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Study on the Seasonal Development of the Secondary Phloem in *Larix leptolepis**

By

Hitoshi IMAGAWA

カラマツ (*Larix leptolepis*) の2次師部の季節的な発達経過に関する研究

今川一志**

CONTENTS

Introduction

The raidal growth of forest tree is accomplished by cell divisions in the cambium and the phellogen. However, most of it is derived from the cells which are newly produced in the cambium. Cambium which is located between xylem and phloem produces xylem and phloem elements, respectively inward and outward. Therefore, in order to clarify the processes of the radial growth, extensive studies about not only the xylem formation but also the cambial activity itself and the phleom formation are very significant. The xylem formation in *Larix leptolepis* has been already studied (IMAGAWA and ISHIDA 1970, IMAGAWA et al. 1976). But the phloem elements which are produced simultaneously with the xylem elements have been little dealt with. Therefore, it appears that many problems about the phloem formation remain still to unresolved.

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Generally, in comparision with morphological studies about the xylem elements of forest trees, such ones about the phloem elements have been less done. Especially, in Japan such ones have been extremely less reported (HIRAI 1941, SHIMAKURA 1936). Therefore, it is considered that many studies about phloem must be advanced hereafter.

From such point of view, in this study the seasonal development of the secondary phloem in Japanese larch is examined. As mentioned above, since phloem elements are derived from cambium, this study seems to be also effective to clarify cambial activity itself. And the results obtained will be described in relation to the xylem formation.

Materials and Methods

About 20 year-old Japanese larchs, *Larix leptolepis*, grown at the nursery in the campus of Hokkaido University, Sapporo, Japan were used. Specimens which included inner bark, cambium and outer wood were periodically punched out from the stems throughout three growth periods (1974, 1976 and 1978). Specimens were fixed in FAA or glutaraldehyde followed by osmic acid. Some of FAA fixed specimens were embedded in celloidine and the remainders in epoxy resin. Sections for light microscopy were cut from celloidine embedded specimens and stained with safranin and fast green. Semi-ultrathin sections (1～5 micron in thickness) for light microscopy, and 0.5 micron sections for ultraviolet microscopy (only from FAA fixed specimens) were cut from epoxy resin embedded specimens on an ultramicrotome. Semi-ultrathin sections were stained with basic fuchsin and methylene blue (HUBER et al. 1968). And also ultrathin sections were cut from the specimens which are fixed in glutaraldehyde-OsO_4, stained with uranyl acetate and lead citrate, and observed by an electron microscope. In all specimens, the relation in regard to the numbers between newly differentiated phloem elements and xylem elements (tracheids) were examined. At each specimen, the measurement for the numbers was done in each ten radial file and the average number was calculated.

Results and Discussions

In the mature bark of *Larix occidentalis* NUTT, the secondary phloem was composed of sieve cells, phloem parenchyma, sclereid-like fibers as a sclerenchyma, and phloem rays (CHANG 1954). Although there are some problems about the definition of sclerenchyme cells as mentioned later, in this paper phloem elements in Japanese larch are conveniently separated into four groups, that is, sieve cells, phloem parenchyma cells, sclerenchyma cells and phloem rays. Therefore, precise definitions about phloem elements in *Larix leptolepis* must be done hereafter. The results obtained will be described to be separated into two, that is, the development of current growth increment and the maturatuion of sclerenchyma cells.

1. The development of current growth increment

Cambium and its vicinity in dormancy are shown in Photo 1. Flattened cells at the middle in photo are dormant cambial cells. Cells at the upper are phloem

elements which were produced in the previous growth period. And ones at the bottom are last formed tracheids. The cambial cells show typically dormant appearance, i. e., their walls are very thick, and their cell contents are relatively dense though plasmolysis occurs to some extent by FAA fixation. Among the phloem elements at the upper of the cambium, there are almost rectangular sieve cells (S) and round phloem parenchyma cells (P). And phloem ray cells are also observed, but their cell contents are not found because of cutting their walls.

Cambium just before the initiation of cell divisions is shown in Photo 2. Although the cambial cells are still flattened and have thick walls such as those in dormancy (Photo 1), and their cell contents appear to become more or less clear. The shapes of one or two sieve cells adjacent to the cambium are very noticable. Their radial diameters are remarkably larger than those in dormancy (Photo 1). Since their walls are considerably thick, it is apparent that they are not cells which are newly produced in this growth period. They seem to be cells which have been produced near the end of the previous growth period, overwintered in immatured state and begin their enlargements prior to the initiation of cell divisions in early spring. ABBE and CRAFTS (1939) reported that in *Pinus strobus* one of the first indications of growth in the spring was the enlargement of sieve tubes which failed to complete their differentiation during preceding fall. And also in other species such cells have been reported and their origins have been discussed (ESAU 1969).

As a result of the enlargement, the other sieve cells at the phloem side of the enlarging cells become radially crushed. While, phloem parenchyma cells at the same position are not influenced by the enlargement. It appears that the crushed cells lose their function but the parenchyma cells maintain it.

In the lumina of the parenchyma cells, deposits are seen along the walls. Although their appearances are not similar to those in dormancy (Photo 1), this difference seems to result from sorts of fixatives used. At the right upper in photo, a transparent squar is observed. This is the trace where crystal has been dropped out. In *Larix decidua*, the crystals were small and rectangular in shape and composed of calcium oxalate (SRIVASTAVA 1963, WATTENDORFF 1969).

Cambium immediately after the initiation of cell divisions is shown in Photo 3. Overwintered sieve cells are further enlarging and the other sieve cells are more crushed. Parenchyma cells maintain to be round. As a result of the initiation of cell divisions, many cambial cells which are newly formed are found. A few thick tangential walls and many thin ones are distinguishable among them of cambial cells. The thick walls seem to belong to the overwintered cambial cells (Photo 1) and the thin ones to the new cambial cells which are formed by the cell divisions of such overwintered cells. Most of the overwintered cells were separated into four cells, so that there are three thin walls between two thick ones. Since newly formed walls (or partitions) are always found in every overwintered cambial cells, it appears that cell divisions in each cambial cell began at almost same time in early spring. And then, the cambial cells which were separated into four occur again cell divisions to become eight. In xylem formation, four of the

eight cells continue cell divisions furthermore and the remainders four begin the differentiation into tracheids (IMAGAWA and ISHIDA 1981). However such pattern of phloem production has been not still clarified at least in *Larix leptolepis.*

At this time, neither phloem nor xylem cells which begin their differentiatoni (arrow) is found.

Cambium and its vicinity at about one month after the initiation of cell divisions are shown in Photo 4. Flattened cells at the middle in photo are cambial cells. In addition to the overwintered sieve cells, phloem cells and tracheids which have been produced in this growth period begin the differentiation. Both differentiating cells and cambial cells are regularly arranged in radial file.

In each radial file, seven or eight sieve cells after the initiation of the differentiation are found. Some of them seem to complete their enlargement and the remainders near the cambium are enlarging. Parenchrma cells adjacent to the cambial cells are located. They have already contained deposits in their lumina and are aligned in nearly continuous tangential band. Such bands of parenchyma cells have been well known (CHANG 1954, SRIVASTAVA 1963, ESAU 1969).

At the xylem side of the cambium, several future tracheids begin their differentiation. Most of them appear to be still enlarging and from no secondary wall. At this time, the numbers of phloem cells after the initiation of the differentiation may be slightly more or nearly same in comparision with those of xylem cells. At first, pseudotransverse division may occur (arrow).

Developing phloem and cambium at about two month after the initiation of cell divisions are shown in Photo 5. Cells between crushed cells at the upper and flattened cambial cells at the bottom are current phloem cells, which are regularly arranged in radial file. Both sieve cells and parenchyma cells are more increased in number than in Photo 4. In each radial file, one parenchyma cell is located every two or three sieve cells.

It is noticable that sieve cells are obviously variable in wall thickness. The walls of three or four sieve cells including the overwintered cells are remarkably thinner than those of sieve cells near the cambium. These thick walled sieve cells appear to be more or less smaller in diameter than those of the thin walled sieve cells far from the cambium. ABBE and CRAFT (1939) also mentiomed that in *Pinus strobus* sieve tubes which differentiate during cooler, moisture part of the year have slightly thinner walls and larger lumina than those found later. And also, in sieve cells of *Pseudotsuga menziesii* (GRILLOSE and SMITH 1959) or even sieve tubes of *Juglans regia* (SCHAAD and WILSON 1970), such morphological differences were reported.

On the base of these observations, it may be considered that such sieve cells which have thin walls and relatively large lumina are comparable to earlywood, and thick walled and small ones to latewood. It is supposed that early sieve cells complete their differentiation during May and late ones not begin to differentiate till June.

