Original

Studies on the Improvement of the Pinning Method for Marking Xylem Growth I. Minute Examination of Pin Marks in Taeda Pine and other Species*

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Abstract——In order to make an accurate estimation of the location of cambium at the time of pin insertion, pin marks left in the xylem tissue were minutely examined. The size and shape of the abnormal tissue induced by pin insertion depended on the size of needle, growth rate of trees, and the tree species. Since the abnormal tissue changed its shape and size as the cross section receded from the center, it was concluded to be necessary to use the cross section obtained from the center of pinning for correct application of the pinning method.

The site relation between cambial initials at the time of pinning and the abnormal tissue was investigated following the modified Wolter's procedure. As a result, the site of cambial initials at the time of pinning was assumed to be at the cambial-side margin of the spindle-like abnormal tissue.

Introduction

Of the various methods for marking xylem growth, the pinning method has recently become very common[1] for its many advantages over others, such as tilting[2] or radiological method[3]. Namely, the pinning method is very simple in practice, and it causes little disturbance to the physiological condition of trees. Moreover, only this method is applicable to big trees.

The pinning method has been applied on the premise that the abnormal tissue induced by needle insertion indicates the site of the cambial initials at the time of the pinning. In contrast to this premise, Wolter[4], an advocate of this method, observed that there was a significant difference between the number of cells counted from the last annual-ring boundary to the mark and to cambial initial at the time of pinning, while there was no significant difference between the number of cells counted to the mark and to the last cell in which secondary wall formation was apparent at the time of pinning. Thus, he concluded that the pin mark indicates the site where secondary

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wall formation was beginning.

In spite of Wolter's proposal, the three dimensional structure of the abnormal tissue induced by pin insertion is very complicated, ranging widely beyond the depth of cambial and enlarging zone, and the site of cambial initials at the time of pinning has not been collated exactly with the restricted place of the abnormal tissue. In these circumstances, the pinning method remains inaccurate and unrefined at present.

The purpose of this experiment is to investigate minutely the shape of the abnormal tissue induced by pin insertion, and to clarify the site relation between the abnormal tissue and cambial initials at the time of pinning.

Materials and Methods

To observe the three dimensional pattern of the abnoraml tissue induced by pin insertion, three vigorous trees of taeda pine (*Pinus taeda* L.), about 12 years old, planted in Kamigamo Experimental Forest, Kyoto University were selected. At the beginning of June in 1979, needles of three different sizes, 250, 400, and 700 μm in diameter, were inserted into the stems from the bark through the cambial zone at 9 points in each tree, and removed immediately. All sample blocks, about the size of 1.5 cm $\times$ 2.0 cm $\times$ 2 annual rings, were harvested using a chisel in the middle of August and fixed in FAA. They were then embedded in celloidin according to the conventional procedure. Serial sections of transverse, radial, and tangential faces, 20 to 25 μm in thickeness, were cut from each set of three blocks respectively. The secitons were double stained with safranin-fastgreen for microscopic observations. Besides, for comparison with the case of taeda pine, needles of three sizes were also inserted into the stems of Sugi (*Cryptomeria japonica* D. Don) planted in Nara Prefectural Forest Experiment Station, and Himalayan ceder (*Cedrus deodara* Loud.) growning in Nara Prefecture in June 1979. These samples were processed in the same way as the case of taeda pine.

To clarify the site relation between the cambial initials at the time of pinning and the abnormal tissue induced by pin insertion, five vigorous trees of taeda pine growing in the same forest as the first experiment were used. A sewing needle, 400 μm in diameter, was inserted into three points of each stem as indicated in Figure 1, once a month from May to September in 1979, changing trees each time. The needle was removed immediately after every pinning. Two or three months after each pinning, sample blocks were harvested from the pinned area. Besides, in order to know the number of tracheids formed in that year before pin insertion, two control samples were taken from both sides 5 cm apart from each pinning point (Fig. 1, R and L) at the time of each pinning. Sample blocks were processed in the same way as mentioned above. With regard to the control samples, the tracheid number was counted along four radial rows from the last annual ring boundary to cambial initials

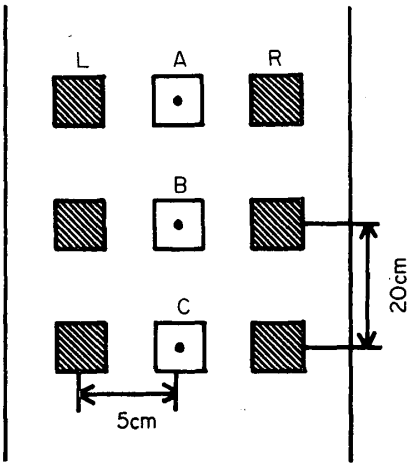

Fig. 1. Diagrammatic representation of the site of pinning and control sample on the stem surface. A, B, and C: Pinning area. R and L: Control block on the right side and left side of the pinning respectively.

for each sample (R and L), in which cambial initials were distinguished by "group of four cells" proposed by Mahmood[5] and the shortest ray cells. The average (N) of the cell number of 8 radial files on both sides (R and L) were applied to the sections from the pinned sample, and the position of the original cambial initials was assumed (Fig. 2).

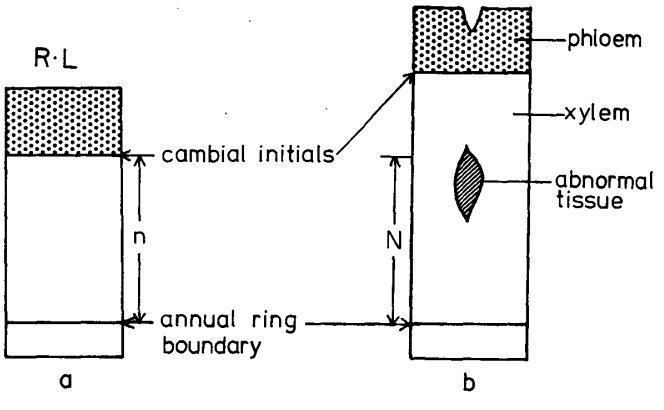

Fig. 2. Diagrammatic explanation to "assume" the site of cambial initials at the time of pinning. a) Cross section from a control sampl (R or L ni Fig. 1.) collected at the time of pinnig. b) Cross section from a pinned block through the center of pinnig. n: The number of tracheids counted in a radial file from the last annual ring boundary to cambial initial. N: The average number of tracheids (n) counted along 8 radial files on both sides (R and L) of the pinned point.

