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New Marking Method by Electrical Stimulation for Studying Xylem Formation*

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*

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Introduction

It is very important to investigate the xylem formation in forest trees. In particular, it has been concerned that the seasonal progress of xylem cell production to form a growth ring is quantitatively clarified. For such reason, not a few method to examine the quantitative progress has been proposed and used. Recently, pin marking method which was originated by WOLTER¹⁾ has been often used because of its simplicity and efficiency^{2~4)}. However, it may still include some unsolved problems^{5,6)}.

In this study, an entirely new marking method by the external application of electrical stimulation was preliminarily examined, and the usable possibility for marking could be suggested to some extent. It seems to be considerably significant

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to show the results obtained hitherto, though this method has not still established sufficiently.

The electrical influence on living trees has been well known by the damages of lightning or contact with electric wires⁷⁻⁹. And in many physiological studies on trees, the electrical resistance was also measured¹⁰⁻¹⁷. However, studies about the influences on xylem cells through which electrical currents flow have been extremely less done, and studies such as the present one in which electrical stimulation is applied to mark or inscribe xylem have been not still encountered. Therefore, in the present paper, the influences on xylem cells as well as the possibility for marking by the application of electrical stimulation are described in detail.

Materials and Methods

As an electric source, two sorts of dry batteries (1.5 and 67.5 volts) were used, and various voltages were applied. In addition to the dry battery, the direct current rectified from alternating current was also applied. As the electrodes setting pins or sewing needles were employed. They were firmly inserted through bark and cambium into wood. Two electrodes were mainly located in vertical orientation on the stems of 8 year-old Japanese firs, *Abies sachalinensis* Fr. SCHMIT, which were grown in pots (Fig. 1). The electrodes were pulled out every application.

After the application of electrical stimulation, the stems were not cut till dormancy. Specimens were collected from the various positions of the stems, i. e. between or near the electrodes. The transverse sections were stained with safranin alone or safranin and fast green. The examinations were done in two growth periods (in 1978 and 1979). Each experimental condition was as follows.

Experiment-1

Electrical stimulation was only once applied at the late of August in 1978. The voltage applied were all 337.5 volts (dry batteries). The electrodes were spaced 1, 3 or 5 cm. apart. In addition, two electrodes were inserted one another at an angle of 90 or 180 degrees at a given height of the stems. The duration of the application of electrical stimulation was very short.

Experiment-2

From May to September in 1979, the electrical stimulation was monthly applied. Two electrodes were always inserted in vertical orientation. They were spaced 6 cm.

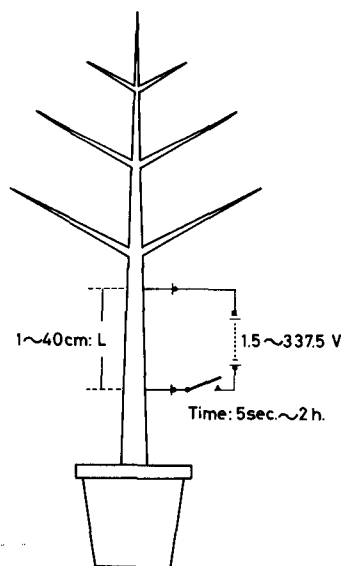


Fig. 1. Diagram of the experimental method. Two electrodes are inserted in vertical orientation to the stem of *Abies sachalinensis* Fr. SCHMID.

apart except for one case (40 cm. apart). The voltages applied were 1.5 or 135 volts by dry battery and 12 volts by rectifier. The duration was varied from 5 seconds to 2 hours.

Results

Experiment-1

Photo 1 shows a part of the current growth ring which was collected from the position between the electrodes, which were spaced 1 cm. apart. At the outer side in the ring, a line which is extended along the growth ring boundary is found. This line is the very mark that is inscribed in the growth ring by the application of the electrical stimulation. This line is composed of the cells which were radially crushed.

