ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XXXIV — nr 1
1965

Annual ring of wood formation and seasonal changes of natural growth-inhibitors in larch

T. J. WODZICKI

The investigation performed by Wareing and Roberts (1956) on *Robinia pseudacacia* led authors to the conclusion that photoperiodic control of cambial activity depend on the light conditions to which the mature leaves are subjected. Similarly, it was found in later experiments with larch (Wodzicki 1961a, 1961b) and spruce (Wodzicki and Witkowska 1961) that photoperiodically-controlled thickening of cell walls of the tracheids depended on the photoperiodic conditions to which the fully grown needles were exposed. These facts substantiated the earlier supposition of Żelawski (1957) and Molski and Żelawski (1958) that thickening of the cell wall of tracheids in larch, and the extension growth of the shoot were probably not related directly. Further experiments on the photoperiodic control of natural growth-substances content and wood formation in larch (Wodzicki 1964) revealed that the formation of thick-walled tracheids was accompanied by accumulation of water-soluble inhibitors (substances which inhibited wheat coleoptile section elongation) in the cortical tissues. On the other hand, photoperiodically-controlled changes in the content of promoters could not be correlated with the thickening of the cell wall of tracheids. The results suggested the possible role of growth-inhibitors in the formation of the secondary wall during the phase of differentiation of tracheids and the probable contribution of this group of substances to the annual ring of wood formation in larch.

If this supposition is correct, a close relation between seasonal changes in growth-inhibitors content in the cortex (the cortical tissues adjacent to the region of wood formation) and the seasonal changes in the type of wood formed in plants growing in natural conditions might be expected. This relation was studied in larch and supplemented by the simultaneous microscopical investigation of the cambial zone because, as found earlier (Wodzicki 1960, 1962, Wodzicki and Peda 1963), seasonal changes in the formation of different types of tracheids in conifers are accompanied by considerable changes in the length of time required for their growth and differentiation as reflected by changes in the width of the respective cell layers in the cambial zone.

MATERIAL AND METHODS

Three-year old *Larix decidua* Mill. trees growing in natural conditions on an experimental plot near the Agricultural College (S.G.G.W.) in Warsaw were examined at different times throughout the year 1963. The dates of plant collection are specified in the subsequent part of the paper. Each time several plants were choosen randomly.

Besides these plants, two-year old larches grown for six weeks under continuous illumination were used. Continuous illumination (about 4500 lux at night) was provided by fluorescent TELAM "white-light" tubes switched on during a 24-hrs diurnal cycle (additionally to daylight during the daytime). This type of light was also provided during the subsequent experimental treatment, except for the plants exposed to short day. The latter conditions were obtained by removal of the plants to a dark chamber for 12 hrs during the night. The temperature regime under the two photoperiodic treatments was kept constant: 23 and 17°C during the day and night hours, respectively in a 24-hrs cycle.

Each time after collection, the plants grown in natural conditions were prepared as follows: roots were cut off and discarded; 2—3 cm long basal part of stems (including the first node) were fixed in ethyl alcohol for anatomical examination; cortical tissues*, removed from the remaining parts of the stem (the last year shoot excepted), fully grown needles, and apical parts (including shoot apex and needles not exceeding 2—3 mm in length) were separated, weighed and immediately frozen at dry ice temperature for further investigation of the growth-controlling substances content. In plants grown under various photoperiods only the cortical tissues were investigated.

The wood and cambial zone were studied on the transverse free-hand sections, stained with safranin and light green and mounted in Canada balsam as advocated by W a r e i n g (1951). Two sections from the stem at and identical level from four plants were investigated every time. Measurement of the radial diameter and cell wall thickness of tracheids was performed according to the method described earlier (W o d z i c k i 1960). The magnification used was 1080 ×. The cambial zone** was examined by determining the number of cells in radial direction (width) across each of the three following layers: cambium, radial diameter

---

* Tissues outside the wood cylinder (periderm, cortex, phloem, cambium and probably part of cells differentiating to xylem elements) which were extracted together are refered to as cortical tissues or shortly cortex in subsequent parts of the paper.

** All the living cells originating from cambial initials being in the process of growth or differentiation to xylem. This definition does not include the cells differentiating to pholoem elements, but it was introduced as a convenient working definition.

growth layer, differentiation layer. The three layers were distinguished as previously described (Wodzicki 1960). Observations were performed under a 2025 × magnification.

Tissues for estimation of the growth-controlling substances were stored at —16°C, or extracted immediately after plant collection. The method of extraction with absolute and 80% aqueous methanol — 1 ml of 80% MeOH/10 mg of fresh weight — was similar to that described by Phillips and Wareing (1958).

Separation of extracts into aqueous-MeOH and ether fractions was performed according to the procedure described previously (Wodzicki 1964). The method was found convenient for removing the resinous material (toxic to the coleoptile sections) to the ether fraction. Known volumes of concentrated aqueous fraction (and in some cases ether fraction) of extracts were strip-loaded on to Whatman's No. 3 and chromatograms were developed by the descending method with isopropanol-ammonia-water (10:1:1 v/v) to 26 cm in darkness at room temperature. The particular R_f strips of chromatograms were eluted for 18 hrs at 0°C with a mixture of phosphate buffer (pH 5.6) and 2% sucrose in Petri dishes 6 cm in diameter, and the eluates were bioassayed.

The wheat coleoptile sections straight growth test was applied after Bentley and Housley (1954). Wheat variety Opolska was used. This variety was selected as providing suitable test material (Wodzicki and Witkowska-Żuk 1964). The initial length of coleoptile sections was 10 mm. In some occasions, the aot coleoptile or first internode sections straight growth tests were followed as described by Nitsch and Nitsch (1956) and Nitsch (1956) with few modifications concerning the incubation method. The aot variety Biały Mazur recommended by Kentzer and Rowicka (1963) was used in these assays.

Methanol and diethyl ether were redistilled and water was double distilled in glass prior to use in any part of the investigation.

The statistical significance of results was tested, when necessary, by Snedecor's variance method, Tukey's Q-test, Student's t-test, or only the standard deviation was computed.

RESULTS

Formation of annual ring of wood

1. Seasonal variation of cell number in the cambial zone

The number of cells in the three layers of the cambial zone (namely: cambium, radial diameter growth layer and differentiation layer) was investigated from mid June to November. Data presented in Table 1 indicate that the width of separate layers (expressed as the number of

Table 1

Number of cells in the cambial zone at different times of the season 1963

Date of plant collection	Cambial zone total	Layer of cambium	Radial diameter growth layer	Differentation layer	Layers of radial diameter growth and differentation, total
June 21	9.5	3.7 ± 0.15	3.1 ± 0.23	2.7 ± 0.28	5.8 ± 0.44
August 1	7.2	3.3 ± 0.15	2.2 ± 0.25	1.7 ± 0.15	3.9 ± 0.38
August 30	12.0	3.8 ± 0.19	4.1 ± 0.25	4.1 ± 0.25	8.2 ± 0.14
September 16	16.1	4.4 ± 0.15	6.3 ± 0.21	5.4 ± 0.22	11.7 ± 0.31
September 30	12.1	3.8 ± 0.16	1.2 ± 0.29	7.1 ± 0.38	8.3 ± 0.16
October 16	7.6	4.7 ± 0.17	0.0	2.9 ± 0.44	2.9 ± 0.44
November 8	5.2	5.2 ± 0.16	0.0	0.0	0.0

cells in radial direction) as well as the width of the whole cambial zone varied markedly during the season. Maximum width of the cambial zone occurred in mid September. It was preceded by a reduction in the number of cells observed at the beginning of August and followed by a gradual decrease to the minimum in November. As it is seen, the former reduction was mainly due to the smaller cell number in the radial diameter growth and differentiation layers, although three was also a detectable tendency to reduction in the cambial layer. The greatest number of cells in the radial diameter growth layer was revealed at mid September, though at the end of August there was also a significant increase. During the last two weeks of September, the number of cells in this layer suddenly diminished and in October the layer of radial diameter growth could no more be distinguished.

The maximum width of differentiation layer was observed at the end of September. However, gradual increase in the cell number of this layer was observed since the end of August. At mid October this layer was still distinguishable, though the number of cells in it was more than twofold reduced. Only the cambial layer could be distinguished at the beginning of November.

2. Seasonal variation in the type of wood formed

Table 2 shows the results of measurement of radial diameter and cell wall thickness of the four most recently formed tracheids (starting from the tracheid closest to the cambial zone in radial direction) on the same transversal sections of the stem of plants investigated for changes in the cambial zone. Each number represents the mean value for 16 tracheids. It may be seen, that the significant increase in cell wall thickness occurred not earlier than at the end of September. A somewhat thickened cell wall was observed, however, in the last tracheid measured in plants collected as early as mid September. This significant increase was evi-

Table 2

Cell wall thickness and radial diameter of tracheids formed at different times of the season 1963

Date of plant collection	Cell wall thickness					Radial diameter					
	1*	2	3	4	F-test**	1*	2	3	4	F-test**	Average
June 21	3.3	3.0	3.0	2.8	-	20.0	20.0	18.7	19.8	-	19.6
August 1	3.2	3.3	3.3	3.3	-	17.2	18.1	19.0	18.9	-	18.3
August 30	3.3	3.3	3.2	3.1	$F_e < F_{0.05}$	18.2	18.8	18.2	18.2	$F_e < F_{0.05}$	18.3
September 16	3.4	3.2	3.3	3.3	$F_e < F_{0.05}$	20.7	21.9	21.3	21.8	$F_e < F_{0.05}$	21.4
September 30	4.2	3.8	3.5	3.1	$F_e > F_{0.05}$	22.2	21.8	23.2	22.0	$F_e < F_{0.05}$	22.3
October 16	4.7	4.6	4.4	4.0	$F_e > F_{0.05}$	15.5	16.2	16.8	17.8	-	16.6
November 8	4.6	4.9	4.6	4.2	-	14.4	16.2	17.6	17.9	-	16.5
F-test**	-	-	-	-		-	-	-	-		$F_e > F_{0.05}$

*Successive tracheids from the cambial zone in radial direction.

**F-test according to Snedecor's method.

denced also by the difference between the cell wall thicknesses of the third and fourth tracheids investigated at the end of September.

Changes in the radial diameter of tracheids followed a different pattern than those of cell wall thickness. The exact moment of change in radial diameter was not established in four tracheids measured, both in plants collected at the end of August and at the end of September. Analysis of variance and Q-test of Tukey (numerical data are not presented) revealed, however, significant differences between the cells formed in June and August. In the plants collected at mid September the diameter of cells was again significantly greater (greater even than that of tracheids formed in June). The radial diameter of tracheids measured at the end of September was greatest, thought the increase was not significantly different from the diameter of tracheids measured at mid September. A very significant decrease in radial diameter of tracheids was found in plants investigated at mid October.

