



Title	Study on the Cell Wall Development of Ray Parenchyma in Genus Pinus Using Ultraviolet Microscopy
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Citation	北海道大學農學部演習林研究報告, 34(1), 79-95
Issue Date	1977-03
Doc URL	http://hdl.handle.net/2115/20975
Type	bulletin (article)
File Information	34(1)_P79-95.pdf



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Study on the Cell Wall Development of Ray Parenchyma in Genus *Pinus* Using Ultraviolet Microscopy*

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紫外線顕微鏡によるマツ属放射柔細胞壁の発達に関する研究*

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Introduction

The xylem ray parenchyma cells in most gymnosperms mature within the year they were formed and continued to live for many years, functioning in storage and translocation of food materials. They are finally faced with death, resulting in the heartwood in which several other changes, such as depletion of stored starch and deposition of polyphenols, usually occur simultaneously.

Numerous investigations into the parenchyma cells have been conducted concerning the act in the process of heartwood formation, from the physiological, histological and chemical points of view.

In connection with the mechanism of heartwood formation, the Genus *Pinus* is notable for diversity of the mature process of ray parenchyma cells. Genus

Received July 31, 1976.

* A part of this paper was presented at the 7th Annual Meeting of the Hokkaido Branch of the Japanese Wood Research Society at Sapporo, Nov. 1975.

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Pinus is classified into two groups, Haploxyton pines and Diploxyton pines, on the basis of the number of fibrovascular bundles of the needles, i.e., single in the former and double in the latter. It is already known that maturation of ray parenchyma cells in Diploxyton pines, unlike those in Haploxyton pines and most other coniferous wood species, lags behind that of other xylem elements, and is completed in the intermediate wood zone where the heartwood forms.

Diploxyton pines are believed to be a favorable material for the study of maturation of ray parenchyma cells in relation to the heartwood formation. BALATINECZ and KENNEDY (1967)¹⁾ reported the delay in maturation of ray parenchyma cells of Diploxyton pines as stated above. They demonstrated that the maturation of ray parenchyma cells takes place gradually with aging of the sapwood in the hard pines with window-like pits, while somewhat more abruptly as the sapwood-heartwood boundary is approached in the hard pines having pinoid pits. BAMBER and DAVIES (1969)²⁾ confirmed these observations through the studies of the ray parenchyma cells of *Pinus radiata* of the Diploxyton pines, using an ultraviolet microscope and sections of 2 microns in thickness. BAMBER (1972, 1973)^{3,4)} also demonstrated a clear difference between the sapwood and heartwood in both Haploxyton and Diploxyton pines, with regard to the secondary thickening and lignification of cell walls of ray parenchyma as well as the resin canal tissue. BAUCH et al. (1974)⁵⁾ considered that maturation in ray parenchyma cell walls of Diploxyton pines might occur during heartwood formation, while Haploxyton pines and all the other coniferous genera examined exhibited the lignification in these cells already in the cambial zone, by using various methods such as light microscopy, histochemical tests, micro-autoradiography and UV-microspectrophotometry.

Although valuable information concerning the maturation of ray parenchyma cells in Genus *Pinus* is already known, there is little information about the detail of the maturation process progressing throughout the sapwood including the intermediate wood zone, except from the observations of BALATINECZ and KENNEDY (1967)¹⁾ who used standard histochemical techniques for their study.

This study aims to clarify the detailed process of secondary wall thickening and lignification of ray parenchyma cells throughout the sapwood including the intermediate wood and typical heartwood in some pine species belonging to both the Haploxyton and Diploxyton groups. Serial radial sections of 0.5 micron in thickness from the cambium to the heartwood were prepared, and ultraviolet microscopy was employed effectively to determine the process of lignifying cell walls of the ray parenchyma examined.

Materials and methods

Disks or increment cores for this study were removed from the breast height of living trees of the following species: *Pinus densiflora* SIEB. et ZUCC., *P. rigida* MILL., *P. banksiana* LAMB., *P. strobus* LINN.. Table 1 summarizes the data for the trees from which wood samples were obtained for the study.

Table 1. Trees from which wood samples were obtained

Species	No.	Age (year)	Height (m)	B. H. D. (cm)	Location	Sampling season
Diploxyylon pine						
<i>Pinus densiflora</i>	1	58	18	28	Sapporo, Hokkaido	May 1974
<i>P. densiflora</i>	2	50*	18	28	Wakayama Experiment Forest, Wakayama, Central Honshu	April 1973
<i>P. rigida</i>	—	43*	12	25	Tomakomai Experiment Forest, Tomakomai Hokkaido	June 1975
<i>P. banksiana</i>	—	56	15	17	"	"
Haploxyylon pine						
<i>P. strobus</i>	1	30*	16	27	"	"
<i>P. strobus</i>	2	46	19	38	"	May 1974

* The number of annual rings at the breast height.

After the removal, strips cut from the disks and increment cores were immediately placed in a fixative F. A. A. (ethyl alcohol : H₂O : formalin : acetic acid = 60 : 30 : 5 : 5) for a week at room temperature. After fixed the wood samples were rinsed with running water for 24 hrs. Small blocks of ca. 2 mm (rad.) × 2 mm (tang.) × 3 mm (long.) were cut out in a serial line from the cambium to heartwood, making sure to keep all the annual rings to be examined continuously. These blocks were then subjected to dehydration through a graded series of ethanol and embedded in a mixture of methyl/butyl methacrylates.

Thin sections of 0.5 micron in thickness were cut by an ultramicrotome (LKB Ultratome III) with a diamond knife and were mounted on a quartz slide glass with a quartz cover glass using glycerin, and examined under an ultraviolet microscope (Carl Zeiss, West Germany, Type MPM-01). UV microphotographs were taken on an ordinary commercial film at a wavelength of 280 milli-microns. The figure of UV-absorption represents directly the lignin distribution in the cell wall. In this paper, the maturation was judged by the secondary wall thickening and lignification.

Results

1. *Pinus densiflora* SIEB. et ZUCC. (Photos 1-14)

Newly formed xylem in the year when the samples were obtained was not found in both sample trees, so the latewood formed in the previous year was directly adjacent to the cambium (Photo 1). From photos 1-14, the process of maturation of ray parenchyma cells can be differentiated between the earlywood and latewood and that their maturation took many years to finish.

In the earlywood, secondary wall thickening and lignification were observed in ray parenchyma cells formed during the previous year, although they were not present in the latewood (Photo 1). Most of ray parenchyma cells next to the ray tracheids in the earlywood were observed to have completed the secondary wall thickening and lignification within one or two rings from the cambium (Photos 3 and 5),

leaving no change in the pit membrane. On the other hand, cells not in contact with the ray tracheids indicated little secondary wall thickening, especially near the cambium (Photo 5). Thickened lignified cells of ray parenchyma generally increased in number gradually toward the central row of cells with aging of the sapwood (Photos 5, 7 and 10). Near the sapwood-heartwoods boundary, the cells exhibited a rather abrupt increase of the completion of secondary wall thickening and lignification, and died by losing their protoplasm (Photos 9 and 10). The UV-absorption was observed in all of the pit membranes of the ray parenchyma cells (Photo 10). Those ray cells which already possessed lignified secondary walls in the outer sapwood, showed weak UV-absorption in the window-like pit membrane in the central part of the sapwood (Photo 7). Other cells without a secondary wall showed little UV-absorption of the pit membrane in the sapwood (Photo 7).

In the latewood, most of the parenchyma cells showed little signs of secondary wall thickening and lignification throughout the sapwood (Photos 2, 4 and 8). When the intermediate wood was approached, UV-absorption appeared at the corners of the ray parenchyma cell walls, and then spread out to the entire end wall and a part of the transverse wall (Photo 9). Here the ray cells seemed to show no indication of wall thickening.

In the heartwood, the entire cell wall had UV-absorption. A minor amount of secondary wall thickening even in the latewood was found there in many ray parenchyma cells (Photos 12 and 13). This is not illustrated in photo 9 which was taken of the intermediate wood on the basis of colour. Therefore, this small amount of secondary wall formation in the latewood ray cells occurred during the heartwood formation within the intermediate wood as also described by BAUCH et al. (1974)⁶⁾. Heartwood substances indicated various strength of UV-absorption, with more amount of them in the earlywood than in the latewood (Photo 11, compare with photos 12 and 13). The window-like pit membranes had a strong UV-absorption in the heartwood and were thicker than those in the sapwood (Photo 14).

Within one ray tissue, secondary wall thickening and lignification began, in most cases, with the ray parenchyma cells in contact with ray tracheids, and gradually proceeded to the cells farther from the ray tracheids (Photo 3). In the early stage of maturation of each ray parenchyma cell, secondary wall deposition on the existing primary wall was irregular in the vicinity of the cell walls as seen in the radial section (Photo 4), while it was quite smooth in the tangential section (Photo 5), suggesting a tangential annular thickening in the wall deposition. Finally the cell walls may attained a smooth surface. Non UV-absorbing (unlignified) thin layers within the cell walls were observed under the secondary wall deposited in the early stage (Photos 2 and 4), which disappeared in the late stage (Photos 9 and 10).

The maturation of an individual cell of a ray parenchyma in the earlywood-latewood boundary within an annual ring is quite different from each other (Photos 6, 8 and 9).

2. *Pinus rigida* MILL. and *Pinus banksiana* LAMB. (Photos 15-24)

The cell of ray parenchyma in *P. rigida* and *P. banksiana* matured almost by the same process, and thus explanation mainly concerns maturation in *P. rigida*. All ray parenchyma cells were free from wall thickening and lignification in the outer sapwood (Photos 15 and 16). In the middle of the sapwood, a few ray cells had secondary wall thickening and lignification (Photos 17 and 18), and contained no cytoplasm. In the specimen examined which was obtained in June, various intermediary phases of secondary wall thickening and lignification of ray cells were not found in any annual ring at all. This fact suggests the importance of the season or period of time during which cell wall development occurs. Although location of these types of cells were not limited in either the earlywood or latewood, they were often found near the ray tracheids. The total number of cells of this type increased towards the intermediate wood (Photos 19, 20 and 21).

Many parenchyma cells remained unthickened and unlignified throughout the sapwood. In the intermediate wood, UV-absorption began to occur from the corners of the cell walls, and then proceeded to the entire end wall and a part of the transverse wall. This was exactly the same as that of the latewood ray parenchyma cells of *P. densiflora* (Photos 19, 20 and 21, compare with photo 9). During UV-absorption was extended to the compound middle lamellae, protoplasm was clearly present and wall thickening was not observed. The various maturing phases of the cell wall were found in a tangential section of a ray (Photo 21). Furthermore, after the completion of maturation of these cells the UV-absorption was somewhat different in strength between individual cell walls, and between pit membranes. UV-absorption of the membranes of the bordered pit in ray tracheids occurred slightly earlier than that of the thin walls in the ray parenchyma cells as shown in photo 21.

