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

Report 3. Seasonal Change of Cell Wall Thickening and Lignification in *Pinus banksiana**

By

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マツ属放射柔細胞の発達に関する研究 (第3報)

バンクスマツの壁肥厚と木化の季節変化

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CONTENTS

Introduction

Maturing process of ray parenchyma cells of Genus *Pinus* varies with Haploxylon, Diploxylon having pinoid pits and Diploxylon having window-like pits in a cross-field.[1,2,23,24,] The ray parenchyma cells of Diploxylon pine having pinoid pits are subdivided into two types, thick-walled and thin-walled ones[5,8,13,14,16,23] according to difference of maturing process. One type of parenchyma has thin and unlignified wall through the sapwood, and then is lignified without wall thickening in the sapwood/heartwood boundary. The other type of parenchyma has thin and unlignified wall in the outer sapwood, but it is then thickened and lignified, and

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soon dies in the inner sapwood.

The maturing process of ray parenchyma cells with aging is revealed from the cambium to the heartwood, but their seasonal development remains unexplained. In the previous paper[24], the seasonal development of cell wall thickening and lignification was observed considering the difference between uniseriate rays and radial resin canal tissue in *Pinus strobus*, one of Haploxylon pines.

The object of the present study is to investigate the seasonal development of the ray parenchyma cells in addition to the aging effect in *P. banksiana*, one of Diploxylon pines, having pinoid pits, using ultraviolet microscopy.

Materials and methods

A sample tree was selected to remove increment cores for this study from a plantation in Hokkaido University College Experiment Forest, Tomakomai. It was 58 years old, 16 m high, and 25 cm in diameter at breast height. Width and number of the rings of the sapwood were about 50 mm and 25 rings or so, respectively. The increment cores were obtained at breast height once in the middle of every month from May to 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 sections were made under an ultraviolet microscope at a wavelength of 280 nm (band width : 5 nm).

Results

1. Uniseriate rays

In about the thick-walled parenchyma cells, the existence of ray parenchyma cells being in the middle stages of wall thickening and lignification was investigated over the samples of every month to know when secondary wall formation occurred. Progressing of the secondary wall formation was judged from whether these cells were on the middle stages or concrete stage of the wall thickening and lignification. Only in September, living parenchyma cells with thickening and lignifying walls were found in the inner sapwood, 25$\sim$46 mm inward from the cambium (Photo 1), but not in the sapwood/heartwood boundary, 50 mm from the cambium. On the other hand, the parenchyma cells of this kind were not observed in the other months (Photo 2). In other words, all thickened parenchyma cells in the sapwood had died without exception. Therefore it was suggested that thin-walled parenchyma cells (Photo 3) of *P. banksiana* were thickened and lignified in September, in the sequel they became thick-walled ones. And these thick-walled parenchyma died soon after the completion of secondary wall formation.

Remained thin-walled parenchyma cells in the sapwood (Photo 3), which showed UV-absorption first on the intercelluler layer of cell corners and then overall the primary wall (Photo 4) and finally died in the sapwood/heartwood boundary (Photo 5), were looked over on every month's sample. Many of them tended to be found in the latewood. In this case, it was difficult to take up the transition just from

the living parenchyma cell to dead one. Therefore, there was little indications to suggest in what seasons these thin-walled parenchyma cells were lignified and/or infiltrated with polyphenols and died.

Bordered pit aspiration and deposition of phenolic compounds that were related to the aging of ray parenchyma, were found in rather outer part from the sapwood/heartwood boundary (about 15 mm outside from the sapwood/heartwood boundary) (Photo 6).

2. Radial resin canal tissue

In the radial resin canal tissue, maturation of the uniseriate portion differed from the multiseriate portion, showing that the parenchyma cells of uniseriate portion took the same maturing process as the parenchyma of uniseriate rays. While parenchyma cells and epitherial cells of multiseriate portion were unthickened and unlignified through the sapwood (Photo 7). In the sapwood/heartwood boundary these unlignified cells in multiseriate portion died, indicating UV-absorption in their walls without secondary wall thickening (Photos 8 and 9).