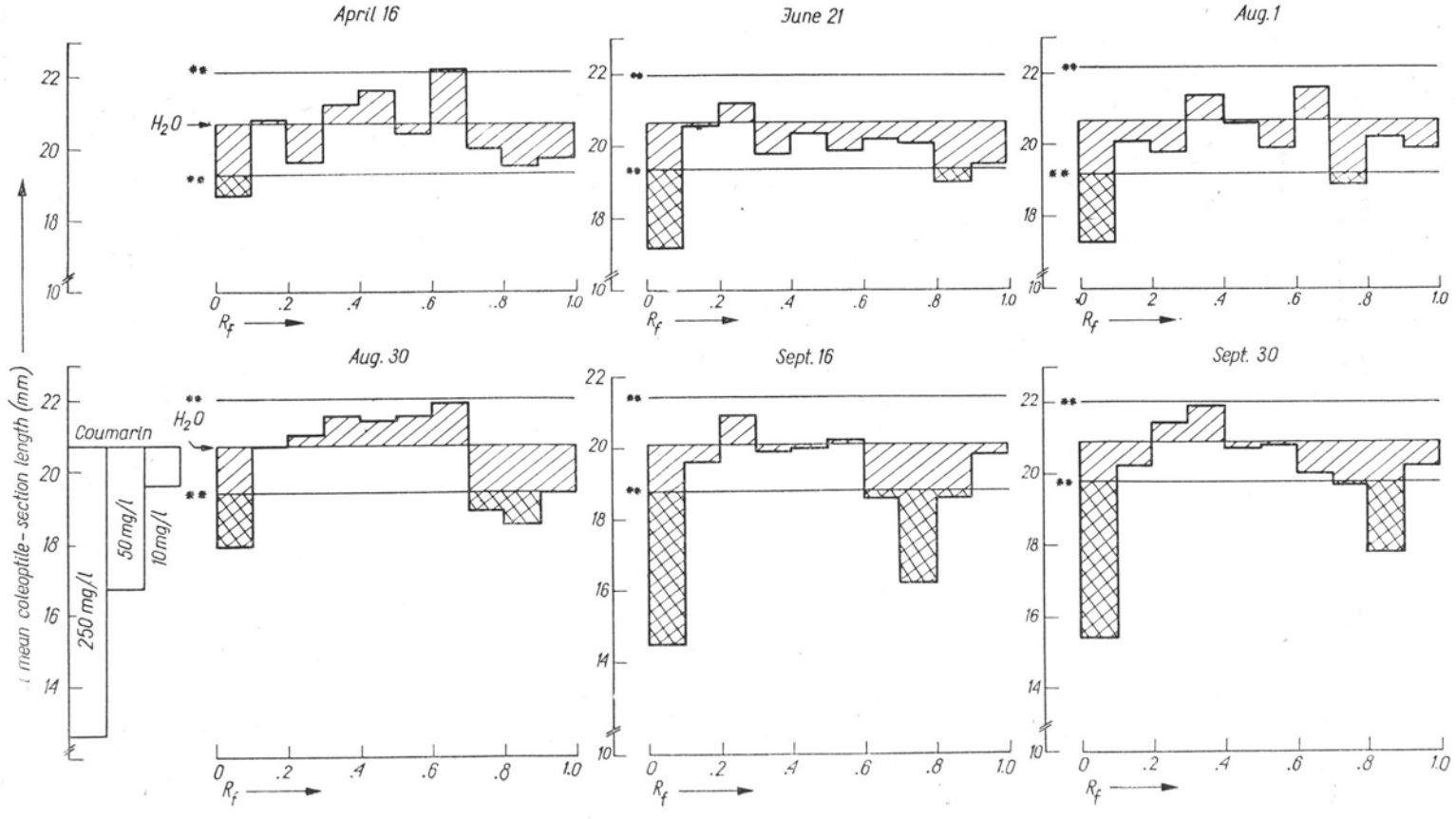
Substances affecting growth of wheat coleoptile section in the tissues of larches growing under natural conditions

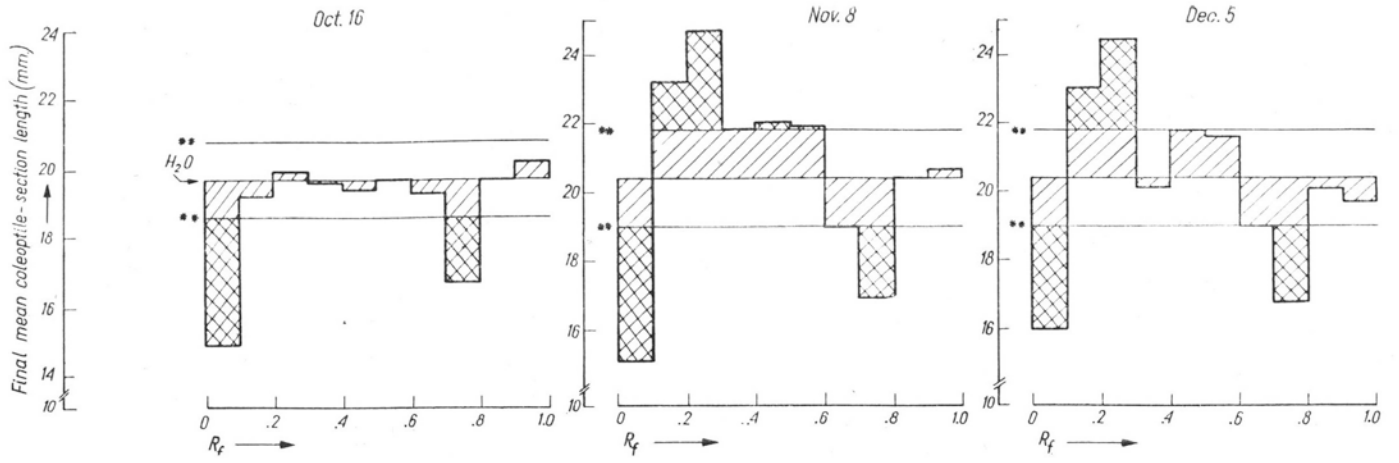
1. Water-soluble substances extracted from cortical tissues

The results presented on histograms (Fig. 1) show that the amount (or activity) of growth-inhibiting substances located at the R_f 0.7—0.9 of the chromatographed aqueous fraction varied throughout the investigated period. It was low in April, June and the beginning of August, slightly surpassing the least significant difference as compared with water control. The analysis of variance between the joint inhibition of

Fig. 1. Assay with wheat coleoptile sections of chromatographed aqueous fraction of extracts of cortical tissues (each equivalent to 1 g fresh weight). Plants collected at different times of the season 1963

** Least significant difference at $P < 1$ per cent




Oct. 16
Nov. 8
Dec. 5
Final mean coleoptile-section length (mm)
24
22
20
18
16
14
10
H2O
0
.2
.4
.6
.8
1.0
Rf

Dec. 20

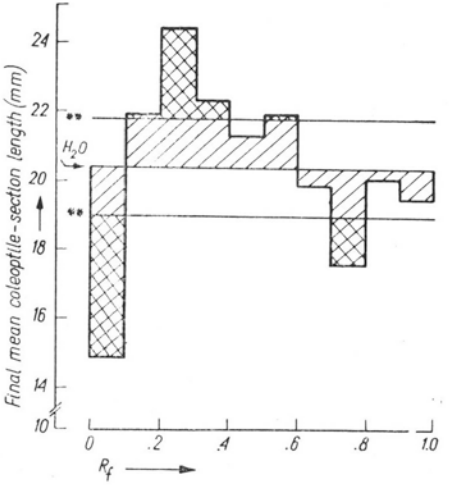

Final mean coleoptile-section length (mm)
24
22
20
18
16
14
10
H2O
0
.2
.4
.6
.8
1.0
Rf

Table 3

Significance of differences between the inhibition of wheat coleoptile-section elongation produced by the substances extracted from larch cortex at different times of the season 1963

Date of plant collection	April 16	June 21	Aug. 1	Aug. 30	Sept. 16	Sept. 30	Dec. 20
April 16		$F_e < F_{0.05}$	$F_e < F_{0.05}$	-	-	-	-
June 21			$F_e < F_{0.05}$	-	-	-	-
August 1				$F_e > F_{0.05}$	-	-	$F_e < F_{0.05}$
August 30					$F_e^* < F_{0.05}$	$F_e < F_{0.05}$	-
September 16						$F_e < F_{0.05}$	$F_e > F_{0.05}$

The bio-assay of eluates from R_f strips 0.8 and 0.9 of chromatograms the aqueous fraction are compared (each equivalent to 1 g fresh wt). F-test after Snedecor.

*If only the inhibition at R_f 0.8 is compared in both cases, the difference is significant ($F_e = 17.09 > F_{0.01} = 8.28$).

coleoptile elongation caused by the substances located at R_f 0.8 and 0.9 of chromatograms showed that these three observations were not significantly different (Table 3). A significant increase of inhibition was found, however, in extracts from plant tissues collected at the end of August. Further accumulation of the inhibitors was observed in mid September. A high inhibitory substances content was still found in extracts from plants collected at the end of September. This inhibition was still not significantly different from that in extracts from plants collected at the two previous dates. In October some decrease of inhibitors content occurred but its level was still high. The decrease was statistically evidenced not earlier than in the second decade of December as compared with the amount of inhibitors estimated in mid September. It was similar to that observed at the beginning of August. Some displacement of inhibitors on chromatograms (towards R_f 0.7) may have been connected with the minute changes in chromatography conditions, as the successive estimations were performed at different times. The high inhibitory effect of the eluate from the R_f 0.1 position (close to the starting line) was disregarded owing to the possibility of a side effect of some residual impurities in this part of chromatogram.

In the plants collected in the first decade of November a great amount of substances strongly stimulating growth of coleoptile sections was revealed. These substances occupied the region 0.2—0.6 R_f of chromatograms with maximum at R_f 0.3. These substances were found also in tissues of plants collected in December. Two different groups of substances could be distinguished in the extracts at this time, at R_f 0.2—0.3 and at R_f 0.5—0.6, respectively. Spraying of the replicate chromatograms

with 2 per cent aniline and 0.2 M oxalic acid mixture and drying at 105°C revealed the two groups of substances giving a coloured reaction: the first with aldehyde group at R_f 0.2—0.3 and the second with ketone group at 0.5—0.6. Detailed investigation of the character of these substances showing properties of sugars were not continued.

2. Ether-soluble substances extracted from cortical tissues

The ether fraction of extracts was also bio-assayed before the essential change in the type of tracheids became apparent (i.e. of plants collected at the beginning and end of August). In the both cases no growth promoting substances were detected (Fig. 2 A and B). To check the sepa-

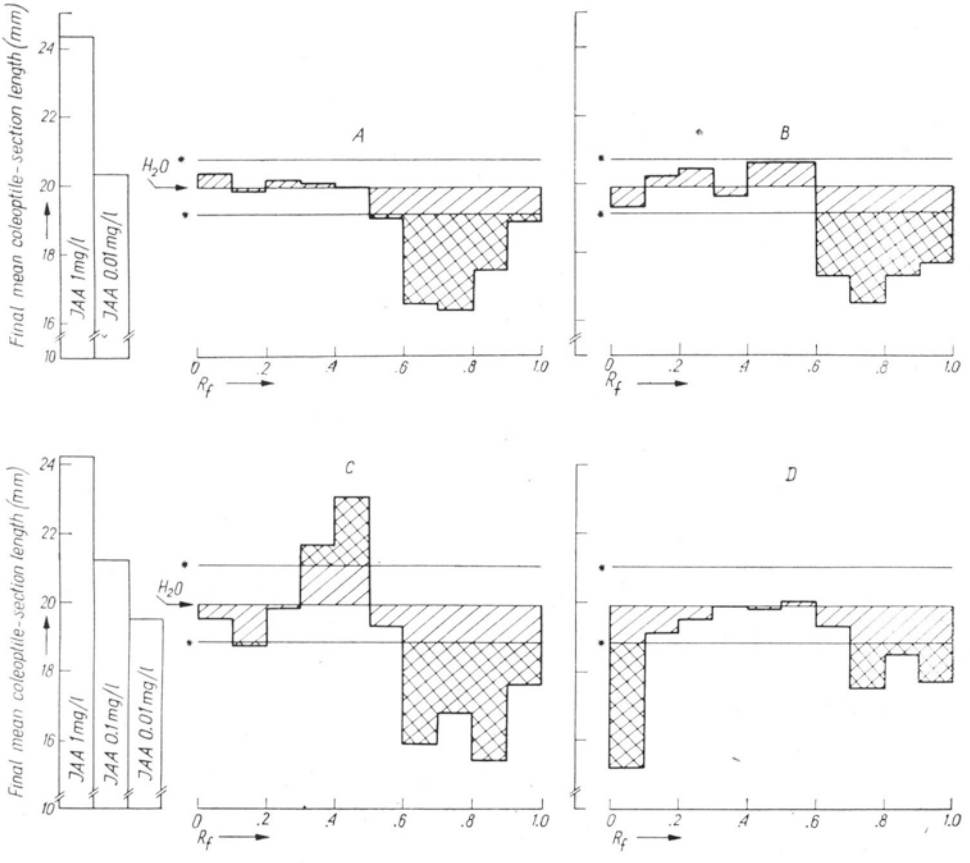


Fig. 2. Assay with wheat coleoptile sections of chromatographed ether fractions (A, B, C) and aqueous fraction (D) of cortical tissues (each equivalent to 1 g fresh weight). Plants collected at the beginning (A) and at the end of August (B, C, D). Assay of ether fraction (C) and aqueous fraction (D) partitioned and chromatographed after the addition of 1 ml IAA solution in conc. 1 mg/l to the crude MeOH-extract

* Least significant difference at 5 per cent level of risk

rating efficiency of the method, 1 ml of IAA solution (conc. 1 mg/l) was added to the crude-MeOH extract of tissue. The extract was subsequently purified, partitioned into two fractions and chromatographed. The results of bio-assay shown in Fig. 2 C and D proved the procedure used to be satisfactory for the detection of substances similar to heteroauxin. Thus it could be presumed, that in both extracts from tissues collected in August the content of growth-substances such as auxins was probably lower than that which could be revealed by the wheat coleoptile section straight growth test.

