Review Article

Wound Effects on Cytodifferentiation in the Secondary Xylem of Woody Plants

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Contents

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Introduction

Wound tissue is widely known to be formed in tree xylem in response to various types of injuries to trunks, such as big injuries caused by pruning[1], pith fleck caused by insects[2], frost ring[3]. Most of the past reports discuss wound response from the stand point of practical problems in forestry, and usually focus on the mass of wound tissue which has healed in the xylem. Therefore, wound response of tree has been roughly explained as the necrosis of cells with discoloration[4], or as abnormal cell formation[5] with callus formation in a broad sense, on the basis of rather macroscopic observations. Cytological researches on the regeneration of secondary tissue of tree[6] are few except those using *in vitro* methods. The observation of the process of wound tissue formation *in situ* seems to be difficult, especially in the case of big injuries, because of the involvement of various contaminants such as microorganisms. From the practical view point in forestry, investigations of wound tissue might be achieved by the macroscopic observations, however a fundamental anatomical approach to this subject may give some clues for the basic problem of cytodifferentiation in the secondary xylem of woody plants.

When wound tissue is observed microscopically, many kind of abnormal cells are found. This fact indicates that the xylem and phloem daughter cells including cambial cells around the injury may behave diversely depending on their differentiation phase at the time of injury, in contrast to simple division into "callus" in the cultured tissue. Although wound tissues have also been called "callus" in a broad sense in forestry, the concept is different from cultured callus.

Many *in vitro* experiments aimed at inducing plantlets from callus[7,8] have been reported. They based on experiments on the regeneration of vascular tissue in injured herbaceous plants[9,10]. On the other hand, the process of wound healing in woody plants, which are significantly different from herbaceous ones in having cambium and secondary xylem has not fully been investigated. In fact, the experimental regeneration of primary tissue or organs such as shoot or root in the callus induced from woody plants have been successful[11], but this is possible only when the materials is juvenile[12]. The direct regeneration of secondary tissue or cambium has never succeeded from the callus tissue. This may be because of the inappropriate assumption that the mechanism of the differentiation of primary tissue of herbaceous plants is similar to the differentiation of secondary tissue of woody plants. It is very questionable whether all living cells in the woody stem can pro-

duce callus cells with "totipotency". Under these circumstances, it would be expected that new information about the mechanism of cytodifferentiation in secondary xylem would be forthcoming from investigations on modified cytodifferentiation *in situ* in the injured cambium of woody plants. The term "cytodifferentiation" which has been used ambiguously and in confusion are defined as the developmental process from the point of determination to the mature of xylem elements[13] in this report.

The development of wound tissue is especially important in respect of its use to mark radial growth. There have been some trials to mark the radial growth of trees by the application of fine artificial injuries to the stem[9,14]. This is called the "pinning method". The foresters have needed a method for the precise measurement of xylem growth for a long time[15,16]. The pinning method has recently become commonly used because of its many advantages. This marking method is very simple in practice and cause little disturbance to the physiological condition of trees. Although the pinning method has been used on the premise that the wound tissue is formed in the cambial region at the time of pin insertion[19,20] and the callus tissue is used as a marker, the site relationships between the wound tissue and the cambial initials at the time of wounding has not yet been confirmed experimentally. If the wound reaction of cambial initials and xylem elements at various phase in differentiation are examined closely, the cells which have been in the cambium at the time of wounding can be detected precisely. Consequently, close examinations of the cytodifferentiation during the wound healing will, not only help obtain informations about the mechanism of cytodifferentiation in secondary xylem, but will also help establish the precise pinning method for marking xylem growth.

Part I of the following reports deals firstly, with the three dimensional shape of wound tissues induced in xylem of several conifers by pin insertion from examination by light microscopy. Then the process of the wound tissue formation in both conifers and hardwood species are examined, especially in relation to the site of cambial initials at the time of pinning, in order to establish the pinning method. As well, traumatic resin canal formation in the pinaceous genera induced by pin insertion was examined as another expression of the modification of cytodifferentiation. The applicability of the traumatic resin canal formation for marking xylem growth was also investigated. For these purpose, fine injuries, which do not cause the discoloration or decay by microorganisms, were made in the cambium by means of penetration through the bark by several sizes of metal pins. Part II deals with much microscopic, cell level observation of wound tissue formation. Investigations were made on how the immature xylem cells or parenchyma cells modify the direction of cytodifferentiation in the disturbed internal environment caused by wounding,

in connection with their phase of differentiation. The mechanism of cytodifferentiation in secondary xylem was discussed with conifer and hardwood species.

PART I Basic observations on wound tissue formation and its use for marking xylem growth

1. Wound tissue induced in conifer xylem by pin insertion

Wound tissues described in the many past reports[5,17,18], are diverse, depending on the way of wounding or wood species. Even when focused on the same kind of minute artificial injury for marking xylem growth, such as pinning[9,14,19,20], the wound tissues are not always the same, and the interpretation of the site of pin marks is in confusion. Although the pinning method has been applied on the premise that the wound tissue induced by needle insertion indicates the site of the cambial initials at the time of the pinning[19,20], Wolter[14], the advocate of this method, concluded that the pin mark indicates the site where secondary wall formation was beginning. Such a confusion may come from the insufficient observations of wound tissues, and it may be settled only if the process of wound healing was observed very carefully.

From this stand point, the author discusses in this chapter about the wound tissue caused in coniferous woods with simpler constituents than of hardwoods, in order to establish the pinning method. After the observations on three dimensional shapes of wound tissue caused by pin insertion in several conifers, the estimation of site relationship between wound tissue and the cambial initials at the time of pinning was firstly made with the control samples harvested at the time of pinning[21], and secondly by the periodical observations on the healing process of wounds after the pin insertion into tree stems[22].

1.1 Characteristics of wound tissue

To observe the three dimensional pattern of the abnormal tissue induced by pin insertion, three vigorous trees of loblolly pine (*Pinus taeda* L.), about 12 years old, planted in Kamigamo Experimental Forest, Kyoto University were selected. At the beginning of June in 1979, needles of three different sizes, 250 μm (specially made), 400 μm (sewing needle), and 700 μm (insect mounting pin) in diamter, were inserted into the stems from the bark through the cambial zone at 9 points in each tree, and removed immediately. All sample blocks ($T \times L \times R = 1.5\ cm \times 2.0\ cm \times$ 2 annual rings) were harvested using a chisel in the middle of August and fixed in FAA. They were then embedded in celloidin according to the conventional procedure. Serial sections of transverse, radial, and tangential faces, 20 to 25 μm in thickness, were cut from each set of three blocks respectively. The sections were double stained with Safranin-Fast Green for microscopic observations.

Photo 1 shows the three dimensional sections from the wounded area where a 400 μm thick needle was inserted two months before. The area where abnormal parenchymatous cells exist will be called wound tissue in this report. In the cross section at the center of pinning, the wound tissue showed a radially-long spindle shape (Photo 1a). In the radial section (Photo 1b), the wound tissue was longitudinally very high, measuring over 5 mm. The radial depth of the wound tissue was greatest at the pinned point, and much deeper than the width of cambial zone at the time of pinning, becoming abruptly narrow above and below that point. The tangential width of the wound tissue was narrower than the pin diameter, and almost constant (300 μm) along its height, ranging about 5 mm as indicated in the tangential section (Photo 1c). This longitudinal height of the wound tissue may be ascribed to a cleavage formed between the immature cells when a pin was inserted.

From the same sample block of Photo 1a, cross sections were cut successively at the interval of 0.5 mm (Photo 2). The wound changes its shape and reduced its size gradually as the section recedes from the center of the pinned point. The abrupt shortening of its radial depth is most remarkable. As the range of wound tissue varied according to the distance from the center of the pinning, is very important to use cross sections from a definite level, the center of the pinned point, when discussing the site of cambial initials at the time of pinning. In this study, the cross section taken from the very point of pining was used in order to assume the site of cambial initials at the time of pinning.

Shape of the wound tissues caused in loblolly pine were somewhat different depending on the needle diameter as shown in Photo 3. In the case of 250 (Photo 3a) and 400 μm (Photo 1a) needle, wound tissue was a radially-long spindle in cross section, and the boundary of the tissue was clear, although it was smaller in the case of 250 μm. In the case of 700 μm needle, wound tissue was big, not spindle-like, and the boundary of this tissue was not clear (Photo 3b). The radial shape of the wound tissue was almost the same in all cases with some difference in size.

For practical use of wound tissue for marking xylem growth, the wounded area is desirable as small as possible and to heal rapidly. However, the needle of 250 μm is sometimes not practical, because this size of needle is so fine and apt to bend that it was difficult to pierce the firm bark, this is useful for young seedlings with delicate tissue. As for the needle of 700 μm the wound seemed to take longer time for healing, and the radial growth was sometimes so surpressed at the pinned area, that this size of needle may not be appropriate in practice.

It was examined whether the shape of abnormal tissue formed in other species is the same with those of loblolly pine or not. Needles of the three sizes were inserted also into the stems of Sugi (*Cryptomeria japonica* D. Don) planted in Nara Pre-

fectural Forest Experiment Station, and Himalayan ceder (*Cedrus deodara* Loud.) growing in Nara Prefecture in June 1979. These samples were processed in the same way as the case of loblolly pine. In the case of Sugi, the shape of wound tissue induced by 250 μm needle (cf. Photo 11) was similar to that by 400 μm needle for loblolly pine. The wound tissue caused by 400 μm needle in Sugi was tangentially wide. Cells of Sugi were broken more excessively than pine probably because of their thin wall, and/or the slow rate of wound healing in Sugi due to its slow growth rate. In the case of Himalayan ceder, although the shape of wound tissues caused by the 250 and 400 μm resembled those of pine, that caused by the 700 μm needle (Photo 4) considerably wide in the tangential direction. In many samples of Himalayan ceder, traumatic resin canals were formed. From these results, it was concluded that the difference in size and shape of the wound tissue depends not only on the histological characteristics of the species or genera such as the size of cell and the thickness of cell wall, but also on the growth rate of trees and the diameter of the inserted needles. Before the application of the pinning method, the most suitable diamter of needles should be determined according to species or age of sample trees.

1.2 Site relationship between cambial initials at the time of pinning and wound tissue

To assume the site relationship between the cambial initials at the time of pinning and the wound tissue induced by pin insertion, a needle, 400 μm in diamter was inserted into three points on a stem of loblolly pine (*Pinus taeda* L.) (Fig. 1), once a month from May to September in 1979, changing trees each time. The needle was removed immediately after every pinning. Two or three months after each pinning, pinned areas were harvested. In order to know the number of tracheids formed before pin insertion, two control samples were taken from both sides

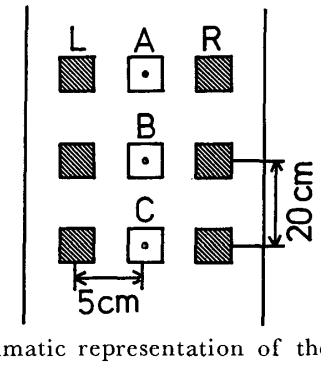

Fig. 1. Diagrammatic representation of the site of pinning and control sample on the stem surface. A, B & C: Pinning area. R & L: Control block on the right side and left side of the pinning, respectively.

of each pinning point (Fig. 1, R & L) immediately after each pinning. Cross sections were cut from sample blocks and were processed for microscopic observations. With regard to the control samples, the tracheid number of the year up to each pinning date (n) was counted along four radial rows for each sample (R & L), in which cambial initials were distinguished by "group of four cells"[23] and the shortest ray cells. The average (N) of tracheids were applied to the cross sections from the pinned sample, and the position of the original cambial initials was assumed (Fig. 2).

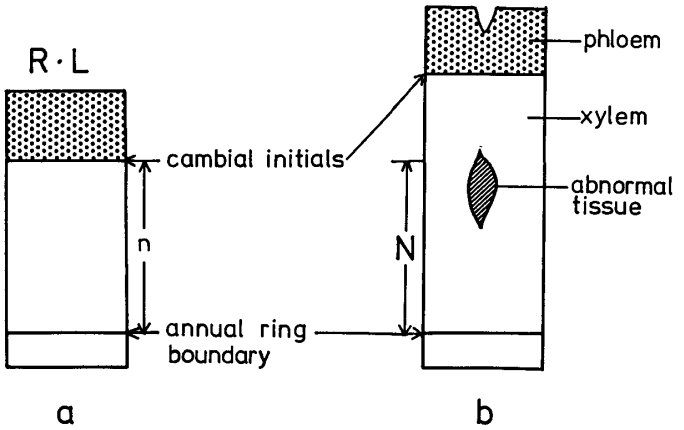

Fig. 2. Diagrammatic explanation to "assume" the site of cambial initials at the time of pinning. Cross sections from a) a control sample (R or L in Fig. 1) collected at the time of pinning and b) a pinned block through the center of pinning. n: The radial number of tracheids in the current annual ring. N: The average radial number of tracheids in control samples counted along 8 radial rows.

The cell number of samples R and L sometimes showed a significant difference. For instance, in case of May-11-A, the average cell number was 72 in R and 49 in L, and the difference was 23, probably because of local variation of growth rate. In such a case, the average does not necessarily indicate the cell number of the pinned area at the time of pinning. The site of the cambial initials was estimated using the sample which had the least difference between R and L among a set of three samples of the same month.

Photo 5 is an example of transverse sections cut from the center of wound tissue caused by pinning in July. The cell number counted from the last annual ring boudary is given on the right side of Photo 5, and the site of cambial initials estimated by the above-mentioned method is indicated by an arrow. There were considerable differences in shape of wound tissue according to the season of pinning or the growth rate of individuals.

With all sample trees pinned from May to September, the sites of cambial ini-

tials at the time of pinning were assumed to be in the neighborhood of the cambial-side margin of the wound tissue. These results differ from those of Wolter[14]. His sections seem to be obtained away from the center of pinning because the trace of pin cannot be seen, and the range of the wound tissue is very shallow in radial direction in his photograph. It should be kept in mind that the range of wound tissue extends deeply from the cambial initials to the lignifying cells at the time of pinning, and that the wound tissue changes its shape and size as the cross section recedes from the center of pinning.

In the present experiment, there were small discrepancies between the assumed cambial initials at the time of pinning and cambial-side margin of wound tissue within ± 5 cells. Some discrepancies had naturally been presumed in advance, since the indirect method, i.e., the use of control samples from both sides of pinning area, was applied to assume the site of cambial initials.

1.3 Process of wound tissue formation in loblolly pine

In order to pursuit the process of wound tissue formation in xylem, 10 vigorous loblolly pine (*Pinus taeda* L.), 15-year-old, growing in the Uji campus of Kyoto University were selected. At the beginning of June, 1979, a pin, 400 μm in diameter, was inserted into 6 stems at three different points on each tree, and removed immediately. On the pinning day and every three or four days thereafter for one month, two sample chips (1.5×2.0 cm $\times 2$ annual rings) were cut from a pinned area with a chisel. As a reiterative experiment, the same pin was inserted into the stems of the remaining 4 trees in the middle of May, 1980. In this case, the pin was inserted at 24 points in one of them, and at 9 points in each of the remainder. On the pinning day and every four days thereafter for about 40 days, sample chips were cut in the same way as in the first experiment. Sample chips were fixed in FAA and embedded in celloidin. They were processed for microscopic observations.

In the case of the samples harvested immediately after the pin insertion, a trace of the pin was observed as indicated in Photo 6a in the cross section around the center of pinning. Cambial initials, which were judged by "the groups of four cells"[23] and the shortest ray-cells, were destroyed only within the tangential width of three or four cells. Xylem mother-cells and enlarging cells were destroyed extensively, and the tangential width of the gap was widest in this area (200 μm). The crushed cells, about 8 radial rows, were pushed aside on both sides of the gap. The thin-walled cambial initials seemed to be protected by phloem. The site of the initiation of wall-thickening was determined by birefringence using a polarizing microscope (Photo 6b). The gap was very narrow in the zone of secondary-wall thickening, and the cells were only separated from each other like the cleavage.

Four days after the pinning (Photo 7), the gap began to close locally within the

zone of xylem mother-cells and enlarging cells. In the zone of secondary-wall thickening, the wound gap did not exhibit any change. About ten days after the pinning, the gap that existed at the time of pinning almost closed along the zone of xylem mother-cells and enlarging cells (Photo 8). The wall of the crushed cells were packed together closely, taking on the appearance of radially-oriented stripes. This stripe-like residue evidently was disconnected at one point (arrow). Such a disconnection of the cell-wall residue suggests that the "stripe" had stretched radially and torn off at the actively-dividing cambial initial-layer. Abnormal parenchymatous cells began to proliferate in the gap (see Photo 20).

By the 21st day (Photos 9), the cut ends of the cell-wall residue had receded from the cambial initials toward the phloem side and the xylem side (arrows). From the vicinity of the xylem-side cut end, the radial rows of tracheids proliferated toward the cambial initials. These tangential proliferations of tracheid rows must have originated, not from the abnormal parenchymatous cells, but from the cambial initials by their anticlinal division on both sides of the wound to adjust for the gap. Therefore, the xylem-tissue zone outside of the point where the tracheid rows began to proliferate is considered to have been formed after the pinning.

On the 36th day (Photo 10), the cell-wall residue on the xylem side had been cut into fragments at a few points and became difficult to distinguish. But, when investigated carefully, among the lignified tracheids which were stained red by Safranin, fragments of this cell-wall residue (arrow 1) could be recognized by being well-stained by Fast Green because of their death at the primary-wall stage. The proliferation of radial rows of tracheid in the vicinity of the outermost cut ends of the cell-wall residue also was observed in this sample. A little inside the site of proliferation, there were some tracheids which were abnormally large or disfigured. It is assumed that these cells had been in the early stage of differentiation, such as xylem mother-cells or enlarging cells, at the time of pinning. As a whole, these cut ends of cell-wall residue on the xylem side coincided with the point where the proliferation of the radial rows of tracheid initiated. On the other hand, the site of the initiation of secondary-wall thickening at the time of pinning could be recognized with the ordinary light microscope even more than a month after the pinning (Photo 10, arrow 2). This was because the cells which had begun wall-thickening at the time of pinning did not show any change other than disfigurations. In addition, the gap within the zone of wall-thickening cells at the time of pinning was extended by the proliferation of parenchyma cells originating from the ray cells.

1.4 Establishment of the pinning method for marking xylem growth in conifers

The sequence of wound healing is schematically illustrated in Figure 3. A

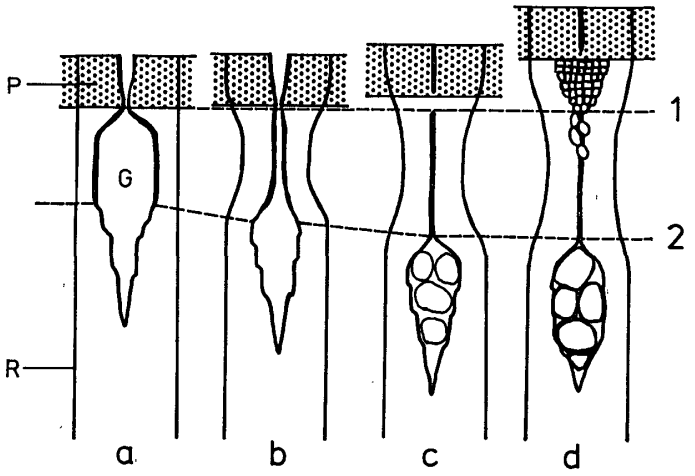

Fig. 3. Diagrammatic illustration of the process of wound tissue formation in loblolly pine. a) Immediately, b) several days, c) about 10 days, and d) about a month after the pinning. 1 and 2: Deduced site of the cambial initials and the initiation of the secondary-wall thickening at the time of pinning, respectively. P: Phloem; R: Ray parenchyma tissue; G: Gap formed by pin.

large gap is formed from the cambial zone to the secondary-wall thickening zone at the time of pinning (Fig. 3a, Photo 6). In a few days, the gap begins to show a tendency to narrow in the zones of xylem mother-cells and enlarging cells (Photo 7), and in about a week, this contracting part of the gap almost closed with stripe-like residue of crushed primary-cell walls (Fig. 3b). About 10 days after the pinning, this stripe-like residue is cut in the vicinity of the cambial initials (Fig. 3c, Photo 8). These cut ends recede from the cambial initials toward the phloem side and the xylem side with time. From the cut end of the xylem side, radial rows of tracheids begin to proliferate toward the cambial initials (Fig. 3d, Photos 9, 10). Meanwhile, abnormal parenchymatous cells are formed, in the gap along the secondary-wall thickening zone. These parenchymatous cells increase in number and size, pushing neighboring tracheids aside, and filled the gap. From the whole process of wound-tissue formation, it is concluded that the place where the proliferation of tracheid rows initiates, as well as the outermost cut-end of the "stripe" of wall residue, coincides with the site of the cambial initials at the time of pinning. This site can be recognized easily any time afterward in the vicinity of the cambial-side margin of the wound tissue in the cross section at the center of pinning. As an indicator of the site of the cambial initials at the time of pinning, it is thought to be more practical to pay attention to the initiation of tracheid row proliferation, because the "stripe" of cell-wall residue is cut into small fragments and becomes difficult to detect before long. The proliferation of tracheid rows is only detectable

within 0.5 mm above or below the center of pinning. Therefore, as mentioned in the preceding section (1.1), it is important to use the cross section obtained from the center of pinning in the application of this method. Besides, it became clear that the cambial-side margin of the wide gap, which was filled up with parenchymatous cells derived from ray cells later can be used as the mark of the site of the secondary-wall initiation at the time of pinning (Fig. 3).

