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The influence of nitrogen nutrition on the dynamics of growth and metabolism of endogenous growth regulators in Scotch pine (*Pinus silvestris* L.) seedlings*

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Abstract

Pine seedlings were cultivated in sterile agar cultures containing nitrogen as NH_4Cl. The most pronounced positive effect on the growth of seedlings was affected by N used at a concentration of 50 ppm. After 4 months was stated that nitrogen had only a slight effect on elongation of shoots but decreased the length of roots. Nitrogen increased the lenght and number of primary and secondary needles as well as the fresh and dry matter of shoots. It stimulated also the number of lateral roots and the fresh and dry matter of the root system.

Stimulation of shoot growth and differentiation as a result of nitrogen treatment was correlated with the increase of free gibberellins and auxins and decrease of the amount of bound gibberellins and ABA-like inhibitor in shoots. However the effect of N on growth of roots was connected with the increase of auxins, cytokinins and ABA-like inhibitor in these organs.

INTRODUCTION

Mineral nutrition significantly influences the growth and development of plants. On the other hand there exists a strict dependence between the intensity of growth and the metabolism of plant hormones. It is also known that mineral fertilization influences the level of plant hormones.

As far as we are aware, the knowledge concerning this problem is very scarce. It is known however that N influences the level of auxins (Avery et al., 1937; Avery and Pottorf, 1945; Gustafson, 1946;

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Gorter, 1954; Bojčuk, 1960; Witt, 1964; Voronina, 1967; Jakuškina and Čurikova, 1967; Rajagopal and Rao, 1974) and gibberellins (Voronina, 1967; Jakuškina and Čurikova, 1967; Rajagopal and Rao, 1974) and NPK (Aleksejev and Starceva, 1950) and Ca (Andreae and Ysselstem, 1960) influence the level of auxins. The amount of auxins and gibberellins were also changed by treatment of plants with some trace elements (Sójkowski, 1971; Kutaček and Jiraček, 1974; Školnik, 1974). On the other hand it was found that the deficiency of mineral substances in nutrient solution increased the level of growth inhibitors (Mizrahi and Richmond, 1972; Rajagopal and Rao, 1974). There are also some data showing a synergism between mineral substances and plant hormones in the influence on growth of plants (Ivanova and Bakrdżieva, 1973; Belova, 1974; Lau and Yang, 1974). However the above mentioned data deal with herbaceous plants and we have no information concerning this problem in conifers.

Among mineral substances necessary for plant growth, nitrogen is especially important. It is also known that pine seedlings are very sensitive to nitrogen nutrition (Gunia, 1967; Etter, 1969). Thus, it is just the reason that the studies concerning the influence of mineral nutrition were started with experiments with nitrogen.

The aim of this work was to learn how the nitrogen nutrition influences the growth and differentiation of pine seedlings and to what extent the changes in growth intensity of shoots and of roots obtained by nitrogen nutrition are reflected by the changes in the level of gibberellins, cytokinins, auxins and growth inhibitors.

MATERIAL AND METHODS

Seeds of pine (*Pinus silvestris* L.) sterilized with 1% bromine water were germinated on 0.8% sterile agar (product of Japan) during 4 days. Then selected germinated seeds were transfered into glass tubes filled to 1/2 volume with sterile agar containing nitrogen at different concentrations. The tubes were placed into wooden devices. In this way the roots of the seedlings were kept in darkness.

The germination of seeds as well as cultivation of seedlings was conducted on 16 hrs day, light intensity about 3000 lx and temperature 25°C.

Basing on the results of Zajączkowska's experiments with Scotch pine seedlings (1973) NH_4Cl was chosen as nitrogen source.

In our preliminary experiments it was stated that nitrogen used at concentration above 200 ppm (calculated on pure substance) strongly inhibited the growth of seedlings and at concentration below 10 ppm did not influence the growth of plants. Basing on these results, in experiments presented here, nitrogen at concentration 10—200 ppm (calculated on pure substance) was used.