A region of strong inhibition (or toxicity) was observed in each chromatogram of the ether fraction at R_f 0.7—1.0. As proved earlier (W o d z i c k i 1964), this inhibition was produced by resinous material.

3. Verification of the results obtained with cortical tissues

a. I n f l u e n c e o f v a r i a t i o n i n d r y m a t t e r c o n t e n t. All estimations of growth-affecting substances refer to the known amount of fresh weight of extracted tissues (1 g fresh weight in the case of cortex, and 0.1 g in other cases). Thus any seasonal variability of dry matter content might affect the results of bio-assays. The results presented below shown that, untill the end of September, the percentage of dry matter in cortical tissues was similar:

June 21,	Aug. 1,	Aug. 30,	Sept. 16,	Sept. 30,	Oct. 16,	Nov. 8,	Dec. 5,	Dec. 20.
36.4	36.8	34.6	38.2	38.4	44.1	49.6	43.8	44.3

Thus the results concerning the accumulation of inhibitor observed during that period were fully comparable in this respect. In the plants collected in October, November and December some increase of dry matter was noticed. This may have influenced the results of bio-assays, making them rather too high as compared to those at the previous terms of investigation.

b. V a r i a b i l i t y a m o n g t h e i n d i v i d u a l p l a n t s. Tissues for one estimation of substances affecting elongation of coleoptile sections were usually collected from 6 to 8 plants and combined before extraction. The variability among the individuals was not known but may have influenced the results obtained for the sample. In October six groups consisting of two plants each were collected and cortical tissues were extracted and bio-assayed separately following the procedure applied earlier *. Data concerning the dry/fresh weight ratio of those

* The amount of tissue collected from one plant was too small for separate estimation.

Table 4

Assay with wheat coleoptile-section of chromatographed aqueous fraction of cortical tissues
(each equivalent of 1 g fresh weight of tissue)

Group of plants	Water control	R_f									
		0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
1	20.2	14.3	18.9	20.1	18.9	19.4	19.1	19.1	16.4	19.9	19.9
2	19.9	14.8	19.8	19.6	19.9	19.8	20.6	19.3	16.5	-	-
3	-	14.2	19.2	20.2	20.0	19.5	19.3	19.4	16.7	19.1	20.1
4	19.3	15.0	19.0	18.8	19.9	19.0	19.6	18.3	16.9	19.8	20.7
5	19.1	15.3	19.1	20.5	19.2	19.5	19.8	19.4	16.5	19.9	20.4
6	19.8	15.9	19.0	20.0	19.6	19.3	20.1	20.2	16.9	19.8	19.7
F-test	$F_e < F_{0.05}$	$F_e > F_{0.01}$	$F_e < F_{0.1}$	$F_e > F_{0.01}$	$F_e < F_{0.1}$	$F_e < F_{0.1}$	$F_e < F_{0.01}$	$F_e < F_{0.01}$	$F_e < F_{0.1}$	$F_e < F_{0.05}$	$F_e > F_{0.01}$
Average	19.7	14.9	19.2	19.9	19.6	19.4	19.7	19.3	16.7	19.7	20.2

Six identical groups of two plants each, collected on October 16, 1963

Final mean coleoptile-section length (mm).

Table 5

Assay with wheat coleoptile-section of chromatographed ether fraction of cortical tissues
(each equivalent of 1 g fresh weight)

Group of plants	Water control	R_f									
		0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
1	20.1	20.1	20.2	19.7	20.7	19.6	20.1	16.7	16.6	17.8	18.6
2	21.0	19.4	19.2	20.4	20.9	21.0	19.9	18.0	16.1	18.1	18.6
3	20.2	19.3	20.2	20.2	20.3	20.6	20.0	19.4	16.6	18.2	19.1
4	21.1	19.8	20.6	19.7	20.7	20.9	20.5	18.1	17.0	18.3	18.9
5	21.2	19.6	20.3	20.4	20.6	20.8	20.3	18.1	17.0	19.1	19.0
F-test	$F_e < F_{0.05}$	$F_e < F_{0.01}$	$F_e < F_{0.1}$	$F_e < F_{0.1}$	$F_e < F_{0.1}$	$F_e > F_{0.01}$	$F_e < F_{0.1}$	$F_e > F_{0.01}$	$F_e < F_{0.1}$	$F_e > F_{0.01}$	$F_e < F_{0.1}$
Average	20.8	19.6	20.2	20.1	20.6	20.6	20.2	18.1	16.7	18.3	18.8

Five identical groups, of two plants each, collected on October 16, 1963. Final mean coleoptile-section length (mm).

plants (presented below) show that material was comparable enough in this respect:

Group of plants	1	2	3	4	5	6
Percentage of dry matter	45.1	45.1	46.0	46.2	43.8	44.2

The results of bio-assays concerning the aqueous fraction (Table 4) and ether fraction of extracts (Table 5) showed the great uniformity of the investigated plant material. Besides the starting line and front of chromatograms in the case of the aqueous fraction and the region occupied by resinous material (R_f 0.7, 0.9) in the ether fraction, the variability among the investigated plants was essentially not different from that of water controls. This fact was proved by analysis of the ratio: individual variability/variability due to the applied method of investigation. In most occasions it was less than 1 at 1 per cent (but in most cases even at 5 or 10 per cent) level of risk. Only at R_f 0.3 in the case of the aqueous fraction and R_f 0.5 in the case of the ether fraction of extracts, the individual variability was found to exceed the error connected with the applied techniques. However, it was due to a single estimation in each case (marked in the Tables).

c. Preservation of plant material. In a few cases the collected tissues were immediately frozen at dry ice temperature and then stored at −16°C for several weeks before extraction. This might have produced changes in the growth-inhibitors content. This question

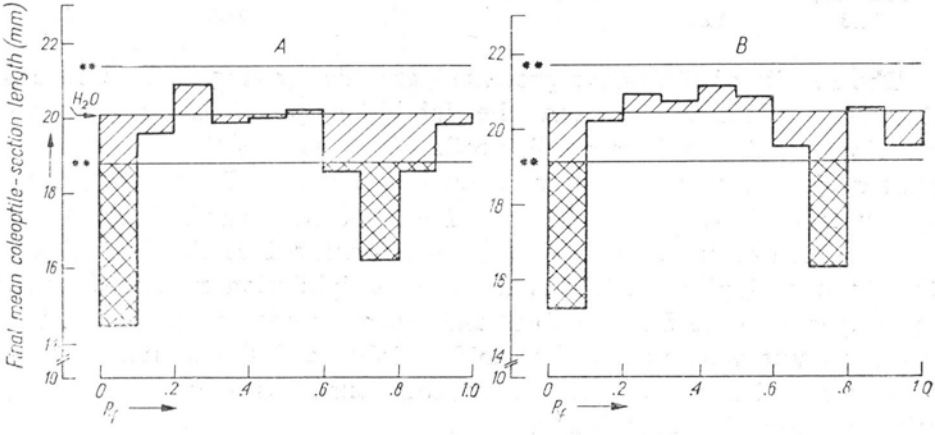


Fig. 3. Assay with wheat coleoptile sections of chromatographed aqueous fraction of extracts of cortical tissues (each equivalent of 1 g fresh weight) collected on September 16, 1963. Tissues extracted and bio-assayed immediately after collection (A), and after 95 days of storage at −16°C (B)

** Least significant difference at 1 per cent level of risk

was investigated using cortex collected in mid September. One part of the tissues was extracted and bio-assayed immediately after collection, another was stored as indicated above for 95 days and than extracted, chromatographed and bio-assayed.

The results presented on histograms Fig. 3 show that even three months of preservation of tissues in the above mentioned conditions did not cause significant differences in the total amount or activity of the inhibitors located at R_f 0.7—0.9 on chromatograms (results were tested statistically). As mentioned earlier, some differences in the position occupied by the inhibitor within this region of the chromatograms were probably connected with slightly different conditions of chromatography (room temperature, different stock of developing solvent etc.) as the extraction and purification proceeded at different times.

The estimations of inhibitors content in cortex during the period of increased accumulation (August — October) were performed immediately after plant collection and these results were not affected by storage but, as it is seen also in other cases, conservation of the material had probably little effect on the results of inhibitors content estimation.

4. Inhibitors extracted from fully grown needles

The needles of plants collected in April were not yet developed. Shed of needles was observed in mid October. During all the investigated time in between, the percentage of dry matter of fully grown needles remained at the same level, as indicated below:

June 21,	Aug. 1,	Aug. 30,	Sept. 16,	Sept. 30,	Oct. 16.
37.8	34.7	38.7	39.0	38.6	37.8

The results of bio-assays presented graphically (Fig. 4) showed considerable differences between the inhibition produced by substances occuring in extracts from needles collected at various times. The results were subjected to analysis of variance and tested by T u k e y' s method (numerical data are not presented). This method showed that the joint accumulation of inhibitors at R_f 0.7—0.8 observed at the beginning of August was significant. The high level of inhibition remained nearly unchanged until mid September and then decreased gradually. This reduction was well advanced in mid October and the inhibition found in extracts of needles collected at that time was not significantly different from that revealed in June.

