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Gas exchange and organic matter production of Scots pine (*Pinus silvestris* L.) seedlings grown in water culture with ammonium or nitrate form of nitrogen*

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Abstract

The reaction of Scots pine (*Pinus silvestris* L.) seedlings to ammonium and nitrate form of nitrogen was investigated. Rates of photosynthesis and respiration, content of chlorophylls, and accumulation of organic substance were determined in 12 and 18 weeks old plants. The results have shown, that the forms of nitrogen does affect the amount of accumulated organic substance as well as its distribution in particular organs.

INTRODUCTION

There are many publications concerning utilization of ammonium or nitrate form of nitrogen, but most of them deal with crop plants. Beside that production of organic matter is usually assumed as a criterion of better utilization by plants of one or the other form of nitrogen, while gas exchange processes in this aspect are less investigated. This was the reason to make an attempt at investigating the effect of nutrition with ammonium or nitrate form of nitrogen on gas exchange processes and accumulation of organic matter in Scots pine seedlings in this work. These experiments are a part of wider collective investigations on the relationship between organic matter production and gas exchange.

MATERIAL AND METHODS

Investigations were carried out on Scots pine seedlings grown under greenhouse conditions at 16-hours day. The experiment was carried out

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during winter months. Natural light was enriched with light from fluorescent lamps giving 3000 lux light intensity. Air temperature inside the greenhouse ranged between 12° and 25°C. Seedlings were grown in 1 l pots. Eight plants were grown in each pot.

Basic nutrient solution was prepared according to Ingestad (1962/63) with ammonium nitrate as nitrogen source. Composition of nutrient solution of experimental variants differed from each other only by the form of nitrogen. There was ammonium chloride introduced into the solution instead of ammonium nitrate in one variant and sodium nitrate in the other one. The two salts were added in equivalent amounts of nitrogen — 50 mg of N in 1 l of solution. The solution was changed every week. Acidity of the nutrient solution in each experimental variants did not change more than within 5.0 to 4.0 pH. Solution containing ammonium chloride was the most acidifying which could result in lower rate of utilization of the ammonium ions.

Photosynthesis and respiration measurements were carried out with the use of gas analyzer, type Infralyt II, in a closed circuit. Measurements were carried out under laboratory conditions: temperature was 25°C, light intensity about 10000 lux and CO_2 concentration in the range 350—300 ppm.

Illumination system consisted of incandescent and fluorescent lamps, surrounding the plant chamber, and of a water screen. Such illumination conditions are quite close to natural diffused light and secure high photosynthetic rate. Photosynthesis measurements were carried out after 1 hour adaptation of plants to the conditions inside the plant chamber. Respiration of shoot was determined after photosynthesis measurements were finished. Root system was cut off after accomplishement of the gas exchange measurements of the shoot; its respiration was measured during 5 minutes after cutting. All measurements were carried out in four replications. There were five plants examined in each replication simultaneously.

Height of plants, number of needles and dry matter of particular organs were determined in plants of each replication.

Content of chlorophyll a and b was determined in needles of plants harvested at the same time as the plants were taken for gas exchange measurements. Needles for chlorophyll determination were taken from the middle part of stem. There were ten replications of each experimental variant. A sample of about 100 mg of fresh needles made one replication. Chlorophyll content was determined spectrophotometrically in 80% acetone extract from fresh needles (MacKinney 1941; Bruinsma 1963).

To estimate the variation of the investigated plant material error of arithmetic mean was determined at the confidence level $p=0.90$.

RESULTS

Seedlings grown in solution with ammonium chloride were found to have highest shoots after 12 weeks as well as after 18 weeks of experiment duration (Table 1). Differences between seedlings grown in solution with sodium nitrate and ammonium nitrate were not distinct. Seedlings grown with ammonium chloride produced the largest number of needles on shoot as well as highest dry matter of single needle at both sampling times. The effect of ammonium chloride, in this respect, was stronger in 18 weeks old seedlings.