In August and September, it was difficult to detect death of the parenchyma cells in the heartwood transition, although the many cells showed the middle stages of UV-absorption (Photo 8). A boundary between living and dead cells was fairly clear except in August and September, suggesting no change of living cell into dead one (Photo 9). Therefore, parenchyma cells of multiseriate portion, especially epitherial cells of radial resin canal tissue, would die suddenly in the sapwood/heartwood boundary between August and September.

Discussion and conclusions

The ray parenchyma cells in Haploxylon pines and Diploxylon pines having window-like pits die almost simultaneously in the sapwood/heartwood boundary. In *P. strobus* (Haploxylon) all ray parenchyma cells which had been thickened and lignified already near the cambium, die simultaneously in the heartwood transition. In *P. densiflora* (Diploxylon) ray parenchyma cells in the earlywood having thickened lignified walls through the sapwood and those in the latewood having thin and unlignified walls in the sapwood, also die almost together in the heartwood transition. However, in *P. banksiana* in the present report, thickened lignified dead parenchyma cells increase in their number gradually through the inner sapwood, and remained living cells die almost together in the heartwood transition. The ratio of dead parenchyma cells to living parenchyma cells was about 1 to 2 through the inner sapwood. Thickened parenchyma cells in *P. banksiana* were found not only in the position adjacent to ray tracheids but in the position remote from the ray tracheids.

This inner sapwood region may be reckoned as "intermediate wood" based upon the presence of some dead parenchyma and aspirated or incrusted bordered pits. The width of the "intermediate wood" in this sample tree amounted almost to half of the sapwood width.

Necrosis of the thick-walled parenchyma cells of *P. banksiana* took place

throughout the "intermediate wood" in September. This corresponds with the information which heartwood formation may occur during the final stage of tree growth season,[10,12] but not with the reports of HARRIS (1954)[9], SHAIN et al. (1973)[20,21], NELSON (1978)[17]. Harris reported that the transition zone of *P. radiata* became evident in late winter and disappeared in late spring or early summer. SHAIN et al. and NELSON stated that dormant season was a time of major heartwood formation in *P. radiata* monitoring ethylene production and respiratory activity, and in walnut and cherry monitoring ethylene production and phenol-oxidizing enzyme activities respectively. We suppose these variances are probably due to the difference of seasonal growing pattern or the presence of real dormancy. It is important to make clear effect of different provenances using same species.

On the other hand, it was not confirmed when remained thin-walled parenchyma died because the middle stages of their death were difficult to detect.

Aspiration and deposition of phenolic compounds in bordered pits of tracheids which caused the decrease of permeability characterizing the heartwood,[15] occurred in the "intermediate wood" stated above, rather outer part from the heartwood boundary. They took place simultaneously in contrast with unevenness of the maturation of each ray parenchyma cell. Many studies were conducted concerning the origin of phenolic compounds, and the connection between phenolic substances of pit membranes and those of parenchyma cells[4,19]. FENGEL (1970)[6] considered from electron microscopic observations in *P. sylvestris* that the heartwood forming compounds within parenchyma cells were passed from the parenchymous pit membranes through the middle lamellae to the bordered pits and from there into the tracheid lumina. PARAMESWARAN and BAUCH (1975)[19] studied the development and localization of phenolic compounds of *Abies alba* using electron microscopy and UV-microspectrophotometry, and stated that there was no indication to suggest that the polyphenols in the cell wall of heartwood were either derived from or identical with the phenolic substance in the lumina of ray cells. In this study, the relation between phenol deposition of the bordered pit membranes and lignification of parenchyma cell walls could not be found.

The transformation of sapwood into heartwood are generally characterized by the death of living parenchyma cells, the loss of starch, and an increase in the extractives content. Death of ray parenchyma cells of *P. banksiana* were observed in September in thick-walled ones, and from August to September in thin-walled ones of resin canal tissue. However, it was not clear when other thin-walled parenchyma died. Indeed, these cells occupied large amount of ray parenchyma tissue. Does the death of these cells occur either in certain season or in every time through the growth season? If former pattern is correct, death of parenchyma, i. e., heartwood formation will be assumed to be conducted exactly in certain controlled process. The fact, some parenchyma cells remote far from the cambium in Genus *Pinus* make secondary wall thickening and lighification, indicates that the heartwood formation is considered to be a regular phenomenon[3]. On the other hand, in the latter case, heartwood formation is presumable to be a rather irregular phenomenon, for instance, parenchyma cells may die one by one with toxic phenolic

substances[22].