In the heartwood, all ray parenchyma cell walls were lignified (Photos 22, 23 and 24). Many of them had smooth and thin cell walls which seemed to be lignified in the intermediate wood, and were recognized to have no secondary wall thickening (Photo 23). Heartwood substances were found within the ray parenchyma cells and some of them coated the cell wall surface giving it a rough appearance (Photo 22).

The rough secondary wall thickening as seen in radial section (Photo 22) were not knob-like but rather had a continuous wavelike appearance.

3. *Pinus strobus* LINN. (Photos 25-35)

Secondary wall thickening and lignification of all of ray parenchyma cells of *P. strobus* were performed near the cambium. This manner of cell wall development differed entirely from that of the Diploxylon pines. It is evident that maturation of all the ray cells takes a duration of one or two years. Parenchyma cells formed in the year when the samples were obtained, had only thin unlignified walls in June (Photo 25). Of the previous year's annual ring, several ray cells in the outer-latewood remained unthickened and unlignified also in June (Photo 26 and 27), while in the inner-latewood there were many ray cells deposited randomly

by secondary wall on their transverse walls, thus making them rough in appearance as shown in photo 28. In the earlywood of this annual ring, it was found to have a rather smooth secondary wall compare with the inner-latewood. In ring No. 3, all the outer-latewood were found to have smooth secondary wall, remaining unchanged ray cells both in the inner-latewood and the earlywood about thickening, and thus maturation appeared to be reached in all ray cells during the second growth season (Photos 29 and 30). In tangential view, this rough surface of cell walls appeared smooth, and all window-like pit membranes were unlignified (Photo 27). Thin unlignified layers appeared within a cell wall at this stage of the secondary wall formation, which were also observed in *P. densiflora*. This layer became unclear as the formation progressed.

Within one ray tissue, the secondary wall formation began first in the ray parenchyma cells contiguous to the ray tracheids. The present study showed that lignification of the secondary wall of the ray parenchyma cells almost coincided with its deposition, differing in this respect from that of the tracheid. Signs of limited lignification in the cell corner was not observed. Secondary wall thickening and lignification were delayed in the ray parenchyma cells which were connected with resin canal tissue. They had thin unlignified cell walls even in the inner sapwood where the other ray parenchyma cells completely matured (Photo 31). After the completion of maturation near the cambium, there was no change in the cell walls through the sapwood (Photo 33). UV-absorption of the window-like pit membranes was observed first in the intermediate wood (Photo 32). Heartwood substances were minor components in the ray of the heartwood (Photos 34 and 35). UV-absorption of the individual window-like pit membranes within a tangential section of a ray was usually of the same strength, in contrast with *P. densiflora* (Photo 35, compare with photo 14).

Discussion and conclusions

This study has made clear differences of maturation among some species of Genus *Pinus*. All species examined had ray parenchyma cells having a secondary wall, though the process and frequency of the secondary wall formation were different from each other. In *P. densiflora* earlywood ray parenchyma cells already showed secondary wall thickening in about half of the cells near the cambium (Photo 3), and these cells increased gradually in number through the sapwood. In *P. rigida* and *P. banksiana* secondary wall thickening of ray parenchyma cells was observed at first in the central portion of the sapwood (Photos 17 and 18). Such cells increased considerably at the sapwood-heartwood boundary (Photos 19, 20 and 21). In *P. strobus* all ray parenchyma cells completed secondary wall thickening near the cambium (Photos 28 and 30). In most gymnosperms, the ray parenchyma cells of the wood continued to live for many years through the sapwood after the completion of secondary wall like in both *P. densiflora* and *P. strobus* examined. In both *P. rigida* and *P. banksiana* (Photos 17-21), on the contrary, they died soon after maturation in sapwood. In *P. densiflora*, *P. rigida* and *P.*

banksiana, some ray parenchyma cells were unlignified in the sapwood, and showed UV-absorption from the cell corners and then died making little secondary wall in the intermediate wood. These cells existed only in the latewood zone in *P. densiflora* (Photos 8, 12 and 13), and random regardless of earlywood and latewood in *P. rigida* and *P. banksiana* (Photos 15, 19 and 23).

Thus the maturing process of ray parenchyma cells in each of the species observed, showed a diversity in secondary wall thickening, lignification process and duration of cell life.

There were two modes of the maturation process of ray parenchyma cells through the sapwood. One mode was accompanied by obvious wall thickening, and occurred in the ray parenchyma cells of earlywood in *P. densiflora* (Photo 3), a part of them regardless of earlywood and latewood in *P. rigida* and *P. banksiana* (Photos 17 and 18) and all in *P. strobus* (Photo 30). In this type of ray cells secondary wall was deposited on the unlignified primary walls, and lignification of the secondary wall was observed to have occurred coincident with its deposition. However it is possible that lignification of the ray parenchyma cell may be more or less delayed with respect to the cell wall deposition like that in the tracheid. Further investigations into the progressive phase of secondary wall formation of ray parenchyma cells in detail are needed to explain this problem. UV-absorption was not found to begin at the corner of the cell walls.

Another mode of maturation was without any sign of wall thickening in the sapwood, and it was observed in the latewood ray parenchyma cells in *P. densiflora* (Photos 2 and 8), and in *P. rigida* and *P. banksiana* except the above mentioned cells belonging to the former mode (Photos 15 and 16). In the intermediate wood UV-absorption began first from the corners of the cell walls, to be exact, from the middle lamella of the corners (Photos 9, 20 and 21). It has been stated that lignification of the tracheid started in the middle lamella or primary wall of the cell corners. In a recent study, KUTSCHA and SCHWARZMANN (1975)¹³⁾ demonstrated that lignification of the tracheid started in the middle lamella between initial pit borders of adjacent tracheids before the cell corners. However there are few reports of the lignification process of ray parenchyma cells in detail.

Formation of secondary wall: The process of secondary wall thickening within a ray usually started in the ray parenchyma cells adjacent to the ray tracheids. In *P. rigida* and *P. banksiana* the secondary wall thickening, however, sometimes started from the ray parenchyma cells which were not in contact with ray tracheids (Photo 19). The deposition of secondary wall within each parenchyma cell was sometimes interspersed randomly in the form of knobs on the unlignified primary wall making the surface rough (Photos 4, 26 and 28). They are considered to be simple pits, and this rough surface is marked off from smooth one. The secondary wall formation of ray parenchyma cells seems to be quite different from that of the tracheids in which cell walls are deposited uniformly from the central portion of cells toward both ends.

Differences of the maturation process between earlywood and latewood in *P.*

densiflora: In the present study the ray parenchyma cells of *P. densiflora* were provided with secondary wall thickening in the earlywood and little in the latewood through the sapwood as FENGEL (1970)²⁰ also stated (Photos 2 and 3). The absence of lignin in the latewood parenchyma cells in the sapwood may be due to a living axial resin canal. This phenomenon corresponds to the data of WU and WILSON (1967)²², SQUIRE, SWAN and WILSON (1967)²⁰ describing respectively that maximum values for lignin and flavonoids occurred within the earlywood zone. This may indicate a difference in lignin biosynthesis or peroxidase activity of the ray parenchyma cells between earlywood and latewood.

The number of years required to complete the maturation of ray cells in *P. strobus*: The ray parenchyma cells in *P. strobus* formed in the year when the sample was obtained in June, were free from the secondary wall and lignification at the time of earlywood formation (Photo 25). At this time, there were found different forms of secondary wall thickening of ray parenchyma cell walls within the previous year's ring, i.e., smooth and rough surface, and non of wall thickening (Photos 26, 27 and 28). The ray cells with rough or smooth secondary wall had matured at this time when examined. It is presumed that most ray parenchyma cells mature in the year when they are formed, but the remains in the next season. The completion of wall development in all ray cells was observed in annual ring of two years before (Photos 29 and 30). BALATINECZ and KENNEDY (1967)¹⁹ described that wall thickening and lignification of ray parenchyma cells in soft pine (involving *P. strobus*) showed no indication of lagging behind other xylem elements. MANN (1972)¹⁴ reported that the formation of the secondary wall in ray parenchyma cells of *P. strobus* was delayed until late summer and was coincident with latewood formation, and deposition of the secondary cell wall occurred first in those cells adjacent to the previous year's latewood and then proceeded along the ray towards the cambium. IMAGAWA, FUKAZAWA and ISHIDA (1967)¹² also stated that lignification of ray parenchyma cells in *Larix leptolepis* lagged behind that of the tracheid. In this study it was found that two growth seasons are required in a part of cells for the completion of secondary wall formation of ray parenchyma cells in *P. strobus*.

Resin canal tissue: Maturation of ray parenchyma cells was affected by the resin canal tissue in all species examined (Photo 31), similar to that reported by BAMBER (1972)⁹. Large numbers of ray parenchyma cells connected with the axial or radial resin canals were unlignified until the sapwood-heartwood boundary even in *P. strobus*. More detailed observation concerning the resin canal is required.

Thin-walled ray parenchyma cells: The thin lignified walls of the mature ray parenchyma cells in the heartwood were observed in *P. densiflora*, *P. rigida* and *P. banksiana*, whether they possessed the secondary wall or not were not examined (Photos 12, 13 and 23). These thin-walled ray parenchyma cells, however, seemed to have no secondary wall in the sapwood. CÔTE and DAY (1969)²¹ demonstrated that in southern yellow pines both thick-walled and thin-walled ray parenchyma cells occurred. THOMAS and NICHOLAS (1968)²¹ described that in

southern yellow pines the pinoid pit membranes and the ray parenchyma cell wall were continuous and had no secondary wall thickening. FUJIKAWA and ISHIDA (1974)⁹ indicated Diploxyylon pine was characterized by ray parenchyma cells in which the wall organization changed from I+P+PL (protection layer) (in the immature state) to I+P+PL+S₁ (in the mature state). The disagreement between these observations may be the result of different parts of the sapwood or of the heartwood. A follow-up survey with electron microscopy on the process of wall formation near the intermediate wood zone is required to determine the change of structure of the ray parenchyma cell wall in detail.

Unlignified primary wall: Non UV-absorbing layers within cell walls were often observed in the early phase of secondary wall formation in *P. strobus* and *P. densiflora* (Photos 4, 26 and 27). It has been described that lignification of the primary wall was delayed behind that of the intercellular layer and the S₁ layer of the tracheid (SCHWARZMANN and KUTSCHA 1973¹⁷, KUTSCHA and SCHWARZMANN 1975¹⁸). In the parenchyma cells this layer may be an unlignified primary wall which disappeared gradually as secondary wall formation progressed.

Duration of life of the ray parenchyma cells: Ray parenchyma cells of *P. rigida* and *P. banksiana* which completed the secondary wall thickening and lignification seemed to have died immediately after completion, in spite of that they were in the sapwood. BALATINECZ and KENNEDY (1967)¹¹, HOWARD and MANWILLER (1969)¹¹ observed this sort of thick-walled ray parenchyma cells in southern pines and others. They did not mention about the life and death of these cells. MANN (1974)¹⁵ demonstrated that in *P. banksiana* the ray parenchyma cell which developed a lignified secondary wall retained distinct cell contents. This dissimilarity may depend on difference of the season of sampling. It is presumed that these cells lose the protoplasm within a rather short period after the completion of secondary wall formation, in relation to the season which so far remains unexplained. It was characteristic of the groups of *P. rigida* and *P. banksiana* that both living and dead cells, even when adjacent to each other coexisted for a long period in the sapwood (16 years of rings Nos. 21~36 in *P. rigida*, 5 years of rings Nos. 21~25 in *P. banksiana*) (Photos 17~21). This zone had a colouration similar to that of the heartwood, and could therefore be easily mistaken for the heartwood. It is considered that this zone is an "intermediate wood" where heartwood formation is taking place individually in each of the ray parenchyma cells.

