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**New Marking Method by Electrical Stimulation
for Studying Xylem Formation II[†]
Application to Broad-leaved Trees***

By

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木部形成研究のための電氣的刺激による
新しいマーキング法 (第2報)[†]
広葉樹への適用*

今川一志** 石田茂雄**

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Introduction

It is significant to clarify the increasing process of xylem cells, i. e., the so-called growth ring formation in forest trees. In such quantitative investigations, some experimental methods to make inscriptions or mark a given date on the xylem or the current growth ring have been devised and used. Among these methods, recently the pinning method which was introduced by WOLTER (1968) has been frequently employed, because of its efficiency and applicability (SHIMAJI and NAKATSUKA 1971, KAWANA et al. 1973, 1974). YOSHIMURA et al. (1981 a, b) have investigated the pinning method in detail for its improvement and establishment.

The authors have attempted a new marking method in which electrical sti-

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mulation was externally applied to the stems. As a result, the possibility of this method for practical employment was suggested in *Abies sachalinensis* (IMAGAWA and ISHIDA 1981) and *Avicennia marina* (IMAGAWA 1981). The latter is one of the species of which mangrove forest is composed. In this study, thus, this electrical marking method was also attempted for estimating its efficiency in usual broad-leaved trees.

Materials and Methods

Electrical stimulation was applied to the mature stems of Hannoki, *Alnus japonica* (BHD: 30 cm, H: 12 m) and Kusunoki, *Cinnamomum camphora* (BHD: 13 cm, H: 7 m) grown at the experimental forests of Ryukyus University in Okinawa. At breast height of the stems, two electrodes were deeply inserted through the outer and inner bark into the mature xylem in a vertical orientation (Fig. 1). The electrodes inserted were spaced 5 cm apart. As the electrodes, commercial dressmaker pins (0.51 mm in diameter, 28.8 mm in length) were used. Electrical stimulation consisting of 154 volts by dry batteries with a duration of 5 seconds was applied to the stems. Immediately after the application of the electrical stimulation, the electrodes were drawn out from the stems.

Electrical stimulation was applied only in late Oct. 1980. The specimens which consisted of xylem, cambium and phloem were collected in late Nov. 1980. They were punched out from the middle portion between the traces of the insertions of these electrodes, in order to avoid traumatic influences arising from insertion of the electrodes. The specimens were immediately fixed in FAA, embedded in celloidin and stained with safranin and fast green.

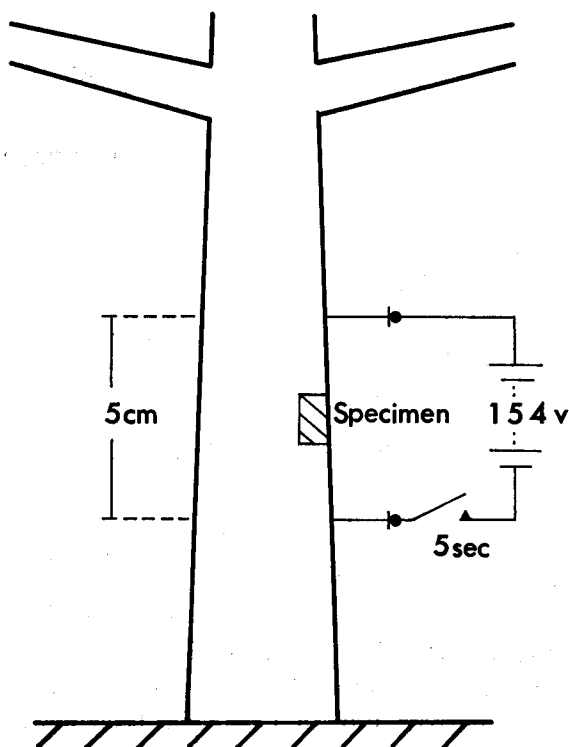


Fig. 1. Schematic diagram of the experimental method.

Results

Since Okinawa island is located in a subtropical region, the cambium in both species examined were active and the current growth rings were developing when the specimens were collected. By the application of the electrical stimulation, some

of the cells in the current growth ring were influenced. Such influences were basically almost similar to those in *Abies* (IMAGAWA and ISHIDA 1981) and *Avicennia* (IMAGAWA 1981). However, the appearances or state of the influenced cells which occurred were somewhat more complex in comparison with those in *Abies*, because of the more complicated organization of xylem cells in broad-leaved trees than those in coniferous trees. Thus, the influences on the cells will be explained in detail in order to estimate the efficacy of this new method.

