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The effect of nitrogen nutrition on growth and on plant hormones content in Scots pine (*Pinus silvestris* L.) seedlings grown under light of different intensity

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Abstract

Pine seedlings were cultivated in the Ingestad nutrient solution containing N as NH_4Cl at concentrations of 0 and 500 ppm, under a 16-hr day, at a light intensity of 1500 and 500 lx and temperature ± 20°C. Measurements of seedlings and determination of plant hormones were performed 8 weeks after sowing. It was found that more intensive light stimulated initiation of needles and lateral roots as well as elongation of needles and roots, and increased the fresh weight and dry matter of these organs. Growth stimulation of needles was correlated with an increase in free gibberellins, cytokinins, an ABA-like inhibitor and with a decrease in auxins and water content of tissues. A similar effect of light on plant hormones (except ABA) was also observed in roots. The level of this inhibitor depended on N nutrition. Nitrogen had a similar effect as light on the growth and initiation of needles and lateral roots. However, it strongly inhibited elongation of roots and increased the water content of the tissues. In needles N increased the level of GAs and auxins, under both light variants, as well as the level of cytokinins, under more intensive light. It decreased the amount of ABA-like inhibitor. In roots the effect of N nutrition on the level of plant hormones depended upon the light intensity. Under light of low intensity N decreased the level of GAs and ABA, increased the level of auxins and had nonsignificant influence on the level of cytokinins. Under more intensive light it had no effect on the GAs and auxin levels and increased the level of cytokinins and the ABA-like inhibitor.

INTRODUCTION

The results of our earlier experiments indicate that nitrogen nutrition significantly increases the growth of pine seedlings and the level of endogenous hormones in these plants (Michniewicz et al., 1976). It is also known that growth intensity of pines depends to a large extent on light conditions (Kramer, Kozlowski, 1960) and that low light intensity arrests seedling development (Kozlowski, Borger, 1971). There are also some data showing that light increases uptake of nitrogen in pine (Giertych, Farrar, 1961) and in other plants (Felippe et al., 1975). It is also known that low light intensity makes impossible a growth response to the N fertilizer. This was found eg. in experiments with oak seedlings (Phares, 1971).

On the other hand it was found that light influences the level of plant hormones in pine (Kopcewicz, 1971).

Taking these facts into consideration we decided to study the effect of nitrogen nutrition on the growth and the level of plant hormones in Scots pine seedlings grown under light of different intensity.

MATERIAL AND METHODS

The pine seedlings (*Pinus silvestris* L.) were cultivated for two weeks in sawdust soaked with distilled water, on a 16-hr day, at a lignt intensity of about 4000 lx and temperature ± 20°C. When the seedlings acquired the stage "free from the seed coat" they were transferred to the Ingestad (1962/63) nutrient solution containing N as NH_4Cl at concentrations of 0 and 50 ppm, on a 16-hr day, at a light intensity of 2500 lx and 500 lx and at a temperature of ± 20°C. The solution was continuously aerated and renewed every 10 days.

The concentration of nitrogen (50 ppm) was chosen basing on the results of our earlier experiments (Michniewicz et al., 1976). However, light intensity was based on the results of preliminary experimants. From the data of these experiments it is clear that light intensity of 500 lx is sufficient for seedling development and that under these light conditions seedlings respond to the N fertilizer. Very clear differences in the growth and development as well as in the response to the N fertilizer between the variants with 500 and 2500 lx were also stated. The use of more light intensities and nutrient variants was impossible for technical reasons.

The measurements of seedlings and determination of plant hormone levels in needles (including the shoot apex) and roots were performed 8 weeks after sowing. Auxins, gibberellins, cytokinins and the abscisic acid-like inhibitor were extracted, fractionated and determined by the methods described earlier (Michniewicz et al., 1976), with the exception of auxin and ABA partitioning. At present auxins, cytokinins and ABA were partitioned by paper chromatography (Whatman No. 3) in the solvent system: isopropanol, ammonia, water (10:1:1).

All analyses were replicated 3 times and the results were elaborated statistically by analysis of variance using the t-test for significance and LSD at P = 0.05.

RESULTS AND DISCUSSION

As seen from the data presented in Tables 1 and 2 light intensity had significant effect on the growth of plants. Increased light intensity stimulated the elongation of needles and main roots as well as the initiation of needles and lateral roots and inhibited the elongation of cotyledons and hypocotyls. This was found in both nutrient variants i.e. with and without N.

