ORIGINAL (論　文)

Cambial Activity and Radial Growth in SUGI Trees (Japanese Cryptomeria)*

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伊東隆夫**・林　昭三**・貴島恒夫**：スギにおける
形成層活動と放射方向の生長について*

Introduction

A number of papers have been presented in relation to the process of diameter growth of trees by the division and multiplication of cambial cells. Since the word "cambium" was given by DU HAMEL at the beginning of eighteenth century[1], different hypotheses on the mechanism of cambial division have been proposed. One of these has been called the initial theory[2], i.e. each division of the initial cells produces two daughter cells of which one remains as an initial while the other develops into a xylem or a phloem mother cell. There have been such other opinions that all cambial cells are originally similar in their capacity for division[2]. However, the former hypothesis seems to be accepted in general at present.

Cell division of coniferous cambium is generally classified as two kinds, i.e. the periclinal and anticlinal. The former type of division brings about xylem and phloem growth, consequently radial growth of tree, and the latter type of division results in circumferential expansion by the multiplication of fusiform cambial initials. Longitudinal elements of wood tissues are primarily produced by both types of division. Observations of the meristematic activity of the cambium and of the process of cell production hitherto been made seem to be still unsatisfactory.

It is technically difficult to obtain suitable sections for observation of cambial zone, because cambial cells, which are soft and flexible, are situated between harder xylem and phloem tissues. In this study, by using sections obtained by the ultra-thin sectioning methods used in electron microscopy, the followings were investigated: (1) usual position of the first divisicn in dormant cambial cells, (2) activity for redivision of each cambial cell, (3) seasonal cambial activity, and (4) phloem development.

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Materials and Methods

Samples were taken from Sugi (*Cryptomeria japonica* D. Don, ca. 50 years old) trees grown in the campus of our Institute, Uji, Kyoto. To observe vernal cambial multiplication, three trunks were chosen, and specimens were collected every other day from the time of inception of cambial division to the time of outer layer deposition of secondary wall. To investigate seasonal change of xylem and phloem development, another trunk was chosen and specimens were taken at a week intervals from March 1. to November 22. in 1967. Each specimen ($t \times l \times r = 10 \times 15 \times 7$ mm in size) containing cambial zone was removed from the tree trunk at 10 cm intervals within a portion of 50 cm above or below breast height of the trunks, and the parts from which specimens were taken were filled with vaseline to protect them. The specimens obtained were carefully chopped with a razor brade into small chips (ca. $0.1 \times 0.3 \times 0.2$ mm). Then the chips were fixed in FAA solution and embedded in epoxy resin according to the Luft's method[3]. To make slides of cambium for observation, each chip was cut into then transverse sections ($3 \sim 5\mu$) by a ultra microtome for electron microscopy, and the sections obtained were mounted on slide-glasses with Biolite*.

In order to know the position of first cambial division, as many slides as possible were prepared from the chips at the stage of incipient division of three trunks and were observed. The results are shown in Table 1. To obtain radial growth curve of the cell amount during the growing season, total number of newly formed xylem cells from

Table 1. Cambial layer and the first cell division.

Cambial layer	Tree No.	D	C_1	C_2	C_3	C_4	O	Total	
3 cells wids	1	2	2	1	0	—	2	7	
	2	3	2	0	0	—	6	11	
	3	32	35	11	0	—	13	91	109
4 cells wide	1	34	36	10	0	0	9	89	
	2	48	52	3	5	7	21	136	
	3	143	71	13	2	1	9	239	464
5 cells wide	1	36	20	0	0	0	8	64	
	2	21	20	0	0	0	3	44	
	3	2	0	0	0	0	0	2	110
6 cells wide	1	3	3	1	0	0	0	7	
	2	1	0	0	0	0	0	1	
	3	3	0	0	0	0	0	3	11

D: Number of radial cambial rows of still dormant state, i. e. without dividing cell.

$C_1 \sim C_4$: Number of radial cambial rows of which only each cell corresponding to C_1, C_2, C_3 or C_4 in Fig. 1 has divided.

O: Others; which include, e.g. cambial layers with 2 or more dividing cells.

* A kind of synthetic resin.

Table 2. Seasonal change of numbers of xylem, cambium and phloem cells.

Sampling day		a	b			c
			min	mean	max	
March	1	3.9	3	3.9	5	
	8	3.9	3	3.9	5	
	15	3.9	3	3.9	5	
	22	4.1	3	4.1	5	
	29	4.9	4	4.9	7	
April	5	10.0	6	7.8	11	
	12	15.1	5	7.9	10	+*
	19	16.6	5	6.5	8	2
	26	25.5	6	7.7	10	2
May	3	26.4	5	6.1	8	4
	10	29.9	3	5.1	7	4
	17	30.4	4	4.6	6	4
	24	37.1	3	4.8	7	4
	31	36.5	2	4.0	6	6
June	7	36.1	2	3.5	5	6
	14	36.2	3	3.9	5	8
	21	47.1	3	4.4	6	10
	28	36.1	3	3.8	6	10
July	5	40.0	4	5.9	8	10
	12	56.0	5	7.0	9	12
	19	54.7	5	6.8	8	12
	26	71.6	5	5.6	7	16
Aug.	2	56.7	3	4.4	6	14
	9	70.3	3	4.2	6	16
	16	77.4	2	3.6	6	14
	23	50.5	3	3.7	5	16
	30	66.8	2	3.3	5	16
Sep.	6	77.6	3	3.8	5	22
	13	51.7	2	3.6	5	20
	20	60.1	3	4.0	5	18
	27	61.7	2	3.3	4	20
Oct.	4	65.3	3	3.4	4	22
	11	55.6	2	3.5	4	18
	18	113.2	3	4.2	5	24
	25	62.6	3	3.7	4	22
Nov.	1	58.5	2	2.7	5	20
	8	49.8	1	2.3	3	16
	15	51.8	3	3.4	4	16
	22	54.6	2	3.3	5	18