Present authors consider that there are two modes in the heartwood formation. One pattern is the phenomenon of ray tissue, and the another is of individual cells, and they overlap each other. In the former case, death of ray parenchyma cells follows the controll system of ray tissue, occurring in a time of the latewood formation, accompaniedby the closure of pits and cell wall formation. In the latter case, death of cells follows a particular physiological condition of each cells, regardless of the season, as suggested by FUKAZAWA et al. (1970)[7] and NOBUCHI et al. (1976)[18]. FUKAZAWA et al. noted that there were two types of ray parenchyma cells, one type could form the heartwood substance by itself and other type could not form the heartwood substance. NOBUCHI et al. stated that in the intermediate wood, the mode of the parenchyma cell contents were not homogeneous, therefore the start of the aging of the parenchyma cells seemed to be not simultaneous.

It is important to make clear differences of function or activity of individual parenchyma cells in the intermediate wood, using methods such as autoradiography or electron microscopy.

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要　　約

マツ属は，Haploxylon pine とピノイド型壁孔を持つ Diploxylon pine および窓状壁孔を持つ Diploxylon pine のそれぞれで放射柔細胞の成熟過程に違いが見られる。

このうち，ピノイド型壁孔を持つ Diploxylon pine には，その放射柔細胞に“薄壁型”と“厚壁型”の２つの型があることが知られている。

第一の型（薄壁型）は，辺材中では薄壁で未木化のままであり，辺心材境界で肥厚せずに，UV 吸収を示して死ぬものである。第二の型（厚壁型）は，辺材中のあるところまでは，第一の型と同様に薄壁で未木化であるが，辺材中央部から内部にかけて壁肥厚と木化がおこり，すぐに死ぬものである。すなわち柔細胞のエイジングによる成熟過程が異なっている。しかし，その成熟過程の季節による変化は明らかではなかった。

前報では，Haploxylon pine であるストローブマツの放射柔細胞の成熟過程の季節的な発達を観察し，それと心材化との関係を考察した。本研究では，前報に引続いてピノイド型壁孔を持つ Diploxylon pine であるバンクスマツを用いて，放射柔細胞のエイジングによる成熟過程および，その季節変化を明らかにしようとした。

供試木は58年生のバンクスマツ1本である。試料は1976年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，F.A.A. あるいはグルタールアルデヒドで固定し，メタクリレート包埋を行った。ダイヤモンドナイフにより0.5 μ 厚の柾目面切片を主に作製し，紫外線顕微鏡 (280 nm) を用いて観察した。

得られた結果を要約すると次のとおりである。

1) 単列放射組織の成熟

第一の型の柔細胞は，辺材中では未木化であり，主に辺心材境界附近で，はじめ細胞間層にその後壁全体にUV吸収を示し，死に至るものである。このステージの柔細胞は，どの季節においても観察されたので，特定の季節に変化がおこることはないと思われる。

第二の型の柔細胞は，辺材中央部から辺心材境界の間で，突然壁肥厚と木化を行い，すぐに死ぬものである。その季節は9月であることが観察され，その月のうちに完了し直ちに死んでしまう。

2) 水平樹脂道組織の成熟

水平樹脂道組織の単列部は，上記の単列放射組織の柔細胞と同様の成熟過程をとる。複列部の柔細胞とエピセリウム細胞は，辺材中では未木化のままであり，心材に移行する際に壁にUV吸収がおこりすぐに死ぬ。その季節は8月から9月の間である。すなわち8月と9月には生細胞から死細胞へ変化していると思われる柔細胞が観察され，その他の月では，生細胞と死細胞の境界がかなり明確に認められた。

心材化と密接に関わっている放射柔細胞の死には，ある特定の季節におこるものと，季節にあまり関わりなくおこるものがあると考えられる。前者では，心材化は，主に晩材形成時におこり，放射柔細胞の壁肥厚と木化や，壁孔壁の閉鎖などの様に，組織として正確にコントロールされた過程としてあり，後者では，心材化は，季節とあまり関わりなくおこり，細胞個々の条件により発現が異なり，有毒物質の蓄積による細胞の死などの様に，細胞個々の生理条件に支配されるかなり不規則な過程としてあると考えられる。

心材化を考えていく上で，今後，細胞単位でその機能や活性の違いを，オートラジオグラフィーや電子顕微鏡などを用いて明らかにしてゆくことが重要であろう。

Explanation of photographs

Photo 1. A radial section from inner sapwood in September, showing a thickening and lignifying parenchyma cell (arrow).