The line is extended only about one fourth of the whole circumference of the current growth ring. The middle portion of the line is considerably thicker than the both ends of it, since the electrodes were inserted above or below the middle. That is, more cells at the middle are influenced and crushed in comparison with ones at the both ends. Furthermore, some cells outwardly adjacent to the line are enlarged and abnormal in shape. Consequently, the radial files of tracheids near the line are considerably disturbed.

As the distance between two electrodes increased, such lines became long even at same voltage. In the case of 3 cm. apart, the line was lengthened throughout about three fourths of the whole circumference of the ring, and in 5 cm. it reached throughout the whole.

In addition to the vertical orientation of electrodes, horizontal orientation were also examined. In the case at 90 degrees, the similar line was found only at the fan shape's portion in the growth ring between the electrodes. However, such line was considerably short because many abnormal cells were located near the both electrodes. In 180 degrees, such line was not found at all. Instead of it, resin cell-like cells were observed along the growth ring boundary.

As mentioned above, the possibility for marking by electrical stimulation was obviously indicated. For marking, it was clarified that the electrodes should be inserted in vertical orientation to the stem and the distance between the electrodes should be carefully determined. And it was considered that the application of electrical stimulation at lower voltages should be examined because abnormal cells may be less produced. Referring to these results, the another experiment was designed and performed.

Experiment-2

Photo 2 shows the whole cross section of the stem and the partially enlarged current growth ring of it, on which the electrodes were spaced 6 cm. apart in vertical orientation and the stimulation at 135 volts was applied for 5 seconds. As the sections were cut from the position near the electrodes, the damage by its

insertion can be also observed. In spite of five times applications, only four marks are found. It is supposed that the stimulation in September failed to mark xylem because the xylem production was not advanced after the application.

Not only groups of abnormally enlarged cells but also traumatic resin canals are observed at these marks. In the case of June, July and August, each line is tangentially extended from the each group. Following these lines, bands which are represented by the differences in cell diameter or cell wall thickness are found. The band in June is most remarkable among them, and it is extended throughout nearly whole circumference. In May, however, even such line does not follow the abnormal cells. It may be reasonable to consider seasonal factor, though it is obscure why such influences do not result from the same stimulation.

Photo 3 shows the whole cross section from the same stem as Photo 2. However, this section was cut from the middle position between the electrodes. Although the influences are not so clearly shown because of the low magnification, one line throughout the whole circumference and two bands at the inner side of it can be found in the current growth ring. The line results from the stimulation in August, and two bands correspond to those in June and July respectively. The influences of the application in May are not observed at all. In comparison with the section near the electrode (Photo 2), the influences are more or less weakly represented. However, the appearance of them are extremely simple because abnormal cells are not seen. Especially, the line in August is very remarkable and it seems to be satisfactory for marking. Although it is obscure why only the influence in August is more remarkable than the others, it is obvious to be able to mark xylem by such experimental condition.

Photo 4 shows a part of the current growth ring from the same position as Photo 3. The cambium is partially observed at the upper side in the photo. Near the growth ring boundary, one or two cells which are radially crushed are located and they are tangentially continuous. This tangential row of the crushed cells corresponds to the line which is found in Photo 3. These crushed cells are extremely thin walled, and their walls are not fully lignified judging from the staining. And also two or three cells inwardly adjacent to the crushed cells are considerably thin walled and low lignified. In addition, they still contain remainders of the protoplasts, though they are gradually lost in the process of usual differentiation. While, one or two cells outwardly adjacent to the crushed cells are radially enlarged, and they present earlywood tracheid-like in shape. Moreover, ray cells adjacent to such effected cells are also influenced by the stimulation. They are locally swollen and bent. Some of them include deposits in their lumina (double arrows).

At the middle in Photo 4, a few of crushed cells are also found (arrows). They may be unsatisfactory as a marking line because they are not continuous tangentially. As well as in August, these crushed cells and two or three cells inwardly adjacent to them are incompletely differentiated. The influences by the stimulation are not represented in the other radial files except for ones in which the crushed cells are located.