Window-like pit membranes: It has been stated that the pit membranes of the bordered pits contained phenolic substances even in the sapwood, and normally ligification took place besides the development of other polyphenols in the heartwood (BAUCH and BERNDT 1973⁹). Some window-like pit membranes also indicated weak UV-absorption in the central part of the sapwood in *P. densiflora* (Photo 7). UV-absorption of various strengths from the cross-field pit membranes at the time of heartwood formation might result from lignin or heartwood phenols (Photos 10, 14, 21, 24, 32 and 35^{5,6,16}). As a result of the deposition of heartwood substances, the pit membranes of the cross-field pitting seemed

to increase some what in thickness in comparison with the unlignified one in the sapwood. The strength of UV-absorption of the cross-field pit membranes was equal within an individual ray parenchyma cell (Photos 11 and 34), and was often dissimilar between adjacent cells (Photo 14). UV-absorbing substances within a ray parenchyma cell, therefore, seemed to penetrate uniformly into its own unlignified cross field pit membranes, which occur independently in each cell. The amount or quality of lignin or heartwood phenols may differ from different parenchyma cells.

Season: HIRAI (1951)¹⁰ stated that the transformation of sapwood-heartwood occurred from July to November in the Japanese larch. SHAIN and MACKAY (1973)¹⁸, SHAIN and HILLIS (1973)¹⁹ also described that heartwood formation in *P. radiata* occurred mainly during the dormant season by monitored respiratory activity and ethylene production. The season of secondary wall formation of ray parenchyma cells may nearly agree with that of heartwood formation, presumed from the reports of SHAIN, et al. (1973)^{18,19}, HIRAI (1951)¹⁰ and MANN (1972)¹⁴. Sampling only in June, therefore, might be the "wrong" season to make clear the exact process of maturation of ray parenchyma cells in relation to heartwood formation. Observation of seasonal changes of the ray parenchyma cells is therefore needed.

The Ultraviolet microscope was mainly used in this investigation. It is impossible to make clear the change of cell wall organization in the maturing process of ray parenchyma cells only by UV observation. Observation by electron microscopy or polarized light microscopy are needed to solve this problem. We have started the work using scanning electron microscopy, which is effective in elucidating three-dimentional picture in the intermediate stage of secondary wall deposition. It is, however, very difficult to determine what substances cause strong UV-absorption the membranes of the cross-field pitting which occurred at the time of heartwood formation but we hope to continue this kind of study.

要 約

マツ属の放射柔細胞は、その成熟の多様性の故に関心が持たれ、その細胞壁や分野壁孔壁に関する研究が今まで数多く行われてきた。それにより個々の細胞の微細な構造や変化がかなり詳しく理解されている。しかしながら、それら個々の細胞が辺材中でどの様な変遷をたどり成熟してゆくのかは、BALATINECZ and KENNEDY (1967)¹¹などの研究により、ある程度明らかにされているが、その詳細はいまだ明らかではない。

そこで本研究では、数種のマツ属を対象に、辺材から心材までの、主に柾目面切片による連続的な観察により、放射柔細胞の2次壁形成と木化の過程を、心材形成との関係において明らかにすることを目的とした。

観察樹種として、Diploxylon pine から窓状壁孔を持つアカマツ (*Pinus densiflora* SIEB. et ZUCC.), マツ型壁孔を持つリギダマツ (*Pinus rigida* MILL.), バンクスマツ (*Pinus banksiana* LAMB.) の3種, Haploxylyon pine からストローブマツ (*Pinus strobus* LINN.) を選んだ。試料

は生立木より採取し (Table 1), F. A. A. で固定し, メタクリレート包埋の後, 0.5 μ 厚さの薄切片を作製した。これらの切片を, 成熟の基準としての木化の判定に有効である紫外線顕微鏡 (Carl Zeiss, MPM-01) を用いて 280 m μ 波長で写真撮影を行った。

得られた結果を要約すると, 次のとおりである。

1. アカマツ 放射柔細胞の成熟は, 辺材中で何年もかかって行われ, しかもその成熟の仕方が, 早材部と晩材部で異なっている (Photo 2, 3)。 早材部放射柔細胞では, 形成の翌年に約半数の細胞において (放射仮道管に隣接する柔細胞のほとんどすべて), すでに2次壁の沈着とその木化が現われ (Photo 1, 3), 辺材内部へと次第にその数を増してゆく (Photo 5, 7, 10)。(試料採取の時期により当年度形成された材は見られなかった, Photo 1)。そして移行材部ですべての早材放射柔細胞の成熟が完了する (Photo 10)。辺材の早材柔細胞はすべて生きている。これに対し, 晩材部の放射柔細胞は, 辺材中で2次壁の沈着がほとんどおこらないまま (Photo 2, 6, 8), 移行材部に至り, そこで細胞のコーナーからUV吸収がおこり始め (Photo 9), おそらくは2次壁をあまり形成せずに死に至り心材化するものと思われる (Photo 11~14)。

2. リギダマツ・パンクスマツ (両樹種は, ほぼ同様の成熟過程をたどる) 両樹種は, アカマツと同様, *Diploxyylon pine* グループに属するが, 放射柔細胞の成熟はアカマツとは異なる。辺材外部では, 2次壁の形成と木化は早晚材ともに見られない (Photo 15, 16)。辺材中央部から一部の細胞に, 2次壁の形成と木化が現われ始め (Photo 17, 18), 移行材部ではその数を増していた (Photo 19~21)。これらの細胞は辺材中で木化と共に死んでしまう。これら以外の辺材中で未木化の放射柔細胞は, 移行材部で急に細胞コーナーの間層からUV吸収が始ま (Photo 20, 21), すぐに細胞壁全体に吸収が見られるようになり原形質を失う (Photo 22~24)。このとき2次壁は, おそらくあまり形成されないとと思われる (Photo 23, 24)。

3. ストローブマツ 放射柔細胞は, 形成層附近で成熟を完了するが, 仮道管の成熟には遅れる。 採取された年に形成された柔細胞では, 2次壁の沈着と木化は見られなかった (Photo 25)。その前年に形成された柔細胞の晩材部の年輪界附近では, 2次壁の沈着と木化の見られない細胞がいくらか見られた (Photo 26, 27)。その内側では2次壁が柵目面で見るとコブ状に沈着しており, 単壁孔と思われる部分とかなり平滑な壁とが観察された (Photo 28)。概して晩材部の方が壁厚のむらが大きいように見えた。次の年輪の細胞では前記の未木化の細胞も2次壁を形成しており, すべての細胞の成熟が完了している (Photo 29, 30)。その後, 細胞壁に変化は見られず (Photo 33), 上記の樹種と同様に移行材部で未木化の壁孔壁に, いろいろな強さのUV吸収が見られ (Photo 32, 35), 死に至る (Photo 34, 35)。また, 樹脂道と連絡している放射柔細胞の成熟は遅れ, 移行材部で2次壁形成と木化が完了する (Photo 31)。

以上のように, マツ属放射柔細胞は樹種により成熟過程に違いがあり, 木化の起り方には二通りある。一つは辺材中で2次壁が形成されるもので, これにはそれが形成層附近で完了するもの (ストローブマツ) と, 辺材中で徐々に行われるもの (アカマツ早材部, リギダマツ・

パンクスマツの一部の柔細胞)がある。この時、辺材中で細胞死を伴うもの(リギダマツ・パンクスマツ)と、伴わないものがある(ストローブマツ、アカマツ早材部)。もう一つは、辺材中でおそらく2次壁を形成しないで未木化のまま辺心材境界に至り、そこで急に細胞コーナーからUV吸収が始まるものである(アカマツ晩材部、リギダマツ・パンクスマツの上記以外の柔細胞)。また、放射柔細胞の2次壁沈着と木化の起こり方は仮道管のそれと異なっているようみえる。2次壁は細胞壁にある間隔をおいて不規則に沈着し、枉目面で見ると壁がデコボコに見える。このデコボコがどのように形成され、またどんな形態であるかは走査型電子顕微鏡などによる観察が必要であろう。2次壁の沈着と木化の時間的な差を、本研究では観察することは出来なかった。これは試料採取の時期によるのかも知れない。今後、更に季節による放射柔細胞の成熟の変化を観察してゆく必要があるだろう。

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Explanation of photographs

Note: All the microphotographs were taken by an ultraviolet microscope at a wavelength of $280 \text{ m}\mu$. Stem axis of the sample trees is vertical in all the photographs. "Ring No." is counted from the cambium, i.e., annual growth layer of xylem directly adjacent to the cambium is counted as ring No. 1. The photographs are arranged in the order from the cambium to the heartwood.

Photos 1-14. *Pinus densiflora* SIEB. et ZUCC.

Photos 5, 7, 10 and 14 are from tree-No. 2. Others are from tree-No. 1. Since none of newly formed xylem in the year when the wood samples were obtained are there, the previous year's xylem is counted as ring No. 1. Sapwood: ring Nos. 1-31, Intermediate wood: ring Nos. 32-33, Heartwood: ring Nos. 34-.

- Photo 1.** The latewood and a part of earlywood ray in ring No. 1. The ray parenchyma cells of the previous year's latewood next to the cambium have thin unlignified walls contrasted with the ray tracheids which are thickened and lignified. The parenchyma cells of the earlywood show secondary wall thickening and lignification (arrows).
- Photo 2.** The latewood ray in ring No. 2. The secondary wall is formed sporadically only in the parenchyma cells adjacent to the ray tracheid. Non UV-absorbing layer which seems to be primary wall is detected under the lignified secondary wall (arrow, see also photo 4).
- Photo 3.** Earlywood ray in ring No. 2. The secondary wall thickening first occurs in those ray parenchyma cells in contact with the ray tracheids, and spreads to next row of parenchyma cells. In the photograph, the central cells show lack of the secondary thickening and lignification. The end walls of ray parenchyma cells show especially little absorption of UV light in this stage (arrows).
- Photo 4.** An enlarged view of a part of photo 2. The secondary wall is deposited randomly on thin unlignified layer which is considered as a primary wall (arrows).
- Photo 5.** Tangential section of the earlywood ray in ring No. 1 (tree-No. 2). Secondary wall is developed only in the ray parenchyma cell adjacent to the ray tracheid (RT) (arrow), but not in the others.
- Photo 6.** The ray in the annual ring boundary (arrow) of Nos. 13 and 14. Two parenchyma cells in the earlywood have lignified secondary wall. Four cells in the latewood over two years have no secondary wall.
- Photo 7.** Tangential section of the earlywood ray of ring No. 17 in the central sapwood (tree-No. 2). The ray parenchyma cells near the ray tracheid have secondary wall and the window-like pit membranes in these cells absorb slightly UV light in this stage (arrow 1). Other cells have no secondary wall, and no UV-absorption in their pit membranes (arrows 2).
- Photo 8.** The ray in earlywood-latewood boundary in ring No. 25. All the latewood parenchyma cells (left) show lack of lignification. In the early wood of their boundary zone (right), secondary wall thickening and lignification are observed only on a row of the ray parenchyma cells adjacent to ray tracheid (arrow).