At this time, however, differentiating future tracheids seem to be transitional from earlywood to latewood, though they can not observed in Photo 5. There-

fore, early or late sieve cells which are compared to those of tracheids seem to disagree with those of actural tracheids in regard to the time of the differentiation. It is obscure why both do not coincide or why sieve cells proceeds ahead of tracheids. It is presumed that occurrence of overwintered sieve cells may be one of the significant factors.

Callose is found in some sieve cells at the upper in Photo 5 (arrows). Generally, it is considered that sieve cells in which callose is accumulated lose their function (ABBE and CRAFT 1939, ESAU 1969). These cells are even crushed to some extent.

As mentioned by GRILLOSE and SMITH (1959), phloem parenchyma cells are rectangular or squar near the cambium, but gradually become enlarge and rounded as a result of progression of their differentiation. Parenchyma cells can be distiguishable at extremely earier stage of the differentiation because of the occurrence of depoisits (Photos 4 and 5).

Photos 6 and 7 are radial sections, in which each cambium is located at the left of both photos. In Photo 6, axially long parenchyma cell (P) near the cambium can be identified with certainty by the accumulation of dark substance. Since its radial dimension is almost same as those of the cambial cells, it may be parenchyma cell immediately after the initiation of the differentiation. Therefore, it is considered that such substance is accumulated prior to the enlargement. Perhaps, the substance may be tanniferous or resinous materials. After the enlargement, transverse divisions occur to become in strand (arrow in Photo 7). Similar proceeding were found in the phloem parenchyma cells in *Pseudotsuga menziesii* by GRILLOSE and SMITH (1959).

Almost matured parenchyma cells contain different types of deposits (Photos 8 and 9). In cross section, globe-like (a in Photos 8 and 9), mud-like (b in Photo 8), star-like (c in Photo 8), completely full (d in Photo 9) and incompletely full deposits (e in Photo 9) are found. Most of them are highly osmiophilic except for b. In addition to these deposits, relatively lower osmiophilic substances of which the lumina are full are found (arrows in Photos 8 and 9). Many kinds of these deposits may result from difference of the penetration of fixatives (OsO4) into lumina. And also, highly osmiophilic deposits (a-e in Photos) seem to be different in chemical composition from lower ones (arrows in Photos). Although these composits seem to be composed of tanniferous or resinous materials (CHANG 1954, GRILLOSE and SMITH 1959, SRIVASTAVA 1963, ESAU 1969), the identification did not done in this study.

Phloem parenchyma cell which include deposit such as e in Photo 9 was observed by an electron microscope (Photo 10). Large amounts of osmiophilic substances in the central large vacuole in the lumen are remarkable. They are isolated in the vacuole. And similarly osmiophilic granules are also located on the tonoplast (arrows). In each small vacuole or on the tonoplasts, similar deposits or granules are also observed. It is supposed that these deposits or glanules are produced in the cytoplasm, migrated through the tonoplast and accumulated in the vacuole. In the cytoplasm, mitochondria, endoplasmic reticulum and the other organelles are found. Spherical bodies which are slightly osmiophilic seem to be

lipid droplets.

Whole current growth increment almost near the end of growth period is shown in Photo 11. Each cells are arranged very regularlly in radial file. The round parenchyma cells which have different diposits are located almost every two or four sieve cells in each radial file. The sieve cells are variable in wall thickness as mentioned in Photo 5. Some of the sieve cells which differentiate in early spring are curshed, and callose is abundantly accumulated in such cells. Some tangential bands of the parenchyma cells are found, but ones which are not arranged in tangential band are also observed. In the lumina of parenchyma cells, starch grains (arrows) in addition to deposits are also seen. Since cambial activity completed at the late of September or the early of October, this section (at the late of September) shows an entire growth increment which has been produced in one growth period. Current growth increment in Larix leptolepis can be regarded to be relatively simple cellular structure or composition.

Fusiform ray is observed at the center in this section and the horizontal resin canal is found in the upper part of it. Although this canal appears to be interruped near the cambium, resin canal runs actually through phloem, cambium and xylem (Photo 12).

In slwoly growing trees, the current growth increment is extremely less formed as shown in Photo 13. Total numbers of the cells in the current growth increment is not reached to 10. In each radial file, only one parenchyma cell is located. The parenchyma cells remain till rectangular and undifferentiaie to become rounded. The walls of sieve cells are all thin and not variable in thickness. Thus early or late sieve cells can not be distinguished as the case of normal current growth incement (Photo 11). However, the sieve cells which have differentiated in early spring are curshed, and callose is accumulated. As well as the current growth increment, there are only several tracheids in the current growth ring, but early- and latewood can be distinctly determined. In spite of being distinguishable between earlywood and latewood, such distinction is impossible in the current growth increment. The reasons is obscure as the case of time lag between phloem and xylem formation as mentioned earlier.

Therefore it is considered that the extent of the growth activity of tree influences significantly on not only the xylem formation but also the phloem formation. In phloem formation, its effects seem to occur quantatively and qualitatively as mentioned above. Without relation to the extent of the growth activity, crushed sieve cells which have callose is found as shown in Photos 11 and 13. Thus it is possible to determine growth increments in the secondary phloem owing to these crushed sieve cells. While, since callose may be temporary (ESAU 1969), it is not useful for the determination. In *Pseudotsuga menziesii* (GRILLOSE and SMITH 1959), *Larix decidua* (SRIVASTAVA 1963) and even *Juglans regia* (SCHAAD and WILSON 1970), growth increment or layers were practically determined.

On the base of the observations which have been described till now, the relation between phloem and xylem cells was quantatively examined. In each specimen throughout three growth periods, each total number of phloem and xylem cells

after the initiation of the differentiation was counted. But it may be troublesome to count the overwintered sieve cells, because they are not formed newly in the growth period.

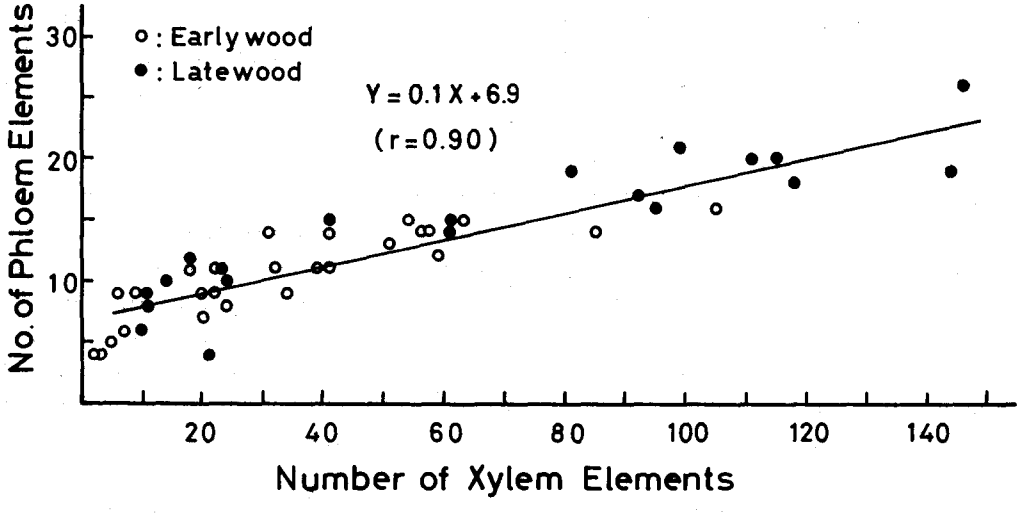

Fig. 1. The relation between phloem and xylem production throughout three growth periods. The vertical and horizontal axis indicate respectively the numbers of phloem and xylem cells which begin the differentiation. A black dot shows the specimen in which latewood tracheids have produced.

The relation is shown in Fig. 1. It is obvious that the numbers of phloem cells are proportion to those of tracheids. The regression line equation obtained is as follows; $Y=0.1\,X+6.9$. The correlation coefficient is 0.9. Y and X indicate the numbers of phloem cells and tracheids respectively. According to this equation, for example, while 100 tracheids are produced, only about 10 phloem cells are produced. This relation is always maintained in spite of the extent of growth activity. As mentioned above, however, this equation is not satisfactory to be applied to the case in which both phloem cells and tracheids undifferentiate. That is, in dormancy it can not be applied. It seems to be very significant how incorporate overwintered cells into such relation or the equation. GRILLOSE and SMITH (1959) reported that during any one season, in Douglas fir there may be over 100 tracheids produced in each tier as compared with only 10 to 12 phloem elements. And they also described that in slowly growing trees the numbers are much lower but the ratio is almost about same. And also, ESAU (1969) introduced many studies about the relation between phloem and xylem production.