Stimulation of growth of needles and roots by more intensive light conditions was correlated with the increase of fresh and dry matter of these organs. In the case of hypocotyls the inhibition of growth was correlated with a small decrease of fresh weight and significant increase of dry matter (Table 1 and 2). Under more intensive light a higher percent of dry matter was found in fresh matter (Table 2).

Table 1

Effect of N nutrition on growth of pine seedlings under different light intensity after 8 weeks (averages from 20 seedlings)

Light intensity (lx)	500		2500	
Concentration (ppm)	0	50	0	50
Length of cotyledons (cm)	2.32	2.42	2.13 b	2.20 b
Length of hypocotyls (cm)	4.60	4.61	4.21 b	4.14 b
Length of primary (juvenile) needles (cm)	1.75	1.95 a	2.37 b	3.41 ab
Number of primary needles	11.00	13.00 a	19.00 b	28.00 ab
Length of roots (cm)	8.00	6.87 a	17.60 b	12.80 ab
Number of first order lateral roots	initiation		11.00 b	18.00 ab

a — significant differences between nutrient combinations at P = 0.05
b — significant differences between light intensities at P = 0.05

Such correlations between light and growth processes are in accordance with the results obtained with white pine seedlings (Mitchel, 1936, cited by Kramer, Kozlowski, 1960). Nitrogen nutrition had a significant effect on growth and development of pine seedlings.

Table 2

Effect of N nutrition on fresh and dry weight of pine seedlings under different light intensity after 8 weeks (average from 30 seedlings, in g/100 seedlings)

Light intensity (lx)		500				2500			
Concentration (ppm)		0		50		0		50	
Measurement		f.wt.	d.wt.	f.wt.	d.wt.	f.wt.	d.wt.	f.wt.	d.wt.
Needles	g	3.36	0.60	4.32 a	0.74 a	5.97 b	1.26 b	8.72 ab	1.59 ab
	%		17.8		17.1		21.1		18.3
Hypocotyls	g	1.31	0.21	1.37	0.22	1.09 b	0.32 b	1.28 ab	0.34 ab
	%		16.2		15.9		29.7		26.5
Roots	g	0.89	0.11	0.87	0.08 a	3.07 b	0.40 b	2.87 ab	0.32 ab
	%		12.4		10.1		13.0		11.1

a — significant differences between nutrient combinations at P = 0.05
b — significant differences between light intensities at P = 0.05

Similarly as light it increased the number of needles and lateral roots and stimulated the elongation of needles. However, in contrast to light, nitrogen had no effect on elongation of cotyledons and hypocotyls and inhibited elongation of roots. These relationships were found in both light variants. Similarly as light nitrogen also increased fresh and dry matter of needles. The stimulation of fresh and dry matter of hypocotyls and inhibition of these parameters in roots was only observed at more intensive light (Table 2). Thus, these results are in accordance with earlier data obtained with 4-month-old pine seedlings in agar cultures (Michniewicz et al., 1976).

It appears from the data presented in Figs. 1 and 2 that free gibberellins were localized in 3 zones corresponding to R_f 0.0-0.3, 0.4-0.7 and 0.8-1.0. From these data it is obvious that the needles and roots of seedlings grown under more intensive light contained more free GAs in the variant with and without N. Thus, these results are in accordance with the data of Kopcewicz (1971) who also found that white light increases the level of GAs in Scots pine seedlings.

However, according to the literature the problem of influence of light conditions on the metabolism of GAs is not clear yet. This effect depends on the light quality and photoperiod (Jones, 1973) as well as the age of plants (Ložnikova, Čajlachjan, 1969). Light conditions influence also the GAs turnover in plants, increasing one kind of GAs and decreasing another (Zeevaart, 1971 a). In our experiments no clear relationship between the influence of light and the kind of GAs was found (Fig. 1).

The data of our experiments show that nitrogen nutrition increased also the level of free GAs in the needles of pine seedlings grown at both light intensities. However, in roots N decreased the level of GAs at light intensity of 500 lx and had only a slight stimulative effect under more intensive light conditions. Thus, it was established that the stimulation of initiation and elongation of needles as an effect of N nutrition was correlated with the increase of the level of GAs. This statement is in accordance with the general opinion concerning the role of gibberellins in plant growth processes (see Michniewicz et al., 1976).