Photo 2. A radial section from inner sapwood in October, showing thickened and lignified parenchyma cells.

Photo 3. A radial section from outer sapwood in September.

Photo 4. A radial section from inner sapwood in November.

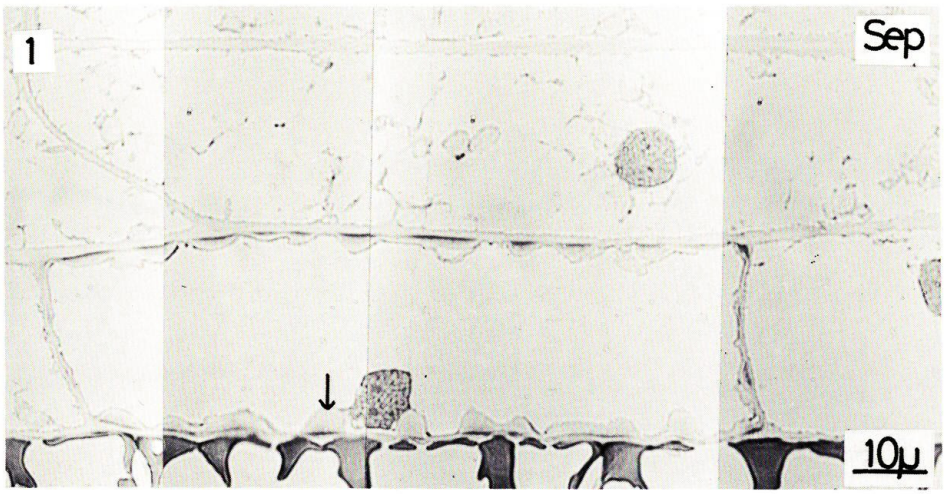
Photo 5. A radial section from sapwood/heartwood boundary in September. Note that the distorted walls reveal in the lumina (arrow 1) and the residual of cytoplasm (arrow 2).

Photo 6. A tangential section from inner sapwood in August, showing strong UV-absorption on the tori and none on the pinoid pit membranes (arrow).

Photo 7. A radial section from sapwood in September.

Photo 8. A radial section from sapwood/heartwood boundary in September.