a : From initial to preceding latewood cell.
b : Cambial zone cell.
c : Newly formed phloem cells.
* Initiation of phloem.

initial and newly formed phloem cells were counted respectively on every sampling day by averaging cell number of 40 radial cell rows (Table 2). On the basis of this Table, growth curves in cell number of xylem and phloem were obtained. The number of cells of cambial zone was also counted by averaging cell number of 40 radial cell rows to inquire into seasonal periodicity of cambial activity (see "b" column of the Table). Actual boundaries between dividing and non-dividing cells were determined by recognizing the part where radial cell enlargement has began. These observation were carried out by using phase contrast microscope and polarization one.

Results and Discussion

1. Vernal Activity of Cambial Division

Before the initiation of division, the width of cambial zones ranged from 3 to 5 cells as can be seen in Photo 1*. In Photo 2, a little larger cell continuing to immature phloem cells which were generally more birefringent than cambial cells might be considered as initial cell. The reason will be discussed later.

As can be seen in Table 1, a cambial zone was most commonly consisted of 4 cells in width. Thus, it was inquired that which cell of the 4 cells of cambial zone would

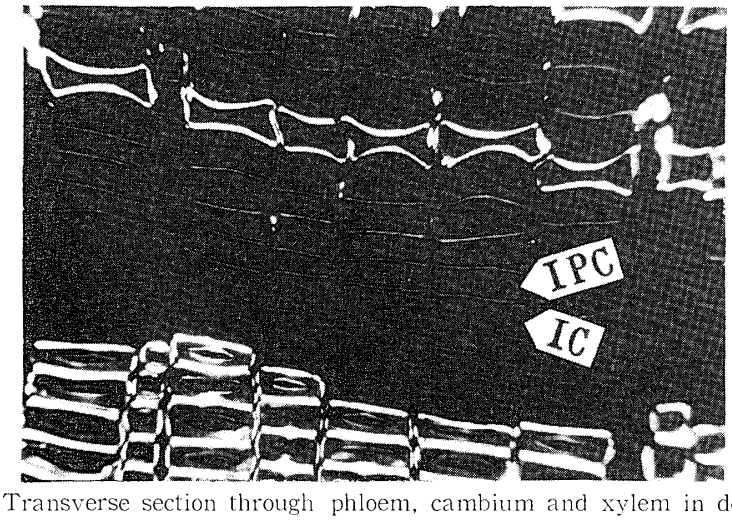

Photo. 1. Transverse section through phloem, cambium and xylem in dormant state. Cambial cell width is variable. (x), ×150.

* Addendum—Explanation of the symbols in Photos 1-14.

IC : Initial cell
IPC : Immature phloem cell
IPF : Immature phloem fiber
PF : Phloem fiber
PMC : Phloem mother cell
PP : Phloem parenchyma cell
SC : Sieve cell

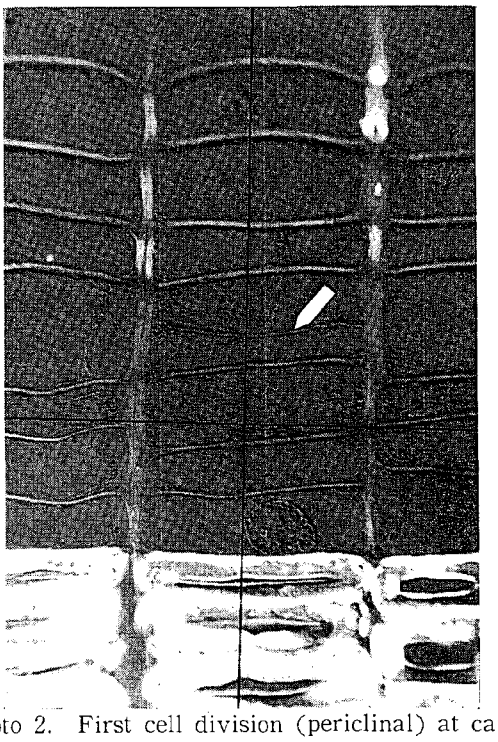

Photo 2. First cell division (periclinal) at cambial initial. Arrow shows new cell wall. (x), ×590.

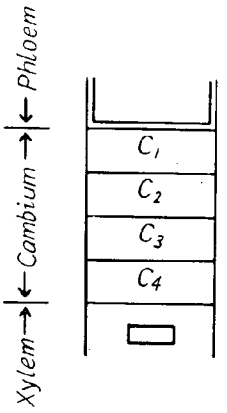

Fig. 1. Model of 4 cambial cells.

divide at first.