Some displacements were observed in the position occupied by inhibitors on chromatograms towards the starting line at the end of the season. In this case, it may probably be assumed as reflecting a change in some chemical properties of those substances occurring in needles

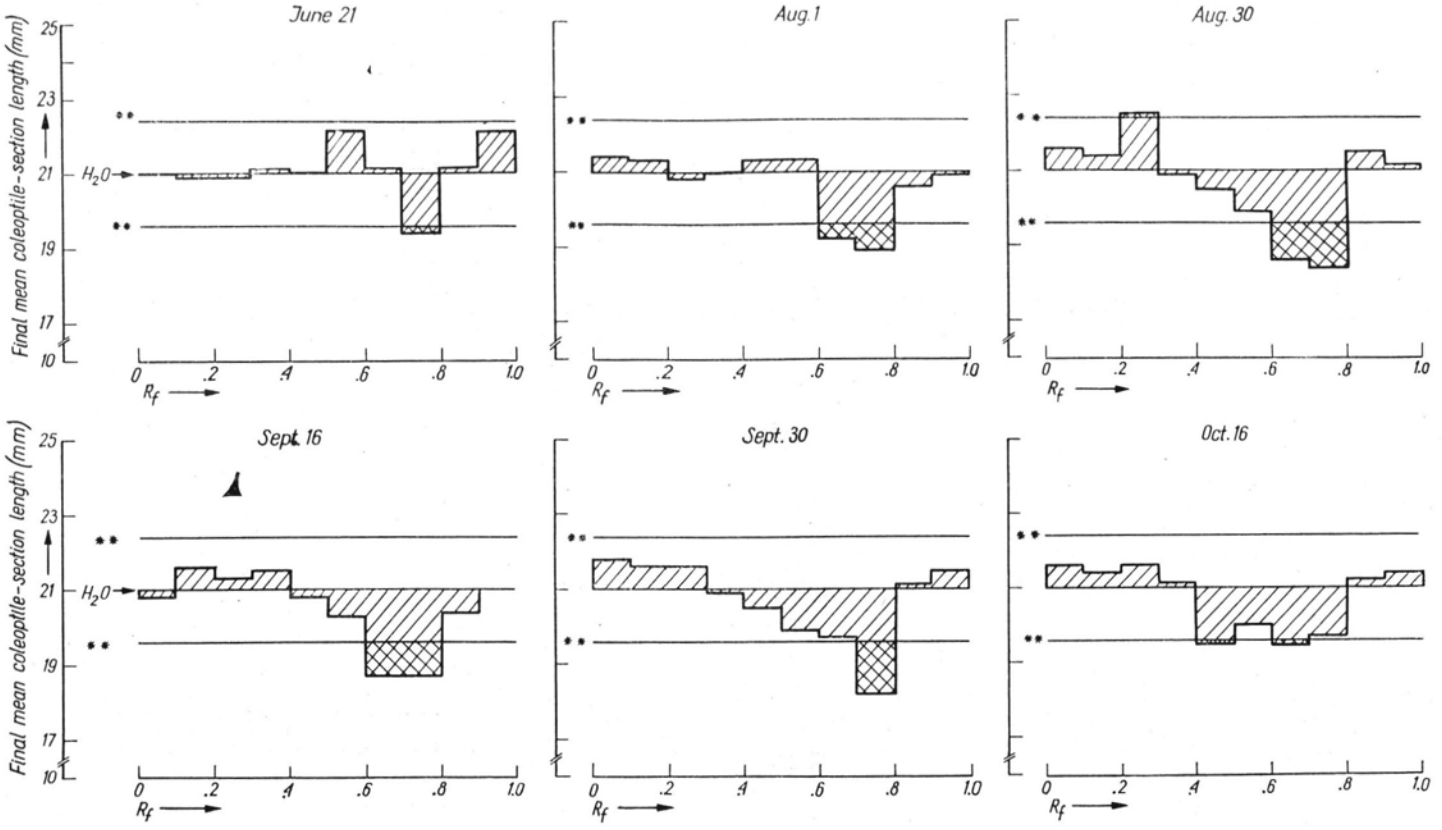
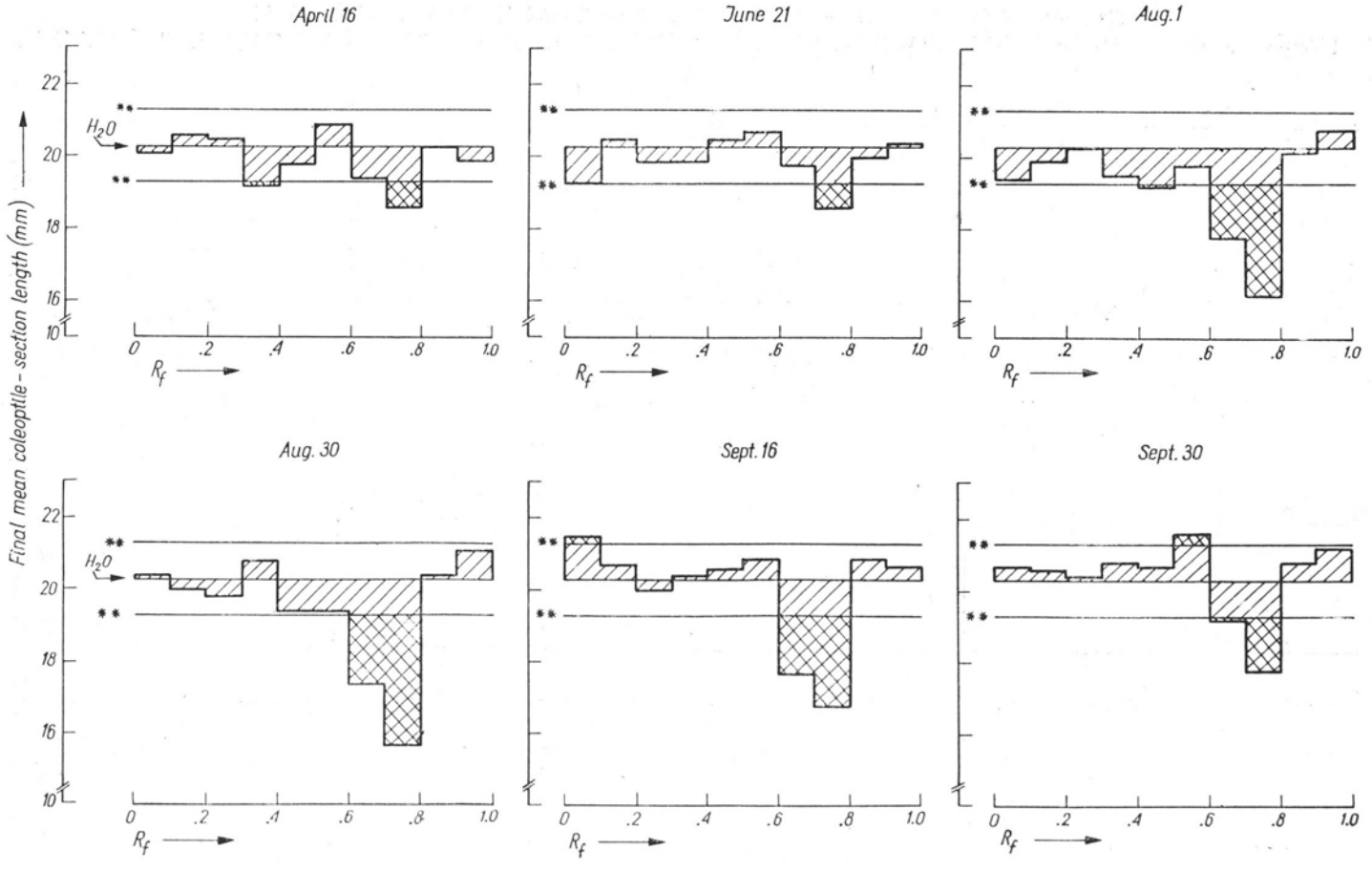


Fig. 4. Assay with wheat coleoptile sections of chromatographed aqueous fraction of extracts of needles (each equivalent to 0.1 g fresh weight). Plants collected at different times of the season 1963

** Least significant difference at $P < 1$ per cent


April 16
June 21
Aug. 1
Aug. 30
Sept. 16
Sept. 30
Final mean coleoptile - section length (mm)
22
20
18
16
10
H_2O
**
**
0
.2
.4
.6
.8
1.0
R_f

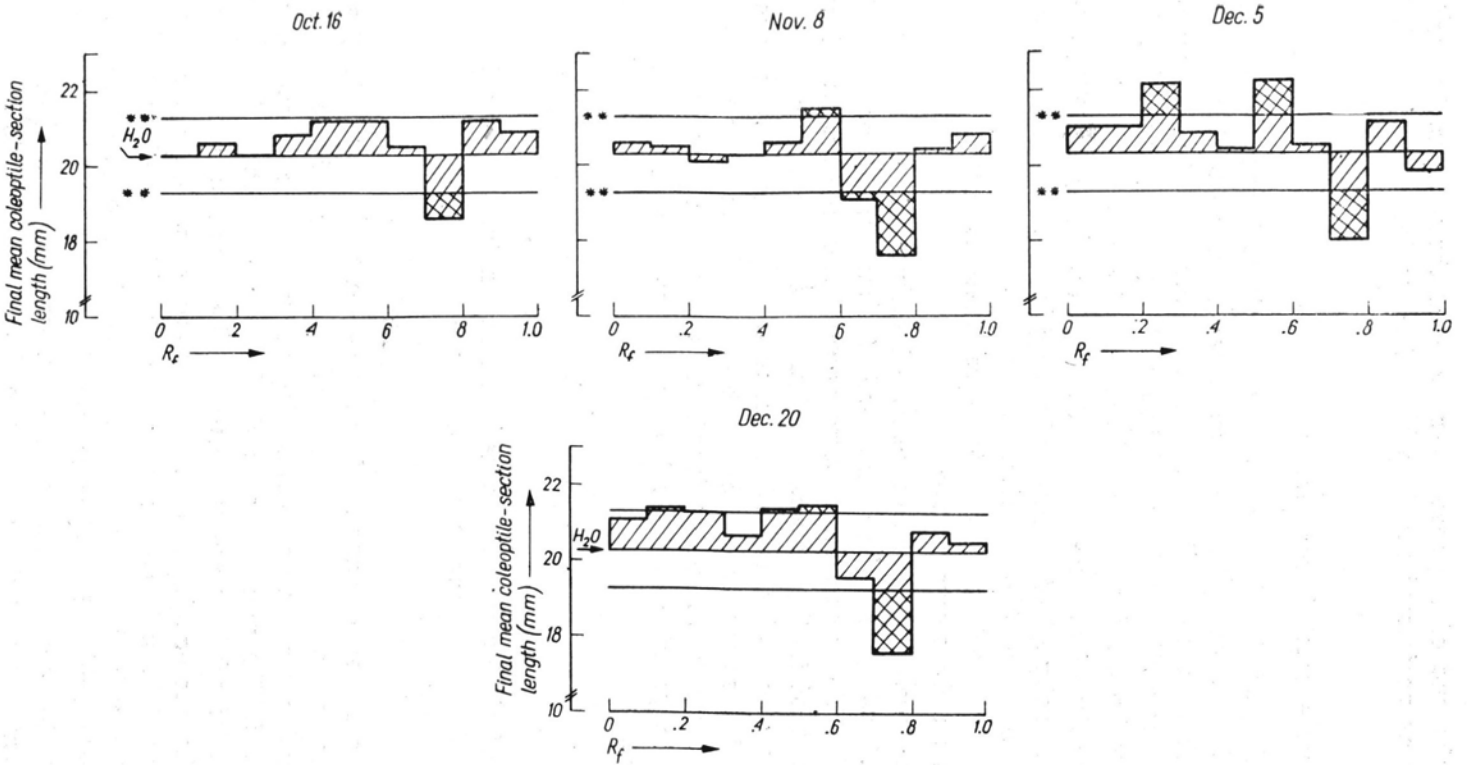


Fig. 5. Assay with wheat coleoptile sections of chromatographed aqueous fraction of extracts of shoot apices (each equivalent to 0.1 g fresh weight). Plants collected at different times of the season 1963

** Least significant difference at $P < 1$ per cent

towards the end of the season (extractions, chromatography and bio-assays were performed simultaneously for needles collected at different times).

5. Inhibitors extracted from apices

Until August the plants grew continuously and formation of terminal buds at the top of main shoot was observed in various plants during that month. At the end of August about 90 per cent of plants set resting buds but their slightly lignified bud scales changed their colour from greenish to brown during the following four weeks.

The results of bio-assays presented in Fig. 5 showed changes in the content (or activity) of water-soluble inhibitory substances in the shoot apex. The position of these substances (R_f 0.7—0.8) on the chromatograms was analogical to that found for substances extracted from cortical tissues and fully grown needles.

The differences at various terms of estimation were tested. This analysis showed a significant increase of inhibition at the beginning of August. Inhibitory substances were present also in April and June but the amount of inhibition was significantly less. At the end of August and mid of September the inhibition produced by these substances was still high but at the end of September a significant decrease occurred. The difference in inhibition found in buds at this term of the season and that in growing apices collected in April and June was no more significant.