- Photo 9.** Ray in annual ring boundary of Nos. 32 and 33. Cell wall development is almost completed in ray parenchyma cells in the side of earlywood, left half. Latewood cells have only thin unlignified walls, but the absorption of UV light starts from the cell corners and spreads out to the end wall (arrow).
- Photo 10.** Tangential section of earlywood ray of ring No. 33 in the intermediate wood (tree-No. 2). All the ray parenchyma cells have secondary wall. The window-like pit membranes indicate weak or strong UV-absorption (arrows).
- Photo 11.** Earlywood ray of ring No. 36. in the heartwood. Heartwood substances absorb UV light in various strength (arrows 1). Strength of UV-absorption is the same in two window-like pit membranes of individual ray parenchyma cells (arrows 2, 3) (Compare with photo 14 where strength of UV-absorption of window-like pit membranes varies among the individual ray cells).
- Photo 12.** Latewood ray of ring No. 35 in the heartwood. The secondary wall is found here in the heartwood (arrows 1), which is not observed in the sapwood. Amount of secondary wall, however, is less than that in the earlywood and is partial. Heartwood substances deposit on the cell wall surface, which strongly absorb UV light (arrows 2). A part of the thin wall remain unlignified (arrow 3).
- Photo 13.** Latewood ray of ring No. 42 in the heartwood. In this case, the wall of some cells is thin and accompanied by no secondary thickening (arrow, compare with photo 12).
- Photo 14.** Tangential section of earlywood ray of ring No. 35 in the heartwood (tree-No. 2). Strength of UV-absorption of the pit membrane is markedly different among individual ray cells (arrows). Pit membrane is thicker than that of the intermediate wood.
- Photos 15-24. *Pinus rigida* MILL.**
- Photo 15.** Tangential section of ray of ring No. 12 in the sapwood. All the ray parenchyma cells both in earlywood and latewood show lack of secondary wall and lignification.
- Photo 16.** Ray of ring No. 13 in the sapwood. All parenchyma cells show lack of secondary wall and lignification.
- Photo 17.** Ray of ring No. 21 in the sapwood. A ray parenchyma cell at the top center of the photograph has irregularly thickened, lignified secondary wall and not protoplasm (arrow). The other cells have thin unlignified walls and protoplasm.
- Photo 18.** Tangential section of ray of ring No. 29 in the sapwood. A ray parenchyma cell contiguous to the ray tracheid (RT) has lignified secondary wall (arrow 1), and non UV-absorption pinoid pit membranes (arrow 2). A part of the primary wall on which lignified secondary wall is deposited, show non UV-absorption (arrow 3). The other parenchyma cells show lack of secondary wall and lignification.
- Photo 19.** Ray of ring No. 35 in the intermediate wood. Dead ray parenchyma cells in a central row have lignified secondary wall. No difference of development of the wall is observed between the earlywood and latewood.

- Photo 20.** Ray of ring No. 36 in the intermediate wood. In the dead ray parenchyma cells, two different strength of UV-absorption are observed with regard to the secondary wall, suggesting that the secondary wall formation occurs at two stages (arrow 1). In the living cells, UV-absorption is detected in the intercellular layer especially noticeable in the cell corner and end wall (arrow 2).
- Photo 21.** Tangential section of ray of ring No. 35 in the intermediate wood. The upper ray parenchyma cell adjacent to the ray tracheid (arrow 1), and the lower one which contains the heartwood substance (arrow 2) have slightly thickened, and lignified wall compared with other unlignified cell wall (The upper cell seems to have secondary wall). The ray cells in no contact with ray tracheid have thin unlignified walls and indicate UV-absorption only in the intercellular layer of the cell corners (arrow 3). The bordered pit membranes of ray tracheids absorb UV light (arrows 4). This phenomenon is observed in ring No. 35 (Photo 19), but is not in ring No. 34 which seems to be the intermediate wood judged based on colour.
- Photo 22.** Ray of ring No. 37 in the heartwood. Some ray parenchyma cells have the rough secondary walls which do not make pairs in the double walls (arrows 1). The lowest parenchyma cell has thin wall coated with non wall substances, probably heartwood substances (arrows 2).
- Photo 23.** Ray of ring No. 37 in the heartwood. Most of the ray parenchyma cells have thin lignified walls which seems to be developed in the intermediate wood (arrow 1). Heartwood substances are observed on the surface of cell wall (arrows 2).
- Photo 24.** Tangential section of ray of ring No. 37. There are found thick-walled ray parenchyma cells (arrows). Secondary wall indicate slightly stronger UV-absorption than that of the longitudinal tracheid (LT).
- Photos 25-35.** *Pinus strobus* LINN. Photos 29, 32, 35 are from tree-No. 2. Others are from tree-No. 1.
- Photo 25.** Latewood ray of ring No. 1 in the sapwood. The ray parenchyma cells even next to ray tracheid (RT) have thin unlignified walls (arrow 1). The ray tracheid of *P. strobus* has smooth cell walls, different from dentate ones in Diploxylon pines (arrow 2).
- Photo 26.** Ray of ring No. 2 in the sapwood. The rough secondary walls are observed only in the ray parenchyma cells near the ray tracheid. Non UV-absorbing layers are detected within the cell wall on which the rough secondary walls are deposited (arrows). The other cells show lack of secondary thickening.
- Photo 27.** Tangential section of latewood ray of ring No. 2, which is the same ring as that of photo 26. Rough secondary walls observed in the former photograph are found smooth here in tangential view (arrows 1). The window-like pit membranes indicate non UV-absorption (arrows 2). Non UV-absorbing layers which are the same with photo 26 seem to be primary wall, as they are continuing to the window-like pit membrane (arrows 3).

- Photo 28.** Ray of earlywood-latewood boundary in ring No. 2. Though in the same ring as photo 26, all ray cells have the rough secondary walls like the knobs. The thin cell walls which are observed to have no secondary wall thickening indicate UV-absorption (arrows 1) or do not (arrows 2).
- Photo 29.** Tangential section of ray of ring No. 3 in the sapwood (tree-No. 2). Secondary wall formation is completed. No window-like pit membranes indicate UV-absorption (arrow).
- Photo 30.** Earlywood ray of ring No. 3 in the sapwood. Cell wall development seems to be finished in the form of the secondary thickening and lignification. Non UV-absorbing layers which are observed in photos 26, 27 are not clear in the mature ray parenchyma cells (arrows).
- Photo 31.** Longitudinal resin canal tissue and ray of ring No. 16 in the inner sapwood. The ray parenchyma cells which are connected with resin canal have thin unlignified walls. Ray cells remote from resin canal have lignified secondary walls.
- Photo 32.** Tangential section of ray of ring No. 6 in the intermediate wood (tree-No. 2). The window-like pit membranes of the cell losing protoplasm absorb strongly UV light (arrow 1), but no UV-absorption revealed in the window-like pit membranes of the cell having protoplasm (arrow 2).
- Photo 33.** Earlywood ray of ring No. 17 in the intermediate wood. The ray parenchyma cell walls are nearly the same appearance as the sapwood ones. Ray cells adjacent to the ray tracheid have little cytoplasm compared with those in central row. End walls have thickened lignified nodular wall (arrows), which is completed near the cambium (Photos 26 and 28).
- Photo 34.** Earlywood ray of ring No. 18 in the heartwood. A few window-like pit membranes (arrows) appear as arrows indicate for its distortion like photo 35.
- Photo 35.** Tangential section of ray of ring No. 9 in the heartwood (tree-No. 2). All the window-like pit membranes absorb UV light. The pit membranes have changed the shape (arrow).

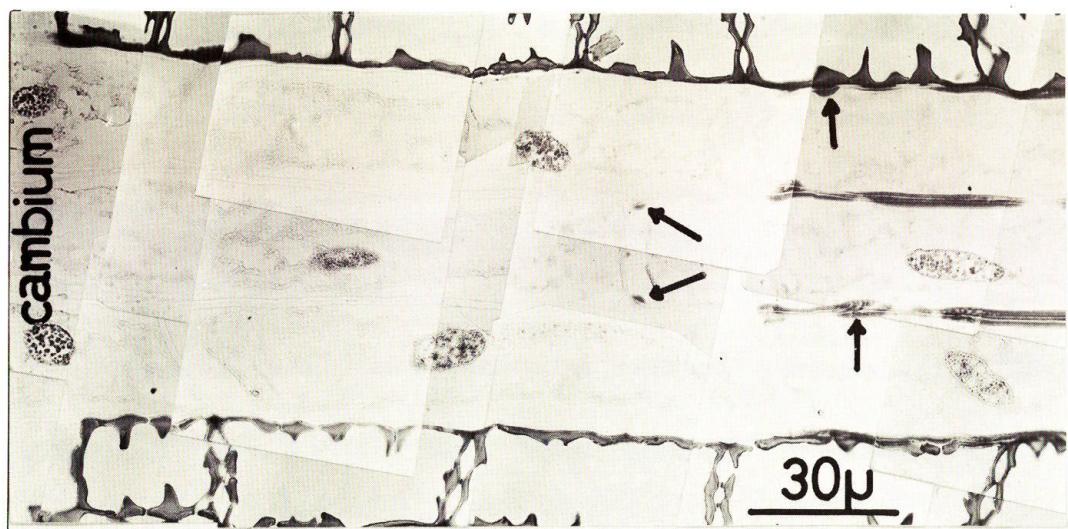


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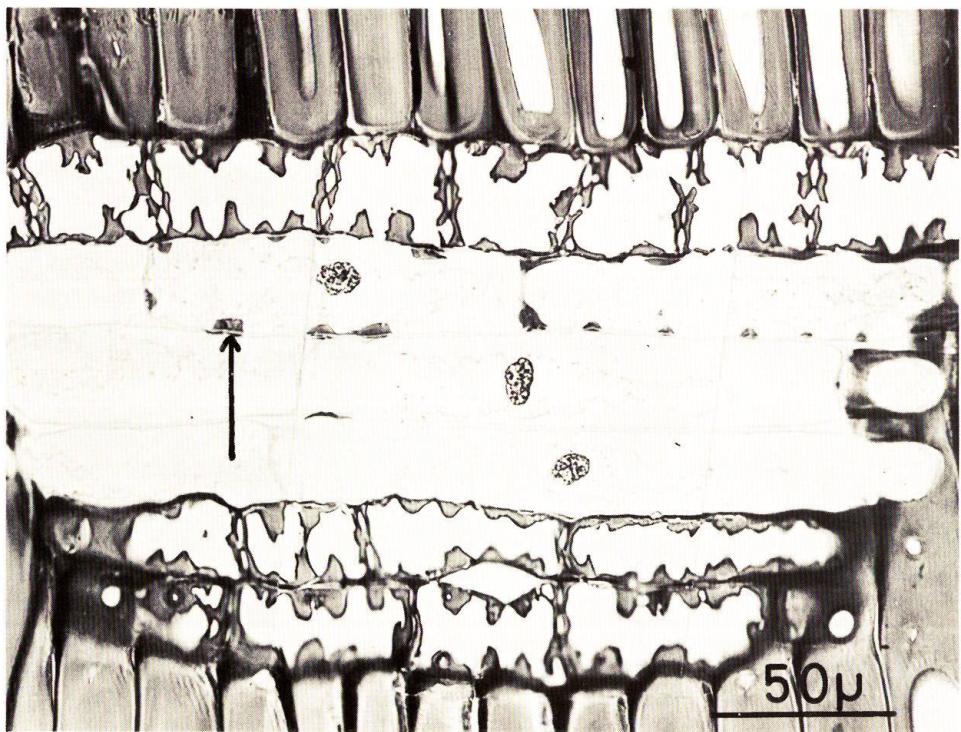