For convenience, these 4 cells are called C_1, C_2, C_3, and C_4 respectively from the phloem side as shown in Fig. 1. In this case, C_1 was considered as the cambial initial and the frequency rate [$C_1/(C_1+C_2+C_3+C_4)$ in Table 1] of first division of C_1 was exceedingly high shown as follows:

The tree No. 1 ..... 78.3%
No. 2 ..... 77.6%
No. 3 ..... 81.6%

BANNAN[4], and GRILLOS and SMITH[5] stated that the inception of division occured generally in cambial cells nearest the mature xylem. As its reason, BANNAN has suggested that water supply might be important on the resumption of growth. However, the results obtained in the present experiment were quite different. The rate of C_1 division was followed by that of C_2 one, but subsequent frequency order of cell division was uncertain between C_3 and C_4.

The following results are about the frequency of division which took place until the cells induced by the division of each C_1, C_2, C_3, and C_4 cell [derivatives of each cell can be identified by thicker (Photo 3, arrows) and the more birefringent (Photo 4, arrows) parent cell walls than others] initiated their secondary wall deposition, and about the

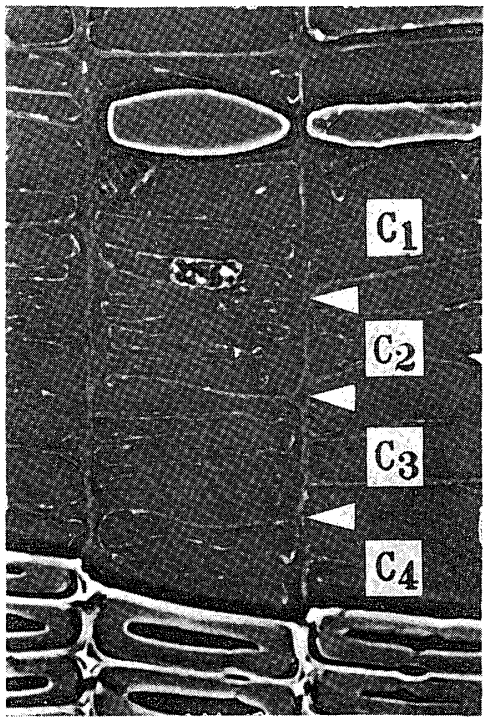

Photo 3. C_1-, C_2-, C_3-, and C_4-derivatives can be distinguished each other from their parent cell walls which are thicker than their daughter ones (arrows). C_3-derivative show 4 cells derived. (x), ×470.

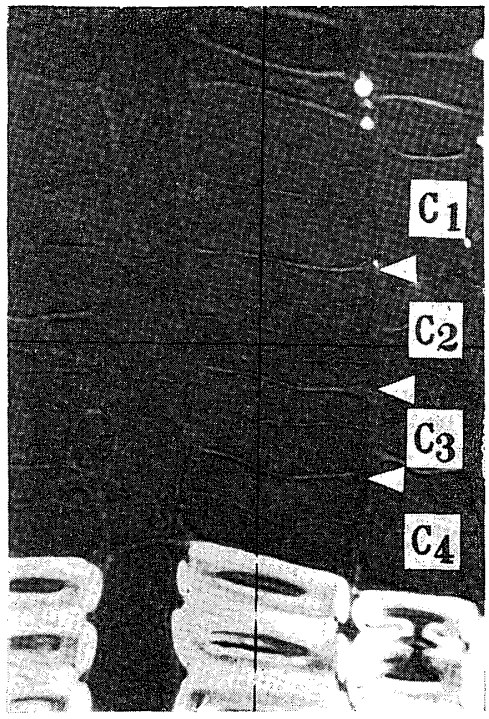

Photo 4. C_1-, C_2-, C_3-, and C_4-derivatives can be distinguished each other from their parent cell walls, wnich are birefringent (arrows). (x), ×590.

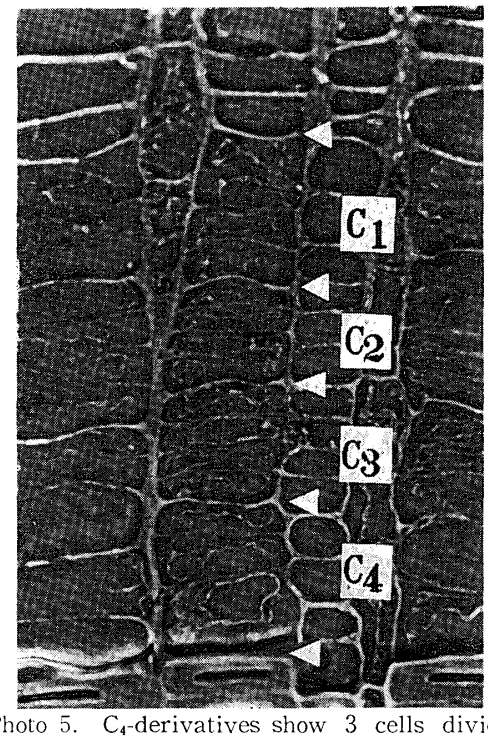

Photo 5. C_4-derivatives show 3 cells divided. (x), ×470.

