The effect of the photoperiod on the level of endogenous growth regulators in pine (*Pinus silvestris* L.) seedlings

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Abstract

The investigations were performed on pine seedlings growing under 12, 16 and 20 hour photoperiods. In 4 successive stages of seedling development i.e. after 2, 12, 18 and 30 weeks of culture morphological characters of the seedlings were measured and the levels of auxins-, gibberellins-, cytokinins- and abscisic acid-like inhibitor were determined. The intensity of growth and development of juvenile leaves, needles and of the shoot was the lowest in plants growing under 12 hour photoperiod conditions. As the length of the photoperiod increased so did the intensity of these processes. Under the 12 hour photoperiod the development of scale leaves, axillary buds and the formation of the terminal bud started earliest. This process reached completion under the 12 hour photoperiod and the bud remained in a state of dormancy. Seedlings growing under the 12 hour photoperiod were characterized by a low level of stimulators, and at the same time by a high level of inhibitors. On the other hand in seedlings grown at 16 and 20 hour photoperiods the content of stimulators was higher and that of inhibitors lower. A high intensity of growth and development processes was correlated with a high level of stimulators while a high level of inhibitors was correlated with a low intensity of these processes.

The obtained results suggest the participation of gibberellins and cytokinins in the processes of regulation of the initiation of scale leaves and axillary buds, and the participation of these hormones and of abscisic acid in the regulation of needle elongation.

INTRODUCTION

The phenomenon of the dependence of pine seedling growth on the photoperiod is well known (Wareing 1950, 1951, Downs and Borthwick 1956, Downs and Piringier 1958). Hormone metabolism is also known to affect pine seedling growth and development (Michniewicz et al. 1974, 1976). The effect of the photoperiod on the level of endogenous regulators has been examined in many plants,

but there is little data available for pine seedlings. They only concern the effects of the photoperiod on the levels of auxins and auxin inhibitors (Wareing 1951). The experiments described in this paper were a continuation of investigations performed for many years in the Department of Plant Physiology of the Institute of Biology of the Copernicus University in Toruń on the role of hormones in the growth and development of the pine. Investigations on the dependence of hormone metabolism on the photoperiod in pine seedlings were examined. The effects of the photoperiod on the growth and development of pine seedlings were determined and the hormone levels were assayed in subsequent phases of development of these plants.

MATERIAL AND METHODS

The subject of these investigations were Scots pine (*Pinus silvestris* L.) seedlings aged from 2 to 30 weeks growing under 12, 16 and 20 hour photoperiods. Appropriate photoperiod variants were obtained using various times of illumination and a different light intensity. For all three variants a 12 hour period of light with an intensity of about 4000 lx was used. The longer, 16 and 20 hour photoperiods were obtained by additional irradiation for 4 or 8 hours with light of a much lower intensity (200 lx). The source of light were "Flora" fluorescent lamps produced by Polam. The temperature of the chambers in which the plants grew was $25 \pm 2^\circ\text{C}$ in daytime and $18 \pm 2^\circ\text{C}$ at night. The basic substrate for cultivation was sandy soil from a pine forest and only the youngest seedlings grew in perlite. The plants were watered once a week with Ingstad's medium (1962/63) and when necessary they were sprinkled with tap water.

Systematical measurements of the morphological characters of the seedlings growing under various photoperiod conditions were made. The length of the cotyledon, juvenile leaves and needles on the shoot were measured as well as the length of the shoot. The number of the following organs were determined: juvenile and scale leaves, fascicles of needles and axillary buds in the axills of juvenile and scale leaves. All measurements were performed on 20 plants from each variant. The results were subjected to statistical analysis calculating LSD with $P = 0.05$.

The material for hormone analysis was collected after 2, 12, 18 and 30 weeks after the seeds were planted. The hormones were determined in the above-ground parts of the seedlings without the hypocotyl. In 30 weeks old seedlings the hormone level was determined in the above-ground part of the seedling and separately in juvenile leaves and needles. The dry and fresh matter was determined in the same part of

the plants. In three parallel samples of which each contained 50 g of plant material the content of auxins, gibberellins, cytokinins and abscisic acid was determined according to the method described by Michniewicz et al. (1976), with the exception of partition of auxins and abscisic acid. In this work auxins and abscisic acid were partitioned with paper chromatography in solvent system: isopropanol, ammonia, water (10:1:1 v/v). The equivalent of the same amount of dry matter was applied to chromatograms. In the case of auxins, gibberellins and abscisic acid it corresponded to the dry matter contained in 2 g of fresh matter of above-ground parts of seedlings which grew under conditions of the 12-hour photoperiod. In the case of cytokinins this equivalent corresponded to 5 g of fresh matter of these seedlings.