The decreasing effect of N on the level of GAs in roots of seedlings grown at 500 lx is correlated with the inhibition of elongation of these plant organs under the above light conditions. Nitrogen inhibited also elongation of roots under more intensive light, but under these conditions it stimulated simultaneously the initiation of lateral roots which could be the sites of gibberellin biosynthesis.

The results obtained in our experiments show that the level of cytokinins was higher in seedlings grown under more intensive light (Fig. 3). Information concerning the influence of light conditions on the level of

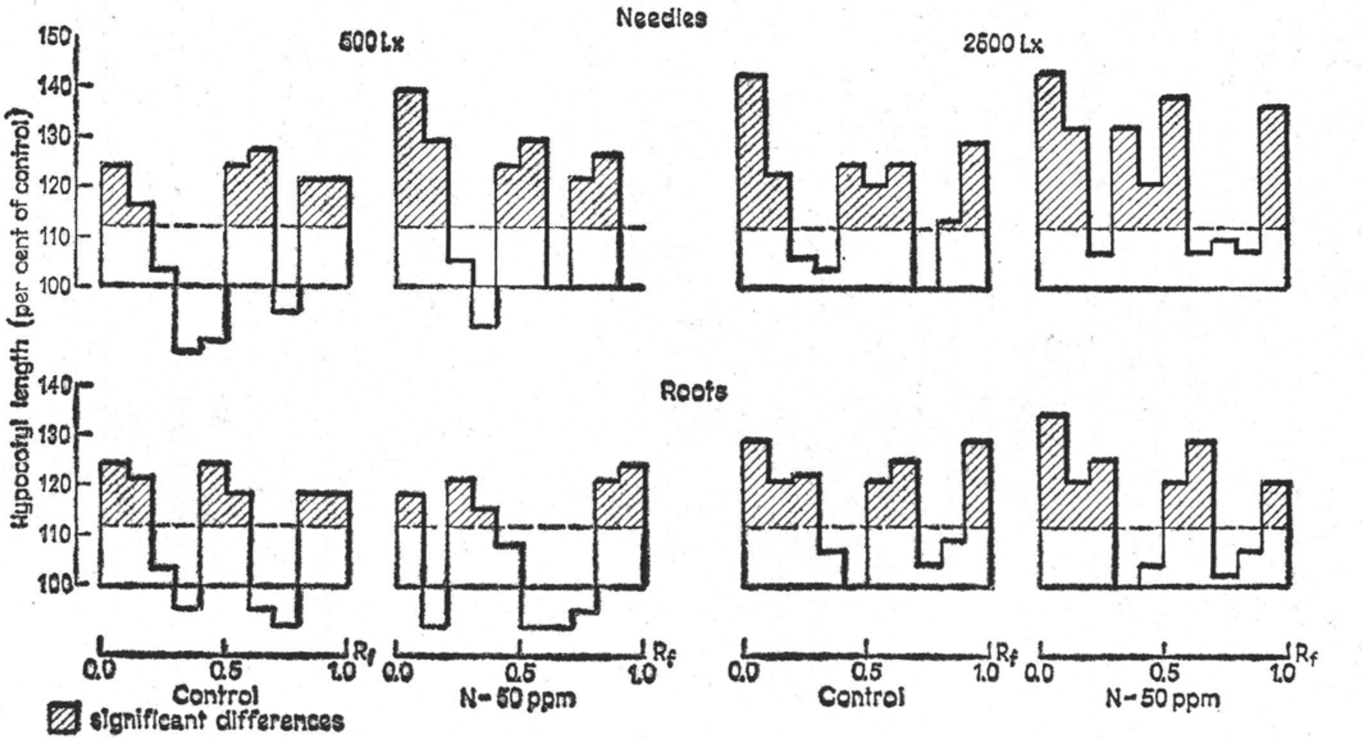

Fig. 1. Chromatographic analysis of free gibberellins in needles and roots of pine seedlings.

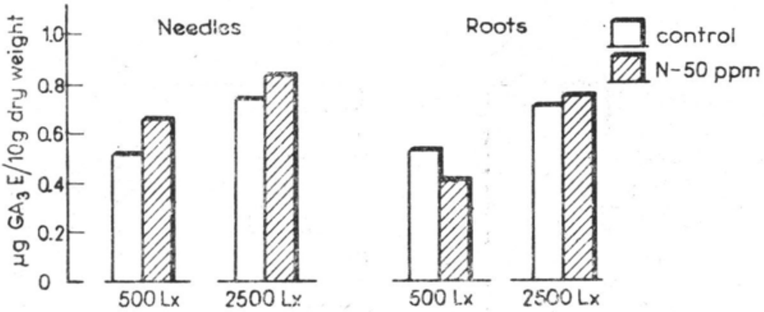

Fig. 2. Total amount of free gibberellins in needles and roots of pine seedlings.

endogenous cytokinins is still very limited. We know however, that red light increases the cytokinin content in poplar leaves (Hewett, Wareing, 1973). In *Xanthium strumarium* short day treatment provokes a rapid decrease of cytokinins (Henson, Wareing, 1974).