2. The maturation of sclerechyma cells

Although the seasonal development of current growth increment has been described, it is not still enough to clarify the actual development of the secondary phloem in *Larix leptolepis*. In Photo 14, three growth increments including current one can be distinguished on the base of crushed cells (arrows). It is noticable that

thick walled sclerenchyma cells are found in the two increments except for the current one. Therefore it is considered that sclerenchyma cells are undifferentiated in the year when they were produced but are differentiated in the next year. Hereafter, the processes of the maturation of sclerenchyma cells are described.

Sclerenchyma cell immediately after the initiation of the differentiation is shown in Photo 15. Cambial cells and enlarging parenchyma cells near it are found at the right bottom in Photo 15. Several large cells above the parenchyma cells are sieve cells which may correspond to earlywood. And, crushed cells above these sieve cells are the boundary between the current increment and the previous one.

In the previous increment, more larger cell (arrow) than the surrounding cells is found. This is a sclerenchyma cell just after the initiation of the differentiation. Such cells could not be found in any specimens which were collected before the late of May. Although it has been known that such sclerenchyma cells (or sclereids) are not direct derivatives of phloic initials but are secondarily modified parenchyma cells (SRIVASTAVA 1963, ESAU 1969), the corresponding parenchyma cells could not be identified in this study. According to GRILLOSE and SMITH (1959), in Oregon future sclereids in *Pseudotsuga mensiesii* began to enlarge at about the middle of May in 1955.

Photo 16 is the enlargement of Photo 15. The cell wall is not adequately clear, but cytoplasm seems to be directly adhesive to the wall before the plasmolysis. A large nucleus is found in the lumen. STERLING (1947) stated that the first indication of a sclereidal idioblast is the increased size of its nucleus. GRILLOSE and SMITH (1959) also mentioned that during the second year after they are differentiated, some of cells in the phloem parenchyma strands lose much of the resins and tannins, increase greatly in size, and differentiated as sclereids.

The sclerenchyma cells which begin the enlargement at the late of May continue to enlarge furthermore radially and tangentially. Such enlargement results in the displacements or the collapses of the surrounding sieve cells, except for parenchyma cells (Photo 17). Secondary wall formation follows the enlargement of the sclerenchyma cells. Seasonal process of the secondary wall formation is shown in Photos 17, 18 and 19, which were collected at the early of June, July and August respectively. Even at the early of September, the secondary wall was not sufficiently completed, though the section are not shown. The secondary wall continues to be gradually formed till the late of September or the early of October, and the lumina of the sclerenchyma cells become very narrow. The secondary wall formation of the sclerenchyma cells advances very slowly throughout almost entire growth period in which they begin the enlargement. Since the original cells, maybe parenchyma cells, from which sclerenchyma cells are secondarily derived have been produced in the previous growth period, therefore, it may be considered that the sclerenchyma cells need two growth periods for the maturation. In *Pseudotsuga menziesii*, also, the deposition of the lamellae composing the thick secondary wall of a sclereid progressed very slowly (GRILLOSE and SMITH 1959).

Generally, sclerenchyma cells are highly lignified (CHANG 1954, MARTIN and CRIST 1970). In order to investigate the process of the lignification, only FAA

fixed specimens were examined by an ultraviolet microscope (at 280 mμ in wave length). Glutaraldehyde-OsO4 fixed specimens can not be used absolutely for ultraviolet microscopy, because such fixatives themselves absorb ultraviolet rays highly.

Photo 20 shows the lignifying walls of the sclerenchyma cells which are taken by an ultraviolet microscope. The walls are still thin considerably, but the lignification has already begun. Although examinations for the first initiation of the lignification in such cell were not done in this study, the lignification seems to sdvance soon after the deposition of lamellae. In the left cell, the secondary wall can be separated into three parts based on the extent of ultraviolet absorption. Since the innermost portion adjacent to the lumen indicates little absorption, it is suggested that the lignification has not still initiated. Outward this portion, there is another one which indicate relatively weak absorption. The outermost portion shows high absorption and occupies about two thirds of the secondary wall thickness. In this portion, thus, the lignification seems to advance considerably or complete nearly. And in the intermediate portion where the absorption is relatively lower, lignification seems to advance actively. Outward the outermost portion, there is a narrow part, in which the absorption is most high. This appears to be compound middle lamella or middle lamella itself. The walls of parenchyma cells which are shown at the bottom in photo indicate no absorption except for the cell corner and the middle lamella (or compound middle lamella).

Photo 21 shows considerably thickened wall of sclerenchyma cell which is taken by an ultraviolet microscope. The secondary wall formation advances extremely to almost embed the lumen. The wall can be separated into three parts, i. e., innermost, intermediate and outermost as in Photo 20. The outermost portion in which absorption is high occupies more than four fifths of the entire secondary wall. And also the compound middle lamella or middle lamella itself indicate most high absorption. In the outermost and the intermediate portion, a number of thin layers which are arranged in concentric circles can be distinguished on the base of difference in ultraviolet absorption. Since it has been known that the wall of sclerenchyma cells are lamellate in structure (ESAU 1969), these thin layers seem to be lamellae. According to SRIVASTAVA (1963), however, the lamellation of sclereid walls were not conspicuous in *Larix decidua*. On the other hand, CHANG (1954) stated that sclereid like fibers in *Larix occidentalis* show distinct laminate layers in the secondary wall. If such difference of the absorption is directly derived from those of lignin deposition, it is interested in order to elucidate the lignification process. It may be assumed that the secondary walls of sclerenchyma cells are unevenly lignified, in spite of even distribution in the secondary wall of the tracheids (IMAGAWA et al. 1967). And also it may be considered that such difference results from another factors, e. g., edge effect between each lamella. It seems to be difficult to conclude distinctly only in this study and necessary to be investigated furthermore by an electron microscope.

Although the differentiating process of the sclerenchyma cells has been considerably clarified, they are variable in number in each growth increment. Photo 22 shows the previous growth increment in which there are extreme abundance

of sclerenchyma cells. Many differentiating sclerenchyma cells are found. Some of cells are even adhesive each other. As described by CHANG (1954), generally sclerenchyma cells occur sporadically in *Larix*. Therefore, such case seems to be rather rare, but the reasons are still obscure.

Photo 23 shows the increment which is the reverse to Photo 22. Three growth increments, current, previous and two years ago, are found. In the two years ago's increment, sclerenchyma cells are sporadically located. But no cells are found in the previous growth increment. Since this specimen was collected near the end of the growth period, newly differentiated sclerenchyma cells outght to be found in the previous increment. Nevertheless, sclerenchyma cells are not observed at all in this increment. Because both current phloem and xylem increment are extremely narrow, this tree seems to be severely suppressed in the current growth period. Therefore, when trees are severely suppressed in the next growth period after future sclerenchyma cells are produced, the differentiation of them may not be initiated. Otherwise, it may be also considered that the cells from which sclerenchyma cells are secondarily derived have not been produced in the previous year. However, it may be unreasonable to assuem so, because sclerenchyma cells are apparently located in the two years ago's increment. Thus, in this paper, it is considered that future sclerenchyma cells do not begin the differentiation when the tree is severely suppressed. But it is obscure whether a number of sclerenchyma cells differentiate when growth is very accelerated.

In this paper, the term "sclerenchyma cell" has been always used. As mentioned earlier, it has been discussed whether sclerenchyma cells in *Larix* are fibers or sclereids, and thus the terminology has been confused to be used. SHIMAKURA (1936) called the sclerenchyma cells in *Larix Kaempferi* and *L. dahurica* "fibrous scleotic cells". CHANG (1954) also termed those in *Larix occidentalis* "sclereid-like fibers", and regarded them as a intermediate group between typical sclereids and fibers. On the other hand, refering to CHANG, SRIVASTAVA (1963) called those in *Larix decidua* "sclereids", and emphasized that the term "fiber" should not be used to describe them. He stated that the term "sclereid" denotes cell types that are not direct derivatives of phloic initials but are secondarily modified parenchyma cells. ESAU (1969) also introduced that one of the evidences for the distinction between sclereid and fiber is based on the different processes of their differentiation. However, she also added that such evidence has not been completely established but some exceptions have been occasionally reported. According to HIRAI (1941), "Bastfasern" or "bastfaserähnliche Steinzellen" in *Larix Kaempferi* were about 1.5 mm in length. It is considered that they are relatively long cells. MARTIN and CRIST (1970) defined briefly that fibers are long and sclereids short.