The activity of the investigated hormones was determined by means of biological tests. For auxins the coleoptile test of oat was performed, for gibberellin — of lettuce hypocotyl, for cytokinins — of soybean callus, and for abscisic acid — of wheat coleoptyl. The tests were repeated 4-6 times. The obtained results were analysed statistically calculating LSD at $P = 0.05$.

RESULTS

The morphological characterization of seedlings in subsequent developmental stages in which hormones were determined is presented in Tables 1-3. The pine seedlings grown under a 12 hour photoperiod were characterized by the shortest juvenile leaves and needles and shoots, while in seedlings growing under the 20 hour photoperiod the length of these organs was the greatest. The number of formed juvenile leaves and fascicles of needles was the lowest in the 12 hour variant and the highest in the 20 hour photoperiod variant (Table 1).

The development of needles was found to start after 12 weeks of culture in seedlings growing under the 20 hour photoperiod, after 16 weeks in seedling grown with a 16 hour photoperiod, and after 18 weeks in the variant of the 12 hour photoperiod. All needles developed from axillary buds in axills of juvenile leaves.

The intensity of growth of scale leaves and axillary buds was different than for needles. In 12 week seedlings the number of these organs was the highest in the 12 hour photoperiod variant, in 18 week seedlings in the 16 hour photoperiod variant and in 30 weeks old seedlings in the 20 hour photoperiod variant (Table 2).

During the whole experiment only in seedlings grown at the 12 hour photoperiod the number of scale leaves reached a stable value after 18 weeks culture (Table 2).

Table 1

Effect of photoperiod on the growth and development of hypocotyls, juvenile leaves and needles in pine seedlings

Age, weeks	Photo-period, h	Length (mm) of				Number of	
		cotyledons	total juvenile leaves	total needles	shoot	juvenile leaves	fascicles of needles
2	12	17.0	—	—	—	—	—
	16	18.7	—	—	—	—	—
	20	20.3	—	—	—	—	—
	LSD P=0.05	1.1	—	—	—	—	—
12	12	24.8	591.8	—	4.8	34.4	—
	16	24.5	790.0	—	5.5	44.2	—
	20	26.2	1002.6	54.0 ⁺	7.0	50.0	6.0 ⁺
	LSD P=0.05	1.7	121.9	—	1.0	6.0	—
18	12	24.9	780.0	58.0 ⁺	8.0	40.7	2.1 ⁺
	16	25.1	1087.0	75.0 ⁺	11.2	46.5	5.1 ⁺
	20	25.1	1440.0	320.0 ⁺	15.6	57.2	9.1 ⁺
	LSD P=0.05	1.4	158.0	—	1.5	5.3	—
30	12	—	655.0	262.5	7.8	34.8	3.0
	16	—	1275.0	830.0	15.9	49.5	6.5
	20	—	1663.0	1588.7	22.5	62.0	11.4
	LSD P=0.05	—	173.2	377.9	2.5	5.6	2.5

(Average of measurements of 20 seedlings)

+—as not all 12 and 18 weeks old seedlings developed needles fewer plants were measured

The fresh and dry matter of seedlings was the lowest in the 12 hour photoperiod variant, higher in the seedlings grown at the 16 hour photoperiod variant and the highest in seedlings grown at the 20 hour photoperiod (Table 3). Such differences were observed in all investigated stages of seedling development.

The results of experiments concerning auxins are presented in Fig. 1. The content of substances corresponding in their location to IAA, with R_f values 0.3-0.5 is given. In all stages of seedling development regardless of the age of plants, the highest level of auxins was observed in seedlings growing under a 20 hour photoperiod. In juvenile leaves from 30 week seedlings and in needles of these plants the auxin contents were similar.