With regard to marking or making an inscription on the xylem, the radially crushed cells are most interesting and noticeable among the cells which were influenced (Photos 1 and 3). One or two cells at almost the same position in each radial file of cells were radially collapsed, so that the crushed cells were almost arranged in tangential series and thus represented a line macroscopically. Since they were nearly completely crushed, their lumina could not be found in the transverse section (Photos 2 and 4 a). Especially, such tangential series were remarkable in Hannoki (Photos 1 and 2). In Kusunoki, however, the crushed cells inclined to be aligned in a radial short series rather than in tangential series (Photo 3). Such radial series existed in a curve along the outline of the ray parenchyma cells or the other cells which were all profoundly influenced to become abnormal in shape. Although most of the crushed cells seemed to be wood fibers judging from their dimensions, a few of them were cells which were destined to become vessels (V in Photo 2). They were not completely but partially crushed radially. In Kusunoki, however, crushed vessels were hardly found. Such difference may arise from the difference in xylem organization, i. e. the existence of paratracheal parenchyma cells in Kusunoki. The walls of the crushed fibers were considerably thin, and not fully lignified judging from the staining results with safranin. On the other hand, the walls of the crushed vessels were relatively thick and more strongly lignified than these fibers. In the transverse section, the walls of both crushed fibers and crushed vessels were brightened between cross nicol prisms (arrows in Photos 4 b and 5 b). It is well known that S-1 and S-3 layer show birefringence. Thus it is considered that the crushed fibers with very thin walls have at least S-1 layers. In the crushed vessels, the layers other than S-1 layer seem to be also deposited because they have relatively thick walls.

On the cambium side of the crushed cells, two or three cells which were abnormal in shape and arrangement were found (Photos 1-3). As shown in the radial section, most of them were strand-like (Photos 6 and 7). And simple pits are frequently observed in their end walls as shown in the transverse section (arrows in Photo 2). Although it is still not clear what they were differentiated from or what they eventually matured to, they seem to be parenchyma-like cells.

On the cambium side of such abnormal cells, almost normal shaped cells were again located (Photos 1 and 3). In such regions, extremely thin walled vessels were rarely found in Hannoki (arrow in Photo 5 a). It is presumed that the secondary wall formation was still not initiated in these vessels, because the birefringence was not observed at all (Photo 5 b). In Hannoki, the vessels which were located

far from the abnormal cells were considerably smaller in diameter than those which were differentiated and matured before the application of the electrical stimulation (Photo 1). Additional studies are necessary to clarify whether these small vessels resulted from the electrical stimulation or from seasonal factors. In Kusunoki, however, such a tendency was not observed (Photo 3).

On the pith side of the crushed cells, thin walled cells were abundantly grouped in a tangential wide-band (Photos 1-4). Most of these cells seem to be wood fibers. In each radial file of cells, usually, there were about 20 fibers located inward from the crushed cells. The walls of these cells were uneven and varied in thickness. Namely, the walls of the fibers which were located nearer the crushed cells became thinner in thickness. Thus, it is considered that they have at least S-1 layers. In the fibers far from the crushed cells, the innermost portion of the relatively thick walls were also brightened (arrows in Photo 4 b). It appears that such cells had S-3 layers. Since their walls were relatively well stained with fast green, however, it is considered that they are not fully lignified. Whereas, their intercellular layers were strongly stained with safranin. In Kusunoki, however, it appears that the intercellular layers in some of the wood fibers are not sufficiently lignified because they were less stained with safranin. In both species, most of the thin walled cells showed the cell contents in their lumina (Photos 1-4 a). They appear to be the residues of the protoplasm.

However, all the cells on the pith side of the crushed cells were not thin walled and less lignified. The vessels in such regions had thick and lignified walls (Photos 1 and 3). In Kusunoki, paratracheal parenchyma cells showed also the same state (Photo 3). In addition to these cells, a few of the cells which were not associated with vessels had thick and lignified walls in both species (arrows in Photos 1 and 3). In Hannoki, some of the vessels near the crushed cells had end walls which were still not completely perforated (Photo 8). Remarkable influences which resulted from the application of the electrical stimulation were not observed in oil cells, which are characterized in Kusunoki (0 in Photo 3).

Rays were also profoundly affected by the electrical stimulation. Most of the ray parenchyma cells at the position adjacent to the crushed cells were considerably extended tangentially (Photos 1-3). Especially, in Kusunoki the rays increased in width (Photo 3) and height (Photo 7). In other words, new ray parenchyma cells were added both tangentially (width) and longitudinally (height). In Hannoki, however, such a tendency was not found (Photo 6).