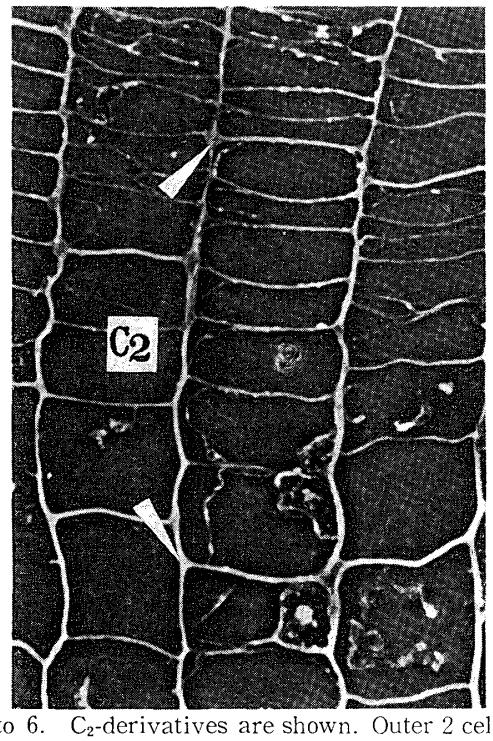

Photo 6. C_2-derivatives are shown. Outer 2 cells seem to have the ability for further division. (x), ×470.

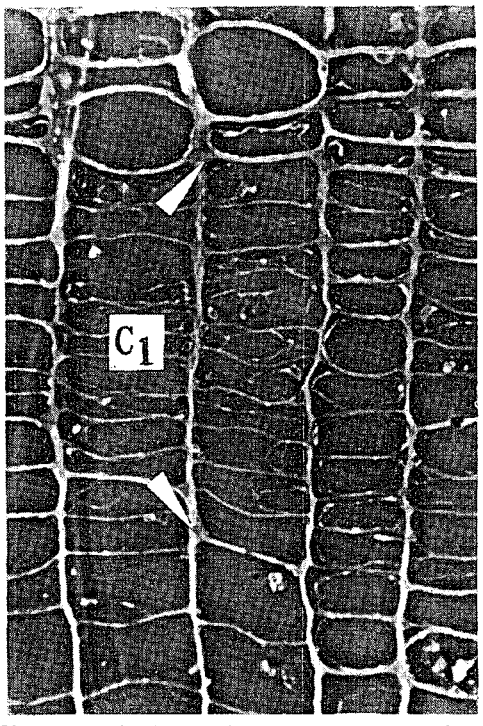

Photo 7. C_1-derivatives produced by 9 divisions, which include 2 immature phloem elements and 8 cambial cells. (x), ×470.

total cell numbers resulted. The differentiation of C_4 cell generally took place after no division, or after 1 division producing 2 derivative cells, or rarely after 2 divisions producing 3 derivative cells (Photo 5). The differentiation of C_3 cell took place after only 1 division, or after 2 divisions producing 3 cells, or rarely after 3 divisions producing 4 cells (Photo 3). In the case of C_2 cell, totally 3 to 7 cells were produced after 2 to 6 divisions. Among them, it was the most common to produce 6 cells in amount after 5 divisions. Supposing the extent of differentiation before the initiation of secondary wall thickening, some of C_2-derivatives might still have the capacity for 1 or 2 divisions (Photo 6). C_1-derivatives became totally 4 to 10 cells after 3 to 9 divisions before the initiation of secondary wall deposition (Photo 7). Then the cell number in radial direction might be controlled in cambial zone in such a way that C_1-derivatives are many in number when C_2-derivatives are few and vice versa.

In dormant condition, cambial initial was to be either C_1 or C_2 in Fig. 1. If C_2 was cambial initial, C_1 had to become a phloem mother cell. However, it is said that phloem mother cell layer is uniseriate or absent[6], which was true in our study. In fact, C_1-derivatives were 4 to 10 cells as mentioned above, and this indicates that C_1 cell divided more than one time. From this discrepancy, C_1 ought to be considered as a cambial initial, and phloem mother cell which could not be seen in dormant condition ought to to be produced by the division of C_1 cell or its first derivative.

Multiplication of C_1 cell of which derivatives include initial cell continued till the cessation of cambial activity. Thus, it would be concluded that most of xylem cells belong to C_1-derivatives. Presenting in number, C_2-derivatives were 6 cells, and C_3-derivatives and C_4-derivatives were 3 cells and 2 cells on an average, respectively. And newly formed xylem cells in this growing season amounted to 60 cells (from Fig. 3). Thus, it would be suggested that about 80% of xylem cells could be produced originally from C_1 cell and the remaining 20% produced from C_2, C_3 and C_4 cell. Besides, all of phloem cells could belong to C_1-derivatives because phloem mother cell was produced by it.

2. Observation of Xylem Development

The observation results obtained during the period from March 1. to November 22.