Effect of photoperiod and decapitation on natural growth-inhibitors and growth-substances content

Besides seasonal changes of growth-inhibitors content in tissues of plants grown under natural conditions, two-year old plants of larch grown under continuous illumination in growth-room conditions were investigated. The plants were divided into four groups (four plants in each) two of which were subjected to short photoperiod (12-hrs ray) during the subsequent 21 days. One group of plants remaining under continuous light and one group under short-day conditions were decapitated. All newly developing buds at the base of the needles were successively removed as they appeared. At the end of experimental treatment the cortical tissues of all groups of plants were extracted and the extracts partitioned to aqueous and ether fractions.

During the time of varied photoperiodical treatment, intact plants

subjected to continuous illumination grew continuously and those under short-day conditions formed resting buds. Estimation of the percentage of dry matter in the corex, as presented below, showed no essential differences between the investigated groups of plants:

Number of plant	1	2	3	4	Mean
Continuous illumination, intact plants	26.3	27.0	27.3	26.0	26.6 ± 0.4
Short-day conditions, intact plants	27.0	25.9	23.4	25.8	25.5 ± 0.8
Short-day conditions, decapitated plants	29.8	29.8	27.1	20.2	26.7 ± 2.3

1. Inhibitory substances in aqueous fraction

Results of bio-assays presented in Fig. 6 showed a different content of inhibitors occurring at R_f 0.8—1.0 of chromatograms among the investigated groups of plants. Analysis of variance (Table 6) revealed the significant effect of both photoperiod and decapitation. Interaction between these two factors was not evidenced. Thus, it may be concluded that short-day conditions caused the additional accumulation of the inhibitors in cortex, regardless of the presence of the growing or resting shoot-apex. On the other hand, removal of the apex produced an increase in the inhibitors content regardless of photoperiodic conditions, it was, however, higher under short photoperiods. Also in decapitated plants a greater amount of inhibitory substances was accumulated at R_f 1.0.

Table 6

Effect of decapitation and photoperiodic conditions on the content of growth-inhibiting substances located at the R_f 0.8 - 1.0 region of chromatograms. The aqueous fraction of extracts of cortical tissues equivalent to 1 g fresh weight having been chromatographed and bio-assayed in each case. Analysis of variance after Snedecor.

Source of variation	Degree of freedom	Sum of squares	Mean square	F_{emp}	F_t
Photoperiodic conditions	1	258.1	258.1	8.37	
Decapitation	1	182.6	182.6	5.92	$F_{0.05} = 3.92$
Interaction	1	14.7	14.7	0.48	$F_{0.01} = 6.88$
Error	116	3578.6	30.8		
Total	119	4034.0			

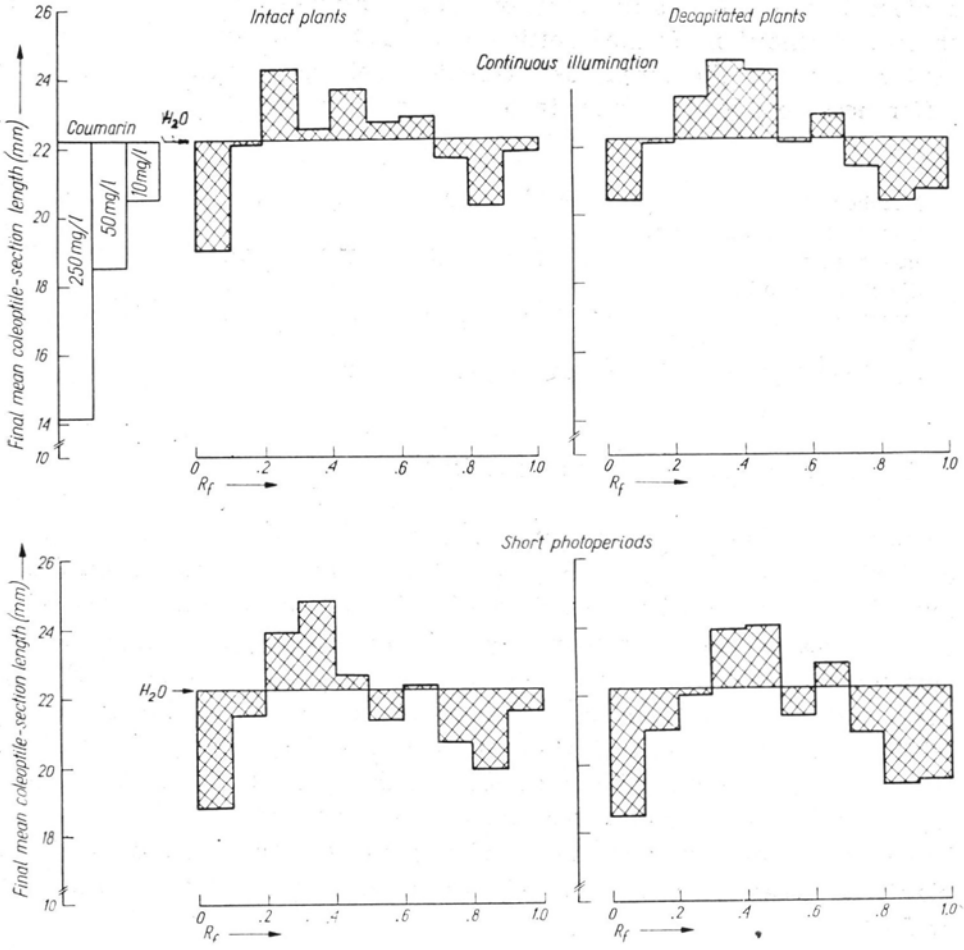


Fig. 6. Assay with wheat coleoptile sections of chromatographed aqueous fraction of extracts of cortical tissues (each equivalent to 1 g fresh weight). Intact or decapitated plants subjected to various photoperiodic conditions

In all groups of plants a distinct zone of growth promotion was observed at R_f 0.3—0.5. This could probably be attributed to the presence of sugar-like substances.

2. Promoters in ether fraction

Bio-assays performed with eluates of the ether fraction showed a grater amount of growth-stimulating substances in intact plants grown under continuous illumination (Fig. 7). The results concerning joint promotion of growth obtained at R_f 0.3—0.5 (analogical to the position of IAA) were subjected to analysis of variance (Table 7). This proved the significant effect of both the photoperiodic conditions and decapita-

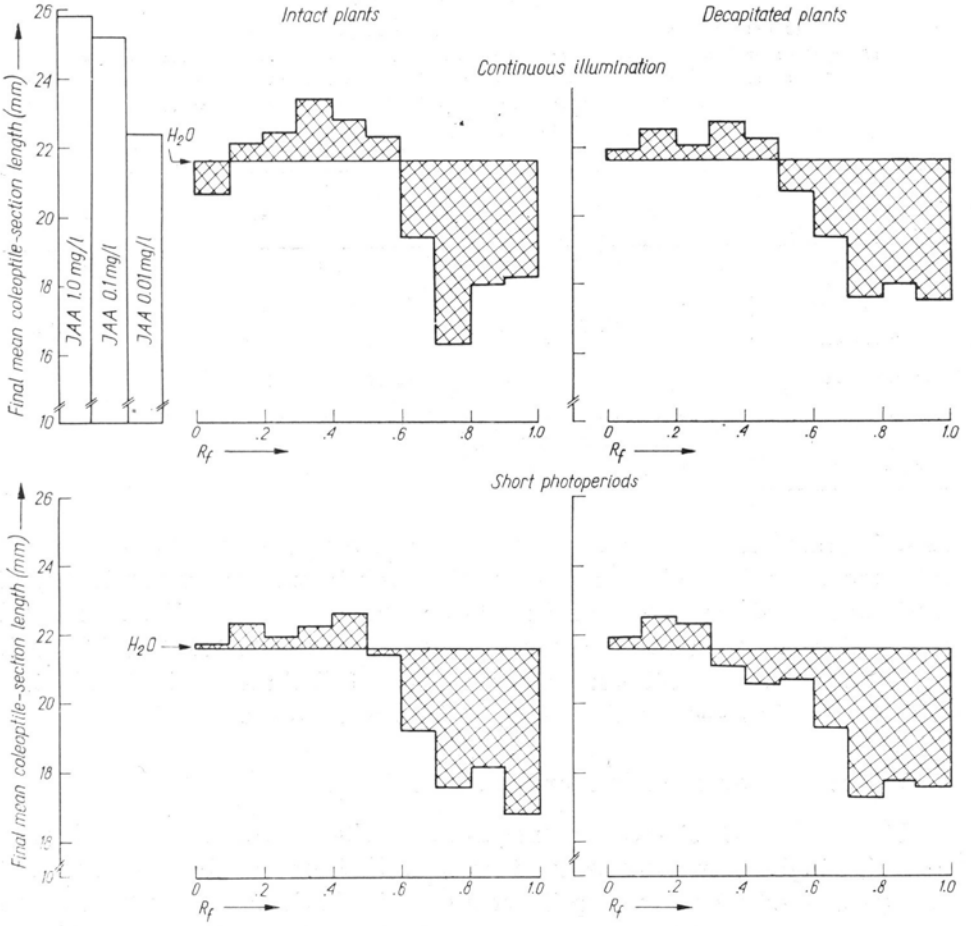


Fig. 7. Assay with wheat coleoptile sections of chromatographed ether fraction of extracts of cortical tissues (each equivalent to 1 g fresh weight). Intact or decapitated plants subjected to various photoperiodic conditions

tion on the content of these substances in cortex. The interaction between these two effects has not been evidenced.

Besides the region of stimulation, the inhibitory substances corresponding to the resinous material position on chromatograms were observed in all groups of plants.

Some biological properties of the inhibitors from larch

Preliminary investigation of some biological properties of the inhibitory substances eluted with water from R_f 0.7—0.9 strips of chromatograms of the aqueous fraction of cortex extracts was performed by adding known amounts of IAA to the tested solutions. The inhibitors

Table 7

Effect of decapitation and photoperiodic conditions on the content of growth-promoting substances located at the region R_f 0.3 - 0.5 of chromatograms. The ether fraction of extracts of cortical tissues equivalent to 1 g fresh weight having been chromatographed and bio-assayed in each case. Analysis of variance after Snedecor.

Source of variation	Degree of freedom	Sum of squares	Mean square	F_{emp}	F_t
Photoperiodic conditions	1	334.4	334.4	9.26	$F_{0.05} = 3.93$
Decapitation	1	240.9	240.9	6.67	$F_{0.01} = 6.88$
Interaction	1	21.4	21.4	0.59	
Error	116	4189.7	36.2		
Total	119	4786.4			

used in this experiment were extracted from the cortex of plants collected in mid October. The amount of inhibitors equivalent to 1 g of fresh weight of tissue was arbitrarily chosen as a comparable unit equal to 1.0. The effect of four concentrations of inhibitors: 1.0, 0.3, 0.1, 0.04, in arbitrary units, and six concentrations of IAA: 0.01, 0.05, 0.5, 1.0, 10.0, 100.0, mg/l — and their interactions were investigated.