Results and Discussion

1. Characteristics of abnormal tissue

1.1 Three dimensional shape of abnormal tissue in taeda pine

Figure 3 shows the three dimensional structure of the wounded area where a 400 μm thick needle was inserted two months before. There were many abnormal parenchymatous cells among the normal or disfigured tracheids. Such area where abnormal parenchymatous cells exist will be called abnormal tissue in this paper.

In the cross section at the center of pinning, the abnormal tissue showed a radially-long spindle shape (Fig. 3a). The range of that tissue was so long in radial direction that it is not possible to determine the site of cambial initials at the time of pinning from the abnormal tissue. In the radial section (Fig. 3b), the abnormal tissue was longitudinally very high, measuring over 5 mm. The radial depth of the abnormal tissue was greatest at the pinned point (Fig. 3b, arrow), becoming abruptly narrow above and below that point. The tangential width of the abnormal tissue was almost constant (300 μm) along its height, ranging about 5 mm as indicated in the tangential section (Fig. 3c). On the basis of these observations, it was concluded that (1) the longitudinal height of the abnormal tissue induced by pin insertion ranges as high as over 10 times of the diameter of the pin; (2) the tangential width of the abnormal tissue is narrower than the pin diameter; (3) its radial depth is much deeper than the width of the cambial zone at the time of pinning. The reason why the longitudinal dimension of the abnormal tissue was so high is considered to be ascribed to a cleavage which was formed between the cells at the stage of differentiation when a pin was inserted.

The origin of the abnormal parenchyma cells in the wounded area is thought to be ray parenchyma cells, judging from the fact that the ray cells changed their direction and shapes in the abnormal tissue as indicated in Figure 4. Ray cells around the gap formed by pin insertion seemed to swell toward the gap and proliferate till the gap was filled with those cells.

Many septa were observed in tracheids through a wide area around the abnormal tissue (Fig. 5a). As suggested by Imamura *et al.*[6], these septa seemed to originate from tylosis-like structures, one of which is demonstrated to be ballooning out from a ray cell into a tracheid in Figure 5b (arrow). Such a structure must have proliferated in the tracheid and resulted in partition walls (septa). The boundary between the wall of the newly formed parenchyma cells in the tracheid and that of the host tracheid could be distinguished with light microscope. These walls did not fuse with each other. Thus, it is clear that these cells are not formed by changing the direction of differentiation from the tracheid toward the axial parenchyma cells, although there is a

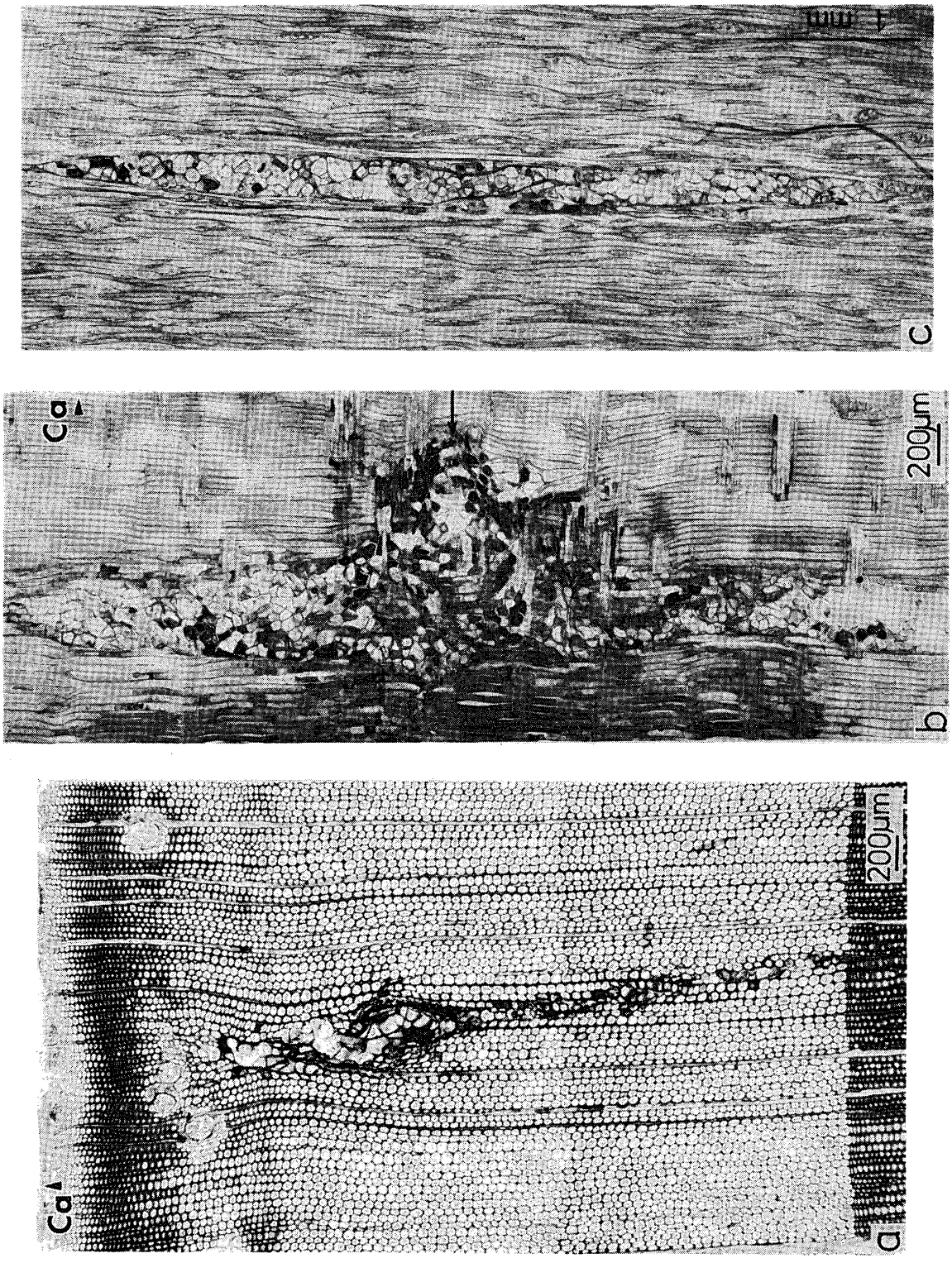

Fig. 3. Three dimensional shape of the abnormal tissue formed two months after the insertion of a 400 μm needle in taeda pine. a) Cross section. b) Radial section. The arrow showing the point of pin insertion. c) Tangential section. Ca: Direction of cambium.

