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Investigations on the kind of *Larix polonica* Rac. wood formed under various photoperiodic conditions

II. Effect of different light conditions on wood formed by seedlings grown in greenhouse

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In the first part of the present investigations published earlier (Wodzicki 1960) a correlation between the time of resting bud formation and late wood formation had been observed in 2- and 3-year old larch seedlings grown in natural conditions. Both these processes occurred towards the end of September when the day length was significantly reduced (to 12 hours). These investigations did not reveal whether there exists some dependence between cessation of extension growth of plants and thick walled tracheid formation but it may be supposed that the naturally shortened photoperiod at the end of summer was the factor which affected both processes. Experiments carried out by Żelawski (1957) showed that after 20 short days resting buds were formed at the top of shoots of larch seedlings. Anatomical examination of shoots revealed the presence of a ring of thick-walled tracheids. At present, an attempt was made to establish what is the time of thick-walled tracheids formation during short day treatment. Some other experiments of Żelawski, showed that there is an important difference between the shoot apex reaction and the reaction that brings about the formation of thick-walled tracheids, which both are affected by photoperiodic conditions. Molski, Żelawski (1958) has established that thick-walled tracheids might be formed also under continuous illumination obtained by using additional light of low intensity (10 lux) at night hours during the 20 days. Therefore, the reaction bringing about cell wall thickening of tracheids under such conditions of continuous illumination was the same as under short day treatment (diurnal 12 hours light and 12 hours darkness). Extension growth of plants growing under the conditions of additional light of low intensity was not ceased, but it was ceased after 20 days of short day treatment. At present, some experiments were carried out in order to examine if the total sum of light hours is an impor-

tant external factor affecting the thick-walled tracheids formation, and where is the locus of perception of the photoperiodic stimulus of this reaction. The elucidation of these problems may be helpful in consideration of the main problem: the causes of early and late wood formation in the larch.

METHODS

Experiments were carried out on the Polish larch plants grown in greenhouse under continuous illumination till the day when they were submitted to some other photoperiodic conditions. Two groups of plants were used: 1) young seedlings from seeds sown in 1300 cm^3 pots, and 2) 1-year old plants from nursery, potted in Wagner's pots. The entire plant material (seeds) originated from the same natural stand of *Larix polonica* Rac. in Bliżyn similarly as those used formerly in investigations carried out in natural conditions.

The additional light in greenhouse was provided by a system of 300 watt incandescent „TELAM" bulbs placed 1 meter distance from each other and about 50 cm above the tops of plants. Light intensity at the plant level during night hours was 1200—1500 lux. Plants were also illuminated by the electric light when the day was cloudy and on the early mornings or late evenings. The exact data concerning light and temperature conditions in the same greenhouse have already been published by Żelawski (1957).

Short day treatment (12 hours night) was generally obtained by putting out the light on one side of the greenhouse and drawing the light-proof curtains. When necessary, dark conditions or various intensity light conditions were gained by using paper boxes of 36 × 80 × 36 cm dimensions. The boxes providing various intensity of light or short day conditions were made of a different number of sheets of white pellucid or light-proof paper. Control series of plants were exposed to continuous illumination during the whole time of experiments.

The means of minimal and maximal air temperatures in the greenhouse during the winter and spring months of 1958 were 18.6°C and 28.6°C, respectively. Under the box in the same time: 19,3°C and 27.9°C, respectively. The air moisture amplitude was on the average 60—78% in the greenhouse, and 67—80% under the box. During the 1959 experiments, the mean day air temperature was 22.6°C and mean night temperature: 19.8°C, but the amplitude was smaller than in the previous year. Taking into account that the deviations of temperature and moisture of air were not significant and rather regular it may be assumed that the results of experiments are due to changes in photoperiodic treatment.

For anatomical examination, plants were killed and preserved in ethyl alcohol. Transversal sections from the basal part of all the examined plants were cut by hand. The method of preparation and staining with safranin and light green was the same as that already used earlier in investigations of plants growing in natural conditions (W o d z i c k i 1960).

All measurements of tracheids were done on the one transversal section of several plants in each experimental series. The radial diameter (between two neighbouring tangential middle lammelas) and lumen of tracheids were measured. The thickness of cell wall was calculated as the difference of radial diameter and lumen of tracheids. Thus, the cell wall thickness of each tracheid is presented as the total thickness of two tangential cell walls.

RESULTS

Experiment 1. Wood formation under short day conditions

The plants had been grown for 42 days under continuous illumination since germination, and then 2/3 of seedlings were exposed to short photoperiods (12 hours night) for 20 consecutive days. At the end of this period, a part of the short day treated plants were transferred back under continuous light and the other part was left under short day treatment for the next 20 days. One series (1/3) of plants remained under continuous light from the beginning to the end of experiment. Samples (each of 7 plants) were taken from each series in 4 days intervals. Radial diameter and cell wall thickness of the last two fully differentiated tracheids (recently formed) were examined and the number of tracheids along each of four examined radiuses (perpendicular each other) was determined on transversal sections of the stem.

Under continuous light, the plants showed uninterrupted height growth till the end of experiment. All the plants exposed to short day treatment ceased to grow and formed a resting-bud * at the top of the shoot after 20 days. This resting bud began to develop after the next 20 days but only when the plants had been transferred back to continuous illumination again.

Anatomical examination showed that greater cell wall thickness of the last fully formed tracheids might be observed not earlier than after 20 — 24 days from the beginning of short-day treatment (Table 1 and Fig. 1).

