Influence of various sources of nitrogen on the growth of Scots pine seedlings and the IAA content in needles

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Abstract

One year old pine seedlings transferred from the nursery and potted in a greenhouse were watered with nitrogen solutions of different form and concentration (urea 0.36 mM and 0.90 mM, ammonium tartrate 0.36 mM and 1.79 mM, calcium nitrate 0.36 mM, 1.79 mM and 3.58 mM). After 10 months the growth and IAA content in needles were compared. Nitrogen fertilization affected shoot growth considerably, but its effects on root growth were less pronounced. The highest shoot weight was obtained after application of ammonium tartrate in 1.79 mM concentration. The lowest concentration of urea (0.36 mM) caused the same effect, as did the most concentrated calcium nitrate solution (3.58 mM). Auxine content increased in the needles after nitrogen fertilization as compared with control. The highest accumulation of IAA was found in seedlings treated with low concentrations of urea.

Key words: ammonium, nitrate, urea, Pinus sylvestris

INTRODUCTION

Various environmental parameters (light, temperature, moisture, soil nitrogen content) have strong effects on all phases of plant growth and development, and it is not surprising that these parameters influence the content of endogenous hormones.

The response of coniferous trees to the application of nitrogen fertilizers is a major area of research in forest ecology, physiology and silviculture, however, hormonal response of conifers to nitrogen nutrition has scarcely been investigated (Michniewicz et al. 1976, Rudawska 1987). Therefore these studies were undertaken.

We consider only the auxin indole-3-acetic acid (IAA), since this compound in herbaceous plants is known to be influenced by nitrogen nutrition (ref. Michniewicz et al. 1976) and because IAA is a major indole substance occurring in Scots pine tissues (Sandberg 1984, Sandberg et al. 1981, 1987, Sandberg and Ericsson 1987).

Our study was designed to determine the effects of varying fertility sources and levels on the IAA pool in the shoots of Scots pine seedlings.

MATERIALS AND METHODS

PLANT MATERIAL

One-year-old Scots pine seedlings from a nursery were transferred into the greenhouse, potted in sand and fertilized twice a week with nutrient solution during 10 months. The nitrogen sources in nutrient solutions were urea, ammonium tartrate or calcium nitrate in various concentrations (Table 1). Concentrations of nitrogen sources were chosen according to the preliminary studies (Rudawska, unpublished data) which revealed that levels of nutrients higher than 1 mM in the case of urea and 2 mM in the case of ammonium tartrate caused a growth inhibition of tested seedlings.

Table 1
Effect of various nitrogen sources and
concentrations on growth of Scots pine seedlings¹

Nitrogen compound	Concentration (mM)	Fresh weight		
		shoot (g)	root (g)	shoot/root
Control	—	18.8a	17.1b	1.1a
Urea	0.36	24.5ab	14.7a	1.7b
	0.90	32.3b	17.4b	1.9b
Ammonium tartrate	0.36	30.4b	19.3bc	1.6b
	1.79	45.8d	19.9c	2.3bc
Calcium nitrate	0.36	28.8b	14.1a	2.0bc
	1.79	27.0b	17.4b	1.6b
	3.58	35.4bc	17.1b	2.1bc

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

ANALYSIS OF IAA

Current year needles from treated plants (10 g for one sample) were frozen in liquid nitrogen, homogenized in 80% methanol, extracted three times with 80% methanol in darkness at 4°C. The extracts were fractionated using the

method adapted from Michniewicz et al. (1976) modified slightly. Extracts were reduced to dryness in vacuo. Residues were dissolved in distilled water, adjusted to pH 3.0 and extracted with diethyl ether. Ether phases were extracted with 3% sodium bicarbonate. The bicarbonate fractions were adjusted to pH 3.0 and extracted with diethyl ether. Ether fractions were reduced to dryness in vacuo. Dried residues were dissolved in 2 ml of anhydrous methanol (IAA fraction). Preparative thin-layer-chromatography (TLC) was carried out on silica gel 60 using isopropanol-ethyl acetate-ammonia (45:35:20 v/v) as a solvent. Successive zones, or the zone corresponding to IAA only, were scraped off the plates and eluted with methanol. Methanol was evaporated *in vacuo* to dryness, the residues dissolved in phosphate buffer 0.01 M, pH 4.7 and bioassayed using *Avena* section straight test (Larsen 1955).

RESULTS AND DISCUSSION

The results presented in Table 1 showed that various nitrogen forms and concentrations had only a slight effect on the fresh weight of roots but significantly increased the fresh weight of shoots. The treatments which resulted in the highest increase of the shoot weight were ammonium as source of nitrogen at 1.79 mM concentration and nitrate solution at 3.58 mM concentration. The shoot/root ratio was high at 1.79 mM ammonium as source of nitrogen and much lower on the same concentration of nitrate. Low concentration of urea nitrogen produced similar growth responses like the highest concentration of nitrate. Nitrogen nutrition significantly increased the amount of auxin in the needles of treated plants (Fig. 1). An increased level of IAA as a results of nitrogen feeding depended both on the source and on the level of nutrient used and in the case of ammonium tartrate and calcium nitrate treatments it was correlated with stimulation of shoot growth. However, the highest level of IAA was revealed in plants grown on the lower concentration of urea (0.36 mM). These results are partly in accordance with the data of Michniewicz et al. (1976) and Rudawska (1987) who found an increased amount of IAA in ammonium fertilized plants (other sources of nitrogen and their effects on IAA level were not tested).