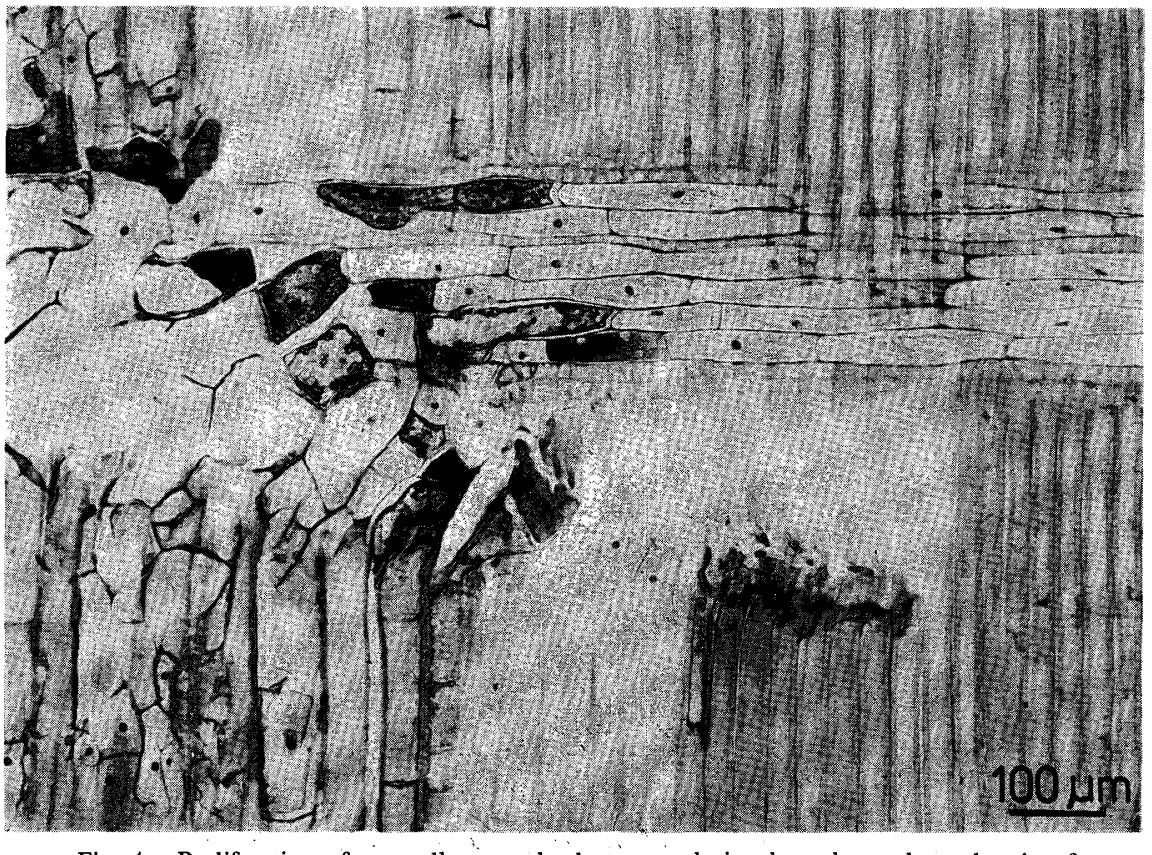

Fig. 4. Proliferation of ray cells near the last annual ring boundary where the tip of the needle has reached at the time of pinning. (radial section)

report[6] assuming such origination. Ray cells were found to keep the potential ability for proliferation even after they had finished the differentiation.

From the same sample block of Figure 3a, cross sections were cut successively at the interval of 0.5 mm (Fig. 6). The wound changes its shape and reduces its size gradually as the section recedes from the center of the pinned point, and among others, the abrupt shortening of its radial depth is most remarkable. As the range of abnormal tissue varied according to the distance from the center of the pinning, it was thought to be very important to use cross sections from a definite level when discussing the site of cambial initials at the time of pinning. From this point of view, the center of the pinned point is most suitable for the definite level. This point is easy to determine, and the abnormal cells are most abundant at this point. Therefore, in this study, the cross section taken from the very point of pinning was used in order to assume the site of cambial initials at the time of pinning.

