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

Report 2. The Season of Cell Wall Thickening and Lignification in *Pinus strobus**

By

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マツ属放射柔細胞壁の発達に関する研究 (第2報)
ストローブマツの壁肥厚と木化の季節*

山本幸一** 深沢和三** 石田茂雄**

CONTENTS

Introduction

The variabilities in the maturing process of the ray parenchyma cells of Genus *Pinus* have been dealt with in many papers, concerning partly relationship between aging of the ray and the heartwood formation, and partly contributions to the classification of Genus *Pinus*. Thickening and lignification of the ray parenchyma cell walls in Diploxylon pines occur somewhere in the sapwood in certain species of the genus, and mainly in the sapwood/heartwood transition in the others[1),5)]. Those of Haploxylon, on the other hand, are attained near the cambium in the same way as in many other coniferous genera. In both the subgenera, it has been

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reported that wall thickening and lignification of the parenchyma cells of radial resin canal tissue[2),8)] occur during the time of the heartwood formation[2),3),4)].

Seasonal studies of aging xylem in *Pinus radiata* demonstrated recently that ethylene production and respiratory activity increased in the sapwood/heartwood transition zone during the dormant season (SHAIN and MACKAY 1973; SHAIN and HILLIS 1973)[13),14)]. Secondary wall thickening and lignification of parenchyma cells in the radial resin canal tissue in Genus *Pinus* which occur for the first time in the sapwood/heartwood transition support this view on the position. This phenomenon occuring during the senescence of the radial resin canal parenchyma is considered as one of the important indications of heartwood formation.

In the previous report of the authors[15)], differences of the maturing process of the ray parenchyma cells were described as observed through the sapwood to heartwood in various species of Genus *Pinus*, using ultraviolet microscopy.

The objective of this investigation is (1) to make clear the seasonal development of the wall thickening and lignification in the parenchyma cells of uniseriate rays near the cambium and that of the radial resin canals in the sapwood/heartwood boundary in *Pinus strobus*, one of Haploxylon pines, and (2) to discuss relationship between maturation of the cell wall of the radial resin canal parenchyma and the heartwood formation in this species.

Materials and methods

A *Pinus strobus* tree planted in the Tomakomai Experiment Forest, Hokkaido University was selected to remove increment cores for this study. It was 49 years old, 20 m high and 37 cm in diameter at breast height, having an average ring width of 0.2 mm for the last 6 years. The width and the annual ring number of the sapwood was 9 mm and 12 years respectively. The increment cores were taken at breast height once in the middle of every month between May and November 1976. The cores were immediately fixed in F. A. A. or glutaraldehyde fixative, and embedded in methyl/buthyl methacrylates. Radial and tangential longitudinal sections of 0.5 μm thickness were cut with a diamond knife on an ultramicrotome. Observations of the sections were made mainly on those near the cambium and the sapwood/heartwood boundary under an ultraviolet microscope (Carl Zeiss, West Germany, Type MPM-01) at a wavelength of 280 milli-microns.

In addition to UV microscope observation, the radial longitudinal surfaces coated with gold were observed with a scanning electron microscope (JSM-2) at 15 kV of acceleration, to examine the form of the cell wall in greater detail.

Results

1) Uniseriate ray

On the samples of May and June, the newly formed xylem in this year was not found yet (Photo 1). The boundary line between the unlignified immature ray parenchyma cells and the lignified mature parenchyma cells, both formed in the last year, was shown as an uneven line in the radial longitudinal section (Photo 1).

In July, the earlywood formation was in progress. Newly formed parenchyma cells in this year had thin unlignified walls. These immature parenchyma cells were distinguished clearly from the mature cells formed in the last year. In August, latewood formation started. Newly formed parenchyma cell walls still remained thin and unlignified (Photo 2).

In September, cell division in the cambium nearly ended. Various developmental stages of walls were found in the ray parenchyma cells formed in this year; namely 1) thickened and lignified walls, 2) thickening and lignifying walls, 3) thickening and unlignified walls, 4) unthickened and unlignified walls (Photos 3, 4 a and 4 b). Any part of the cell wall of an individual ray parenchyma was in an even developmental stage. The adjacent cells occasionally showed rather different maturing stages from each other, suggesting that the maturation of the ray parenchyma cells tended to occur independently (Photo 4 b). Some double walls were not symmetrical in their secondary wall, as the wall thickening occurred in one wall but not in the other (Photo 4 b). Within a ray, the secondary wall thickening and lignification occurred first in the ray parenchyma cells next to the mature parenchyma cells of the previous year and then proceeded to the newer cells, i. e., towards the cambium (Photo 3). Maturation of ray parenchyma cells connected to a vertical resin canal was delayed (Photo 4 a). In October, the parenchyma cells were somewhat in an advanced stage of maturation as compared with those of September's sample. In November, maturation of the ray parenchyma cells had completed except for a few cells in the outer-latewood which had unthickened and unlighified walls (Photo 5). It seemed that these parenchyma cells near the cambium passed the winter in an immature stage and matured in September of the next season.

The secondary wall of the ray parenchyma cells in the inner-latewood formed during the last stage of maturation revealed rough features in contrast with the smooth wall of earlywood parenchyma (Photo 5). It was comfirmed that the rough features seen as alternating projection and depression in radial longitudinal sections, reported in the previous paper by the authors, were the simple pits by scanning electron microscopy (Photo 6).

2) Radial resin canal tissue

Fusiform rays of this species consisted of epitherial cells, "sheath-like cells", ray parenchyma cells, and ray tracheids. In this paper, we use the term "sheath-like cell" to the thin-walled lignified dead cell which made distinctions between the epitherial cell and the surrounding ray parenchyma cell. The epitherial cell, the "sheath-like cell", and the ray parenchyma cell of the multiseriate portion corresponded to epitherial cell, intermediate cell, and outer cell in the report of Kibblewhite and Thompson respectively[8]. The ray parenchyma cells were distinguished between multiseriate portion and uniseriate portion according to the difference of the maturing process in the secondary wall, as well as to their position. Multiseriate parenchyma cells and epitherial cells had thin unlignified walls throughout the sapwood, and then formed the secondary walls abruptly when they approached the sapwood/heartwood boundary. The progress of the secondary wall formation

was judged by that these cells were found to be in the intermediate stages of the wall thickening and lignification.