Therefore, it appears to be unsatisfactory to term the sclerenchyma cells in *Larix leptolepis* "sclereids", only on the base of the differentiation. Because, the cell shapes are completely neglected, though the matured sclerenchyma cells in *Larix leplolepis* were not examined in this study. In order to determine the most suitable term, it seems to be necessary to investigate on the cell shapes of the matured sclerenchyma cells in *Larix leptolepis*.

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要　　約

北海道大学構内の実験苗畑に植栽されていた約 20 年生のカラマツ, *Larix leptolepis* の樹幹から定期的に試料を採取し, その 2 次師部の季節的な発達経過を 3 生長期にわたって調べた。得られた結果は 2 つに分けて述べられている。1 つは当年生部の 2 次師部の発達経過についてであり, もう 1 つは厚壁細胞の成熟経過についてである。

1. 当年生師部の発達
   1) 未成熟の状態で越冬した師細胞が春先に最初に分化を始めた。その後, 形成層細胞の分裂が始まった。
   2) 形成層における分裂再開は, 各細胞でほぼ同時に始まると思われた。
   3) 新生, 分化した師部要素細胞は整然とした半径列を呈して配置していた。各半径列において, 連続する 2～4 個の師細胞毎に 1 個の師部柔細胞が存在しており, また柔細胞は接線方向にほぼ連続して並び, 全体的には帯状を呈して配列していた。

4) 師細胞の壁は一定の厚さではなかった。春先に分化した師細胞の壁は薄く，それ以降のものは厚かった。したがって，前者は早材に，後者は晩材に相当すると見なされたが，両者の分化時期は同じではなかった。

5) 分化開始直後の師部柔細胞は矩形であるが，分化が進むにつれ円形になった。また分化の極く初期から，その内腔に沈着物を保持しながら拡大し，横断分裂を行い，ストランド状になった。

6) この沈着物には種々の形状のものが見られた。その中の1つが電顕観察され，それは細胞質中で生産され，トノプラストを通過し，液胞中に集積されるものと推定された。

7) 生長期の終り頃，春先に分化した師細胞は半径方向に押しつぶされた。そのような細胞にはカロースの沈着が見られた。

8) 形成層活動は9月下旬または10月上旬に終わると推定された。当年生の2次師部もその頃までには完成した。その細胞構成や細胞配列は比較的単純であった。

9) 樹木の生長が抑圧された場合，その当年生の2次師部の発達は質的にも量的にもかなり影響された。

10) 押しつぶされた師細胞群の存在に基づいて，2次師部中に各年の生長層を識別することが可能であった。

11) 生長期の各時期における分化開始以降の師部細胞総数と木部細胞総数（仮道管総数）との関係が調査された。その結果，師部細胞総数と仮道管総数は比例関係にあることが明らかになった。その回帰直線式は $Y=0.1X+6.9$ （Y: 師部細胞総数，X: 仮道管総数）であり，その相関係数は0.9であった。

2. 厚壁細胞の成熟

1) 厚壁細胞はそれが新生された年の翌年になって初めて，厚壁細胞としての分化を始めた。それは5月下旬に始まった。

2) その分化はほぼ全生長期間にわたって徐々に進行し，新生から成熟までに2生長期にわたる時間を要した。

3) その壁層は強く木化した。また，その2次壁中には同心円状に配向した多数のラメラが見出された。各ラメラの木化の程度は異なるらしく思われた。

4) 2次師部中の各生長層によって，存在する厚壁細胞数は一定していなかった。極めて多数存在する場合と，全く見出されない場合とがあった。後者の場合は，樹木の生長が抑圧されたことが，その原因の一つと考えられた。

5) *Larix leptolepis* の厚壁細胞の呼び方について若干の論議がなされた。最も適当な名称を決定するには，その分化過程だけでなく，その成熟した時の形状についても十分に考慮されなければならない。したがって，成熟した厚壁細胞の形状についての研究がなされてからその名称を決定するのが妥当は思われる。

Explanation of Photographs

Photo 1. Cambium and its vicinity in dormancy. In the previous growth increment above the flattened cambial cells, sieve cells (S) and phloem praenchyma cells (P) are shown. Collected at the early of November in 1976.

Photo 2. Cambium just before the initiation of cell divisions in early spring. One or two sieve cells adjacent to the cambium are radially enlarging. The trace where crystal has been dropped out is shown (arrow). Collected at the late of April in 1978.

Photo 3. Cambium immediately after the initiation of cell divisions. Thick tangential walls belong to the overwintered cambial cells, and thin ones to the newly formed cambial cells. Cambial ray cell begins the division (arrow). Collected at the early of May in 1978.

Photo 4. Cambium and its vicinity at about one month after the initiation of cell divisions. Differentiating phloem and xylem elements are seen. Phloem parenchyma cells have already contained deposits in their lumina. Pseudo-transverse division occurs (arrow). Collected at the late of May in 1978.

Photo 5. Developing phloem at about two month after the initiation of cell divisions. Sieve cells are apparently variable in wall thickness and diameter. Callose is found (arrow). Collected at the middle of July in 1978.

Photo 6. Radial section of axially long parenchyma cell near the cambium. This parenchyma cell (P) has already accumulated dark substances in the lumen. Collected at the early of June in 1978.

Photo 7. Radial section of differentiating parenchyma cells. Transverse division occurs (arrow) after the deposition and the enlargement. Collected at the early of June in 1978.

Photos 8. and 9. Different types of the deposits in the parenchyma cells. Glove-like (a), mud-like (b), star-like (c), completely full (d) and incompletely full deposits (e) are found. Relatively lower osmiophilic subustances (arrow) are also seen. Collected at the middle of June in 1978.

Photo 10. Electron microscopical observation of phloem parenchyma cell. Osmiophilic substances are seen in the large or small vacuole. On the tonoplasts, osmiophilic granules are located (arrows). Collected at the late of June in 1978.

Photo 11. Current growth increment near the end of growth period. Each cell is arranged regularlly in radial file. In each file, one parenchyma cell is located every two or four sieve cells. Starch grains are seen (arrows). Collected at the middle of September in 1978.

Photo 12. Cross section of the horizontal resin canal. Canal runs through the phloem, cambium and xylem. Collected at the middle of November in 1978.

Photo 13. Narrow current growth increment. Numbers of cells in both current growth increment and ring are remarkably less. Crushed cells and callose are observed. Collected at the late of October in 1974.

Photo 14. Three growth increments including current one. Each one is distingushed by the crushed cells (arrows). Thick walled sclerenchyma cells (S) are located in the two increments except for the current one. Collected at the early of November in 1978.

Photo 15. Sclerenchyma cell immediately after the initiation of the differentiation. More larger cell (arrow) than the surrounding cells is a enlarging sclerenchyma cell. Collected at the late of May in 1978.

Photo 16. The enlargement of Photo 15. Large nucleus is found in the lumen.

