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Photosynthesis and respiration of Scots pine *(Pinus silvestris L.)* seedlings of various provenance grown under different light conditions*

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Being a shade-intolerant species of tree, Scots pine suffers seriously in conditions of light deficiency and shows the symptoms of growth reduction. Nevertheless, it happens sometimes that pine seedlings develop successfully under the canopy of an old stand. Therefore better knowledge of the adaptability of the assimilatory organs to shading and of the photosynthesis and respiration rates in plants developed under different light intensity seemed to be of interest especially when the problem is considered under the aspect of ecotype differences.

MATERIAL AND METHOD

The experiments were carried out with pine seedlings grown from seed originating from the same three sites in Poland (Dłużek, Spała, Nowy Targ) which have been investigated in our previous works (Żelawski, Góral 1966; Żelawski, Kinelska 1967). Plants grown in pots under full natural light intensity were compared with those cultivated in half shade (60% of full light) and in deep shade (20% of full illumination). Paper-tape screens provided this 40 or 80% reduction of natural illumination over the whole period of growth, i.e. from germination in May 1965 to the beginning of photosynthesis measurements in Summer 1966. The detailed description of the greenhouse pot experiments as well as the characteristic of seedlings after the first vegetation season are given in our previous works (Żelawski, Góral 1966; Żelawski, Żelawska 1967).

For gas exchange determinations plants were always transferred from natural into the laboratory conditions. Three experiments were carried out during the second vegetation season: at the beginning of July, during the first half of August, and in mid September. In view of the number of replications (usually 5—7), it was impossible to measure

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all the experimental variants simultaneously, therefore each experiment was extended in time (to about two weeks).

The photosynthesis and respiration rates were measured under constant laboratory conditions. Uniform conditions of light (combined fluorescent and incandescent illumination exceeding 10,000 lux), temperature (plant chamber connected with an ultrathermostat 25°C), and CO_2-concentration (measurements always within range 350—300 ppm) secured the comparability of the experimental data obtained with plants of various origin and different shade influence. Photosynthesis and respiration were measured similarly as in our experiments with seedlings grown in water culture (Żelawski, Kinelska 1967). The only difference was the use of cut shoots instead of intact plants. Such a simplification was admissible after obtaining proof that short-exposure data, soon after the cutting, do not differ from those obtained with intact plants.

Measurements of photosynthesis rate were always carried out after the "steady state" of the process had been reached. Before the beginning of the experimental procedure plants (not detached from roots) were adapted to the conditions of measurements by standing for at least one hour under the illumination chamber.

The investigation was carried out on young shoots with paired needles in fascicles; the juvenile needles of the first year were not the object of research. However, it should always be remembered that the data obtained in measurements made on the whole shoot do not represent the rates of processes which take place in needles only, but also reflect the ratios of the rates occurring in various organs exposed in the chamber.

The chlorophyll content was determined spectrophotometrically on samples of needles from each plant subjected to photosynthesis measurements. Calculations of the chlorophyll ($a + b$) concentrations were based on measurements of extinction at 645 and 663 mμ in samples exctracted with 85% acetone (Mackinney 1941; Bruinsma 1960).

RESULTS

Cultivation of plants under the illumination of various intensity caused a typical change of their habitus: the control unscreened plants, developed needles of sun-type, the plants under 60% of illumination were of the shade-type, whereas seedlings grown in deep shade (20% of light) showed signs of starvation and a strong reduction of growth.

There was a considerable difference in the average weight of needles in the particular variants of illumination; the difference was noticeable over the whole vegetation season and was of nearly the same order of magnitude in each of the investigated pine strains (tabl. 1).

Table 1

Average dry weight of a single needle and concentration of the chlorophyll ($a+b$) in needles of Scots pine grown under various experimental conditions

Experiment No	Provenance	Mean characteristics of needles					
		Dry weight (mg)			Chlorophyll content (mg per g of dry weight)		
		Light conditions during culture of the plants (%)					
		20	60	100	20	60	100
Exp. I (June 20 — July 8)	Dłużek	1.0	2,6	4.1	6,6	5.9	2.8
	Spała	1.3	2.1	4.3	6.8	5.2	3.8
	Nowy Targ	1.3	1.9	4.3	6.7	5.4	3.2
Exp. II (July 20 — August 9)	Dłużek	1.8	3.7	7.3	7.2	5.9	1.4
	Spała	1.5	3.3	8.2	6.5	4.7	2.1
	Nowy Targ	1.9	3.5	7.9	6.6	5.0	2.0
Exp. III (Sept. 7 — Sept. 20)	Dłużek	3.0	6.4	8.0	7.7	5.0	1.7
	Spała	1.8	5.0	9.5	6.6	5.4	1.6
	Nowy Targ	2.0	5.3	8.0	6.4	5.5	1.7

