Distribution of radioactive products of photosynthesis in Scots pine (*Pinus silvestris* L.) seedlings during the first vegetation season

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Abstract

Dynamics of translocation and distribution of radioactive photosynthetic products in one year old pine (*P. silvestris* L.) seedlings was investigated at different growth stages. It was found that the utilization of photosynthetic products in particular organs depends greatly on the growth stage. Losses of photosynthetic products as a result of respiration were the smallest at the stage of intensive needle growth and the greatest at the stage of approaching dormancy.

INTRODUCTION

Investigations on the formation of photosynthetic products, their translocation and distribution among particular plant organs is essential for understanding of the entire complex of problems related to wood biogenesis. The seasonal course of these processes in trees is still very little known as a result of the rather complicated experimental techniques used in such investigations.

This paper describes studies carried out on Scots pine seedlings at different growth stages during their first season of vegetation. The plants were exposed to $^{14}\text{CO}_2$ and the processes of translocation, distribution and incorporation of radioactive photosynthetic products were investigated.

MATERIAL AND METHOD

Seedlings of Scots pine (*Pinus silvestris* L.) grown in sand culture during the first vegetation season were used in these studies. In each pot 12.5×12.5×14.5 cm in size, 36 plants were grown. The nutrient solution recommended by Ingsted (1962/63) for the water culture

of the pine was administered twice at sowing 470 ml per pot, and four weeks later, 150 ml per pot. The moisture content of the sand was maintained at the level of 65% of the total capillary capacity by watering the pots to a constant weight at 1—2 day intervals.

Exposure to $^{14}\text{CO}_2$ took place in a plexiglass chamber $60 \times 60 \times 60$ cm in size, standing out-of-doors. Natural sun illumination during the morning hours, was used in the experiments.

The temperature in the chamber was about 24—28°C. 16 pots were exposed simultaneously at each exposure. Radioactive $^{14}\text{CO}_2$ was produced from $\text{Na}_2^{14}\text{CO}_3$ using 30% lactic acid. The carbon dioxide concentration during the exposure was maintained at a level near 0.03% by the gradual evolution at ten-minute intervals, of additional doses of non-radioactive carbon dioxide from the 1.50% solution of Na_2CO_3 . At the end of the exposure, the chamber was flushed out with air and the non-assimilated radioactive carbon dioxide was absorbed with KOH. 15—20 minutes after the end of exposure, 6—8 plants from each pot were cut off. In further analysis and calculations the activity of needles from this harvest was assumed as the maximum activity taken by the plants; changes in activity in the following harvest were calculated in relation to this first sample. The following harvest took place after several hours or days, and 14—16 plants were taken in each harvest. Immediately after cutting the plants, the needles were separated from the lignified stems, the weight was taken again, and the material was frozen in dry ice (CO_2). The plant material was stored under refrigeration at a temperature of -20°C until the time of chemical analysis.

Before homogenization, the plant material was thoroughly crushed, and after mixing, two identical samples containing about 20 mg of dry matter each, were taken. The samples were homogenized in glass homogenizer in 80% ethanol (v/v). The specific activity of the dry matter of the tissues of various organs was determined. After the complete sedimentation of the nonsoluble fraction, the specific activity of the soluble fraction was determined. Activity measurements were carried out by means of a G—M counter with a low background (2.7 imp./100s) and of window thickness mg/cm^2 . The efficiency of the counter was about 4%.

In the experiments described, an attempt was made to study the course of translocation as well as the degree of photosynthate utilization by the aerial parts of plants in three phases of growth: during the intensive growth of needles, at the time of thickening of the stem, and after the setting of the apical bud, when no visible change in the growth of the aerial parts is observed.

One of the main difficulties in studies of this type appears at creating in the same experiment comparable conditions of plant growth and comparable conditions of plant exposure. If an attempt is made to expose

the plants at different periods of vegetation in identical conditions, such plants must be sown at different dates, and then, their growth takes place in conditions deviating from natural course of vegetation season. If, however, the plants are sown at the same time, then, the exposures must be made at different times in the course of vegetation, and the conditions in which photosynthesis takes place are not fully comparable. In order to avoid some of these difficulties, two experiments were made.