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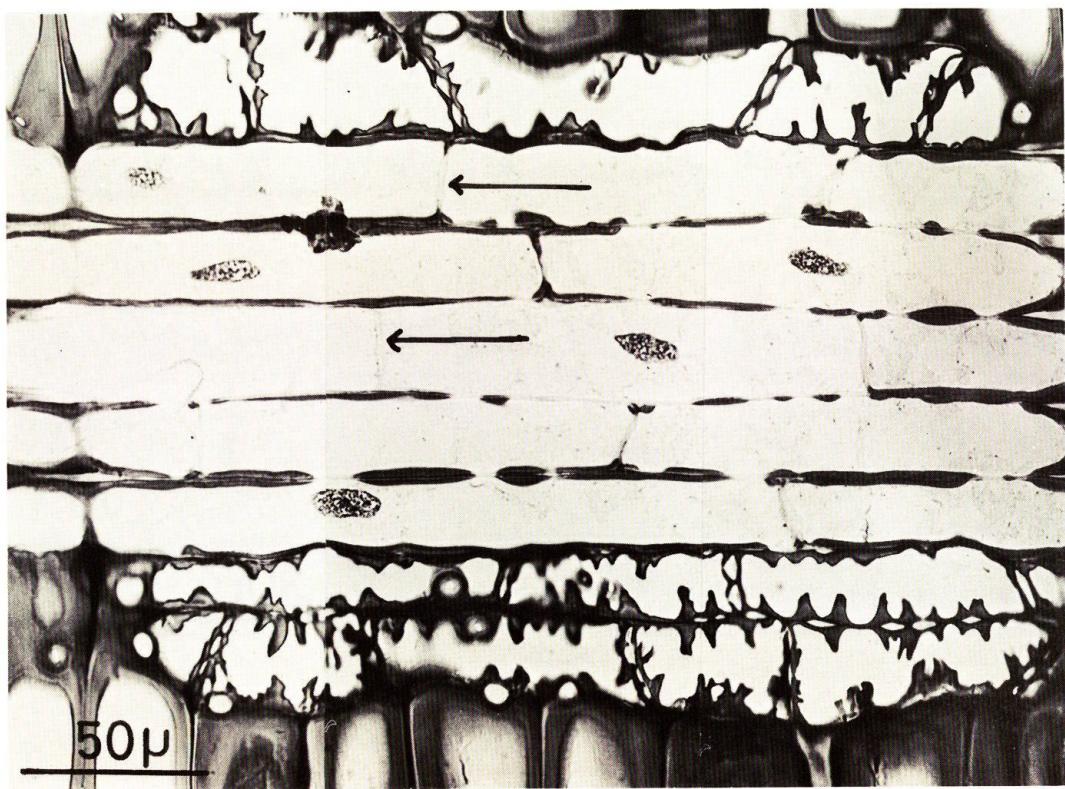


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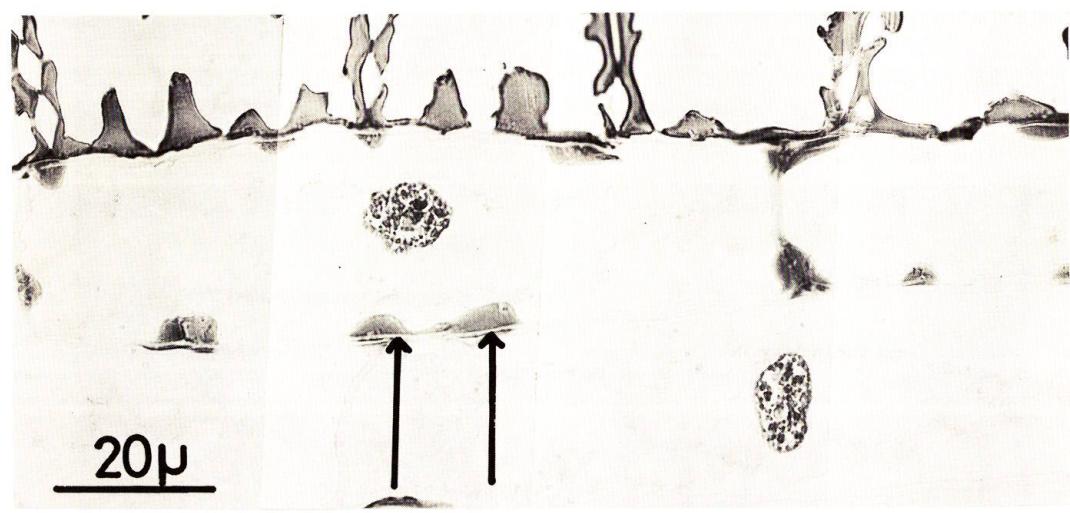


Photo 4



Photo 5

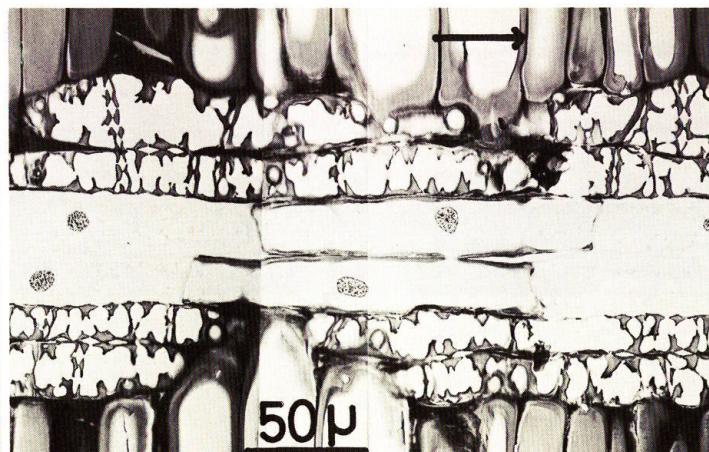


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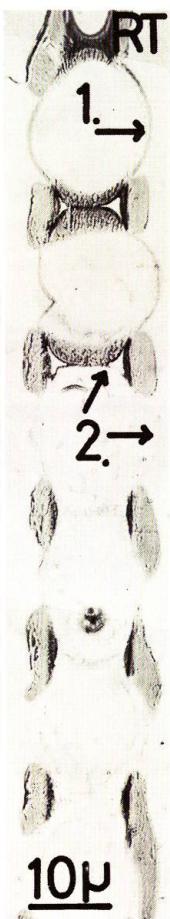


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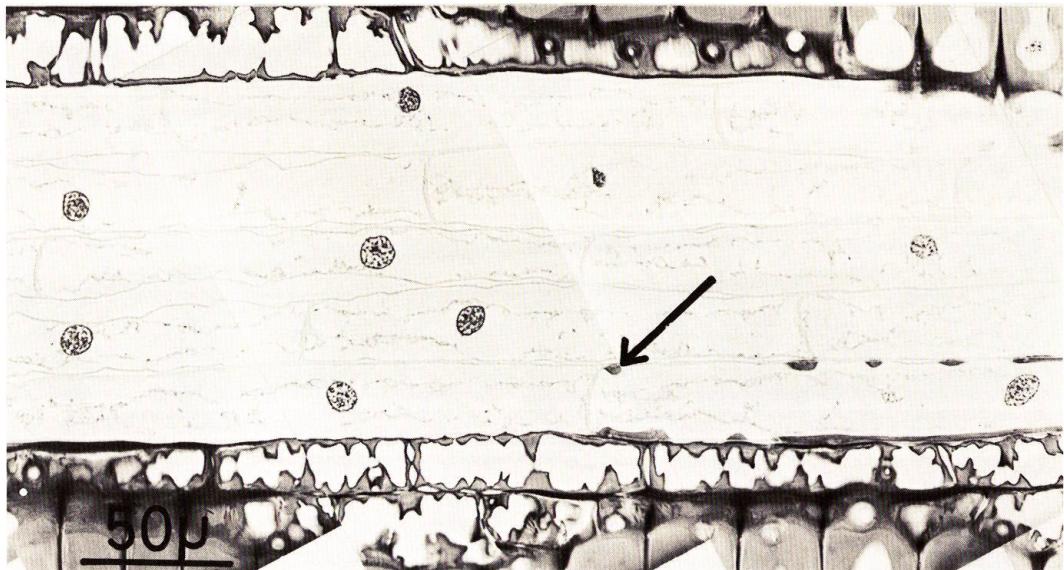


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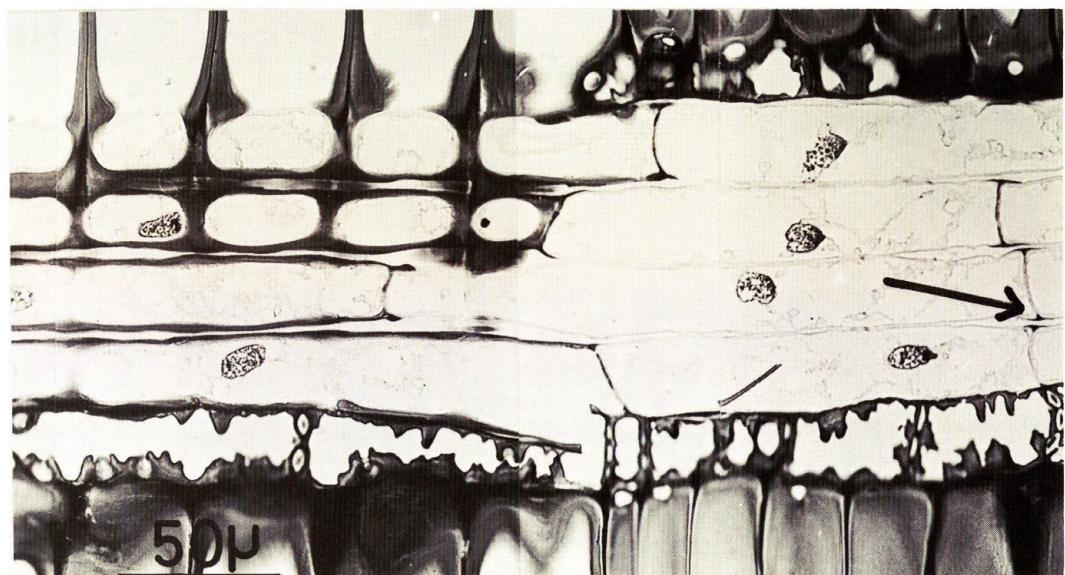


