Investigation on the annual ring of wood formation in european silver fir (*Abies pectinata* D. C.)

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Microscopical examination of the transversal sections of stem of young larch or older pine trees (Wodzicki 1960, 1962) showed remarkable correlation between the type of wood formed (early- or late-wood) and the changes in width of the separate growth layers in the cambial zone*. This correlation was considered as possibly reflecting some dependance of the type of differentiated xylem elements upon the duration of their differentiation in the cambial zone. The paper reports some further results of investigation on the annual ring of wood formation in Conifers, dealing with the problem of mentioned above correlation. The following questions were considered:

1. Seasonal changes of the width and structure of the cambial zone and of the duration of differentiation period of xylem elements.
2. Seasonal changes in the type of wood formed considering the radial diameter and cell wall thickness of tracheids.

MATERIAL AND METHODS

5-year old European silver fir trees (*Abies pectinata* D. C.) grown in Experimental Forests of SGGW in Rogów were collected at different times during the 1960 vegetation season. The dates of collection were as follows:

April 22, May 16, June 2, June 16, July 3, July 26, August 25, October 10. Each time, the basal part of stem from ten selected trees was cut off and preserved in ethyl alcohol.

The transversal, free-hand sections cut from the stem at comparable hight were examined after staining with safranin and light green and mounting in Canada balsam.

Measurement and observations were performed under the optical microscope (magnification: 1200×).

Within the cambial zone three layers were distinguished, namely: a) cambium — as a complex layer (Esau 1960), b) layer of the radial

* All the living cells which originated from cambial initials by means of longitudinal tangential divisions and are in the process of growth or differentiating for woody elements. This definition of the cambial zone does not include the cells differentiating for phloem elements but it was introduced as a working definition for convenience during the ealier research work on the formation of wood.

diameter growth of cells, and c) layer of differentiation — according to the earlier work on larch (Wodzicki 1960).

Two sections of each tree were examined. On each section, the number of cells in the separate layers of cambial zone and mature tracheids were determined along two perpendicular to each other radial files of cells formed during the last year.

For determination of the type of wood being actually formed, radial diameter and cell wall thickness of the four last, fully differentiated, consecutive tracheids were measured.

All the results are presented as the averages based on the data obtained from 40 radial files of xylem cells examined at the each term of plant collection.

Wherever necessary, standard deviation or t-test according to Snedecor's method was computed.

RESULTS

1. Structure of the cambial zone

The formation of new xylem elements was first observed in the trees collected on May 16. Width of the separate layers as well as whole cambial zone varied somewhat throughout the vegetation season (Tab. 1).

Table 1

Number of cells in the cambial zone and its separate layers at the different dates of plant collection

Date of plant collection		Number of cells			
		in the layer of			total in the cambial zone
		cambium	radial diameter growth of cells	differentiation	
May	16	4.6	2.4	4.7	11.7
June	2	4.3	1.6	4.3	10.2
June	16	3.4	1.4	4.8	9.6
July	3	3.8	1.3	6.2	11.3
July	26	3.9	1.1	7.1	12.1
August	25	4.1	0.2	6.6	10.9
October	10	4.3	0.0	0.0	4.3

Changes in the number of cells in the layer of cambium were rather small and only at the beginning and the end of season.

Width of the layer of radial diameter growth of cells gradually diminished as from the beginning towards the end of the season.

The number of cells in the differentiation layer was similar during May and June but a significant increase of the cell number was revealed at the beginning of July. Further rise in the cell number of this layer was

noted at the end of July. This wide differentiation layer was still observed at the end of August.

The total number of cells in the cambial zone was rather little varying during the season, although a visible decrease in the width of the zone was found during the first part of June which was followed by the subsequent increase up to the maximum observed at the end of July.

Plants collected in October showed the cambial layer matching fully differentiated tracheids. The layers of radial diameter growth and differentiation were no more observed.

2. Rate of xylem cell formation

Considering the radial increment of the total number of mature tracheids and cells in the cambial zone at different periods of the vegetation season, the rate of xylem cell formation from cambial fusiform initials in these periods may be deduced. Similarly, the increment of mature tracheids provides the information about the rate of completion of cell differentiation in the cambial zone.