In the samples of May and June, it seemed that the formation of the secondary wall should not begin because the intermediate stages of wall development were scarecely observed (Photos 7 a and 7 b), though few parenchyma cells showed the intermediate stages, probably remaining from the previous year. Most parenchyma cells, which finished maturation of the secondary wall in the previous year, were dead. In July, August, and September, the parenchyma cells showing the intermediate stages of wall thickening and lignification were found in abundance between the lignified dead cells of the "heartwood tissue" and the unlignified living cells of the "sapwood tissue" (Photos 8, 9, 10 a and 10 b). The time of vigorous secondary wall formation seemed to be August, as photo 10 b showed many thickened unlignified walls compared with the other months' photographs. Lignification of the secondary wall was nearly complete in September, but pit membranes were not lignified yet (Photo 9). The parenchyma cells were living for some time after the completion of secondary wall formation. It was not clear how many cells completed their maturation in the radial resin canal tissue within one year, because of a difficulty of confirmation of initiation and end of the secondary wall formation, and besides a difficulty of preparation of the radial longitudinal sections containing radial resin canals. In October, the parenchyma cells showed little sign of the intermediate stages of wall thickening and lignification (Photo 11). These cells ended their secondary wall formation and showed UV-absorption in the pit membranes which had remained in unlignified until August or September. In November, the parenchyma cells did not show the intermediate stages, and matured cells were dead (Photo 12). Immature unlignified living cells directly adjoined to the lignified dead cells. From the seasonal observation with UV microscope, it was presumed that thickening and lignification of the secondary wall in the parenchyma cells of the radial resin canal tissue took place during July to September in the sapwood/heartwood boundary, and that necrosis of these cells and UV-absorption of the pit membranes were observed from September to November.

It was suggested from photo 12 in November that most cells of resin canal tissue died within the same year as the occurrence of the wall thickening and lignification. But it was not clear when uniseriate rays died. The boundary line between living cells and dead cells in radial resin canals was situated about one annual ring outside than that in uniseriate rays from the cambium; it laid in about 11 rings from the cambium in the resin canals, and 12 rings in the uniseriate rays.

Discussion and conclusions

Wall thickening and lignification of uniseriate rays in *P. strobus* started in the parenchyma cells adjacent to previous year's matured parenchyma and then proceeded towards the cambium (Photo 3). Maturation of those cells occurred from September to October, corresponding to Mann's result[9]. The sections of September

showed the most vigorous stage of the wall development in this species of tree (Photos 3, 4 a and 4 b). The greater part of the parenchyma cells were complete in maturation within the year when they were formed. But a few cells near the cambium remained immature and they matured in September of the next year. Thus, the partial parenchyma cells completed their maturation in the next growth season. Details of the transition of maturation within an annual ring were not made clear because of the markedly narrow width of the newly formed ring examined in this study. These results may be somewhat different from those of normal growth trees.

Thickening and lignification of secondary walls in parenchyma cells of radial resin canal tissue took place mainly from July to September in the sapwood/heartwood boundary, and then most of the parenchyma cells died mainly during the time from September to November in the same year, judging from UV-absorption in the pit membranes. The pit membranes were encrusted by polyphenols, and additionally lignification might occur[5]. BAMBER (1972, 1973, 1976)[2),3),4)] observed that secondary wall formation in the radial resin canal parenchyma cells of Haploxylon pine occurred during heartwood formation. They did not refer to the season of the secondary wall formation. There was a short time lag between the secondary wall formation and the occurrence of UV-absorption in the pit membranes. HIRAI (1951)[6)] and NOBUCHI et al. (1977)[12)] studied the seasons of additional formation of heartwood from a point of darkening of the tissue by the use of increment cores. HIRAI reported that the inmost side of sapwood was transformed into the outermost of heartwood from July to November in the Japanese larch, but July was the failing season of cambium activity and November was the beginning season of the hibernation of the cambium in the Tomakomai district, Hokkaido. NOBUCHI et al. stated that the heartwood transition occurred from July to September in *Cryptomeria japonica* in the Kyoto district. ISHIDA et al. (1976)[7)] studied seasonal development of tyloses in *Robinia pseudo-acacia* in Tomakomai. They found that the tyloses in the earlywood began to form in August and ended at least their surface growth within September in the annual ring next to the latest. NAKAGAWA et al. (1976)[10)] described that occurrence of the tyloses in *R. pseudo-acacia* was recognized in the latest ring from September to October when it was in the late stage of tree growth season. The season of maturation in the radial resin canal tissue almost agreed with that of the heartwood formation and tyloses development stated above. It must be noticed here that encrusting of the pit membranes in both uniseriate rays and fusiform rays would be generally one of the significant indications of heartwood formation.

SHAIN et al. (1973)[13),14)], however, suggested that heartwood formation took place mainly in the dormant season by measurement of ethylene production and respiratory activity in the xylem tissue of *Pinus radiata* planted in Australia. The present authors were confronted by the problem whether heartwood formation occurred in about the time of latewood formation or mainly in the dormant season. This disagreement may be due to differences of climates, especially temperature

of the winter, and differences of the tree species and tree ages, and the time lag between the increase of these physiological activities and the occurrence of wall thickening and lignification.

Parenchyma cells of the radial resin canals died earlier by about one year than those of uniseriate rays, in the sapwood/heartwood boundary in the present observation. NOBUCHI et al. (1976)[11] suggested that lipid droplets in the epitherial cells degenerated faster than the ray parenchyma cells in the intermediate wood of *Pinus densiflora*. This time lag was an interesting problem.

Lignification slightly lagged behind secondary wall thickening in both uniseriate rays and resin canal tissue.

UV-absorption was not found yet in window-like, simple, and blind pit membranes when secondary wall formation of resin canal tissue had already completed (Photo 9). UV-absorption in the pit membranes was presumed to occur while these parenchyma cells died.

The detailed process of secondary wall thickening and lignification or a transition of protoplasm should be made clear using a combination of UV and electron microscopy. Enzyme histochemistry of peroxidase and autoradiography of lignin precursors within individual cells in consideration of the season and the position of heartwood formation are a promising area for a further study on heartwood formation.

References

1) BALATINECZ, J. J. and R. V. KENNEDY: "Maturation of ray parenchyma cells in pine." For. Prod. Jour., **17**, 57–64 (1967).
2) BAMBER, R. K.: "Properties of the cell walls of the resin canal tissue of the sapwood and heartwood of *pinus lambertiana* and *Pinus radiata*." Jour. Inst. Wood Sci., **6**, 32–35 (1972).
3) BAMBER, R. K.: "The formation and permeability of interstitial spaces in the sapwood of some *pinus* species." Jour. Inst. Wood Sci., **6**, 36–38 (1973).
4) BAMBER, R. K.: "The occurrence of secondary walls in the resin canal tissue in the genus *pinus*." Jour. Inst. Wood Sci., **7**, 15–17 (1976).
5) BAUCH, J., W. SCHWEERS and H. BERNDT: "Lignification during heartwood formation: Comparative study of rays and bordered pit membranes in coniferous woods." Holzforschung, **28**, 86–91 (1974).
6) HIRAI, S.: "Study on the process of heartwood-growth in the Japanese Larch stem." Trans. 59th Meet. Jap. Forest. Soc., 231–234 (1951).
7) ISHIDA, S., J. OHTANI and T. KAWARADA: "Study of tyloses by the scanning electron microscopy (report 2). Yearly and seasonal development of tyloses in Harienju, *Rabinia pseudo-acacia*." Trans. 8th Meet. Hokkaido Branch Jap. Wood Res. Soc., 6–9 (1976).
8) KIBBLEWHITE, R. P. and N. S. THOMPSON: "The ultrastructure of the middle lamella region in resin canal tissue isolated from Slash Pine holocellulose." Wood Sci. Technol., **7**, 112–126 (1973).
9) MANN, R. T.: "Ray parenchyma cell wall ultrastructure and formation in *Pinus banksiana* and *Pinus strobus*." Dissertation Abs. Int., **33**, 1890–B (1972).
10) NAKAGAWA, K., M. FUJITA and H. HARADA: Trans. 26th Meet. Jap. Wood Res. Soc., 290 (1977).