1. Wheat coleoptile section straight growth test

The results of bio-assays presented in Fig. 8 and Table 8 showed that the highest two concentrations of inhibitors in all cases reduced the growth of wheat coleoptile sections. In both cases the promotion of growth produced by IAA at concentrations greater than 0.05 ppm

Table 8

Effect of interaction of inhibitors from cortical tissues of larch and IAA on the elongation of wheat coleoptile-sections
Experiment 1

Inhibitors eqv.g fr wt \ IAA mg/l	0.00	0.01	0.05	0.10	1.00	10.00	100.00
0.00	18.80	18.80	18.85	19.75	24.37	28.92	25.9
0.04	18.40	18.57	19.30	20.00	24.15	-	-
0.10	18.70	18.65	19.85	20.35	23.50	28.67	27.2
0.30	17.65	17.45	18.37	19.52	22.60	26.22	25.22
1.00	16.40	-	17.42	18.12	22.55	24.35	25.00
F-test	$F_e > F_{0.05}$	$F_e > F_{0.05}$	$F_e > F_{0.05}$	$F_e > F_{0.05}$	$F_e > F_{0.05}$	$F_e > F_{0.05}$	$F_e > F_{0.05}$

The results present the final mean length of sections (mm).

could be observed, but it was always smaller than that of controls without inhibitors added. There was significant decrease in promotion of coleoptile growth at the 100.0 ppm of IAA which was not found if the inhibitors were present in the same solution. The lowest concentration of IAA which produced visible promotion of growth was 0.1 ppm, however if the inhibitors were present in any of the studied amounts,

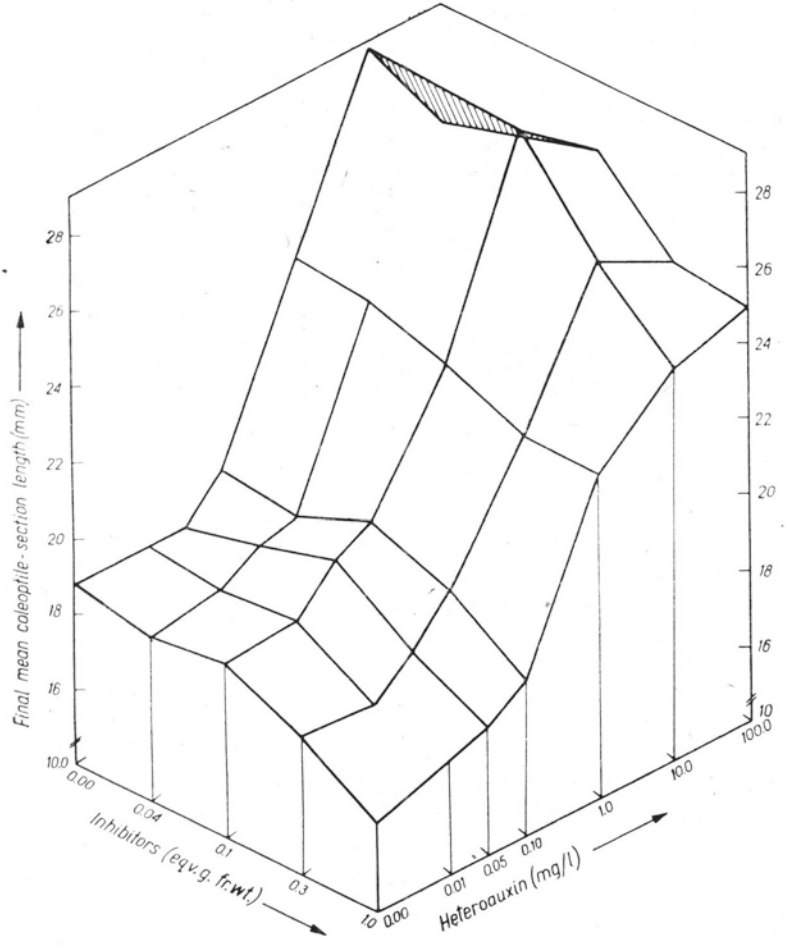


Fig. 8. Effect of interaction of inhibitors from cortical tissues of larch and IAA on the elongation of wheat coleoptile sections

this stimulation was observed also at 0.05 ppm of IAA. There was no suppression of growth of sections by inhibitors at 0.1 concentration of the arbitrary unit. Interesting slight stimulation of growth of coleoptile sections was observed, when the interaction of inhibitors and IAA in concentrations 0.1 of the arbitrary unit and 0.05 ppm respectively (both

Table 9

Effect of interaction of inhibitors from cortical tissues of larch and IAA on the elongation of wheat coleoptile-sections
Experiment 2

IAA mg/l	Replicates	Inhibitors eqv.g fr wt of tissue				F-test
		0.00	0.05	0.10	0.15	
0.00	1	19.77	19.92	19.37	19.05	
	2	20.35	19.27	19.50	19.80	
	3	19.62	20.22	19.47	19.42	
	mean	19.91	19.80	19.45	19.42	$F_e > F_{0.05}$
0.01	1	20.45	20.12	20.40	-	
	2	19.65	19.65	19.52	-	
	3	20.00	20.35	20.52	-	
	mean	20.03	20.04	20.15	-	$F_e < F_{0.05}$
0.05	1	20.57	21.00	21.40	21.10	
	2	21.25	21.47	21.10	21.65	
	3	22.12	22.67	20.62	21.57	
	mean	21.31	21.71	21.04	21.44	$F_e < F_{0.05}$
0.10	1	22.52	22.67	20.82	-	
	2	22.12	21.62	21.67	-	
	3	22.07	22.35	21.92	-	
	mean	22.24	22.21	21.47	-	$F_e < F_{0.05}$

The figures denote the final mean length of sections (mm).

concentrations ineffective if the substances were given separately) was tested. However, analysis of variance did not proved this interaction to be significant.

The experiment was repeated three times using only the low concentrations of inhibitor and promoter. It is seen from data listed in Table 9 that in this case, as previously, a slight synergistic action could be observed in the three tests at the concentration of 0.05 of arbitrary unit of inhibitors and of IAA 0.05 ppm. As previously, however this interaction was found to be nonsignificant.

2. Oat coleoptile and mesocotyl section straight growth tests

Since the information about the possibility of synergistic action of the inhibitors extracted from larch cortex and auxin at low concentrations was considered important, tests were performed with the use of test material more sensitive to auxin stimulation. Firstly, oat coleoptile sections, and secondly sections of the first internode were used. Assays were perfomed twice. Mean results of two coleoptile section (and of two first internode section) tests are presented in Table 10. The results revealed once more some increase of elongation of coleoptile sections

Table 10

Effect of interaction of inhibitors from cortical tissues of larch and IAA on the elongation of oat coleoptile and first internode sections

Inhibitors eqv.g fr wt	Coleoptile sections						First internode sections					
	IAA mg/l						IAA mg/l					
	0.000	0.001	0.005	0.01	0.05	0.1	0.000	0.001	0.005	0.01	0.05	0.1
0.00	13.37	13.94	14.06	14.54	16.47	17.30	5.25	5.60	6.37	7.02	8.31	8.26
0.01	13.54	13.57	13.97	14.71	-	-	5.19	5.65	6.09	-	-	-
0.05	13.69	13.37	14.04	14.92	16.46	-	5.19	5.60	6.49	7.04	-	-
0.10	13.42	13.61	14.50	15.00	16.75	17.15	5.25	5.44	5.86	6.74	8.22	-
0.15	13.42	13.67	14.14	14.67	16.61	17.70	5.25	5.46	6.10	6.99	8.25	8.34
0.30	13.42	-	13.72	13.80	15.95	17.02	5.24	-	6.12	6.69	7.91	-
0.50	13.25	-	-	14.40	16.06	16.66	5.07	-	-	6.04	7.26	8.01
F-test at P<5 per cent	$F_e < F_t$	$F_e > F_t$	$F_e > F_t$	$F_e > F_t$	$F_e < F_t$	$F_e < F_t$	$F_e < F_t$	$F_e < F_t$	$F_e > F_t$	$F_e > F_t$	$F_e > F_t$	$F_e < F_t$
$t_{0.05}$	-	0.32	0.41	0.61	-	-	-	-	-	-	-	-

The figures denote final mean length of sections (mm).

F-test according to Snedecor and t-test after Student.

at the respective concentrations of the inhibitors 0.1 of arbitrary unit and auxin 0.005, 0.01, 0.05 ppm. The synergistic promotion observed at concentration of IAA 0.005 ppm surpassed even the least significant difference at the 5 per cent level of risk calculated on the basis of Student's t-test. Taking into account the great tolerance of the error which is attributed to that method if more than two averages are compared, and the negative result of the Q-test according to Tukey's method more competent in this type of investigation, also in this case the observed synergism should probably be assumed statistically non-significant.

The results of two bio-assays following the oat first internode section straight growth procedure did not indicate any synergistic action of inhibitors and promoter.

DISCUSSION

As in the earlier works performed on three coniferous species (Wodzicki 1960, 1962; Wodzicki and Peda 1963) the attention was focussed on the correlation between changes in the cambial zone and the seasonal changes in the type of wood formed. It was suggested in those papers that the radial diameter and cell-wall thickness of tracheids depended mainly upon the duration of growth and differentiation of cells originating from cambial fusiform initials, and in a lesser degree upon the intensity of these processes. These time periods varied

throughout the vegetation season and could be calculated on the basis of changes in the rate of woody elements formation and the width of the radial diameter growth and differentiation layers in the cambial zone, which could be observed under the microscope. The correlation (which can be conveniently followed in Fig. 9) was observed also presently. The maximum width of the radial diameter growth layer was noted two weeks before the greater increase in width of the differentiation layer. Similarly, the maximum radial diameter of tracheids was observed two weeks before the tracheids with significantly thickened cell walls became apparent. In this respect, it cannot be excluded that the small radial diameter of tracheids found in August was connected with the reduction of width of the radial diameter growth layer observed in the preceding period. In the late part of the season the layer of differentiation in the cambial zone came close to the meristematic layer (radial diameter growth layer being strongly reduced). This should result in earlier inception of thickening of the cell wall and probably, lignification of xylem elements.

The changes in the cambial zone of conifers can be compared to those accompaning primary xylem differentiation during the suppression of root growth described by Torrey (1953). The suppression of growth caused extension of the differentiation zone in the roots towards the meristematic region and speed up the formation of xylem elements. The inhibitor of growth in Torrey's experiments could be an auxin at high concentration. On this basis, Torrey suggested that auxin plays an important role in differentiation, secondary wall formation, its lignification and finally desorganisation of protoplasm in the newly forming elements. Similarly, Wardrop (1957) pointed out the possibility that auxin is involved in maintenance of the specific properties between the size-growth and differentiation of fibres and tracheids to secondary xylem.

The auxin control of cambial activity, especially its initiation at the spring time was reported by many authors (Snow 1935; Söding 1936; Brown and Cormack 1937; Gouventak and Maas 1940; Reinders-Gouwentak 1941). Recently, Wareing (1958a) and Larson (1960, 1962) showed also that auxin may influence the dimensional growth of the cambial fusiform initials derivatives. The results of experiments on the regeneration of tissue after wounding the stem of a woody plant (Jacobs 1952, 1954) and of the investigations performed in vitro by Wetmore and Rier (1963) would suggest the important role of auxin in differentiation of the conducting elements of xylem.

All these facts would seem to support the early hypothesis of Priestley and Scott (1936) and Oppenheimer (1945), developed later by Wareing (1958b) that auxin may control the differentiation of annual ring in trees to early- and late-wood.

However, the correlation of seasonal changes of auxin content in shoot and the seasonal pattern which follows the cambial activity in trees of the temperate climatic zone appears rather difficult. The experiments of Söding (1937) and Zimmermann (1936/37) showed that, after the high accumulation in spring, the amount of auxin in shoots of many tree species decreases rapidly and usually about mid June it is many times less than in May, and does not rise again in the season. Cambial activity, on the other hand, after the burst in the spring slows down in many tree species in the mid of summer and after a few weeks it increases again (Mischke 1890; Jost 1892; Brown 1915; Lodewick 1925; Kienholtz 1932; Wodzicki 1962). The late-wood tracheids are formed in the late part of summer and finally the cambial cells stop division in the autumn when no significant changes in the auxin content in shoots can be detected.