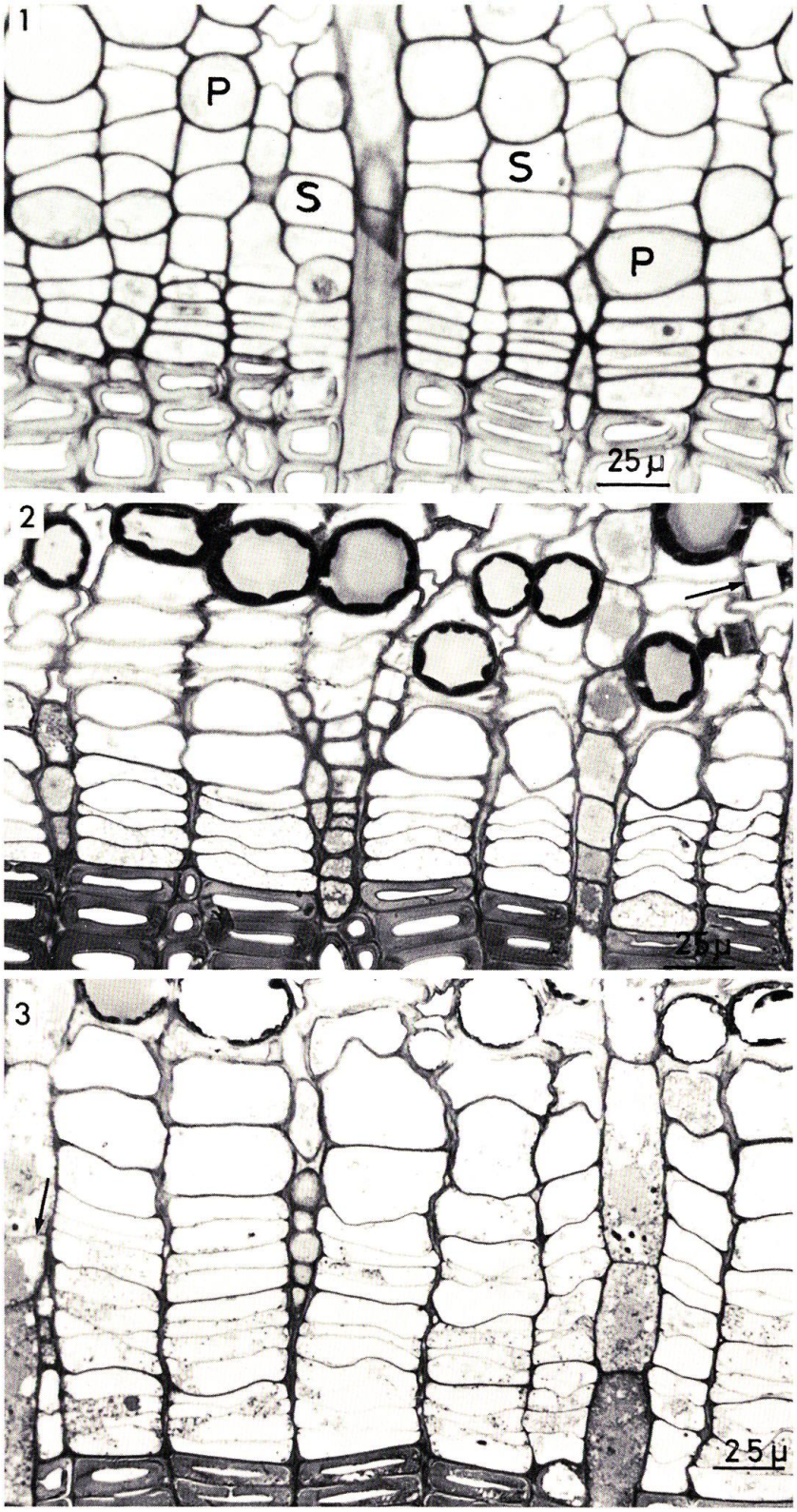
Photos 17, 18 and 19. Seasonal process of the secondary wall formation of sclerenchyma cells. Collected at the early of June, July and August in 1974.

Photo 20. Ultraviolet microscopical observation of differentiating sclerenchyma cells. The secondary walls are still thin, but the lignification has already begun. The secondary wall can be separated into three parts based on the extent of ultraviolet absorption. Collected at the late of June in 1974.

Photo 21. Ultraviolet microscopical observation of the thickened wall of the sclerenchyma cell. The secondary wall formation advances to almost embed the lumen, and it can be also separated into three parts. Many thin layers in concentric circles are observed in the secondary wall. Collected at the late of September in 1974.

Photo 22. Many sclerenchyma cells in the previous growth increment. An abundance of differentiating sclerenchyma cells are found. Collected at the middle of July in 1978.

Photo 23. Three growth increments (current, previous and two years ago). Sclerenchyma cells are not found at all in the previous growth increment. Collected at the early of November in 1976.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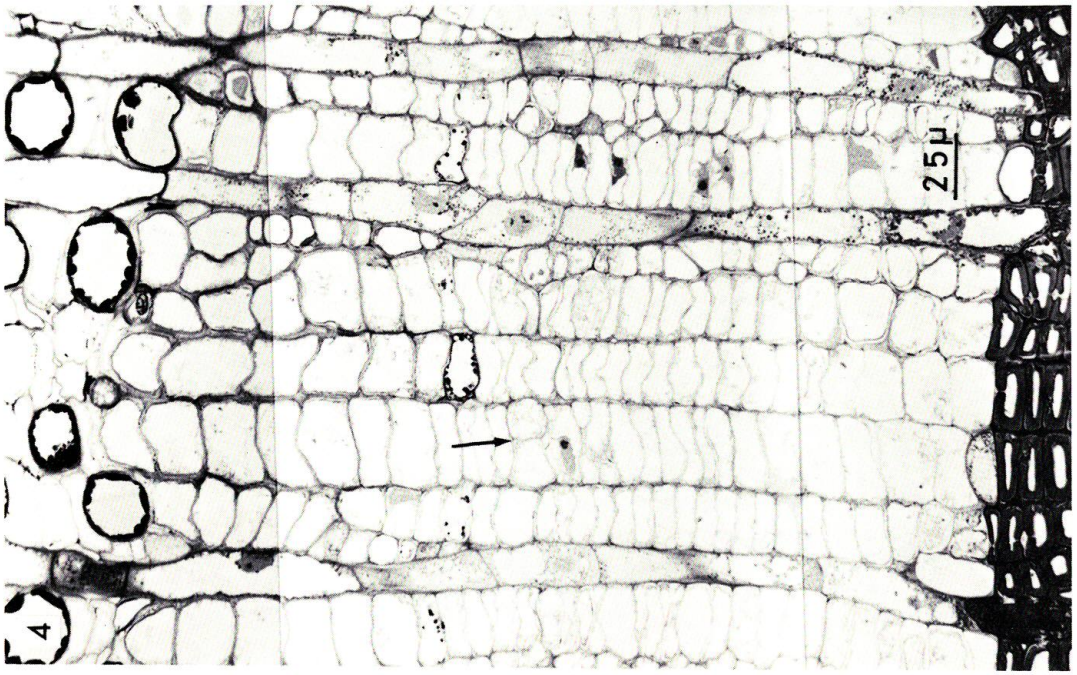
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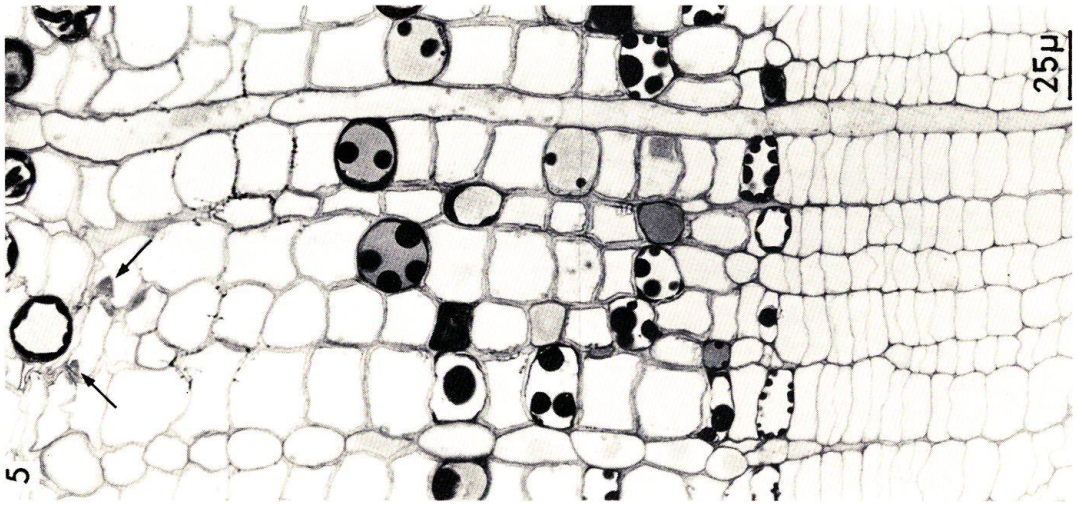
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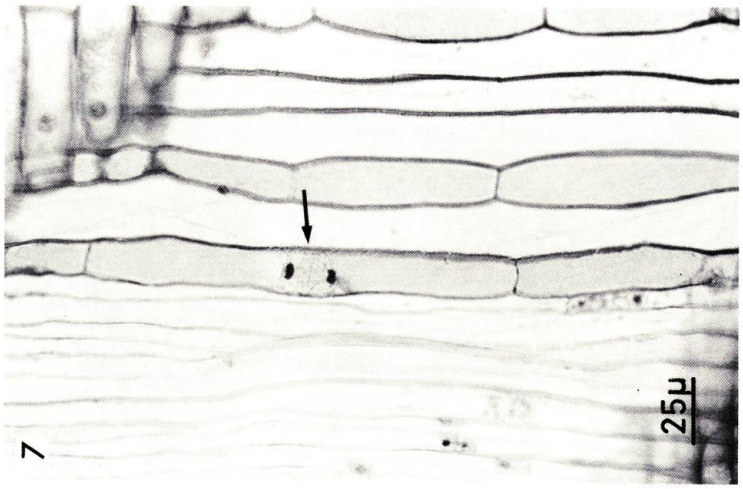
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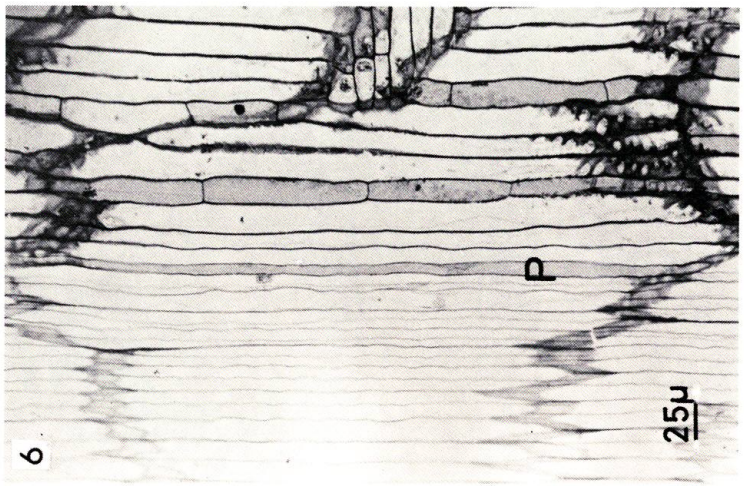
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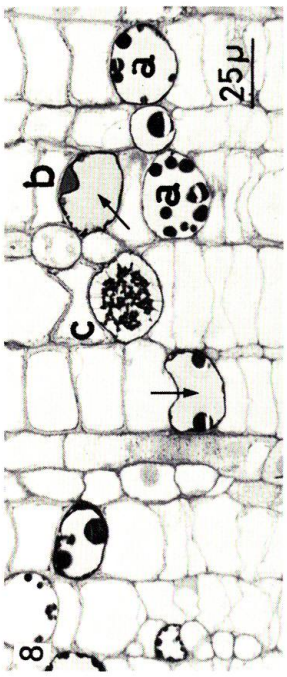