As demonstrated in the previous section (1.1), the wound tissue formed in Sugi (*Cryptomeria japonica* D. Don) by a 250 μm pin is quite similar to that formed in loblolly pine by a 400 μm pin (Photos 10, 11). Therefore the above-mentioned criteria for locating the site of the cambial initials and that of the secondary-wall initiation at the time of pinning in loblolly pine also are applicable to Sugi as shown by arrow 1 and 2 respectively in Photo 11. Kawana et al.[19,20] conjectured using Sugi that the site of the cambial initials was at the outer margin of the wide pin-gap filled with parenchymatous cells, on the basis of "the appearance of collapsed tracheids and the size of collapsed area". The present study, however, indicates that the site of the cambium conjectured by them apparently corresponds to the site of secondary-wall initiation (photo 11). Kawana et al.[19,20] seem to have over-looked the residue of crushed primary-walls farther outside.

The investigations reported in this chapter greatly improved the accuracy of the pinning method in pin-pointing the site of the cabial initials and that of the initiation of secondary-wall thickening at certain times in the past, for coniferous trees.

2. Wound tissue induced in haedwood xylem by pin insertion

The wound responses of hardwoods are supposed to be much different from conifer, judging from the already reported anatomical structures of large wound tissues such as caused by mechanical injuries[5,18,24] or by radial cracks[25]. The method for marking xylem growth in hardwood species also is much needed today. However, only crude applications such as nailing have been tried[26]. In addition, no intensive analysis of pinning wounds have been made, except for reports discussing the large wounds mentioned above. The purpose of this chapter, therefore, is to clarify the difference, if any, between conifers and hardwood species in the wound response, and to test the applicability of pinning method to hardwood species. For this purpose, the process of abnormal tissue formation in poplar was periodically examined after pin insertion, with the same procedure reported for the conifer (1.3), and the structure of wound tissue was compared with that of conifers[27]. Supplemental observations were made on black locust.

2.1 Process of wound tissue formation in poplar

In order to pursuit the wound tissue formation in hardwood, 3 vigorous poplar trees (*Populus euramericana*), about 12 years old, growing in the Uji campus of Kyoto

University, were selected. In the middle of May, 1980, metal pins were inserted into the stems through the cambium at about 15 points (7 cm intervals) for each tree, and were removed immediately. The diameter of the pin was 400 μm for two trees, and 250 μm for the third. One or two sample blocks ($1.5 \times 2 \times 1$ cm) per tree were harvested from the pinned area on the pinning day and every three or four days thereafter for about a month. The remaining samples were harvesed in November. They were fixed in FAA and embedded in celloidin. Sections, 20 μm thick, were cut from the center of pinning, stained with Safranin-Fast Green, and observed by light and polarizing microscopy. For comparison, a 250 μm diameter pin was inserted into the stem of black locust (*Robinia pseudoacacia* Linn.) in the middle of June. Sample blocks were harvested five months later and observed as described above.

Observations were mainly made with the 400 μm pin specimens because their wound tissues were more easily detected than those induced by a 250 μm pin. When observed immediately after the removal of pin, the cells on the both sides of pinned area were extensively crushed within the zone of xylem mother cells and enlarging cells, and somewhat crushed in the vicinity of the cambial initials (Photo 12a). In the zone of secondary wall thickening (Photo 12b), cells were not crushed completely, but rather separated from each other. These are as same with the case of loblolly pine (1.3). By the forth day (Photo 13), cells within the zone of xylem mother cells and enlarging cells began to enlarge and proliferate on both sides of the gap resulting in contraction of the gap. Such enlargement and proliferation of cells extended to about 300 μm tangentially on either side of the gap. Some of the ray tissues became untraceable because of the enlargement and/or proliferation of cells. Abnormal swelling of cambial initials themselves around the injury also occurred within the range of about 300 μm. These cells within the abnormal area were slower to differentiate and tended to remain parenchymatous. Wall residue of cells which were completely crushed at the time of pinning was packed into dark colored stripes on both flanks of the gap (Photo 13, arrowhead). Such residue was lacking within the zone of cambial initials and xylem mother cells (Photo 13). Within this short period of four days, this stripe of wall residue, which once continued from xylem to phloem through cambium, was probably stretched and thereby broken at the actively dividing cambial initials. Therefore, the xylem side broken end of the cell wall residue (Photo 13, arrow) was considered to have been the site of cambial initials at the time of pinning with a probable error of a few cells, because some xylem mother cells next to the cambial initials might have divided once or twice in the first four days after pinning. The fact that it took only four days for the cell residue to be broken after pinning, in contrast to about 10 days in the case of coniferous species

(1.3), suggests more active cambium in poplar. In the radial section of the specimens at day 4, many swelling cells were found around the gap formed by pinning (see Photo 31a). Tyloses were formed widely from the enlarging zone to the last annual ring boundary around the pinned area (see Photo 31b). In addition, many crystals (druses) were found in the ray cells near the cambial initials. These cellular phenomena, none of which are seen in normal poplar xylem, might have resulted from changed cellular physiology caused by pinning.

After the forth day, cambial initials and newly initiated xylem mother cells gradually attained the normal flat cell shape. The gap closed completely within the xylem mother cell and enlarging cell zones at the time of pinning. Within the zone of secondary wall thickening, the gap was left open, and then gradually filled with parenchyma cells derived from ray tissue facing the gap (Photo 14, arrowheads). By the 19th day (Photo 15), abnormal cells around the pinned area formed a diamond or elliptical area in cross section, being enclosed completely in the normal xylem tissue. The abnormal cells began secondary wall thickening and lignification. From the center and inward of the abnormal cell area, the wall residue was observed as a radially oriented stripe (Photo 15). The broken end of the stripe at the center of the abnormal cell area (Photo 15, arrow) can be used as a marker for the position of the cambial initials at the time of pinning. This unfragmented stripe in poplar made it much easier to discern, compared to conifers (1.3), where the stripe fragments. The stripe probably did not fragment in poplar because wood fibers, the principal constituent of hardwood xylem, do not radially enlarge so much as conifer tracheids.

Even six months after the pinning (Photo 16), the shape of the abnormal cell area was like the shape at 19 days after pinning, and the cell wall residue was unchanged. The abnormal cell area consisted of large numbers of parenchymatous cells[5], rarely containing small vessel elements[18]. The site of cambial initials and secondary wall initiation at the time of pinning was easily determined (Photo 16, arrow 1, 2).

In the experiment using black locust, the shape of the abnormal cell area in cross section was somewhat different from that of poplar. However, the stripe of wall residue from cells crushed at the time of pinning was similar to that of poplar, and the gap formed by pinning also was filled with parenchyma cells inward from the zone of secondary wall thickening at the time of pinning.

2.2 Establishment of the pinning method for marking xylem growth in hardwoods

The wounding effects of pinning and subsequent wound healing for a hardwood species are summarized as follows: a) A large gap is formed inward from the cam-

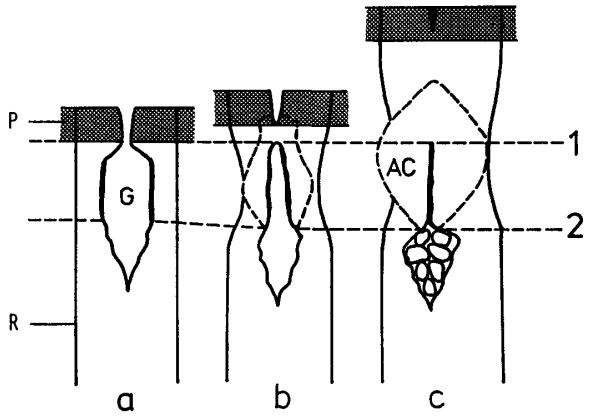

Fig. 4. Schematic illustration of abnormal tissue formation in hardwood species (*Populus euramericana*) after pin insertion. Immediately (a), several days (b), and more than two weeks (c) after pinning. Deduced sites of the cambial initials (1) and initiation of secondary wall thickening (2) at the time of pinning. P: Phloem; R: Ray parenchyma cell; G: Gap formed by pin; AC: Abnormal cell area.

bium at the time of pinning, especially within the zone of xylem mother cells and enalarging xylem cells. The gap is lined with cell wall residue of the crushed cells (Fig. 4a). b) Within a few days, xylem mother cells and enlarging xylem cells further enlarge and proliferate abnormally around the gap, resulting in narrowing the gap within these zones. Closely packed residual walls of the crushed cells, which look like radial stripes in cross section, is broken at the cambial region because of cambial divisions. As a result, the stripes are left behind within the xylem. The gap eventually closes within the above-mentioned zones (Fig. 4b). c) After two or three weeks, the mass of abnormal cells becomes completely enclosed in normal xylem tissue. At this time, this abnormal cell area appears diamond shape or elliptical in cross section. The cell wall residue is packed together into a radial stripe through the center of the inner half of this area (Fig. 4c). The site of cambial initials at the time of pinning is determined to be at the broken end of the stripe of wall residue, clearly distinguishable around the center of the abnormal cell area (Fig. 4, broken line 1).

In comparison, the gap formed inward from the zone of secondary wall thickening at the time of pinning remains open for a fairly long time. By two or three weeks after pinning, this open gap is gradually filled with parenchyma cells which originate from neighboring ray cells. Cells that were thickening secondary wall at the time of pinning do not abnormally enlarge around the gap. Therefore, the earliest site of secondary wall thickening at the time of pinning was identified as being located at the inner margin of the abnormal cell area around the outer

margin of the gap which is later filled with parenchyma cells (Fig. 4, broken line 2). The present tests indicated that the pinning method for marking xylem growth in conifers (see Chapter 1) is also satisfactory for hardwoods.

3. Traumatic resin canal formation in pinaceous genera

Traumatic resin canals are known to be formed in some pinaceous genera, notably, *Tsuga, Abies,* and *Larix*[28,29]. In the process of resin canal foramtion in such genera, epithelial cells are formed among the tracheids which are the basic component of the xylem of conifers. This implies that certain cells which are to differentiate into tracheids in noraml condition changed the course of differentiation to epithelial cells because of the stimuli of injury. This is very interesting phenomenon in discussing the cell differentiation in xylem.

The site of traumatic resin canal formation was assumed to coincide with the site of cambium at the time of wounding and it has been used for marking xylem growth[9]. However, this is not experimentally determined. If such a positive relationship were established, traumatic resin canals might also be used as markers of xylem growth. Traumatic resin canals are knwon to be formed constantly through the growing season in *Tsuga* and *Abies* as a response to pin insertion[9], but occasionally do not form under the same conditions in other pinaceous species and a few species of Taxodiaceae[30]. In this chapter, the author investigated the timing and placement of traumatic resin canal formation in *Tsuga sieboldii* after pin insertion, and assessed the validity using traumatic resin canals as a marker of xylem growth[31].

3.1 Process of traumatic resin canal formation

Three Japanese hemlock trees (*Tsuga sieboldii* Carr.), about 15 cm dbh, were selected at Kamigamo Experimental Forest, Kyoto University. In early June 1980, metal pins were inserted at 16 points at 10 cm intervals for each stem and removed immediately. Pins used on two stems were 250 μm, and on a third stem were 400 μm in diameter. Two or three specimens ($1.5 \times 2 \times 1$ cm) from each tree were harvested, beginning from the day of pinning and every 3 or 4 days thereafter for 20 days. The rest of the specimens were harvested on the 36th day. Trimmed specimens ($1 \times 1 \times 0.5$ cm) were fixed in 2.5% glutaraldehyde and embedded in celloidin. Mainly, cross sections 20 μm in thickness were cut, stained with Safranin-Fast Green, and investigated by light microscopy.

Traumatic resin canals were formed, irrespective of pin diamter, on both sides of the wounded area in a tangential row, parallel to the annual ring boundary, as indicated in Photo 17. The process of resin canal formation observed in cross section was as follows: Three days after the pinning (Photo 18a), some cells within the zone of xylem mother cells (3–5 cells inward from the cambial initials), could be distin-

guished from the other cells by their larger size and more rounded shape. Four to six such rounded cells formed a group (Photo 18a, broken lines). This is the first symptom of traumatic resin canal formation. It is considered that some groups of cells among uniform xylem mother cells stopped to divide periclinally, and became round in these 3 days. Such groups of rounded cells were formed within 2 to 3 mm on both sides of the wound. On the 6th day, groups of rounded cells had already transferred to the enlarging zone (Photo 18b). At the center of the rounded cell groups, small rhombic gaps had been generated at cell corners or, in some cases between tangential walls (Photo 18b, broken lines). On the 10th day, the gaps between the rounded cells enlarged (Photo 18c). The rounded cells around the gap with dense cytoplasm and conspicuous nuclei indicate the horizontal cellular septation (Photo 18c, arrows). These septated cells were to become epithelial cells. By the 20th day, the gaps had become larger, and thin-walled cells containing a number of small resin-like droplets were observed surrounding the epithelial cells (Photo 18d). Thirty-six days after pinning, an irregular line of resin canals tangentially traversed the 1 cm width of the cross sections (Photo 17). Tangential fusion of canals also was observed.

3.2 Traumatic resin canals of pinaceous genera as a marker of xylem growth

Juding from the fact that symptoms of traumatic resin canal formation (i.e., groups of rounded cells) was found within the zone of xylem mother cells only 3 days after pinning, and that the gap of resin canals subsequently originated and enlarged at the centers of those anatomical symptoms, the site of cambial initials at the time of pinning is near the center, or a little outside of the traumatic resin canals. However, the number of cells derived from an initial during such a 3-day period is unknown, nor is it certain whether the formation of resin canals in all cases begins immediately after pinning. Accordingly, the pinning method (Chapter 1) was applied in order to learn whether traumatic resin canals are always formed at a definite distance from the cambial initials.

Wound tissue induced in *Tsuga* by 250 μm pin insertion (Photo 19) was very similar to that formed in *Pinus taeda* (see Chapter 1). Thus, the sites occupied by cambial initials at the time of pinning can be assumed in *Tsuga* (Photo 19 arrow). The mean distance from the assumed site of cambial initials (Photo 19, arrow) to outside (Fig. 5, open circles) or inside (Fig. 5, solid circles) of total 60 resin canals in three specimens, 10th, 20th, and 36th day after the 250 μm pin insertion was calculated. The means of 60 values plus 95 percent confidence intervals were 0.0034 ± 0.0067 mm and -0.077 ± 0.0083 mm for outside and inside of resin canals, respectively. Positive or negative sign of the mean values indicate outside or inside

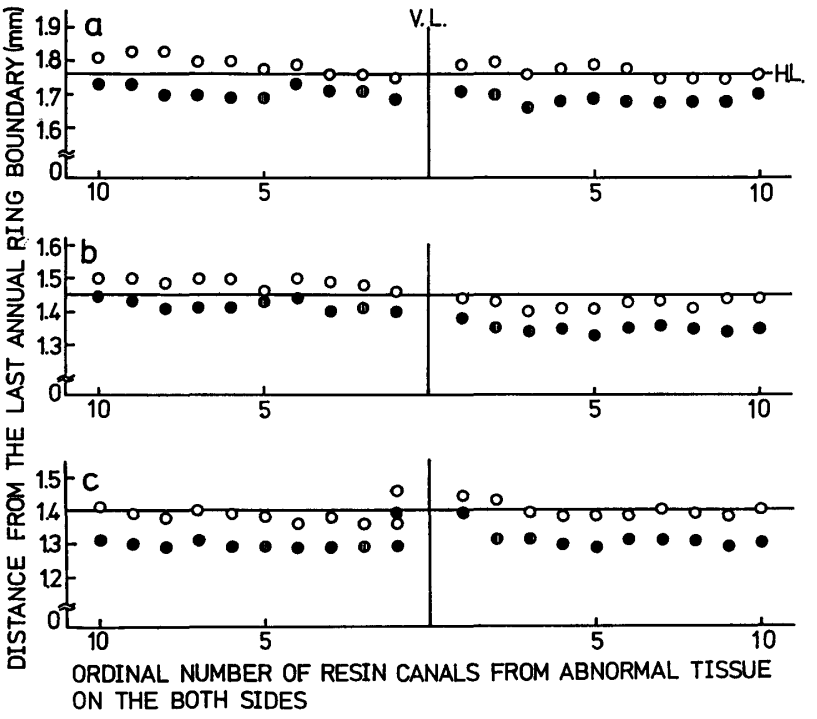

Fig. 5. Site relationships between traumatic resin canals and cambial initials at the time of pinning in *T. siebeldii*. (a), (b), and (c) represent the samples on the 10th, 20th, and 36th day (Photo 19), respectively, after insertion of 250 μm pin. V.L.: Center of wound tissue. H.L.: Assumed site of cambial initials. Outside (○) and inside (●) of each resin canal were measured from the last annual ring boundary.

of the assumed site of cambial initials at the time of pinning (Photo 19, arrow, and Fig. 5, horizontal line). This statistical analysis shows that the outside of traumatic resin canals coincides with the site of cambial initials at the time of pinning. This supports the hypothesis that the traumatic resin canals are always formed in the zone of xylem mother cells very close to the site that was occupied by cambial initials at the time of pinning. In some cases, traumatic resin canal formation was a little delayed in the close vicinity of the pinning point (Photo 19, Fig. 5c).

The radial width of traumatic resin canal differed in individual samples, in particular, the number of epithelial cells in a radial row (2 to 8 cells). Such variations might be attributable to difference in radial width of the xylem mother cell zone, wherein cells can differentiate into epithelial cells.

Traumatic resin canal formation as related to the assumed cambial initials at the time of pinning has been schematically summarized in Figure 6. a) During the first 3 days after pinning, many groups of large rounded cells are formed in the zone of xylem mother cells. b) Within 6 days after pinning, intercellular spaces begin to develop at the centers of these cell groups. The cells surrounding these

gaps differentiate into the epithelial cells. c) In the final stage, the gaps enlarge and thin-walled cells containing resin-like droplets surround the epithelium. At this final stage, when the enlargement of the gaps has been completed, the site of the cambial initials at the time of pinning appears to have been in the vicinity of the cambial side of the epithelial cells of the resin canal.