Nitrogen influenced the level of cytokinins in pine seedlings grown under light of higher intensity (Fig. 3). These results concerning the needles are in accordance with the data of Wareing (1974) who found that addition of N increased the level of cytokinins in the leaves of *Betula* and *Helianthus*. Also Göring and Mardanov (1976) mention similar results obtained in their not yet published experiments.

The increase of cytokinins in roots in the variant with higher light intensity as an effect of N nutrition may be explained by assuming that under these light conditions nitrogen increases the number of lateral roots. According to the general opinion roots are the sites of cytokinins biosynthesis. It should be underlined that Wagner and Michael (1971) also found that N increased the level of cytokinins in the roots of sunflower.

Under light of low intensity the effect of N on the amount of cytokinins was not clear, probably because of the low level of these plant hormones in this light variant.

The needles and roots of seedlings grown under more intensive light contained less auxins in both variants of N nutrition (Figs 4 and 5). Probably a high level of auxins in the roots of seedlings grown under light of lower intensity is needed for lateral root initiation which took place in this period. On the other hand, the low level of auxins in roots of seedlings grown under more intensive light favours the elongation of both main and lateral roots.

The higher level of auxins in needles from plants grown under weak light correlated with the intensive elongation of hypocotyls and with the inhibition of initiation of needles and their elongation. Probably concentration of auxins under these light conditions is optimal

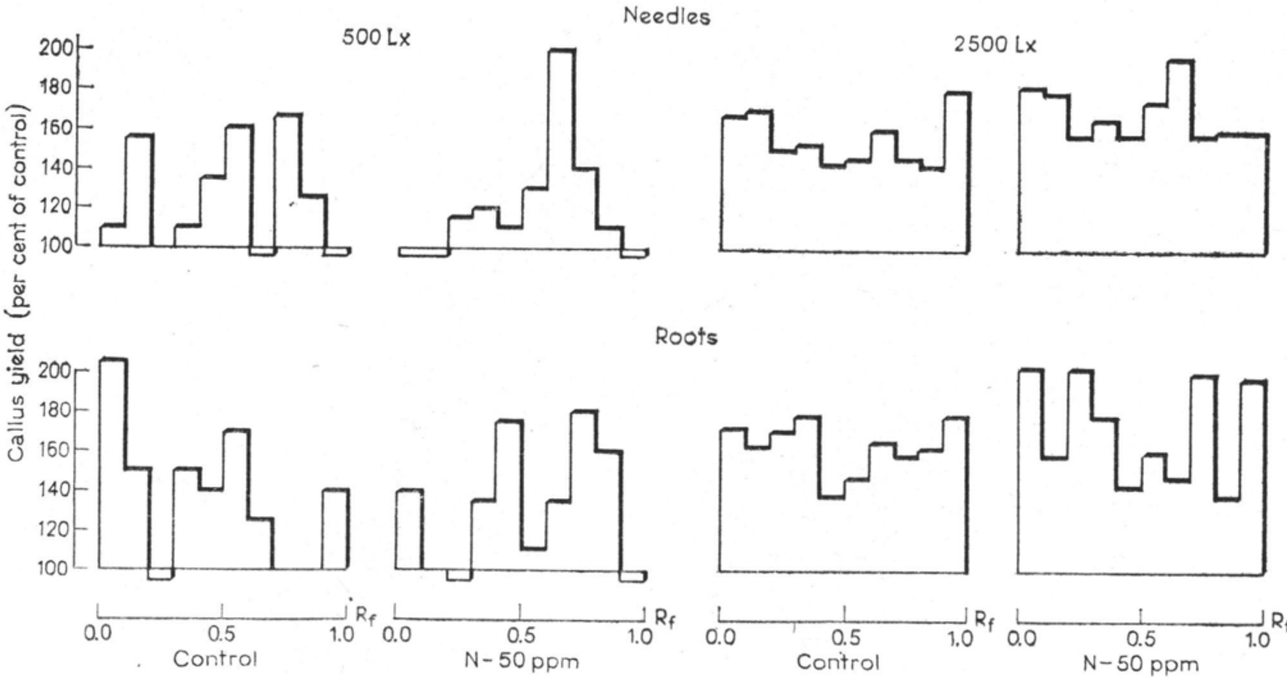

Fig. 3. Chromatographic analysis of cytokinins in needles and roots of pine seedlings.