During the next 16 — 20 days (following the first 20 days of short photoperiod) the cell wall thickness of the newly formed-tracheids was also greater, independently whether the plants were still exposed to short

* Resting bud formation was observed externally by means of the bud scales formation instead of the needles.

Table 1

Cell wall thickness at different times of photoperiodic treatment (microns)

upper row — last but one tracheid in radius
lower row — last tracheid in radius

Means of 28 measurements

Number of days from the beginning of changed photoperiodic treatment										
0	4	8	12	16	20	24	28	32	36	40
			*CL**					*CL*		
2.6					3.1			3.1		3.1
2.7					3.1			3.0		3.1
			*SD***					*CL*		
			2.7	2.8	3.3	3.4	3.9	5.2	4.8	4.3
			2.8	3.0	3.8	4.4	4.9	5.2	4.8	3.7
			SD					*SD*		
						3.5		4.7		5.0
						4.5		4.7		4.6
μt***								0.4		

* *CL* — continuous light. ** *SD* — short day. *** Statistical computations by Snedecor's method, *t* — test at 5 percent level.

day treatment or were transferred back under continuous light. In that last series some thinner cell wall of tracheids were observed at the end of this 20 days continuous illumination (Fig. 1 B).

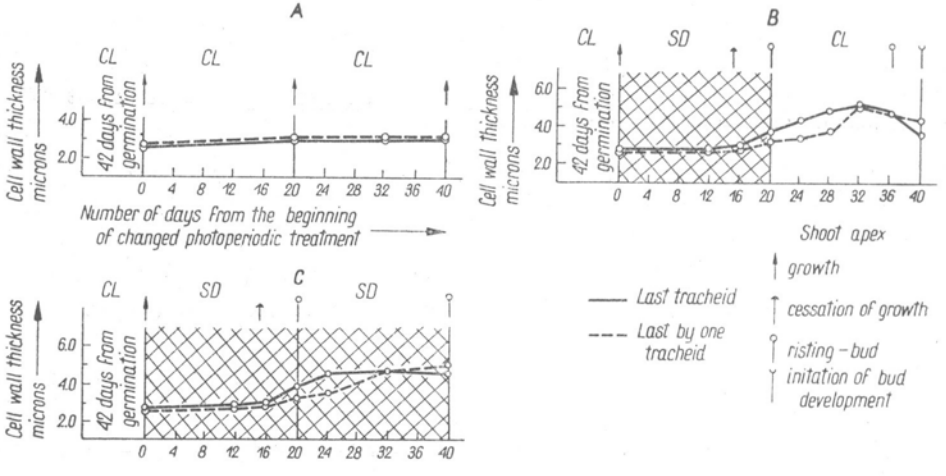

Fig. 1. Cell wall thickness of tracheids at different times of photoperiodic treatment *A* — Control series; *B, C* — Experimental series (*CL* — continuous light conditions, *SD* — short day conditions)

Table 2

Radial diameter of tracheids at different times of photoperiodic treatment
Means of 28 measurements (microns)

upper row — last but one tracheid in radius
lower row — last tracheid in radius

Number of days from the beginning of changed photoperiodic treatment										
0	4	8	12	16	20	24	28	32	36	40
			CL					*CL*		
10.0					10.1			8.9		9.2
9.6					9.5			9.3		9.1
			SD					*CL*		
			9.2	9.1	8.9	9.3	8.5	9.3	9.3	8.1
			8.0	8.8	9.0	8.0	8.4	8.8	8.3	7.4
			SD					*SD*		
						9.7		9.0		9.0
						9.0		8.1		8.3

Control plants growing all the time under continuous illumination formed only the thin-walled tracheids (Fig. 1 A). Thus, it is clear that the final effect of daylength on cell wall thickening may be observed not earlier than about 20 days after the photoperiodic treatment was changed, i.e. immediately after resting-bud formation.

In this experiment no influence of photoperiodic conditions on the radial diameter of tracheids was observed (Table 2).

The number of tracheids in radial direction (from pith to cambial zone) was not significantly lower in plants that had received 20 or 40 short days than in the plants subejcted all the time to continuous illumination (Table 3). But it should be kept in mind that in the plants grown

Table 3

Number of tracheids along radiuses at different times of photoperiodic treatment
Means of 28 radiuses

Number of days from beginning of changed photoperiodic treatment										
0	4	8	12	16	20	24	28	32	36	40
			CL					*CL*		
6.0					8.5			10.4		11.5
			SD					*CL*		
			7.4	6.8	9.2	8.2	8.8	9.9	9.5	10.1
			SD					*SD*		
						9.1		9.1		9.7
μt								0.7		0.6

under uninterrupted illumination an active cambium and a few layers of growing and differentiating cells in the cambial zone could be observed. On the other hand in the plants that had been during 40 days subjected to short-day conditions the typical symptoms of dormancy of the cambium were found.

In order to obtain more information about the nature of the influence of illumination on the wood formation the three following experiments were carried out. Plants used in these experiments grew 52 days under continuous illumination from germination till the beginning of changed photoperiodic treatment.

Experiment 2. Effect of different intensity of additional light

The plants were divided into 5 series each consisting of 60 seedlings. 4 series were exposed to one of the following light conditions during the 12 night hours as a supplement of 12 hours daylight:

a) about 1300 lux (as formerly)
b) about 500 lux
c) about 200 lux
d) about 10 lux

Thus, these 4 series of plants grew under continuous illumination of different intensity of light during night hours.