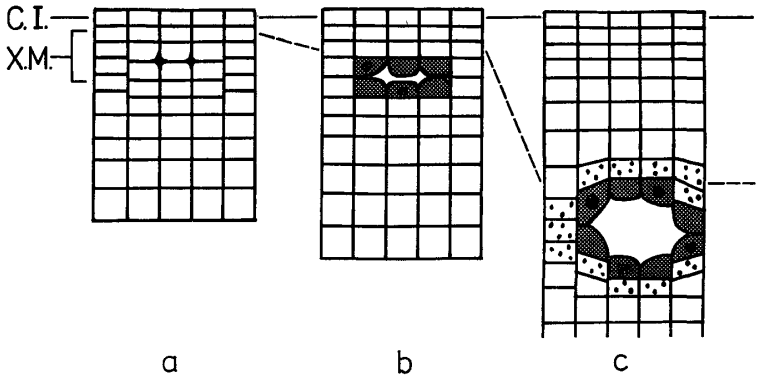

Fig. 6. Diagrammatic illustration of the process of traumatic resin canal formation in relation to the assumed site of cambial initials at the time of pinning. a) Three days, b) about 10 days, and c) more than a month after the pin insertion, respectively. C.I.: Cambial initial. X.M.: Xylem mother cells. Solid line: Cambial initials at the time of sampling. Broken line: Assumed site of cambial initials at the time of pinning. Small dotted cell: Epithelial cell. Large dotted cell: Thin-walled cell.

Traumatic resin canals do not discern the site of cambial initials as precisely as does the wound tissue (Chapter 1), and the initiation of secondary wall thickening cannot be determined from the resin canals. On the other hand, traumatic resin canals are much easier to detect than is wound tissue. This is because wound tissue is available only when the cross section was cut from the very center of the pinned area (section 1.2), while the range of traumatic resin canal formation covers more than 0.5 cm around the pinned area. For practical application, therefore, traumatic resin canals are very useful when attention is given to the following: Traumatic resin canals might be formed by natural or artificial injuries such as frost, wind, or pressure[32]. A control specimen should be taken from unpinned area for comparison to eliminate the confusion with those traumatic resin canals caused by injuries other than pin insertion.

PART II Wound effects on cytodifferentiation in the secondary xylem

4. Effects of pinning injury on cytodifferentiation in conifer xylem

The experiments aiming at the redifferentiation or regeneration of vascular

tissue around the injury of plant stems or roots[33,34], or induction of the tracheary elements or cambium artificially in the *in vitro* system[7,8] explain the process of wound healing in plants as the formation of dedifferentiated callus tissue, followed by vascular tissue formation within it[35]. There are controversial discussions about various plant hormones needed for such vascular reorganization or tracheary element formation[36,37].

In the studies described in Part I, the author found that the wound response of tree xylem is more complicated than in herbaceous species[38]. In addition to the callus like proliferation of ray cells, and instead of the regeneration of vascular tissue, various modifications of cytodifferentiation were observed in the secondary xylem of woody plants.

Close examinations on the wound healing process in tree xylem from another point of view than the *in vitro* study of callus will add new information about the mechanism of cytodifferentiation in xylem. The usefulness of the *in vivo* system is, firstly, the effect of naturally-caused environmental disorder to cytodifferentiation can be studied by wounding immature xylem, although the control of cellular environments in the intact plant is thought to be difficult[41]. Secondly, the origin of each abnormal cell are traceable only in this *in vivo* condition. The origin of each cell in the cultured callus tissue is never known, although the callus cells are treated as homogeneous[42] ignoring the difference in their origin. In this chapter, the author examined how xylem cells change at each differentiating stage, after minute wounding in the cambial zone of a coniferous species. The differences in wound responce between the xylem element were compared in order to discuss the differentiation of xylem cells in conifers[43].

4.1 Anatomical changes of xylem cells after pin insertion in loblolly pine

To make minute injuries, a needle, 400 μm thick, was inserted at 51 points into the stem of a loblolly pine (*Pinus taeda* L.), 15 years old, in the Uji campus of Kyoto University on May 20, 1981. The needle was removed immediately. Each one specmen ($T \times L \times R = 3 \times 3 \times 1$ cm) containing three pinned points was harvested on the pinning day and every one to four days thereafter for about a month, and the last harvest was postponed until three months later. Each of them were divided into three, trimmed ($1 \times 2 \times 1$ cm), and fixed in FAA. All the fixed samples except the last harvest were embedded in celloidin, and serial sections of the three planes, about 20 μm in thickness were cut from the pinned areas. They were stained with Safranin-Fast Green, and observed by light microscopy. The samples of the last harvest were sectioned radially into about 40 μm thick sections in frozen condition, treated with antiformin-water (1 : 1v/v) to remove the cell contents, and observed by scanning microscopy (SEM).

On the seventh day, some anatomical changes were conspicuous in the xylem tissue around the wound gap (Photo 20). Among the ray cells, some facing the wound gap were swelling into the gap (Photo 20a, arrows 1), and some around the wound divided radially (Photo. 20b)[17,44]. In addition, ray cells invaded into the neighboring tracheids through the pinoid type pit like tylosis formation[45], or elongated longitudinally intruding between the immature tracheids. Yellow-brown colored droplets were seen in several ray cells near the cambium around the wound (Photo 20a, arrowheads). A part of ray cells derived from ray initials are to differentiate into ray tracheids later. Regardless of their destination (ray parenchyma cells or ray tracheids) immature ray cells in xylem-mother-cell zone have divided repeatedly by the injury. The injury seems to stimulate the mitotic activity of immature ray cells. In contrast, the maturation of the cells derived from fusiform initials proceeded almost normally, although some swelling of primary pit fields or sporadic transverse divisions were observed. Fusiform and ray initials have not noticeably changed. The subsequent changes will be described for ray cells mainly, and changs in fusiform cells will be added if relevant.

In the wall thickening zone, or in the mature xylem, ray parenchyma cells were also found to have invaded into the tracheids through the pinoid pits or destroyed wall on the 9th day (Photo 21, arrows). Inward from the wall thickening zone, only ray parenchyma cells keep the ability to divide because the cells destined to be ray tracheids show rapid wall thickening followed by autolysis, while ray parenchyma cells of loblolly pine develop a secondary wall very gradually and do not lignify until the shift to heartwood[46~48].

Ray parenchyma continued active proliferation in the gap formed by pin insertion until the gap was completely filled with them (Photos 22 & 23). Ray cells containing many yellow-brown droplets increased in number and scattered over 7 mm longitudinally on the 12th day (Photo 22, arrowheads). These droplets, which are assumed to be phenolic substances[49,50], may indicate metabolic changes in the cells around the woud, similar to those involved in heartwood formation.

When ray cell derivatives almost filled the gap on the 26th day (Photo 23a), these derivatives indicated an irregular shape. Yellow-brown droplets appeared also in these cells (Photo 23a, arrowheads). The most marked changes in this period are the wall thickening and lignification of ray tracheids (Photo 23b, asterisks), which divided repeatedly for a while to produce "ray tracheid derivatives" before the initiation of wall thickening and lignification which were slightly delayed as compared with the noraml ones. Some of them produced yellow-brown material and kept their cytoplasmic contents for a relatively long time. In that case, the development of dentate thickenings which is characteristic of ray tracheids in diploxylous Pinus

was rather weak, and have modified pits transitional between bordered and simple. Most of the cells derived from immature ray tracheids differentiated to ray tracheids, and in some cses, such "ray tracheid derivatives" might be modified by the change of the rate or duration of cell wall thickening, being affected by metabolic or genetic changes in the cell[51]. The above mentioned tylosis-like cells, which had the same origin as the proliferating ray parenchyma cells in the wound gap, also divided several times in the host tracheid (Photo 23a, arrow, Photo 24).

The proliferated ray parenchyma derivatives, as well as tylosis-like cells, began wall thickening and lignification as early as the 30th day (Photos 24 & 25). This is quite abnormal, because these phenomena are not seen until heartwood formation. The wall thickening and lignification of ray parenchyma derivatives initiated when there was no more room for cells to enlarge. This supports the idea that the trigger for the initiation of wall thickening might be external pressure on the enlarging cells[52,53]. Ray parenchyma derivatives in the gap produced remarkable reticulate walls, which are never found even in the heartwood. Wall thickening and heavy lignification were followed by autolysis (Photo 25). The same type of wall-thickenings are observed in the intact pith cells of *Abies* (unpublished data). They resemble so-called tracheary elements which are induced in cultured callus tissue[54] or pith segments[42,55], or from a cultured isolated mesophyll cells[56,57].

Within a strand of tylosis-like cells derived from a certain ray parenchyma cell (Photo 24a), wall thickening and lignification of each cell initiated not necessarily in the order of its formation in the host tracheid, but in the random order. When observed with SEM (Photo 24b), the boundary between tracheids and tylosis-like cells was very clear. Their simple pitting did not correspond to the bordered pits in the host racheid, while simple pit pairs were distinct between the tylosis-like cells in the same tracheid. In the cross section (Photo 26), this tylosis-like structure was seen as if a part of the tracheid. These structures have been misunderstood as abnormal tracheids with additional thick wall layers formed by repeated apposition of S2 and S3 layers[17,39,58] when these cells have thick walls as shown in Photo 24. They also have sometimes been misinterpreted as abnormal "fossilized" tracheids[40], or, judging from the cytoplasmic contents in that structure (Photo 26), as parenchyma cells formed by a shift of differentiating direction in developing tracheids after wounding[30]. From the author's observation, however, it is clear that these structures are the result of the invasion of living parenchyma cells into mature tracheids, and that the "extra additive wall" is not formed by tracheids, but by ray parenchyma derivatives which invaded into the tracheids.

Regarding the longitudinal elements, only a few cells near the wound divided once or twice, but differentiated into normal tracheids. Some of them in the zones

of xylem-mother cells or cell enlargement showed excessive diameter enlargement. Besides, septate tracheids with bordered pits on the septa were found in rare case. These results show that the ability of proliferation does not increase in the case of immature axial element, and once or twice of their division do not shift the once destined course of differentiation to tracheid. Abnormal pitting was found in the 30th day's section (Photo 27) a few millimeter longitudinally away from the pinned points: a group of several very small bordered pit pairs, each of which was typical, were formed in one primary pit field of fusiform cells which had initiated secondary wall thickening at the time of pinning. In spite of Kučera's report[17], so-called callus cells, presumably derived from fusiform elements, were not found by the author.

Fusiform cambial initials seemed to make radial division around the injury in order to supply new initial cells to the gap formed by pin insertion when observed a cross section of the sample which finished healing. Cell production by periclinal divisions in the cambium was normal after injury except for some decrease in the rate of cell division. Cambial initials, ray and fusiform, did not show any change (Photo 22 & 23) such as a callus-like proliferation as the ray parenchyma cells did. Thus, cambial initials, as a whole, seemed to be very stable in the case of this coniferous species.

4.2 Interpretation of modified cytodifferentiation in conifer xylem

The abnormalities found in the differentiating xylem cells around the wounded area are summarised in Table 1, for the different xylem elements at each phase of differentiation. The prominent phenomenon caused by injury is the high activation of ray parenchyma cells. They not only showed repeated proliferation, but also excessively promoted growth (wall formation) and ageing (lignification to necrosis) processes. These ray cell proliferation were explained as the origin of callus tisse[35,53]. Ray cell derivatives with reticulately thickened wall may be, in a sense, a new type of element differentiated from ray parenchyma cells[13] reminiscent of tracheary el-

Table 1. Abnormalities observed around the wound in loblolly pine

Stage[a]	Axial tracheid	Ray tracheid[b]	Ray parenchyma[b]
Initial	Radial division[c]	Yellow droplets Elongation Proliferation	Yellow droplets Proliferation: Multiseriate Tylosis-like Disordered
Xylem-mother cells			
Cell enlargement	Extra enlargement[c]		
Wall thickening	Abnormal bordered pits	Modification of wall thickening and bordered pits	Reticulate wall thickening Lignification Necrosis

a) At the time of pinning. b) Including their derivatives.
c) These are found in a few cases.

ement formation from cultured mesophyll cells[56,57]. Although, in the experiments using parenchymatous explants or callus derived from herbaceous species, the so-called tracheary elements may be thought to be the substitutes for tracheids or vessel elements, it is inappropriate to assume this in the case of secondary xylem of woody plants. The shape of ray cell-derivatives, or "tracheary elements", does not resemble that of tracheids in the secondary xylem at all. From the results of this experiment, the ray parenchyma derivatives in the wounded area are thought to have followed an accelerated, as well as excessive ageing process, followed by an early death, without differentiating into any other element or retaining meristematic capacities.

Beside the ray parenchyma cells, some immature ray tracheids also proliferate. Their behaviour, such as excessive enlargement or production of yellow material, is almost the same as that of the ray parenchyma derivatives for a certain period, and is completely different from that of the immature axial tracheids. After several times of division, they initiated wall thickening and lignification earlier than ray parenchyma derivatives. Judging from the fact that almost all of them differentiated into ray tracheids after the repetition of division, the fate of a ray cell whether to parenchyma or to tracheid may be decided as early as at the stage of cambial initials or in the zone of xylem-mother cells.

In the case of fusiform elements, division of immature cells were not frequent, and even after the division, they also differentiated into tracheids. The modification of pit pairs are thought to be caused by the disturbance of cell-wall thickening.

Influenced by the idea that callus cells are being dedifferentiated and have a totipotency for redifferentiating to any type of cells[59], a number of observations have been interpreted in a manner to suggest that immature cells or parenchyma cells easily change the course of differentiation by the effect of injury[17,30]. The present experiment, however, clearly demonstrated that immature cells or parenchyma cells neither differentiate to cambial initials nor shift their course of differentiation so easily in nature. Regarding the potential of cells in the callus tissue, including the wound tissue reported here, it is thought to be more natural that each cell does not directly redifferentiate into the elements of the secondary xylem. If any secondary xylem elements develop in the callus, the formation of meristematic cells in the callus tissue is a prerequisite for the differentiation of such elements.

5. Effects of pinning injury on cytodifferentiation in hardwood xylem

The studies on the xylem cell differentiation in the wounded stem of conifers (Chapter 4)[43] have revealed several facts which are a little different from the commonly accepted concepts. That is, the main constituents of the wound tissue were the new derivatives of the revived ray parenchyma cells, and do not show any toti-

potency. The ray cell derivatives with reticulately thickened walls are considered to be in the last stage of ageing, even though they are similar to tracheary elements in the cultured tissue of herbaceous species[34,55]. Immature xylem cells do not change the course of differentiation by the wounding, although some investigators have reported such a reorientation of differentiation in conifer[17] by misunderstanding the nature of ray cell proliferation.

In the case of hardwood species, cytodifferentiation in xylem of the wounded stem seemed to be somewhat different from that of coniferous species, because of much diversity in the longitudinal elements. In previous reports on large wounds in hardwood stems, the abnormalities in the cytodifferentiation were reported fragmentarily[5,18,24,25] and not discussed systematically, because past observations were confined only to the wound tissue of the already healed zone of injuries. However, this author's observations on the wound tissue formation in hardwoods (Chapter 2) suggests significant modifications in the pattern of differentiation of fusiform elements. Cytological observations of wound tissue formation will yield informations on cytodifferentiation also in the secondary xylem of hardwood.

This chapter examines the mechanism of cytodifferentiation in hardwood xylem, with the same procedures in Chapter 4. The difference between woody and herbaceous dicotyledons in respect to wound reaction are compared[60].

5.1 Abnormal cytodifferentiation in wounded poplar xylem

On June 15 in 1981, a needle (400 μm thick) was inserted at 60 positions into the cambium of a poplar (*Populus euramericana*), 15 years old, in the Uji campus of Kyoto University. This species, with diffuse porous wood, is known to react to wounding by pin insertion in the same way as black locust, a ring porous wood (Chapter 2). Each one or two specimens ($3\times3\times1$ cm) containing three pinned points were harvested on the pinning day and every day for a week, and then every two to ten days for three weeks thereafter. Each specimen was divided into three, then trimmed ($1\times2\times1$ cm), fixed in 2.5% glutaraldehyde, and embedded in celloidin. Serial sections of the three planes, 20 μm thick, were cut, stained with Safranin-Fast Green, and observed by light microscopy. The anatomical changes of the cells around the injury were studied, and compared with the same tissue in coniferous species.

In the last harvested specimens (29 days after wounding), the wound tissue had been completely enclosed within normal xylem, and the cambium in the injured region was normal again (Photo 28). The sites of both cambial initials and secondary wall initiation at the time of pinning were precisely deduced in the cross section through the center of the wound tissue as shown by the solid line and broken line in Photo 28a, respectively, by the pinning method (Chapter 2). Both of the deduced sites

of cabial initaials and secondary wall initiation in the cross section (Photo 28a) were observed in the radial section of the same date (Photo 28b, solid- and broken-line). The wound tissue in the radial face was divided into three zonse by these lines: A) Zone of secondary wall formation at the time of pinning. B) Zone of primary wall formation, including cambial initials, xylem mother-cell zone and enlarging zone at the time of pinning. C) Zone of tissue formed after wounding.

The center of this section was occupied by a mass of abnormal parenchymatous cells (Photo 28b, arrowhead) (zones A and B), and surrounded by many abnormal or discolored cells. This callus-like area resulted from the proliferation of ray cells into the wound gap (Chapter 2). Abundant tyloses were found around this wound from the zone of xylem mother cells to the mature zone (zone B to A).

The reaction of the cells around the mass of parencymatous cells was different in these 3 zones, respectively. In the zone of secondary wall thickening and mature zone (zone A), only ray cells proliferated actively not only filling the gap fomred by pin but also invading into the neighboring fibers (Photo 29, arrow 1) or vessels (Photo 29, arrow 2) from their broken ends, and looked as if the septate fibers. This phenomenon is similar to the case of coniferous species (Chapter 4). On the other hand, in the area around the solid line in Photo 28b (arrow 1), many fusiform cells with septate walls were found. These are strand cells formed by the transverse diivsion of themselves (Photo 30, arrows). The strand cells were distributed at least for several millimeters above and below the pinned pint. In addition, the differentiation of vessel element in this area was seldom observed. The more the stage of differentiation had developed at the time of pinning (that is, as the site apart from solid line inward toward broken line in Photo 28b), the less the frequency of transverse division probably because of cell ageing, and the more limited the distribution of strand cells in the vicinity of the wounded area.

In the zone of xylem mother cells and enlarging cells (zone B), there were also numerous abnormal "septate" cells (Photo 28b, arrow 2). Some may be strand cells formed by the transverse division in this area, but most of them were thought to be cells proliferating from ray cells and invading other cells as observed in zone A. These derivatives were easily confused with strand fusiform cells (Photo 30), and it is very difficult to distinguish one from the other as in the case of coniferous species. The invaded cells have been incorrectly described as modified cells with septate walls[25], parenchymatous cells[17], or tracheids with additive thick walls[39,40] in the past reports.

A group of vessels with abnormally small diameter were found just outside the solid line in Photo 28 among the strand cells but a little apart from the wound (Photo 34, arrows). Outside this region differentiated xylem elements normally,

although vessel elements (Photo 34a, asterisks) and fibers were short, and there are some ray tissues only one cell high.

Ray cells with yellow-brown contents, assumed to be the phenolic substances[49], were scattered widely in and around the wound tissue similarly to the case of conifers. Many ray cells which have been in the zone of xylem mother cells to initials at the time of pinning contained druse type crystals.

Above observations indicated that the most significant difference from coniferous species was the reaction of fusiform cells (Photo 30). On the other hand, the mitotic reactivation of ray cells was the same with conifer. Therefore, ray cells will not be discussed in detail as they have been discussed in Chapter 4. Based on these results, the reaction of the xylem cells in the various phases of differentiation was pursued with samples periodically harvested from the day of pinning to about a month later.

5.2 The timing and nature of abnormal cell formation around the injury

The reaction of cells around the wound gap was very rapid in poplar. Only two days after pinning, several ray cells were found swelling around the injury (Photo 31a, arrow 1) in the similar pattern with the case of conifers (Chapter 4). Some ray cells had already invaded the fiber lumina through their broken ends. Such prompt reactions may be ascribed to the high mitotic activty in poplar. Tylosis-buds were also formed in the mature vessels inside the wound (Photo 31b, arrows). Immature fusiform cells as well as adjacent cambial initials had begun to divide once or twice transversely in the restricted area very close to the wound gap (Photo 31a, arrow 2). This site coincides with the strand cells of the 29th-day sample observed a just inside the solid line in Photo 28b. These are apparently strand cells and not derived from ray cells.