Table 2

Rate of the new xylem elements formation from cambium and the rate of tracheid formation at different parts of the vegetation season 1960

Date of plant collection	Number of days between two successive dates of plant collection	Number of cells in the cambial zone plus tracheids	Increment of number of cells in the cambial zone plus tracheids	Rate of the new cell formation from cambium (cells per day)	Number of tracheids	Increment of tracheids	Rate of the completion of differentiation of tracheids (cells per day)
May 16		11.7			0.0		
	17		3.3	0.19		4.8	0.29
June 2		15.0			4.8		
	14		3.6	0.26		4.2	0.30
June 16		18.6			9.0		
	17		4.2	0.25		2.5	0.15
July 3		22.8			11.5		
	23		3.4	0.15		2.6	0.11
July 26		26.2			14.1		
	30		10.2	0.34		11.4	0.38
August 25		36.4			25.5		
	46		6.7	0.14		13.3	0.29
October 10		43.1			38.8		

The data brought up in Table 2 show that the rate of adding of the new xylem elements from cambium decreased by half in July, and then rose again reaching its seasonal maximum in August.

Changes in the rate of formation of the fully differentiated tracheids were somewhat different. Within the period of time: May 16 to June 2, there were formed at least 0.29 tracheids per day (the rate corresponding to this period of time cannot be exactly established due to the lack of information about the time of completion of differentiation of the first tracheid formed that year). During the next period (June 2 to June 16) the rate of formation of the mature tracheids kept at the same level. Since the mid of June until the end of July, however, a significant decrease was observed. During August, and very likely during September, the rate of completion of differentiation of tracheids rose very significantly again.

Data concerning the rate of xylem-cell formation during the last period (later than August 25) are less accurate and probably lowered as the mean time of completion of the differentiation process in the last tracheid formed that year was not established.

This also renders difficult the carrying out of any further analysis of the duration of cell differentiation in the cambial zone in this part of the vegetation season.

3. Duration of cell growth and differentiation in the cambial zone

Considering the number of cells in the separate layers of cambial zone, the increment of fully differentiated tracheids, and the rate of their formation, the attempt was made to determine the duration of the growth and differentiation of cells in the cambial zone during the successive investigated periods.

The duration of radial diameter growth and differentiation processes in the cambial zone is almost for sure somewhat different for each cell cut off from the cambium, as the continuous changes in the number of cells in both growth and differentiation layers throughout the season were observed. For the sake of clearing up the general seasonal pattern, however, only the duration of growth and differentiation of one particular cell in the cambial zone may be considered at each period under investigation. Calculations were performed for the most extreme (outer) cell in the layer of radial diameter growth observed at the each time of plant collection. The following formula was applied:

$$T = t + \frac{n - i}{r}$$

where:

T — period of time necessary for the completion of growth and differentiation of the most outer cell in the layer of radial diameter growth of cells, observed at any particular date of plant collection,

t — period of time between the particular date of plant collection and the following one,

- n — number of cells in the layers of radial growth and differentiation, at any particular date of plant collection,
 i — increment of the fully differentiated tracheids formed during the considered part of the season,
 r — rate of completion of radial growth and differentiation of cells in the cambial zone in the following part of the season.

In the other words, the formula represents the most probable time in which the cell which just passed from cambium to the radial diameter growth of cells layer was still alive in the cambial zone before it completed the processes of growth and differentiation and became the mature tracheid.

The analysis of variability of the cell number in the two layers of the cambial zone of different plants, by means of standard deviation, showed that the error produced by this variability does not influence the results much (Tab. 3).