In the investigated material 3 groups of gibberellin-like substances were determined with R_f values of 0.0-0.2, 0.5-0.6 and 0.8-1.0. Regardless of the age of the investigated plants the lowest gibberellin content was obtained in seedlings growing at a 12 hour photoperiod and the

Table 2

Effect of photoperiod on the development of scale leaves and axillary buds in pine seedlings

Age, weeks	Photo- period, h	Number of	
		scale leaves	axillary buds
12	12	12.1	18.5
	16	8.9	13.3
	20	6.7	10.4
	LSD P=0.05	—	4.1
18	12	26.6	33.6
	16	37.5	44.3
	20	29.7	33.5
	LSD P=0.05	5.5	5.7
30	12	26.7	30.4
	16	48.7	56.0
	20	56.5	60.2
	LSD P=0.05	8.2	8.6

(Average of measurements of 20 seedlings)

Table 3

Effect of photoperiod on the fresh and dry weight of pine seedlings

Age, weeks	Photo- period, h	Weight of 100 seedlings ⁺ , g		Dry weight, %
		fresh	dry	
2	12	1.33	0.18	13.5
	16	1.40	0.19	13.6
	20	1.56	0.21	13.5
12	12	4.68	1.19	25.4
	16	8.46	2.03	24.0
	20	9.34	2.10	22.5
18	12	7.53	2.22	29.5
	16	12.83	3.45	26.9
	20	16.04	4.01	25.0
30	12	8.55	3.07	35.9
	16	24.73	8.60	34.8
	20	38.67	12.98	33.6

⁺—above-ground parts of the seedlings without hypocotyls

Fig. 1. Effect of photoperiod on the level of auxins in pine shoots

highest gibberellin level was found seedlings aged 2, 12 and 18 weeks grown under the 16 hour photoperiod and 30 weeks old seedlings which grew under 20 hour photoperiod (Fig. 2). In 2 and 18 weeks old seedlings the differences in gibberellin content between variants which grew under 16 and 20 hour photoperiods were not considerable. Juvenile leaves of 30 weeks old seedlings contained less gibberellins than needles.

Fig. 2. Effect of photoperiod on the level of gibberellins in pine shoots

Fig. 3. Chromatographic analysis of cytokinins in pine shoots at different photoperiods

The cytokinins could be found in two main zones of activity on chromatograms — with R_f values 0.1-0.5 and 0.6-0.9 (Fig. 3). In all examined stages plants grown at 12 hour photoperiods contained the lowest amounts of cytokinins. The level of these hormones was much higher in seedlings grown under 16 and 20 hour photoperiods. In seedlings 2, 18 and 30 weeks old the cytokinin content was the highest in the 20-hour photoperiod variant and in 12 weeks old seedlings in the 16 hour photoperiod variant. In needles from 30 weeks old seedlings the level of these hormones was higher as compared to juvenile leaves from these seedlings.

Fig. 4. Effect of photoperiod on the level of ABA-like inhibitor in pine shoots

The main group of inhibitors were substances located at R_f values 0.5-0.7 corresponding to ABA in their location. In 2, 12 and 30 weeks old seedlings the level of inhibitors was the highest in plants grown under the 12 hour photoperiod, and the lowest in plants grown under a 20 hour photoperiod (Fig. 4). Different results were obtained for 18 weeks old seedlings where the highest amounts of inhibitors were detected in seedlings grown under a 20 hour photoperiod and the lowest in plants grown at 16 hour photoperiods. However, the level of inhibitors at this stage of seedling development was low, and the differences in their contents in different photoperiod conditions were not large. In 30 weeks old seedlings more inhibitors were found in juvenile leaves than in needles.

DISCUSSION

The differences in the growth and development of seedlings and in the level of hormones were already detectable in the earliest stages of plant development. The fact should be noted that in all photoperiod variants the plants were illuminated by light of the same intensity (4000 lx) for the first 12 hours. The longer, 16 and 20 hour photoperiods were obtained by additional illumination with light of a much lower intensity (200 lx) which should not have caused an increase in photosynthesis. Thus the conclusion may be drawn that the differences in growth and in hormone levels in seedlings were primarily caused by the differences in length of the photoperiod. However, as photoperiods longer than 12 hours distinctly stimulated the intensity of growth and of needles development, and thus increased the size of the main photosynthesizing organs, the differences detectable in subsequent stages of ontogenetic development must have been a consequence of interaction of the photoperiod and photosynthesis.