Photo 9



Photo 10

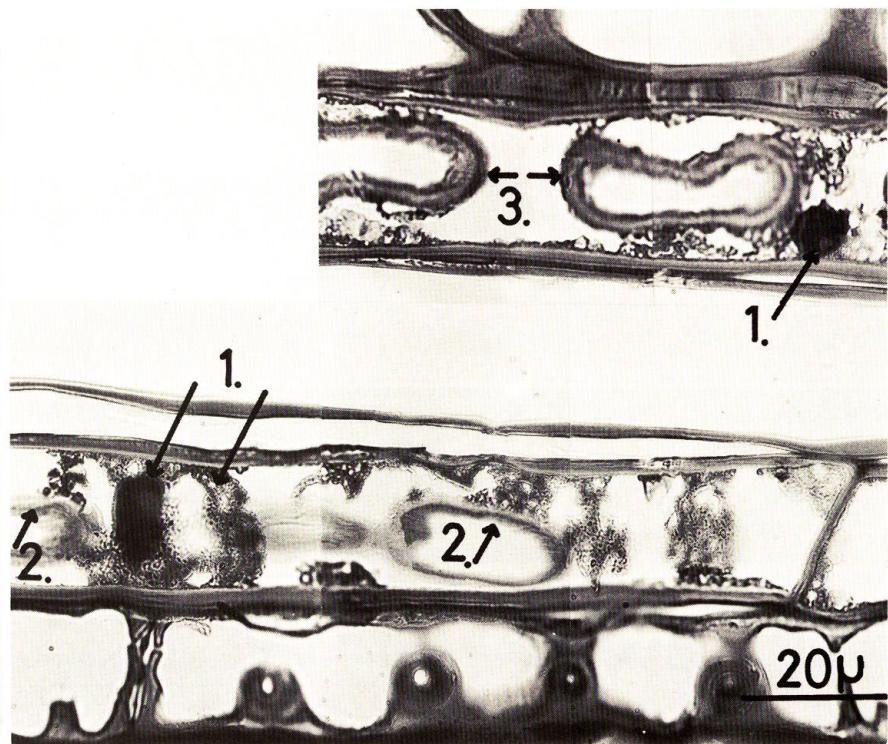


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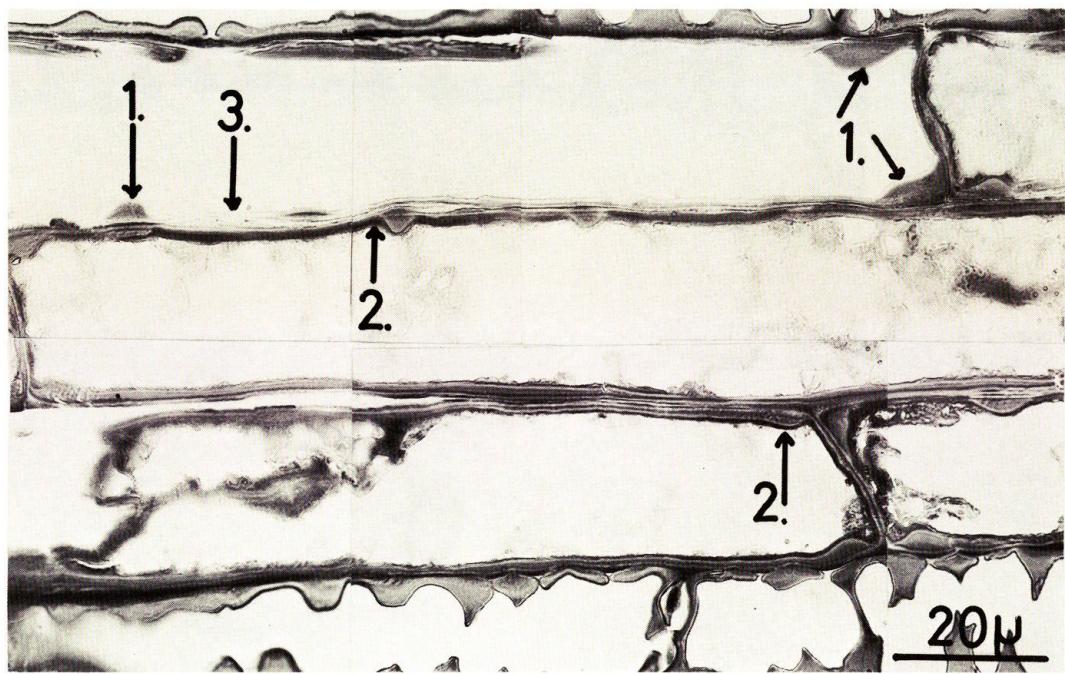


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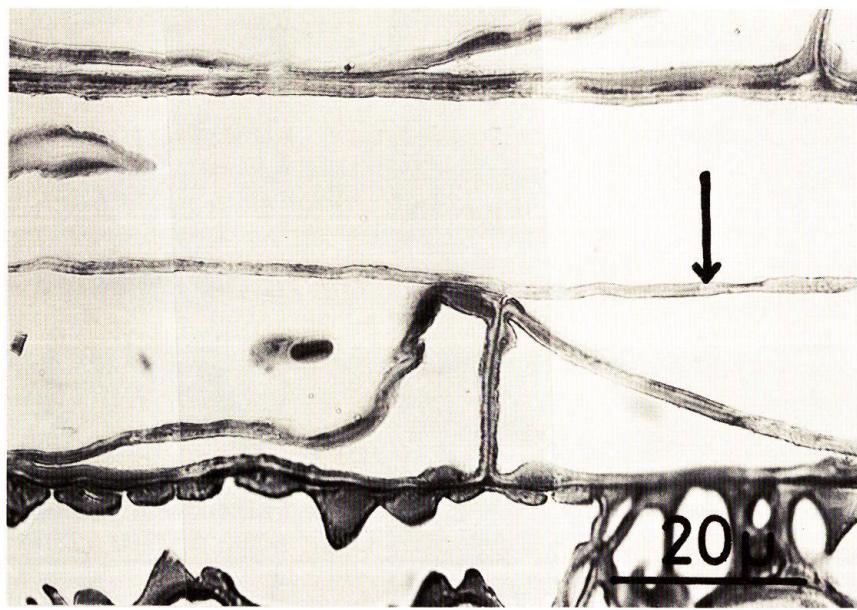


Photo 13

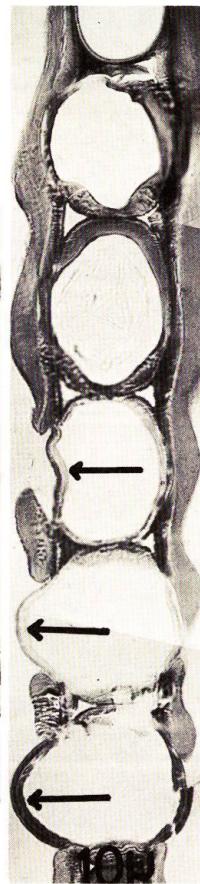


Photo 14

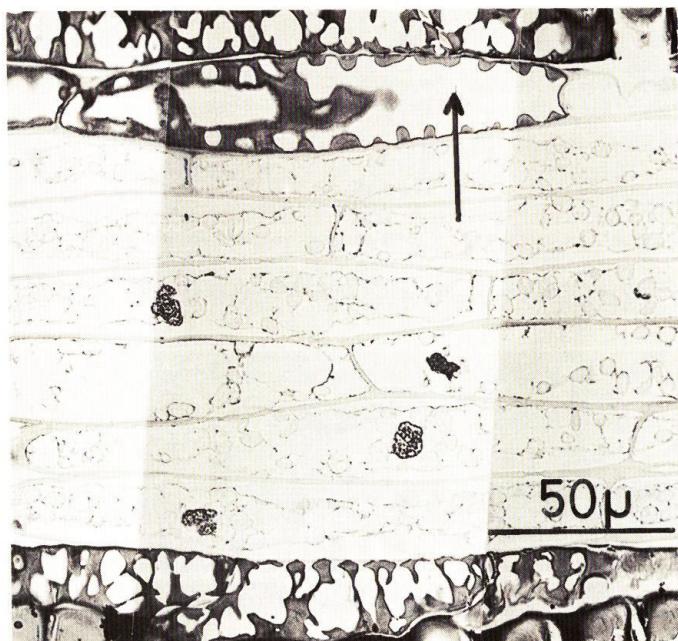
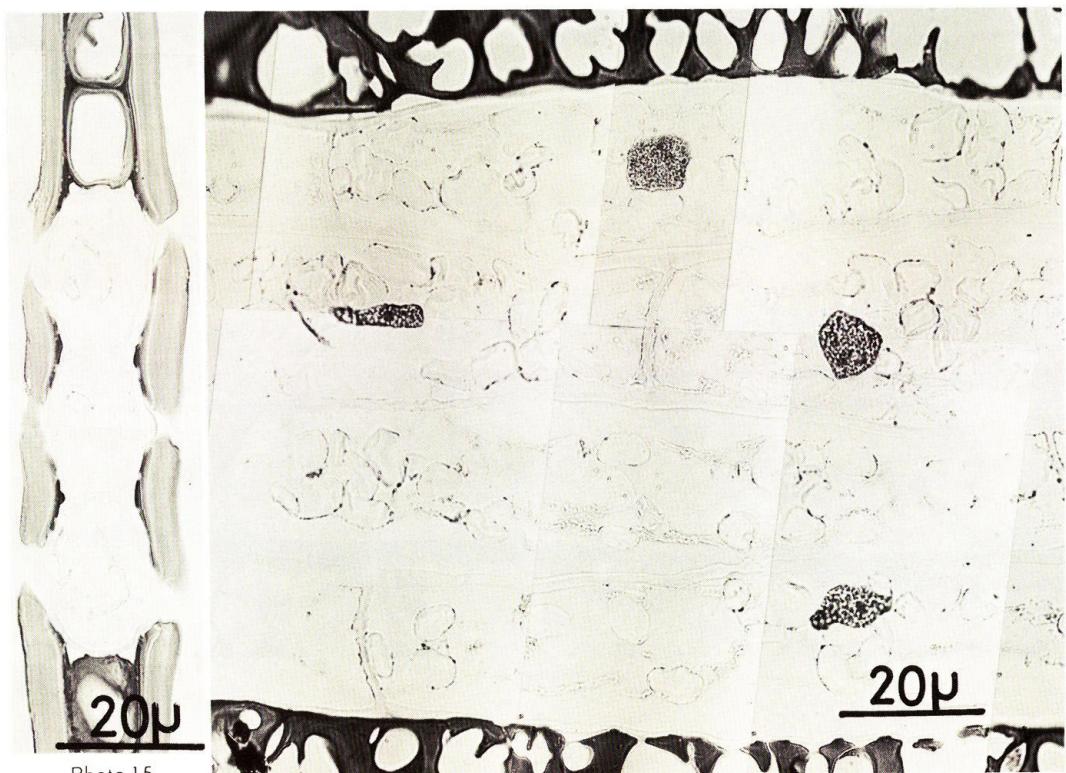