At the lower in Photo 4, one or two crushed cells are also found. Some of them are tangentially continuous but such continuities are very short. Two or three cells outwardly adjacent to the crushed cells are somewhat flattened and relatively thick walled. It is still obscure why the influences are reverse to those in August.

Judging from these results, it is deduced that the electrical stimulation at some level acts to kill the living cells at some stage during differentiating processes. Consequently, the cells in which wall formations are most insufficient become radially crushed by some pressure produced after their death. And when crushed cells are tangentially continuous, a marking line is distinctly inscribed in the current growth ring. Although some influences except for such collapse of cells are also observed in the growth ring, for the sake of marking it seems to be most important to establish the experimental condition under which the tangential row of crushed cells are certainly occurred. And also for its sake, it seems to be necessary and significant to show the other results obtained under the extremely different conditions. Thus, the followings are described.

When the electrical stimulation at 1.5 volts was applied for 10 minutes, crushed cells were not found at all but influenced cells were observed. Their walls were insufficiently lignified and the remainders of their protoplasts were also seen in their lumina. In addition, some enlarged cells almost tangentially aligned. Therefore, it is considered that such experimental condition is inadequate to mark xylem.

When the electrical stimulation at 12 volts was applied for an hour, the tangential rows of traumatic resin canals developed remarkably. Most of them were extended more than half of the whole circumference of the current growth ring. The bands mentioned above were always following these tangential rows. Some of the epitherial cells surrounded the canals included deposits. Thus, it is considered that such condition is also unsatisfactory to mark xylem because of the remarkable development of traumatic resin canals.

In addition, when the electrical stimulation at 12 volts was applied for two hours on the electrodes which were spaced 40 cm. apart, many resin cell-like cells were found only at the position near the electrode in 2 year-old internode. They were concentrically aligned and each concentric row corresponded to each stimulation. Consequently, it is considered that such condition is also not enough to mark xylem.

Discussion and Conclusion

As mentioned above, the measurements of electrical resistance have been done in many physiological studies on trees. According to STONE⁷⁾, next to the cambium the phloem had the least resistance, following by the sapwood. In addition, SHORTLE et al.¹⁰⁾ reported that least electrical resistance was determined to be the wood-bark interface (the cambial zone). Moreover, GLERUM and KRENCIGLOWA¹¹⁾ mentioned that the cytoplasm and vacuoles have a very low resistance, as a result of the examinations in *Picea glauca*, *Pinus resinosa*, *Larix laricina*, *Populus deltoides*,

Betula papyrifera and *Acer saccharium*. In this study, therefore, it is also supposed that most of the electrical stimulation, i.e. electric currents flow mainly through the cells in the cambial zone, in which full protoplasts function actively.

While, it can be deduced that the presence of an externally applied electrical flow through plants cause an electrical reaction since they are alive¹⁰. According to PEACE⁸, experiments with electric currents on small trees have shown that they can be killed by a current too small to cause any burning, and also that trees subjected to a current insufficient to kill them often develop abnormal tissue of the type found in the affected branches of the trees surrounding the dead trees by lightning. And also, STONE⁷ examined the influence of direct current on the stem of *Rhus taxicodendron* L., and found that the current burned out the cambium or vital layer of it. Consequently, he deduced that all the injuries were due to the effect of heat generated by the current. In this study, therefore, it may be is also considered that when direct currents flow through the cells in the cambial zone, they may be killed by the heat, i.e. JOULE's heat generated in them. It is obvious that the dead cells remain thin walled because the followed differentiations such as secondary wall formation or lignification are not occurred. Therefore, by some pressure produced later, such thin walled cells become radially crushed to inscribe xylem as a marking line. While, some of the dead cells in which secondary wall formation or lignification has advanced to some extent are not crushed because of their rigidities. But they also remain unchanged in their differentiating stages at the time of their death. Although it is still obscure which cells in the cambial zone are killed by the stimulation, it is supposed that at least a part of cells with the capacity of cell divisions are not killed, because many new cells were produced after the application of the electrical stimulation.