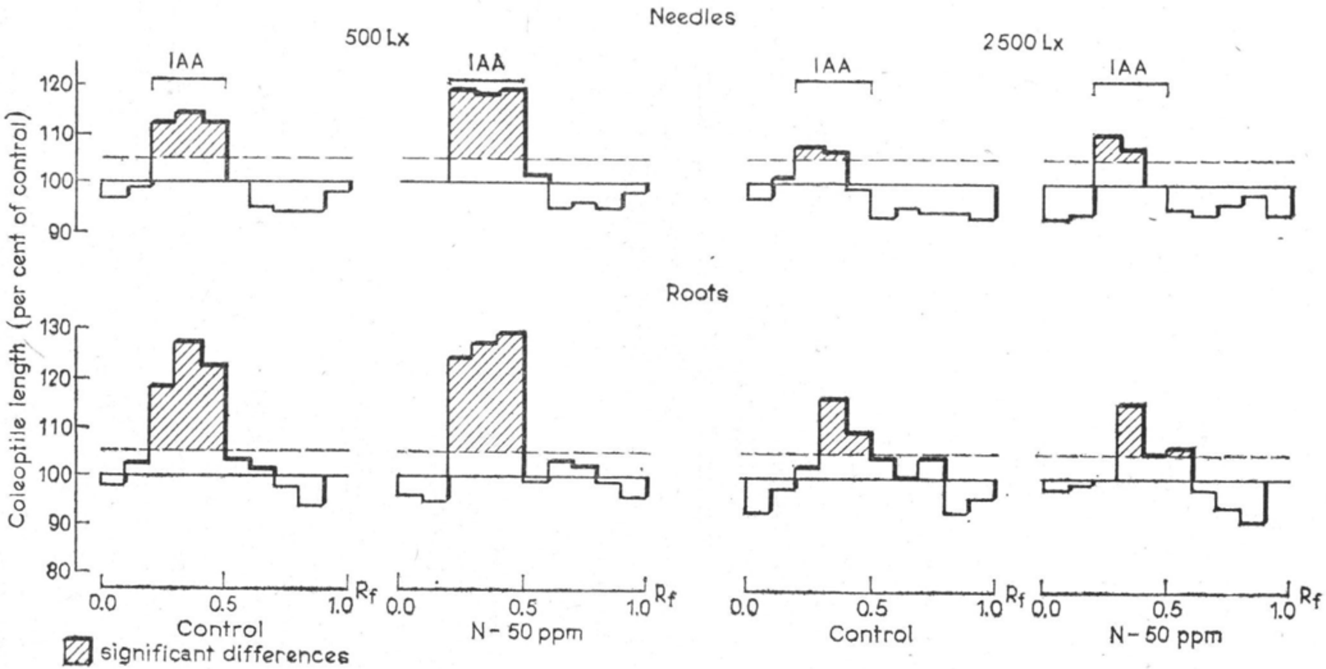

Fig. 4. Chromatographic analysis of auxins in needles and roots of pine seedlings.

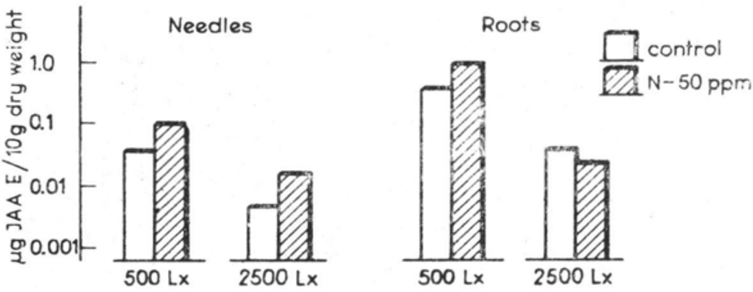

Fig. 5. Total amount of auxins in needles and roots of pine seedlings.

for elongation of hypocotyls and overoptimal for growth and development of needles.