Using sterile agar culture we were able to eliminate the influence of microorganisms on plant growth. It is known that microorganisms of the rhizosphere and rhizoplane of pine seedlings are capable of producing considerable amounts of plant growth regulators like auxins and gibberellins (Kampert et al., 1975 a, b; Strzelczyk et al; 1975). From our earlier experiments (unpublished) we know that pine seedlings can grow in sterile agar cultures even for one year. It must be underlined that solubility of oxygen in soft agar is similar to that of water (Rovira, 1965).

Every variant of the experiment included 10 tubes. The measurement of seedlings and determination of growth regulators were performed after 4 months.

Plant growth regulators were extracted from frozen samples with aqueous methanol during 48 hr.

Gibberellins were fractionated according to the method of Rappaport et al. (1967) presented in Fig. 1. Then they were partitioned chromatographically using thin layer chromatography (Silica gel G, solvent system: benzene, acetic acid 10:3 v/v). The lettuce hypocotyl test was used for determination of gibberellin activity.

Auxins and ABA-like inhibitor were fractionated according to the method presented in Fig. 2 and partitioned chromatographically using the column chromatography with Sephadex LH-20. The solvent was 70% ethanol to which HCl was added to make a final concentration of 0.001 M (Steen and Eliasson, 1969). Succesive fractions (10 ml each) were bioassayed using *Avena* section straight growth test for auxins and by wheat coleoptile test for ABA.

Cytokinins were extracted and purified according to the method of Hewett and Wareing (1973), except that the polyvinylpyrollidine step was omitted (Fig. 3). The cation exchange column (Dowex 50, H^+ form) was applied, which was washed with 70% methanol and distilled water. Extracts were eluted with 2 and 5 N NH_4OH, and chromatographed on Whatman's 3 MM paper with butan-2-ol : 25% NH_4OH (4 : 1 v/v). The soybean tissues were used in the growth test (Miller, 1968).

All results were analysed statistically by estimating LSD at P=0.01 and 0.05.

RESULTS AND DISCUSSION

As seen from the data presented in Tables 1 and 2 and in Fig. 4 nitrogen had only a slight effect on the elongation of shoots but significantly increased the length and number of primary and secondary needles as