The experiments performed with larch provided similar results. Auxin-like substances could not be detected in cortex as long as 60 days before the late-wood formation became apparent (although the sensitivity of test was high enough for detecting auxin at the physiological concentrations). Also changes in growth-promoters (assumed to be auxins) in cortex of larch plants grown under various photoperiodic conditions could not be correlated with changes in thickening of the secondary wall of tracheids, though they were to some extent in agreement with changes in radial diameters (Wodzicki 1964). In this respect the influence of auxin on reaction wood formation which used to be the main argument that auxin controls thickening of secondary wall, deserves special attention. Usually reaction wood was formed in experimental conditions if higher concentrations of auxin were applied (Wershing and Bailey 1942; Onaka 1949; Fraser 1949). In such conditions the effect of auxin could be inhibitory like in Torrey's experiments on roots. Such a possibility would be in agreement with Nečesany's (1958) supposition that the inhibitor could be involved in the formation of reaction wood. In this case, it seems to be interesting that formation of reaction wood in conifers is accompanied by considerable extension of the layer of differentiation resembling analogical changes in the cambial zone during late-wood formation. The facts were repeatedly observed in plants investigated over the last few years by the author (unpublished data).

All the facts discussed above seem to indicate that seasonal changes in the activity of cambium and the full differentiation of the annual ring of wood in trees (at least in larch and probably some other investigated conifers) cannot be explained solely by the control of auxin-like substances. This conclusion would be in agreement with the opinion of many authors who suggested that cambial activity and normal differentiation of xylem elements may be controlled or requires, besides auxin,

also some other substances regulating growth (Jost 1931/2, 1940; Czaja 1935; Snow 1935; Dagys 1936; Rehm 1937; Söding 1940/1; Künning 1950. It was shown by Wareing (1958a) that also gibberellins might be involved in the processes connected with the complex differention of the annual ring of wood in deciduous trees.

The increased accumulation of inhibitors in the cortical tissues of larch was found at the end of the season. The substances, presumably, were similar to those which accumulated under controlled shor-day

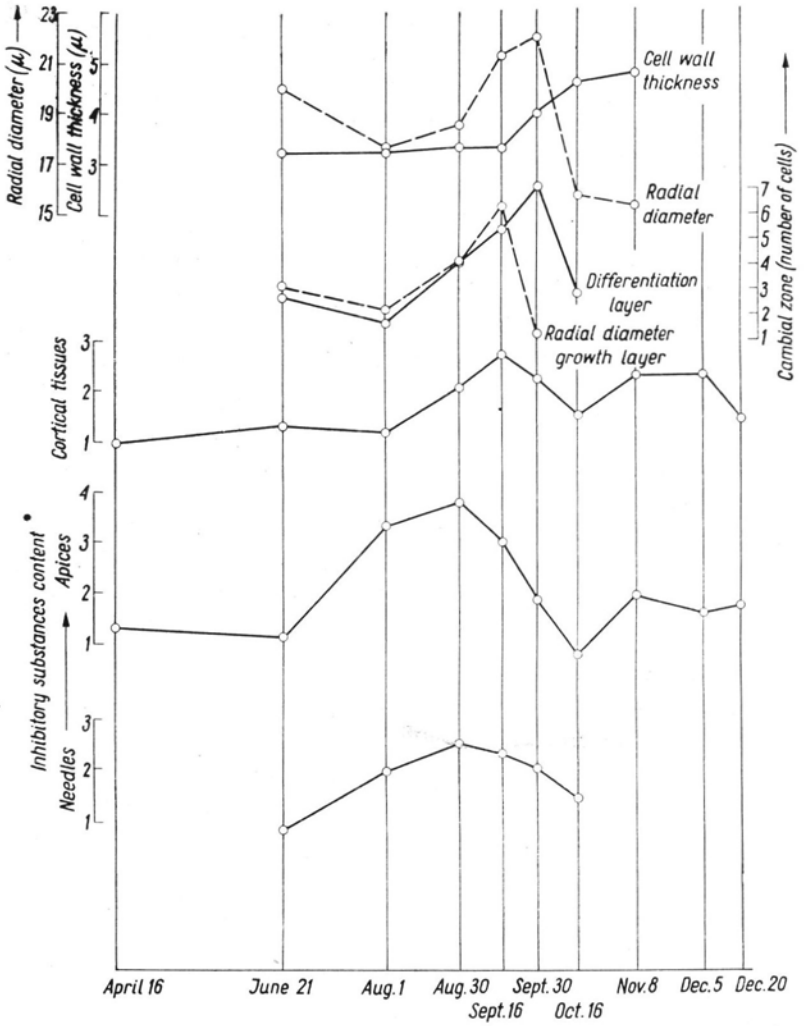


Fig. 9. Seasonal course of: inhibitory substances content in fully grown needles, apices, and cortex; width of radial diameter growth layer and differentiation layer in the cambial zone; radial diameter and cell wall thickness of tracheids in larch

• Inhibition expressed as a difference between the final mean length of coleoptile-section incubated in control solutions and those in eluates from R 0.8 and 0.9 strips of chromatographed aqueous fraction of plant extracts (mm)

conditions. The increased appearance of inhibitors in cortical tissues was preceded by significantly greater accumulation of these substances in fully grown needles and shoot apices (Fig. 9). Decapitation of shoots in plants subjected to short-day conditions resulted in the increase of accumulation of growth-inhibitors (contrary to promoters formed under continuous illumination). These facts led to the conclusion that the inhibitory substances in larch were formed in the fully grown needles in the second part of summer and transported to growth apices and cortical tissues. It seem possible to assume that the growing shoot apex was a strong acceptor of these substances but probably did not mediate their supply to the cortex. The present results would be in accordance with findings of others that the inhibitors form under suitable conditions (especially short-photoperiods) mainly in the mature leaves (W a r e i n g 1954; N i t s c h 1957; P h i l i p s and W a r e i n g 1959; K a w a s e 1961).

Similarly as in earlier experiments (W o d z i c k i 1964), the growth--inhibitory substances content in cortex could be related to the thickness of the cell wall of tracheids, and besides, to the width of the differentiation layer in the cambial zone (Fig. 9). It is seen that accumulation of inhibitors in the cortical tissues in the second part of the season was accompanied, or rather followed closely, by the increase of cell number in the differentiation layer and this was followed by the thick-walled tracheids formation. The time lapse (about 30 days) between the increased accumulation of inhibitors in cortex and the observable production of thick-walled tracheids possibly reflects the time necessary for full differentiation of cells in the cambial zone. This would suggest that the essential effects determining thickening of the secondary wall occur in the early stage of cell differentiation. A similar conclusion was drown from the results of earlier experiments (W o d z i c k i 1961a, 1964). Although there is no direct evidence, this correlation and the facts discussed above seem to allow the possibility that the substances extracted from larch cortex which inhibit elongation of wheat coleoptile sections are involved in the processes controlling changes in the cambial zone and differentiation of tracheids. The action of these substances has not been studied in the original tissues, but it is possible that they may influence or control also other processes of cell differentiation than elongation growth.

Taking into account: the well known influece of auxin on the division of cambial initials and radial diameter growth of cells after division, the correlation between the time of accumulation of inhibitors in cortex, changes, in the cambial zone and formation of thick-walled tracheids, and, finally, the results of preliminary investigation of the biological properties of inhibitors extracted from larch tissues (the slight synergistic action with auxin) — it seems likely that there exists an interaction

between promoters and inhibitors in the differentiation of the annual ring of wood in larch (and probably also in some other conifers).

One of the possible ways in which this interaction might operate seems to be control of specific proportions between duration of size--growth and differentiation processes of cells in the cambial zone (and connected with them primary and secondary wall formation). Probably the synergistic action of these two groups of substances (being both in low concentration) might help to understand the increase of cambial activity of certain tree species in the second part of summer. No more precise hypothesis can be suggested, however, before further possibly direct investigation will elucidate whether the observed correlations reflect really the causal relation.

SUMMARY

Seasonal changes in the natural growth-controlling substances content, especially inhibitors in various tissues of 3-year old larches and its relation to microscopically observable changes in the cambial zone and the type of wood differentiated were studied. Investigation was performed at 10 different dates from April till December, and, besides, under controlled photoperiodic conditions in the growth-room during winter.

As in the earlier investigation, a correlation between changes in the relative width of the radial diameter growth layer and the differentiation layer in the cambial zone, and the radial diameter and cell wall thickness of tracheids actually formed was observed.

A considerable accumulation of growth-inhibitory substances (inhibitors of elongation of wheat and oat coleoptile sections) was found in the cortical tissues at the end of the season. This was preceded by a significant increase of the amount (or activity) of those substances in the fully grown needles and apices of shoots. The 80% MeOH--extracted water-soluble inhibitory substances (in contrast to resinous toxic materials which were soluble in diethyl ether) occupied the R_f 0.7—0.9 region on chromatograms if developed with isopropanol--ammonia-water (10:1:1 v/v) by the descending method. Separate experiments were devoted to some methodical points of the investigation.

The results obtained with plants growing in natural conditions and with those subjected to various photoperiodic treatments (short-day and continuous illumination) and decapitated in growth-room conditions indicated that the inhibitory substances form in fully grown needles, and probably the shoot apex does not mediate the supply of these substances to the cortex in larch.

In the preliminary experiments, interaction of the inhibitory substances extracted from larch tissues with known concentrations of IAA was studied. High concentration of inhibitors reduced the effect of auxin. At low concentrations of both substances, a slight synergistic action was noted.

By comparing the results of anatomical investigation and the results of bio-assays, a distinct correlation between the seasonal changes in the inhibitory substances content in cortex and changes in the cambial zone and various types of tracheids forming during the season was observed.

The possibility of interaction of auxins and growth-inhibiting substances in the differentiation of the annual ring of wood in larch and some other conifers is discussed.

Author aknowledges the very helpful assistance of Mrs. L. Witkowska-Żuk, M. Sc., during the large single bio-assays.

Department of Forest Botany
Central College of Agriculture (S.G.G.W.)
Rakowiecka 8, Warsaw 12, Poland

(Entered: 12.X.1964.)

REFERENCES

Bentley J. A., Housley S., 1954, Bio-assay of plant growth hormones Physiol. Plant. 7(2):405—419.

Brown H. P., 1915, Growth studies in forest trees. II. *Pinus strobus* L., Bot. Gaz. 59: 197—241.

Brown A. B., Cormack R. G. H., 1937, Simulation of cambial activity, locally in the region of application and at a distance in relation to wound, by means of heteroauxin, Canad. Journ. of Res. C. 15: 433—441.

Czaja A. Th., 1935. Wurzelwachstum, Wuchsstoff und die Theorie der Wuchsstoffwirkung, Ber. d. deutsch. bot. Ges. 53(2): 221—245.

Dagys J., 1936. Die Hefewuchsstoffe in Knospen und Blättern, Protoplasma 26(1): 20—44.

Fraser D. A., 1949, Production of spring wood with β-indole acetic acid (heteroauxin), Nature (London) 164: 542.

Gouwentak C. A., Maas M. L., 1940. Kambiumtätigkeit und Wuchsstoff. II Meded. van de Land. Wageningen 44(1).

Jacobs W. P., 1952, The role of auxin in the differentiation of xylem around a wound, Am. J. Bot. 39: 301—309.

Jacobs W. P., 1954, Acropetal auxin transport and xylem regeneration — a quantitative study, American Naturalist 88(842): 327—337.

Jost L., 1892, Beobachtungen über den zeitlichen Verlauf des secundaren Dickenwachstums der Bäume, Ber. deut. bot. Ges. 10: 587—607.

Jost L., 1931/32, Die Determinierung der Wurzelstructur, Zeitschrift für Botanik 25: 481:522.

Jost L., 1940, Zur Physiologie der Gefäsbildung, Zeitschrift für Botanik 35(3/4): 114—150.

Kawase M., 1961, Growth Substances related to dormancy in *Betula* Proc. Amer. Soc. Hort. Sci. 78: 532—544.