The 5th series of plants e) was exposed to short day conditions (darkness during 12 hours at night).

After 20 days of such treatment all the plants were transferred for the next 36 days under continuous illumination (of about 1300 lux at night) to enable further differentiation of the cells in cambial zone.

In order to estimate the fresh and dry weight of the plants and the number of needles, a part of the plants was harvested directly before and the other part after 20 days of different additional illumination. For anatomical examination, plants were once more harvested at the end of experiment. Each series comprised 8 seedlings which were examined at the basal part of the stem (just below the cotyledonary node).

All the tracheids of each examined plant were counted along 4 radiuses perpendicular to each other and radial diameter and cell wall thickness of all the tracheids were measured. Thus, the wood formed during the whole period of life of plants in each series were characterized by 32 rows of figures. One of the rows of figures gained by measurements of tracheids in plants that had been exposed to short day conditions is presented below, for illustration of the method:

	11*	14	15	15	12	17	15	13	15	14	13	18	20	18	19	20	20	
pith																		cambium
	3**	4	3	3	3	5	5	7	10	9	8	8	7	4	5	5	3	
					I					II							III	

(values in micrometer units; one micrometer unit — 0.67 microns)

* Radial diameter of tracheids.

** Cell wall thickness of tracheids.

Wood that had been formed during the first period of growth under uniform conditions of uninterrupted illumination (part I of radius), was characterized in all series by the values of radial diameter and cell wall thickness of the 3-rd and 4th tracheids from the pith. The ring of thick-walled tracheids was observed in some series. This zone of the radius was distinguished as part II of radius. Tracheids with cell wall thickness above 4 μ * were icluded in it.

For characterizing the wood of part II of the radius, the sizes of two central-situated tracheids of this zone were taken into account.

In the series where the thick-walled tracheids did not occur, the cell wall thickness and radial diameter along the radius were significantly uniform. For comparison however, two tracheids were distinguished in „part II" of the radius in these plants. As criterion for the distinction of these tracheids served their position analogical to the position of thick-walled tracheids in other series.

The wood formed in the last period of experiment was characterised in all plants of all series by the sizes of the last two fully differentiated tracheids (nearest the cambial zone). This wood was distinguished as part III of the radius.

Plants exposed to continuous illumination, independently of the intensity of additional light at night grew in height continuously till the end of experiment. The plants that had received short photoperiods, began to form after 16 — 18 days the resting-buds at the top of shoots. These buds developed in the next 19 — 28 days after the plants had been

Table 4

Characteristic of plants before and after experimental treatment with additional light of differen intensity during night hours

Series	Number of tracheids along the radius	Number of needles per 1 pot (5 seedlings)		Dry weight in mg per 1 pot (5 seedlings)			
				aeriel part of plants		roots	
		before	after	before	after	before	after
		experimental treatment					
SD plus 1300 lux	8.3	112	202	64	162	27	68
SD plus 500 lux			216		178		55
SD plus 200 lux			210		178		55
SD plus 10 lux			205		145		52
SD	8.1		146		118		62
μt			23		16		—

brought back under continuous light. In all the series, excluding short day treatment, the number of needles formed during the experimental treatment was the same (Table 4). The differences in dry weight increment

* The value of 4 μ was assumed as the limit of cell wall thickness of early and late wood tracheids in one-year old larch seedlings (Budkiewicz 1956; Molski, Żelawski 1958).

among these series were not significant, although some lower dry weight of plants was observed in the series which received 10 lux additional illumination. Only the dry weight of plants exposed to short days was significantly lower.

From the data summarized in Table 5 it can be seen that the plants of the series receiving 1300 lux additional light did not form any thick-walled tracheids. Mean cell wall thickness of tracheids in part II of the radius was greater, as the intensity of additional light was lower. The most thickened tracheidal cell walls were formed by the plants which received 20 short photoperiods. A similar relationship could be observed also in the number of thick-walled tracheids, although the differences were not so pronounced among the individual series and in the plants exposed to 500 lux additional light no separate zone of thickwalled tracheids could be distinguished.

Table 5

Effect of the various intensity additional light on the wood formation

Series	Number of tracheids in the				Cell wall thickness in the				Radial diameter of trach. in the			
	I	II	III*	whole radius	I	II	III	μt	I	II	III	μt
	part of radius				part of radius				part of radius			
					microns				microns			
SD plus 1300 lux				14.6	2.7	3.3	3.4	—	9.6	10.5	11.8	0.6
SD plus 500 lux				14.6	2.5	3.7	3.7	—	10.9	11.4	12.4	—
SD plus 200 lux	9.2	2.5	3.7	15.4	2.7	4.5	3.4	0.2	10 8	11 9	12.2	—
SD plus 10 lux	8.9	3.8	2 6	15.3	2.6	5.0	3.8	—	9.9	11.8	12.6	—
SD	7.0	5.4	3.9	16.3	2.7	5.8	2.9	—	10.2	10.3	13.4	—
μt	—	1.26	—	—	—	0.3	—	—	0.5	—	—	—

Average values based on 64 measurements

* See text.

Differences between the mean diameter of tracheids of part I of the radius are significant. This variability renders a comparison of mean radial diameter of tracheids of the other parts of the radius difficult. Nevertheless, it may be seen that radial diameter of tracheids representing part II of the radius mostly did not differ significantly in individual series.