11) NOBUCHI, T., Y. KAMIZONO and H. HARADA: "Cytological changes of parenchyma cells associated with heartwood formation —On three soft wood species, Sugi, Momi and Akamatsu—." Bull. Kyotó Univ. Forests, Kyoto Univ., **48**, 178–186 (1976).
12) NOBUCHI, T., M. NAKAGAMI and H. HARADA: Trans. 27th Meet. Jap. Wood Res. Soc., 306 (1977).
13) SHAIN, L. and J. F. G. MACKAY: "Seasonal fluctuation in respiration of aging xylem in relation to heartwood formation in *Pinus radiata*." Can. J. Bot., **51**, 737–741 (1973).
14) SHAIN, L. and W. E. HILLIS: "Ethylene production in xylem of *Pinus radiata* in relation to heartwood formation." Can. J. Bot., **51**, 1331–1335 (1973).
15) YAMAMOTO, K., K. FUKAZAWA and S. ISHIDA: "Study on the cell wall development of ray parenchyma in Genus *Pinus* using ultraviolet microscopy." Res. Bull. Coll. Exp. Forests, Hokkaido Univ., **34**, 79–96 (1977).

要　　約

マツ属の放射柔細胞の成熟過程は樹種により多様な変化を示し，放射組織と心材化との関係やマツ属分類等の点から関心が持たれ，多くの研究がなされている。Diploxylon pine の放射柔細胞はおおむね心材化の時点で壁肥厚と木化を完了する。それに対して，Haploxylon pine のそれは他の多くの針葉樹と同じく形成層附近で完了するが，このグループの水平樹脂道組織の柔細胞は心材化の時点で2次壁肥厚と木化を完了することが知られている。

本研究の目的は，Haploxylon pine の一樹種であるストローブマツ (*Pinus strobus* LINN.) を用い，形成層附近での単列放射組織と，辺心材境界での水平樹脂道組織の細胞壁の成熟過程の季節的な経過を明らかにし，更に水平樹脂道の成熟と心材化の関係を考察する事である。

試料は5月から11月まで毎月中旬に1回ずつ生立木の胸高部より生長錐でコアを採取し，メタクリレート包埋を行い，主に紫外線顕微鏡 (280 mμ 波長) を用いて柾目面切片の観察を行った。

得られた結果を要約すると次の通りである。

1) 単列放射組織の成熟　単列放射組織の成熟は形成層附近で完了する。5月から8月の試料では，当年度形成された柔細胞は薄い壁で未木化のままである (Photo 1, 2)。そして，供試木生育期の最終段階とみなされる9月から10月にかけて，前年度形成され未成熟のまま残った一部の晩材柔細胞と当年度形成された柔細胞に，はじめて2次壁形成と木化が見られる (Photo 3, 4 a, 4 b)。2次壁形成と木化は，未成熟のまま残った前年の柔細胞から始まり形成層に向かって進行する。11月の試料では，ほとんどの細胞は成熟を完了しているが，形成層に近い一部の柔細胞は未成熟のまま残されており翌年の秋に成熟を完了する (Photo 5)。柾目面切片でコブ状に見える肥厚は，SEM 観察により単壁孔であることがわかった (Photo 6)。

2) 水平樹脂道組織の成熟　樹脂道組織のエピセリウム細胞と多列部の放射柔細胞は辺心材境界で成熟を完了し，その季節は主に7月から9月の間である。すなわち，5月，6月，10月，11月の試料では肥厚し木化した柔細胞と薄い壁で未木化のままの未成熟の柔細胞が隣り合っ

ており，2次壁形成の途中段階の細胞はほとんど見られない (Photo 7 a, 7 b, 11, 12)。これに対し，7月から9月の試料では，壁肥厚と木化の途中段階にある柔細胞が数多く見られる (Photo 8, 9, 10 a, 10 b)。

心材化の一つの大きな現われであると思われる水平樹脂道組織の辺心材境界での成熟の季節は，心材化に伴う材の着色という観点から得られた針葉樹の心材化の季節や広葉樹におけるチロース形成の季節とほぼ一致した。しかし，呼吸活性やエチレン生合成の変化などの観点から得られた心材化の季節（おもに休止期におこる）とは異なっている。

心材化の経過や機構については不明な点がいまだ数多く存在する。今後，UV 顕と電顕を併用した壁形成と木化の観察，パーオキシダーゼの酵素組織化学，あるいはリグニン前駆物質取り込みのオートラジオグラフィー等，辺心材境界での柔細胞ごとの季節的な研究を更に行ってゆくことが必要である。

Explanation of photographs

Note: All the microphotographs except photo 6 were taken by an ultraviolet microscope at a wavelength of 280 mμ. Stem axis of the sample tree is vertical in all the photographs. Left side of all the photographs of the radial longitudinal sections is outside direction of the tree.

Photos. 1–6 Uniseriate ray

Photo 1. May 10th. The latewood of the last year is directly adjacent to cambium. Left side is phloem. Boundary line between lignified and unlignified parenchyma cells is uneven.

Photo 2. August 16th. The boundary line lies near the annual ring boundary. Newly formed parenchyma cells are not lignified yet.

Photo 3. September 10th. Secondary wall formation is found in many parenchyma cells formed in this year. UV-absorption on the secondary wall of some newer parenchyma cells is weak compared with the other mature cells, showing a lignification in progress.

Photo 4 a. September 10th. Ray connected to a vertical resin canal. Previous year's parenchyma cells connected to a vertical resin canal remain unthickened, unlignified in their walls. Only newly formed parenchyma cells near the previous year's annual ring boundary are lignified (arrow).

Photo 4 b. An enlarged view of a part of the same ray as photo 4 a. Various developmental stages of walls are found, i.e., thickened and lignified wall (arrow 1), thickening and lignifing wall (arrow 2), thickening and unlignified wall (arrow 3), unthickened and unlignified wall (arrow 4).

Photo 5. November 11th. Parenchyma cells in the outer-latewood are unthickened and unlignified (arrow 1), and those in the inner-latewood have rough secondary wall (arrow 2). This photograph shows the same stage of wall development as that of May.

Photo 6. A scanning electron micrograph of a radial surface in the inner-latewood shows the horizontal walls of ray parenchyma cells with many simple pits which are found as a rough surface of wall in the radial sections under the light microscope.

Photos 7 a–12 Radial resin canal tissue

Photo 7 a. June 16th. Boundary line between lignified and unlignified cells is uneven. This photograph shows the same stage of wall development as that of October and November. One or two parenchyma cells show intermediate stages of wall development (arrow 1), which remain from the previous year. Though lignified epitherial cells are dead without exception (arrow 2), lignified ray parenchyma cells of the multiseriate portion are dead or partly still living (arrow 3), and those of the uniseriate portion are still living (arrow 4).

Photo 7 b. An enlarged view of a part of photo 7 a. Dead parenchyma cells indicate strong UV-absorption on the pit membranes (arrow 1), and living cells having secondary wall do not show yet UV-absorption on the pit membranes (arrow 2). Unlignified cells next to lignified cells have thin walls (arrow 3).