Ray parenchyma cells included abundant deposits, which were probably produced after the application of the electrical stimulation (Photos 6 and 7). Because such deposits were not present in the rays of the previous growth rings. Such deposits were also accumulated even in the current ray parenchyma cells which were produced before the application of the electrical stimulation (Photos 1 and 3). In addition, deposits were also observed in vessels or paratracheal parenchyma cells (Photos 1 and 3).

In Kusunoki, bud tyloses were rarely found in the lumina of the vessels near

the crushed cells (Photo 9). It is well known that tyloses can be artificially formed by any traumata. It is interesting that tyloses can also be formed by electrical stimulation.

Discussion

PEACE (1940) mentioned briefly that abnormal tissues formed by externally applied electrical currents. However, the purpose of his examination was not to attempt to mark or make an inscription on the xylme by electrical currents, but to elucidate the influences on trees exerted by lightning.

In both species examined, some influenced cells as mentioned above occurred in the current growth rings. Judging from the state of their appearance, it is presumed that the differentiating cells were strongly influenced by the application of the electrical stimulation and consequently resulted in necroses or abnormal differentiations. The exact causes from which the necroses or abnormal differentiation resulted were not clarified in this study. However, it seems to be reasonable to consider that the electrical stimulation, i. e. electrical current, flows mainly through the cells near or in the cambium, in which the protoplasts are abundantly contained (IMAGAWA and ISHIDA 1982). Based on the state of their appearances in the current growth rings, therefore, it may be possible to form an assumption of the processes in which the influenced cells were produced.

It can be considered that both the crushed cells, and the thin walled and unignified cells died during the course of their differentiation. Because such influenced cells had at least S-1 layers and most of the intercellular layers were more or less lignified, depending on polarizing microscopy and staining result respectively.

Among such dead cells, the crushed cells were located at the position nearest the cambium and had the thinnest walls. Therefore, they seem to be the cells immediately after the initiation of the secondary wall formation. And they appear to be radially collapsed by pressure which occurred after their necroses, because of their thinnest and weakest walls. As a result, since they were located at almost the same position in each radial file of cells, they were aligned in tangential series or linearly. Clearly, the crushed cells are most suitable for marking xylem or making an inscription, because such series or a line of them can be easily identified microscopically or macroscopically. The crushed cells occurred more remarkably in tangential series in Hannoki than in Kusunoki. The difference seems to arise from the difference in xylem cell organization, especially the existence of paratracheal parenchyma cells. In Kusunoki, even if the crushed cells were not arranged in regular tangential series, it is apparent that they are most suitable for marking the xylem.

The walls of the crushed cells indicated the birefringence slightly. Usually, such birefringence implies an accumulation of S-1 or S-3 layer. However, the walls were severely folded by the collapse of the cells. It is well known that the bulked portions in the walls, e. g. compression failures, also show unusual birefringence, because of the disorder in the native orientation of the fibrils. There-

fore, it may be doubtful whether such birefringence results only from the deposition of S-1 layer. Additional observations by electron microscopy appear to be necessary to elucidate exactly the wall structure of the crushed cells.

Generally, the differentiation of a vessel inclines to advance faster than the surrounding cells (IMAGAWA and ISHIDA 1972). In fact, the walls of the crushed vessels were relatively thick and more lignified compared to those of the surrounding cells. Therefore, one of the reasons why their vessels were collapsed seems to arise from their large lumina. In other words, even such thick and lignified walls may be not sufficient to maintain large volumes that would resist any pressure, which occurs after the necroses. While, on the cambium side of the crushed cells, thin walled and unlignified vessels were observed. There were also found almost normal cells which surrounded such vessels. Since such vessels initiated the differentiation to some extent and the surrounding cells had not begun, the former was left in an immature state and the latter was usually differentiated after the stimulation. The occurrence of both kinds of vessels seems to be highly suggestive from a view point of the elucidation of the differentiation of vessels.

In the cells other than the crushed cells among the dead cells, such cells were left in an immature state at the time of their death in the current growth ring. However, they were not collapsed, because their walls were thicker than those of the crushed cells. This is reasonable based on the fact that the cell wall formation of the cells far from the cambium advances furthermore than those near it. In Kusunoki, some of the intercellular layers were not lignified. Whereas, in Hannoki such phenomena werenot found. This difference may possibly reflect the difference in the process of the lignification in both species.