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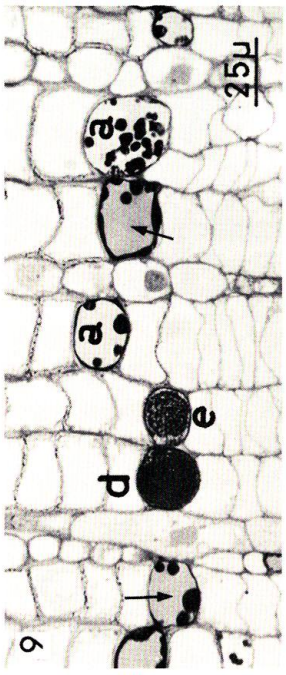

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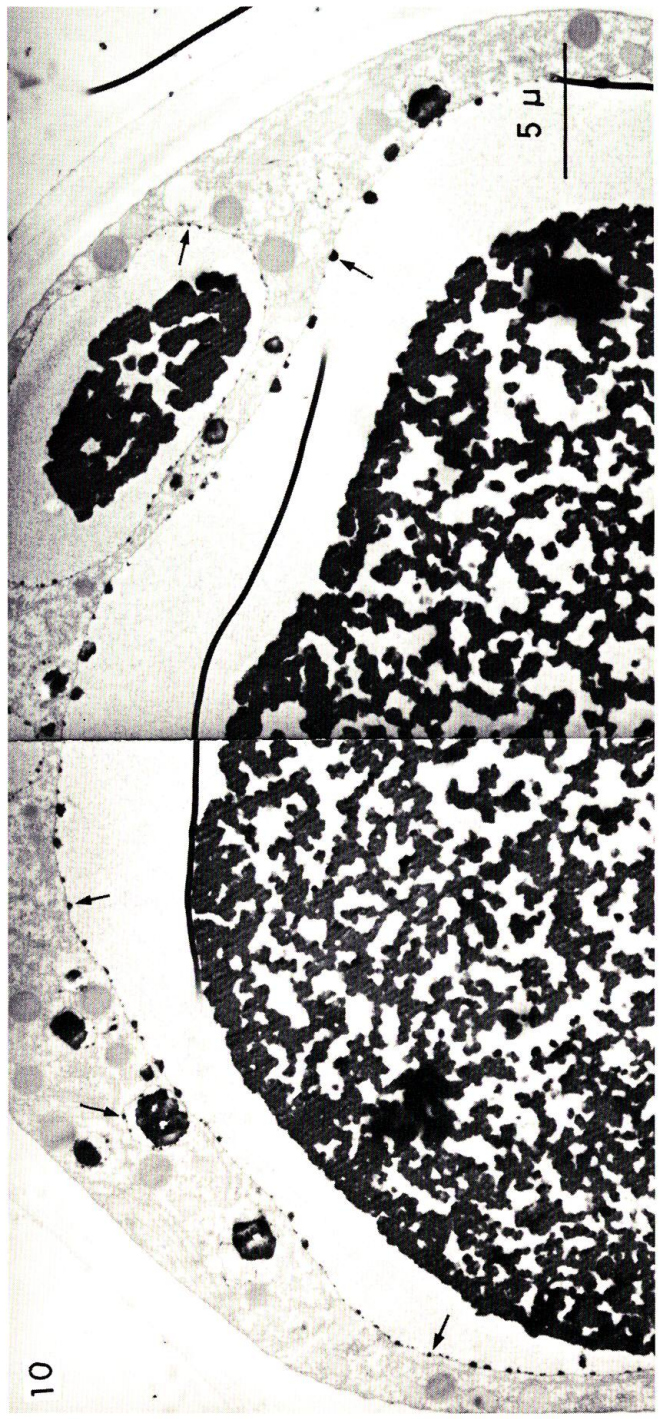