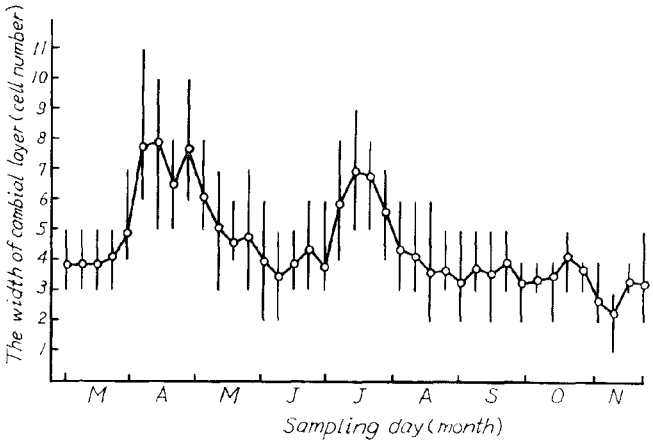

Fig. 2. Seasonal change of cambial activity based on "b" column in Table 2.

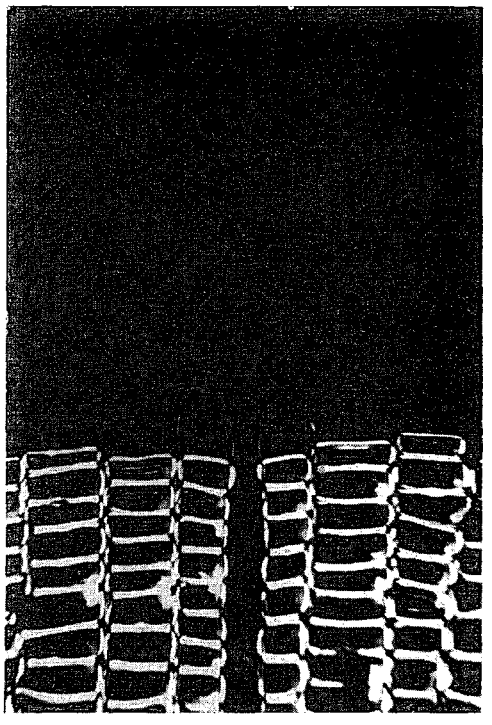

Photo 8. Initiation of secondary wall thickening. A few cells adjacent to precedingly formed latewood are birefringent. (x), ×250.

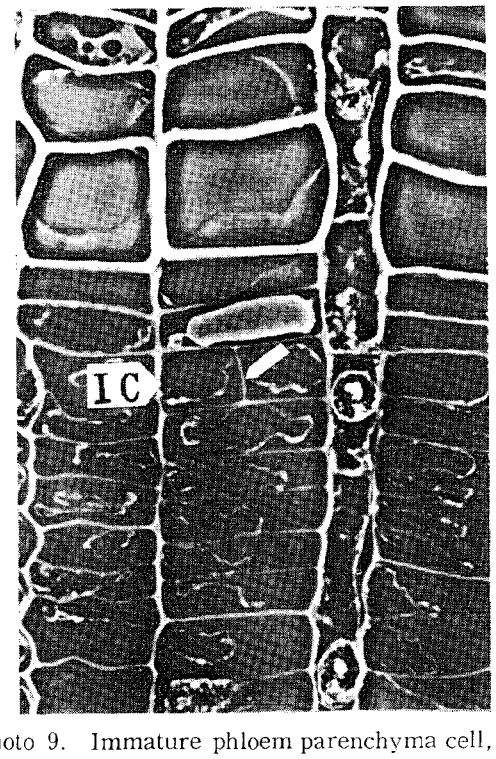

Photo 9. Immature phloem parenchyma cell, filled with resinous material, adjacent to initial cell whick has newly formed radial wall by anticlinal division (arrow). (x), ×470.

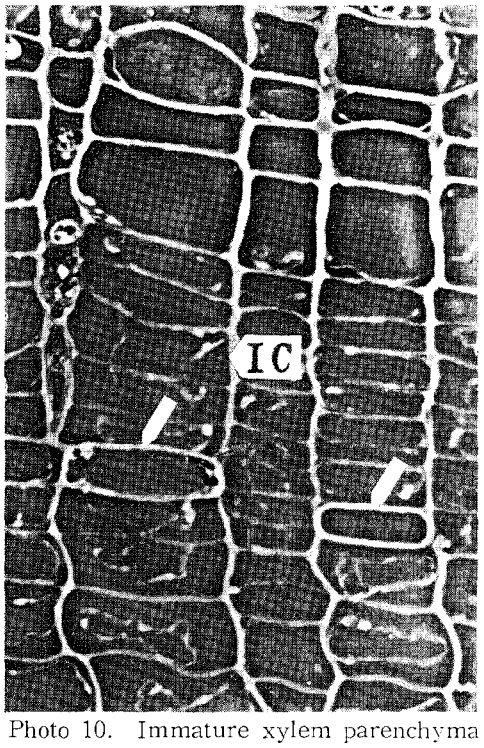

Photo 10. Immature xylem parenchyma lined or filled with resinous material (arrows). (x), ×470.

1967 were as follows. Cell division in cambial zone began on March 29., and the number of cambial cells which is regarded as the criterion of cambial activity increased acceleratedly in radial direction within one or two weeks. On April 5., it almost amounted to maximum i.e. 6 to 11 cells, average 8 cells, in width (Fig. 2). On April 19., the outer layer of secondary wall was first deposited in this season (Photo 8). Then cambial activity decreased and the firstly formed xylem and phloem parenchyma cells filled with brown resinous material could be seen simultaneously on May 10 (Photos 9 and 10).