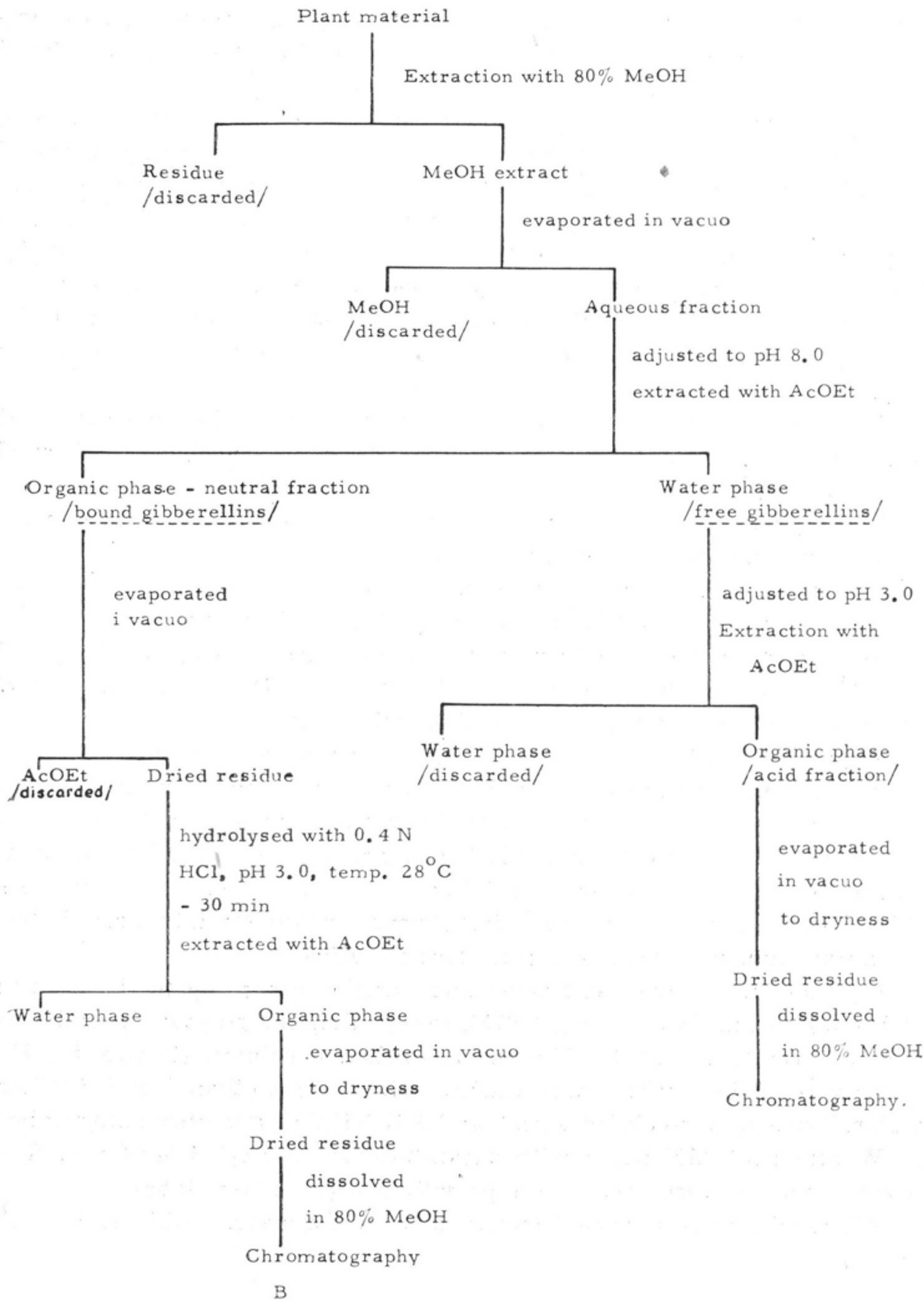

Fig. 1. Extraction of gibberellins

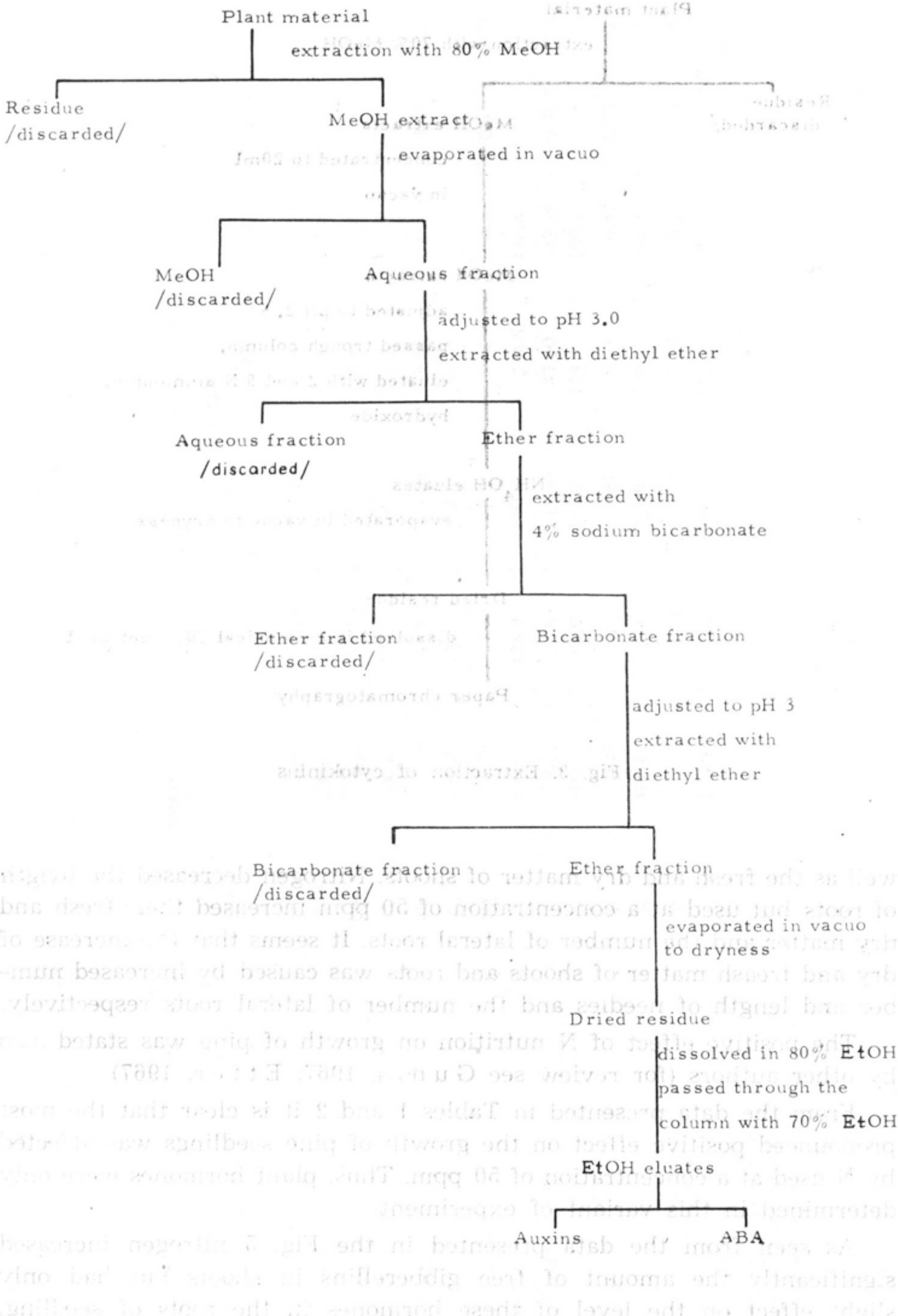

Fig. 2. Extraction of auxins and ABA

Plant material

extraction with 70% MeOH

Residue discarded/

MeOH extracts

concentrated to 20ml in vacuo

MeOH extracts

adjusted to pH 2.5

passed trough column,

eluated with 2 and 5 N ammonium hydroxide

NH_4OH eluates

evaporated in vacuo to dryness

Dried residue

dissolved in ammonical 70% methanol

Paper chromatography

Fig. 3. Extraction of cytokinins

well as the fresh and dry matter of shoots. Nitrogen decreased the length of roots but used at a concentration of 50 ppm increased their fresh and dry matter and the number of lateral roots. It seems that the increase of dry and freash matter of shoots and roots was caused by increased number and length of needles and the number of lateral roots respectively.

The positive effect of N nutrition on growth of pine was stated also by other authors (for review see Gunia, 1967; Etter, 1967).

From the data presented in Tables 1 and 2 it is clear that the most pronounced positive effect on the growth of pine seedlings was affected by N used at a concentration of 50 ppm. Thus, plant hormones were only determined in this variant of experiment.