Table 3

Approximate time of radial diameter growth and differentiation of cells in the cambial zone during the successive parts of the vegetation season 1960 in fir trees

Date of plant collection		Number of days between two successive dates of plant collection	Number of cells in radial diameter growth and differentiation layers of the cambial zone	Number of cells in differentiation layers of the cambial zone	Increment of tracheids	Rate of new tracheid formation	Time necessary for the completion of radial growth and differentiation of the most outer cell in the layer of radial diameter growth	Time necessary for the completion only of the differentiation of the most outer cell in the differentiation layer
		(t)	(n)	(n ₁)	(i)	(r)	(T)	(T ₁)
May	16		7.1 ± 0.4	4.7 ± 0.4			$17 + \frac{7.1 - 4.8}{0.30} = 25$	$17 + \frac{4.7 - 4.8}{0.30} = 17$
	17				4.8	0.29		
June	2		5.9 ± 0.2	4.3 ± 0.2			$14 + \frac{5.9 - 4.2}{0.15} = 26$	$14 + \frac{4.3 - 4.2}{0.15} = 15$
	14				4.2	0.30		
June	16		6.2 ± 0.3	4.8 ± 0.3			$17 + \frac{6.2 - 2.5}{0.11} = 50$	$17 + \frac{4.8 - 2.5}{0.11} = 38$
	17				2.5	0.15		
July	3		7.5 ± 0.3	6.2 ± 0.3			$23 + \frac{7.5 - 2.6}{0.38} = 46$	$23 + \frac{6.2 - 2.6}{0.38} = 32$
	23				2.6	0.11		
July	26		8.2 ± 0.3	7.1 ± 0.3				
	30				11.4	0.33		
August	25		6.8 ± 0.3	6.6 ± 0.3				
	46				13.3	0.29		
October	10		0.0	0.0				

It is obvious that the most outer cell in the layer of radial diameter growth of cells was still alive during the whole investigated period of time if the increment of number of mature tracheids during this period was smaller than the total number of cells in the layers of radial diameter growth and differentiation. The second part of formula may introduce

some error to the calculation as it is based on the mean rate of tracheid formation in the following investigated part of the season. This error is smaller as the difference between cell number in the examined layers of the cambial zone and the number of tracheids produced during the first investigated period is smaller. Thus, it could be reduced by a suitable frequency of plant collection.

In the present investigation, this could not be fully achieved during the latter part of season. Nevertheless, the results are considered very probable as during the first part of summer plants were collected frequently enough, and during the second — mean rate of tracheid formation was not differing significantly — at least during the two subsequent periods: June 16 — July 3, and July 3 — July 26 (Tab. 3).

Analogical calculations of the period of time required only for the differentiation in the cambial zone may be performed. This time, expressed in Table 3 as T_1 , and the number of cells in the layer of differentiation — as n_1 , are introduced to the formula instead of T and n .

As follows from Table 3, until mid of June, the radial diameter growth and differentiation of cells in the cambial zone were completed within about 25 days. The completion of these processes during July and very probably during August required twice as long time. This difference is even more pronounced when the duration of cell differentiation only is considered. Cells were differentiating about 15—17 days and about 38 days respectively at the two above mentioned periods of the season.

4. Seasonal Changes in the type of wood formed

As mentioned in the methodical part, for the purpose of determining of the type of wood being actually formed, the radial diameter and cell wall thickness of four lastly differentiated tracheids were measured. However, at the first term of plant collection there were not yet formed all four mature tracheids along each of the radial files of cells. The type of wood of these plants was determined considering only two last fully differentiated tracheids.

It is seen from Table 4 that until the mid of June only the thinwalled tracheids were formed, but at the beginning of July the cells with significantly thicker cell walls were observed. The cell walls of tracheids formed during July were increasingly thicker and at the end of August it was found that the tracheids formed during this month were of the maximal thickness of cell walls.

* It means, the period of time required for completion of the different stages of cell differentiation as from the time when no visible dimensional changes of the cell could be observed until the protoplasm completely disorganises inside the xylem conducting element.

Results dealing with the radial diameter of tracheids showed that only the cells formed at the very beginning and the end of vegetation period differ significantly.