Experiment 3. Effect of daylength

Experiment was carried out simultaneously with the experiments 2 and 4. Two series of plants were exposed to photoperiods of 15 or 17 hours, respectively during 20 days.

Table 6

Characteristic of plants before and after experimental treatment with different daylength

Series	Number of tracheids along the radius	Number of needles per 1 pot (5 seedlings)		Dry weight in mg per 1 pot (5 seedlings)			
				aerial part of plants		roots	
		before	after	before	after	before	after
		experimental treatment					
Continuous light*	8.3	112	202±7	64	162	27	68
17 hrs. light a day			172±6		165		74
15 hrs. light a day			178±9		163		67
12 hrs. light a day*	8.1		146±6		118		62

* Control series as in the previous experiment.

Plants under 15 hours illumination had formed resting-buds after 16 — 18 days. These buds began to develop in 12 — 15 days after the plants were transferred back to continuous light. Under 17 hours photoperiods the resting buds were formed in 16—21 days but 5 plants (of 56) did not form the bud at all — and grew continuously. The resting buds were not so well formed as under the 15 hours photoperiod and started to develop almost 9 days after the plants were brought back under continuous illumination.

Table 7

Effect of day-length on the wood formation

Series	Number of tracheids in the				Cell wall thickness of tracheids in the			Radial diameter of tracheids in the		
	I	II	III	whole radius	I	II	III	I	II	III
	part of radius				part of radius			part of radius		
					microns			microns		
Cont. light				14.6	2.7*	3.3	3.4	9.6	10.5	11.8
17 hrs. light a day	7.2	3.4	4.3	14.9	2.7	5.2	3.4	10.3	11.0	12.3
15 hrs. light a day	7.1	4.9	4.4	16.4	2.7	5.7	3.0	11.0	11.8	14.1
12 hrs. light a day	7.0	5.4	3.9	16.3	2.7	5.8	2.9	10.2	10.3	13,4
μt	—	1.2	—	—	—	0.3	—	—	—	—

* Mean values based on 64 measurements.

Data presented in Table 6 shows that number of needles in the plants of these two series was lower than of plants grown under uninterrupted light, while it was higher than that of plants exposed to short (12 hours) photoperiods. In spite of some differences in the number of needles, the dry weight of the plants in these two series and the plants grown

under continuous illumination were the same, but markedly higher than that of plants under short photoperiod.

The influence of 15 and 17 hours photoperiods on the formation of thick-walled tracheids is presented in Table 7. As may be seen, the cell wall thickness of tracheids and especially their number was lower under a 17 hours photoperiod than under 15 and 12 hours and the cell wall thickness in the latter two series did not differ significantly.

As formerly, no significant differences were observed in the mean dimater of tracheids in part II of radius of individual experimental series.

Experiment 4. Effect of alternating short cycles of light and darkness

Four series of plants were exposed to one of the following photoperiods, respectively:

a) 12 hours light — 12 hours darkness
b) 6 hours light — 6 hours darkness (twice a day)
c) 2 hours light — 2 hours darkness (six times a day)
d) continuous illumination: daylight supplemented by artificial light (about 1300 lux from sunset till sunrise).

Thus, the total number of hours of light and darkness in the 3 first series was the same. The time of covering the plants was arranged so that all the plants of these series received the same number of hours of daylight and artificial light daily.

Plants exposed to the 12 hours photoperiods formed resting-buds after 15 — 18 days. These buds developed 15 — 28 days after the plants had been transferred back under continuous illumination. The plants under the 3 remaining photoperiods continued to grow. Only under 6 hours cycles of light and darkness, 3 plants (of 67) formed resting-buds after 20 days of such conditions, and after the next 7 days (when the plants were transferred back to continuous illumination) two other plants formed the resting-bud. One of these buds began to develop 2 days after it had been formed, but the others developed only after 10 to 20 days.

In the "6+6" and "2+2" series there were obviously lower numbers of needles and particularly dry weight of the plants were visibly lower as compared with those which had received continuous illumination (Table 8). However, the dry weight of plants exposed to "6+6" and "2+2" treatment was not greater than of those exposed to a 12 hours photoperiod, although the latter had the lowest number of needles.

As in former experiments, the plants under continuous illumination formed only thin-walled tracheids, and thick-walled tracheids were formed under short day (12 hours) conditions (Table 9). Plants that had received "6+6" treatment formed fewer thick-walled tracheids, although the cell wall thickness was the same as under short photoperiod. Plants

Table 8

Characteristic of plants before and after experimental treatment with short cycles of light and darkness

Series	Number of tracheids along the radius	Number of needles per 1 pot (5 seedlings)		Dry weight in mg per 1 pot (5 seedlings)			
				aerial part of plants		roots	
		before	after	before	after	before	after
		experimental treatment					
12 hours light +12 hours darkness	6.7		160		121		36
6 hours light +6 hours darkness			178		119		31
2 hours light +2 hours darkness			191		124		39
Continuous light	7.1	117	225	71	193	29	73
μt			23				

Table 9

Effect of short light and dark cycles on the wood formation

Series	Number of tracheids in the				Cell wall thickness in the				Radial diameter of trach. in the			
	I	II	III	whole	I	II	III	μt	I	II	III	μt
	part of radius			radius	part of radius				part of radius			
					microns				microns			
12 hours light +12 hours darkness	7.1	4.6	4.0	15.7	2.9*	5.8	3.1	—	10.0	10.6	11.7	—
6 hours light +6 hours darkness	7.5	3.6	3.0	14.1	2.7	5.6	3.3	—	10.3	11,2	10.7	—
2 hours light + 2 hours darkness	7.6	1.9	3.8	13.3	2.7	4.9	3.0	0.2	10.6	11.0	11.7	—
Continuous light				14.3	2.8	3.3	3.5	—	10.9	9.8	9.5	0.6
μt	—	0.9	—	—	—	0.3	—	—	0.6	0.7	—	—

* Mean values based on by 64 measurments

exposed to "2+2" experimental treatment, formed only few if any of the thick-walled tracheids and the cell wall thickness was significantly lower.