It seems likely that temporary midseason pause takes place from May 24. to 31 (Fig. 3). In this study, as main environmental factors relating with tree growth, temperature and rainfall were recorded through the growing season as shown in Figs. 4 and 5. By comparing these Figures with seasonal change of cambial activity (Fig. 2), the

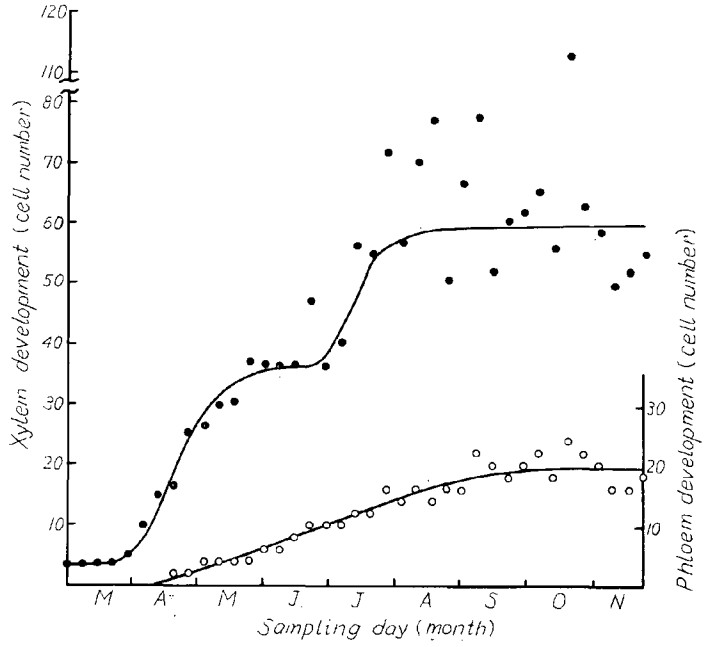

Fig. 3. Growth curves of xylem and phloem development based on "a" and "c" columm in Table 2. That of phloem doesn't show temporary midseason pause.

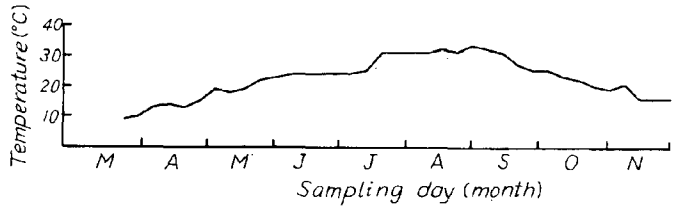

Fig. 4. Temeperature during the growing season.

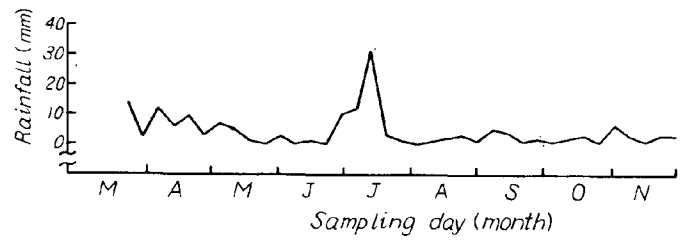

Fig. 5. Rainfall during the growing season.

occurence of a midseason pause seemed to be more dependent on the rainfall but less to the temperature. Midseason pause became almost perfect on June 28. In this case, the width of cambial zone layers was similar to that as before the inception of division as seen in Photo 11. On July 5., the activity of cambial division initiat ed again, which is mainly due to rainfall begun from a week before. Also in this case, the width of cambial zone layers increased acceleratedly at first as similar to the inception of vernal division and no secondary thickening of their cells could be seen in a first week.

Cambial activity almost ceased on October 11., when imperfectly thickened cells could be seen in 3 to 5 layers. Until November 22., secondary wall thickening did not completely stop in some of the last formed xylem cells.

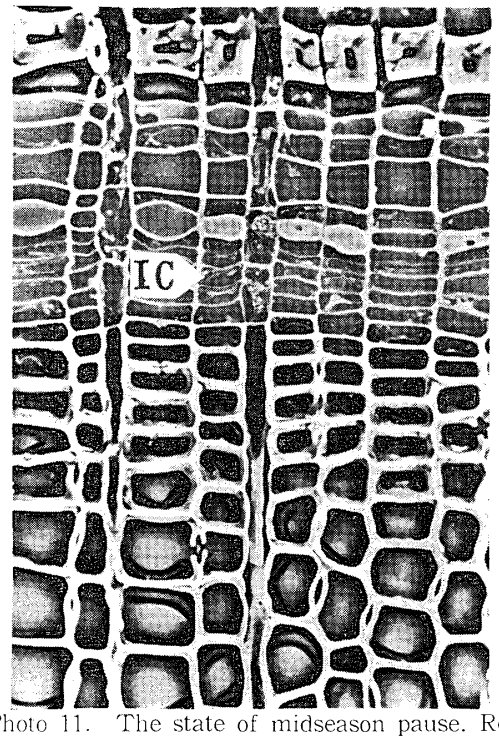

Photo 11. The state of midseason pause. Recently formed xylem elements do not show earlywood but latewood type. (x), ×250.