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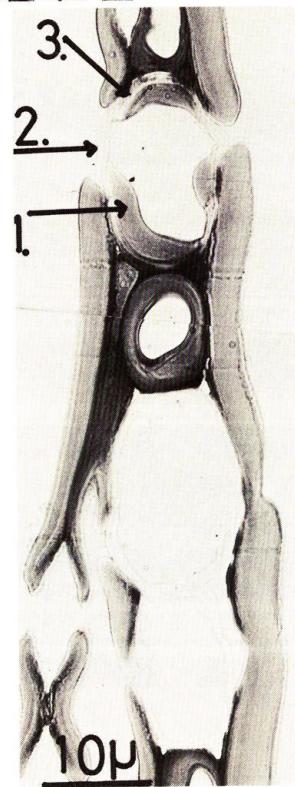


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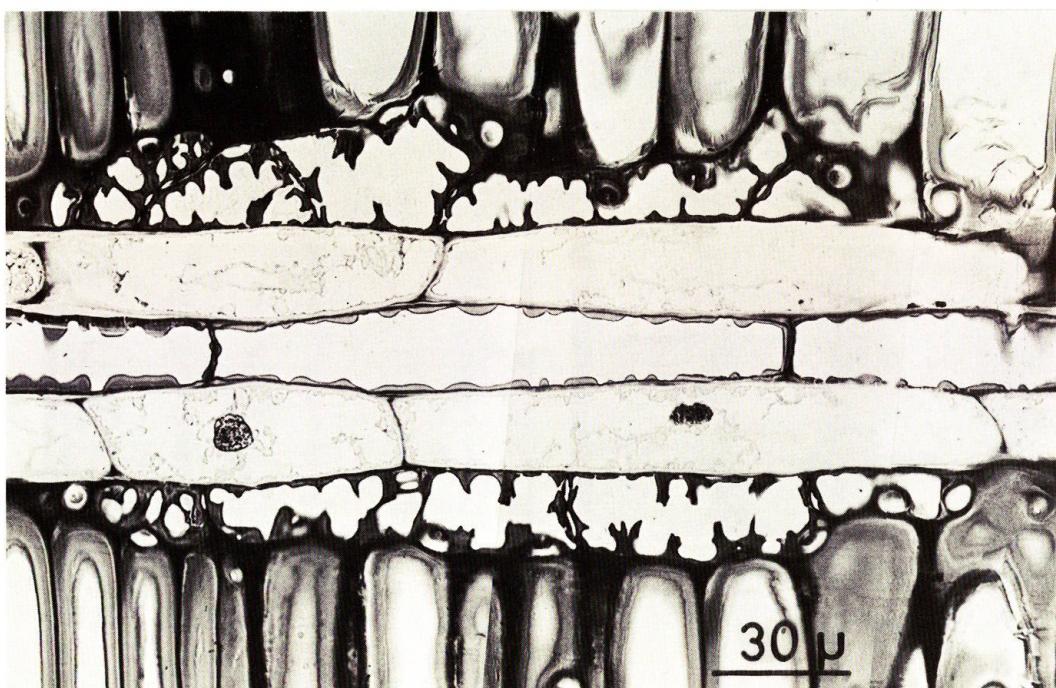


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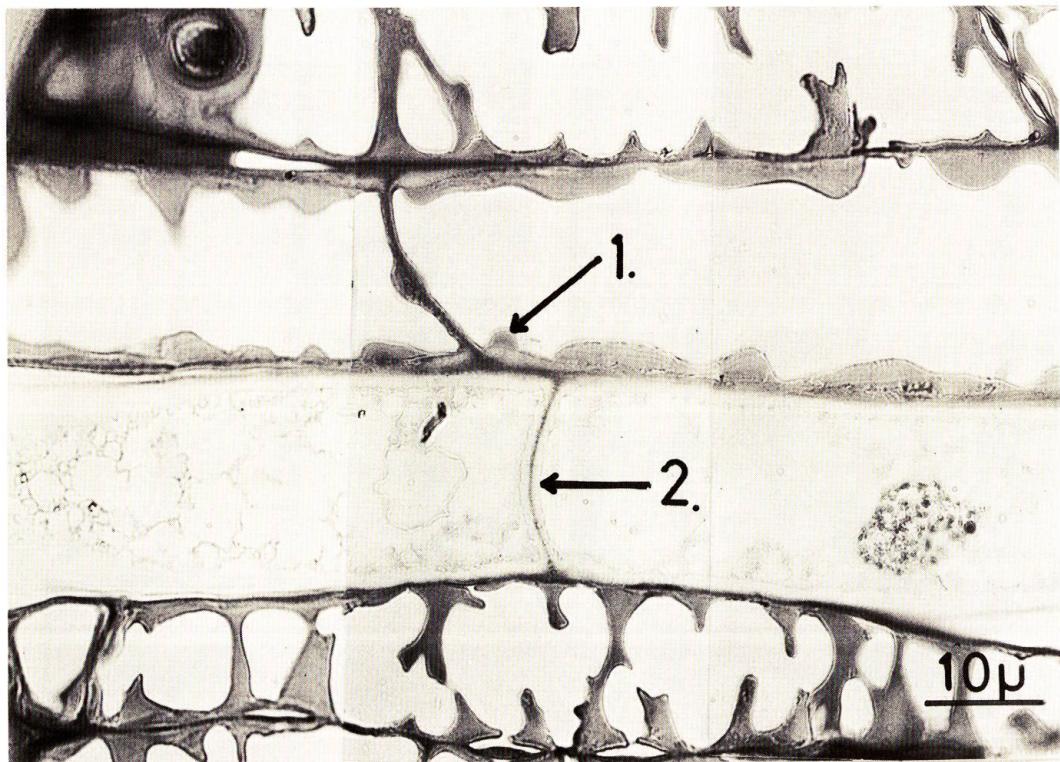


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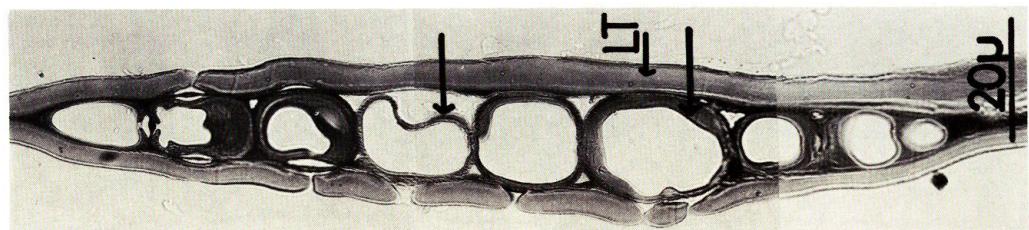


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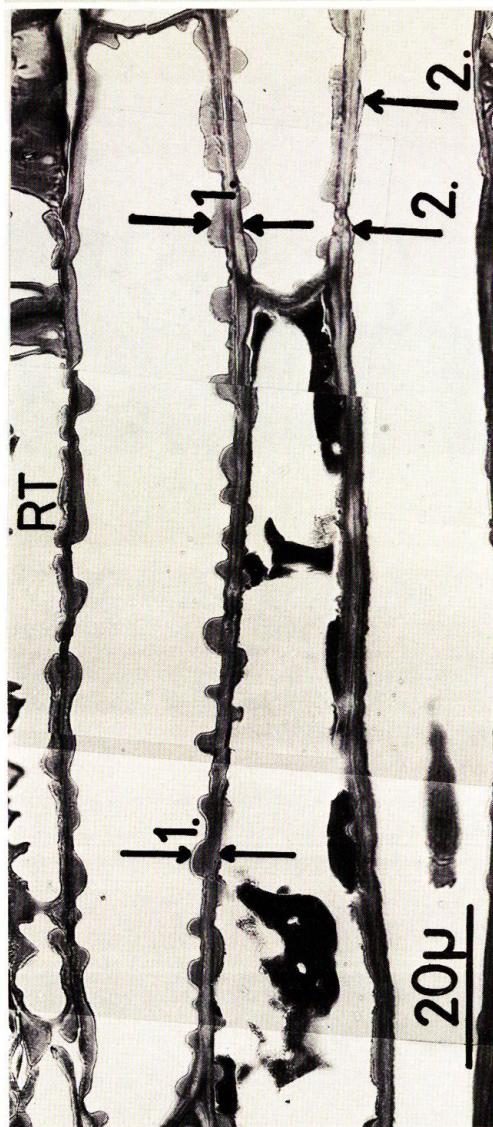


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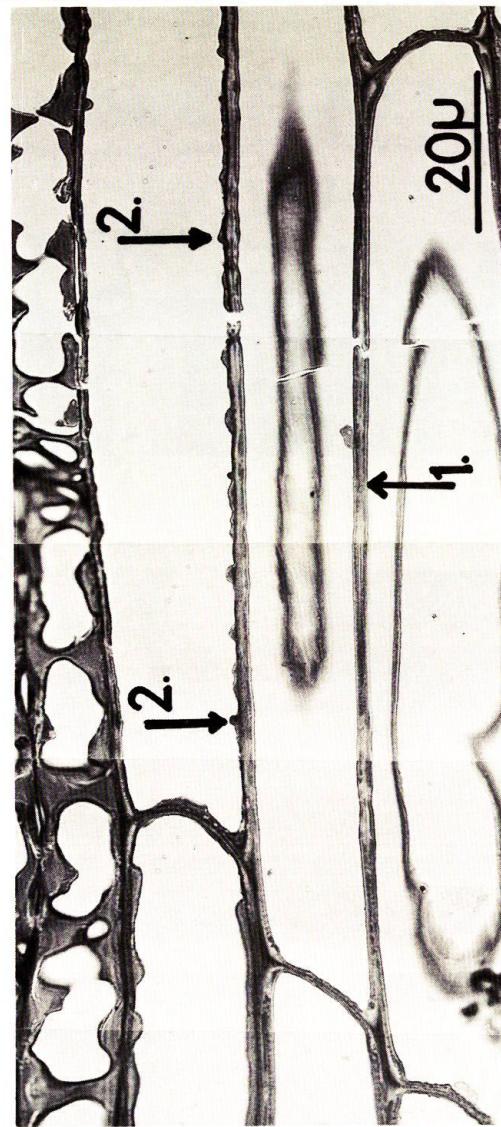