According to WILNER¹³, in *Mulus robusta* the electrical resistance increased as the growing season advanced from summer to fall. And also DAVIS et al.¹⁷ mentioned that in *Acer rubrum* L., *Quercus rubra* L. and *Pinus strobus* L. the measurements of electrical resistance were lowest in the summer when metabolic processes are most active, and the highest in the winter when metabolic processes are least active. Such difference of electrical resistance may be one of the reasons why the appearances of the influences on the xylem are not always same but various in the each application throughout one growth period as shown in the experiment-2.

In this preliminary study, the usable possibility to mark or inscribe xylem by an electrical stimulation was obviously suggested to some extent. Practically, the stimulation at 135 volts for 5 seconds could inscribe the xylem by the radially crushed cells. But it can not be concluded that this experimental condition is most effective for marking. Therefore, in order to confirm the most effective condition, various points such as the extent of electrical stimulation, the distance between electrodes, the sorts or the shapes of the electrodes, or the duration of the application must be extensively investigated hereafter. In addition to direct currents, the application of alternating currents may be necessary to be examined.

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摘 要

樹木の木部細胞生産の量的経過を明らかにするための実験手段として、電氣的刺激を与える新しい方法が予備的に試みられた。8年生のトドマツ樹幹に刺激を与えた結果、その材中には二・三の影響が見出された。すなわち、幾つかの細胞は分化途中で殺され、未成熟のままであった。そのような未成熟のままの細胞のなかで、その壁が最も薄い細胞はその後に生じた圧力により半径方向に押しつぶされた。押しつぶされた細胞が接線方向に連続して生じた場合には、その年輪中に一本のラインが認められ、それは印づけとして用いることができると思われた。その他にも刺激により影響を受けた細胞は見出されたが、印づけとしては十分でないように思われた。本実験の結果では135ボルトで5秒間刺激した場合に生じたラインが印づけのためには最も適しているように思われた。電氣的刺激による印づけの方法、特にその処理条件はまだ検討しなければならない点は多くあると考えられるが(例えば、刺激の強さ及びその時間、電極間の距離、電極の質や形状など)、その実用的可能性は十分に示唆され得たと思われる。

Explanation of photographs

- Photo 1.** A part of the current growth ring. The electrical stimulation at 337.5 volts were only once applied at the late of August in 1978.
- Photo 2.** Partially enlarged current growth ring (upper) and the whole section of the position near the electrode in the stem. The electrical stimulation at 135 volts were monthly (month-day) applied for 5 seconds from May to September in 1979.
- Photo 3.** Whole cross section between the electrodes in the same stem as Fig. 3.
- Photo 4.** A part of the current growth ring from the same position as Fig. 4. Arrows show the crushed cells and double arrows the ray cells including deposits.