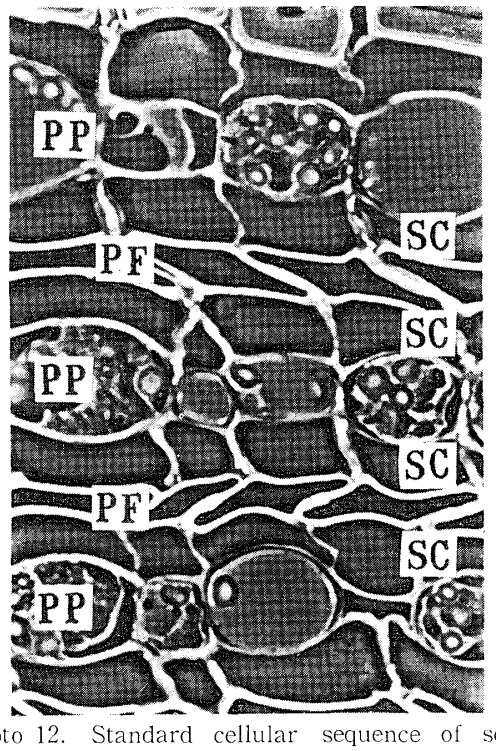

Photo 12. Standard cellular sequence of secondary phloem. (x), ×470.

3. Observation of Phloem Development

Phloem mother cells which were not seen before the inception of cambial division were produced by the first division of the initials or their first derivatives, i.e. their daughter cells. Phloem cells began to be produced from these mother cells on April 12. A regular sequence of secondary phloem cells through a growing season was sieve cell – parenchyma cell – sieve cell – fiber – sieve cell – parenchyma cell – sieve cell – fiber – and so forth (Photo 12). Therefore either classes of a sieve cell (outward) and a fiber (inward) or a sieve cell (outward) and a parenchyma cell (inward) was produced by

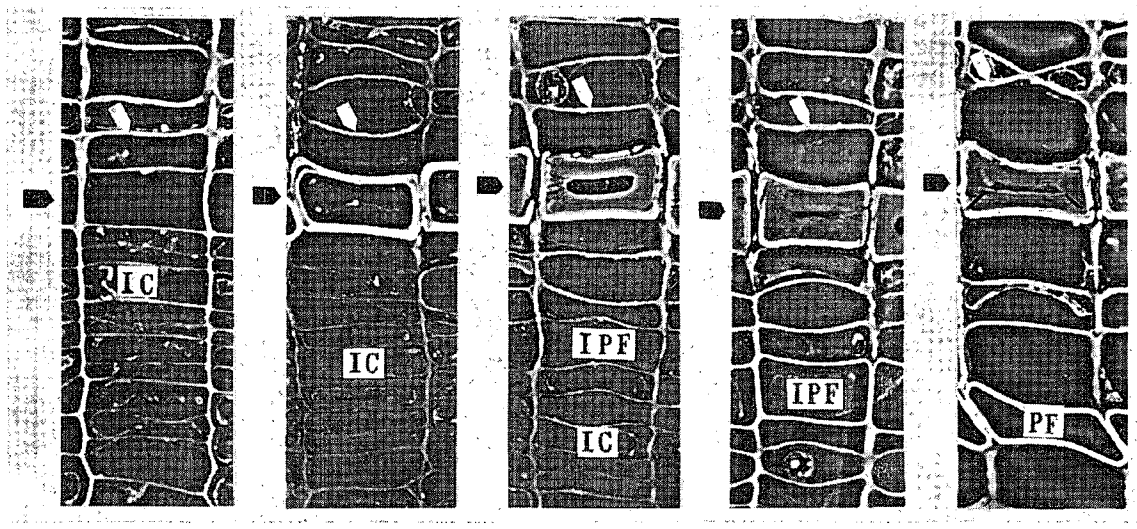

Photo 13. Developing stage of phloem fiber.
First formed phloem fiber developes into thick-walled cells (black arrows). The second one becomes common phloem fiber which is deformed to mature. White arrows show the boundary of phloem increment. (x), ×360.

the division of phloem mother cell in almost uniform rate (Fig. 3), hence the layer of sieve cells was always arranged outward. Photo 13 shows the development of phloem fibers. The first layer of phloem fibers developed in the growing season had always thicker walls than the others and it is said that the layers of these thicker walled cells are useful to detect phloem increments in SUGI trees[7]. And it was more likely that the true boundary of a phloem increment situated between sieve cell layer adjacent to outward the layer of thicker walled phloem fibers and parenchyma cell layer produced at the end of the preceding season, as the sieve cells were the first formed elements in every growing season.