As seen from the data presented in the Fig. 5 nitrogen increased significantly the amount of free gibberellins in shoots but had only slight effect on the level of these hormones in the roots of seedling. Probably the hormones move from the roots, which are the sites of GA biosynthesis, to the shoots stimulating their elongation.

Table 1

Effect of N nutrition on the growth of shoots of pine seedlings (plants grown in agar cultures for 4 months)

Kind of measurements*	Values in:	Concentrations of N in ppm					LSD	
		0	10	50	100	200	P=0.05	P=0.01
Length of shoots	cm	4.98	4.66	5.43	5.61	5.15	0.69	0.93
	%**	100.0	93.6	109.0	112.6	103.4		
Length of secondary (dwarf shoot) needles	cm	5.10	6.25	10.14	12.40	13.58	1.58	2.12
	%	100.0	122.5	198.8	243.1	266.3		
Number of secondary needles	cm	5.38	6.88	9.00	9.88	10.50	3.47	4.66
	%	100.0	127.9	167.3	183.6	195.2		
Length of primary (juvenile) needles	cm	2.35	3.03	3.31	3.50	3.45	0.35	0.47
	%	100.0	128.9	140.8	148.9	146.8		
Number of primary needles		38.50	34.63	45.50	47.50	42.13	9.28	12.45
	%	100.0	89.9	118.2	123.4	109.4		
Fresh weight of shoots	g	0.27	0.34	0.61	0.73	0.80	0.09	0.12
	%	100.0	125.9	225.9	270.4	296.3		
Dry weight of shoots	g	0.11	0.15	0.24	0.25	0.28	0.03	0.05
	%	100.0	136.4	218.2	227.3	254.4		

* average of 10 seedlings in each variant.
** per cent in relation to control.
Significant differences at: — P = 0.05, ... P = 0.01.