In the first experiment, the plants were sown on three different dates at 6 and 4 weeks intervals. The plants were grown in the greenhouse conditions at 16-hours day, with additional fluorescent light of the intensity of about 6000 lux

One month before exposure the plants were transferred to an open space covered only by a roof of polyethylene sheet. Two exposures were made in this experiment in the beginning of July — the first on a cloudless day with very good insolation; the second one on a cloudy day. The exposures lasted 60 min. each. The activity taken to each exposure was 0.4 mCi. At that time, the youngest plants were about 9 weeks old and were beginning the period of intensive growth of needles. In the older, 15-weeks old, plants, the intensive growth of needles was already ended. The oldest, 20-weeks old, plants had their apical buds well developed and were beginning their dormancy period. The first harvest was made immediately after the exposure, and the next harvests were made after 24, 48, 96, and 240 hours. 4 pots were taken for each harvest. The plants from each pot were divided into two parallel samples, 14—15 plants each.

In the second experiment all the plants were sown simultaneously at the end of April. Till the time of exposure they grew in the open space covered only by a roof of polyethylene sheet. Three exposures were made at one-month intervals. The plants taken for the first exposure 11 weeks old and were in the final phase of the intensive growth of needles. Second exposure was made for 15-weeks old plants and a third one for 20-weeks old plants. Thus, the vegetation phases of the 15 and 20-weeks old plants were more or less the same as in plants of the same age in the first experiment. The exposures lasted 30 min. each. The activity taken to each exposure was 1 mCi. Harvesting was done immediately after the exposure, and after 6, 24, 48 and 96 hours. In each harvest, except the first one, 5 parallel samples were taken. There were 16 plants in each sample.

RESULTS

As Table 1 shows, the total dry weight of plants taken for experiment one, was lower than that of plants used in experiment two. The difference mainly concerned the dry weight of needles and was the

greatest in the oldest plants. Higher accumulation of dry matter by the plants cultivated during the natural period of vegetation can be explained by their better conditions of growth.

Experiment 1

Simultaneous exposure of plants at various stages of growth allowed relative comparison of the intensity of photosynthesis. The specific activity of needles of the first harvest after exposure may be taken as a measure of the relative intensity of photosynthesis. Needles of the youngest plants showed the greatest photosynthesis capacity (Table 1). The photosynthesis capacity of needles decreased with age towards the end of the vegetation period, it was reduced by about a half compared to the youngest plants.

The difference in the photosynthesis capacity of needles between the oldest and the youngest plants was more distinct in the case of exposure carried out on a cloudless day.

In the first hours following exposure the decrease in the activity of the needles could be characterized by an exponential function (Table 1). Within 24 hours the specific activity of the needles decreased to about one half of the specific activity of the first harvest, the greatest decrease in activity being found in the older and the oldest plants. The specific activity of the youngest needles, after 24 hours, was about 55-57% of the specific activity of the first harvest. During the following days the decrease in the specific activity of needles of the older and oldest plants exhibited a different character than that of the youngest plants. In the youngest plants, when they are in the initial period of intensive growth, the specific activity changed very little during the first day and after 10 days it amounted to 47—50% of the specific activity of needles of the first harvest after exposure. The specific activity of the needles of the older and oldest plants distinctly decreased in the following harvests, and after 10 days is stabilized at the level of 27 to 30% of the specific activity of the first harvest.

The activity of the soluble fraction from needles of the youngest plants, an hour and a half after the application of $^{14}\text{CO}_2$, amounted to about 91% of the total activity contained in needles of that harvest (Table 2). After 24 hours the activity of the soluble fraction was about 50%, and after 10 days it amounted to about 32 to 34% of the total activity contained in needles of the corresponding harvest.

The soluble fraction in the needles of the older plants contained 95% of the total activity of the needles, and in the oldest it contained 98%. After 24 hours the activity of the soluble fraction of needles decreased in the older plants to about 64% and in the oldest plants to 68—77% of the total activity of needles of the corresponding harvest. In the successive

Percent of specific activity of needles and stems in relation to the specific activity of needles from the first harvest

[illegible]

harvests the largest percent of activity was found in the soluble fraction of needles of the oldest plants, a smaller percent of activity was contained in the soluble fraction of needles of younger plants, and the smallest, in the youngest plants.