1.2 Difference in the abnormal tissues due to the needle sizes and tree species

Abnormal tissues caused in taeda pine by the needle of different diameters, 250

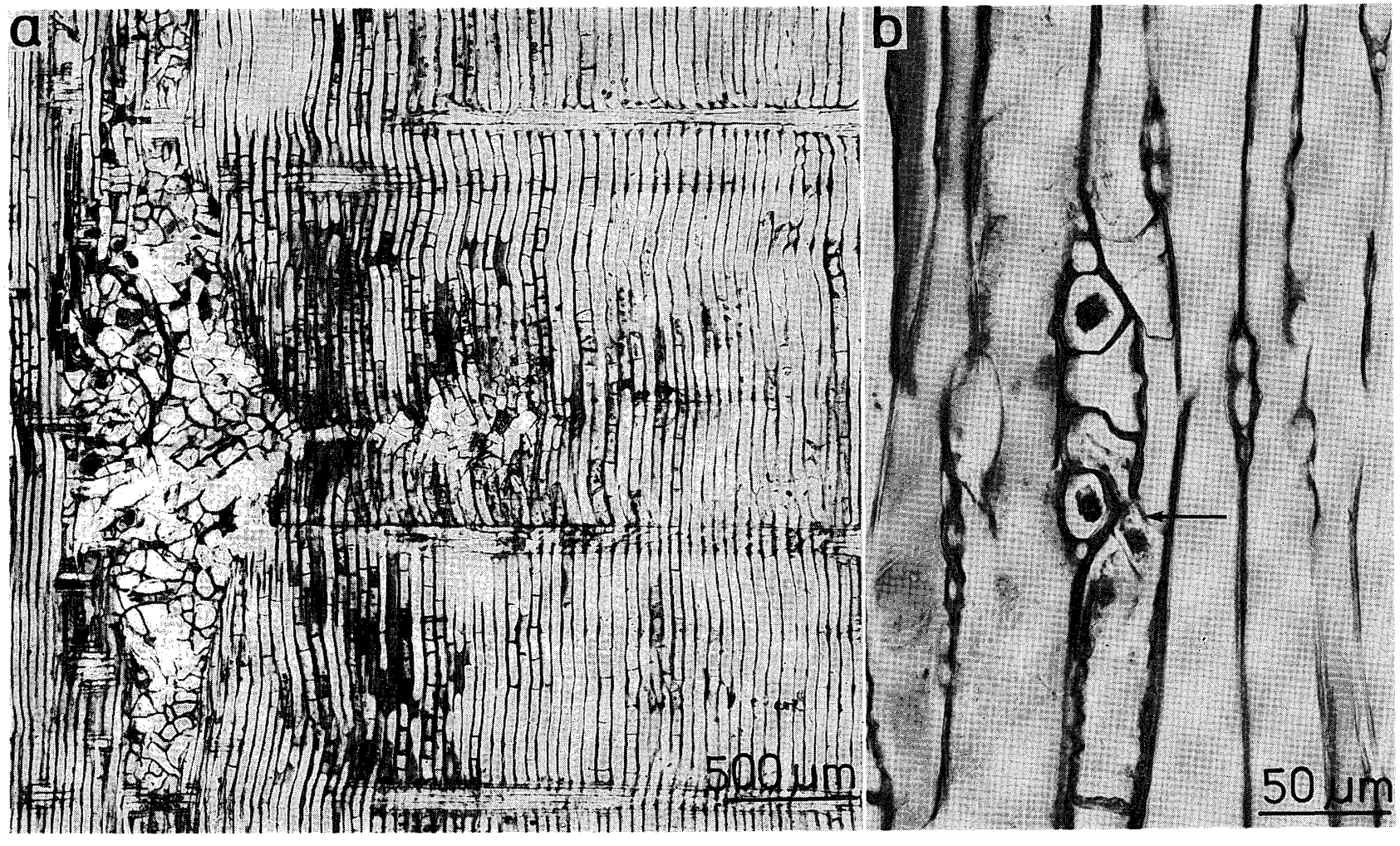

Fig. 5. a) Septa formed in the tracheids around the abnormal tissue. b) Tylosis-like structure (arrow) ballooning from a ray cell. (tangential section)

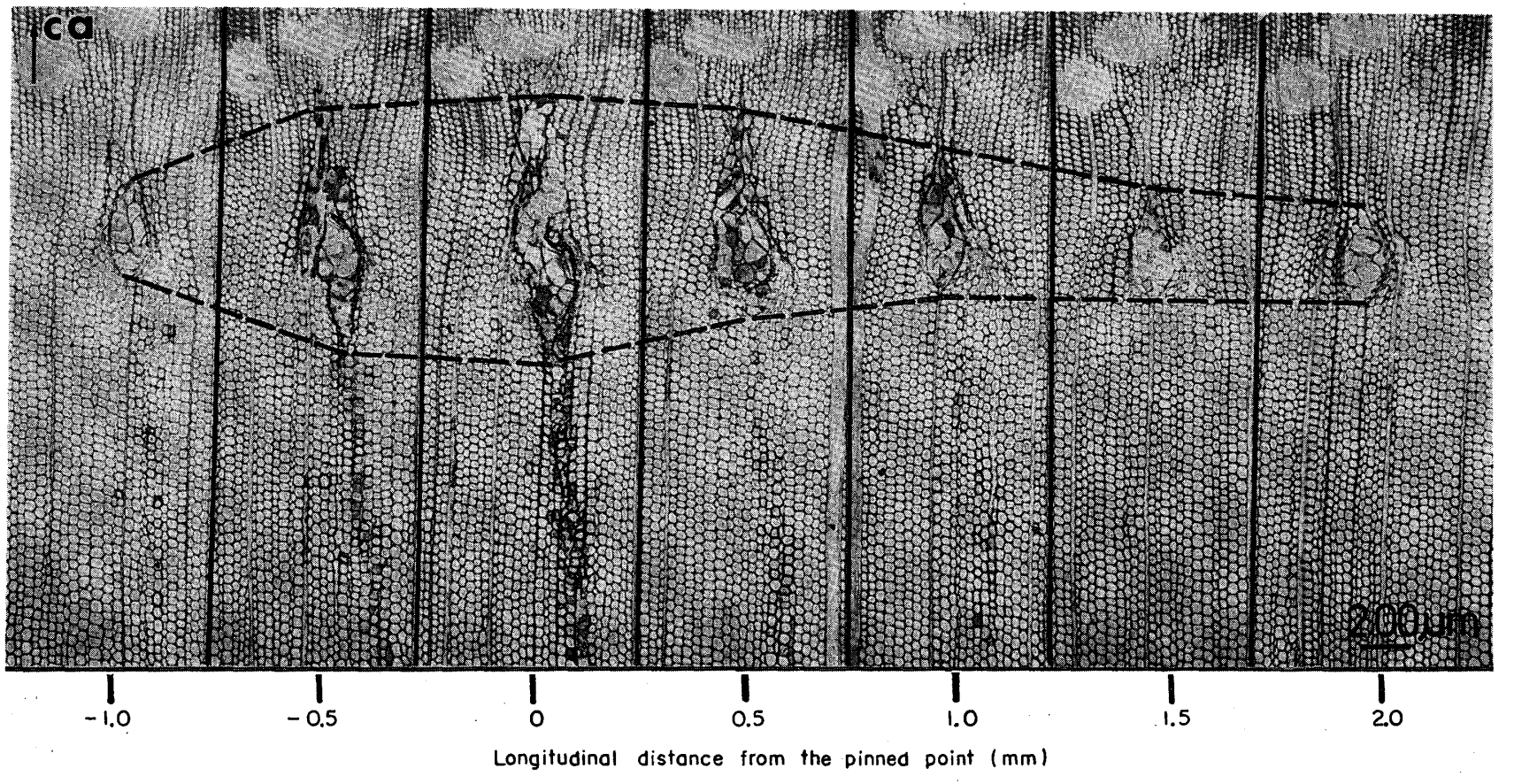

Fig. 6. Serial cross sections of the abnormal tissue in taeda pine. The interval of the sections is 0.5 mm.

and 700 μm are shown in Figure 7a, 7b, which are both cross sections from the center of abnormal tissues. In the case of 250 and 400 μm (Fig. 3a) needle, the shape of abnormal tissue was a radially-long spindle, and the boundary of the tissue was clear, although abnormal parenchyma cells were formed in a smaller area in the case of 250 μm. In the case of 700 μm needle, abnormal parenchyma cells were formed over a wide range, the shape was not spindle-like, and the boundary of this tissue was not clear. The radial shape of the abnormal tissue was almost the same in all cases, though there were differences in size according to the diameter of the needles inserted.