Dry matter determination of particular organs showed that seedlings grown in solution with ammonium chloride exhibited highest dry matter of needles, stem and root (Table 2). Mean dry weight of these plants

Table 1

Growth characteristics of seedlings

Experimental variant	Lenght of the shoot (mm)	Number of needles	Dry weight of a single needle (mg)
first sampling at the age 12 weeks			
$NaNO_3$	43±2	37±2	1.09
NH_4NO_3	44±2	36±2	1.02
NH_4Cl	49±2	40±2	1.32
second sampling at the age 18 weeks			
$NaNO_3$	60±3	47±3	— 1.78
NH_4NO_3	60±2	42±2	1.60
NH_4Cl	65±3	54±4	2.13

Table 2

Dry weight of needles, stem and root (mg/plant) and the ratio shoot/root

Experimental variant	Needles	Stem	Root	Whole plant	Shoot / root
first sampling at the age 12 weeks					
$NaNO_3$	40.3± 3.8	8.8±0.8	14.4±1.2	63.5± 5.8	3.4
NH_4NO_3	36.8± 1.8	9.1±0.5	11.8±1.3	57.7± 3.0	3.9
NH_4Cl	52.7± 4.4	12.5±1.1	17.8±2.0	83.0± 7.3	3.7
second sampling at the age 18 weeks					
$NaNO_3$	83.5±11.2	33.7±4.5	69.0±6.9	186.2±20.6	1.7
NH_4NO_3	67.1± 5.9	30.0±1.5	52.7±3.6	149.8± 9.5	1.8
NH_4Cl	115.2±18.3	43.1±5.9	76.3±8.8	234.6±32.1	2.1

was at first harvest 44% and at second harvest 57% higher than that of plants grown in solution with ammonium nitrate. The lowest dry weight of whole plant as well as of particular organs, except for stem dry weight, was found in plants grown with ammonium nitrate. Comparing dry weight of plant organs of the three variants it shows that largest differences appeared between dry weight of needles.

Table 3

Per cent of increase of dry weight in particular organs and in the whole plant between the first and second sampling

Experimental variant	Needles	Stem	Root	Whole plant
$NaNO_3$	107	283	379	193
NH_4NO_3	82	230	347	160
NH_4Cl	118	245	329	183

Mean dry weight increment of a single plant between the two harvests amounted 123 mg in plants of the nitrate variant, 152 mg in the ammonium variant and only 92 mg in plants which were supplied with NH_4NO_3. Results shown in table 3 indicate, however, that per cent of increase of dry weight in whole plant grown with $NaNO_3$ was during that period slightly higher than the increase of plant grown in solution with NH_4Cl.

Net assimilation rate values estimated for the period between the two harvests were for nitrate, control, and ammonium variants respectively: 45.0, 40.3, 41.1 mg/day/g dry weight of needles. Percentage participation of organic substance in particular organs at the first harvest was similar in all three variants (Table 4).

Table 4

Percentage distribution of dry matter in particular organs

Experimental variant	Needles	Stem	Root
first sampling at the age 12 weeks			
$NaNO_3$	63	14	23
NH_4NO_3	64	16	20
NH_4Cl	63	15	21
second sampling at the age 18 weeks			
$NaNO_3$	45	18	37
NH_4NO_3	45	20	35
NH_4Cl	49	18	32

Table 5

Photosynthesis rate, photosynthetic activity per plant and per 1000 needles, assimilation number and the ratio: photosynthesis/respiration of the whole plant and of the shoot

Experimental variant	mg CO_2/hour/g of dry wt.	mg CO_2/hour/1 plant	mg CO_2/hour/1000 needles	mg CO_2/hour/mg of chlorophyll	photosynthesis/respiration of the whole plant	photosynthesis/respiration of the shoot
first sampling at the age 12 weeks						
$NaNO_3$	18.8±2.4	0.75	20.3	2.0	4.7	6.3
NH_4NO_3	19.4±1.9	0.71	19.7	1.8	5.1	7.1
NH_4Cl	20.8±0.8	1.09	27.3	2.3	5.7	7.8
second sampling at the age 18 weeks						
$NaNO_3$	14.9±2.1	1.22	26.0	1.4	2.9	5.3
NH_4NO_3	16.3±0.8	1.10	26.2	1.3	3.3	6.1
NH_4Cl	15.5±0.6	1.78	33.0	1.4	3.2	5.2