Chlorophyll concentration calculated per dry matter of needles differed widely between the shade and sun plants and was also not quite the same in the strains of different origin (tabl. 1). It is remarkable that chlorophyll concentration was increased not only in plants under rather light shading (60% of full light) but also in starving plants, growing under deep-shade conditions (20% of full light). The difference between the illumination variants became even more distinct towards the end of the vegetation season, since, during the growth of needles, the concentration remained at constant level in both shade types but showed a decreasing tendency in sun leaves.

Table 2 presents the average net photosynthesis rates in seedlings grown under different light intensities. It is seen that the photosynthesis rate expressed per fresh or dry weight unit is always greater in shade plants than in sun plants. In the experiment carried out during the first days of July the difference was not large. It was distinct only in August, but it became most pronounced in the September experiment when the needles were already fully developed. During the first experiment, both shade types (60% and 20% of full light) exhibited nearly the same photosynthesis rate but at the end of the vegetation season the light-starved plants showed somewhat lower values than the half-shaded ones. Towards the lond of the vegetation season, in each variant of culture conditions, a relatively faster drop of photosynthesis rate was noticeable in the highland (Nowy Targ) pine than in the two lowland (Dłużek and Spała) pines. Besides this there does not seem to exist any major difference in the photosynthesis rates in pines of

Table 2

Average net photosynthesis rates in Scots pine seedlings of various provenance

Experiment No.	Provenance	Net photosynthesis rate in mg CO_2 per hour											
		per 1 g. of needles fresh weight			per 1 g. of needles dry weight			per 1000 needles			per 1 mg. of chlorophyll (assimilation number)		
		Light conditions during culture of the plants (%)											
		20	60	100	20	60	100	20	60	100	20	60	100
Exp. I (June 20 — July 8)	Dłużek	4.67	4.38	3.59	15.3	16.5	11.9	14.7	37.6	49.9	2.1	2.9	4.2
	Spała	4.53	4.64	4.24	15.2	18.4	14.9	19.4	37.9	59.7	2.5	2.8	3.9
	Nowy Targ	3.96	4.45	4.49	18.2	17.3	14.4	24.9	32.1	61.1	2.2	2.7	4.6
Exp. II (July 20 — Aug. 9)	Dłużek	3.96	3.87	2.41	14.3	14.7	6.6	25.8	52.6	49.5	2.1	2.4	4.7
	Spała	4.72	4.69	2.74	17.3	16.3	8.7	26.1	54.0	72.3	2.5	3.5	4.6
	Nowy Targ	4.73	4.55	2.49	17.8	15.8	7.4	34.8	55.0	61.9	2.8	3.1	4.6
Exp. III (Sept. 7 — Sept. 20)	Dłużek	4.17	4.57	2.81	13.6	14.0	7.6	40.1	87.9	62.3	1.7	2.9	4.6
	Spała	3.93	5.20	2.60	14.0	16.8	6.8	25.8	81.1	58.6	1.8	3.2	4.2
	Nowy Targ	3.44	4.30	2.25	10.7	14.3	5.6	20.7	66.3	42.8	1.7	2.5	3.6

different provenance and the reaction to shade was similar in each of them.

When the photosynthesis rate was expressed per 1000 needles the smallest values occured in the heavily shaded plants, the needles of which are strongly reduced in size. However, the differences in photosynthetic activity are only partly due to the reduction of needle size, since the rates of photosynthesis (per 1000 needles) in ful-light plants and in half-shade plants are not quite proportional to the change in needle dry matter. The full-light plants showed high photosynthetic activity already in the first experiment and later, during August and September, their photosynthesis was maintained at a similar level, despite the further considerable growth of needles. In half-shade plants the photosynthesis rates increased during the development of assimilatory organs and at the end of the vegetation season they reached the highest values, exceeding the rates of much bigger sun--needles.