For practical application as a marking method of xylem growth, it is desirable that the wounded area is as small as possible and the wound heals rapidly. However, the needle of 250 μm is sometimes not practical, because this size of needle is so fine and apt to bend that it was difficult to piarce the firm bark, though it may be useful for young seedlings with delicate tissue. As for the needle of 700 μm, the wound seemed to take so much time for healing, and the radial growth was sometimes so supressed at the pinned area, that this size of needle may not be appropriate in practice. In case of taeda pine, 250 and 400 μm needles are considered to be the most appropriate size to use for the practical pinning method.

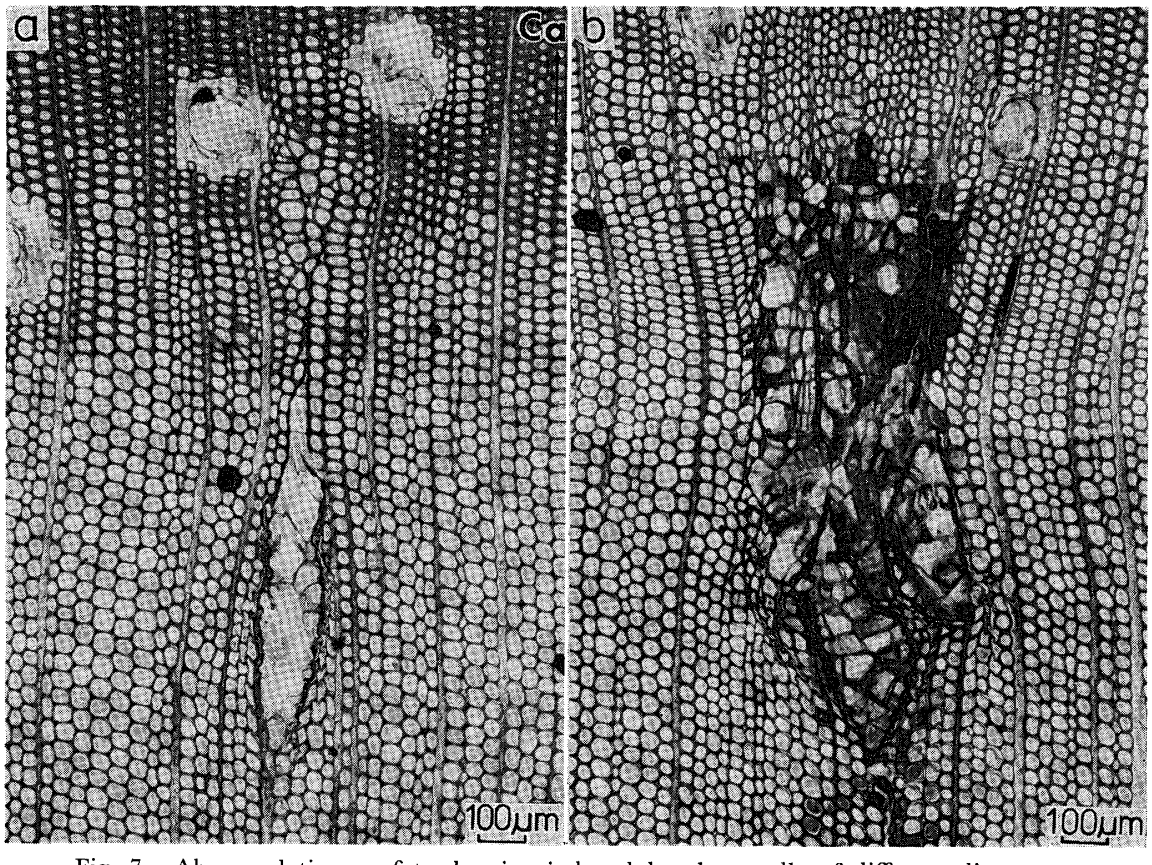

Fig. 7. Abnormal tissues of taeda pine induced by the needle of different diameters; a) 250 μm b) 700 μm. Compare with Fig. 3a (400 μm).

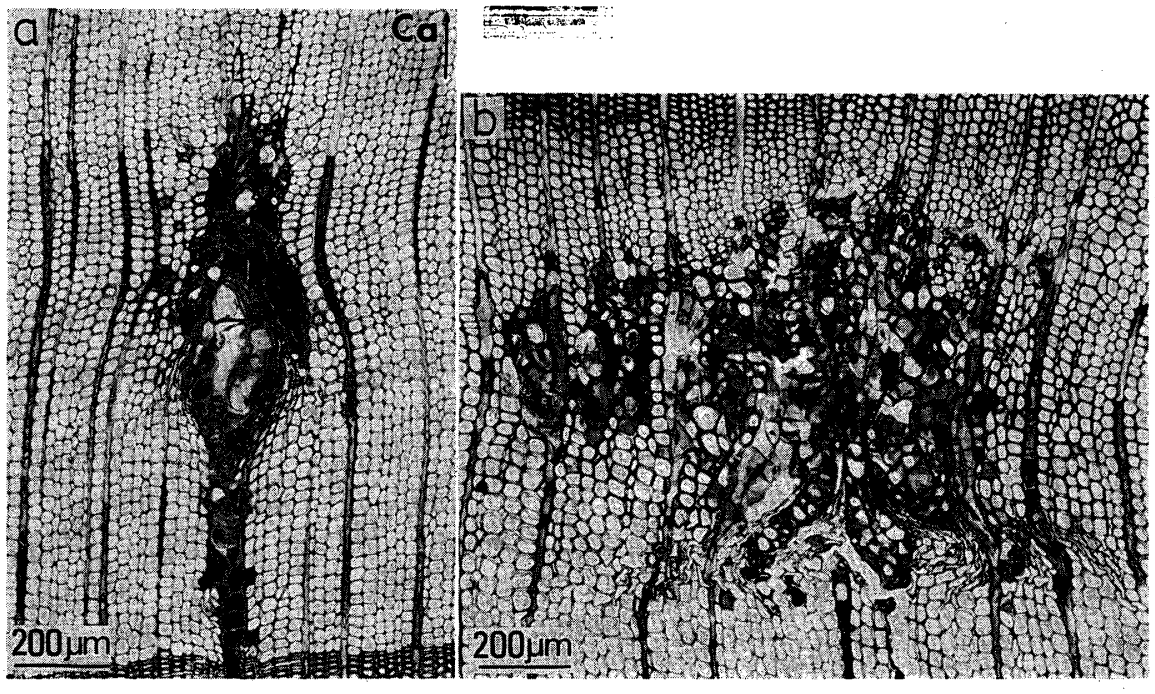

Fig. 8. Abnormal tissue of Himalayan ceder induced by the needle of different diameters; a) 400 μm b) 700 μm.