The effect of N on the level of auxins in the needles was opposite to that of light. Thus, stimulation of initiation and elongation of needles as an effect of N treatment was correlated with the increase of auxins. The latter observation agrees with the point of view of many authors (see Michniewicz et al., 1976).

The effect of nitrogen on the level of auxins in roots also depended on the light conditions. Thus, inhibition of growth, fresh weight and dry matter of roots under light of 500 lx was correlated with an increase of the level of auxins. However, under light of 2500 lx N also inhibited growth, fresh weight and dry matter of roots, but had no significant effect on the level of auxins. The more intensive light increased the level of the ABA-like inhibitor in needles in both nutrient variants (Figs 6 and 7). Thus, the stimulation of growth and development of needles as an effect of light was correlated with the increase of the level of growth inhibitors.

It is known that biosynthesis of ABA takes place in the leaves (Milborrow, 1970). According to Dörffling (1971) light of high intensity increases the amount of ABA in plant tissues. Thus, our results are in accordance with the data from the literature.

The effect of N on the level of growth inhibitors in the needles was opposite to the effect of light. Thus, the stimulation of initiation and elongation of needles as an effect of N treatment was correlated with the decrease of the amount of ABA. A similar effect of N treament was stated by Michael and coworkers (1974) who experimented with sunflower leaves.

The influence of nitrogen on the level of growth inhibitors in the roots also depended on the light conditions. Thus inhibition of growth, of fresh and dry matter accumulation in roots under light of 500 lx was correlated with a decrease of growth inhibitors. However, under

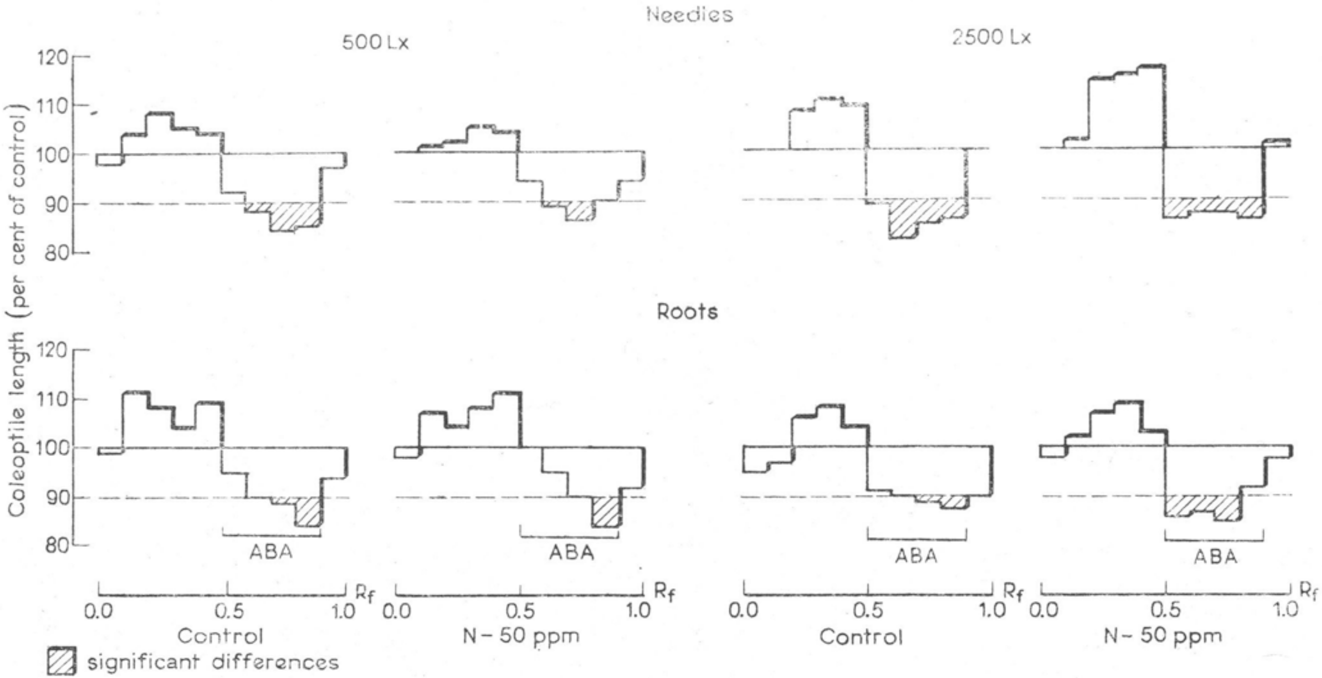

Fig. 6. Chromatographic analysis of ABA-like inhibitor in needles and roots of pine seedlings.