Table 2

Effect of N nutrition on the growth of roots of pine seedlings (plants grown in agar cultures for 4 months)

Kind of measurements*	Values in:	Concentrations of N in ppm					LSD	
		0	10	50	100	200	P=0.05	P=0.01
Length of roots	cm	12.00	12.43	7.61	5.74	6.19	2.23	2.99
	**	100.0	103.6	63.4	47.8	51.6		
Number of the first order of lateral roots		20.00	22.50	27.50	19.88	16.13	7.43	9.97
	%	100.0	112.5	137.5	99.4	80.7		
Fresh weight of roots	g	0.28	0.36	0.41	0.28	0.16	0.12	0.16
	%	100.0	128.6	146.4	100.0	57.1		
Dry weight of roots	g	0.05	0.06	0.06	0.04	0.03	0.008	0.01
	%	100.0	120.0	120.0	80.0	60.0		

* average of 10 seedlings in each variant.
** per cent in relation to control.
Significant differences at: — P = 0.05, ... P = 0.01.

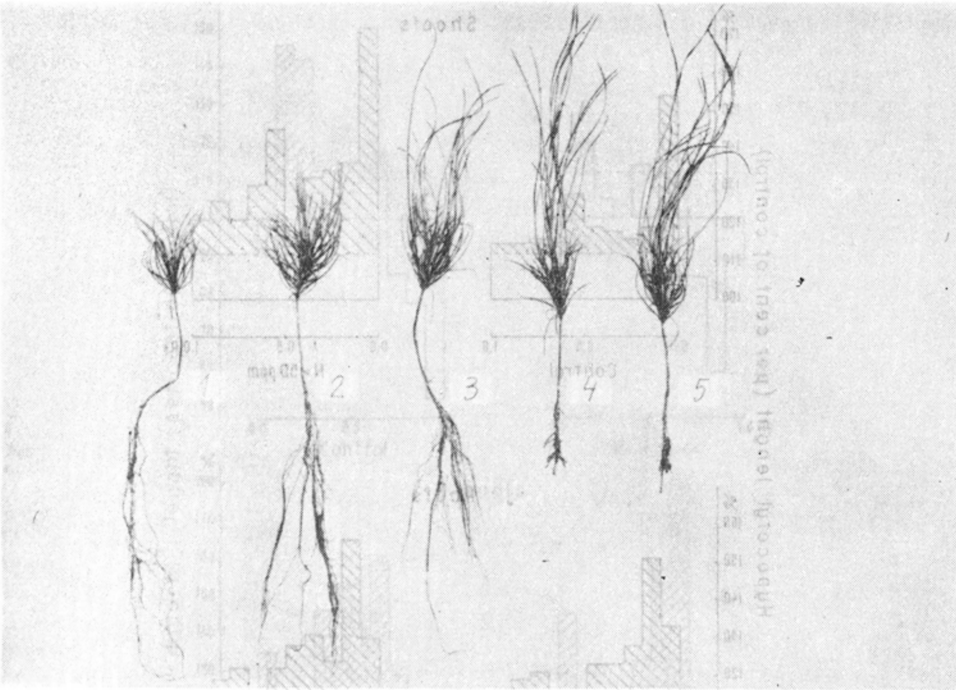

Fig. 4. Effect of N nutrition on growth of pine seedlings (plant grown in agar cultures for 4 months). Nitrogen concentrations in ppm: *1* — 0, *2* — 10, *3* — 50, *4* — 100, *5* — 200

Nitrogen strongly decreased the level of bound gibberellins in shoots but had no significant effect on the level of these compounds in the roots (Fig. 6).

The increase of free gibberellins as an effect of nitrogen nutrition is in accordance with results of other authors who studied this problem in herbaceous plants (W i t t, 1964; V o r o n i n a, 1967). The data presented here confirm also the results of our earlier experiments showing that the increase of growth intensity of pine shoots are correlated with the increase of free gibberellins in these organs (M i c h n i e w i c z et al., 1974).