The 16 and 20 hour photoperiod caused an increase in the intensity of growth and development of juvenile leaves and needles and of the shoot. In seedlings growing under these photoperiod conditions the process of formation of the terminal bud was not completed whereas under the 12 hour photoperiod this process was completed and the bud remained dormant until the end of the experiment.

The obtained results confirm the observations of Wareing (1950) and Downs and Borthwick (1956) performed on pine seedlings grown under conditions of short and long photoperiods. We should stress that in this work in order to catch the physiological state of seedlings appropriate for physiological determinations, a detailed analysis of more morphological characters was made in the works of the quoted authors.

In general we found that a long, 16 or 20 hour photoperiod caused an increase in the level of auxins, gibberellins and cytokinins in pine seedlings and a concomitant decrease of the level of inhibitors in relation to the content of these hormones in seedlings growing under a 12 hour photoperiod. Also other authors have noted a higher content of stimulators in trees grown under long photoperiod. A higher auxin content was observed in the pine (Wareing 1951) and birch (Digby and Wareing 1966) and of gibberellins in the maple (Lenton et al. 1972).

A higher level of auxins, gibberellins and cytokinins in seedlings growing under long, 16 and 20 hour photoperiod as compared with the level of these hormones in seedlings grown under the 12 hour photoperiod was associated with a higher intensity of growth processes of the shoot, juvenile leaves and needles. A high level of inhibitors con-

tained in seedlings grown under 12 hour photoperiod was correlated with low intensity of these growth processes. A similar interdependence between the level of auxins and gibberellins and the intensity of growth was shown by our earlier investigations performed on coniferous trees — *Pinus* and *Larix* (Kopcewicz et al. 1967, 1970). In experiments performed so far this interdependence was not, however, so distinct within long day variants of the 16 and 20 hour day. Thus for example in some stages of seedling development the highest level of stimulators was detected in plants under conditions of the 16 hour photoperiod, when growth was less intense than under a 20 hour photoperiod.

An additional experiment in which the level of hormones was examined separately in juvenile leaves and needles of 30 weeks old seedlings sheds some light on the matter. The results indicate that a higher level of gibberellins and cytokinins and lower level of abscisic acid is found in needles than in juvenile leaves. No differences in auxin level were detected. As needles are in the elongation stage the conclusion may be drawn that gibberellins, cytokinins and abscisic acid participate in the regulation of this process.

The results obtained in this work also indicate that the process of regulation of the inhibition of scale leaves and axillary buds depends on gibberellin and cytokinin levels. This is shown by a high level of these hormones in 12 weeks old shoots grown under a 16 hour photoperiod i.e. in the period of intensive growth of these organs. Such a conclusion is confirmed by the results of Varnell and Vasil (1978) who found that when kinetin is applied to apical shoots of *Pinus elliottii* it causes the formation of scale leaves. These authors did not observe any effects of GA₃ on this process, which might be due to the low sensitivity of the pine to exogenously applied gibberellins (Michniewicz 1976).

The results of experiments performed on 30 weeks old seedlings grown under conditions of the 12 hour photoperiod give some information on the role played by plant hormones in bud dormancy. They indicate that bud dormancy is correlated with a high level of inhibitors and a low level of stimulators. It is commonly believed that this state is caused by ABA and other inhibitors produced in leaves under short day conditions (Wareing and Saunders 1971), but recent data on this subject indicate that ABA does not always induce dormancy in plants. Alvim et al. (1978) in experiments with willow trees have shown the level of this hormone was not affected by the photoperiod. Earlier Lenton et al. (1972) discovered in the birch, less ABA under conditions of a short photoperiod. The results obtained by these authors may have been due to different methods of ABA determination.

