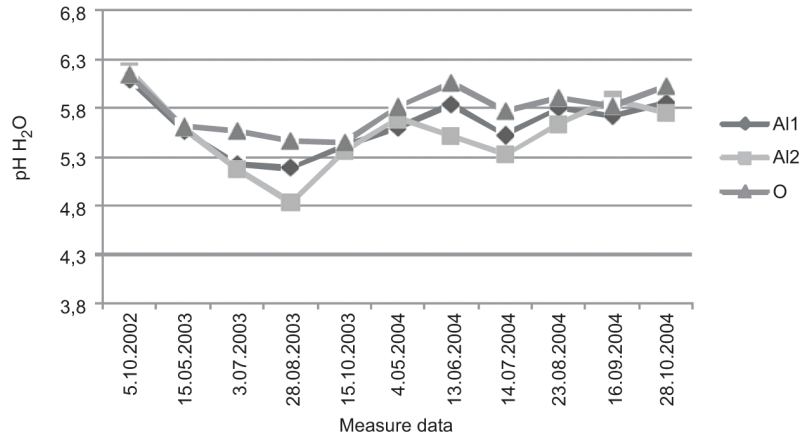
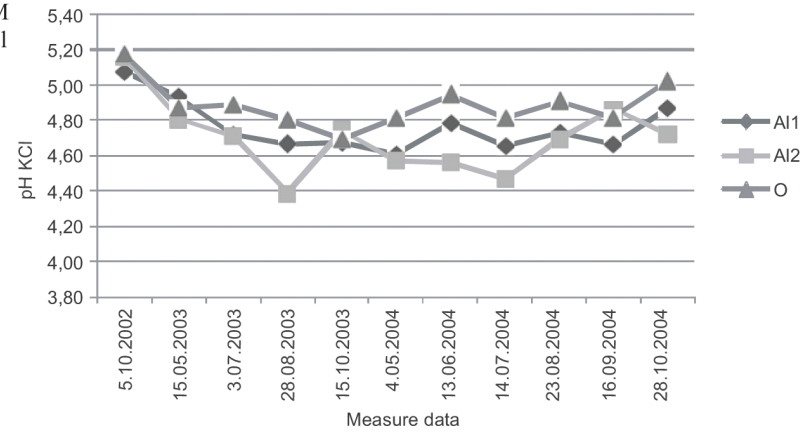


Figure 2. The average values (n = 5) pH in 1M KCl of soil in the cultivated horizon of control variant (O), with a smaller (Al1) and higher (Al2) dose of aluminium sulphate



acidity (3.95 and 3.86 cmol (+) kg^{-1} of soil, respectively), but smaller amount of base cations (2.92 and 3.13 cmol (+) kg^{-1} of soil, respectively) and a lower degree of base saturation (40.78 and 42.17, respectively).

The enzymatic activity of the soil

There were no significant differences in the activity of invertase and phosphatases in the soil of examined variants in the first and second year of the study. In 2003, the highest average invertase activity was found in the variant Al1 (1.231 mg glucose/1 g soil/1 h), and the lowest in the control variant (1.048 mg glucose/1 g soil/1 h). The highest average phosphatase activity was determined in the soil of control plots (3.322 mg phenol/5 g soil/2 h), and the lowest in the variant Al2 (2.644 mg phenol/5 g soil/2 h). In 2004, the highest average invertase activity was measured in the variant Al2 (0.458 mg glucose/1 g soil/1 h) and the lowest in the control variant (0.366 mg glucose/1 g soil/1 h). Average phosphatase activity was highest in the variant Al1 and lowest in the control variant (2.722 and 1.774 mg phenol/5 g soil/2 h, respectively).

The acid phosphatase activity (APhA) area the ends of the roots

There were no statistically significant differences between the studied variants in terms of the activity of acid phosphatase (APhA) area on the ends of the roots of Scots pine seedlings (Table 2). Among 1-year-old seedlings, the highest APhA was found in the variant Al2 and the lowest in the control variant. Among 2-year-old seedlings, the highest activity was found in the variant Al1 and the lowest in the control variant (Table 2). In the first year of the study, the values of APhA in pine seedlings were lower (an average of 28.856 mg of p-NP 50 mg of $roots^{-1}$ $1h^{-1}$) than in the second year of the research (43.846 mg of p-NF 50 mg of $roots^{-1}$ $1h^{-1}$).