Table 4

Cell wall thickness and radial diameter of 2 or successive tracheids from cambial zone in radial direction at different dates of the vegetation season

Successive tracheids from the cambial zone	Dates of plant collection											
	June 2		June 16		July 3		July 26		August 25		October 10	
	<i>t</i> *	<i>d</i> **	<i>t</i>	<i>d</i>	<i>t</i>	<i>d</i>	<i>t</i>	<i>d</i>	<i>t</i>	<i>d</i>	<i>t</i>	<i>d</i>
4			3.0	18.4	3.8	15.0	4.9	14.7	6.9	15.8	6.9	14.1
3			3.1	16.7	4.1	14.9	5.2	14.7	7.0	15.8	7.0	13.6
2	2.6	18.5	2.9	16.3	3.4	14.3	5.6	15.6	6.8	15.6	6.8	13.4
1	2.6	17.5	3.0	15.4	4.7	14.4	5.6	15.3	7.0	15.3	7.0	13.2
Averages	2.6	18.0	3.0	16.7	4.2	14.6	5.3	15.1	6.9	15.8	7.1	13.6

$$\mu t_t = 0.3, \quad \mu t_d = 1.0$$

* Cell wall thickness.

** Radial diameter.

Occasionally, during the whole season, the differences among the average radial diameter of tracheids exceeded somewhat over the lowest significant difference. However, taking into account the high level of variability, this observation was rather of limited value for the conclusions.

DISCUSSION

The investigation showed that the seasonal changes of the width of cambial zone in 5-year old European silver fir trees were connected with the different rate of adding of the new cells from cambium and the rate of fully differentiated tracheids formation.

Until the mid of June, completion of the differentiation was somewhat faster than adding of the new cells from cambium. It produced narrowing of the cambial zone observed in plants collected at the mid of June. During the second part of June, however, the rate of mature tracheid formation decreased by half while the mean rate of adding of the new cells from cambium was not visibly changing until July. Owing to this situation, a repeated increase of cell number in the cambial zone was observed in plants collected at this time. During July a very significant decrease of adding of new cells from cambium was observed with nearly no change in the rate of tracheid formation. The cambial activity as well as the rate of tracheid formation increased markedly again and kept stable at the high level during August, so that only

a small change in the width of cambial zone occurred. At the end of season, cambial activity greatly decreased but the rate of tracheid formation was found to be very high. This produced the completion of differentiation process within all the cells in cambial zone except the layer of cambium and at the first decade of October no differentiating cells in the cambial zone were observed, and the cambium very probably became dormant.

The results show the temporary depression of cambial activity in young trees of fir occurring in the mid of summer. The same depression was earlier observed with 44 — year old *Pinus silvestris* (Wodzicki 1962) and still earlier reported for other tree species by different authors (Mischke 1890, Jost 1892, Brown 1915, Korstian 1921, Lodewick 1925).

The analysis of changes in the width of cambial zone and the rate of xylem cell formation made it possible to take a further step in determination of the duration of the radial diameter growth and differentiation processes in the cambial zone. The period of time required for completion of both the radial diameter growth and differentiation of cells in the cambial zone was proved to be twice as long in the second part of summer as at the beginning of the vegetation season. It took about 50 and 25 days respectively in the trees under study.

It is noteworthy, that not only the general period of life of the cell in the cambial zone was extended during the second part of summer, but the part of it corresponding to differentiation of the cell was extended as well. Thus, the cell differentiation period occupied about 58—68 per cent (15—17 days) of the total life period of the cell after adding from cambium in the first part of the season, but it increased up to 70—76 per cent (32—38 days) in the second.

The change of the duration of differentiation occurred in the second part of June which was the time when first tracheids with significantly thicker cell walls formed. At the same time only the diminution of the rate of mature tracheid formation was observed and no decrease of the addition of the new cells from cambium occurred. The depression of cambial activity was revealed subsequently in July. The facts seem to show that the thickwalled tracheid (late-wood) formation was not directly connected with the fall in the activity of cambium as it was earlier suggested by Lodewick (1925), and Kienholz (1934). This becomes apparent when the fact is considered that the activity of cambium (as well as the rate of mature tracheid formation) increased very significantly again in the second part of summer but no change in the cell wall thickness of tracheids occurred and all the tracheids formed at that time were increasingly thickwalled.