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REFERENCES

- Alvim R., Thomas S., Saunders P. F., 1978. Seasonal variation in the hormone content of willow. *Plant Physiol.* 62: 779-780.
- Digby J., Wareing P. F., 1966. The effect of applied growth hormones on cambial division and the differentiation of the cambial derivative. *Ann. Bot.* 30: 607-622.
- Downs R. J., Borthwick H. A., 1956. Effects of photoperiod on growth of trees. *Bot. Gaz.* 117: 310-326.
- Downs R. J., Piringer Jr A. A., 1958. Effects of photoperiod and kind of supplemental light of vegetative growth of *Pinus*. *Forest Sci.* 4: 185-195.
- Ingestad T., 1962/63. Macroelement nutrition of pine spruce and birch seedlings in nutrient solutions. *Medd. J. St. Skogsf.* 51: 1-131.
- Kopcewicz J., Michniewicz M., Kriesel K., 1967. Dynamics of gibberellin-like substances and growth inhibitors in pine (*Pinus silvestris* L.) and larch (*Larix decidua* Mill.) in relation to age and season. *Bull. Acad. Pol. Sci. ser. biol.* 15: 427.
- Kopcewicz J., Michniewicz M., Kriesel K., 1970. Auxins and growth inhibitors in pine (*Pinus silvestris* L.) and larch trees (*Larix decidua* Mill.) of different age. *Acta Univ. Nic. Copernici, biol.* 23: 139.
- Lenton J. R., Perry V. M., Saunders P. F., 1972. Endogenous abscisic acid in relation to photoperiodically induced bud dormancy. *Planta* 106: 13-22.
- Michniewicz M., 1976. Outline of Physiology of Scots Pine. Ed. For. Scient. Public. Depart. Nat. Cent. Scient., Technic. Econ. Informat. Warsaw, Poland. pp. 269-301.
- Michniewicz M., Galoch E., Rozej B., Zatorska Z., 1974. Dynamics of endogenous plant growth regulators in the early stages of growth development of scotch pine seedlings (*Pinus silvestris* L.). *Bull. Acad. Polon. Ser. Sci. biol.* 22: 625-630.
- Michniewicz M., Rozej B., Stopińska J., 1976. The influence of nitrogen nutrition of the dynamics of growth and metabolism of endogenous growth regulators in scotch pine (*Pinus silvestris* L.) seedlings. *Acta Soc. Bot. Pol.* 45: 495-510.
- Varnell R. J., Vasil J. K., 1978. Experimental studies of the shoot apical meristem of seed plants. II. Morphological and cytochemical effects of kinetin applied to the exposed meristem of *Pinus elliotii*. *Amer. J. Bot.* 65: 47-49.
- Wareing P. F., 1950. Growth studies in woody species. I. Photoperiodism in first-year seedlings of *Pinus silvestris*. *Physiol. Plant.* 3: 258-276.
- Wareing P. F., 1951. Growth studies in woody species III. Further photoperiodic effects in *Pinus silvestris*. *Physiol. Plant.* 4: 41-56.
- Wareing P. F., Saunders P. F., 1971. Hormones and dormancy. *Ann. Rev. Plant. Physiol.* 22: 261-288.

*Wpływ fotoperiodu na poziom endogennych regulatorów wzrostu
w siewkach sosny zwyczajnej (Pinus silvestris L.)*

Streszczenie

Badania przeprowadzono na siewkach sosny w wieku od 2 do 30 tygodni, rosnących w warunkach 12-, 16- i 20-godz. fotoperiodu. Stwierdzono, że intensywność procesów wzrostu i rozwoju szpilek juwenilnych, krótkopędowych oraz pędu najniższa była u roślin rosnących w warunkach 12-godz. fotoperiodu. W miarę jak wzrastała długość fotoperiodu intensywność tych procesów zwiększała się. W warunkach 12-godz. fotoperiodu najwcześniej rozpoczynał się rozwój liści łuskowatych i pąków pachwinowych oraz formowanie pąka szczytowego. Tylko w warunkach 12-godz. fotoperiodu proces ten został zakończony i pąk pozostał w stanie spoczynku. Siewki rosnące w warunkach 12-godz. fotoperiodu charakteryzował niski poziom stymulatorów, a jednocześnie wysoki poziom inhibitorów. Natomiast w siewkach rosnących w warunkach 16- i 20-godz. fotoperiodu zawartość stymulatorów była większa, a inhibitorów mniejsza. Z wysokim poziomem stymulatorów skorelowana była duża intensywność procesów wzrostu i rozwoju siewek, natomiast wysoki poziom inhibitorów łączył się z małą intensywnością tych procesów. Uzyskane wyniki sugerują udział giberelin i cytokinin w procesach regulacji inicjacji liści łuskowatych i pąków pachwinowych, oraz udział tych hormonów i kwasu abscysynowego w regulacji elongacji szpilek krótkopędowych.