It was examined whether the shape of abnormal tissues formed in other species, Sugi and Himalayan ceder, is the same with those of taeda pine or not. In the case of Sugi, the shape of abnormal tissue induced by 250 μm needle was similar to that by 400 μm needle for taeda pine. The abnormal tissue caused by 400 μm needle in Sugi was not spindle-like in cross section and the range was tangentially wide. The cause of this difference is thought that cells of Sugi were broken more excessively than pine because of their thin wall, and/or the healing of wound may be delayed in Sugi because of its slow growth rate. In the case of Himalayan ceder, although the shape of abnormal tissues caused by the 250 and 400 μm (Fig. 8a) resembled those of pine, that caused by the 700 μm needle (Fig. 8b) differed considerably because of abnormal parenchyma cells formed widely in the tangential direction. In many samples of Himalayan ceder, traumatic resin canals were formed. From these results, it was concluded that the difference in size and shape of the abnormal tissue depends not only on the histological characteristics of the species or genera such as the size of cell and the thickness of cell wall, but also on the growth rate of trees and the diameter of the inserted needles. Before the application of the pinning method, the most suitable diameter of needles should be determined according to species or age of sample trees.

2. Site relation between cambial initials at the time of pinning and abnormal tissue

The probable site of cambial initials at the time of pinning in the pinned sample

block was estimated by tracheid numbers produced in the control sample up to each pinning date as mentioned in materials and methods.

Cell numbers counted on all control samples and the averages of them (N) are shown in Table 1. In some cases, in spite of their same sampling date, the cell number of samples R and L showed a significant difference. For instance, in case of May-11-A, the average cell number was 72 in R and 49 in L, and the difference was 23.

Table 1. Cell number from the last annual ring boundary to cambial initials in control samples.

Sampling date	Material[a]		Cell number					
			radial file				average	average of R and L (N)
			1	2	3	4		
May 11	A	R	74	74	67	71	72	60
		L	49	51	48	47	49	
	B	R	77	74	70	74	74	68
		L	69	58	58	66	63	
	C	R	74	73	72	75	74	68[b]
		L	61	60	66	61	62	
June 14	A	R	68	66	73	75	71	60
		L	48	49	53	47	49	
	B	R	63	64	67	67	65	57
		L	46	47	51	48	48	
	C	R	58	58	56	58	58	57[b]
		L	56	60	56	51	56	
July 11	A	R	106	98	101	107	103	104[b]
		L	102	101	110	103	104	
	B	R	113	116	109	111	112	108
		L	99	110	101	106	104	
	C	R	118	120	124	131	123	116
		L	117	108	105	103	108	
August 13	A	R	162	150	151	151	153	140[b]
		L	128	131	122	124	126	
	B	R	184	186	179	175	181	158
		L	132	142	126	137	134	
	C	R	166	187	180	188	180	163
		L	148	130	146	155	145	
September 13	A	R	148	149	150	150	149	151[b]
		L	156	148	157	150	153	
	B	R	151	150	151	145	149	157
		L	169	162	165	164	165	
	C	R	170	172	171	178	173	—
		L	—	—	—	—	—	

a) See Figure 1.
b) Used for the site estimation of the cambial initials.

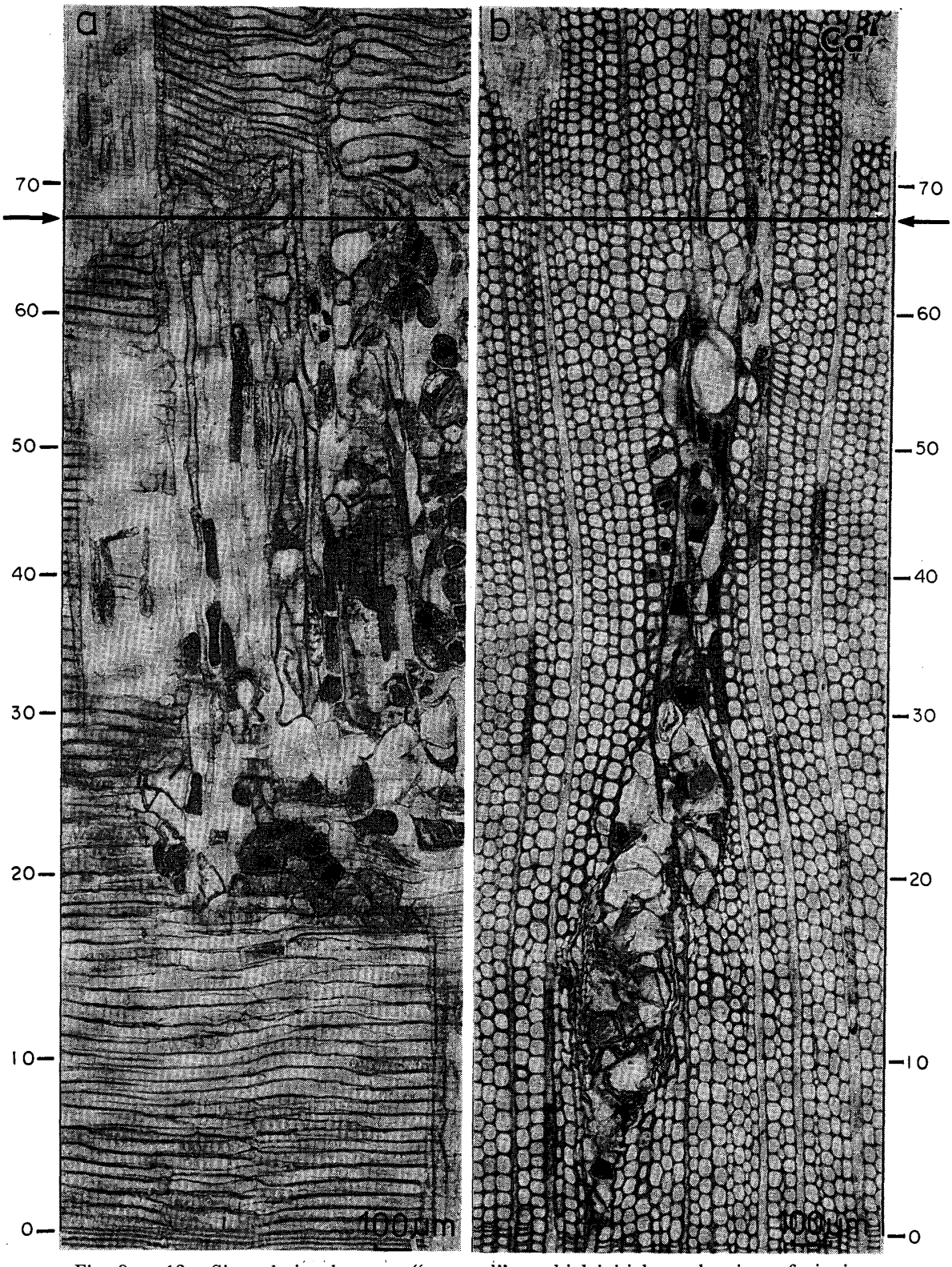

Fig. 9 to 13. Site relation between "assumed" cambial initials at the time of pinning and abnormal tissue. Figures on the side line of each photograph indicate the number of tracheids in radial file counted from the last annual ring boundary. The "assumed" site of cambial initials is indicated by an arrow.