This conclusion is in accordance with the results of earlier work carried out on larch on different experimental basis (Wodzicki 1961).

Those experiments showed no direct dependance of the thickening of cell walls of differentiating tracheids upon the cambial activity in the young larches.

The correlation between the type of wood formed and the changes in width of the cambial zone observed earlier in larch was found to occur also in the young trees of fir. Furthermore, the partial correlation between the type of wood formed (early- and late-wood) and the duration of cell growth and differentiation was showed. On the basis of the present investigation, it is still extremely difficult to establish how close is the correlation between the cell wall thickness of each tracheid and duration of its differentiation in the cambial zone. Nevertheless, the provisional calculation showed that the cell observed as the most outer in the differentiation layer at June 2, corresponds to the most outer tracheid measured in plants collected at June 16, which reached the cell wall thickness 3.0 microns. The cell wall thickness of the analogical tracheid observed in June reached 5.6 microns as it corresponds to the last or last but one tracheid measured in plants collected on July 26. The duration of differentiation of these cells was 15 and 38 days respectively. This would indicate that the intensity of the processes of thickening of cell walls of the second cell was somewhat lower during its differentiation. Thus, the intensity of cell wall thickening varied during the season as well as the duration of cell differentiation in the cambial zone.

Radial diameter of tracheids is assumed to characterise the type of wood produced during the season. It is commonly accepted as a feature which makes it possible to distinguish between the early- and late-wood of many species. Even taking into account the high level of the variability of the results obtained by measuring the radial diameter of tracheids, it is seen, that there was nearly no positive correlation between the calculated probable time corresponding to the period of radial diameter growth in the cambial zone and the mean radial diameter of tracheids produced in the two parts of season.

It took about 9 days to accomplish the radial diameter growth of cells in the cambial zone during the period: mid of May — mid of June, and about 11 days in the second part of June. The mean radial diameters of tracheids produced during these periods were 16.7 microns and 14.6 microns respectively. Radial diameters of tracheids produced during July and August were on average 15.1 and 15.8 microns (almost significantly smaller than those produced during the first part of June) but the time of growth in radial diameter of these tracheids was calculated about 12 and 14 days. The only conclusion which may be reached from these observations is that the intensity of radial diameter growth of cells in the cambial zone during the second part of the season was significantly lower than during the first.

These conclusions throw a new light on the possibility of dependence of the type of wood formed upon the duration of growth and differentiation of tracheids as implied by the correlation between the seasonal changes in the width of different layers of the cambial zone and the seasonal changes in the wood formation. The type of tracheid formed would be not only related simply to changes of the duration of growth and differentiation but to the changes of intensity of thickening of cell walls and intensity of radial diameter growth of cells in the cambial zone as well. There is still nearly no information about the seasonal course of these changes.

SUMMARY

The seasonal changes of the width and structure of the cambial zone and the duration of differentiation period of xylem elements were investigated in 5-year old *Abies pectinata* D. C. trees.

The cell number in the cambial zone was varying due to the different rate of adding of new xylem cells from cambium and the rate of completion of tracheid differentiation throughout the season. The correlation between the width of cambial zone and the type of wood formed (taking into account mainly the cell wall thickness of tracheids of the early- and late-wood) was observed as in the earlier work on larch and pine.

Considering the number of cells in separate layers of the cambial zone, the increment of fully differentiated tracheids and the rate of their formation, the attempt was undertaken to determine the duration of growth and differentiation of certain cells in the cambial zone after adding from cambium. In this way, further partial correlation between the type of wood formed and the duration of radial diameter growth and differentiation of cells in the cambial zone was shown. The provisional calculation showed, however, that the correlation was probably not very close. The type of tracheid formed, even if related to the duration of its differentiation (what might be considered to be reflected by the correlation) would be very probably conditioned by the seasonal changes of the intensity of cell wall thickening and especially the seasonal changes of the rate of radial diameter growth in the cambial zone as well.

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