Four days after pinning, at the stage when the wound gap was already closed in the zone from cambium to enlarging cells, highly frequent but somewhat random transverse divisions were observed around the pinned point within the zone of xylem mother cells and probably including cambial initials (Photo 32, arrow). This zone of active transverse division extended longitudinally more than several millimeters above and below the wounded area. The site was probably formed at the cambium immediately before to after wounding. Around the wound in the 2nd day's sample, no one which had been in the zone of xylem mother cells at the time of pinning showed the enlargement for the differentiation of normal vessels even entering into the enlarging zone. Extensively proliferating ray cells filled many vessels and fibers in the area close to the zone of cell "septation". The cambial initials were not rectangular in the cross section.

After a week from the pin insertion, above mentioned strand cells, which had

been in the zone of xylem mother cells at the time of pinning (Photo 28b), entered the last stage of differentiation, wall thickening and lignification, without differentiating into vessel elements. The walls of these cells were thicker than normal fibers, especially in the neighborhood of the wound gap (Photo 33). In the center of pinned site however they remained somewhat parenchymatous. The pitting of these thick-walled cells was very similar to fibers, i.e., sparse and very small. Within but towards the outside of the zone of enlarging cells, and above and below from the pinned area, a mass of immature strand-cells longitudinally extended (see Photo 28, arrow 1). Differentiation of abnormal vessel-like cells were found amongst these cells. Many strand cells, some of which contained cytoplasm, surrounded those abnormally narrow vessels like paratracheal parenchyma. Normally, poplar only possesses terminal. These cells which differentiated into narrow vessels and strand cells were those formed immediately or within several days after the wounding.

The abnormal cell production by the cambium lasted less than 14 days. By the 9th day, the transverse division of fusiform cells in the zone of the xylem mother cells had almost ceased. Twelve days after the pinning, the cambial initials around the pinned area had regained the normal flattened rectangular in cross sections and seemed to be dividing normally. Vessels differentiated almost normally near the current cambium, although the elements were short, apparently because of transverse division of the fusiform initials. Ray tissues also regained the normal width, i.e., uni- or bi-seriate. The zone of abnormal cells, elliptical to diamond shaped in transverse section, was completely included in xylem, and was followed by normally differentiated xylem cells. From the 9th to the 29th day after the pinning, the development of abnormal tissue was not conspicuous.

By the 12th day, the wall thickening of the abnormal narrow vessels was completed. As well as being narrower, the abnormal vessel-like elements showed modifications in morphology (Photo 34a, b). Some relatively near the wound, formed a strand similar to the axial parenchyma, but occasionally with bordered pits (Photo 34a, b, arrows 1). Some were imperforate like the tracheids (Photo 34a, arrow 2) and some had a single perforation on the side wall (Photo 34b, arrow 3). Such structure indicated successive modification to normal in longitudinal direction from injury. Vessels differentiating after the injury gradually reverted to their normal structure.

The strand cells around the narrow vessels continued to show abnormal differentiation in respect of wall thickening and lignification until after the 12th day. Some did not show the synchronized maturation. Although the mass of strand cells had the appearance of axial parenchyma, observation at high magnification revealed

that there was also successive modifications in cell morphology from inside to outside (Photo 35). In the innermost zone very near to the wound, fusiform cells had less frequent transverse divisions and were thick-walled with small sparse pits like fibers (Photo 35, arrow 1). The shape and size of pits changed in successive cells (Photo 35, arrow 2) becoming large and round like the pits of axial parenchyma cells (Photo 35, arrow 3) towards the outer regions of the abnormal tissue. The strand cells and narrow vessel-like cells, both indicate a variation in differentiation, longitudinally from the center to above or below, and radially from inside to outside.

The behavior of ray cells was almost the same as with conifers (Chapter 1 & 4) except for the extent of wall thickening and the presence of crystals. In poplar, reticulate thickening was not so intensive as conifers.

5.3 Interpretation of modified cytodifferentiation in hardwood xylem

The abnormalities observed in the xylem around the wounded area for the different xylem elements at each phase of differentiation are summarized in Table 2.

Table 2. Abnormalities observed around the wound in popular

Stage	Fusiform element	Ray parenchyma[b]
Cells formed after wounding	Transverse→Axial parenchyma to fiber-like division →Tracheid- and narrow-vessel-like	Crystal formation Colored material
Initials	Transverse division→Short initial	None
Xylem-mother cells[a]	Transverse→Fiber-like division Sporadic vessel	Proliferation Colored material Crystal formation
Cell enlargement[a]	Abnormal perforation	Proliferation Invasion into other cells Tylosis Colored material
Wall thickening[a]	None	

a) At the time of pinning. b) Including their derivatives.

The ray cell derivatives, especially in the zone of xylem mother cells to enlarging cells at the time of pinning, were mingled with the strand cells derived from fusiform cells, and were difficult to distinguish one from the other. Careful observations by the author clarified that the conspicuously abnormal fusiform cells occurred only in the limited portion around the wound. The area with strand cells was limited to a zone 200 to 300 μm radially, more than 5 mm longitudinally (Photo 28b), and 500 μm wide tangentially. Most of the strand cells were transitional between fiber and axial parenchyma cells. Some of them differentiated into vessel-like elements, a little distant longitudinally from the wounded area.

These facts indicate that the disturbance of internal environment — such as changes in the concentration of hormone or some other substances, diversity in pH,

or exposure to oxygen — caused by the injury promotes mitotic activities both in fusiform cells (transverse and radial division) and ray parenchyma cells (random division), prevents the enlargement of vessel elements, and modifies the morphological differentiation in xylem cells resulting in the cell types variously different from the typical xylem elements.

Except for the mitotic reactivation of ray parenchyma cells, the injury significantly affected only those cells which had been in the zone of xylem mother cells at the time of pinning, and those which were formed from cambial initials within several days after wounding. Moreover, the cells in the zone formed after wounding indicated much diversity in cytodifferentiation in contrast with those in the zone formed before wounding. Therefore, the course of cytodifferentiation sequence seemed to be determined in the cambial initial and/or the neighboring xylem mother-cells, and not subject to further change.

In general, the term "differentiation" has been broadly used when the occurrence of the morphological changes such as cell elongation, wall thickening or lignification are observed in a cell[61]. But in this experiment, the direction of cytodifferentiation sequence seemed to be determined much earlier than the appearance of such morphological changes. Once the course is determined, there is no further significant change in the direction of cytodifferentiation, at least in the case of fusiform cells in secondary xylem, even though possibility of reorientation has been proposed[17]. The concept that "cytokinines regulate the initiation of cytodifferentiation"[13] is doubtful in that the initiation of cytodifferentiation in this case is indicated only from morphological changes. The mitotic reactivation of ray parenchyma cells and immature fusiform cells seems to be independent of the direction of cytodifferentiation, and that ability is kept until the initiation of wall thickening. However, in the case of the organ formation in callus[62] which is known to be derived mainly from ray parenchyma cells[35], a kind of rejuvenation may take place by the frequent repetition of mitosis.

The versatility in hardwood observed in this experiment with vessel elements, tracheid-like cells (including strand ones), axial parenchyma cells, and fibers is completely different from conifers. The morphological variation was observed continuously as a function of distance from the wound (center — below and above), and of time (inside — outside). Based on the criteria for normal xylem, the various elements to which the modified cells belong cannot be clearly distinguished. This suggests that cytodifferentiation in secondary xylem is controlled by certain substances, some of which increase and disperse around the injury, and then decrease with time after wounding. These modified cells resemble those which were induced in the stem of *Salix* by the morphactin or Ethrel treatment.[63] Phelps et al.[63] sug-

gested the participation of auxin in such a modification in xylem cytodifferentiation. The stability observed in the cytodifferentiation of conifer xylem after injury (Chapter 4) may due to its simpler constituents than hardwoods.

The xylem cell formation can be roughly classified into three phenomena: the production of wall materials, cell enlargement, and lignification. The balance among the biochemical reactions which rule these three phenomena in cell formation eventually seem to bring about the final structure of cells. For instance, when cell enlargement is faster than cellulose synthesis, the cell differentiates into vessel element, and the reverse condition brings about fiber. In this experiment, the changes in the level of substances in cambium caused by wounding might have accelerated some of the biochemical processes in the cambial cells producing the intermediate pattern in xylem elements. The variation in the distribution-pattern of xylem elements in the intact wood, which characterize the species or the season of cell production, may also be considered to be the result of the local control of substances in the cambium, by analogy with above instance.

In herbaceous dicotyledons, the regeneration of vascular system after injury is indicated by a rather simple phenomenon, i.e., the formation of wound vessel elements or tracheary elements with reticulately-thickened wall among the homogeneous primary tissue such as pith[64] or cortex. On the contrary, in the secondary tissue of woody dicotyledons (hardwoods) which has originated from cambium, the wound reaction of living cells is not so simple. The reaction of ray parenchyma cells and fusiform cells was completely different between. The former proliferated randomly, and had reticulately thickened walls similar to "tracheary elements", while the latter showed a successive modification in the cytodifferentiation without proliferating randomly. In the normal tree, the first step of cytodifferentiation seems to take place when the cambium is constructed, i.e., differentiation into fusiform initials or ray initials. In the second step, the cells derived from cambial initials differentiate into the respective xylem element under the subtle control by some substances in the vicinity of cambial initials.

Conclusion

The purpose of this study was firstly to establish the pinning method for marking cambial growth based on observations on wound tissue formed in tree stems, and secondly to clarify the wound effect on the cytodifferentiation in secondary xylem.

The wound tissues formed as a result of pin insertion into the stem of conifers in general were shaped like a radially-elongated spindle in cross section and were more than several millimeters high longitudinally, although there was some variation in the size and shape depending on the size of the needle, tree species, or pin-

ning season. Because the cross sectional area of wound tissue was greatest at the center of pinning, it is very important to use the sections from the center of the pinned point in discussing pinning method. The comparison of wound tissue with control material harvested immediately after pinning suggested that the outside margin of the wound tissue coincides with the site of cambium at the time of pinning. In order to determine this site relationship more precisely, the formation of wound tissue was pursued microscopically by the periodic observations of wound healing after pin insertion in *Pinus taeda*. Just outside the site of cambial initials at the time of pinning, the radial row of tracheid increased after pin insertion. In the zone of secondary wall thickening, the cleavage formed by the pin became wider because of the active proliferation of ray parenchyma cells in it. Observations of increasing tracheid-row and proliferating parenchyma made it possible to determine both the sites of cambial initials and the initiation of secondary wall thickening in conifers at the time of pinning.

Wound tissue formation in *Populus euramericana* was also pursued in order to establish the pinning method for hardwoods. Immature cells in the primary wall zone enlarged abnormally and ray parenchyma tissue divided into multiseriate rays around the wound gap, and by these means the gap closing within this zone. Meanwhile, the stripes of wall residue of immature cells which had been crushed and packed together on both flanks of the gap were broken within 4 days by cambial divisions. The broken end of the stripe, which became increasingly distant from cambial initials, marked the site of cambial initials at the time of pinning. The initiating site of secondary wall thickening also was detectable similarly to the case of conifers. The wound tissue in transverse section was elliptic or diamond shape and different from conifers.

The process of traumatic resin canal formation in pinaceous genera was clarified by using *Tsuga sieboldii*. Evidence of traumatic resin canal formation was first observed in the zone of xylem mother cells within three days after wounding by pin insertion. Enlargement of the traumatic resin canals was completed in a month. There was a close correlation between these resin canals and the site of cambial initials at the time of pinning which was deduced from the wound tissue formed by pin insertion. This indicated that the site of cambial initials at the time of pinning was in the vicinity of the epithelial cells on the cambial side of the traumatic resin canals. In some pinaceous genera, e.g., *Tsuga, Abies,* and *Larix,* the traumatic resin canal can be used instead of the minute wound tissue as a xylem growth markers.

Following tissue-level observations which established the pinning method, microscopic observations of the wound effects on the mechanism of cytodifferentiation in xylem were made. In the case of conifers, ray parenchyma cells were found

to become highly activate in the mature xylem as well as in the cambial zone. They not only proliferated randomly in the wound gap, but also invaded mature tracheids through the pinoid pits like the tylosis and formed many septa in the host tracheids. The latter phenomenon has been misinterpreted as being formed by the reorientation of differentiation course from immature tracheids to parenchymas. They thickened and lignified their walls much earlier than the normal ray parenchyma cells and formed thick reticulate walls. Some immature ray cells, which are to be ray tracheids based on the position in the ray tissue laid down before pinning, differentiated into ray tracheids even after the several abnormal divisions, although some had modified pits. Immature axial elements differentiated into tracheids normally after sporadic transverse divisions around the injury. These observations clarified that there was no change in the course of differentiation for instance from immature tracheid to parenchyma cell, as a result of injury. Cambial initials, both ray and fusiform, did not show any change. The ray cell derivatives with reticulately thickened walls are considered to be cells in the last stage of ageing through an accelerated ageing process, althoush they were very similar to so-called tracheary elements.

The wound effects on the cytodifferentiation of hardwood xylem were significantly different from those of conifers. In addition to the reactivation of ray parenchyma cells in the similar way to conifers, there found conspicuous abnormalities in the fusiform cells in the difined area which had been in the zone of xylem mother cells at the time of pinning and which formed from initials during several days after wounding. Wounding promoted transverse divisions in both areas, but only suppressed vessel formation in the former area, while in the latter area, morphological diversities in cytodifferentiation were observed as transitional variations from vessel element through tracheid to fiber, or axial parenchyma cell. These facts suggest that the course of cytodifferentiation is determined at the cambial initials or neighboring xylem-mother-cells, and is controlled with certain substances e.g., plant hormones, which may change their concentration or balance by the stimulus of the wounding, bringing about alteration in patterns of xylem elements. The wound reaction of hardwood was completely different from that observed in the regeneration of vascular system in the injured herbaceous dicotyledons.

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References

1) M.H. ZIMMERMANN and C.L. BROWN: "Trees: Structure and function", pp.105–114, Springer-Verlag (1971).
2) K. TANAKA and S. MATSUZAKI: J. Jap. For. Soc., **65**, 262–267 (1983) (In Japanese with English summary).
3) C. GLERUM and J.L. FARRAR: Can. J. Bot., **44**, 879–886 (1966).
4) W. SHORTLE and E.B. COWLING: Phytopathology, **68**, 609–616 (1978).
5) D.E. SMITH: Wood Sci., **12**, 243–250 (1980).
6) A.R.A. NOEL: Ann. Bot., **32**, 347–359 (1968).
7) K.E. FOSKET and J.G. TORREY: Plant Physiol., **44**, 871–880 (1969).
8) R. PHILLIPS and J.H. DODDS: Planta, **135**, 207–212 (1977).
9) K. SHIMAJI and Y. NAGATSUKA: J. Jap. Wood. Res. Soc., **17**, 122–128 (1971).
10) K.E. PATERSON and T.L. ROST: Amer. J. Bot., **68**, 965–972 (1981).
11) K.E. WOLTER: Nature, **219**, 509–510 (1968).
12) C.L. BROWN and H.E. SOMMER: "Tissue culture in forestry", ed J.M. Bonga and K.J. Durzan, pp.109–149, The Hague: Martin Nijhoff/Dr. W. Junk Publishers (1982).
13) L.W. ROBERTS: "Cytodifferentiation in Plants", Cambridge University Press (1976).
14) K.E. WOLTER: For Sci., **14**, 102–104 (1968).
15) R.W. KENNEDY and J.L. FARRAR: "Cellular Ultrastructure of Woody Plants", ed. W.A. Côté, pp.419–453, Univ. Press, Syracuse, New York (1965).
16) Y. WAISEL and A. FAHN: Physiol. Plant., **18**, 44–46 (1965).
17) L.J. KUČERA: Vjschr. naturf. Ges. Zurich, **116**, 445–470 (1971).
18) J. MULHEREN, W. SHORTLE and A. SHIGO: For Sci., **25**, 313–316 (1979).
19) A. KAWANA, M. DOI and Y. MOTOYAMA: J. Japan For. Soc., **55**, 202–206 (1973).
20) A. KAWANA, M. DOI and Y. MOTOYAMA: J. Japan For. Soc., **56**, 16–19 (1974).
21) K. YOSHIMURA, S. HAYASHI, T. ITOH and K. SHIMAJI: Wood Research, **67**, 1–16 (1981).
22) K. YOSHIMURA, T. ITOH and K. SHIMAJI: Mokuzai Gakkaishi, **27**, 755–760 (1981).
23) A. MAHMOOD: Aust. J. Bot., **19**, 177–195 (1968).
24) J.E. ARMSTRONG, A.L. SHIGO, D.T. FUNK, E.A. MCGINNES, Jr. and D.E. SMITH: Wood and Fiber, **13**, 275–291 (1981).
25) J.E. PHELPS, E.S. MCGINNES Jr. and P.J-Y. LIEU: Wood Sci., **8**, 397–405 (1975).
26) T. SHIOKURA: Tokyo Univ. Agric., J. Agric. Sci., **25**, 27–35, (1980) (In Japanese with English summary).
27) K. KURODA and K. SHIMAJI: For. Sci., **30**, 548–554 (1984).
28) M.W. BANNAN: Ann. Bot., **18**, 857–868 (1934).
29) M.W. BANNAN: New Phytol., **35**, 11–46 (1936).
30) Y. IMAMURA, K. SHIMAJI and M. SUZUKI: Summary of the 29th annual meeting of the Japan Wood Research Society, p.59, (1979) (In Japanese).

31) K. KURODA and K. SHIMAJI: For. Sci., **29**, 653–659 (1983).
32) K. ESAU: "Plant anatomy", 2nd ed. pp.245–265, John Wiley & Sons, New York (1965).
33) E.W. SINNOT and R. BLOCH: Amer. J. Bot., **32**, 151–156 (1945).
34) P.J. ROBBERTSE and M.E. MCCULLY: Planta, **145**, 167–173 (1979).
35) F. YAMAMOTO, T. NAKAYAMA and T. SUZAKI: IAWA Bull. n.s., **4**, 32–38 (1983).
36) R. ALONI: Planta, **150**, 255–263 (1980).
37) R. ALONI: Plant Physiol., **70**, 1631–1633 (1982).
38) S.R. RUGENSTEIN: Amer. J. Bot., **69**, 519–528 (1982).
39) L.J. KUČERA: IAWA Bull., 1977/1, 10–11 (1977).
40) M.P. DENNE: IAWA Bull., 1977/3, 49–50 (1977).
41) J.H. DODDS: "Xylem Cell Development", ed. J.R. Barnett, pp.153–167, Castle House Publ., Tunbridge Wells, Kent (1981).
42) G. DALESSANDRO and L.W. ROBERTS: Amer. J. Bot., **58**, 378–385 (1971).
43) K. KURODA and K. SHIMAJI: IAWA Bull. n.s., **5**, 295–305 (1984).
44) L. LENEY and L.D. MOORE: IAWA Bull., 1977/2, 23–24 (1977).
45) W.J. PETERS: Bot. Gaz., **135**, 126–131 (1974).
46) J.J. BALATINECZ and R.W. KENNEDY: For. Product. J., **17**, 57–64 (1967).
47) J. BAUCH, S. SCHWEERS and H. BERNDT: Holzforschung, **28**, 86–91 (1975).
48) K. YAMAMOTO: Res. Bull. College Exp. For. College of Agr. Hokkaido Univ., **39**, 254–296 (1982).
49) A.B. WARDROP: Nature, 193, 90–92 (1962).
50) M.J. HOWARTH, P.L. PETERSON and D.T. TOMES: Can. J. Bot., **61**, 507–517 (1983).
51) K.M. TRAN THANH VAN: Ann. Rev. Plant Physiol., **32**, 291–311 (1981).
52) C.L. BROWN and K. SAX: Amer. J. Bot., **49**, 683–691 (1962).
53) R. MAKINO, H. KURODA and K. SHIMAJI: Wood Research, **69**, 1–11 (1983).
54) R.A. JEFFS and D.H. NORTHCOTE: Biochem. J., **101**, 146–152 (1966).
55) A.E. COMER: Plant Physiol., **62**, 354–359 (1978).
56) H. FUKUDA and A. KOMAMINE: Plant Physiol., **65**, 57–60 (1980).
57) H. FUKUDA and A. KOMAMINE: Plant Physiol., **65**, 61–64 (1980).
58) H.R. HÖSTER: Holzforschung, **24**, 4–6 (1970).
59) V. RAGHAVAN: "Plant Cell and Tissue Culture", ed. W.E. Sharp *et al.*, pp. 155–178, Ohio State Univ. Press (1979).
60) K. KURODA and K. SHIMAJI: IAWA Bulletin n.s., **6**, 107–118 (1985).
61) H. FUKUDA and A. KOMAMINE: Planta, **155**, 423–430 (1982).
62) V.N. RONCHI: Can. J. Bot., **59**, 1969–1977 (1981).
63) J.E. PHELPS, E.A. MCGINNES, Jr., M. SANIEWSKI, J. PIENIAZEK and M. SMOLIHSKI: IAWA Bull. n.s., **1**, 76–82 (1980).
64) I.M. SUSSEX, M.E. CLUTTER, and M.H.M. GOLDSMITH: Amer. J. Bot., **59**, 797–804 (1972).