A representative chromatogram of the ether extract obtained from needles of tested plants is shown in Fig. 2. Chromatography revealed four growth promoting substances, one of which corresponds in R_f with IAA. The nature of the other substances was not tested, however, several indole constituents were identified in Scots pine tissues by Sandberg et al. (1981). Using gas chromatography-mass spectrometry and high-performance liquid chromatography he demonstrated the presence of indole-3-ethanol (IET) in needles of *Pinus sylvestris* and showed the possible role of IET in the metabolism of indoles in the tissue which has previously been shown to contain IAA, indole-

Fig. 1. The content of endogenous auxin in the needles of Scots pine seedlings treated with various nitrogen forms and levels (TLC; — Rf 0.5). Bars sharing the same letter do not differ significantly

-3-carboxylic acid and the conjugate, indole-3-aspartic acid (Sandberg et al. 1981, Sandberg 1984, Andersson and Sandberg 1982). The results presented here showed that a very similar physiological effect (increased level of IAA) can be achieved by using various sources and levels of nitrogen. Such a response may be a result of the differentiated tolerance of pine seedlings to various nitrogen sources. In our experiment by using the method with systematically renewed nutrient solutions and watering the pot to prevent accumulation of the ions, it became possible to minimize the variation of pH in the root medium and to keep a physiological, constant concentration of various nutritive elements. Under such conditions the hydrolysis of urea to ammonium and carbon dioxide as well as nitrification of ammonium to nitrate were significantly limited. Thus the tested parameters of pine metabolism really reflected the influence of various sources of N (NH_4^+ , NO_3^- , urea).

Fig. 2. Representative chromatogram of methanol extract obtained from Scots pine needles, assayed by *Avena* straight growth test. I – Color reaction of chromatogram by Salkowski reagent modified by Pilet (1957). II – Color reaction of IAA control

In general, the tolerance of plants to an ample supply of NH_4^+ is low, whereas the tolerance for NO_3^- is high (Hayness 1986). Plants can tolerate high levels of soil NO_3^- and the concentration of this ion may rise to several percent in the plant, before phytotoxicity becomes apparent (Maynard and Barker 1971). This is exactly the case of young seedlings which even at a high concentration of nitrate (3.58 mM) have not revealed any toxic effects and responded with a high IAA level. In contrast, severe toxic reactions occur when NH_4^+ accumulates in plants and its translocation to shoots is especially deleterious (e.g. Puritch and Barker 1967). This may explain why the very high concentration of ammonium (3.58 mM, results not considered in the present paper) induced toxicity in pine seedlings.

However, it should be admitted that at 1.79 mM of N in ammonium form the positive effect of nitrogen on the growth rate of the seedlings clearly exceeded that of nitrate at the same N concentration. Urea nutrition seems to play, in conditions of the present experiment, a very special role which probably depends on the mechanism of urea uptake and transformation by the pine seedlings. Intact molecule absorption or hydrolysis of urea to ammonia and carbon dioxide can be involved in the metabolism of urea-fed plants (Bollard 1959, Steward and Pollard 1957). Ammonia accumulation inside

the roots as well as some intermediates of urea hydrolysis are considered to be toxic to the plant (ref. Court et al. 1962). Besides, the urea molecule has no charge. This means that the molecule very easily penetrates the free space and possibly even through the membrane of the surface of the cytoplasm. This can be an explanation why in present experiments the growth of plants and IAA level so quickly responded to the relatively low level of urea (0.36 mM) and also why in the case of urea, the phytotoxic effect was so quickly pronounced.

Therefore manipulating with various sources and levels of nitrogen in fertilizing of coniferous plants seems to be important. However, it should be considered that coniferous seedlings differ greatly in their ability to absorb and utilize various forms of nitrogen (Evers 1964). The most favourable nitrogen sources also change often with the age of plant, pH of the soil, the concentration of some other nutrients and with the carbohydrate content in the plants.

But it seems that at least for the young pine seedlings, great attention should be paid to the fact that various nitrogen sources used at the same concentration can cause very different physiological effects.

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Wpływ zróżnicowanego nawożenia azotowego na wzrost siewek sosny pospolitej i zawartość kwasu indolilo-3-octowego w igłach

Streszczenie

Jednoroczne siewki sosny przeniesiono ze szkółki leśnej do szklarni i podlewano pożywkami zawierającymi azot w różnych formach i stężeniach (mocznik 0,36 mM i 0,90 mM, winian amonowy 0,36 mM i 1,79 mM, azotan wapniowy 0,36 mM, 1,79 mM i 3,58 mM). Po upływie 10 miesięcy porównywano wzrost siewek oraz poziom zawartości kwasu indolilo-3-octowego (IAA) w igłach. Zróżnicowane nawożenie azotowe wpłynęło silnie na wzrost pędów, słabiej na wzrost korzeni. Największy przyrost masy pędów nastąpił w obecności winianu amonowego o stężeniu 1,79 mM. Najmniejsze stężenie mocznika (0,36 mM) wywołało podobny efekt jak największe stężenie azotanu (3,58 mM). Nawożenie azotowe znacznie zwiększyło ilość auksyny w igłach sosny. Największą akumulację IAA stwierdzono w siewkach traktowanych mocznikiem w małych stężeniach.