A different picture was obtained in experiments with cytokinins (Fig. 7). Nitrogen only slightly influenced the level of these hormones in shoots but significantly increased their level in roots. Thus, contrary to gibberellins we did not find any clear correlation between the influence of nitrogen on growth intensity of pine seedlings and the level of cytokinins in these plants. Lack of correlation between growth intensity of Scotch pine seedlings and the level of cytokinins was demonstrated also in our earlier experiments (M i c h n i e w i c z et al., 1974).

Unfortunately we have no information from the literature concerning the influence of nitrogen on the metabolism of cytokinins. Thus, we

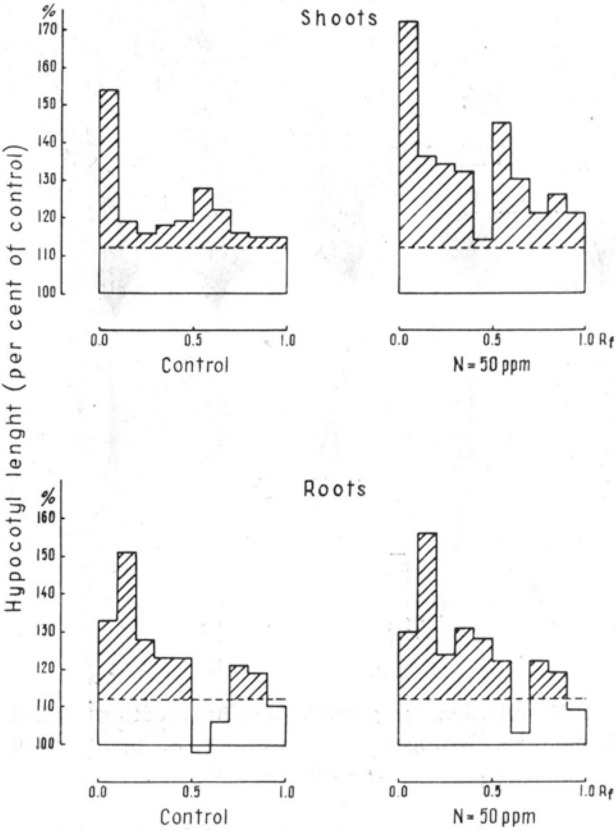

Fig. 5. Effect of N nutrition on the level of free gibberellins in the shoots and roots of pine seedlings (plants grown in agar cultures for 4 months)

cannot compare the results of our experiments with the data of other authors.

The results of the analyses presented in Figs 8 and 9 show that N increased significantly the amount of auxins in shoots as well as in roots. It decreased ABA-like inhibitor in shoots but increased this inhibitor in roots of plants.

Thus, the stimulation of growth and differentiation of shoots was correlated with the increase of auxins and decrease of growth inhibitor. These data are in accordance with the results of Bojčuk (1960), Witt (1964), Voronina (1967), Jakuškina and Čurikova (1967) and Rajagopal and Rao (1974) who stated the increase of auxins in the shoots of some herbaceous plants as an effect of nitrogen nutrition. They are also in accordance with the data of Rajagopal and Rao (1974) who in experiments with tomatoes found that the shoots of plants treated with N contained less growth inhibitors than the untreated ones. The data presented here support also our earlier results