Table 6

Respiration of the shoot, root, and whole plant

Experimental variant	Shoot (needles+stem)		Root		Whole plant
	mg CO_2/hour/g of dry wt.	mg CO_2/hour/shoot	mg CO_2/hour/g of dry wt.	mg CO_2/hour/root	mg CO_2/hour/plant
first sampling at the age 12 weeks					
$NaNO_3$	2.9±0.3	0.12	2.8±0.1	0.04	0.16
NH_4NO_3	2.7±0.2	0.10	3.1±0.5	0.04	0.14
NH_4Cl	2.7±0.2	0.14	3.0±0.3	0.05	0.19
second sampling at the age 18 weeks					
$NaNO_3$	2.8±0.1	0.23	2.8±0.2	0.19	0.42
NH_4NO_3	2.6±0.3	0.18	2.8±0.2	0.15	0.33
NH_4Cl	2.9±0.1	0.34	2.9±0.2	0.22	0.56

There was a considerable decrease of needle weight participation in plants investigated after 18-weeks growing period with a distinct increase of root weight participation in these plants at the same time. Also a slight increase of stem weight contribution in the plants was found. At second harvesting it was also observed that seedlings grown in solution with ammonium chloride showed larger participation of needle weight than those in other variants. The largest contribution of root weight was found in plants which obtained nitrogen in the form of $NaNO_3$.

Photosynthesis rate and assimilation number of 12 weeks old seedlings grown with NH_4Cl was found to be higher as compared to other variants (Table 5). Photosynthesis rate of 18 weeks old seedlings grown with NH_4NO_3 was slightly higher than that of other variants. Photosynthetic activity of one plant supplied with NH_4Cl was 53% higher than that of one plant supplied with NH_4NO_3 at first harvest time, and 62% higher at second harvest time.

Respiration rate in all experimental material at both harvest times was similar (Table 6). However, respiration rate calculated per one seedling showed differences between variants in accordance to differences in growth.

Table 7

Chlorophyll content in mg/g of needles dry weight and the ratio chlorophyll a : b

Experimental variant	Chlorophyll a	Chlorophyll b	Chlorophyll a+b	Ratio a : b
first sampling at the age 12 weeks				
$NaNO_3$	6.7	2.6	9.6	2.6
NH_4NO_3	7.6	2.9	10.9	2.6
NH_4Cl	6.3	2.3	8.9	2.7
second sampling at the age 18 weeks				
$NaNO_3$	7.4	2.8	10.4	2.6
NH_4NO_3	8.5	3.2	12.3	2.6
NH_4Cl	7.7	2.9	11.1	2.6

Chlorophyll content was the highest, at both harvest times, in needles of plants grown in solution with ammonium nitrate (Table 7). Chlorophyll a to b ratio was in all variants at both harvest times similar.

DISCUSSION

The results obtained in the described investigations, on pine seedlings grown in water culture, indicate that ammonium form of nitrogen supplied as NH_4Cl favours higher organic substance accumulation comparing to nitrate form of nitrogen supplied as $NaNO_3$. These results are in agreement with other authors data obtained for coniferous species. Leyton (1952) found, in experiments with 5-months old sitka spruce seedlings grown in water culture, that there was higher organic substance accumulation in shoots of plants grown in solution with ammonium form than in those with nitrate form of nitrogen. Hoffman (1964) found that when ammonium form was the only source of nitrogen spruce on the forest plantation showed best growth. McFee and Stone

(1968) also found higher weight increment of 3 and 5 week old spruce (*Picea glauca*) and pine (*Pinus radiata*) seedlings fertilized in water and sand culture with nitrogen in ammonium form.

The experiments described in this paper did not show distinct differences in the rate of photosynthesis between the experimental variants. However, slightly higher photosynthesis rate could be found after 12 weeks of growth in seedlings grown in solution with ammonium form. The results suggest that this stimulating effect of ammonium form disappears at later stages of growth.