Thus, under applied conditions of illumination not a total sum of light hours, but the suitable length of light and dark periods was an essential factor affecting the formation of thick-walled tracheids.

Table 10

Mean values of cell wall thickness and radial diameter of tracheids in the second part of radius in the experimental series in which distinclty thickened cell walls occurred

Experimental series	Cell wall thickness		Radial diameter of tracheids of the	
	whole II part of radius	2 central tracheids of the II part of radius	whole II part of radius	2 central tracheids of the II part of radius
	microns			
Experiment 2				
Short day	5.6	5.8	10.7	10.3
Short day+10 lux additional light (continuous illum.)	5.2	5.0	11.8	11.8
Experiment 3				
17 hours light a day	5.2	5.2	11.1	11.0
15 hours light a day	5.8	5.7	12.0	11.8
Experiment 4				
Short day	5.8	5.8	10.7	10.6
6 hours light+6 hours darkness twice a day	5.6	5.6	11.2	11.2

Table 11

Mean values of the cell wall thickness and the radial diameter of tracheids in the whole radius and of 2 chosen cells as representative of the part II of radius (see text)

Experimental series	Cell wall thickness		Radial diameter of tracheids of the	
	whole radius	2 chosen tracheids as representative of the II part of radius	whole radius	2 chosen tracheids as representative of the II part of radius
	microns			
Experiments 2 *and* 3				
Continuous illumination	3.1	3.3	10.2	10.5
Experiment 4				
Continuous illumination	3.1	3.3	10.2	9.8

In order to verify the manner of characterization of wood in part II of the radius (corresponding to the period of experimental photoperiodic treatment), mean values of cell wall thickness and radial diameter of all tracheids in this zone and mean values of the two chosen, central tracheids of this zone were compared (Table 10). Moreover, in the Table 11 mean values of cell wall thickness and radial diameter of tracheids along the whole radiuses and mean values of two chosen tracheids in control series of plants exposed to continuous illumination have also been compared. It is clear from these comparisons that the characteristic of wood in part II of the radius on the basis of the average size of two centrally situated tracheids in this zone is quite satisfactory.

Experiment 5. Different photoperiodic treatment of various part of the plant

The results of experiment 2 and others confirmed the earlier known fact that formation of thick-walled wood may proceed during extension growth (Żelawski 1957). The author's intention at present was to establish whether the effect of light conditions on the cell wall thickening is independent from the shoot apex behaviour also when the extension growth of the shoot has ceased and when all other parts of the plant except the shoot apex are submitted to uninterrupted illumination. In this way the locus of photoperiodic stimulus perception for these two reactions could be ascertained.

1-year old plants of *Larix polonica* (Rac.) were used in this experiment. After 62 days of continuous growth under uninterrupted illumination (1300 lux at night), the plants were divided into 6 series, each consisting of 4 plants, and submitted to one of the following experimental treatments:

a. plants under continuous illumination (about 1300 lux at night), only the shoot apex covered for 12 night hours daily. Tops of lateral shoots inctact.

b. plants under continuous illumination, only the shoot apex covered (together with young growing needles) for 12 night hours daily. Tops of lateral shoots removed.

c. Whole plants under continuous illumination. Tops of lateral shoots intact.

d. Whole plants under continuous illumination, Tops of lateral shoots removed.

e. Whole plants under short day conditions (12 hours darkness). Tops of lateral shoots intact.

f. Whole plants under short day conditions. Tops of lateral shoots removed.

The tops of main shoots were covered with 40 mm × 40 mm × 36 mm boxes made of light-proof photographic paper — black on one side and silver on the other. These boxes of the shape of a reversed truncated pyramid could easily hold the apical part of the shoot together with a bundle of young growing needles, which every time were slipped into them through the outlet about 0.5 cm^2. After the top of shoot was covered, the outlet was loosely tightened with dark cotton.

In order to prevent the boxes weighing down on the shoots, they were hung on wooden props.

During 20 days of the above-mentioned treatment all the newly appearing buds in the three decapitated series of plants were systematically removed at a quite early stage of development. After this period all the plants were transferred back under continuous illumination for the next 20 days and then harvested for anatomical examination.

All the plants of the two series where the top of the main shoot was covered (*a* and *b*) formed resting-buds after 17 to 20 days. The lateral shoots of series *a*, where the tops of lateral shoots remained intact, continued growth. Resting-buds formed in plants of the two short day series (*e* and *f*) in the same time as in the first ones. The lateral shoots in series *e*, (where they were not decapitated) also formed resting-buds in these conditions.

Plants exposed to continuous illumination (series *c* and *d*) grew during all the time of experiment.