Photo 8. July 12th. Tangential section shows clearly epitherial cells (EP), "sheath-like cells" (SL) and ray parenchyma cells (RP). Secondary wall of parenchyma cells of the multiseriate portion shows weak UV-absorption compared with that of the uniseriate portion, suggesting that wall thickening and lignification are in progress.

Photo 9. September 10th. Tangential section shows that parenchyma cells which have completed lignification of secondary wall, indicate UV-absorption on the pit membranes (arrow 1) or none of it (arrow 2). The former cells are dead and the latter still living.

Photo 10 a. August 16th. Secondary wall formation takes place extensively. Many thickened unlignified parenchyma cells are found (arrow 1), and lignified cells are still living in this stage (arrow 2).

Photo 10 b. An enlarged view of a part of photo 10 a. Various developmential stages of walls are found same as photo 4 b of the uniseriate ray.

Photo 11. October 1st. Though some lignified parenchyma cells adjacent to unlignified immature cells are yet alive (arrow 1), most cells are dead and show strong UV-absorption in the primary wall (arrow 2).

Photo 12. November 11th. All the unlignified parenchyma cells next to lignified dead cells are living, and remain thin wall (arrow 1). All the lignified matured cells of multiseriate portion are dead (arrow 2).

Photo 1

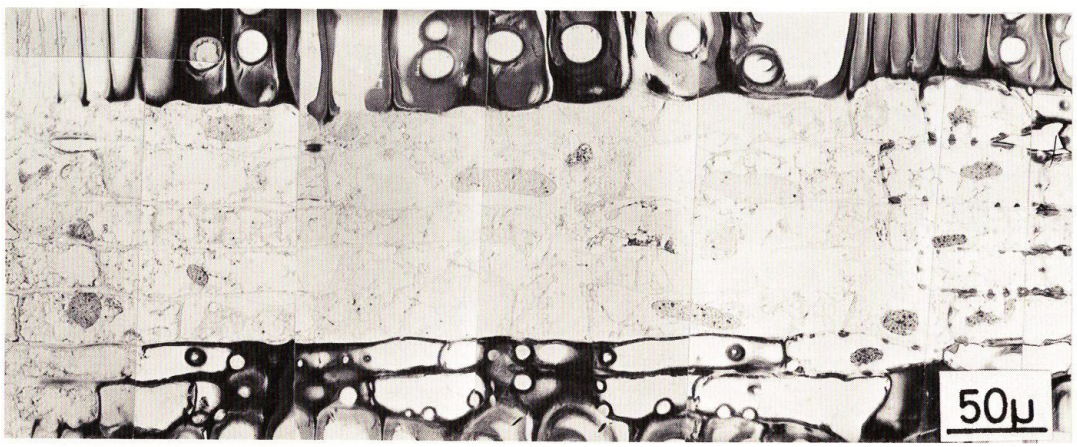
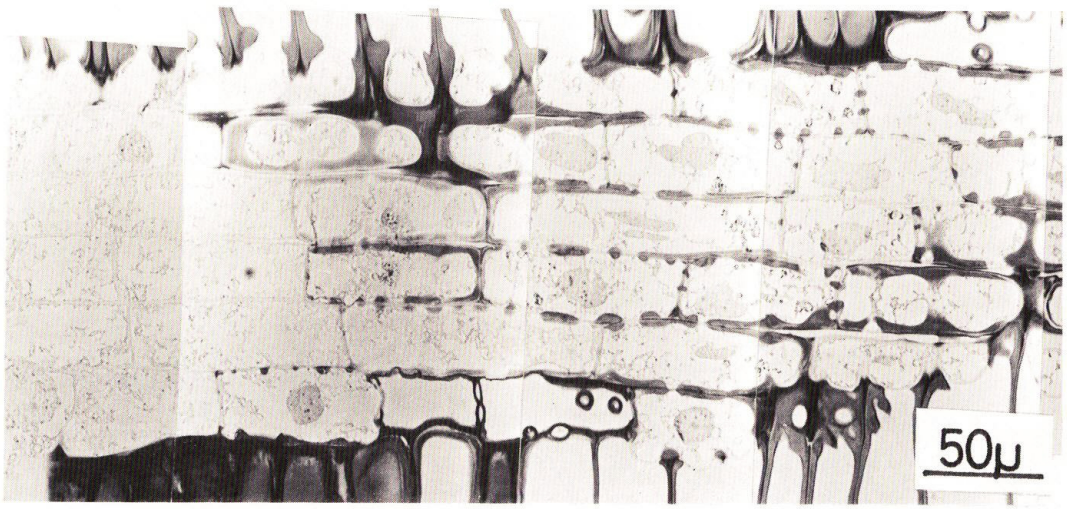


Photo 2



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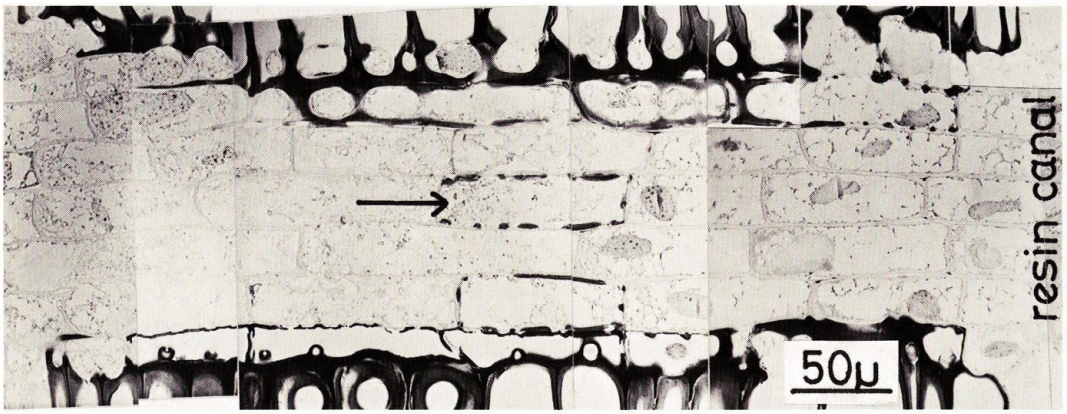