It appears that the abnormal-shaped cells do not result from the death of cells. It is considered that the abnormal-shaped cells derive form the unusual differentiation. Or, unusual cell divisions may have occurred. However, it is presumed that the cambial cells themselves were not directly influenced by the electrical stimulation. Because, the abnormal-shaped cells were extremely scarce and almost normal cells soon occurred again. If the cambial cells are not directly influenced, this marking method would seem advantageous as a experimental method for studying xylem formation, since the normal production of cells is expected after the application of the electrical stimulation.

The processes of the occurrences of the influenced cells can be assumed as mentioned above. As these assumptions are based only on the observations regarding the influenced cells which were already in existence in the growth ring, the actual state of the influenced cells at the time of the application of the electrical stimulation are more or less obscure. With regard to marking the xylem, especially, it seems to be significant to clarify the state of the crushed cells at the time of the stimulation.

In this study, the electrical stimulation was one kind of the experimental condition. In *Pinus luchuensis*, the quality and quantity of the influenced cells were considerably varied due to the experimental conditions, e. g. the voltage, the dura-

tion or the distance between the electrodes (IMAGAWA unpublished). Therefore, it can not be concluded that the experimental condition (154 volts, 5 sec., 5 cm) employed in this study is most effective to mark or make an inscription in the xylem, or to produce the crushed cells in the growth ring. It seems to be important and necessary to pursue the most suitable conditions. The electrical stimulation was applied only once throughout the growth period. Since the processes of xylem formation seasonally vary, the application of the electrical stimulation may be necessary to be examined several times throughout a growth period.

Conclusion

The possibility of new marking method by the application of the electrical stimulation for practical employment was suggested to some extent, though some unresolved problems still remained. By the electrical stimulation, some influenced cells occurred in the current growth ring. Among these influenced cells, the crushed cells seem to be most suitable to mark or make an inscription in the xylem, because of their tangential series. However, the state of the crushed cells at the time of the application of the electrical stimulation is still somewhat obscure in this study. Therefore, additional studies for the identification of the crushed cells at that time must be done. In addition, the experimental conditions to produce effectively the crushed cells must be pursued.

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要 約

樹木の木部形成経過を定量的に把握するための新しい実験手法を検討した。ハンノキとクスノキの成木樹幹に電氣的刺激 (154 DC ボルト, 5 秒間) を 5 cm 離れた電極に与え, それによって材中に何らかのマーキングが可能かどうかを調べた。その結果, その当年生の木部中には種々に影響された細胞が見出された。その主なものは, 押しつぶされた細胞や未成熟なままの細胞や異常な形状の細胞などであった。それらの細胞の状態を詳細に調べ, またその由来したであろう経過についても考察した。その結果, 材中のマーキングとしては, 接線方向に列をなして出現した押しつぶされた細胞が最も適していると考えられた。この実験手法はその電気刺激の条件などの点から, 十分には確立されていないが, その実用に向けての可能性は示唆されたものと考えられる。

Explanation of Photographs

- Photo 1.** Transverse section of Hannoki showing the influenced cells. The crushed cells (large arrow) are arranged in tangential series. The abnormal cells and the immatured cells are seen at the upper and the lower of the crushed cells respectively. The cambium is located upward. arrow: thick walled cell. d: deposit.
- Photo 2.** Enlargement of Photo 2 showing the partially crushed vessel (V). The immatured cells contain the cell contents. The end walls with simple pits are seen in the abnormal cells (arrows). large arrow: crushed cell.
- Photo 3.** Transverse section of Kusunoki showing the influenced cells. large arrow: crushed cell, arrow: thick walled cell, 0: oil cell.
- Photo 4.** Transverse section of Hannoki showing the crushed cells (large arrow) and the immatured cells. Photo 4 b is the polarizing microscopical photograph of Photo 4 a. arrow: S-1 layer.
- Photo 5.** Transverse section of Hannoki showing the thin walled vessel (large arrow) and the crushed vessel with thick wall (V).
- Photo 6.** Radial section of Hannoki showing the influenced ray. Deposits are found in the ray cells. The cambium is located at the right side. large arrow: crushed cell.
- Photo 7.** Radial section of Kusunoki showing the influenced ray. Deposits and extraordinarily elongated ray cell are seen.
- Photo 8.** Transverse section of Hannoki showing the incomplete perforation.
- Photo 9.** Radial section of Kusunoki showing the bud tyloses formed by the electrical stimulation (arrow).