The degree of photosynthate utilization in formation of needle tissues was the greatest in the youngest plants. Already in the first harvest about 9% of activity was found in the biopolymer fraction, insoluble in 80% ethanol solution (Table 3). The incorporation of radioactive products of photosynthesis into the compounds of the insoluble fraction of needles proceeded rather quickly during the first 24 hours after exposure. After 24 hours the insoluble fraction of needles contained about 1/4, and after 10 days over 1/3 of the total activity of needles in the first harvest. The insoluble fraction of needles of older plants contained in the first harvest 5%, after 24 hours — about 17%, and after 10 days about 19% of the total activity of needles in the first harvest. The lowest percent of activity incorporated by the insoluble fraction of the needles was found in the oldest plants, for each examined harvest time. In the first harvest only about 2% of activity was found in the insoluble fraction of this needles, after 24 hours about 6%, and after 10 days 15–16% of the activity of the first harvest.

In the insoluble fraction of the hypocotyl, in the first harvest, no radioactivity was found. After 24 hours the insoluble fraction of the hypocotyl of the youngest plants contained 2.2 and 2.3%, of older plants 2.3 and 2.7%, of the oldest plants 1.5 and 1.7% of the total activity of plants from the first harvest (Table 3). In the hypocotyl of the youngest plants, the further increase of activity of the insoluble fraction was not great after the first day, and after 10 days this activity amounted to 2.5 and 2.8% of the total activity of the plants from the first harvest. In older plants, the incorporation of radioactive products into the compounds of insoluble fraction of the hypocotyl proceeded still rather intensively after the first day, and during the next 9 days the activity of the insoluble fraction increased twofold in comparison with the activity found after the first 24 hours. After 10 days, the activity in the insoluble fraction was 4.2% and 4.6% of the total activity of the plants from the first harvest. In all the harvests, the lowest activity was in the insoluble fraction of the hypocotyl of the oldest plants. After 24 hours it contained only 1.5 and 1.7% and after 10 days 2.3 and 2.4% of the total activity of the plants from the first harvest. The activity incorporated into the compounds of the insoluble fraction of the aerial parts of a plant was greatest in the youngest plants and amounted to about 40% of the activity of plants from the first harvest. The insoluble fraction of the aerial parts of the older plants contained about 1/4, and of the oldest less than 1/5 of the total activity of plants from the first harvest.

Table 2

Percent of activity in compounds of the ethanol soluble fraction from needles in relation to the total activity of needles of the corresponding harvest

		Experiment I					Experiment II				
		Harvest time after exposure					Harvest time after exposure				
		Imme- diately	24	48	96	240	Imme- diately	6	24	48	96
The youngest plants	Exposure I	91	49	47	41	34	95	76	60	52	40
	Exposure II	91	53	42	34	32					
Older plants	Exposure I	95	64	49	45	43	95	79	63	43	34
	Exposure II	95	64	52	40	34					
The oldest plants	Exposure I	99	78	64	57	47	98	89	79	66	52
	Exposure II	98	68	58	40	38					

Experiment 2

The specific activity of needles decreased most quickly immediately after exposure (Tab. 1). 6 hours after the end of exposure the specific activity of needles of the youngest plants amounted to 70% of the specific activity of needles from the first harvest. The specific activity of needles of the older and oldest plants after the same period of time was 85% and 82% of the specific activity of the needles from the first harvest respectively. After 4 days, the lowest specific activity was found in the needles of the oldest plants, and the highest in the needles of the youngest plants.

The percent of the activity of the ethanol soluble fraction of needles in relation to the total activity of the needles of the same harvest was during the first hours after exposure the lowest in the youngest plants (76%) and the highest in the oldest plants (89%) (Table 2). After 4 days the activity of the needles soluble fraction in the youngest plants amounted to 40%, in the older plants to 34%, and in the oldest plants to 52% of the total activity. The insoluble fraction of the youngest and

Table 3

Percent of activity in compounds of the ethanol insoluble fraction from needles and stems in relation to the total assimilated activity

Experiment I										Experiment II											
Harvest time after exposure										Harvest time after exposure											
Immaturity		24		48		96		240		Immaturity		6		24		48		96			
Plant organ										Plant organ											
Needles		Stems		Needles		Stems		Needles		Stems		Needles		Stems		Needles		Stems			
The youngest plants	Exposure I	9.5	0	28.3	2.3	27.2	2.3	29.6	2.4	33.7	2.5	4.7	0	16.5	0.3	18.9	1.3	21.7	1.8	20.6	1.9
	Exposure II	8.6	0	23.5	2.2	29.0	2.2	32.5	2.6	38.7	2.8										
Older plants	Exposure I	4.8	0	16.6	2.3	19.8	2.6	22.2	4.3	18.8	4.2	5.0	0	17.7	0	18.8	0.8	22.7	1.5	22.5	1.2
	Exposure II	4.8	0	18.3	2.7	21.8	2.9	22.5	4.2	—	4.6										
The oldest plants	Exposure I	1.5	0	6.1	1.5	14.5	1.9	14.3	2.2	15.4	2.3	2.2	0	9.3	0	12.3	0.2	16.1	0.5	15.7	0.5
	Exposure II	2.0	0	6.6	1.7	12.3	1.9	17.4	2.3	16.6	2.4	2.2	0								