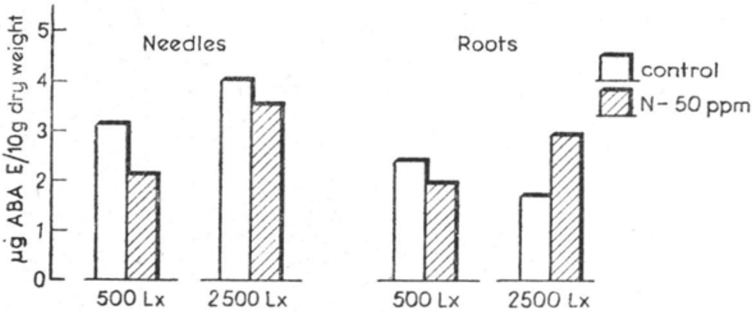

Fig. 7. Total amount of ABA-like inhibitor in needles and roots of pine seedlings.

light of 2500 lx, N also inhibited root growth and fresh and dry matter accumulation in them but significantly increased the level of the ABA inhibitor. These dependences on the light conditions are probably caused by the strong influence of light on the development of roots (Table 1).

From data presented in Tables 1 and 2 it is also evident that nitrogen, in contrast to light, increases the water content in needles and roots. These results are in accordance with data from the literature (Etter, 1969; Morizet, 1975). Except for roots of seedlings grown under more intensive light, this increase of water content in plant tissues was clearly correlated with the increase of auxins and the decrease of the ABA-like inhibitor.

This statement is in accordance with data from the literature. It is known that auxins influence the water content in plant tissues (Davies, 1973). It is also known that plants being under water stress conditions are characterized by a high level of ABA (Zeevaart, 1971b; Dörffling et al., 1974).

No clear correlation between the water content and the level of gibberellins and cytokinins in plant tissues could be established.

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REFERENCES

Davies P. J., 1973. Current theories on the mode of action of auxins. Bot. Rev. 39: 139-171.

Dörffling K., 1971. Recent advances in abscisic acid research. In: Hormonal Regulation in Plant Growth and Development: Ed. H. Kaldewey, J. Vardar. Izmir: 281-295.

Dörffling K., Šonka B., Tietz D., 1974. Variation and metabolism of abscisic acid in pea seedlings during and after water stress. Planta 121: 57-66.

Etter H. M., 1969. Growth, metabolic components and drought survival on lodgepole pine seedlings at three nitrate levels. Can. J. Plant Sci. 49: 393-402.

Felippe C. M., Dale J. E., Marriott C., 1975. The effect of irradiance on uptake and assimilation of nitrate by young barley seedlings. Ann. Bot. 39: 43-55.

Giertych M. M., Farrar J. L., 1961. The effect of photoperiod and nitrogen on the growth and development of seedlings of Jack Pine. Can. J. Bot. 39: 1247-1254.

Göring H., Mardanov A. A., 1976. Beziehungen zwischen dem K^+/Ca^{++} — Varhältnis im Gewabe und der Wirkung von Zytokininen in höheren Pflanzen. Biol. Rdsch. Jg. 14: 177-189.

Henson J. E., Wareing P. F., 1974. Cytokinins in *Xanthium strumarium:* A rapid response to short day treatment. Physiol. Plant. 32: 185-187.

Hewett E. W., Wareing P. F., 1973. Cytokinins in *Populus* × *robusta.* (Schneid): Light effects on endogenous levels. Planta 114: 119-129.

Ingestad T., 1962/63. Macroelement nutrition of pine, spruce and birch seedlings in nutrient solutions Medd. f. St. Skogsf. 51: 1-131.

Jones R. L., 1973. Gibberellins: Their physiological role. Ann. Rev. Plant Physiol. 24: 571-598.

Kopcewicz J., 1971. Effect of white light irradiation on the endogenous growth regulators content in seeds and seedlings of pine (*Pinus silvestris L.*). Acta Soc. Bot. Pol. 40: 431-438.

Kozlowski T. T., Borger G. A., 1971. Effect of temperature and light intensity in ontogeny on growth of *Pinus resinosa* seedlings. Can. J. Forest Res. 1: 57-65.

Kramer P. J. Kozlowski T. T., 1960. Physiology of Trees. Mc Graw-Hill Book Company Inc. New York, Toronto, London: 468-535.