Table 3

Average dark respiration rates in Scots pine seedlings of various provenance

Experiment No.	Provenance	Dark respiration rate in mg CO_2 per hour								
		per 1 g. of needles fresh weight			per 1 g. of needles dry weight			per 1000 needles		
		Light conditions during culture of the plants (%)								
		20	60	100	20	60	100	20	60	100
Exp. I	Dłużek	0.78	0.59	0.45	2.6	2.2	1.5	2.5	5.0	6.1
(June 20 —	Spała	0.68	0.54	0.43	2.4	2.1	1.6	3.3	4.3	5.9
July 8)	Nowy Targ	0.60	0.47	0.52	2.7	1.8	1.7	3.4	3.2	7.1
Exp. II	Dłużek	0.48	0.37	0.43	1.8	1.4	1.2	3.2	5.1	8.7
(July 20 —	Spała	0.53	0.41	0.41	2.3	1.4	1.3	3.2	4.7	10.4
Aug. 9)	Nowy Targ	0.43	0.34	0.43	1.7	1.2	1.2	3.2	4.0	10.8
Exp. III	Dłużek	0.57	0.40	0.52	1.9	1.2	1.4	5.9	8.7	10.7
(Sept. 7 —	Spała	0.64	0.39	0.52	2.1	1.2	1.3	4.0	6.3	12.3
Sept. 20)	Nowy Targ	0.76	0.37	0.51	2.3	1.1	1.2	4.7	5.9	10.0

It appears that the assimilation numbers (phosynthesis rates expressed per 1 mg of chlorophyll) are similar, if the same growth conditions are compared during the investigated growth period. Yet, the photosynthesis rate gradually decreases as the light conditions of the culture deteriorate. Between the most efficient sun plants and both shade types the difference is clear and significant. However, there is no marked difference of the assimilation numbers between the examined pines of various provenance, though a tendency towards a faster decrease of photosynthetic activity in the Nowy Targ pine toward the

end of the vegetation season can also be recognized especially in sun plants and in half-shade plants (60% of illumination).

From the data on dark respiration rates (table 3), it is seen that the relations are here different from those in net photosynthesis rates. In terms of weight units the respiration rates are higher in the heavily-shaded plants than in the half-shaded and sun-plants. The respiration rate calculated per 1000 needles seems to be more or less proportional to the dry weight of the needles, but it changes during the summer time not quite parallel with the dry matter increment. There is a slight but systematic difference between the plants of different provenance marked by lower rates of respiration in the half-shade plants from Nowy Targ, in every period of investigation.

Table 4

Ratio: true photosynthesis/dark respiration

Experiment No.	Provenance	Light conditions during culture of the plants (%)		
		20	60	100
Exp. I (June 20 — July 8)	Dłużek	7.0	8.5	9.0
	Spała	7.3	9.7	10.7
	Nowy Targ	7.9	10.7	9.6
Exp. III (July 20 — Aug. 9)	Dłużek	9.1	11.5	6.6
	Spała	9.5	12.5	7.8
	Nowy Targ	11,8	14.4	6.8
Exp. III (Sept. 7 — Sept. 20)	Dłużek	8.1	11.9	6.6
	Spała	7.4	14.6	6.1
	Nowy Targ	5.1	12.9	5.4

The comparison of photosynthesis and respiration data leads to the conclusion that the seasonal changes as well as the infuence of shade conditions are not identical for both processes. The ratio of the estimated true photosynthesis to dark respiration exhibits a tendency to seasonal change, which is not the same in each of the experimental variants. In our previous study (Żelawski, Góral 1966) it was found that the A+R/R ratio varies considerably during the vegetation season and reaches its highest values (more than 8) in July. In this work the ratio was as high as 10.7 in sun-plants and 14.6 in shade-plants (table 4). However, the sun plants showed a gradual decrease of the index in seasonal course, whereas the 60%-illumination plants exhibited a tendency to increase it towards the end of the vegetation season; the same tendency was observed in deep-shade plants, with the exception of the autumn-months when the value dropped again. The pines of various provenance seem to differ in this respect: in those of highland origin

(Nowy Targ), grown in full light conditions, the ratio decreases more rapidly than in the others; on the other hand, the highland pine seemed to be more efficient in both shade variants of the experiments I and II (as long as the earlier coming of winter depression does not overlap this regularity).

DISCUSSION

Scots pine needles of the sun and shade-types were investigated by Stålfelt (1921) who established that the photosynthesis rate expressed per 1 g of fresh weight is higher in the shade than in the sun needles when the process at low light intensity was compared. Ivanoff and Kossowitsch (1929) have found, in calculations per fresh weight unit, that needles of shade type, as compared with the sun plants, photosynthesize approximately twice as much in conditions of low light intensity, over 50% more at disperse light, but considerably less in conditions of full, direct insolation. The data calculated per surface unit were similar, but in conditions of disperse light the photosynthesis rate of both needle types was equal and in full insolation amounted in shade plants to only one half that found in sun needles.