At the end of the experiment, i.e. 20 days after the plants were transferred back to continuous illumination, the resting-bud on the main shoot of three plants of series *b* (where the tops of lateral shoots had been removed), began to develop rapidly. The bud in the fourth plant was swollen. Resting-buds on the main shoot of plants of series *a*, however, (where the lateral shoots were not decapitated) remained undeveloped in this time and only the lateral buds in the angles of needles on the main shoot began to grow. Similar behaviour of plants was observed when they had received short photoperiods before they were transferred back to continuous light. Where the lateral shoots were decapitated (series *f*) the apical bud on the main shoot of two plants began to develop after 20 days of continuous illumination and the apical buds of two other plants were significantly swollen. In series *e*, where the lateral shoots were intact, only a beginning of development of buds on the lowest lateral shoots but not the buds on the main shoot was observed.

Anatomical examinations were carried out on transversal sections of the basal part of the stem. Radial diameter and cell wall thickness of the last ten tracheids (from cambial zone towards the pith) were measured along 4 radiuses perpendicular to each other. It was decided to measure only the ten last fully differentiated tracheids after ascertaining that into these limits the thick-walled wood had been formed in control plants exposed to short day treatment (Table 12).

It is seen from Table 12 and Fig. 2 that cell wall thickness of tracheids did not change when the top of the main shoot was covered and its extension growth ceased both in partly decapitated and intact plants. There was a marked concurrence of results of measurements of cell wall thickness of tracheids in series *a*, *b*, *c* and *d* where the plants had been exposed to continuous illumination during all the time of experiment.

Table 12

Experiment with top of main shoot covered. Cell wall thickness of 10 last, successive tracheids in radial direction from cambial zone. (microns)

Successive tracheids from cambial zone in radial direction	Experimental series					
	Continuous illumination				Short day	
	*a**	*b*	*c*	*d*	*e*	*f*
10	2.9	2.7	3.0	3.0	3.2	2.8
9	3.0	2.9	2.9	3.1	4.0	2.8
8	3.0	2.9	2.9	3.1	5.1	2.9
7	3.0	3.1	2.9	2.9	5.2	3.0
6	2.9	3.0	3.0	3.1	5.1	3.0
5	3.0	2.9	3.1	2.9	4.2	3.2
4	2.9	2.9	2.9	2.9	3.6	3.8
3	3.1	2.9	2.7	2.9	3.7	4.5
2	3.0	3.0	2.9	3.1	3.2	4.8
1	3.1	3.1	2.9	3.0	3.1	3.9
Average**	3.0	2.9	2.9	3.0		
μt					0.54	

.* See text.

** Each average based on 160 measurements.

The plants which had received 20 short photoperiods, however, had formed a distinct zone of thick-walled tracheids. Besides, in the plants where the lateral shoots remained intact (series *e*), thin-walled tracheids

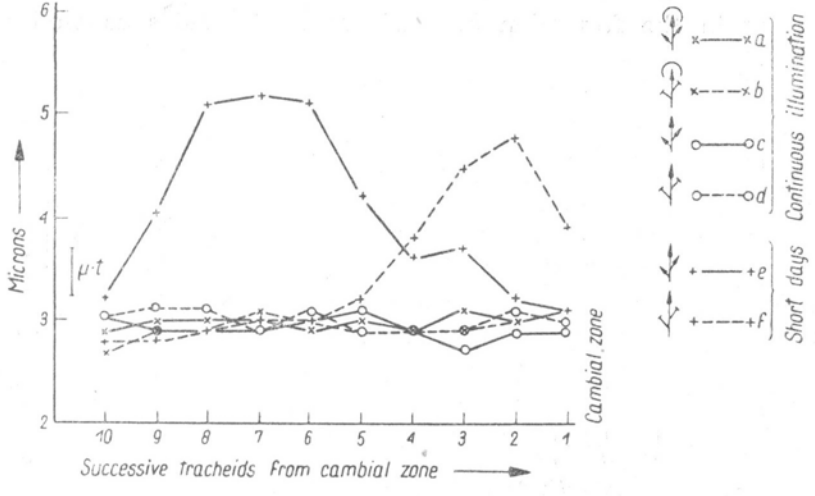

Fig. 2. Experiment with top of main shoot covered. Cell wall thickness of 10 last, successive tracheids in radial direction from cambial zone. Description of experimental series: *a*, *b*, *c*, *d*, *e*, *f* — see text. (Mean values based on 16 measurements)

Table 13

Experiment with top of main shoot covered. Radial diameter of 10 last, successive tracheids in radial direction from cambial zone (microns)

Successive tracheids from cambial zone in radial direction	Experimental series					
	Continuous illumination				Short day	
	*a**	*b*	*c*	*d*	*e*	*f*
10	15.7	14.7	18.0	13.5	13.5	14.3
9	15.0	15.7	18.7	13.8	12.8	14.1
8	17.2	14.8	19.4	14.9	12.3	14.9
7	16.7	14.4	17.5	14.3	12.5	16.5
6	16.5	14.5	19.4	14.9	10.9	15.1
5	16.3	14.9	19.7	14.5	11.2	13.5
4	17.8	15.4	19.2	15.2	15.1	13.5
3	17.0	14.2	17.8	14.7	15.9	12.0
2	18.0	14.1	20.0	15.3	18.1	10.9
1	18.5	15.7	19.0	16.7	19.3	13.1
μt				2.3		
Average**	16.9	14.9	18.9	14.8		
μt	0.6					

* See text.