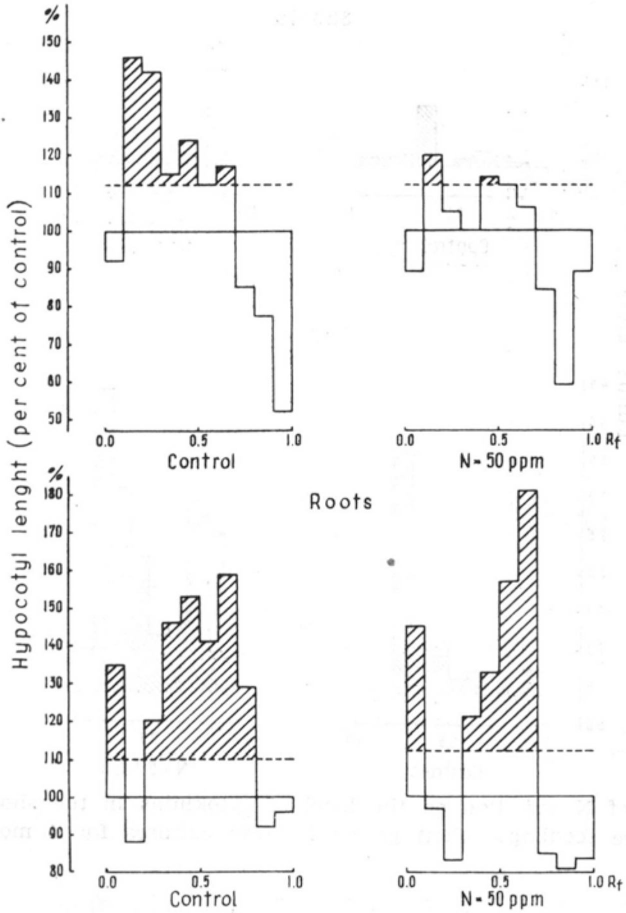

Fig. 6. Effect of N nutrition on the level of bound gibberellins in the shoots and roots of pine seedlings (plants grown in agar cultures for 4 months)

of experiments with Scotch pine showing that the intensive growth of shoots is correlated with the high level of auxins and low level of growth inhibitors (Michniewicz, 1967).

The results presented here show that inhibition of roots elongation is correlated with the increase of auxins. This is clear when taking into consideration the well known fact that roots are more sensitive to auxins than shoots and that the increase of auxins stimulates the roots formation. Thus, nitrogen used at the concentration of 50 ppm increasing the level of auxins simultaneously stimulated the lateral roots formation. Increased number of lateral roots influence the increase of fresh and dry matter of the root system.

The above presented data are in discordance with the results of Bojčuk (1960) who in experiments with tomato plants found that roots treated with nitrogen were characterized by a decrease of auxins.

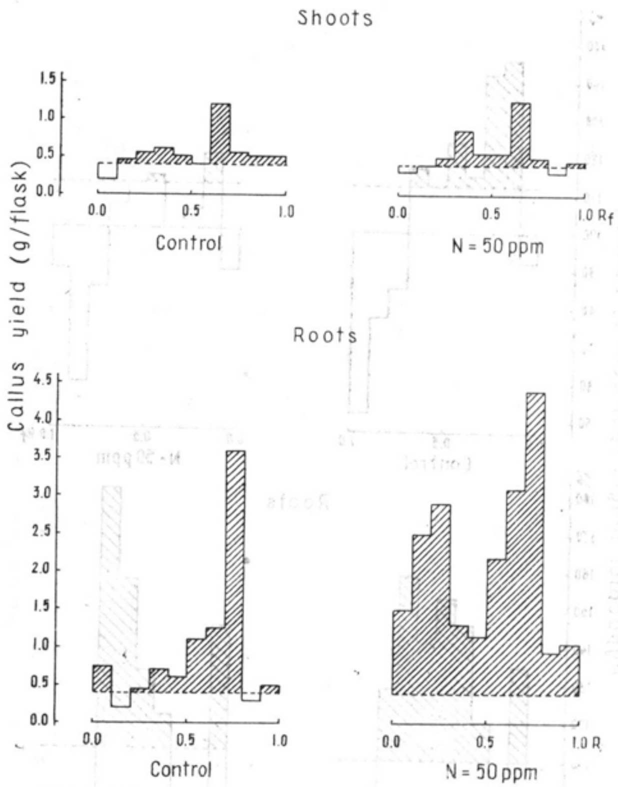

Fig. 7. Effect of N nutrition on the level of cytokinins in the shoots and roots of pine seedlings (plant grown in agar cultures for 4 months)

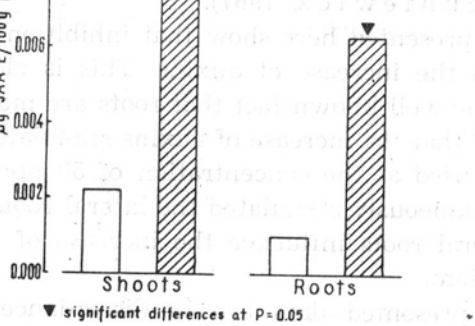

Fig. 8. Effect of N nutrition on the level of auxins in the shoots and roots of pine seedlings (plants grown in agar cultures for 4 months)