Seedling efficiency and spectrum of pathogens causing damping-off

There were statistically significant differences between the studied variants in seedling efficiency. The highest mean number of seedlings was observed in the variant Al2, while a significantly lower (mean 45%) in the control variant. The average number of seedlings in the variant Al1 did not differ significantly from the value of this parameter in other variants of the experiment (Table 2).

From seedlings with symptoms of damping-off, there were isolated a total of 15 taxa of fungi and fungi-like organisms of the genus *Phytophthora* and *Pythium* and non-sporulating cultures. Among the potential tree seedlings pathogens, detected were *Cylindrocarpon* spp., *Fusarium* spp., *Phytophthora* spp., *Pythium* spp. and *Rhizoctonia solani*. The most common were *Fusarium oxysporum*, the genus *Pythium* and *R. solani*. Frequency of these pathogens differed between the studied variants. *F. oxysporum* was isolated more often from seedlings taken from the control variant (frequency 68%) than the ones taken from the variant Al2 (48%). Also the frequency of *Pythium* spp. was significantly higher in the control variant (52%) than in the variant Al2 (28%). In turn, *R. solani* was isolated at the same frequency (48%). Pathogens of the genus *Phytophthora* were isolated from seedlings significantly less frequently (10%) and only in the control variant. Other potential seedling pathogens of the genus *Cylindrocarpon* and *Fusarium* were isolated sporadically.

The parameters of seedlings

Statistically significant differences were noted in the average length of the main root, length of lateral root of I, II and III order, the number of short roots and root collar diameter of seedlings in tested variants, in both the first and the second years of the study (Table 2).

Among the 1-year-old seedlings, the longest main root was observed in the variant Al1, and the shortest in the variant Al2. In turn, among the 2-year-old seedlings, the longest main root was the one on control plots. Compared with control variant, the primary root length of 1-year-old seedlings from the variant Al1 was higher by 11.2%, and from the variant Al2 smaller by 10%. When it comes to 2-year-old seedlings, in both variants Al1 and Al2, primary root length was smaller by 4.8% and 11.3% than in the control (Table 2). The length of lateral roots of I and II order of seedlings in the variant Al2 was lower than in the control variant (16% and 20%, respectively). The same was the case for 2-year-old seedlings whose lateral roots of I, II and III orders were shorter by 21%, 41% and 66% in the variant Al2 (Table 2). The case of lateral roots of the III order of 1-year-old seedlings was different: in the variant Al2, their length was significantly higher (about 37%) than in the control variant (Table 2).

The number of short roots of 1- and 2-year-old seedlings in the variant Al2 was significantly lower than in the control variant, respectively, by 18.6% and 19.2% (Table 2).

Among the 2-year-old seedlings, the greatest diameter of root collar was observed in the control variant, while the lowest in the variant Al2 (Table 2). The root collar diameter of 1-year-old seedlings did not differ statistically between the studied variants of fertilisation. There were no statistically significant differences in the height of aboveground part of 1- and 2-year-old seedlings between the studied variants of fertilisation.

Mycorrhizae

Percentage of mycorrhizae in 1-year-old seedlings taken from control and Al2 variants were equally high (99.2% and 98.2%) (Table 2). Both ectomycorrhizae and ectendomycorrhizae were present. However, ectomycorrhizae dominated in both experimental variants. The frequency of their occurrence in seedlings was almost two times higher in the control variant and almost five times higher in the variant Al2 compared with ectendomycorrhizae (Table 2). In 1-year-old seedlings, as well as in 2-years-old ones, the percentage of mycorrhizae was high (95.7% and 98.2%) (Table 2). Also, ectomycorrhizae and ectendomycorhizae were found in these seedlings, with a clear dominance of the former. This dominance was stronger than in case of the 1-year-old seedlings (Table 2).