Photo 4-a

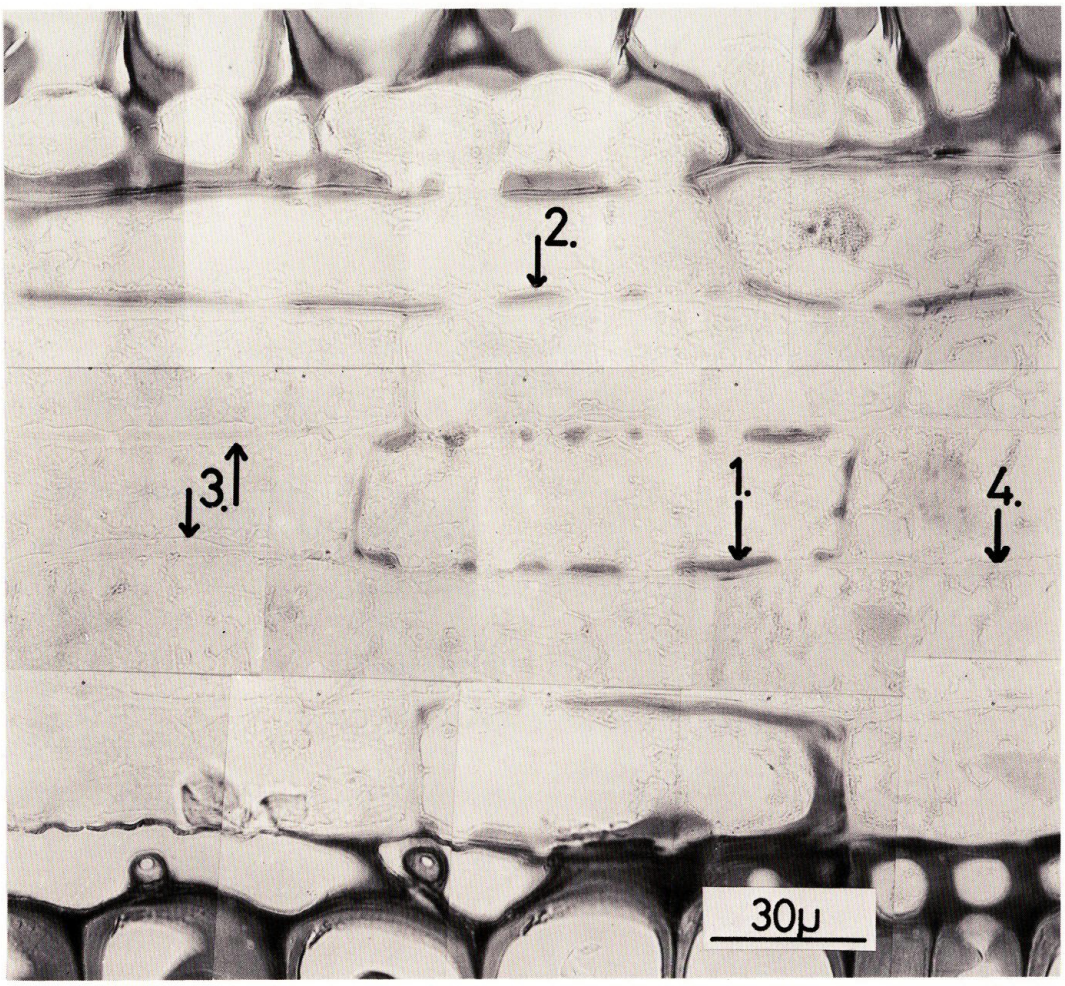

Photo 4-b

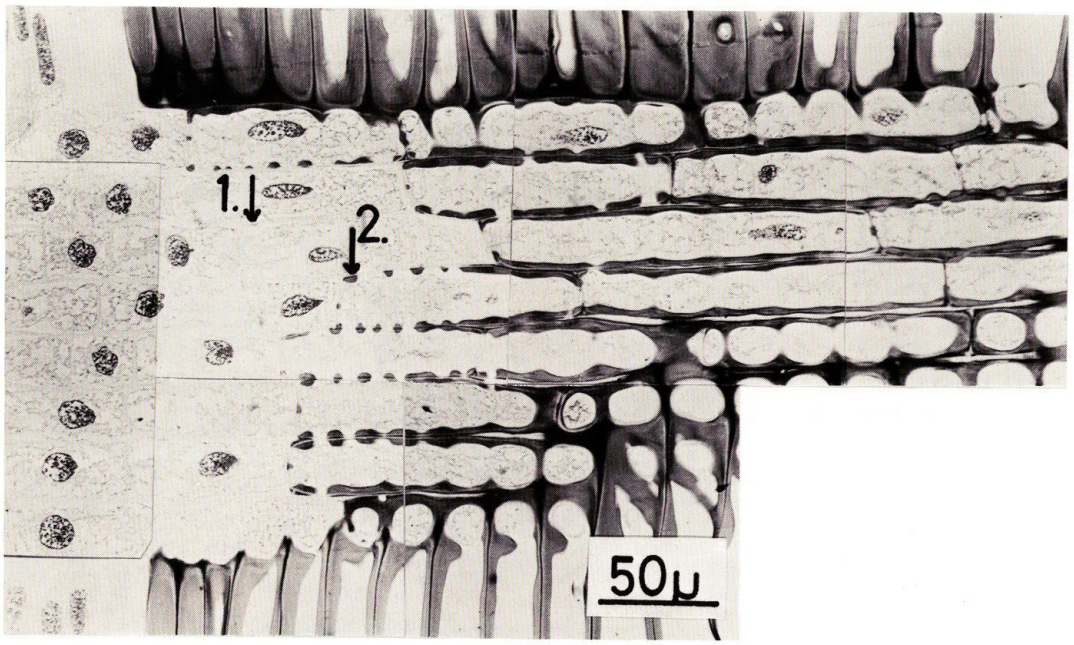

Photo 5

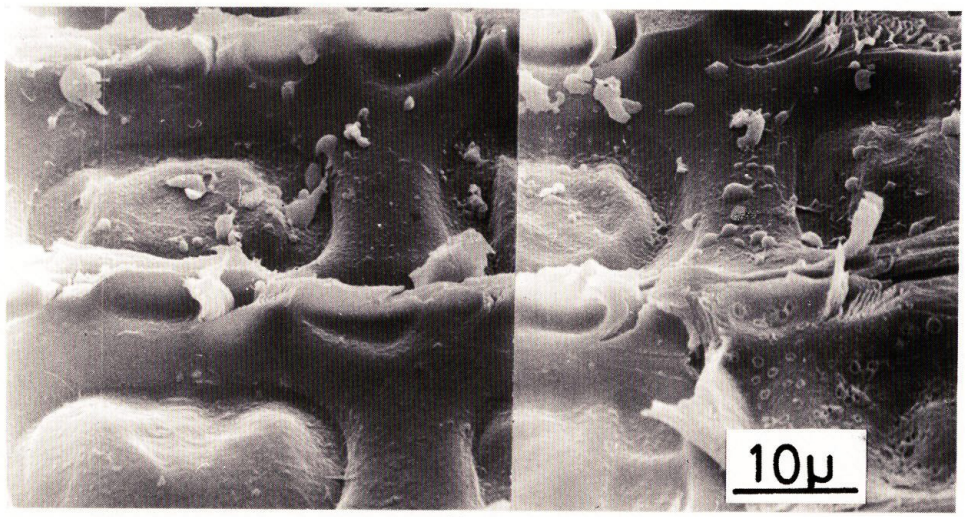