Explanation of photographs

Table of abbreviations

AR : Annual ring boundary	Cam : Direction of cambium
CI : Cambial initials	EN : Enlarging cells
G : Wound gap formed by pin insertion	P : Phloem
R : Ray parenchyma tissue	T : Tracheid
TH : Cells undergoing secondary wall thickening	W : Site of pin insertion
X : Xylem	XM : Xylem mother-cells

Photo 1. Three dimensional sections of the abnormal tissue formed two months after the insertion of a 400 μm needle in loblolly pine (*Pinus taeda*). a) Cross, b) radial, and c) tangential section.

Photo 2. Serial cross sections of the wound tissue in *P. taeda*. The interval of the sections is 0.5 mm.

Photo 3. Cross section of the wound tissue in *P. taeda* induced by the needle of different diameters; a) 250 μm, b) 700 μm. Compare with Photo 1 a (400 μm).

Photo 4. Cross section of the wound tissue in Himalayan ceder (*Cedrus deodara*) induced by the needle, 700 μm thick.

Photo 5. Transvers figure of wound tissue formed in *Pinus taeda* in July. Figures on the side line indicate the average radial number of tracheids in the current annual ring. The "assumed" site of cambial initials is indicated by an arrow.

Photo 6. Trace of the pin observed in the stem of *P. taeda* immediately after the removal of the inserted pin. Cross section observed with a) light-, and b) polarizing microscope.

Photo 7. Cross section of the wounded area four days after the pinning in *P. daeda*. The gap has begun to narrow in the zone from the xylem mother-cells through the enlarging cells during this period.

Photo 8. Cross section of the wounded area 12 days after the pinning in *P. taeda*. The crushed cells along the contracted part of the gap are packed together closely. Note the disconnection of the radial stripe of cell-wall residue (arrow).

Photo 9. Cross section of the wounded area 21 days after the pinning in *P. taeda*. Note the cut ends of the cell-wall residue on the phloem and xylem sides (arrows).

Photo 10. Cross section of the wounded area 36 days after the pinning in *P. taeda*. Cell-wall reside (arrow 1) is cut into fragments. Arrow 2 indicates the site of the initiation of secondary-wall thickening at the time of pinning.

Photo 11. Cross section of wound tissue induced in Sugi (*Cryptomeria japonica*) by a 250 μm pin. Arrows 1 and 2 indicate the deduced site of the cambial initials and the initiation of secondary-wall thickening at the time of pinning, respectively.

Photo 12. Center of pin insertion in *Populus euramericana* immediately after removing the pin as viewed with transmitted (a) and polarized (b) light (cross section).

Photo 13. Wounded area four days after pinning in *P. euramericana*. Cross section showing broken end (arrow) of the wall residue from crushed cells (arrowhead).

Photo 14. Radial section of wounded area 12 days after pinning in *P. euramericana* showing proliferating cells (left, arrowheads) derived from ray tissue facing the gap and the center of pin insertion (right, arrow).

Photo 15. Cross section of the wounded area 19 days after pinning in *P. euramericana* showing broken end of the stripe of cell wall residue included in the xylem (arrow), and gap almost completely filled with parenchyma inward from the zone of secondary wall thickening (arrowhead).

Photo 16. Cross section of wounded area 6 months after pinning in *P. euramericana* showing the assumed sites of cambial initials (arrow 1) and secondary wall initiation (arrow 2) at the time of pinning.

Photo 17. Traumatic resin canals and wound tissue (center) 36 days after a 400 μm pin was inserted into a stem of *Tsuga sieboldii* (cross section).

Photo 18. Sequence of traumatic resin canal formation in *T. sieboldii*. (a) Three days after pinning: Swelling, rounded cells are found within the zone of xylem mother cells (encircled by broken lines). (b) Six days after pinning: Small gaps (intercellular spaces) are observed among the swelling cells (encircled by broken lines). (c) Ten days after pinning: Nuclei and protoplasm are conspicuous in the cells around the gap (arrows). (d) Twenty days after pinning: Gaps are enlarging, and thin-walled cells are formed surrounding the epitherial cells (Cross sections).

Photo 19. Wound tissue induced in a stem of *T. sieboldii* by insertion of a 250 μm pin (36 days after pinning). The arrow indicates the site of the cambial initials at the

time of pinning (Cross section).

Photo 20. Wounded area seven days after pinning in *Pinus taeda*. Radial section (a) shows ray parenchyma cells (arrows 1) ballooning into the gap (*) formed by pin insertion. Arrow 2 shows the site of pin insertion. Tangential section (b) shows the bi- to multiseriate rays (arrowheads) around the gap (*).

Photo 21. Ray parenchyma cells invading into tracheids (arrows) in the mature xylem of wounded area, 9 days after pinning in *P. taeda*.

Photo 22. Radial section of wounded area 12 days after pinning in *P. taeda*, showing proliferating ray cells (arrow) in the gap and widespread ray cells containing droplets (arrowheads).

Photo 23. Radial sections of the wounded area 26 days after pinning in *P. taeda*. (a) Ray cell derivatives completely filling the gap, some of which contain droplets (arrowheads), and tylosis-like cells dividing repeatedly in the tracheids (arrow). (b) Disfigured ray tracheids and their derivatives (*), after wall thickening.

Photo 24. Tylosis-like cells in the tracheids (arrows) 30 days after pinning in *P. taeda*, observed by (a) light and (b) scanning electron microscopy, both in radial section. See also Photos 21 and 26.

Photo 25. Radial sections of the wounded area three months after pinning in *P. taeda*, observed by (a) light and (b) scanning electron microscopy. The walls of ray parenchyma derivatives in the gap have thickened reticulately and are heavily lignified.

Photo 26. Cross section of the tylosis-like cells (arrowheads) in the host tracheids, 21 days after pinning in *P. taeda*.

Photo 27. Abnormal pitting (arrowheads) of tracheids observed on the 30th day after pinning in *P. taeda*. (Radial section).

Photo 28. Wound tissue observed in the stem of *Populus euramericana* 29 days after wounding. Solid line and broken line in the cross section (a) indicate the deduced sites of cambial initials, and the initiation of secondary wall thickening at the time of wounding, respectively. A, B and C in the radial section (b) indicate the zones of secondary wall thickening, xylem mother- and enlarging cells at the time of pinning, and the tissue formed after wounding, respectively. Arrowhead: ray cell derivatives which have filled the gap of pin. Arrow 1: fusiform cells which have divided transversely. Arrow 2: abnormal strand cells probably derived from ray parenchyma cells. Arrow 3: abnormally narrow vessels.

Photo 29. The cells derived from ray parenchyma cells and having invaded into fibers (arrow 1) and vessels (arrow 2) from their broken end (*P. euramericana;* enlargement of Photo 28b area C).

Photo 30. The strand cells formed by the transverse division of fusiform cells (arrows), observed in the 29th day's sample of wounded *P. euramericana* (enlargement of Photo 28b around solid line).

Photo 31. Wounded area two days after pinning in *P. euramericana* (radial section). Note the ray parenchyma cells (a, arrow 1) ballooning into the gap (*), unusual transverse division of fusiform cells (a, arrow 2), and tylosys-buds (b, arrows) in the vessels inside of wound gap.

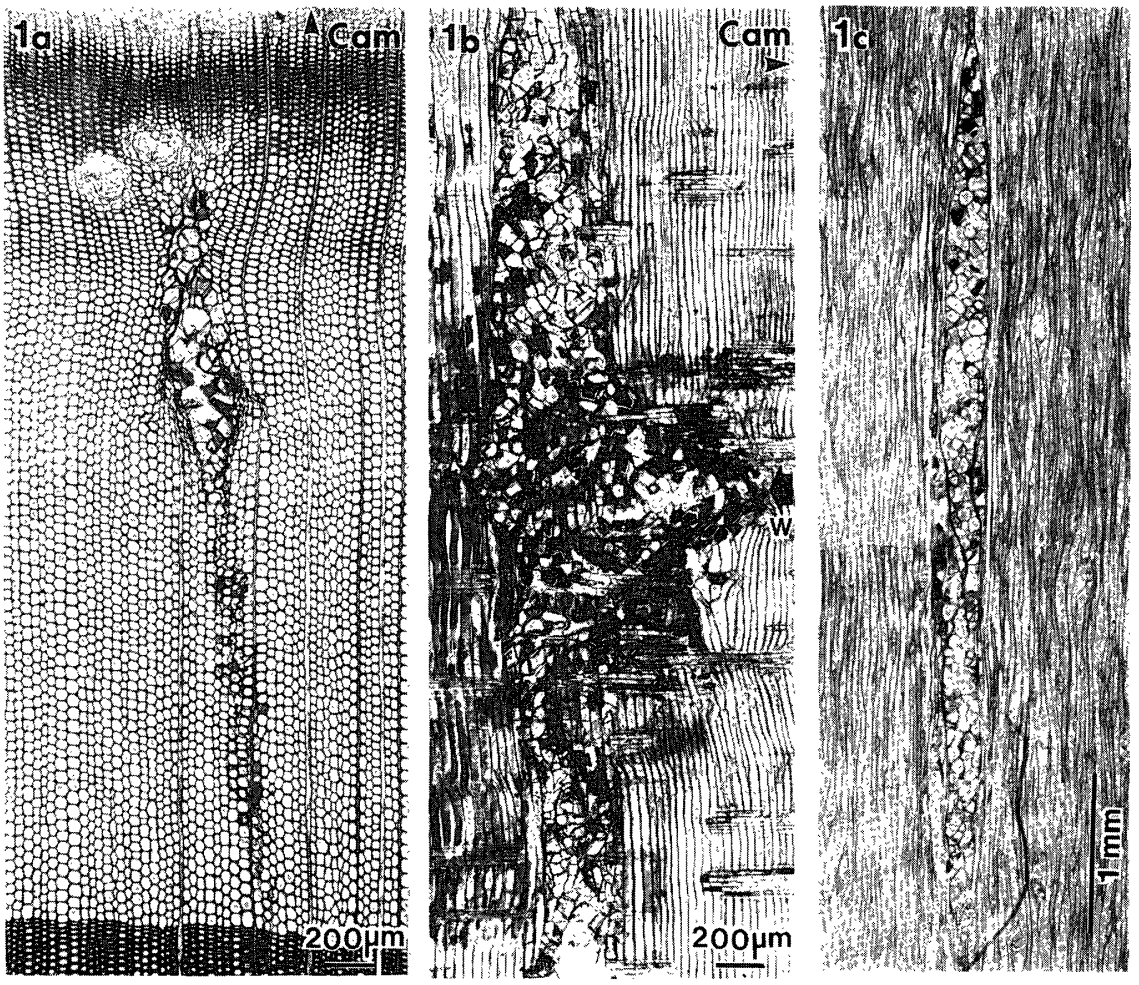
Photo 32. Wounded area four days after pinning in *P. euramericana*. Tangential section from the cambial region (a) shows frequent transverse divisions of young fusiform cells very close to the pinned point (arrow). Radial section (b) shows the lack of the vessel-element differentiation in the area of abnormal transverse division (double-headed arrow).

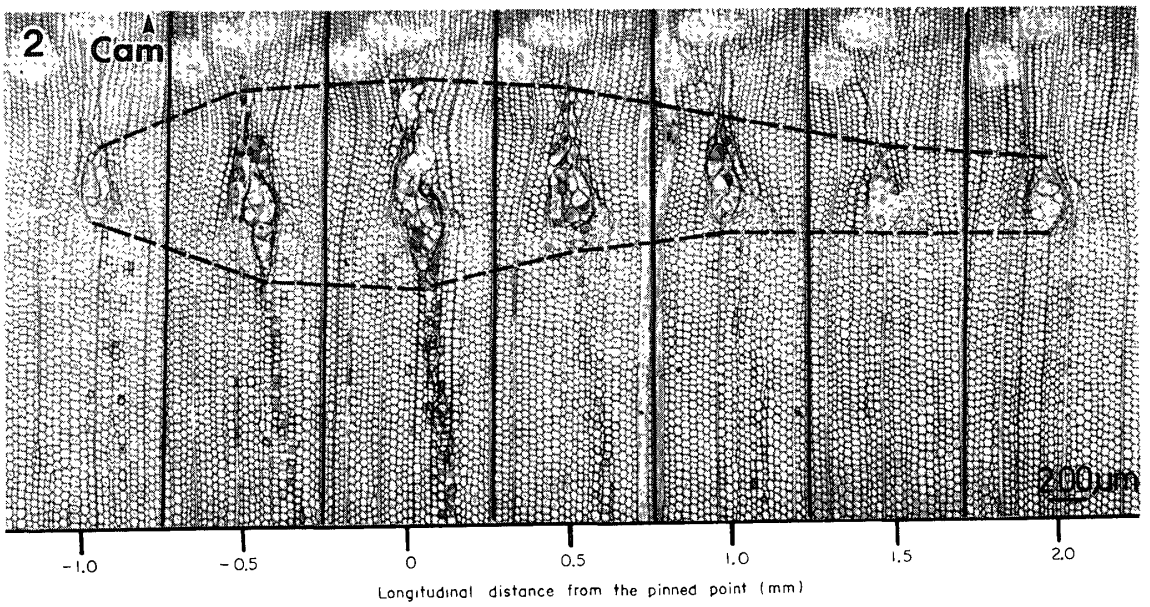
Photo 33. Tangential section of the wounded area 7 days after pinning in *P. euramericana*, showing that strand cells have entered the secondary-wall thickening zone and

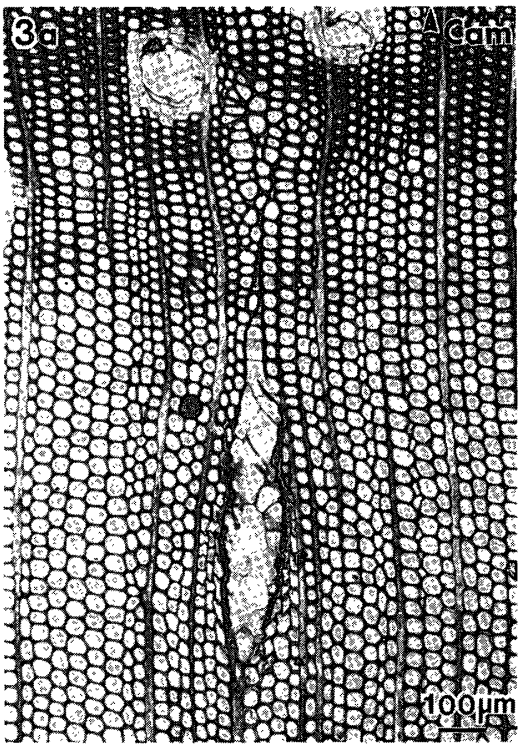
indicated the fiber-like wall thickening with few pits (arrow) around the pinned point (W).

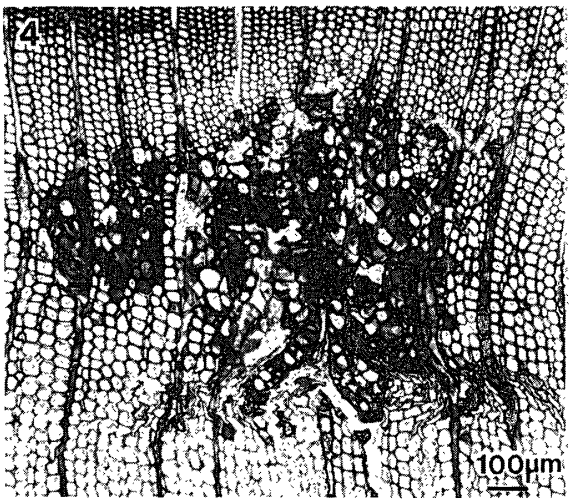
Photo 34. Abnormally narrow vessel-like cells observed in the area formed after wounding in *P. euramericana* (12 days after pinning, radial section). Among them, morphological varieties, such as axial parenchyma (arrow 1) or strand tracheids, and narrow vessel without perforation (=tracheid) (arrow 2) or with only one perforation (arrow 3) were observed. Note the short vessel elements (*) outside the narrow vessels.

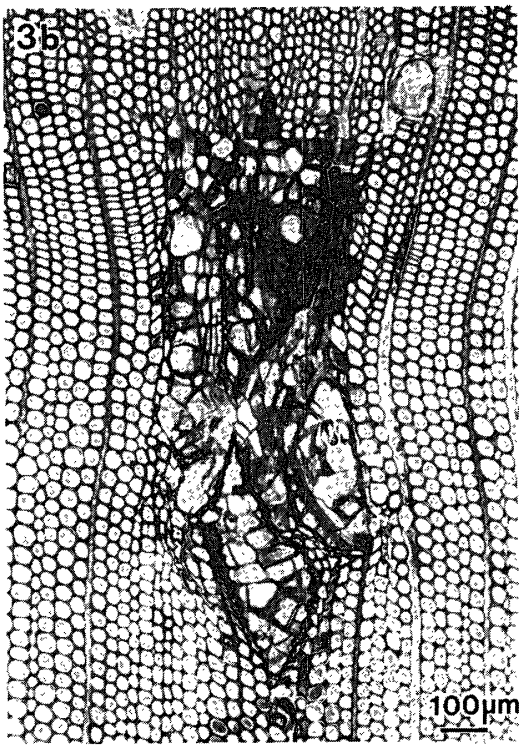
Photo 35. Successive modifications of strand cells derived from fusiform cells in the area formed after wounding in *P. euramericana*. Variations are from thick-walled strand-fibers in the left, i.e., inside (arrow 1) through the intermemdiate pattern (arrow 2) with groups of small pits, to axial-parenchyma-like cells in the right, i.e., outside (arrow 3).