older plants, after 6 hours already contained over 16% of the activity of needles from the first harvest. During the successive days the increase in the activity of the insoluble fraction was slight, and after 96 hours, the activity attained the level of 21% in needles of the youngest plants and 23% in the needles of the older plants. The insoluble fraction of the needles of the oldest plants after six hours contained only 9% and after 96 hours 15.7% of the activity of needles from the first harvest.

The specific activity of the hypocotyl of the youngest plants was, after 6 hours, about $1/3$, that of older plants about $1/4$, and that of the oldest plants about $1/5$ of the specific activity of needles from this harvest. During the next two days an increase in the specific activity of the hypocotyl took place. The specific activity of the hypocotyl of the youngest plants increased by about 10%, of the older plants by about 23%, and of the oldest plants, by about 17% in relation to the specific activity found in the hypocotyl 6 hours after the exposure. During the successive two days, a decrease in the specific activity of the hypocotyl took place, the activity of the hypocotyl of the youngest plants decreasing the most.

The specific activity of the insoluble fraction of the hypocotyl was the greatest in the youngest plants for each harvest. Already after six hours 0.3% of the total activity of the first harvest was found in the insoluble fraction of the hypocotyl of the youngest plants, whereas in the older and oldest plants the insoluble fraction of the hypocotyl at that time did not contain radioactivity. After 4 days, the insoluble fraction of the hypocotyl of the youngest plants contained 1.9%, of the older, 1.5% and of the oldest, 0.5% of the activity of the first harvest.

The youngest plants incorporated into their aerial parts 22% of the assimilated ^{14}C , older plants 24%, and oldest plants 16%.

In the plants harvested after the end of the vegetation season, activity in the roots was determined. The roots of the youngest plants contained only slight amount of activity, the older plants contained only 2.1% of the total activity of the first harvest. The highest activity i.e. 8.4% of the total activity of the first harvest was found in the roots of the oldest plants.

DISCUSSION

The experiments showed that the course of the examined processes of distribution and incorporation of photosynthetic products into different organs of seedlings of Scots pine, similarly as in other coniferous plants (Neish 1958; Nelson 1964; Shiroya et al. 1962, 1966; Gordon, Larson 1968) depends on the phase of growth.

The two experiments revealed essentially the same pattern despite not quite the same growth conditions and not completely comparable

conditions of exposure. Taking into consideration some quantitative differences caused by the different growth conditions of the plants, it is possible to discuss the results of both experiments jointly. However, it should be remembered that in both experiments, the growth phases of the youngest plants did not coincide entirely: the youngest plants in the second experiment were 2 weeks older at time of exposure than the youngest plants in the first experiment, and their assimilatory organs were somewhat more developed. Thus, the growth phase of these plants corresponded to an intermediate stage between the youngest and the older plants in the first experiment. This may have caused a somewhat greater deviation in the results of both experiments at the early stage of vegetation.

A simultaneous exposure to $^{14}\text{CO}_2$ of plants which were at different phases of growth showed that the photosynthesis capacity of needles gradually decreased with the plant age, the differences in the photosynthetic activity being more distinct when the process of photosynthesis occurs in better conditions of illumination. The data concerning the decrease in the photosynthetic activity in the course of vegetation confirm the earlier results for pines, obtained by the use of a gasometric method (Żelawski, Góral 1966; Żelawski et al. 1969). The distribution and incorporation of the photosynthetic products into various organs also showed a close relationship with the phases of plant growth. From a comparison of the decrease in the activity of needles and the increase in the activity of stems, it can be concluded that in the first few hours after exposure a decrease in the amount of photosynthetic products in needles is mainly the result of respiration. In the youngest plants the lowest percent of losses during the first day after exposure as well as the relatively negligible changes in the specific activity of needles during the successive day can be explained by the high rate of metabolization of photosynthetic products in the needles at that period of growth. This is also confirmed by the ratio of the activities of the soluble to insoluble fractions in the harvest after the first day when a half of the activity was in the soluble fraction. A slight increase of the activity in the stems during that time, as well as the fact that at that phase no incorporation of radioactive products into the insoluble root fraction was found, indicate that the translocation of photosynthetic products from needles to other organs was very limited during the period of intensive growth of needles. A rather high percentage of the activity in the soluble fraction, as well as slight changes in the specific activity of needles after the first day from exposure suggest in agreement with the present views (Kursanow 1969) that the photosynthetic products may have been temporarily preserved and accumulated, thus making a reserve of substances for biosynthesis of the material for newly formed cell elements. At the phase of intensive growth of needles these organs