Photo 6

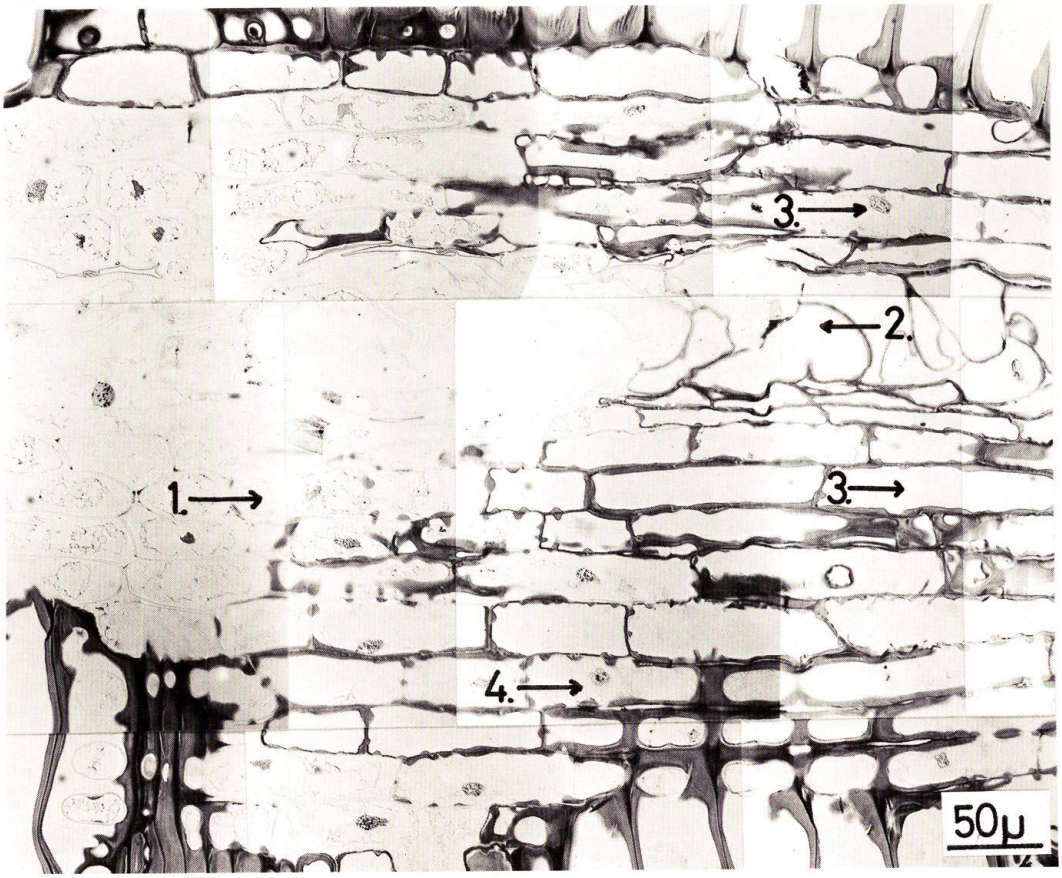

Photo 7-a

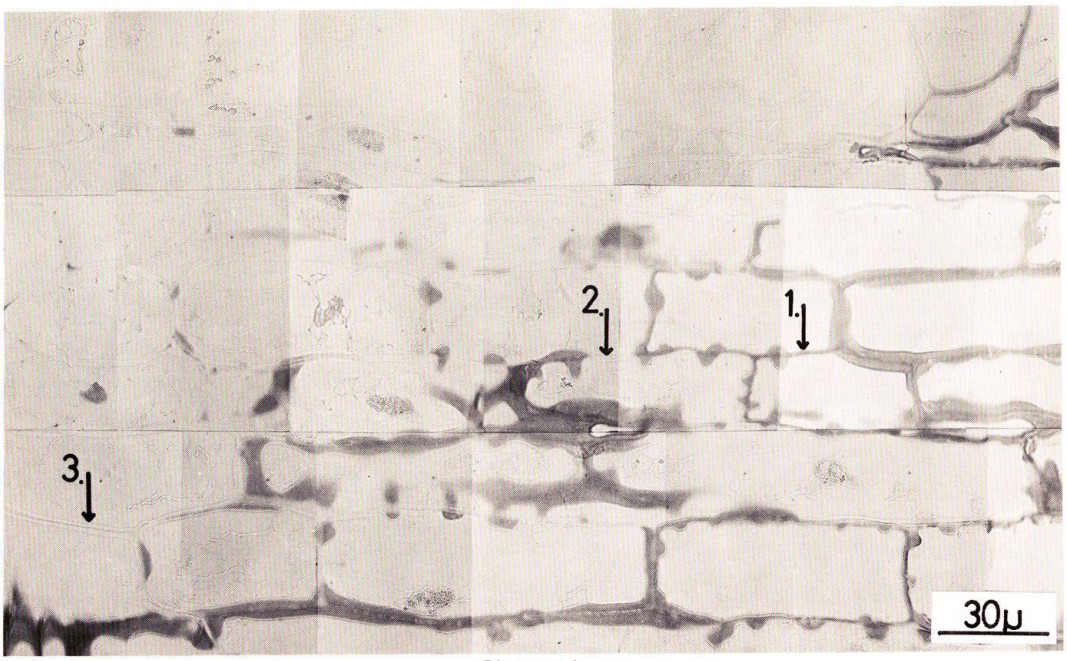

Photo 7-b