Kentzer T., Rowicka K., 1963, Przydatność różnych odmian owsa jako materiału testowego do oznaczeń regulatorów wzrostu roślin, Acta Agrobotanica 13: 117—130.

Kienholz R., 1934, Leader, needle, cambial and root growth of certain Conifers and their interrelatins, Bot. Gaz. 96: 73—92.

Künning H., 1950, Untersuchungen über die Wirkstoffregulation der Kambiumtätigkeit, Plant 38(1): 36—64.

Larson P. R., 1960, A physiological consideration of the spring-wood-summer-wood transition in Red pine, Forest Sci. 6(2): 110—122.

Larson P. R., 1962, The indirect effect of photoperiod on tracheid diameter in *Pinus resinosa*, Am. J. Bot. 49(2): 132—137.

Lodewick J. E., 1925, Growth studies in forest trees. III. Experiments with dendrograph on *Fraxinus americana*, Bot. Gaz. 79: 311—323.

Mischke K., 1890, Beobachtungen über das Dickenwachstum der Coniferen, Bot. Zentbl. 44.

Molski B., Żelawski W., 1958, Wstępne badania anatomiczne procesu kształtowania się drewna późnego w słoju rocznym u siewek modrzewia (*Larix europea* D. C.) w związku z warunkami długości oświetlenia dziennego, Acta Soc. Bot. Pol. 27(1): 83—102.

Nečesaný V., 1958, Effect of β-indoleacetic acid on the formation of reaction wood, Phyton 11(2): 117—127.

Nitsch J. P., 1956, Methods for the investigation of natural auxins and growth--inhibitors, p. 3—31 in: The Chemistry and mode of action of plant growth substances, London.

Nitsch J. P., 1957, Growth responses of woody plants to photoperiodic stimuli, Proc. Amer. Hort. Sci. 70: 512—525.

Nitsch J. P., Nitsch C., 1956, Studies on the growth coleoptile and first--internode sections. A new sensitive, straight-growth test for auxins, Plant Physiol. 31(2):94—111.

Onaka F., 1949, Studies on compression and tension wood, Bull. Wood Res. Inst. Kyoto Univ. 1: 88 (the work was not orginally seen).

Oppenheimer H. R., 1945, Cambial wood production in stems of *Pinus halepensis*, Palestine Journ. Bot. and Hort. Sci. 5: 22—51.

Phillips I. D. J., Wareing P. F., 1958, Studies in dormancy of Sycamore. I. Seasonal changes in the growth-substance content of the shoot, Journ. Exp. Bot. 9(27): 350—365.

Phillips I. D. J., Wareing P. J., 1959, Studies in dormancy of Sycamore. II. The effect of daylength on the natural growth-inhibitor content of the shoot, Journ. Exp. Bot. 10(30): 504—514.

Priestley J. H., Scott L. J., 1936, A note upon summer wood production in the tree, Proc. Leeds Phil. and Lit. Soc. (Sci. Sect.) 3: 235—248.

Rehm S., 1937, Zur Entwicklungsphysiologie der Gefässe und des Trachealensystems, Planta 26(2): 255—274.

Reinders-Gouwentak C. A., 1941, Cambial activity as dependent on the presence of growth hormone and nonresting condition of stems, Proc. Nederl. Akad. van Wetenschappen 44(5): 654—663.

Snow R., 1935, Activation of cambial growth by pure hormones, The New Phyt. 34(5): 347—360.

Söding H., 1936, Über den Einflus von Wuchsstoff auf das Dickenwachstum der Bäume, Ber. d. deutsch. bot. Ges. 54(3): 291—304.

Söding H., 1937, Wuchsstoff und Kambiumtätigkeit der Bäume, Jahrb. f. Wiss. Bot. 84(4): 639—670.

Söding H., 1940/41, Weitere Untersuchungen über die Wuchsstoffregulation der Kambiumtätigkeit, Zeitschr. f. Bot. 36: 113—141.

Torrey J. G., 1953, The effect of certain metabolic inhibitors on vascular tissue differentiation in isolated pea roots Am. J. Bot. 40(7): 525—533.

Wardrop A. B., 1957, The phase of lignification in the differentiation of wood fibres, Tappi 40: 225—243.

Wareing P. F., 1951, Growth studies in woody species. III. Further photoperiodic effect in *Pinus silvestris*, Physiol. Plant. 4(1): 41—56.

Wareing P. F., 1954, Growth studies in woody species. VI. The locus of photoperiodic perception in relation to dormancy, Physiol. Plant. 7(2): 261—277.

Wareing P. F., 1958a, Interaction between indoleacetic acid and gibberellic acid in cambial activity, Nature 181(4625): 1744—1745.

Wareing P. F., 1958b, The physiology of cambial activity, Journ. Inst. Wood Sci. 1: 34—42.

Wareing P. F., Roberts D. L., 1956, Photoperiodic control of cambial activity in *Robina pseudacacia* L., The New Phyt. 55: 356—366.

Wershing H. F., Bailey I. W., 1942, Seedlings as experimental material in the study of "redwood" in conifers, Journ. Forestry 40: 411—414.

Wetmore R. H., Rier J. P., 1963, Experimental induction of vascular tissues in callus of angiosperms, Amer. J. Bot. 50(5): 418—430.

Wodzicki T., 1960, Investigation on the kind of *Larix polonica* Rac. wood formed under various photoperiodic conditions. I. Plants growing in natural conditions, Acta Soc. Bot. Pol. 29(4): 713—730.

Wodzicki T., 1961a, Investigation 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, Acta Soc. Bot. Pol. 30(1): 111—131.

Wodzicki T., 1961b, Investigation on the kind of *Larix polonica* Rac. wood formed under various photoperiodic conditions. III. Effect of decapitation and ringing on the wood formation and cambial activity, Acta Soc. Bot. Pol. 30(2): 293—306.

Wodzicki T., 1962, A contribution to the rate of xylem cell formation and differentiation of tracheids in *Pinus silvestris* L., Acta Soc. Bot. Pol. 31(2): 219—227.

Wodzicki T., 1964, Photoperiodic control of natural growth-substances and wood formation in larch (*Larix decidua* D. C.), Journ. Exp. Bot. 15(45):584—599.

Wodzicki T., Witkowska L., 1961, On the photoperiodic control of extension growth and wood formation in Norway spruce (*Picea abies* (L.) Karst.), Acta Soc. Bot. Pol. 30(3/4):755—764.

Wodzicki T., Peda T., 1963, Investigation on the annual ring of wood formation in european silver fir (*Abies pectinata* D.C.), Acta Soc. Bot. Pol. 32(3):609—618.

Wodzicki T., Witkowska-Żuk L., 1964, Growth response of coleoptile sections of several Polish varieties of wheat to some growth-regulators, Acta Soc. Bot. Pol. 33(2):323—333.

Zimmermann W. A., 1936/7, Untersuchungen über die räumliche un zeitliche Verteilung des Wuchsstoffes bei Bäumen, Zeitschr. f. Bot. 30, 209—252.

Żelawski W., 1957, Dalsze badania reakcji fotoperiodycznej siewek modrzewia (*Larix europea* D.C.), Acta Soc. Bot. Pol. 26(1):80—103.

Różnicowanie słoja rocznego drewna u modrzewia, a zmiany w zawartości naturalnych inhibitorów wzrostu w ciągu okresu wegetacyjnego

Streszczenie

Na podstawie wcześniejszych badań (Wodzicki 1960—1964) wysunięto przypuszczenie, że w procesach różnicowania drewna mogą uczestniczyć substancje o charakterze inhibitorów wzrostu. Zagadnienie to było w dalszym ciągu przedmiotem niniejszej pracy.

Przeprowadzono badania w 10 terminach (od kwietnia do grudnia) na 2-letnich roślinach modrzewia europejskiego rosnących w warunkach naturalnych. Ponadto badano rośliny rosnące w warunkach laboratoryjnych w okresie zimowym.

Badania anatomiczne dotyczyły:

1) ustalenia zmian szerokości warstw wzrostu średnicy promieniowej komórek i warstwy różnicowania ksylemu w strefie kambialnej,

2) ustalenia typu tworzącego się drewna pod względem wielkości średnicy promieniowej i grubości błony komórkowej cewek.

Podobnie jak w badaniach wcześniejszych stwierdzono, że istnieje korelacja między zmianami szerokości poszczególnych warstw strefy kambialnej a zmianami średnicy promieniowej i grubości błon komórkowych cewek w okresie wegetacji.

W roślinach, które były obiektem badań anatomicznych oznaczano zawartość regulatorów wzrostu w trzech rodzajach tkanek:

1) w tkankach korowych (tj. bezpośrednio przyległych do kambium i obejmujących część strefy kambialnej);

2) w pełni wyrośniętych igłach;

3) w wierzchołkach pędów.

Substancje wyekstrahowane z tych tkanek przy pomocy metanolu rozdzielano na frakcję wodną i eterową. Frakcję wodną (a w niektórych przypadkach eterową) poddawano chromatografii bibułowej. Substancje eluowane oddzielnie z 10 pasków R_f każdego chromatogramu poddawano testowi biologicznemu na zawartość substancji czynnych w procesach wzrostu wydłużeniowego. Stosowano test prostoliniowego wzrostu odcinków koleoptile pszenicy, a w niektórych przypadkach testy wzrostu prostoliniowego odcinków koleoptile lub mezokotylu owsa.

W wyniku tych badań w tkankach modrzewia stwierdzono występowanie substancji hamujących wzrost wydłużeniowy, których zawartość zmieniała się istotnie w okresie wegetacji. Substancje te akumulowały się najpierw w igłach i wierzchołkach pędów, a następnie w tkankach korowych. Były to substancje rozpuszczalne w wodzie, zajmujące obszar R_f 0,7 do 0,9 chromatogramu (przy zastosowaniu fazy rozwijającej o składzie: izopropanol, amoniak, woda, w stosunku 10 : 1 : 1).

Uzyskane wyniki sprawdzono w dodatkowych badaniach dotyczących:

1) wpływu zmian w zawartości suchej masy w badanych tkankach w okresie wegetacyjnym;

2) wpływu przechowywania tkanek;

3) zmienności badanego materiału roślinnego na wynik oznaczeń inhibitorów wzrostu.

Rośliny, które rosły w warunkach laboratoryjnych, poddano działaniu nieprzerwanego oświetlenia i krótkiego dnia oraz dekapitacji. Po 21 dniach oddziaływania doświadczalnego oznaczono zawartość substancji stymulujących i hamujących wzrost w tkankach korowych.

Stwierdzono, że wykryte substancje typu inhibitorów najprawdopodobniej tworzą się w pełni wyrośniętych igłach, a wierzchołek pędu przypuszczalnie nie odgrywa roli przy zaopatrywaniu tkanek korowych w te substancje.

Wykonano wstępne badania niektórych właściwości biologicznych wykrytych substancji hamujących wzrost. W tym celu wyekstrahowane inhibitory poddawano testom biologicznym w obecności kwasu indolilooctowego w różnym wzajemnym stosunku koncentracji.

Inhibitory w wysokich koncentracjach obniżały działanie heteroauksyny. W niższych stężeniach obie substancje wykazywały minimalny synergizm działania.

Uzyskane w badaniach wyniki liczbowe poddawano analizie statystycznej z zastosowaniem testów o możliwie najmniejszej tolerancji błędów.

Na podstawie powyższych badań stwierdzono istnienie korelacji między zawartością substancji hamujących wzrost w tkankach korowych, a zmianami zachodzącymi w strefie kambialnej i typem tworzącego się drewna. Daje to podstawy do przypuszczenia, że wykryte substancje o charakterze inhibitorów wzrostu mogą być również, obok substancji stymulujących, odpowiedzialne za proces różnicowania słoja przyrostu rocznego drewna.

Wniosek ten przedyskutowano, uwzględniając istniejące hipotezy o udziale hormonów roślinnych w procesach związanych z różnicowaniem drewna.