** Each severage based on 160 measurements.

began to form as a result of replacing the plants under continuous illumination. Where the lateral shoots were decapitated, only 1 to 2 of the last formed tracheids had somewhat thinner walls. This might be caused by a delay in the formation of thick-walled tracheids as well as by

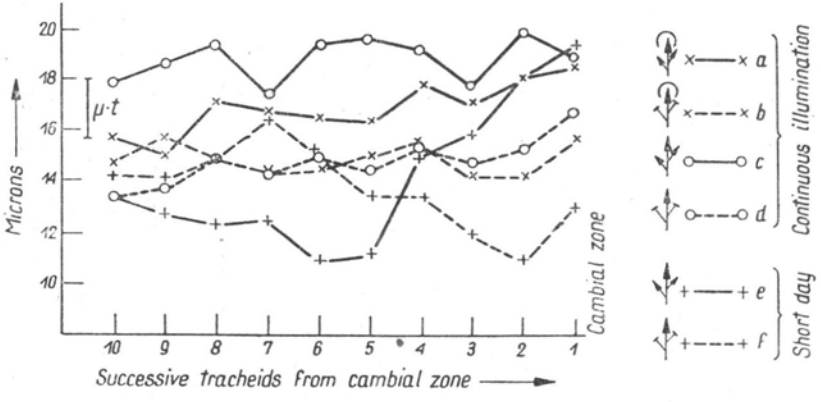

Fig. 3. Experiment with top of shoot covered. Radial diameter of 10 last, successive tracheids in radial direction cambial zone. Description of experimental series: *a*, *b*, *c*, *d*, *e*, *f* — see text. (Mean values based on 16 measurements)

an inhibition of new thin-walled tracheids. formation (as the result of decapitation of the lateral shoots).

The data presented in Table 13 and Fig. 3 show that the radial diameters of the tracheids in decapitated plants or in plants exposed to short day conditions were considerably smaller. Some little decrease of the radial diameter of tracheids was also observed in the plants of series *a*, where only the top of the main shoot was covered but the lateral shoots remained intact under continuous illumination.

The strong accordance of the results in this experiment leads us to believe that these differences observed in the thickness of cell walls and radial diameter of tracheids among the various series of plants are very probably due to experimental treatment, although only 4 plants were used in each series.

DISCUSSION

It has been shown that changes of the cell wall thickness of tracheids under the influence of alternation of photoperiodic conditions may be observed not earlier than after 20 days of experimental photoperiodic treatment. 2 to 3 new thin-walled tracheids were still formed during the 20 days following the beginning of short day treatment. The thick-walled tracheids were formed during the next 16 to 20 days after the plants had already been transferred back to continuous illumination. This fact, probably indicates an inductive character of the reaction or the photoperiodic effect concerned only the celles that were in an early stage of differentiation in the cambial zone.

The inductive character of the photoperiodic reaction is known generally to involve a qualitative change of morphological differentiation of the shoot apex (flowering, resting-bud formation etc.). Cell wall thickening or the change of radial diameter of tracheids under the influence of some photoperiodic conditions, however, may be treated rather as a typical quantitative reaction. This character of reaction has also been shown in experiments where the additional light of low intensity or long photoperiods or short cycles of light and darkness were used. The cell wall thickness of tracheids was always smaller, if the experimental treatment differed widely from the optimal conditions for thick-walled tracheids formation (found to be about 12 hours light and darkness daily). Moreover, a greater number of thin-walled tracheids had formed up to the time when the thick-walled tracheids began to form under such conditions (most widely differing from 12 hours day conditions, especially when additional light of 10 and 200 lux intensity, "2+2"

light-darkness, and 17 hours daylength were applied). In other words, under such conditions thick-walled tracheids began to form later after the beginning of the experimental treatment than under short photoperiod. Thus it may be supposed that some inductive period is required for the initiation of the change in wood formation. This supposition is corroborated by the fact that the thick-walled tracheids are still fermed after the plants had been replaced under continuous illumination and that there were only few or no tracheids of intermediate cell wall thickness between zones of the thin and thick-walled tracheids. Thus, the reaction bringing about the cell wall thickening of tracheids as the result of changed light conditions had probably both a quantitative and inductive character. This problem requires however further study.

Unfortunately the present experiments could not provide any conclusions on the yield of photosynthesis upon the varying light conditions which were applied. The question requires also further investigation but it may be seen from the results of experiment in which dry weight was measured that there was no relationship between dry weight increment, number of needles and cell wall thickness of tracheids. Nevertheless it is of interest that the dry weight of plants which had been subjected to 15, 17 and 24 hours photoperiods during 20 days, was similar.

These experiments support the recently revealed fact (Żelawski 1957) that thick-walled tracheid formation is not dependent on the photoperiodic reaction of the shoot apex of larch seedlings and inhibition of extension growth. Żelawski has shown that thick-walled tracheids may be formed during uninterrupted extension growth of the shoot apex under low intensity additional light (as described earlier). It has now been shown that also the thin-walled tracheids may be formed even when the extension growth of the shoot has ceased (in experiment where only the top of main shoot was covered). This experiment disclosed, moreover, the so far unknown fact that also locus of perception of the photoperiodic stimulus is different in these two reactions. Most important for the processes bringing about cell wall thickening was the kind of photoperiodic treatment to which full grown needles were subjected. These facts seem to show that there is no direct dependence between extension growth of shoots and cell wall thickening of tracheids in larch seedlings.