Our research, carried out at constant light intensity, corresponding however, to the linear part of the photosynthetic light curve, gave results consistent with the previous state of knowledge as far as weight units are concerned. In all the experiments the net photosynthesis rates were considerably higher in the shade type and in several cases the difference exceeded 100%. This resulted in changes of the photosynthetic activity expressed per number of assimilatory organs: the value being distinctly lower in half-shade plants at the begining of needle growth, became nearly the same or even higher than the photosynthesis of sun needles as the size of needles increased. However this was not the case for the deep shade type, growing under starvatian conditions. These plants showed a strong reduction in dry weight of needles, and though their photosynthesis rate per weight unit was similar to that of the half-shade type, the photosynthesis rate per number of assimilatory organs was the lowest in every period of investigation.

Differences in chlorophyll content between the shade and sun types of leaves have often been reported for various species of plants (see literature Simonis 1960). However, information still lacks on the assimilation numbers of both plant types. When using the data of Björkman and Holmgren's work (1963) for *Solidago virgoaurea* L. clones adapted to two different light intensities, one can see that the photosynthesis rate expressed per 1 mg of chlorophyll is usually lower in plants grown under worse illumination conditions. From the results of our work it follows that the efficiency of the chlorophyll unit considera-

bly decreases as the conditions of growth deteriorate; the relation of the assimilation number to the light intensity of the previous treatment appears to be nearly linear when comparing the data at the end of the vegetation period. Perhaps needle tissues adapted to shade, despite their increased concentration of pigments, are not able to augment the photosynthesis activity proportionally to the chlorophyll content because other limiting factors e.g. structural differences may be involved. From the numerous literature data it is well known that chlorophyll content in leaves is rather in excess (Gabrielsen 1960) and the linearity of the photosynthesis rate and chlorophyll content can hardly be observed (see also: Šestak 1966).

It would be interesting to know how these relations look at a light saturation level, but — as seen from many papers — to attain such a light intensity is extreemly difficult for this species. No saturation level occurred even in full natural insolation in Stålfelt's (1921, 1924) and Polster's (1950) investigations.

The only paper which gives assimilation numbers for Scots pine (Keller and Wehrman 1963) is not fully comparable with our results, since the experiments were carried out on first year seedlings grown in water culture. However, it is worth stressing that in the above mentioned work the photosynthesis rates calculated per 1 mg of chlorophyll hardly exceed 5, in seedlings with low chlorophyll content, although 50,000 lux illumination was applied, whereas our highest values were close to 5 despite the lower light intensity. In our previous work, carried out with young seedlings grown in water culture (Żelawski, Kinelska 1967) the assimilation numbers were lower and amounted on the average to 2.6 (under experimental conditions such as in this work). This may indicate that the photosynthetic efficiency of the chlorophyll unit is greater in older seedlings developing on fascicles the double needles typical for pine, than the efficiency of the first year juvenile leaves. The efficiency of the chlorophyll unit in juvenile seedlings seems to be similar to that of the shade plants in the second vegetation season.

The dark respiration rates (table 3) are not in full agreement with the literature data. It has been reported for Scots pine that shade--needles respire apprioximately two times less (per fresh weight or per surface unit) than sun-needles (Oskretkow 1959) and that the former have lower temperature maximum than the latter (Chlebnikowa 1962). From our data, expressed per dry and fresh weight, one can see that this is not always the case: the respiration rate of half-shade needles decreases distinctly during the vegetation season, and being at the beginning higher than the sun needles respiration becomes equal or even lower towards the end of the vegetation season. On the other hand the respiration rate of deep shade plants is always the highest in

calculations per dry and fresh weight unit. The situation is reversed when the number of assimilatory organs is taken as the reference unit: in this case the respiration of shade needles is always lower than that of sun ones. Further discussion of the problem of gas exchange processes occuring in shade and sun-needles of Scots pine will only be possible after detailed investigation of the structural differences between these types of assimilatory organs.

The essential problem of this study — ecotypic differences in photosynthetic efficiency seems to find some further elucidation. The seasonal differences in photosynthesis rate between the examined pines of various provenance have been confirmed, as far as the earlier entering of the highland pine into the winter depression is concerned. This phenomenon was manifest not only in the full light culture plants, but also in the shade-type seedlings, irrespective of the reference unit applied (see experiment III). Thus, the results contained in this work as well as the data of the previous papers (Żelawski, Góral 1966; Żelawski, Kinelska 1967) seem to support the thesis that the investigated plants of various provenance differ in their seasonal course of photosynthesis, at least as regards the lowland and highland pines.