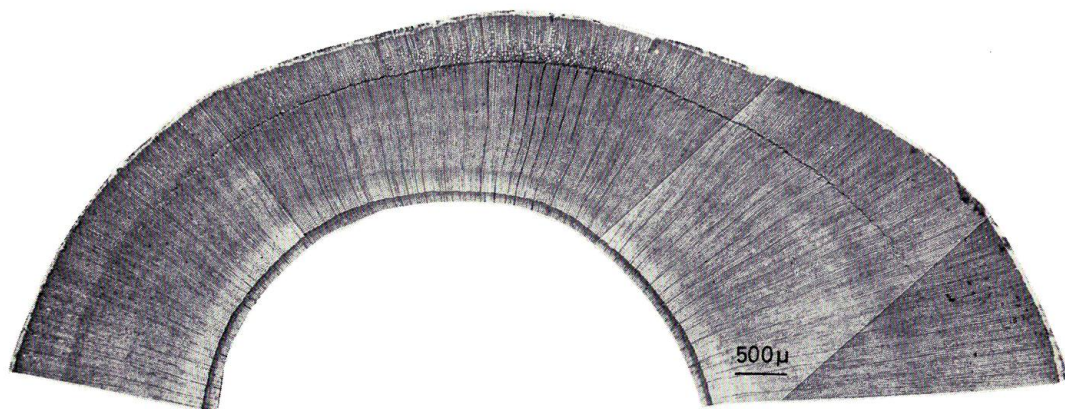


Photo 1

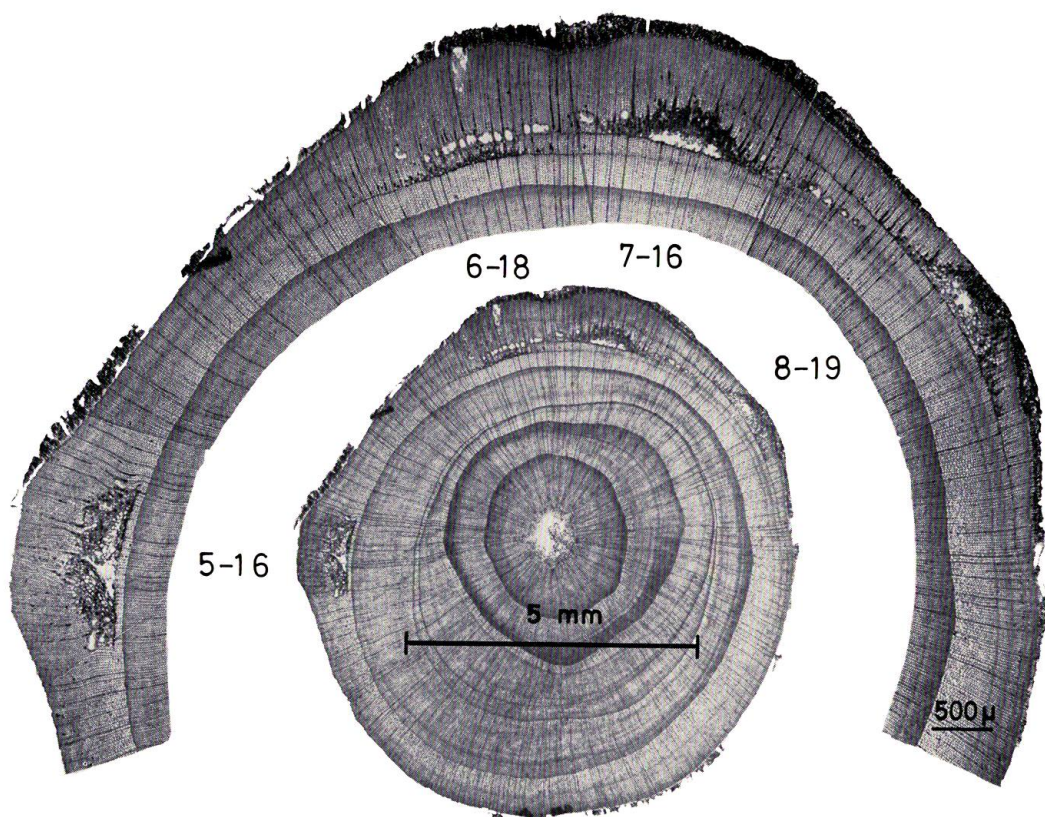


Photo 2