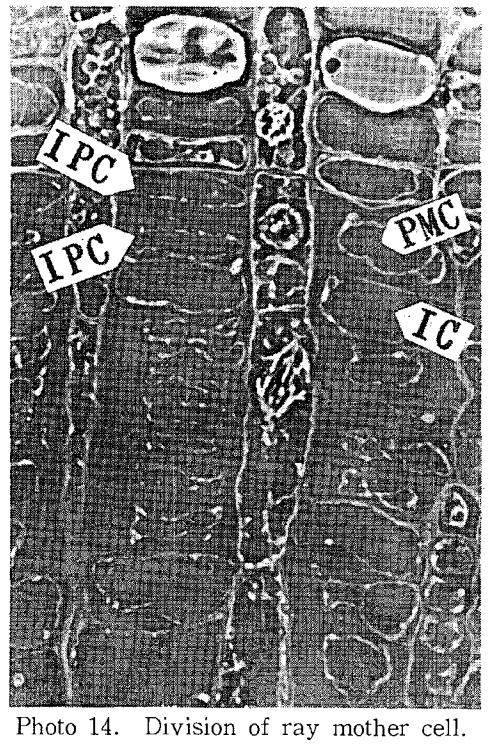

Photo 14. Division of ray mother cell. (x), ×470.

4. On the Type of Cell Division

It has been known that two general types of cell division, periclinal and anticlinal, occur in fusiform cambial cells.

The former (Photo 2) was very common in cambium, while the latter (Photo 9) could not be seen so often, especially in the sample trees examined.

On the other hand, transverse elements,

i. e. ray cells, have been said to increase their number by the division of ray initials in radial direction[8]. As shown in Photo 14, the division could be seen in somewhat longer ray parenchyma cells over the tangential layer of cambial initials. This demonstrates that the dividing cell is not a ray initial but a ray mother cell. Division could not be seen in the center of the cell but in slightly phloem side of it.

Summary

Cambial activity and radial growth of SUGI (Japanese Cryptomeria) trees during a whole growing season in 1967 were investigated by the microscopic observation of the ultra-thin sections obtained by applying sectioning technique of electron microscopy. The tangential wall of parent cambial cells before division in transverse sections was more birefringent than that of their derivative cells produced preceding the initiation of secondary wall thickening. From these facts, vernal activity of cambial division is discussed histologically in detail.

The results obtained are summarized as follows:

1. In dormant condition, the cambial zone was mostly consist of 4 cells including no phloem mother cell.
2. The first division of cambial cells occurred mostly in fusiform cambial initials.
3. Within a growing season, 80% of xylem cells and all of phloem cells were derived from the division of the outermost cambial cell (initial) adjacent to immature phloem cells in dormant condition.
4. Cambial activity reached maximum from April 5 to 26 during which the outer layer of secondary wall began to deposit, i. e. on April 19.
5. Xylem and phloem parenchyma cells were produced simultaneously accompaning the decline of cambial activity on May 10. This may mean a close correlation between the decline of cambial activity and the food storing capacity of parenchyma cells in this species, SUGI.
6. Boundary of phloem increment situated between the layer of phloem parenchyma cells produced at the end of preceding season and that of newly formed sieve cells continuous outward to the first formed layer of phloem fibers having thicker walls.
7. Besides ray initials, ray mother cells might be existent as the source of producing ray cells.

要　　約

電子顕微鏡用の超薄切片作成技術を光学顕微鏡に適用し，スギの形成層活動および放射方向の生長について研究した。分裂開始前における形成層細胞の接線方向の膜は木口切片で観察した場合，２次膜の肥厚が開始するまでに生成される細胞の膜よりもいくぶん複屈折率が大きかったが，この事実に基づいて，とくに春季の形成層の活動状態が組織学的に一層詳しく論じてある。得られた結果は次のとおりである。

1. 分裂開始前において，形成層帯の細胞数は4個の場合が圧倒的に多く（仮に，これら各細胞を師部側から順に C_1, C_2, C_3, C_4 と名づける），このとき形成層には師部母細胞は存在しなかつた。

2. 形成層の細胞が最初に分裂を開始するとき形成層帯のうち最外側の細胞，すなわち，形成層始原細胞（C. I.）で分裂する割合が圧倒的に多かつた。

3. 分裂開始前において，未成熟な師部の細胞に木部側に隣接している形成層細胞 C_1（C. I. にあたる）の分裂によつてその年に生産される全木部要素の80%，師部要素のすべてが生み出される。C_2 は2～6回分裂して3～7細胞となつた後に分化するが，さらに1～2回分裂能力を有する細胞もあると考えられる。C_3 は1～3回分裂して2～4細胞となつた後に分化する。C_4 は分裂せずにそのまま分化するかあるいは1～2回分裂して2～3細胞となつた後に分化する。

4. 形成層活動は4月5日から4月26日にかけて最大となりその間に2次膜外層の形成が開始する。すなわち4月19日である。

5. 形成層活動が衰退する過程において樹脂様物質のつまつた木部柔細胞と師部柔細胞が同時に出現した（5月10日）。これはスギの場合，形成層活動の減退と柔細胞の物質貯蔵能力との間に密接な関係があるからのように思われる。

6. 師部の生長輪界は生長期の始めに生成される厚膜の師部繊維に隣接する師細胞列と，これに接して配列しているところの前年に生成された師部柔細胞列との間と考えた方が正確である。

7. 放射組織を生み出す細胞として，放射組織始原細胞の他に放射組織母細胞なる細胞の存在が考えられる。

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