Fig. 9. Abnormal tissue induced in May. a) Radial section. b) Cross section.

In such a case, the average does not necessarily indicate the cell number of the pinned area at the time of pinning, because such differences occurring within 10 cm in a stem may be caused by local variation of growth rate. For this reason, the site of the cambial initials was estimated using the sample which had the least difference (Table 1, b) between R and L among a set of three samples.

Figure 9b to 13 are transverse sections cut from the center of pinning for each sample from May to September respectively. There were considerable differences in shape according to the season of pinning or the growth rate of individuals. On the right side of each figure, the cell number counted from the last annual ring boundary is given, and the site of cambial initials estimated by the above-mentioned method is indicated by an arrow. In the case of the sample May-C (Fig. 9, a and b), the average cell number was 74 in R and 62 in L, and the average of both sides was 68. After the

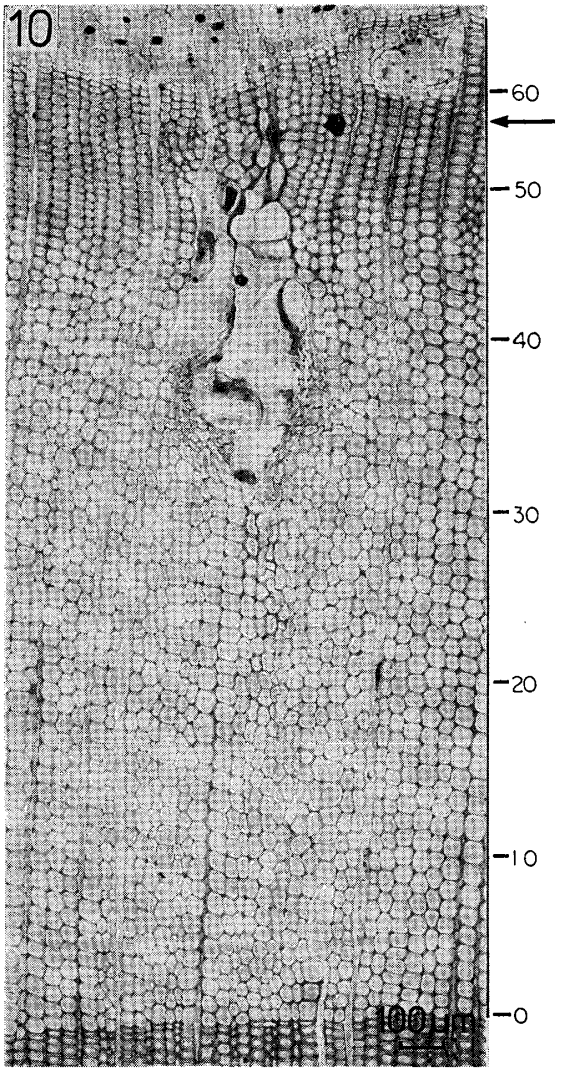

Fig. 10. Abnormal tissue induced in June.

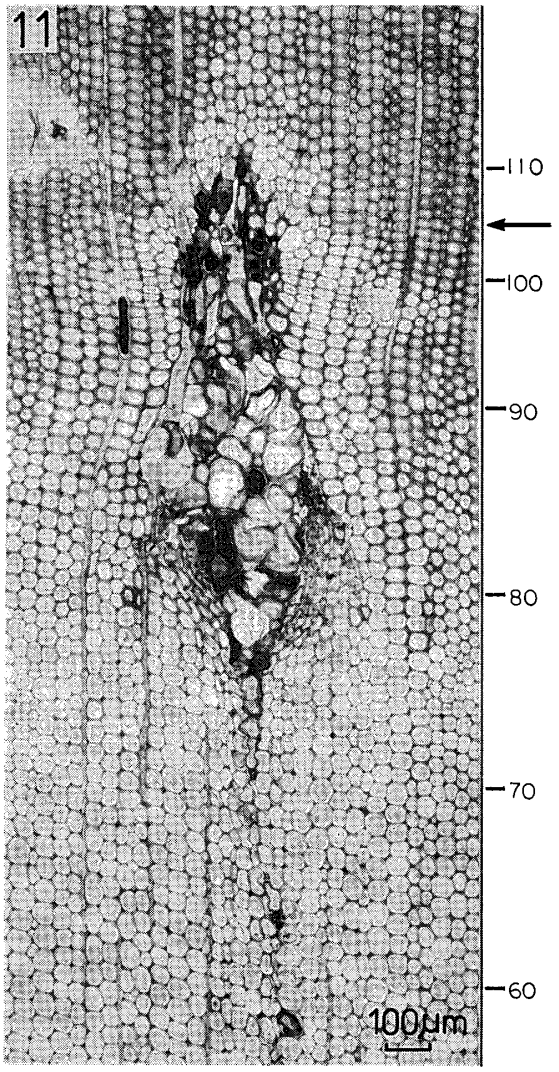

Fig. 11. Abnormal tissue induced in July.