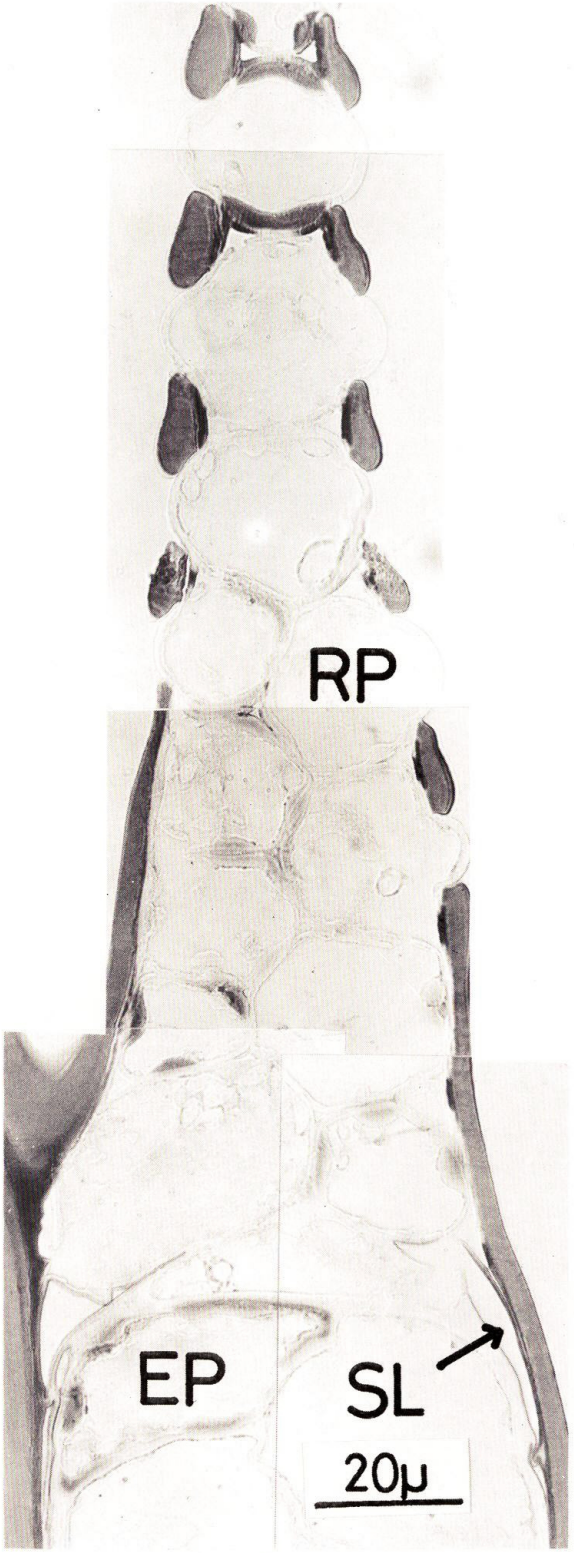

Photo 8

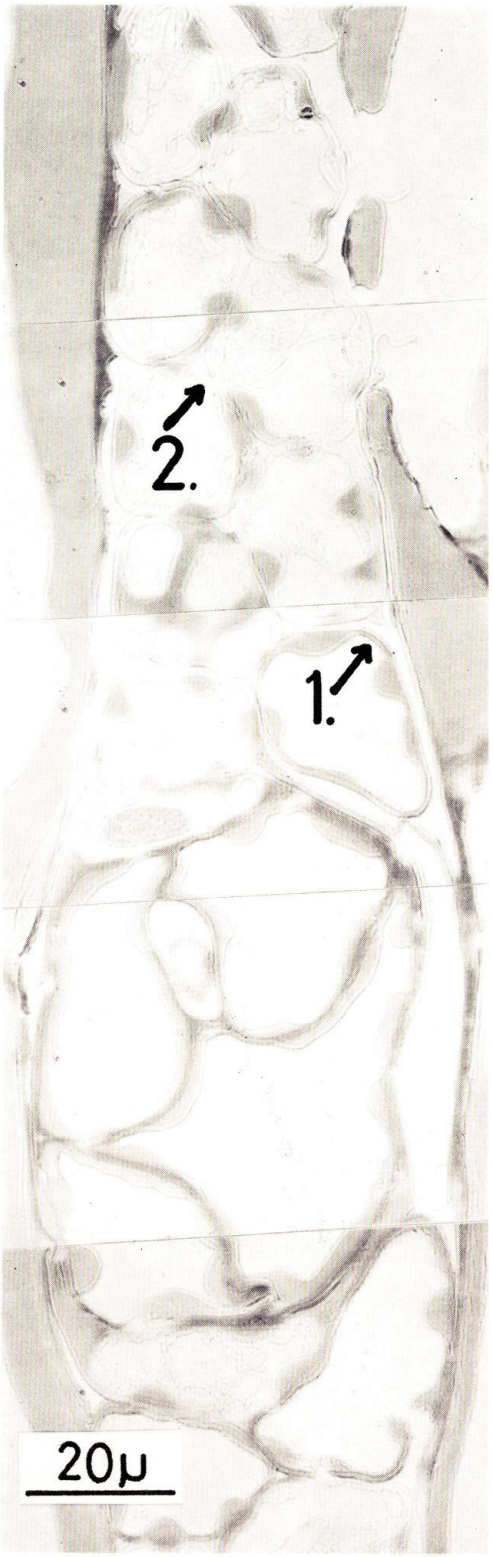

Photo 9

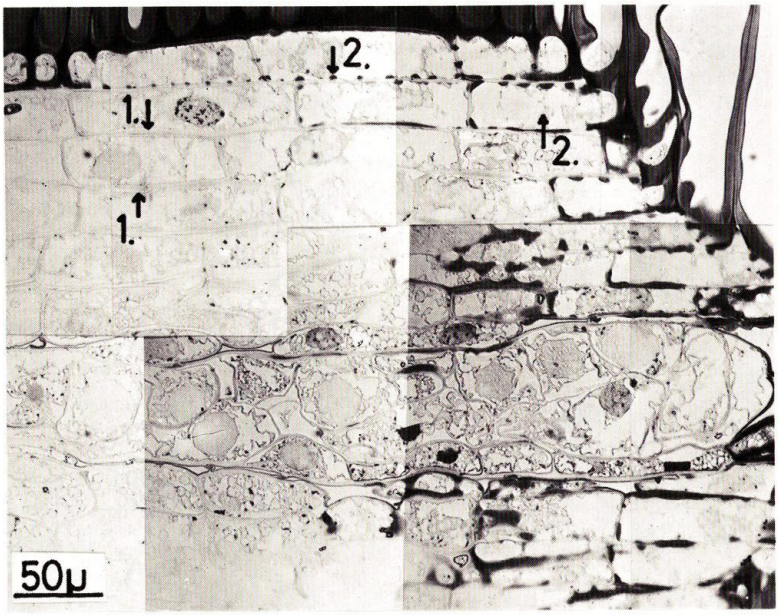

Photo 10-a