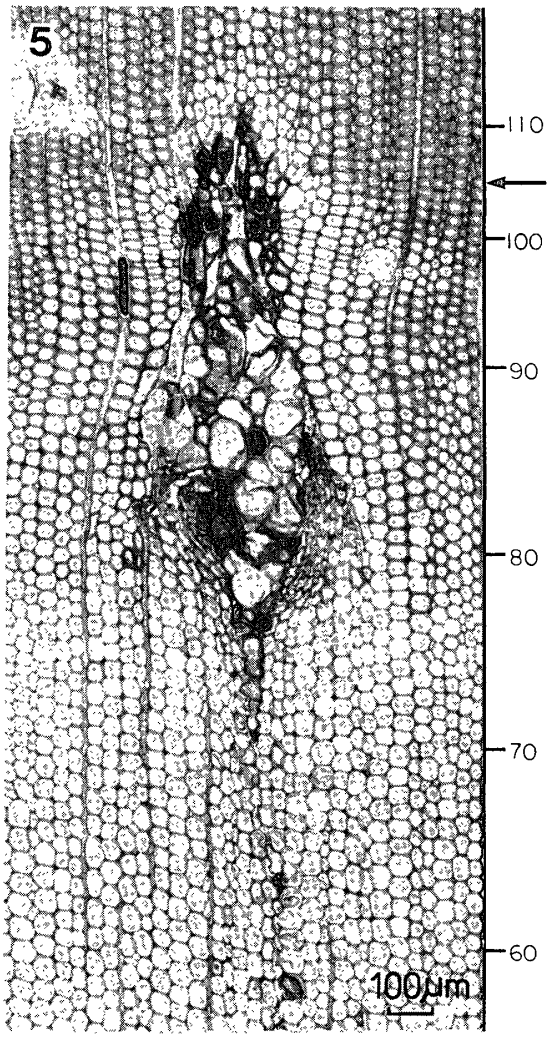

1a
Cam
200μm
1b
Cam
W
200μm
1c
1mm

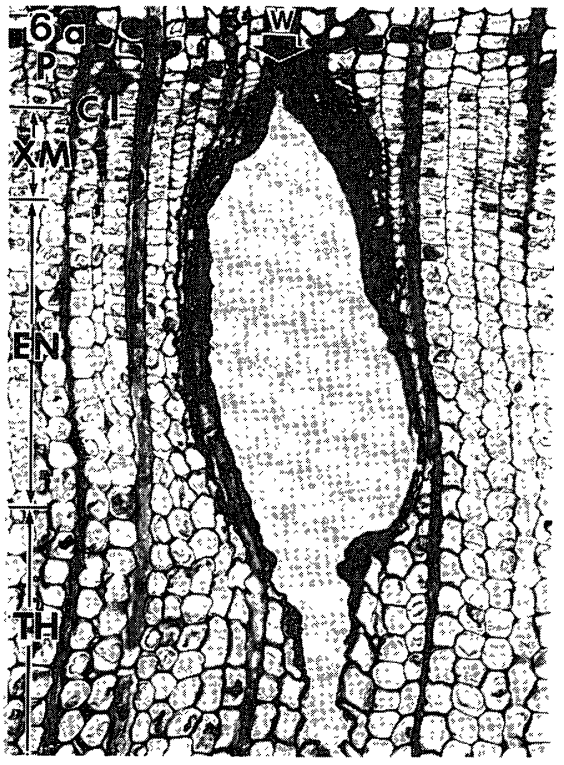

2
Cam
200μm
-1.0
-0.5
0
0.5
1.0
1.5
2.0
Longitudinal distance from the pinned point (mm)

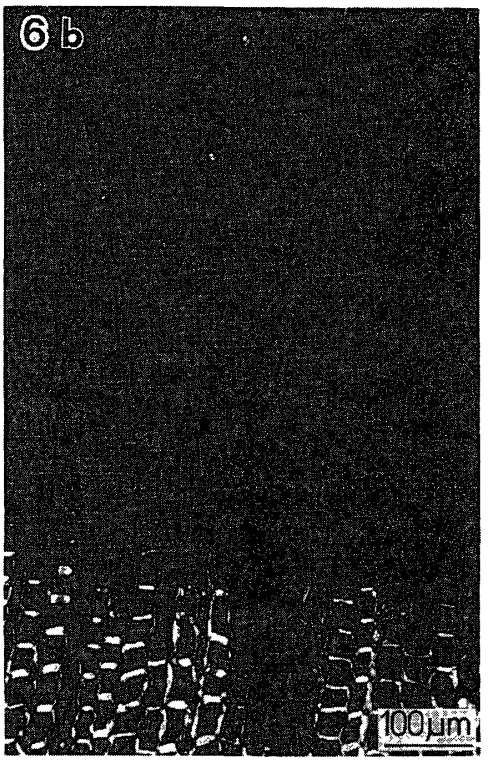

3a
Cam
100μm


4
100μm

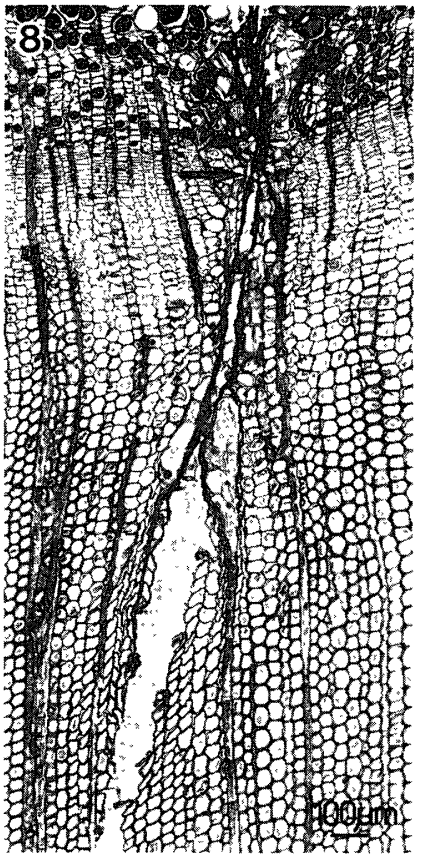

3b
100μm

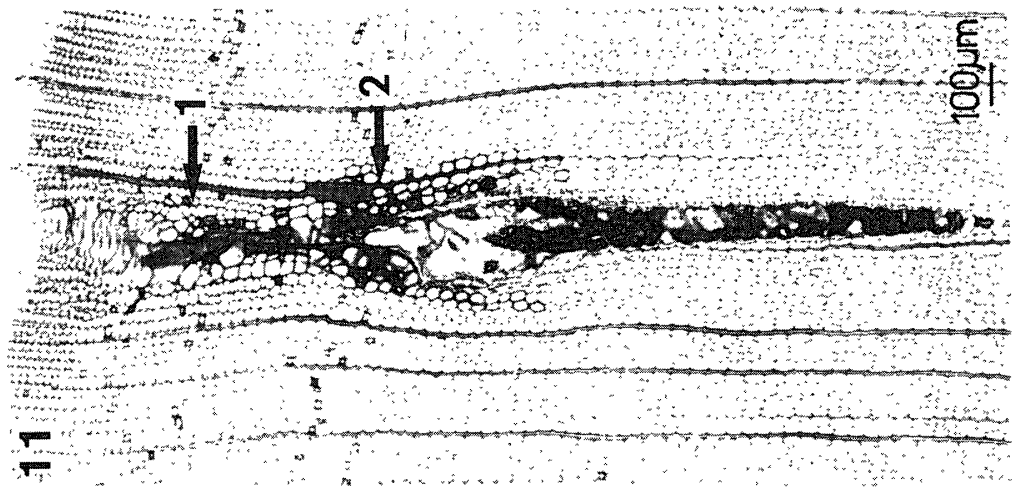

5
110
100
90
80
70
60
100μm

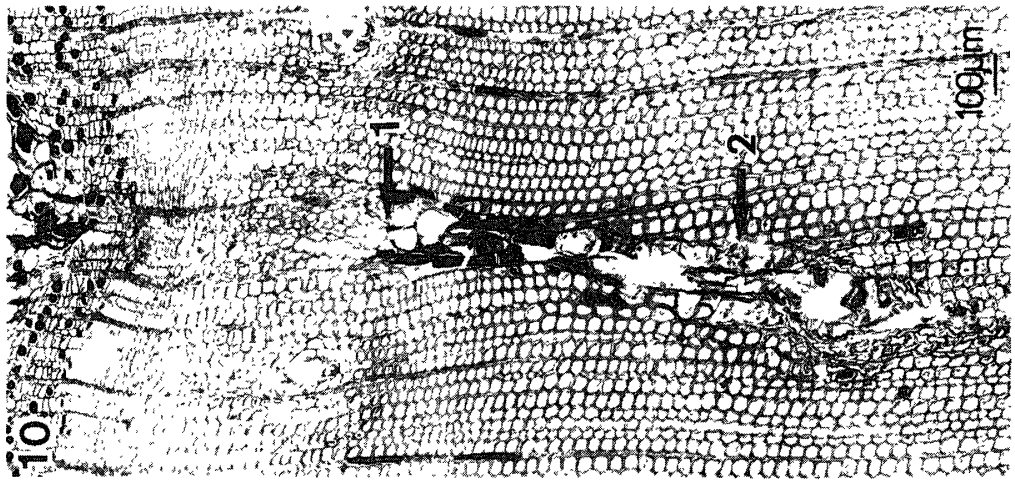

6a
W
P
C
XM
EN
TH

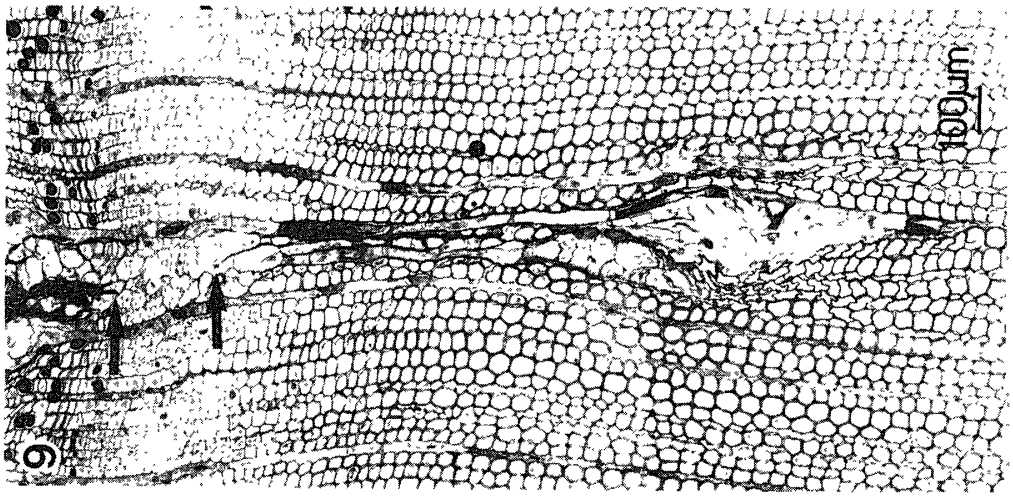

6b
100μm

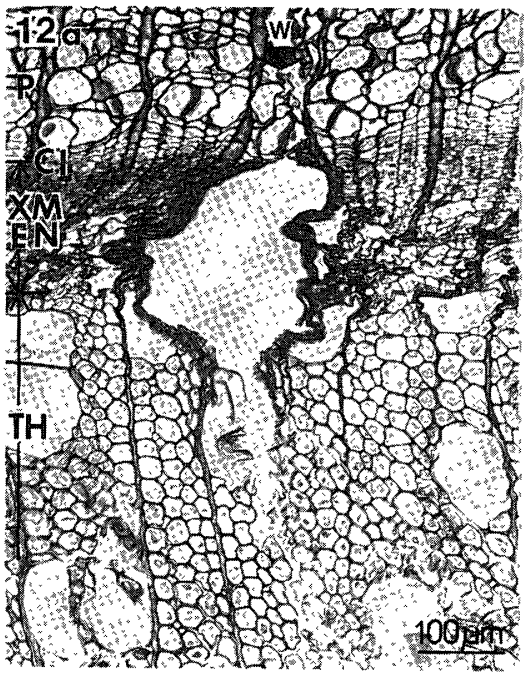

7
100μm

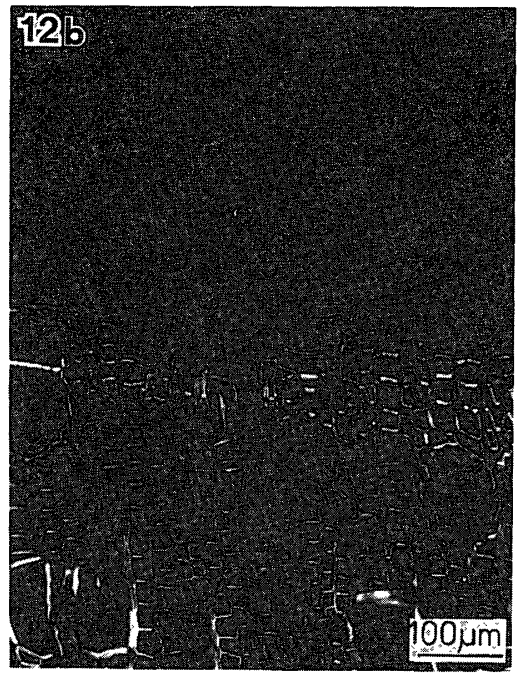

8
100μm

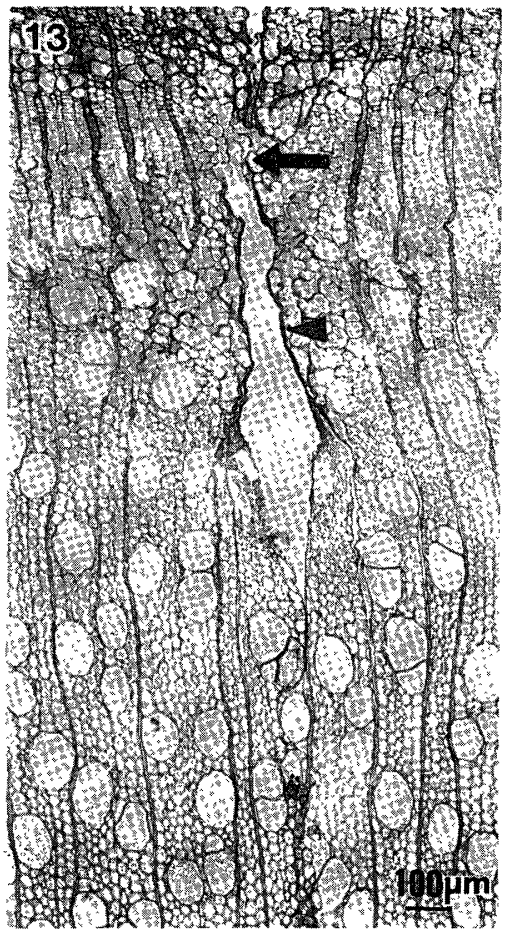

1
2
100μm
11


1
2
100μm
10

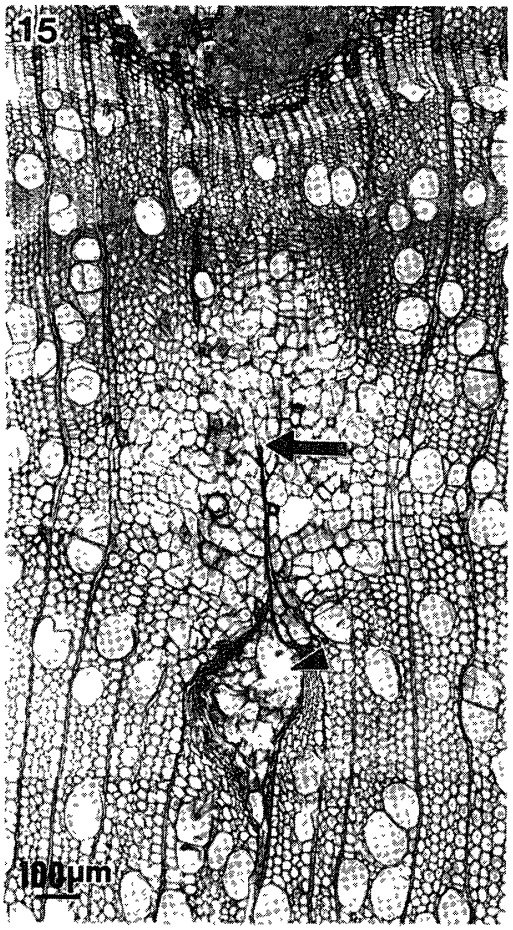

100μm
9

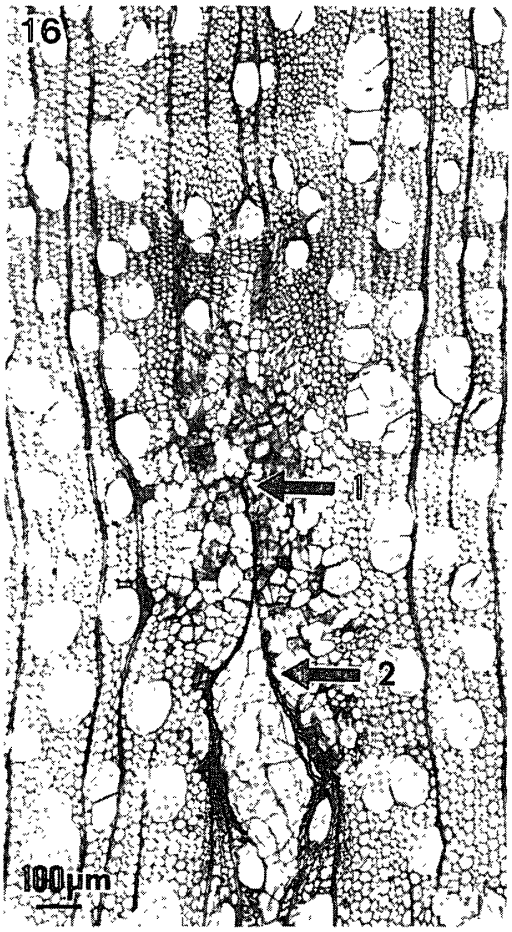

12a
W
VH
R
Cl
XM
EN
TH
100μm