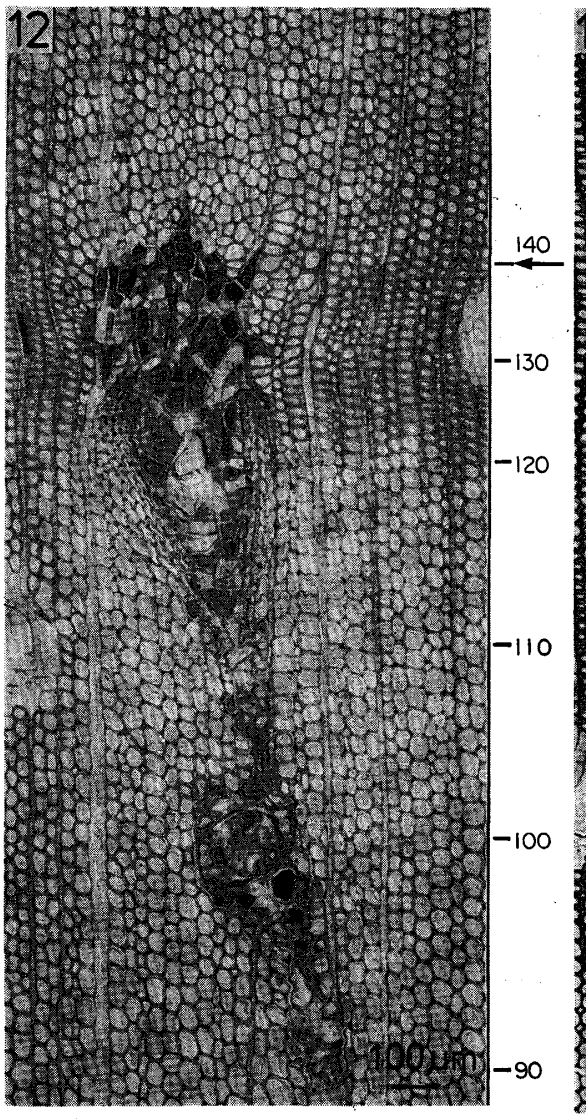

Fig. 12. Abnormal tissue induced in August.

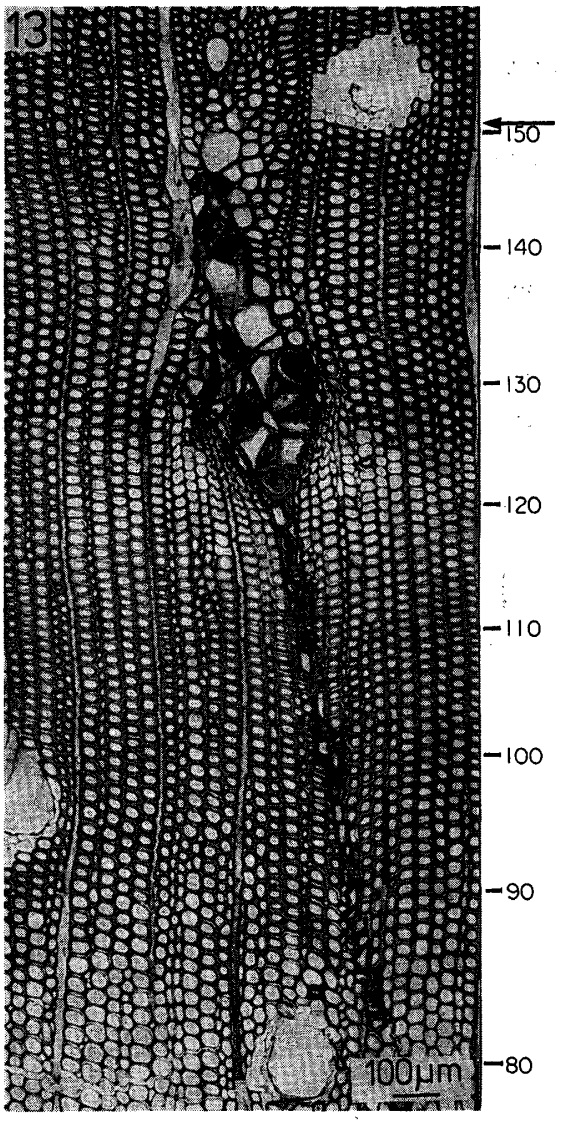

Fig. 13. Abnormal tissue induced in September.

cross section (Fig. 9b) had been cut from the center of the pinning, the radial section (Fig. 9a) was cut to see the relation between two planes. As indicated by the arrow, the site of the cambial initials at the time of pinning was assumed to be in the neighborhood of the cambial-side margin of the abnormal tissue. The growth rate of the sample tree of June 14th was apparently slow as is seen in Figure 10, and the radial dimension of this abnormal tissue was shorter than the case of May. The cell number at the time of pinning was 58 in R and 56 in L, and the average of them was 57. The estimated site of the cambial initials at the time of pinning was a little outside the cambial-side margin of the abnormal tissue, and coincided with where the radial file of tracheid was disturbed. In the sample of July 11th (Fig. 11), the cell number was 103 in R and 104 in L, and the average was 104. The site of cambial initials was

assumed to be a little inside the cambial-side margin of the abnormal tissue (Fig. 11, arrow). In the case of the sample taken in August 13th (Fig. 12), the cell number was 153 in R and 126 in L, and the average was 140. Although the site of cambial initials was assumed to be in the vicinity of the cambial-side margin of the abnormal tissue, this result is not so reliable as other months, for the difference of the cell number in R and L is large. In this sample, the shape of the abnormal tissue was different from the case of May, June, and July; the tangential width of this tissue was wider than others, and was not spindle-like. In addition, flat cells resembling those of late wood were seen in parallel with the annual ring boundary on both sides of this abnormal tissue. The stimulus of pin insertion seemed to be transmitted tangentially on both sides of the wounded area. Regarding the sample of September 13th (Fig. 13), the cell number was 149 in R and 153 in L, and the average was 151. In this case, the site of cambial initials at the time of pinning was assumed to be in the vicinity of the cambial-side margin of the abnormal tissue again.

These results indicate that the site of cambial initials at the time of pinning coincides with the cambial-side margin of the abnormal tissue in all the cases. These results, however, differs from those of Wolter[4]. His sections seem to be obtained away from the center of pinning because the trace of pin cannot be seen, and the range of the abnormal tissue is very shallow in radial direction in his figure. We should keep in mind that the range of abnormal tissue extends deeply from the cambial initials to the lignifying cells at the time of pinning, and that the abnormal tissue changes its shape and size as the cross section recedes from the center of pinning.

In the present experiment, there were small discrepancies between the assumed cambial initials at the time of pinning and cambial-side margin of abnormal tissue with ± 5 cells. Some discrepancies had naturally been presumed in advance, since the indirect method, *i.e.* the use of control samples from both sides of pinning area, was applied to assume the site of cambial initials. Judging from the data obtained from control samples (Table 1), this difference, about ± 5 cells, is rather small.

Thus, we concluded that the site of cambial initials at the time of pinning is the cambial-side margin of the abnormal tissue. In order to locate the place of cambial initials at the time of pinning more exactly, close examinations, pursuing the time sequence of the formation of the abnormal tissue, are extending.

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