The accumulation of macro- and microelements in the needles of seedlings

One- and two-year-old seedlings showed no symptoms of nutrient shortage. Concentration of examined macro- and microelements in the needles (Table 3) was in the range not indicating their deficit (Baule and Fricker 1973, Fober 1993). The concentration of macronutrients and iron in needles of studied plants was significantly higher than in the needles of 4-year-old seedlings grown in the podzolic soil (Prusinkiewicz and Krzemień 1974) or in pine needles from pine stands of I bonitation and age class III (Prusinkiewicz et al. 1974). The accumulation of zinc in the needles of 1-year-old seedlings from variants Al1

Table 2. The number of seedlings and their growth and qualitative parameters in experience variants.

Variant	Value	Number of seedlings per 1 m	H [cm]	Ø [mm]	L [cm]	The length of lateral roots of row [cm]:				Number of short roots	Frequency of mycorrhizae (%)			AFK
						I	II	III	IV		total	ectendo-mycorrhizae	ectomyco-rrhizae	
1–year–old seedlings														
O	śr	33.8[a]	9.04[a]	2.13[a]	16.99[ab]	87.84[a]	63.56[a]	2.35[a]	0.0	482.8[a]	99.16	34.52	64.64	27.09[a]
	min–max	2–84	8.22–9.64	2.0–2.3	15.0–18.7	33.0–181.2	3–307.5	0–14.8	0–0	56–1381	93.97–100	0.00–97.54	2.46–100	5.09–93.34
	sd	20.6	0.64	0.2	3.3	37.19	65.39	4.43	0	333.0	1.55	30.94	30.76	17.01
	N		150	150	150	30	30	30	30	30	30	30	30	50
Al1	śr	47.5[ab]	10.21[a]	2.26[a]	18.94[a]	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	27.73[a]
	min–max	8–78	9.16–11.50	2.1–2.6	17.6–19.8									7.68–51.43
	sd	20.6	0.85	0.22	0.89									11.90
	N		150	150	150									49
Al2	śr	61.5[b***]	9.28[a]	2.10[a]	15.31[b**]	73.49[b*]	51.02[b]	3.23[b]	0.0	349.0[b*]	98.22	16.78	81.44	29.12[a]
	min–max	7–135	7.60–10.40	1.5–2.2	12.3–16.8	26.7–138	1.9–154.4	0–29	0–0	52–852	90.32–100	0.00–59.41	39.85–100	5.10–67.83
	sd	27.3	1.18	0.38	1.76	31.24	41.36	7.24	0	199.9	2.45	16.36	16.15	13.47
	N		150	150	150	30	30	30	30	30	30	30	30	50
2–year–old seedlings														
O	śr	n.o.	26.6[a]	4.7[a]	23.1[a]	26.17[a]	50.72[a]	24.47[a]	1.59[a]	657.7[a]	95.73	25.83	69.90	44.52[a]
	min–max		12.4–43.2	2.1–10.9	15.1–46.9	17.1–41	18–114.7	1.9–75.5	0–16.2	405–920	88.35–100	5.47–43.19	49.50–92.07	15.57–70.24
	sd		6.3	1.4	5.0	26.17	50.72	15.09	3.57	113.5	3.16	10.61	11.95	13.63
	N		125	125	125	30	30	30	30	30	30	30	30	50
Al1	śr	n.o.	27.2[a]	4.3[ab]	22.0[a**]	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	47.65[a]
	min–max		14.4–42.2	2.4–8.5	12.0–35.5									26.10–71.96
	sd		6.0	1.1	4.2									8.77
	N		125	125	125									50
Al2	śr	n.o.	25.9[a]	4.2[b]	20.5[b***]	20.61[b]	29.78[b]	8.28[b]	0.12[a]	531.3[b*]	98.15	2.12	96.03	45.01[a]
	min–max		11.3–40.4	1.2–7.4	9.0–34.0	10.1–30.9	10.2–46.9	2.2–18.3	0–1.4	325–768	94.33–100	0.00–17.32	82.68–100	25.27–76.03
	sd		5.1	1.3	5.8	4.75	29.78	4.42	0.31	123.0	1.79	3.47	3.73	12.96
	N		125	125		30	30	30	30	30	30	30	30	50

Explanation of the Table 2: H – height the aboveground part; Ø – thickness in neck root; L – length of the main root; AFK – acid phosphatase of the root faces ends [µg p-NF 50 mg roots$_{-1}$1h$_{-1}$]; śr – mean value; min–max – minimum and maximum value; sd – standard deviation; N – number of repetitions, the min–max and sd values of H, Ø and L 1-year-old seedlings are mean values obtained from 30 repetitions of each plot, different small letters of the alphabet in superscript at the data mean significant differences in the distribution of the data with a probability: * <0.05, ** <0.01, *** <0.001 (U Mann–Whitney test); n.o. – not determined; $_{1}$ – as in Table 1.