Nevertheless it was shown that cambial activity (i.e. division of cambial cells) may cease after some period of short day treatment. In this respect results are in accordance with the observations of other authors (Wareing 1951, Wareing, Roberts 1956) concerning *Pinus* and *Robinia*. It is still not clear, however, whether the inhibition of cambial activity as a result of short day influence was induced directly through the needles or mature leaves (as Wareing suggested) or was due to

the cessation of extension growth and resting-bud formation which occurred earlier in the present investigation.

The results of experiments concerning the influence of photoperiodic conditions on the radial diameter of tracheids were different in seedlings and in 1-year-old plants. This influence was observed only in older plants where the mean diameter of tracheids was larger. This divergence may probably be due to the difference between the mean sizes of radial diameter of tracheids in younger and older plants. The effect of photoperiodic treatment on the radial diameter of tracheids may supposedly be observed only when the mean diameter of tracheids is sufficiently large. The mean diameter of tracheids increases gradually with the age of woody plants in their youth. Wichrow 1949 has also shown that the difference between the radial diameter of tracheids of early and late wood increases in the individual annual rings, as the width of the rings increases with the age of the plant. This fact was also shown in the first part of present investigation (Wodzicki 1960). Distinct difference between the size of radial diameter of tracheids of early and late wood might be observed only in older plants where the mean width of the annual ring and the mean radial diameter of tracheids are greater.

Taking all this into account it may be concluded that the changes of radial diameter of tracheids observed under continuous light and short day conditions in older plants was due to the influence of the photoperiodic factor on the radial diameter of tracheids.

Some decrease of the radial diameter of tracheids was observed when the top of the main shoot only was subjected to short day conditions while the lateral shoots remained intact. A visible decrease of the radial diameter of tracheids was moreover observed after decapitation of the lateral shoots. Simultaneously no change of cell wall thickness of tracheids as the result of decapitation or shoot apex covering was observed. This fact may suggest that the radial diameter of tracheids is dependent on the extension growth of shoots, contrary to the cell wall thickening.

Additional evidence that the processes of radial diameter growth and of cell wall thickening of tracheids were not controlled by the same reaction of plant to photoperiodic conditions seems to be provided by the fact that the decrease of radial diameter of tracheids was observed somewhat later than cell wall thickening. This delay of the decrease of radial diameter of tracheids as compared with thick-walled tracheids formation had been also observed earlier at the beginning of late wood formation in plants grown under natural conditions (Wodzicki 1960).

Results of the present experiments are insufficient for establishing, whether the influence of the shoot apex on the radial diameter growth of tracheids was direct on growing cells in the cambial zone or intermediate through its effect on the activity of cambium. Some light is

shed on the latter supposition by the fact that the radial diameter of the last few layers of tracheids in the annual ring in plants grown under natural conditions decreases gradually i.e. the most flattened tracheids are situated nearest the cambium, (a fact known also in other woody plants (Sokołowski 1927). This pattern of radial diameter is specific for cells growing after division in the cambial zone. Therefore, the gradual decrease of radial diameter of the last layers of tracheids towards the cambium may be understood as the result of simultaneous cessation of radial growth of all cell layers in the cambial zone (or first of those neighbouring the cambium). For the elucidation of this question further investigations of the influence of the apical growth on the cambial activity during the vegetation season are required.

The present experiments provide also some new observations concerning the photoperiodic reaction of the shoot apex. It has been shown that second-year larch plants may be induced to resting-bud formation as result of 20 short days treatment and to a second period of extension growth after being transferred again to continuous light. Thus the reaction of older plants is the same as that observed earlier on a few-months-old seedlings of the European larch (Żelawski 1956). Moreover, it was shown that the apex of the main shoot may be induced to dormancy separately by covering only the apical part of the shoot during extension growth of all the lateral shoots. Therefore, the kind of photoperiodic treatment to which were subjected young growing needles or the growth apex itself, was the most important factor, regardless of the photoperiodic treatment to which were submitted other parts of the plant (together with all the other growing apices of lateral shoots).

It is known from Wareing's experiments (1954) that the shoot apex of *Betula pubescens* plants may be also induced to formation of the apical bud by covering only the top of the shoot but it was not clear from Wareing's experiments, if there also occurred some growth of the lateral shoots.

Results of further investigations concerning the influence of extension growth on wood formation in larch seedlings will be published in the next paper of this series.

SUMMARY

The influence of different conditions of illumination on the wood formation and the extension growth of seedlings and 1-year-old plants of larch were studied. It has been observed that resting-bud formation and first signs of the thick-walled tracheids formation occur not erlier than after 20 short photoperiods. Similarly new thin-walled tracheids were observed 16 to 20 days after the plants had been replaced under continuous illumination. It was also observed that under additional light of low intensity during night hours (as supplement to daylight), thick-walled

tracheids were also formed as well as under the alternating short 2 and 6 hours periods of light and darkness, or 15 and 17 hours photoperiods. The smallest cell wall thickness of tracheids, however, and fewest thick-walled tracheids were observed under conditions most widely differing from the 12 hours treatment. No correlation between dry weight, number of needles and thick-walled tracheids formation under different conditions of illumination has been demonstrated. The influence of photoperiods on radial diameter of tracheids was observed in 1-year-old plants. Simultaneously no influence of the cessation of main shoot extension growth on the thickening of cell walls of tracheids were detected. Moreover, it was revealed that the apex of the main shoot could be induced to dormancy, when only the top of the shoot was covered, while all the lateral shoots went on growing under continuous illumination.

The results are discussed.

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