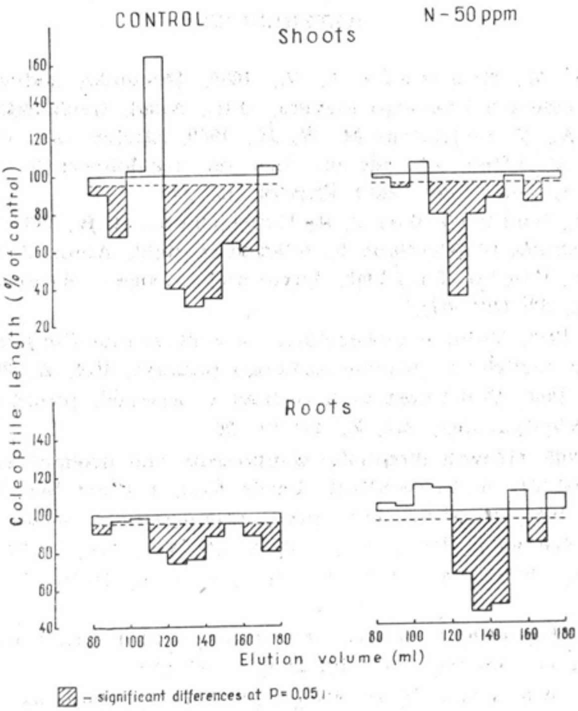

Fig. 9. Effect of N nutrition on the level of ABA-like inhibitor in the shoots and roots of pine seedlings (plants grown in agar cultures for 4 months)

The comparison of the data presented in Table 2 and Fig. 9 show that the increase of ABA-like inhibitor as a result of nitrogen treatment is correlated with the inhibition of roots elongation but not with the process of lateral roots formation.

In experiments of Michniewicz and Galoch (1972) with willow cuttings it was stated that adventitious roots are not the sites of ABA synthesis. Taking this into consideration it could be supposed that in our experiments nitrogen influenced the transport of ABA from shoots to the roots. Thus, it decreased the level of the inhibitor in shoots but increased in roots.

CONCLUSIONS

Stimulation of shoot growth and differentiation of pine seedlings as a result of nitrogen treatment was correlated with the increase of free gibberellins and decrease of the amount of bound gibberellins and ABA-like inhibitor in shoots.

The effect of nitrogen on the growth of roots was connected with the increase of auxins, cytokinins and ABA-like inhibitor in these organs.

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Wpływ azotu na dynamikę wzrostu siewek sosny zwyczajnej (Pinus silvestris L.) oraz metabolizm endogennych regulatorów wzrostu roślin

Streszczenie

Siewki sosny hodowano przez okres czterech miesięcy w sterylnych kulturach agarowych zawierających azot w formie amonowej. Po tym czasie mierzono wzrost

pędów i korzeni oraz oznaczano w nich wolne i związane gibereliny, cytokininy, auksyny i inhibitory o właściwościach kwasu abscysynowego.

Najistotniejsze zmiany wzrostowe stwierdzono pod wpływem N w stężeniu 50 ppm (w przeliczeniu na czystą substancję), dlatego też oznaczenie substancji wzrostowych ograniczono tylko do tego wariantu doświadczenia.

Azot wywierał tylko niewielki wpływ na wzrost elongacyjny pędów, natomiast zwiększał ilość igieł młodocianych i krótkopędowych oraz świeżą i suchą masę pędów. Wpływ azotu na wzrost korzeni ujawnił się obniżeniem wzrostu elongacyjnego, zwiększeniem ilości korzeni bocznych oraz zwiększeniem świeżej i suchej masy systemu korzeniowego.

Stymulacja wzrostu i dyferencjacji pędu jako efekt nawożenia azotem łączyła się ze zwiększeniem ilości wolnych giberelin i auksyn oraz z obniżeniem poziomu giberelin związanych i inhibitora wzrostu w pędach. Wpływ azotu na procesy wzrostowe korzeni łączył się natomiast ze zwiększeniem ilości auksyn, cytokinin i inhibitora wzrostu w tych organach.