Table 3. Mean values, standard deviation (from five replicates), and the minimum and maximum (min–max) values of the concentration macronutrients (in%), and some micronutrients (in mg kg^{-1}) in needles of pine seedling one (1) and two years (2) of control variant (O), with a smaller (Al1) and higher (Al2) doses of aluminium sulphate.

Variant	Value	N		P		K		Ca		Mg		S	
		%											
		Age of seedlings											
		1	2	1	2	1	2	1	2	1	2	1	2
O	śr	2.19[a]	1.30[a]	0.21[a]	0.19[a]	0.72[a]	0.87[a]	0.47[a]	0.61[a]	0.087[a]	0.12[a]	0.15[a]	0.10[a]
	min–max	1.89–2.68	0.98–1.54	0.20–0.22	0.18–0.20	0.59–0.86	0.78–0.96	0.43–0.58	0.59–0.67	0.08–0.10	0.11–0.13	0.11–0.20	0.047–0.139
	sd	0.291	0.256	0.012	0.008	0.125	0.073	0.065	0.041	0.008	0.006	0.042	0.043
Al1	śr	1.83[a]	1.33[a]	0.19[a]	0.18[a]	0.74[a]	0.88[a]	0.46[a]	0.64[a]	0.091[a]	0.11[ab]	0.15[a]	0.08[a]
	min–max	1.17–2.34	0.63–1.58	0.17–0.20	0.18–0.19	0.60–0.88	0.80–0.95	0.41–0.55	0.56–0.71	0.08–0.10	0.09–0.11	0.09–0.20	0.03–0.15
	sd	0.472	0.401	0.009	0.003	0.120	0.053	0.050	0.067	0.007	0.008	0.040	0.045
Al2	śr	2.07[a]	1.51[a]	0.18[a]	0.18[a]	0.68[a]	0.89[a]	0.47[a]	0.71[a]	0.089[a]	0.10[b]**	0.19[a]	0.07[a]
	min–max	1.86–2.59	1.31–1.71	0.14–0.21	0.16–0.20	0.32–0.84	0.83–0.95	0.33–0.55	0.61–0.78	0.07–0.10	0.09–0.11	0.15–0.20	0.02–0.12
	sd	0.302	0.153	0.025	0.017	0.212	0.046	0.087	0.065	0.011	0.007	0.023	0.039

Variant	Value	Mn		Cu		Zn		Fe		N:S		N:P	
		mg kg^{-1}											
		Age of seedlings											
		1	2	1	2	1	2	1	2	1	2	1	2
O	śr	164.20[a]	152.95[a]	9.23[a]	6.95[a]	88.55[a]	92.61[a]	365.50[a]	364.05[a]	16.04[a]	15.47[a]	10.55[a]	6.88[a]
	min–max	45.2–280.0	103.8–204.3	8.43–10.3	5.7–11.92	56.5–115.8	79.09–99.53	345.0–410.0	265.6–474.1	10.60–25.11	7.66–22.49	8.55–13.35	5.31–7.82
	sd	88.95	40.27	0.92	2.78	24.49	8.11	26.01	82.36	6.09	6.35	1.80	1.21
Al1	śr	395.00[ab]	185.01[ab]	8.99[a]	5.53[a]	132.10[b]*	97.05[a]	376.50[a]	311.94[a]	12.58[a]	21.60[a]	9.79[a]	7.27[a]
	min–max	198.2–521.3	129.9–226.1	8.35–9.93	4.81–6.31	122.5–139.8	91.09–104.7	280.0–470.0	278.5–349.1	6.91–17.36	5.84–43.44	5.84–13.39	3.45–8.73
	sd	138.28	43.62	0.68	0.57	6.94	5.78	71.67	25.75	4.25	14.81	2.90	2.19
Al2	śr	548.25[b]*	250.50[b]*	8.73[a]	5.70[a]	127.20[ab]	98.03[a]	426.00[a]	344.87[a]	11.24[a]	32.45[a]	11.35[a]	8.25[a]
	min–max	380.3–735.0	202.3–288.1	6.65–9.6	5.03–7.23	105.3–145.0	86.38–110.4	285.0–545.0	284.8–536.0	9.48–13.61	12.31–87.46	9.66–12.92	7.40–9.09
	sd	164.29	34.05	1.24	0.89	16.17	10.07	96.20	107.85	2.09	31.02	1.48	0.69