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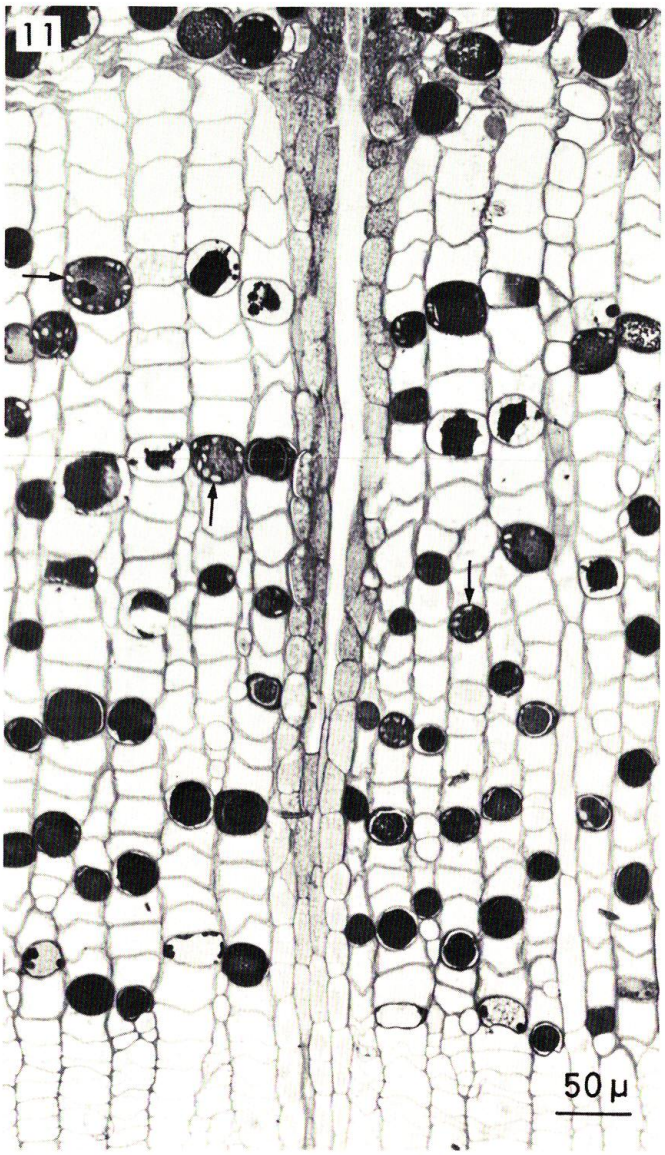
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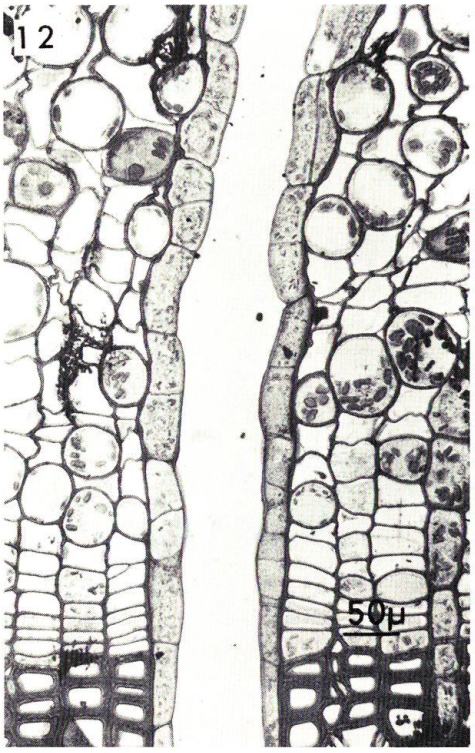
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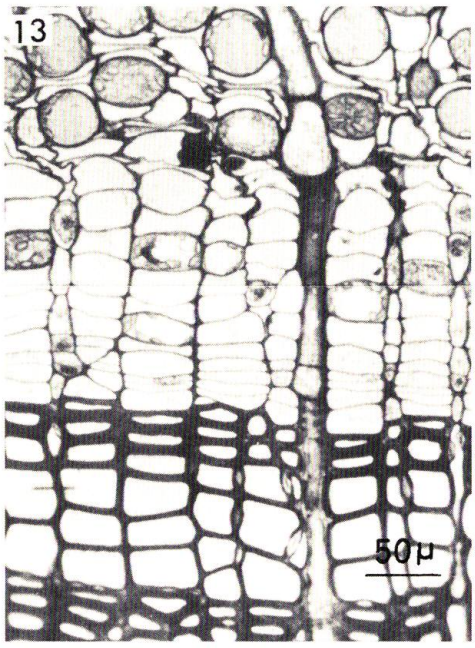
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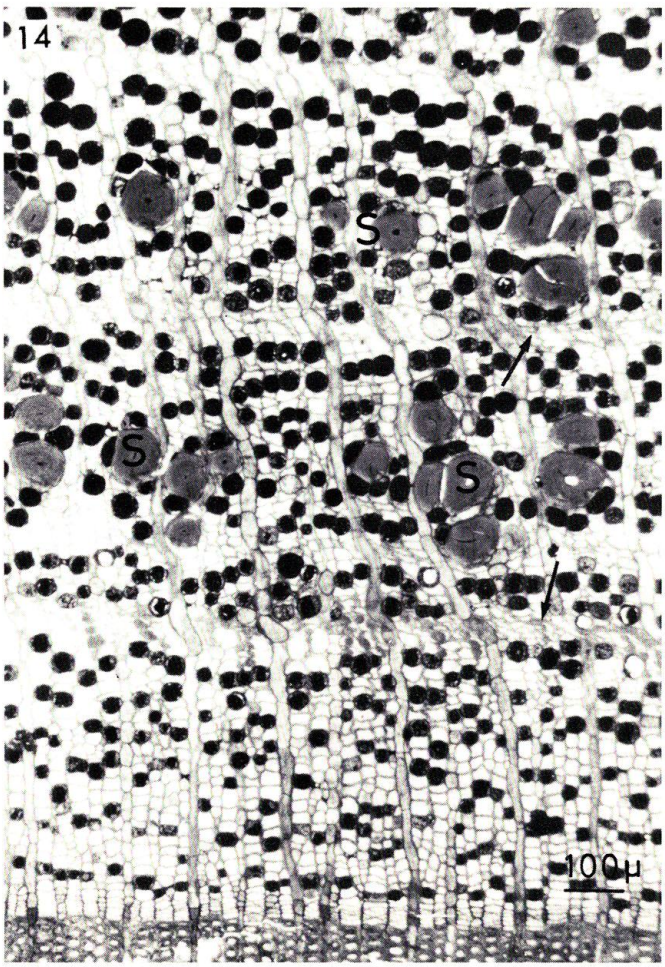
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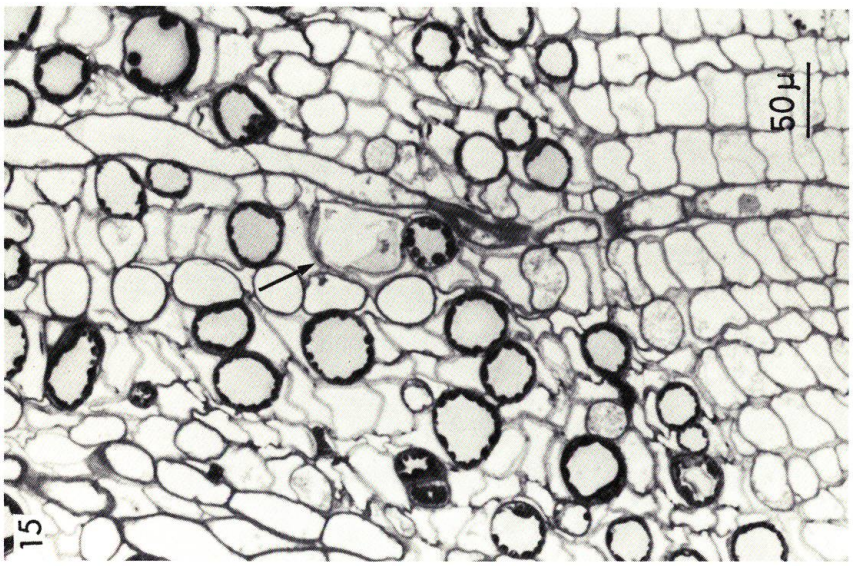
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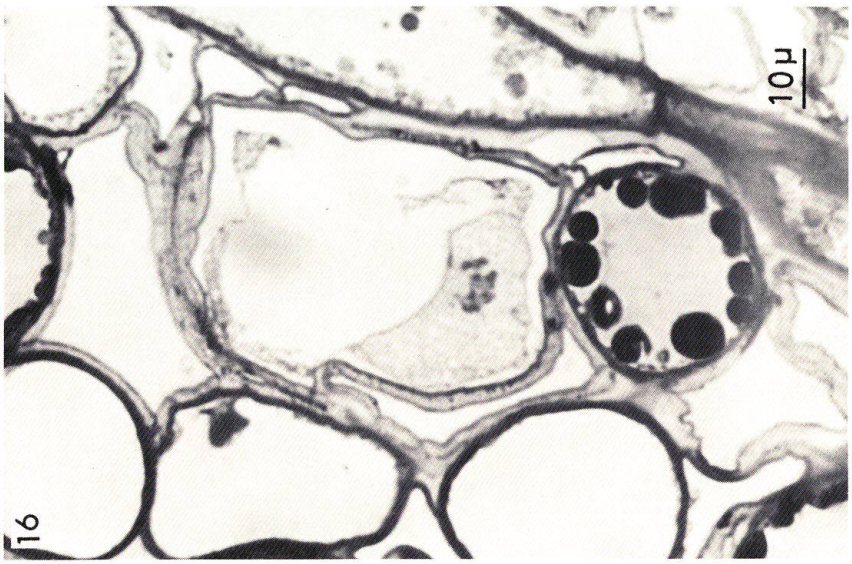
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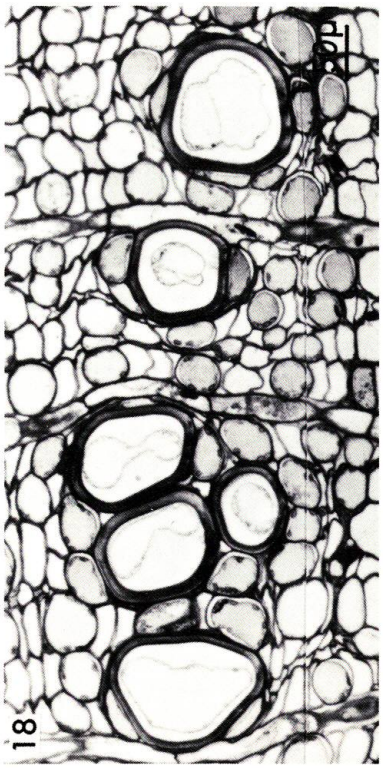