Photo 9. A radial section from sapwood/heartwood boundary in November. Boundary of living epitherial cells into dead ones is definitive (arrow).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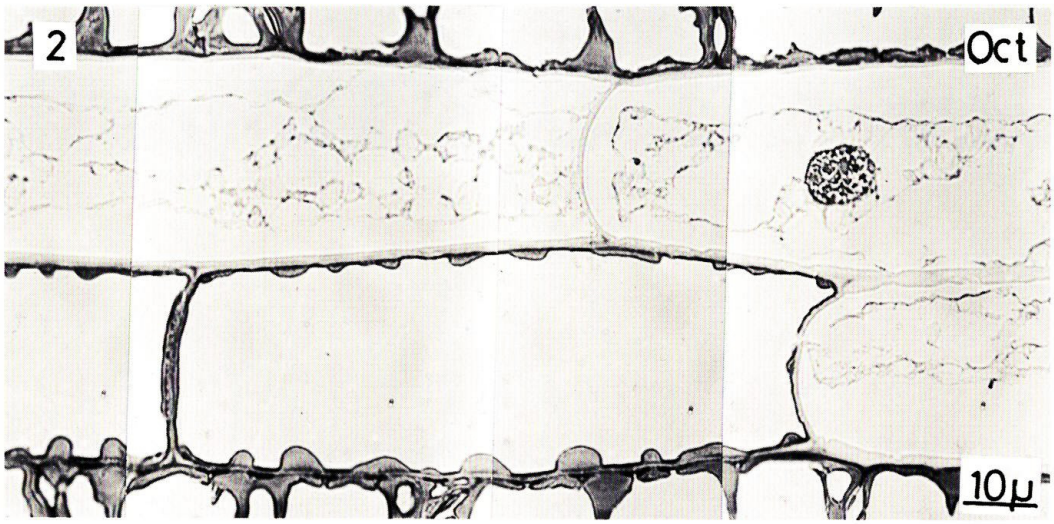
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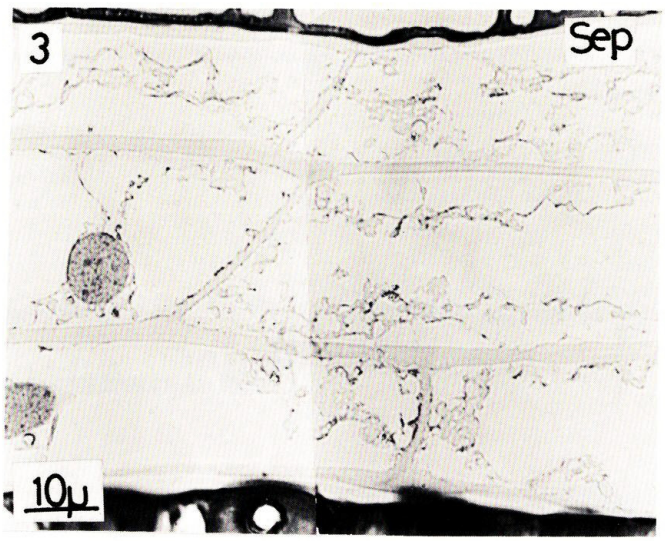
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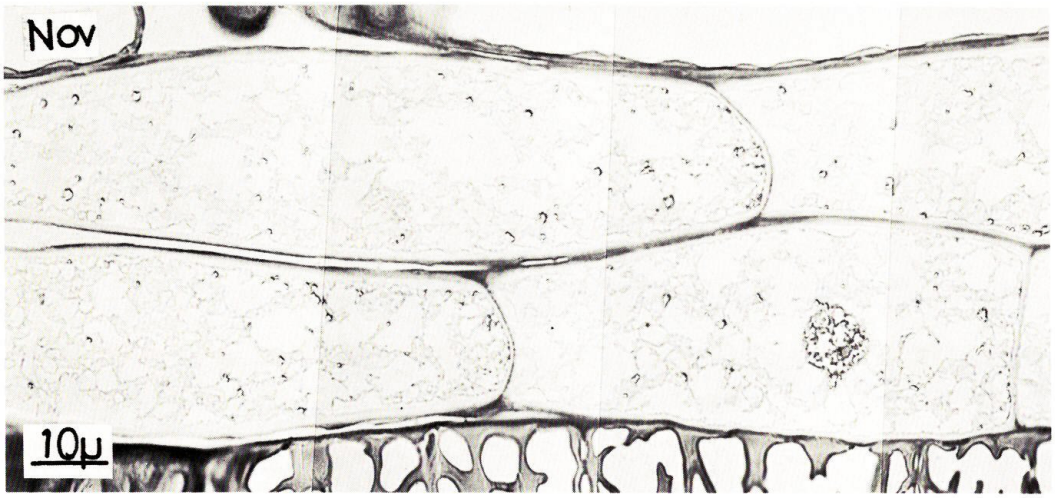
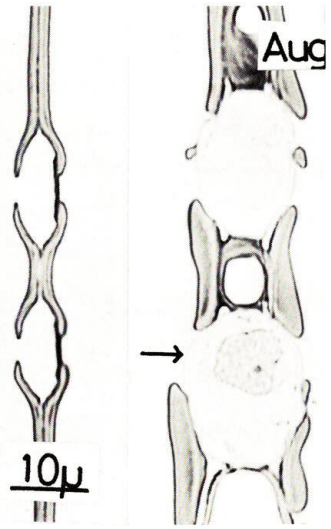