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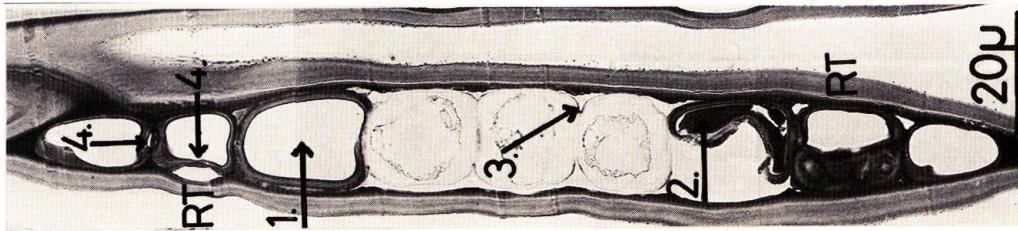


Photo 21



Photo 25

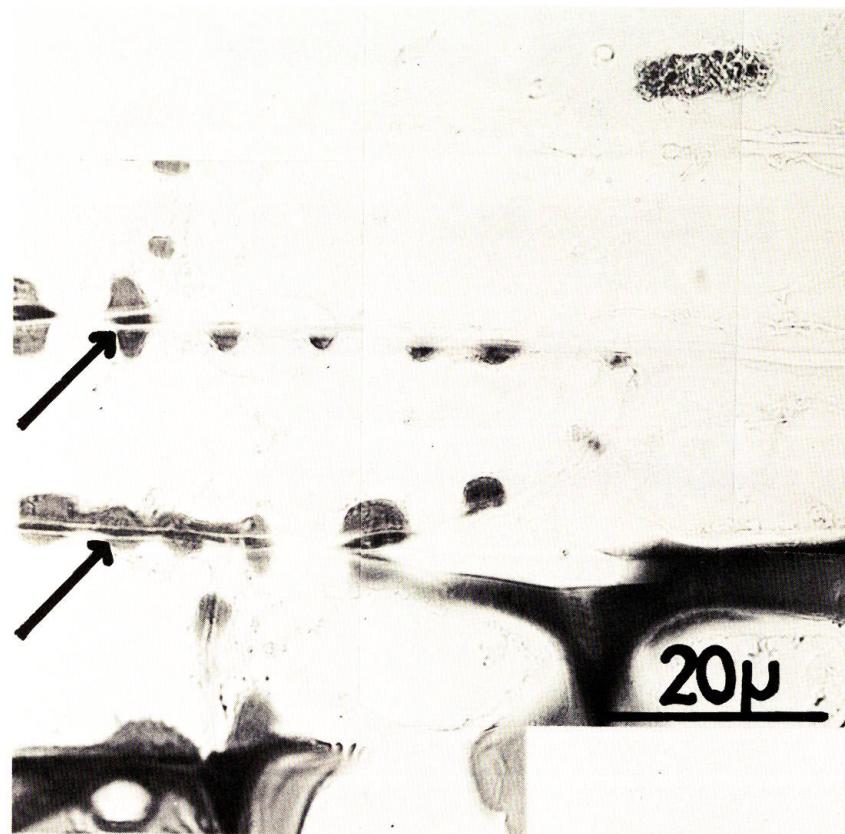


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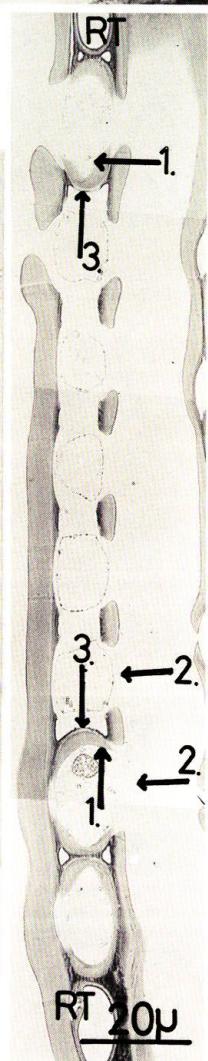


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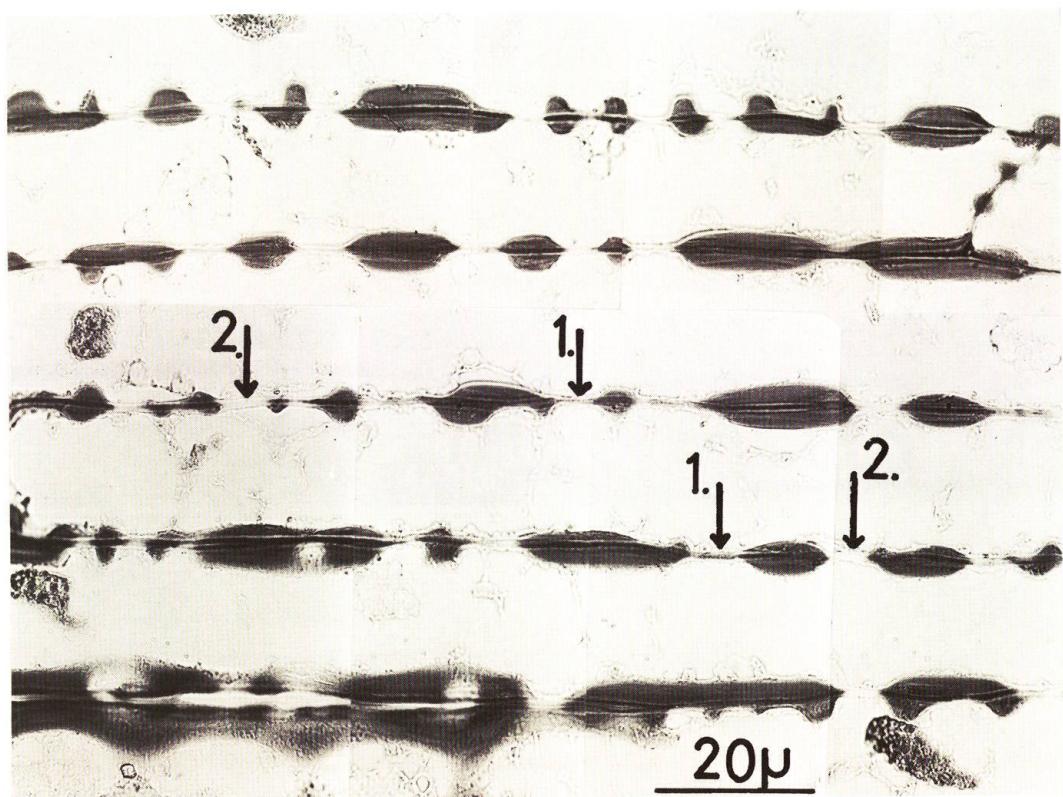


Photo 29

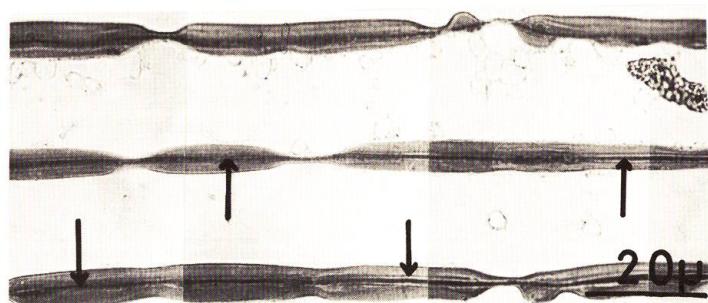


Photo 30



Photo 31

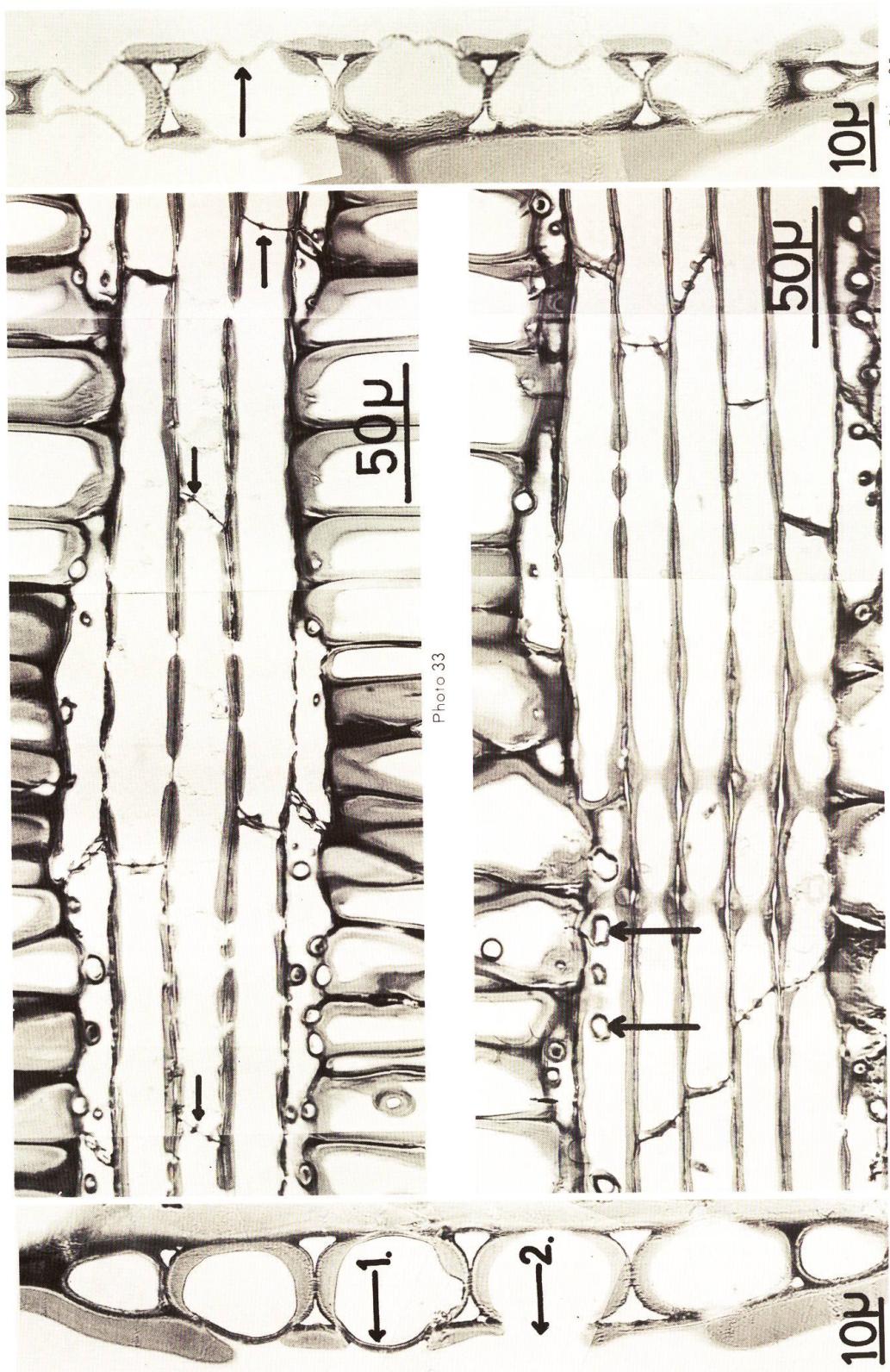


Photo 32

Photo 33

Photo 34

10μ

10μ

50μ

10μ