Explanation: little different letters of the alphabet mean differences in pH values between experience variants with probability: * <0.05, ** <0.01 and *** <0.001 (Kruskal–Wallis test).

and Al2, as in needles of 2-year-old seedlings, slightly exceeded the range of the optimal values (Fober 1993). In needles of 1-year-old seedlings, there were no statistically significant differences in concentration of the analysed nutrients between different variants of experiment, with the exception of manganese and zinc. Significantly higher accumulation of manganese was found in needles of the seedlings in the variant with a higher dose of aluminium sulphate (Al2), and zinc in the variant with a lower dose of aluminium sulphate (Al1) with regard to the concentration of these elements in the needles of seedlings from the control variant (Table 3). In the case of needles of 2-year-old seedlings, statistically significant differences of accumulation of magnesium and manganese were observed between the researched variants. Needles of the seedlings from the variant Al2 contained significantly more manganese and less magnesium in comparison with control variant (Table 3). There were no significant differences in molar ratios of N:S and N:P in needles of seedlings examined, neither 1-year-old nor 2-year-old (Table 3).

4. Discussion

Negative impact of aluminium on plant growth in soils with low pH values is considered to be the rule (Królikowski, Ciok 1968; Prusinkiewicz, Krzemień 1974, Filipek 1994; Marschner 1995; De Wit et al. 2010). The applied dose of aluminium sulphate has contributed to lowering the pH of the studied soil. The lowest average pH in H_2O was recorded in August in 2003 and amounted to 5.5, 5.2 and 4.8 in O, Al1 and Al2 soil variants, respectively. Aluminium concentrations toxic to plants can occur in the soil below pH 4.5 (Filipek 1994). Studies by Schöll et al. (2004) showed that aluminium toxicity depends to a large extent on the accumulation of Al directly in the soil solution and the sensitivity of the plant species to the concentration of toxic aluminium ions in the soil solution (Nowak, Friend 1995). The studies on soil with high concentration of aluminium are particularly difficult to interpret in terms of physiological responses of plants since a large part of or almost the entire amount of added aluminium is lost presumably due to precipitation (e.g. in the form of phosphate) or by polymerisation and complexation (Marschner 1995).

The study shows that in the soil with a higher dose of aluminium (Al2), the concentration of free aluminium in the soil solution, both in 2003 and 2004, was too high, contributing significantly to shorten the length of the roots, especially the lateral roots, and to reduce the diameter of the root collar of 2-year-old seedlings. Lateral roots are more sensitive to aluminium than the main roots and accumulate more of it (Silva et al. 2000). In studies on toxicity of high doses of aluminium to plants, it is commonly found that roots are more sensitive than stems (Nowak, Friend 1995).

Too low concentration of potassium in the needles of seedlings collected from the variant Al2 confirms that the concentration of toxic Al ions in the soil solution was too high (Filipek 1994).

The toxicity level of aluminium is also modified by occurrence of ectomycorrhizae (Moyer-Henry et al. 2005). Seedlings grown in the variant with a higher dose of aluminium were characterised by higher frequency of ectomycorrhizae and lower frequency of ectendomycorrhizae than in the control variant. Study conducted by Moyer-Henry et al. (2005) shows that the tolerance to aluminium of pine seedlings is associated both with the removal of aluminium from the apical part of the root and absorbing it in the peripheral areas, in cells outside the meristem, as well as with the accumulation of aluminium in the hyphae of the mycelium and in the Hartig net area of lateral roots colonised by mycorrhizal fungi (*Pisolithus tinctorius*). Clearly, more numerous occurrences of ectomycorrhizae than ectendomycorrhizae in seedlings from the variant with a higher dose of aluminium compared with the control may suggest that the presence of aluminium at lower pH of the soil determines the occurrence of ectendomycorrhizal and ectomycorrhizal fungi. Commonly known is the interspecific and intraspecific variability of response of mycorrhizal fungi in relation to aluminium ion (Garcidueñas-Piña, Cervantes 1996; Majewska, Werner 2001).