Photo 4

Photo 5



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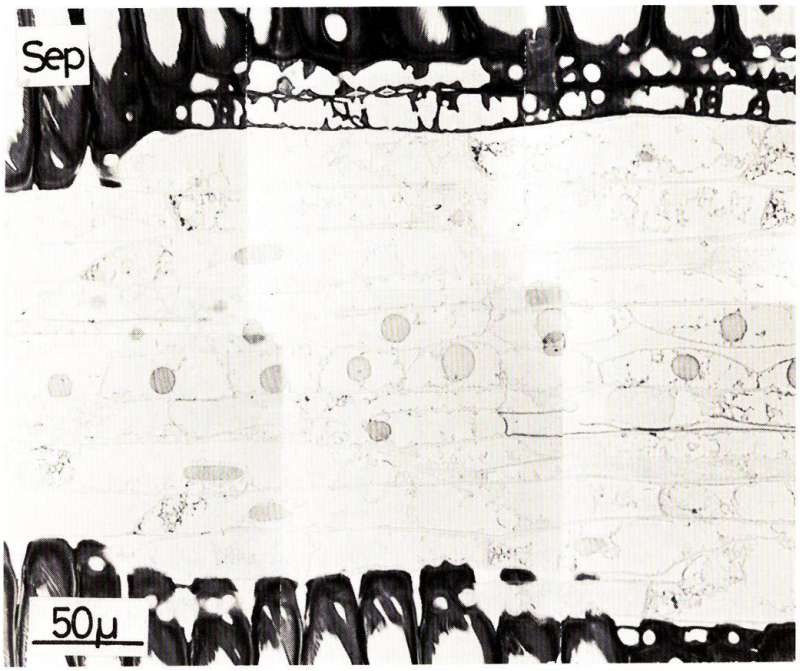

Photo 7

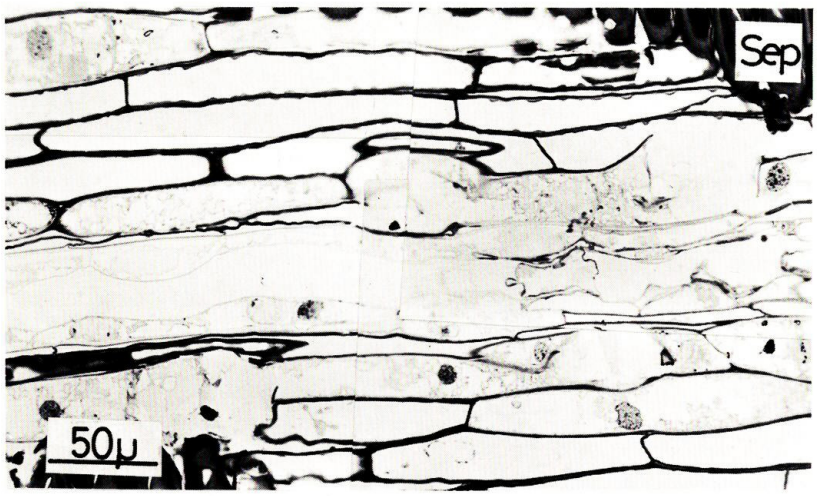

Photo 8

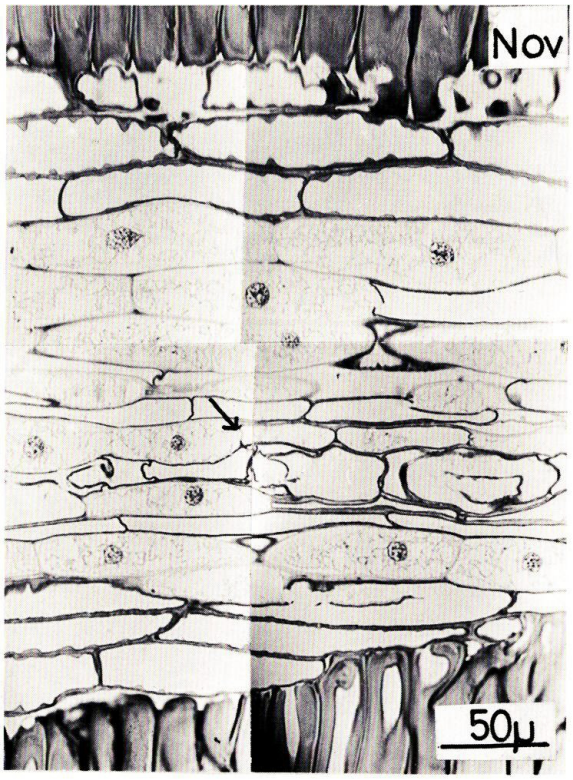

Photo 9