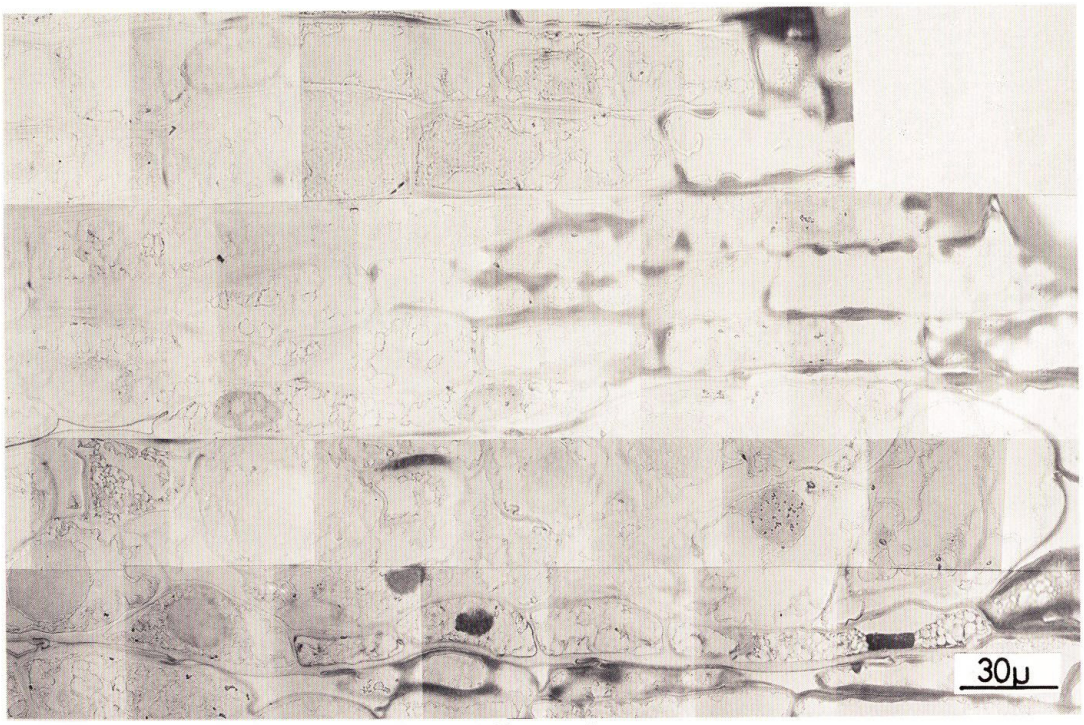

Photo 10-b

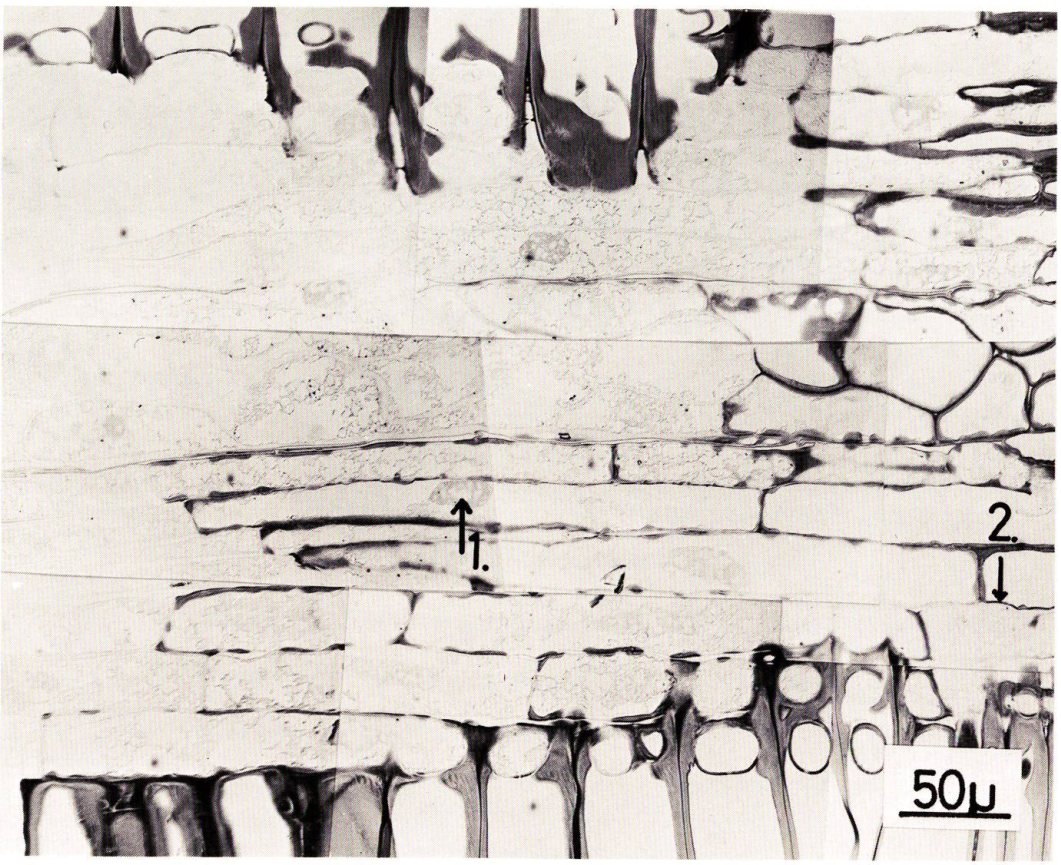

Photo 11

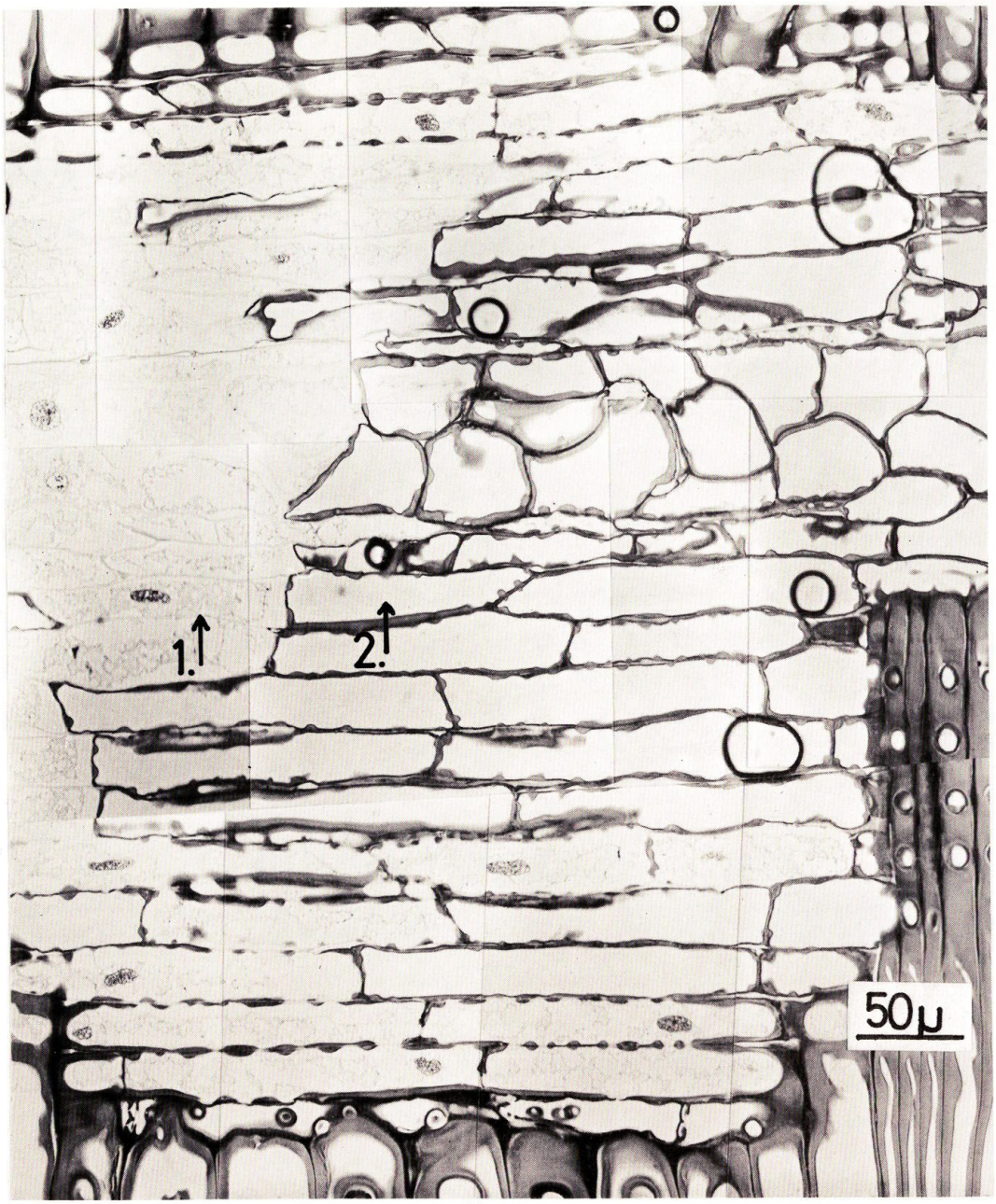

Photo 12