The use of aluminium sulphate has contributed to more efficient emergence of seedlings. The reason could be a reduced disease predisposition of seedlings by improving conditions for growth – favourable soil pH, which is a well-known phenomenon. Another reason could be inhibiting the development of pathogens that cause damping-off as a result of changes in the population of soil microorganisms or as a result of changes in physico-chemical properties of the soil. Based on experiments conducted, it is difficult to say whether this is a direct effect of aluminium ions or indirect, by lowering the pH. In the study by Fichtner (2003), isolates of *R. solani* from pine seedlings with damping-off symptoms were more inhibited in an environment with the addition of $Al_2(SO_4)_3$ buffered to pH 4 than to pH 6. At pH < 4, the most significant form of aluminium is free Al^{3+} ion. Predominance of $Al(OH)_2^+$ ions and Al com-

plexes with organic matter of peat enriched by a solution of $Al_2(SO_4)_3$ with a pH of 6 indicates that for the suppression of *Phytophthora parasitica* other than Al^{3+} ions are responsible (Fichtner 2003).

The presence of aluminium at low pH may limit the growth of fungal hyphae and inhibit the germination of spores of fungi of the genus *Fusarium* (Dursun, Boddy 2002). This corresponds to the results of this study, as after the use of aluminium sulphate, the frequency of *F. oxysporum* as the cause of damping-off of pine seedlings was lower than in the control variant. In the study by Huang and Kuhlman (1991a), usage of SF-21 formulation, containing mainly aluminium sulphate, reduced losses in pine done by damping-off (*Pinus elliottii* Engelm.) caused by *R. solani*, *P. aphanidermatum* and *F. moniliforme* var. *subglutinans*, and the pathogen population in the soil was also reduced. At the same time, an increase in the population of saprotrophic fungi of the genus *Trichoderma*, *Penicillium* and *Gliocladium* was noted. These fungi are known for their antibiotic effects against pathogens (Domsch et al. 1980). In laboratory tests, Huang and Kuhlman (1991b) showed that soil enrichment with SF-21 inhibits the development of *P. aphanidermatum* and *R. solani* hyphae. The density of *Trichoderma* spp. and *Penicillium* spp. population in soil enriched with SF-21 was negatively correlated with the pH value in the range 4–6. The $Al_2(SO_4)_3$ ability of inhibiting the growth of *R. solani* and *P. aphanidermatum* in aqueous agar was much greater at pH 4 than at pH 6. Huang and Kuhlman (1991b) on the basis of their study came to the conclusion that the mechanism of *R. solani* inhibition was of an indirect nature and was the result of stimulation of the development of other microorganisms, in particular *Trichoderma harzianum* and *Penicillium oxalicum*. The development of *Pythium aphanidermatum* was inhibited both directly, through the inorganic and organic components of the formulation, and indirectly, by lowering the pH of the soil and stimulating the growth of microorganisms.

Although aluminium is not included in the elements essential to plants, low concentration of it in soil can favourably influence the growth of plants that have a high tolerance for this element and the ability to absorb it (Marschner 1995).

Gawliński (1978) in his sand-water cultures experiment noticed the stimulating effect of aluminium on the growth of pine seedlings at a dose of 10 ppm in the medium. Also, in the present study, the seedlings grown in the variant with a lower dose of aluminium (Al1) had better growth characteristics, although the average values of the studied parameters did not differ significantly from the parameters of seedlings in the control variant. The applied doses of aluminium sulphate contributed significantly to the increased concentration of manganese in the needles of the surveyed plants. This may mean that the accumulation of aluminium in the soil solutions does not contribute to the inhibition of manganese absorption, which was observed by Marschner (1995), in soils with high concentrations of Al but results from a reduction in soil pH (Lityński, Jurkowska 1982).