Some authors point out that long-lasting nutrition with ammonium form of nitrogen causes accumulation of ammonia in leaves, what has unfavourable effect on chloroplast structure resulting in decrease of net photosynthesis (review — Nátr 1972). On the other hand, however, one can assume that slight differences in the rate of photosynthesis between plants supplied with ammonium and nitrate form of nitrogen can be caused by different nutritional requirements of older seedlings comparing to younger ones. According to other authors, preferences of plants as to the form of nitrogen change with their age (Clark, Shive 1934; Chandler 1952; Durzan and Steward 1967).

Higher photosynthesis rate during earlier stage of growth of plants grown in water cultures with ammonium chloride as nitrogen source comparing to those grown with sodium nitrate and ammonium nitrate could be caused not only by NH_4^+ ions but also by closer to optimal chlorine content. Arnon (1959) reported that chlorine uptaken in moderate amounts could stimulate photosynthesis. It seems, however, that chlorine effect was in our case insignificant, because Ingestad's nutrient solution contains chlorine in the form of KCl, $CaCl_2$, $MgCl_2$, $FeCl_3$, $MnCl_2$ and $CuCl_2$ and NH_4Cl introduced into the solution only slightly changes the total content of chlorine.

Lack of considerable differences in respiration rate between seedlings grown in nutrient solution with ammonium or nitrate form of nitrogen was also confirmed by the results of investigations by Łotocki and Żelawski (1973). On the other hand, Warburg and Negelein (1920), Gilbert and Shive (1945), Gumiński et al. (1957) found stimulating effect of nitrate form of nitrogen on respiration rate in some other species.

One can suppose, according to these data, that higher accumulation of organic matter in seedlings supplied with nitrogen in ammonium form comparing to those supplied with nitrate nitrogen can by caused partly by formation of larger needles, as well as by formation of larger number of needles on shoots. Gas exchange measurements carried out in earlier growth stages of plants indicate that also increased photosynthesis rate and high photosynthesis to respiration ratio could be of considerable importance in accumulation of organic matter in seedlings. Though

photosynthesis and respiration rates of plants supplied with both forms of nitrogen were similar during their later growth stages, the photosynthetic activity of plants supplied with ammonium form to nitrogen was distinctly higher as compared to that of plants supplied with nitrates — this being in connection with strong enlargement of assimilating organs of seedlings supplied with nitrogen in ammonium form. Organic substance, owing to that, was still accumulated in larger amounts by plants supplied with ammonium form of nitrogen.

CONCLUSIONS

1. Scots pine seedlings supplied with nitrogen in NH_4Cl form produce more organic substance during first growing season comparing to those supplied with nitrogen in $NaNO_3$ form.

2. Larger number of needles on shoots and greater weight of a single needle of plants supplied with NH_4Cl comparing to those supplied with $NaNO_3$ indicate that form of nitrogen nutrition affects differentiation processes of assimilating organs.

3. Value of ratio of the aerial to underground parts of seedling is dependent on the form of nitrogen nutrition. Higher values of this ratio are characteristic for plants supplied with nitrogen in NH_4Cl.

4. Higher accumulation of organic matter in seedlings supplied with NH_4Cl form of nitrogen to those supplied with $NaNO_3$ form results from higher photosynthesis rate during their earlier growth stages — and during later growth stages it is also secondary effect of larger assimilating organs.

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Wymiana gazowa i produkcja masy organicznej siewek sosny zwyczajnej (Pinus silvestris L.) wyhodowanych w kulturze wodnej z amonową lub azotanową formą azotu

Streszczenie

Badano reakcję siewek sosny zwyczajnej na amonową i azotanową formę azotu. Siewki hodowano w kulturze wodnej w obecności NH_4NO_3, NH_4Cl lub $NaNO_3$ jako źródeł azotu. U 12- i 18-tygodniowych roślin oznaczano intensywność fotosyntezy i oddychania, zawartość chlorofilu oraz akumulację substancji organicznych. Uzyskane wyniki wskazują, że forma azotu wpływa istotnie na wielkość gromadzenia masy organicznej i na rozmieszczenie jej w poszczególnych organach.

Nie stwierdzono wyraźnych różnic w intensywności wymiany gazowej siewek różnych wariantów nawożeniowych. Jedynie w wieku 12 tygodni obserwowano nieznacznie większą intensywność fotosyntezy roślin wariantu amonowego w porównaniu z intensywnością fotosyntezy roślin wariantu azotanowego.