Lożnikova V. N., Čajlachjan M. Ch., 1969. Vliyaniye sveta i temnosti na soderżaniye gibberellinov v prorostkakh i v zraslykh rastieniyakh bobov. Dokl. Akad. Nauk SSSR 188: 715-717.

Michael G., Mounla M. A. K., Goldbach H., 1974. Nitrogen fertilizer application, hormon activity and crop production. In: Plant Analysis and Fertilizer Problems Vol. 2, Proceed. 7 Intern. Coll. Plant Analysis and Fertilizer Problems. Ed. J. Wehrmann, Hanover: 307-316.

Michniewicz M., Rożej B., Stopińska J., 1976. The influence of nitrogen nutrition on the dynamics of growth and metabolism of endogenous growth regulators in Scots pine (*Pinus silvestris* L.) seedlings. Acta Soc. Bot. Pol. 45: 495-510.

Milborrow B. V., 1970. The biosynthesis and degradation of abscisic acid. In: Plant Growth Substances 1970. Ed. D. J. Carr, Springer-Verlag, Berlin, Heidelberg, New York: 281-290.

Morizet J., 1975. Etude des transferts de l'eau dans la plante. Action d'une alimentation carencee en azote sur l'evolution de la teneur en eau des differents organes du tournesól (*Helianthus annuus*). C. R. Acad. sci. 281: 1593-1596.

Phares R. E., 1971. Growth of red oat (*Quercus rubra* L.) seedlings in relation to light and nutrients. Ecology 52: 669-672.

Wagner H., Michael G., 1971. The influence of varied nitrogen supply on the production of cytokinins in the roots of sunflower plants. Biochem. Physiol. Pflanzen 162: 147-158.

Wareing P. F., 1974. Endogenous cytokinins as growth regulators. In: Biochemistry and Chemistry of Plant Growth Regulators. Proceed. Intern. Sympos., Cottbus, GDR: 243-257.

Zeevaart J. A. D., 1971a. Effects of photoperiod on growth rate and endogenous gibberellins in the long-day rosette plant spinach. Plant Physiol. 47: 821-827.

Zeevaart J. A. D., 1971b. Abscisic acid content of spinach in relation to photoperiod and water stress. Plant Physiol. 48: 86-90.

Wpływ odżywiania azotowego na wzrost siewek sosny zwyczanej (Pinus silvestris L.) rosnących przy różnej intensywności światła i zawartość w nich hormonów roślinnych

Streszczenie

Siewki sosny hodowano na pożywce Ingestada zawierającej N (jako NH_4Cl) w stężeniu 0 i 50 ppm, przy 16-godzinnym dniu, intensywności światła 2500 i 500 lx i temperaturze ± 20° C. Pomiarów wzrostu i określenia zawartości hormonów roślinnych dokonano po 8 tygodniach po wysiewie. Wyniki doświadczeń wskazują, że zarówno światło jak i N istotnie wpływają na wzrost i na poziom hormonów w tkankach roślin. Światło stymulowało inicjację igieł i korzeni bocznych, oraz elongację igieł i korzeni a także zwiększało suchą i świeżą masę tych organów. Stymulacja wzrostu igieł była skorelowana ze zwiększeniem poziomu wolnych giberelin, cytokinin i inhibitora o właściwościach ABA oraz ze zmniejszeniem poziomu auksyn i zawartości wody w tkankach. Podobny wpływ wywierało także światło na poziom hormonów w korzeniach za wyjątkiem ABA którego poziom zależał od obecności N w pożywce. Azot wywierał podobny wpływ jak światło na wzrost igieł oraz na inicjację igieł i korzeni bocznych, jednakże w przeciwieństwie do światła silnie hamował elongację korzeni i zwiększał zawartość wody w tkankach. W igłach N zwiększał poziom giberelin i auksyn w obu wariantach świetlnych a na świetle o wyższej intenswności także poziom cytokinin. Zmniejszał natomiast poziom ABA. W korzeniach wpływ N na poziom hormonów zależał od warunków świetlnych. Na świetle o niższej intensywności zmniejszał poziom giberelin i ABA, zwiększał poziom auksyn i nie wywierał istotnego wpływu na poziom cytokinin. Na świetle o wyższej intensywności nie miał wpływu na poziom giberelin i auksyn oraz zwiększał zawartość cytokinin i inhibitora o właściwościach ABA.