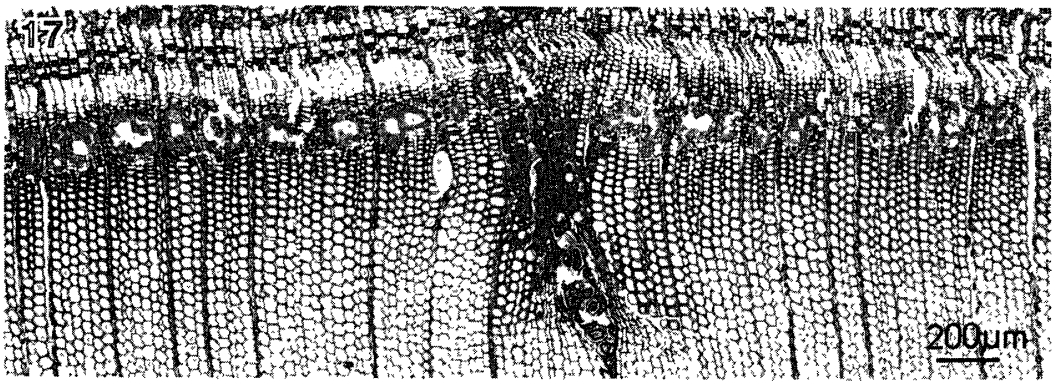

12b
100μm

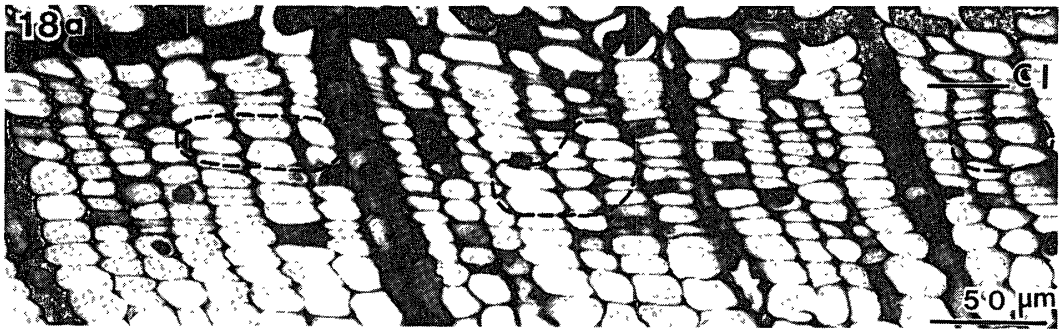

13
100μm

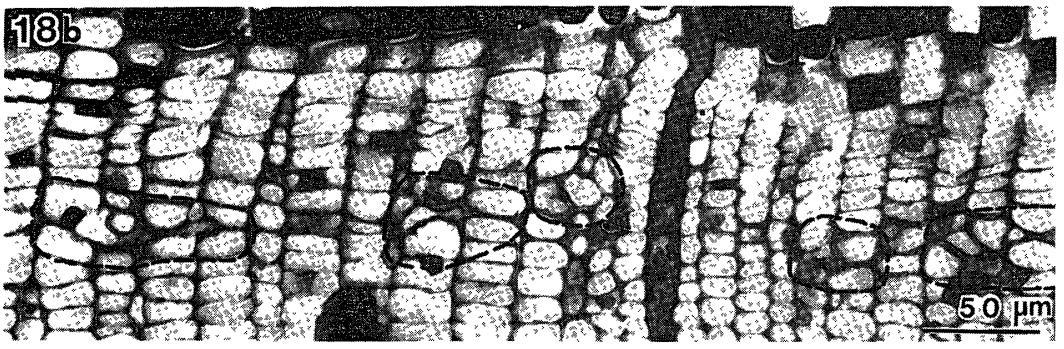

14
Cl
W
200μm

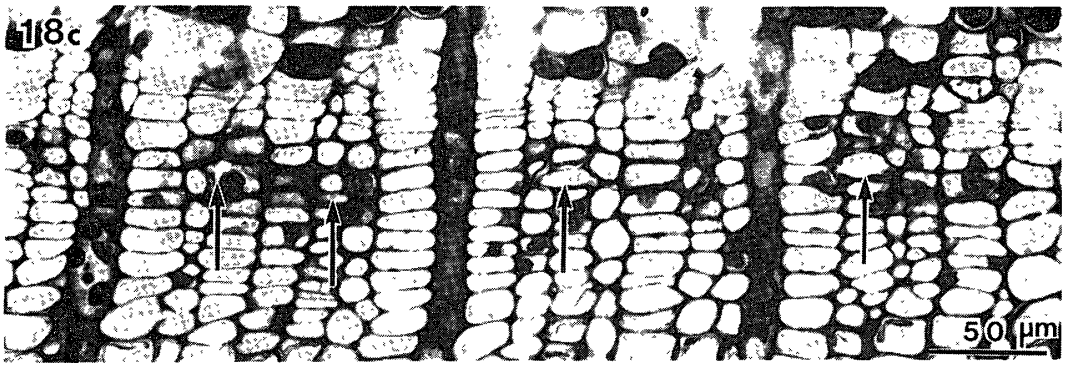

15
100μm

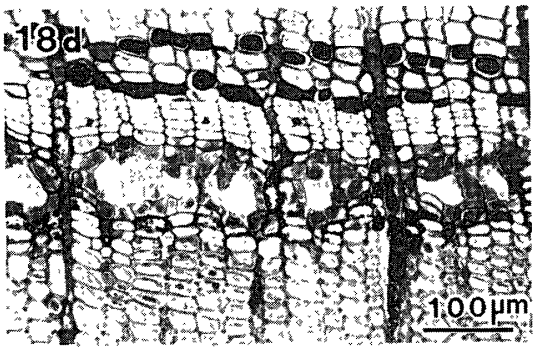

16
1
2
100μm

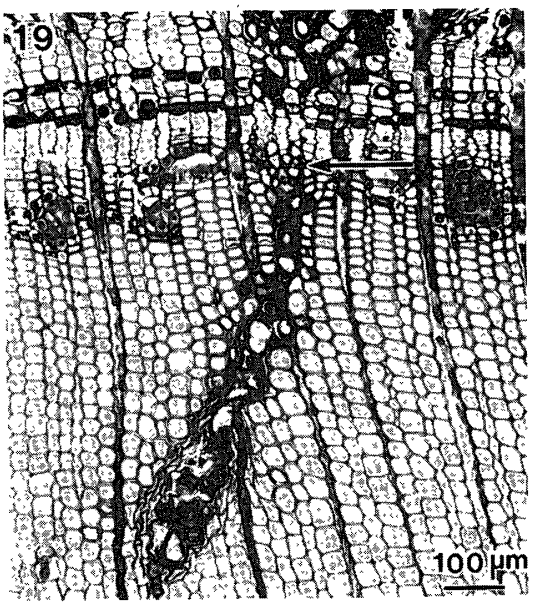

17
200μm

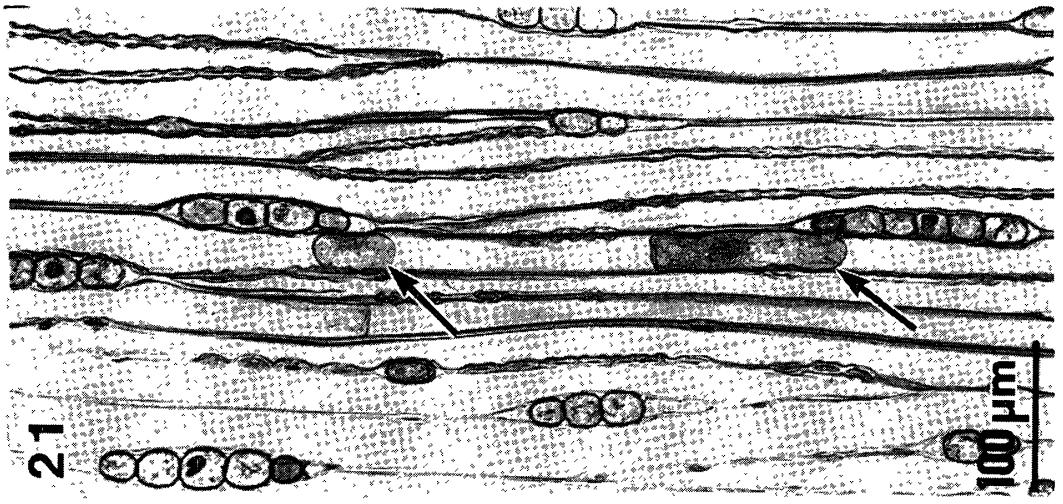

18a
C
50 μm

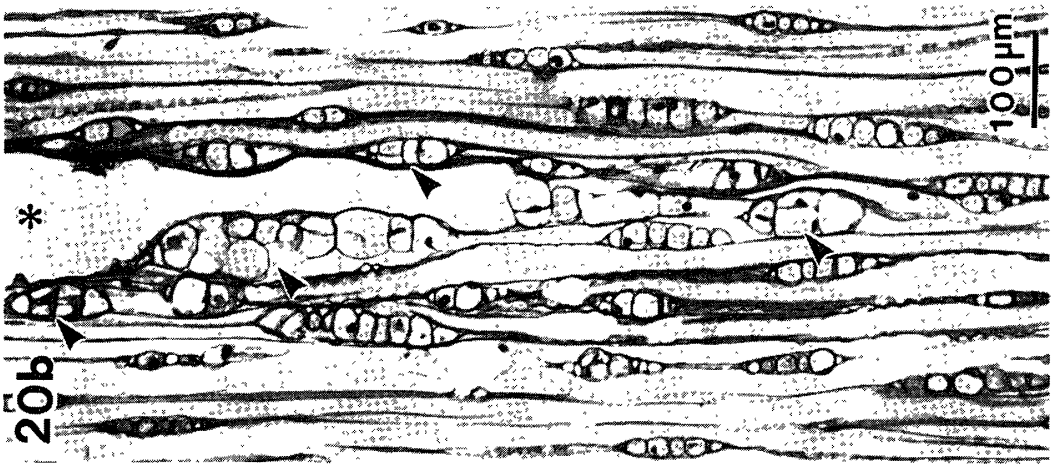

18b
50 µm


18c
50 µm

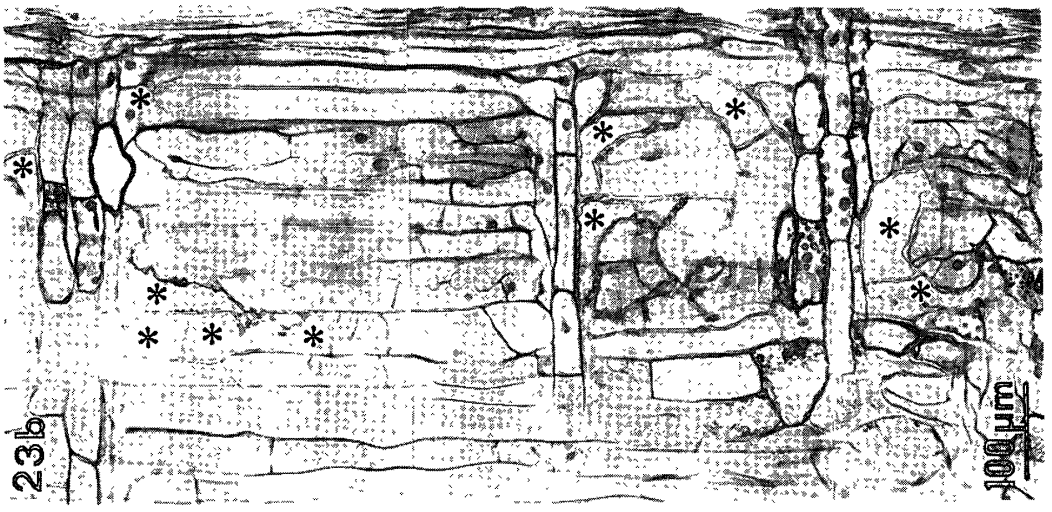

18d
100 µm

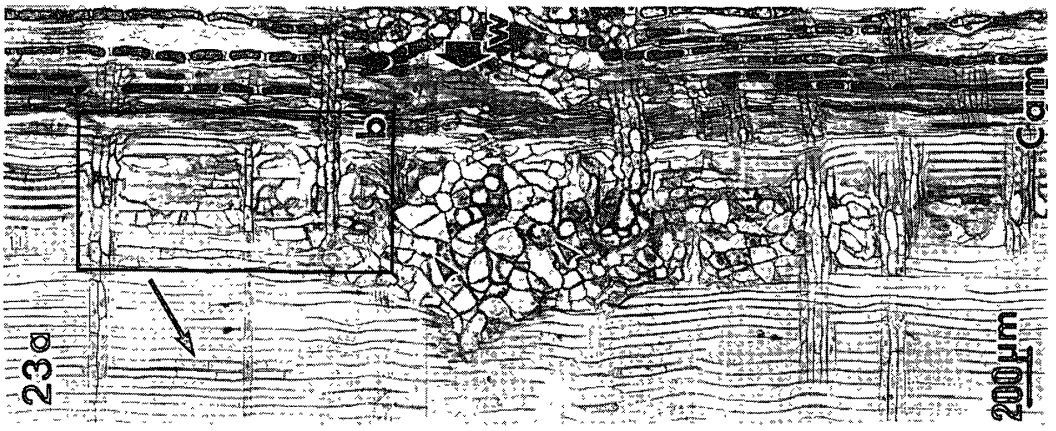

19
100 µm


21
100 μm

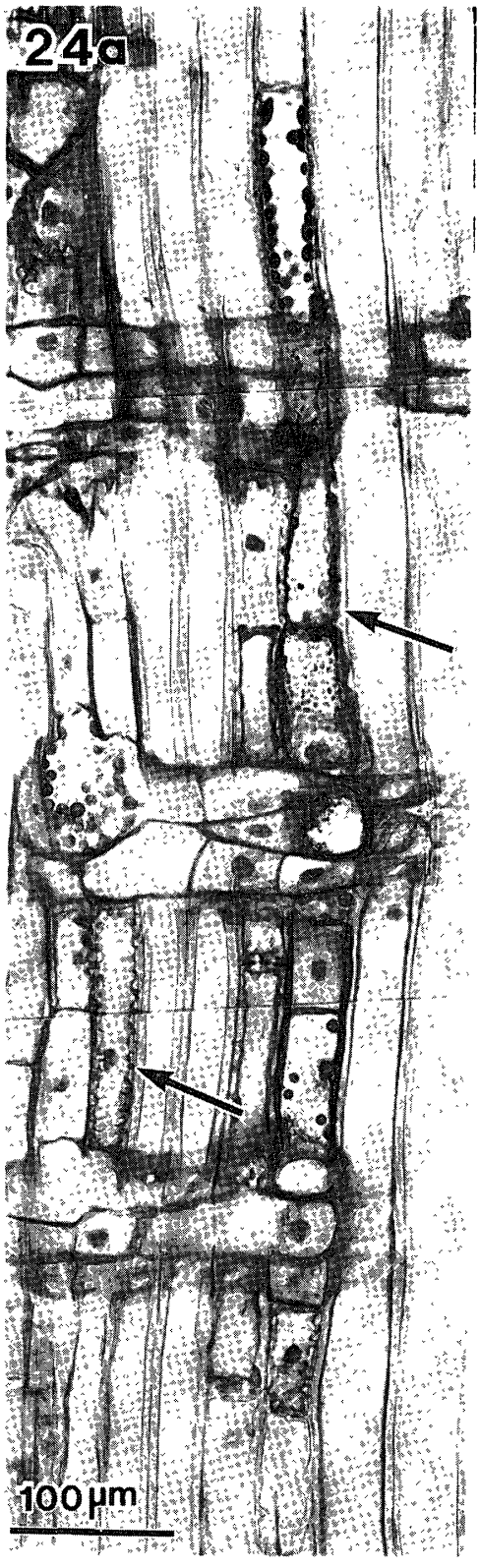

*
20b
100 μm

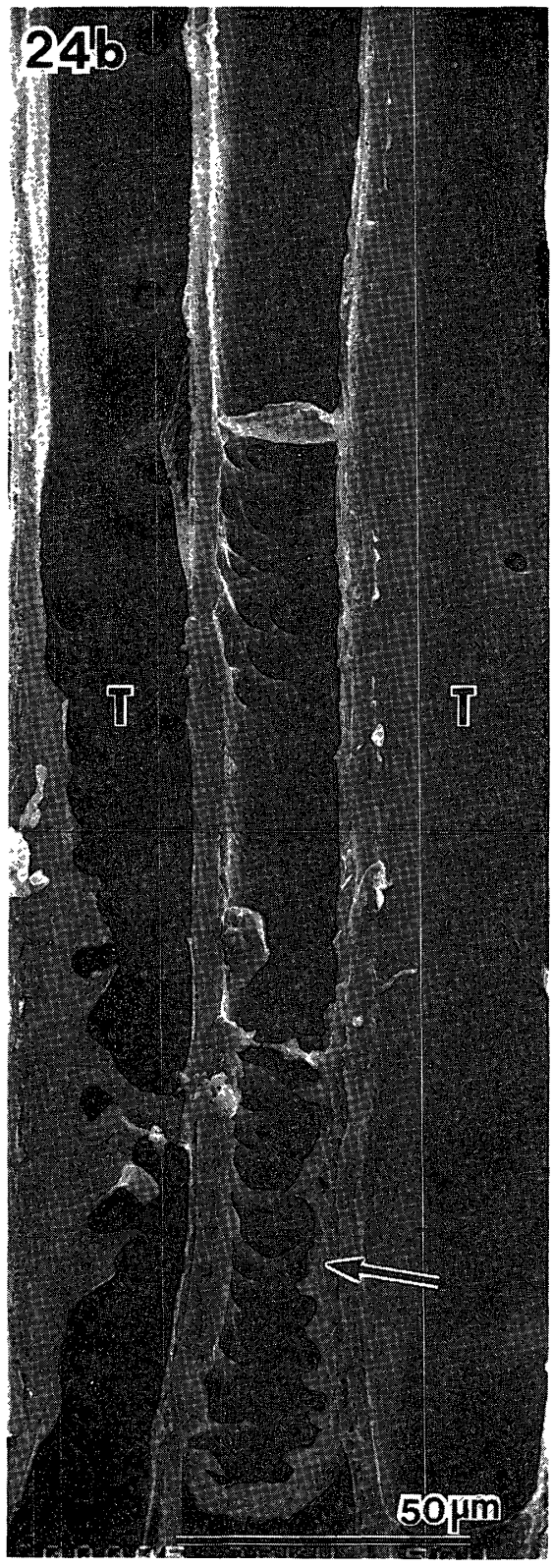

Cam
*
20a
200 μm

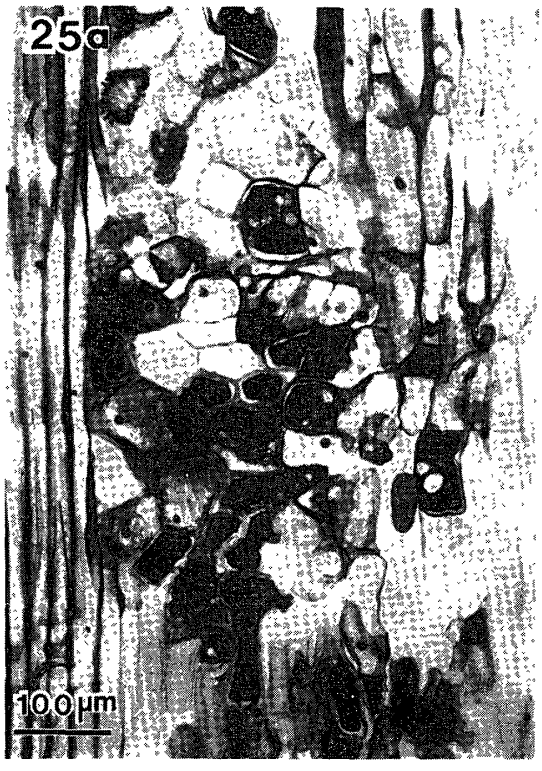

23b
100μm

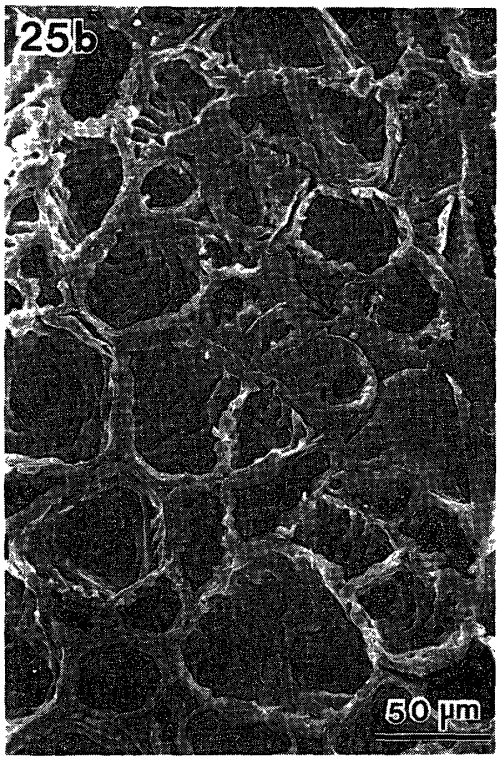

23a
200μm

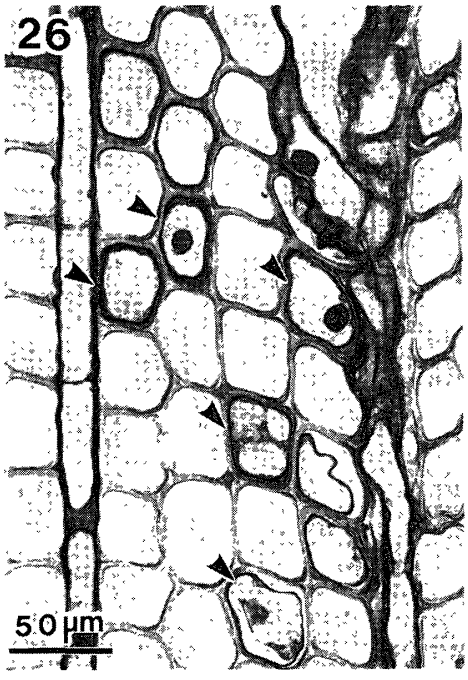

22