In our study, the needles of 2-year-old seedlings from the variant Al2 had significantly lower concentration of magnesium than in the control variant, while there were no significant differences in the case of calcium. Reduced concentration of phosphorus along with addition of doses of aluminium can also be observed. In the case of other macro- and microelements in the needles of 1- and 2-year-old seedlings, no significant differences of accumulation were recorded. Their content was in the range of optimal values (Fober 1993). There was no observable change in needle colouration of pine seedlings after the application of both doses of aluminium sulphate.

The toxic effect of aluminium (with a concentration of up to 500 μmol L^{-1}) on the growth of fine roots and growth of plants, noted in the hydroponic studies and pot experiments, has not been confirmed in long-term field experiments conducted in a mature stand of spruce (De Wit et al. 2010). However, the magnesium content in needles decreased significantly and steadily in the plots with increased concentration of Al, while the content of Ca in needles did not change and there were no other changes indicating the reduction of stand vitality (De Wit et al. 2010).

The reduced phosphorous concentration in needles of seedlings from the variants Al1 and Al2 with respect to the control variant, though statistically insignificant, can cause increase in chemical sorption of phosphates with decreasing soil pH and formation of phosphates of aluminium, although this was not confirmed by the results of Gawliński's study (1978).

The reduced concentration of magnesium in the needles of analysed seedlings may be due to increased rate of magnesium leaching from the soil with increasing acidity level and easier aluminium penetration into the cell (compared to magnesium) and the blockade of absorption locations (Filipek 1994).

Tree species of the genus *Pinus* and *Picea* grow on very acidic soils with a high concentration of aluminium. They could therefore be classified as the so-called aluminium accumulators or tolerant to high concentrations

of aluminium in the soil due to the numerous mechanisms of adaptation to grow under the harmful influence of this nutrient (Filipek 1994, Gruba 2004, Moyer-Henry et al. 2005). Research results of Moyer-Henry et al. (2005) clearly indicate that pine seedlings (*Pinus taeda* L.) are highly resistant to Al, and the elongation growth of primary root is not reduced until the concentration of Al^{3+} is close to 40 μmol L^{-1}. This growth was reduced only by 30% when activity of Al^{3+} reached 580 μmol L^{-1}.

5. Summary and conclusions

1. There were statistically significant differences in the number of 1-year-old seedlings in different variants of experiment. The highest average number of seedlings was recorded in the variant with a higher dose of aluminium sulphate and the lowest in the control variant.

2. *Fusarium oxysporum* and *Pythium* spp. were isolated less frequently from seedlings collected from the variant with a higher dose of aluminium sulphate than from the seedlings of control variant. Insertion of aluminium sulphate to soil did not affect the frequency of isolation of *Rhizoctonia solani* from pine seedlings.

3. The use of aluminium sulphate in a higher dose had a positive impact on the spectrum of pine seedlings mycorrhizae; the share of ectomycorrhizae in relation to ectendomycorrhizae increased.

4. The applied doses of aluminium sulphate did not affect the phosphatase and invertase activity in analysed soil nor on the phosphatase activity of the root ends.

5. The use of a higher dose of aluminium sulphate contributed significantly to reducing the length of main and lateral roots as well as the number of short roots of pine seedlings.

6. The applied dose of aluminium sulphate contributed significantly to increase accumulation of manganese and zinc in needles of pine seedlings, but reduced the magnesium concentration.

7. The lower applied dose of aluminium sulphate can be considered as the threshold for the analysed soil due to the inhibitory effect on the growth of pine seedlings of a higher dose of the compound.

8. The study confirmed the beneficial effects of low doses of aluminium on the performance of emergence and growth parameters of pine seedlings. On the basis of experiments carried out, it is difficult to say whether this beneficial effect results from the direct impact of aluminium on the emergence of seedlings, or from an indirect effect – the development of pathogens and forming of the structure of mycorrhizae by lowering the pH of the soil.

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Author contributions

Kazimierz Januszek and Hanna Stępniewska – concept, assumptions, interpretation of results, writing, coordination and review of the literature; Ewa Błońska – statistical analysis, preparation of figures, editing and preparation of manuscript; Joanna Molicka and Krzysztof Kozieł – sampling of soils and plants, preparation of research materials, collation of data and review of the literature; Anna Gdula and Anna Wójs – collection and analysis of the roots of plants and mycorrhizae and summarising data.

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