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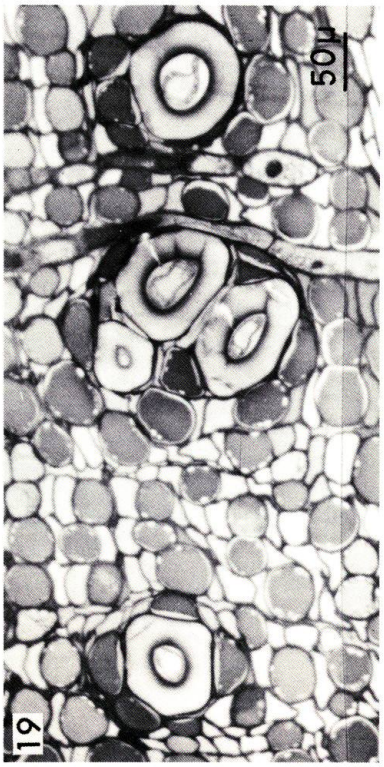
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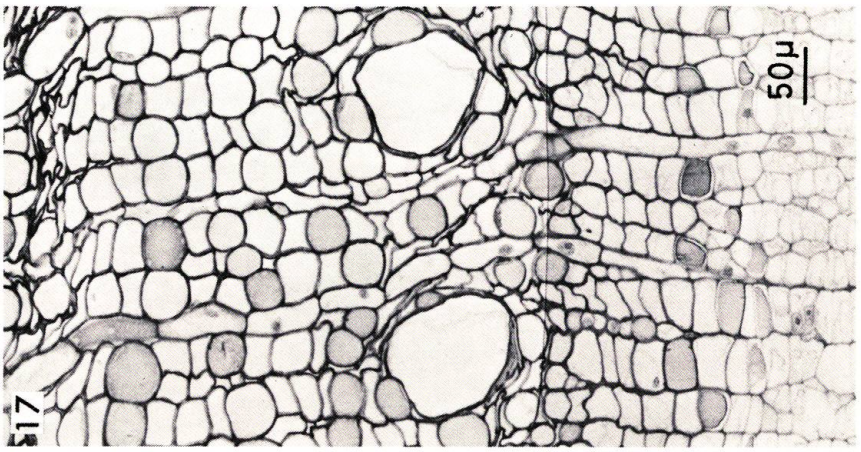
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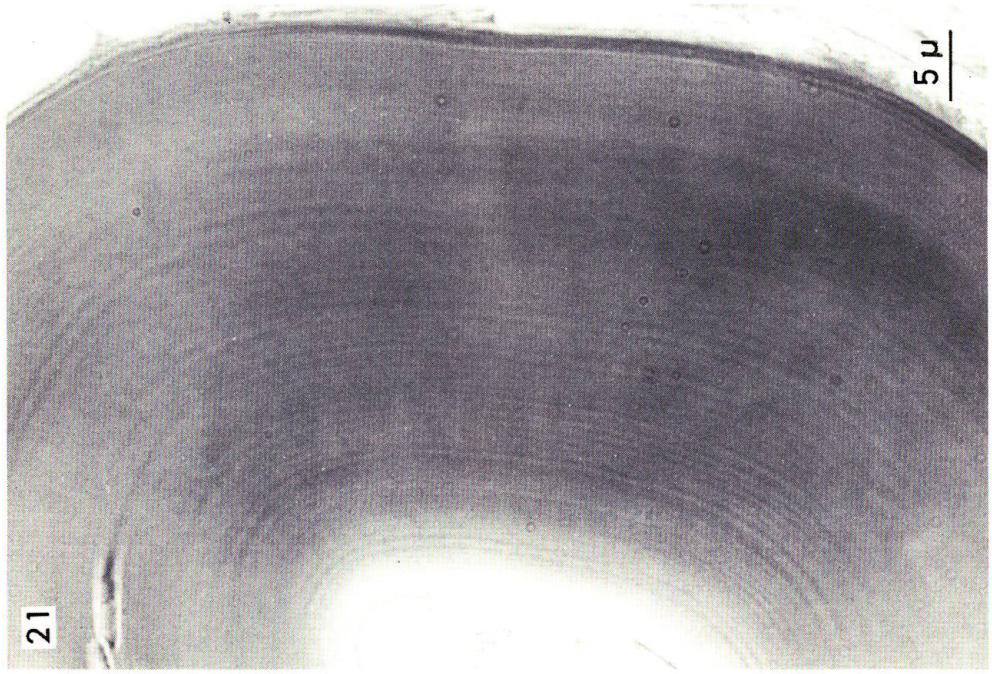
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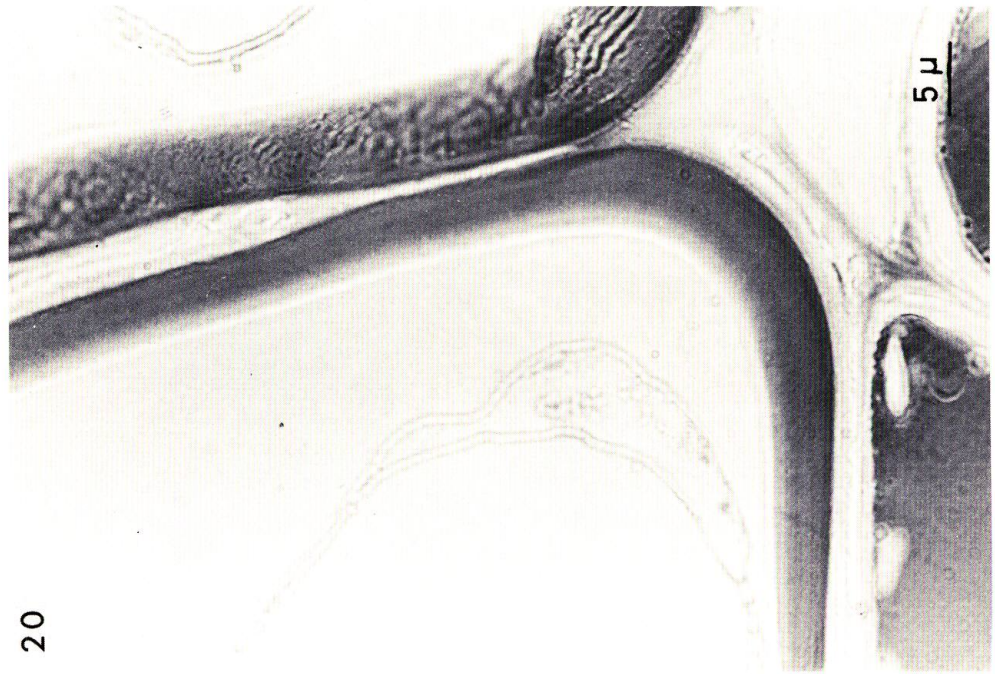
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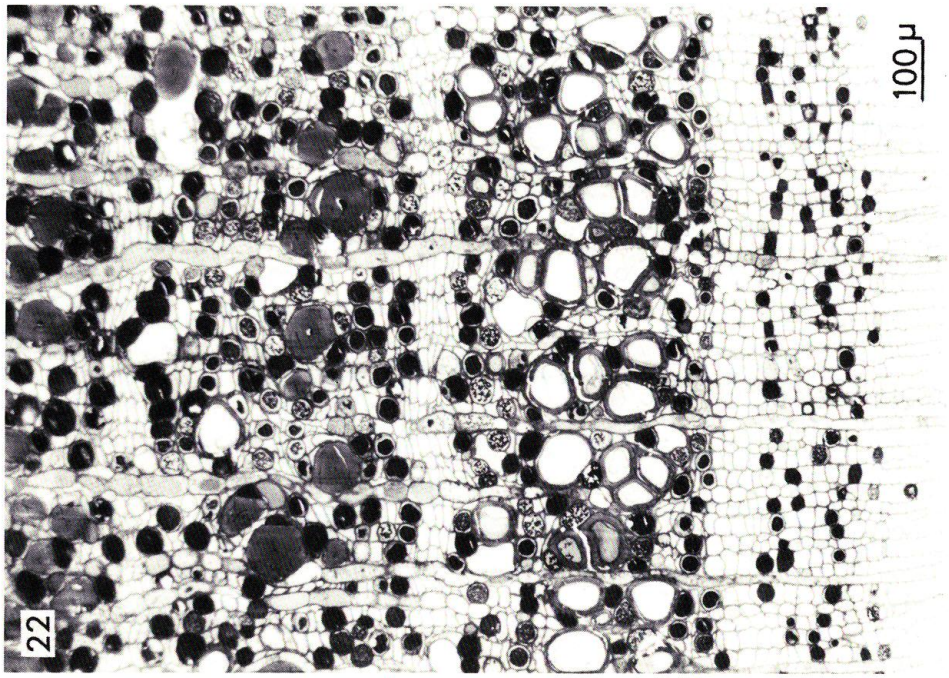
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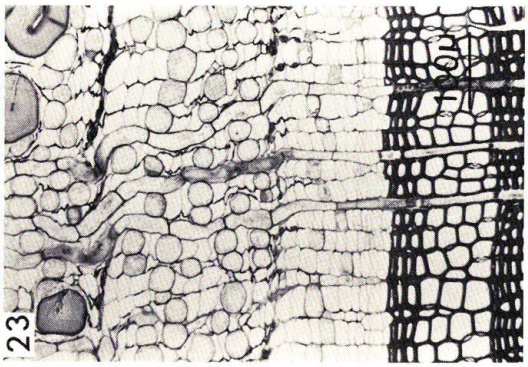
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