200μm

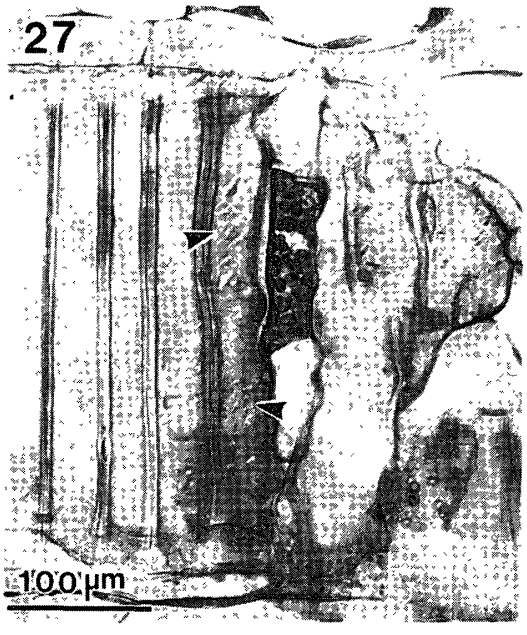

24a
100 µm

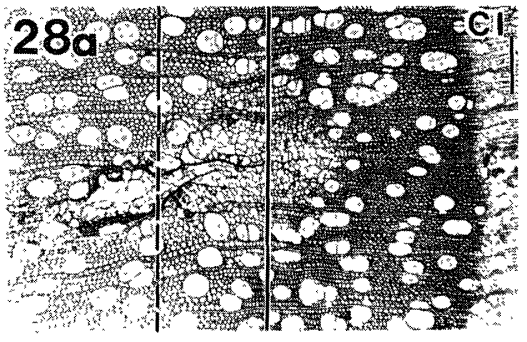

24b
T
T
50µm

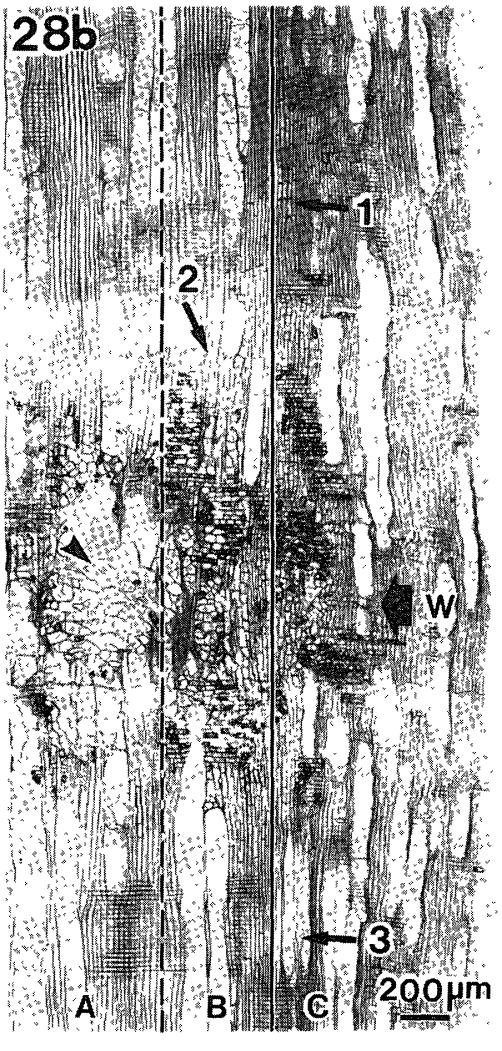

25a
100 µm

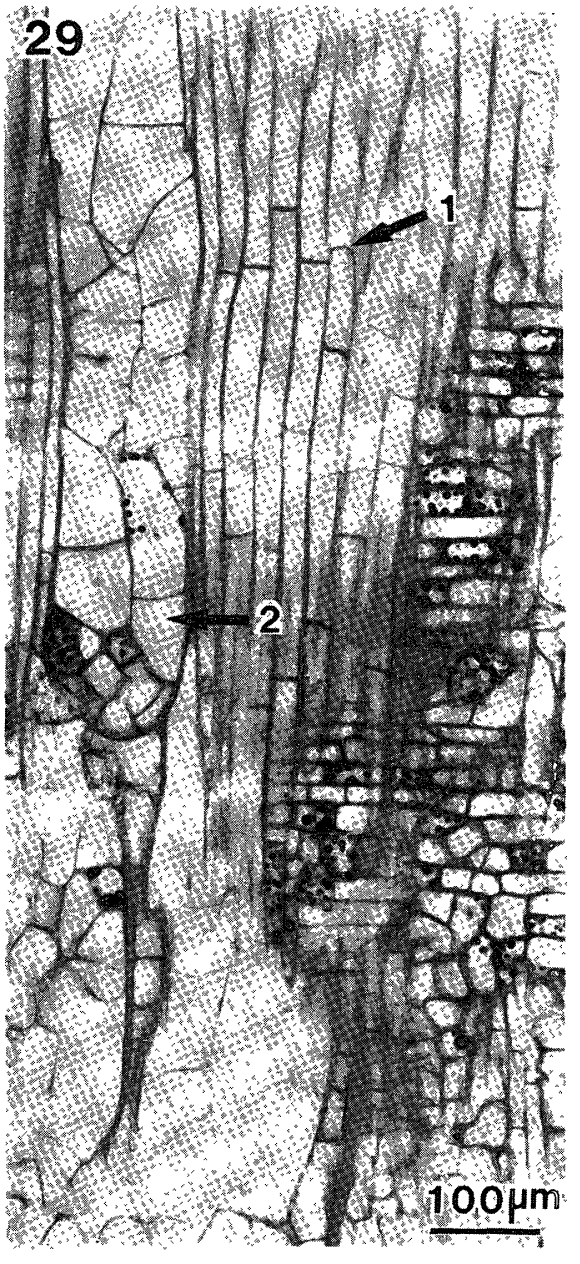

25b
50 µm

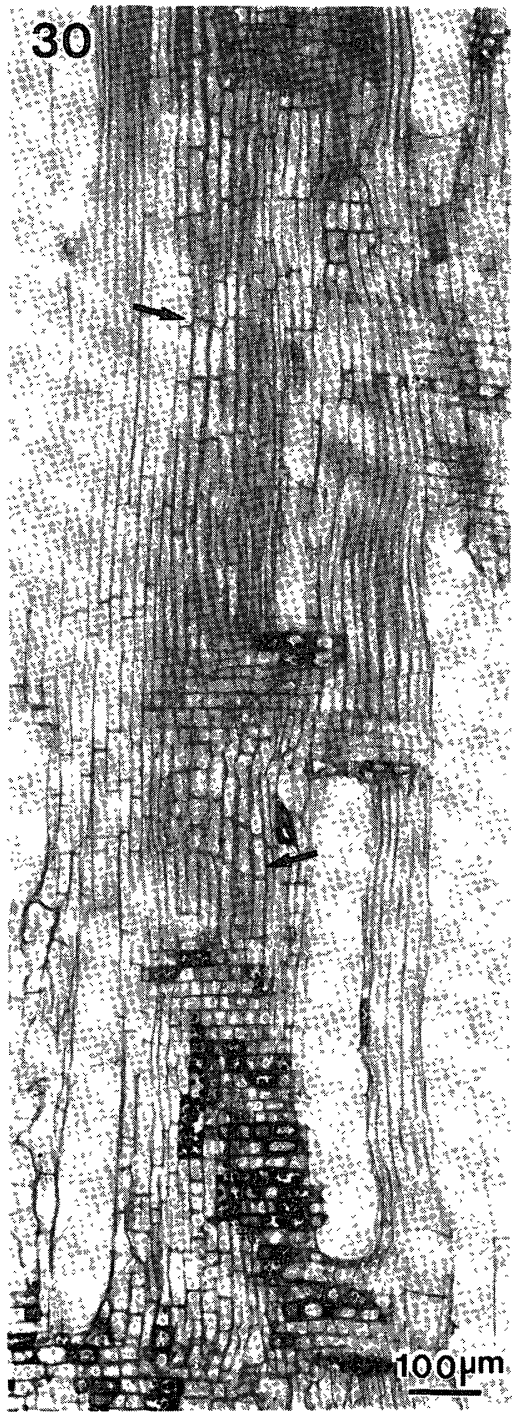

26
50 µm

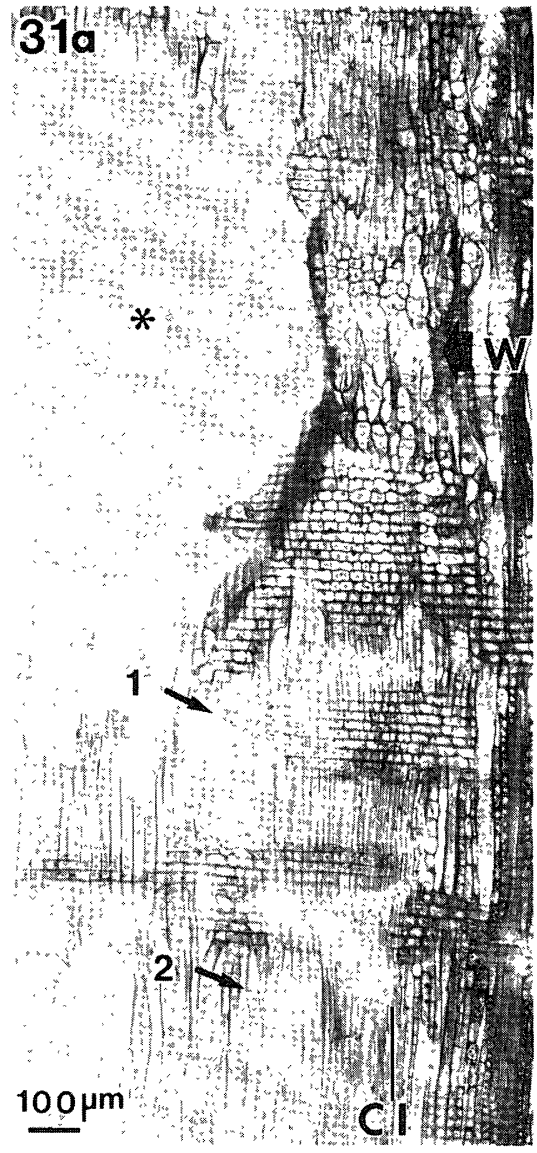

27
100 µm

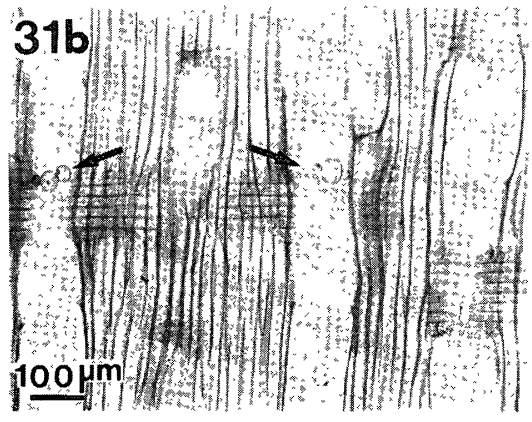

28a
C

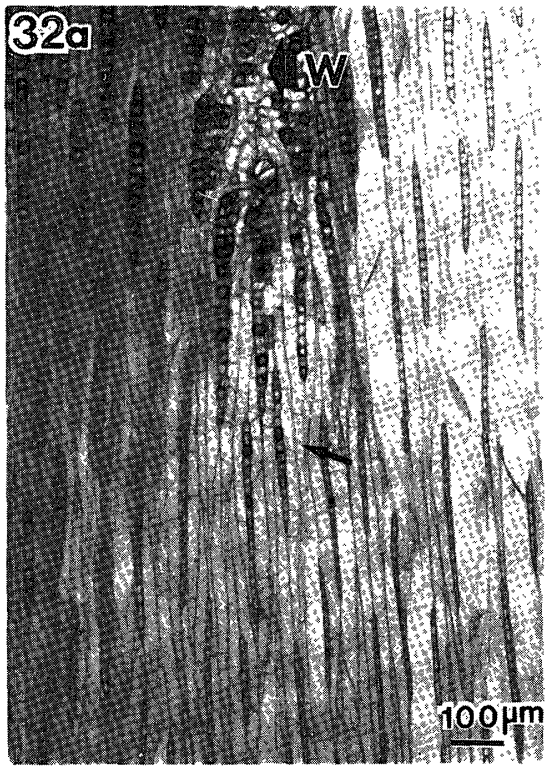

28b
1
2
W
3
A
B
C
200μm

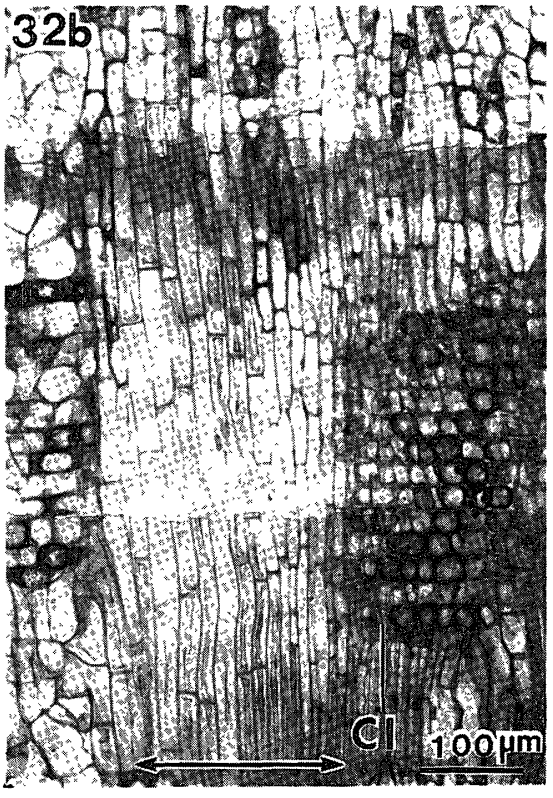

29
1
2
100μm

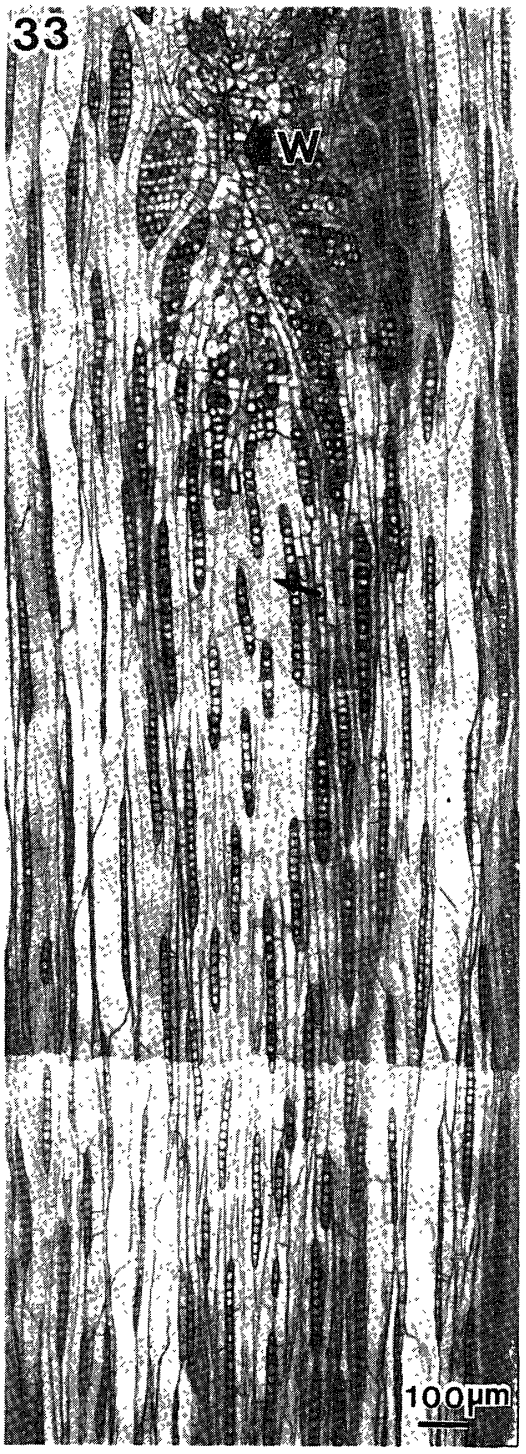

30
100μm

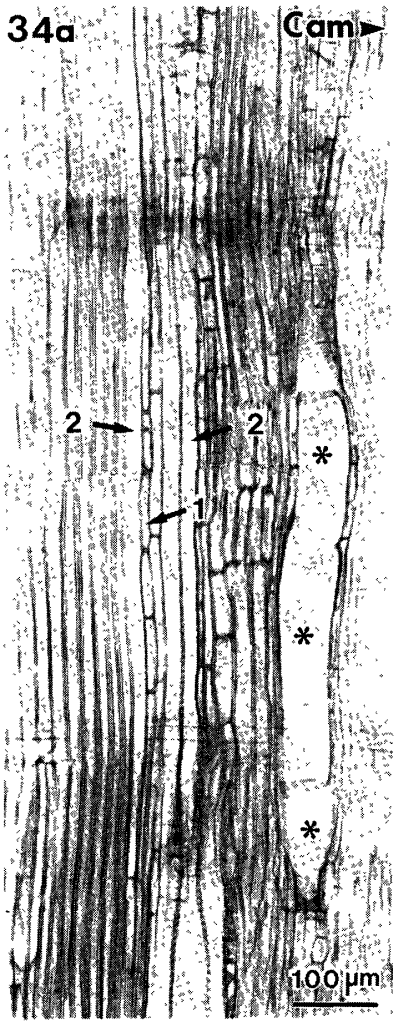

31a
*
W
1
2
100μm
CI

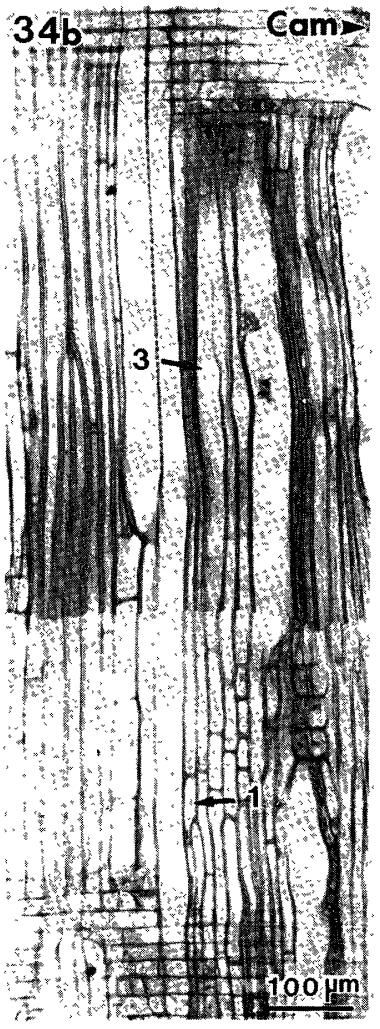

31b
100μm

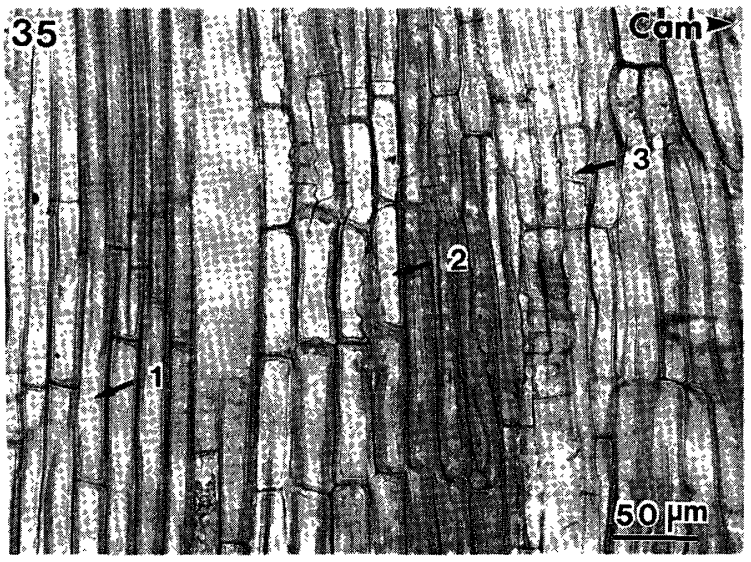

32a
W
100μm


32b
Cl
100μm


33
W
100μm


34a
Cam
2
2
1
*
*
*
100 µm


34b
Cam
3
1
100 µm


35
Cam
3
2
1
50 µm