retained the greatest amount of the photosynthetic products which they needed for their growth.

It can be concluded from the course of changes in the specific activity of the insoluble fraction of the needles and stems that the rate of incorporation of photosynthetic products into the biopolymers of various organs, and the time of its occurrence are the parameters characteristic for various phases of plant growth. At the time of the intensive growth of needles, incorporation into the compounds contained in insoluble fraction of needles proceeded fairly intensively during a few days after the exposure to $^{14}\text{CO}_2$. Whereas the incorporation into stems was very small.

After the completion of the intensive growth of needles a change in the course of incorporation occurred. The incorporation into the compounds of insoluble fraction of needles proceeded intensively only during the short time after exposure, and after one day no significant changes in the specific activity of the insoluble fraction of needles was noted. On the other hand the process of incorporation into the compounds of insoluble fraction of the stems still continued to proceed fairly intensively for the next few days. This confirms the results of Niziołek et al. (1969), who found in Scots pine a fairly intensive incorporation of ^{14}C into lignin and cellulose even several days after the exposure.

The final period of vegetation was characterized by a relatively low and slow incorporation of photosynthetic products in stems and needles; this process proceeded gradually for a few days after exposure.

It can be concluded, on the basis of the amount of radioactive products found in the roots after completion of the vegetation season, that during the period of intensive growth of needles and stems, the part played by the root in the incorporation of photosynthetic products is quite small. Only toward the end of the vegetation season does the root accept more significant amounts of photosynthetic products.

In contrast to the results of Balatinez et al. (1966) who, in experiments carried out on 9-month old pine plants, found no radioactivity in the soluble fraction already after three days from exposure — in the experiments described above, a fairly high percentage of the activity in the soluble fraction was still detectable even 10 days after the exposure.

A comparison of the amount of activity incorporated into the insoluble fraction in the whole plants of different growth stages indicates that during the period of the intensive growth of needles plants utilized for polymer biosynthesis almost twice as much photosynthetic products as during the further periods of growth.

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*Dystrybucja radioaktywnych produktów fotosyntezy w siewkach sosny zwyczajnej
(Pinus silvestris L.) podczas pierwszego okresu wegetacji*

Streszczenie

Badano dynamikę przemieszczania i rozdział radioaktywnych produktów asymilacji w jednorocznych siewkach sosny zwyczajnej po półgodzinnej lub jedno-godzinnej ekspozycji roślin w obecności $^{14}\text{CO}_2$.

Stwierdzono, że wykorzystanie asymilatów przez poszczególne organy (igły, strzałki), jak też przez całą roślinę silnie zależy od fazy wzrostu. W okresie intensywnego wzrostu igieł w roślinie pozostawało około 2/5 zasymilowanego węgla, podczas gdy pod koniec okresu wegetacji ilość ta malała do 1/5. Największe straty asymilatów, spowodowane głównie oddychaniem, obserwowano w ciągu pierwszej doby po ekspozycji.

Wbudowywanie asymilatów do polimerów zachodziło najintensywniej w ciągu pierwszej doby po ekspozycji, przy czym dynamika i czas wbudowywania zależały od fazy wzrostu i organu rośliny: najintensywniejszą inkorporację obserwowano w igłach w okresie ich intensywnego wzrostu, najwolniejszą zaś w roślinach wchodzących w stan spoczynku. We wszystkich fazach wzrostu igły wbudowywały do frakcji polimerów znacznie większe ilości asymilatów niż strzałki:

Po 10 dniach od ekspozycji, kiedy już nie obserwowano większych zmian radioaktywności właściwych poszczególnych frakcji, frakcja rozpuszczalna w 80% etanolu zawierała jeszcze znaczne ilości radioaktywnych asymilatów (ponad 1/3 radioaktywności ogólnej w igłach i około 1/5 radioaktywności ogólnej w strzałkach).