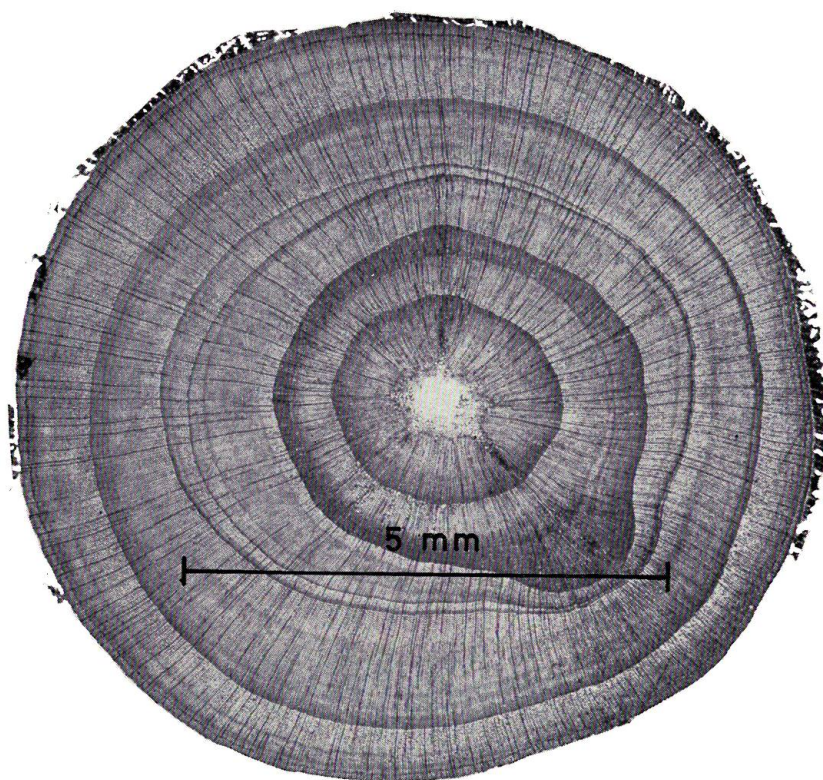


Photo 3

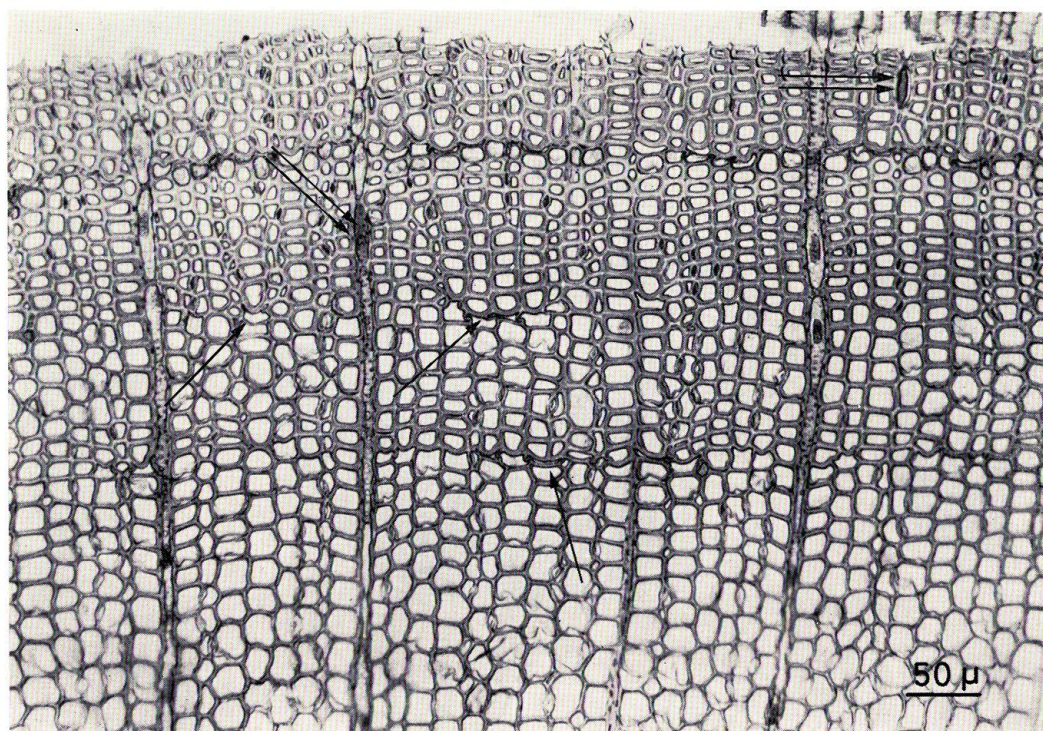


Photo 4