As to the adaptability of the ecotypes to the shade conditions, which was postulated to be slightly different in the pine of highland provenance (Żelawski, Żelawska 1967), the data presented gave some additional information. Although the photosynthesis rates of plants adapted to shade did not differ significantly in the examined seedlings of various origin, the trends may not have been the same at various times of the vegetation season. Thus, for instance, during the full summer time (experiment II) the Nowy Targ plants grown in heavy shade (20% of full illumination), seem to photosynthesize somewhat more efficiently than seedlings of other provenance. However, this was not the case in determinations carried out in September when the earlier winter depression overlapped the previous picture of relationship.

The differences in the respiration rates were also not very pronounced though the distinctiveness of the highland pine from the pines of other origin can often be observed (for instance the difference between full light and 60% illumination plants was the greatest in calculations per needles' number and the lowest in relation to dry matter of needles in the seedlings from Nowy Targ).

During the period of needle growth the ratio — true photosynthesis: respiration — considerably decreases in sun needles and distinctly increases in the shade ones. If the adaptation of the growing leaves to shade or sun conditions is connected with an entirely different change of the discussed index, the value of this ratio might be considered as a relative measure of the adaptability. From this point of view the plants originating from Nowy Targ, can be considered to be perhaps more shade-

tolerant than other investigated pines. The phenomenon could be observed in both summer experiments and was probably partialy masked at the end of the vegetation season, owing to the difference in the time of the winter depression occurrence.

SUMMARY

Scots pine seedlings of various provenance, cultivated for two vegetation seasons under various light intensities were examined in constant laboratory conditions. The results of measurements show that the photosynthesis rate of shade plants, as compared with sun plants, is higher when the rate is expressed per 1 g of dry or fresh weight of needles, but it is considerably lower when the amount of chlorophyll is used as the reference unit. In seedlings grown under natural light intensity, the typical for pine paired needles on fascicles exhibit markedly higher assimilation numbers than those found previously in the first year juvenile needles.

During the three experiments carried out in summer and autumn months further evidence was found for the difference between the lowland and highland pines as regards the winter depression advance. Some new facts were also noted, indicating that plants of various origin may differ in shade adaptability: the highland pine from Nowy Targ seems to be somewhat more tolerant to shade than the lowland pines, at least at the end of spring and in full summer time.

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Fotosynteza i oddychanie siewek sosny zwyczajnej (Pinus silvestris L.) różnego pochodzenia, wyhodowanych przy różnym oświetleniu

Streszczenie

W stałych warunkach laboratoryjnych przeprowadzono badania porównawcze siewek sosny zwyczajnej różnego pochodzenia. Do badań użyto materiału roślinnego wyhodowanego w doświadczeniu wazonowym, założonym w hali wegetacyjnej, w zróżnicowanych warunkach ocienienia (20, 60 i 100% naturalnego światła).

Wyniki pomiarów wymiany CO_2 przeprowadzonych w układzie zamkniętym z analizatorem CO_2 w podczerwieni wskazują, że intensywność fotosyntezy netto u roślin z ocienienia w porównaniu z roślinami z pełnego światła jest wyższa w przeliczeniu na g świeżej lub suchej masy igliwia, natomiast jest ona wyraźnie niższa, jeśli za jednostkę odniesienia przyjąć zawartość chlorofilu. Stwierdzono również, że u roślin wyrosłych w pełnym świetle naturalnym typowe dla sosny igły podwójne na krótkopędach wykazują znacznie wyższe liczby asymilacyjne w porównaniu z igliwiem młodocianym pierwszego roku wegetacji (Żelawski i Kinelska 1967).

W toku trzech serii doświadczeń przeprowadzonych w miesiącach letnich i jesiennych stwierdzono powtórnie (Żelawski i Góral 1966) różnice czasu następowania zimowej depresji asymilacyjnej u sosny pochodzenia górskiego (Nowy Targ) w porównaniu z sosnami niżowymi (Dłużek i Spała).

Niektóre wyniki zdają się wskazywać, że badane sosny różnej proweniencji mogą nie jednakowo reagować na warunki ocienienia. Wysoki stosunek fotosyntezy do oddychania u roślin wyrosłych w warunkach częściowego ocienienia, wyróżnia sosnę nowotarską od sosen pochodzenia niżowego. Może to być dowodem nieco lepszego przystosowania siewek tego pochodzenia do warunków ocienienia.