Preliminary Experiment of Relationship between Electrical Resistance and State of Cells in *Pinus luchuensis* Stems*

By

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リュウキュウマツ樹幹の電気抵抗と細胞の状態との関係の予備的実験*

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Introduction

For studying the processes of xylem formation of forest trees, new marking method in which some cells were influenced by externally applied electrical stimulation was examined, and the possibility for marking xylem was suggested (IMAGAWA and ISHIDA 1981). In a series of these experiments, it was very interesting that the electrical current or resistance measured was almost usually variable at each position even in the same stems.

In recent years, electrical measurements of trees are receiving much attention as useful indicators of various pathological states of trees (TATTER 1974, TATTAR et al. 1974, SHIGO and BERRY 1975, TATTAR and BLANCHARD 1979, NEWBANKS and TATTAR 1977, MALIA and TATTAR 1978, SYLVIA and TATTAR 1978, BLANCHARD and CARTER 1980). While, electrical resistance had been often measured to evaluate the extent in growth or vigor of forest trees by ZHURAVLEVA (1972),

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WARGO and SKUTT (1975) and SMITH et al. (1976). And in relation to phloem or inner bark width, electrical measurements were also taken (CARTER and BLANCHARD 1978, COLE and JENSEN 1979, DAVIS et al. 1979, COLE 1980). Most of these studies were done by the employment of commercially available field ohmmeter, Shigometer (SHIGO and BERRY 1975).

Judging from these investigations, especially about growth or vigor, it is presumed that the variations of electrical current or resistance measured in our experiments may be derived from any differences in the states of cells in or near the cambium. To our knowledges, however, the variations of electrical readings have never been investigated from such view point. In this study, therefore, the electrical resistances were preliminarily examined in relation to the states of cells, especially the numbers of cells, at each position in the stems measured.

Materials and Methods

Electrical measurements were taken on the stems of Ryukyu-Matsu, *Pinus luchuensis*, grown at the campus of Ryukyu University, Okinawa, Japan. Six trees measured ranged from 12 cm. to 30 cm. in diameter at breast height, and from 5 m. to 8 m. in height.

Two electrodes in vertical orientation were deeply inserted through the bark into xylem at a right angle to the stem axis. The electrodes were spaced 5 cm. apart, and connected via alligator clips to the wires from a D. C. ammeter (Fig. 1). Maximum readings of the electrical current were determined for 5 seconds. The electrodes were commercial dressmaker's pins (0.51 mm. in diameter, 28.8 mm. in length) as used by BLANCHARD and CARTER (1980). Depending upon Ohm's law, electrical resistances between the two electrodes were calculated from the maximum readings of the electrical currents determined and the voltages applied by dry batteries (about 150 volts).

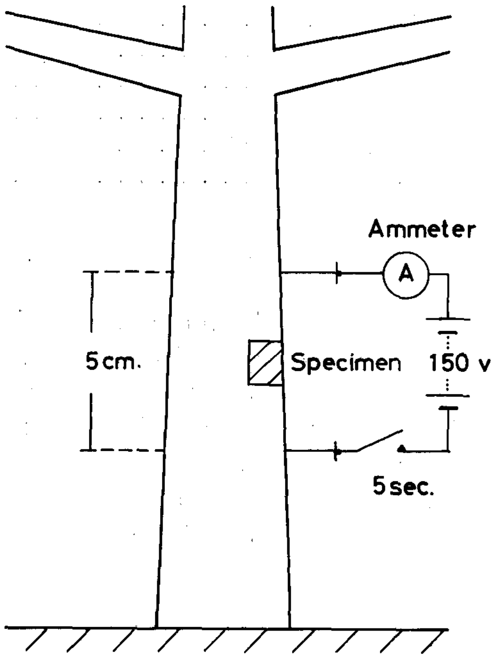

Fig. 1. Diagram of measuring the electrical current of stem.

Electrical measurements were taken five times (Oct. 17, 30; Dec. 4 in 1980 and Jan. 6, 17 in 1981). At first, measurements were done at two portions in each of three stems; second, at four in two; third, at four in three; fourth, at four in two; fifth, at four in two. Immediately after the each measurement of electrical currents, one specimen obtained xylem, cambium and phloem was punched out between two electrodes. The specimens were fixed in FAA, and embedded in celloidin. Transverse sections were cut and stained with safranine and fast green.

In each of ten radial files of cells through xylem, cambium and phloem in transverse section, each number of immatured tracheids, cambial cells and phloem cells was counted, and the average numbers were calculated. Immatured tracheids are cells during differentiation, and have unsatisfactorily lignified thin walls and contain protoplasms in their lumina. Cambial cells are tangentially flattened cells between immatured tracheids and phloem cells. Phloem cells are located between cambial cells and radially crushed phloem cells which were produced in the previous growth period. And morphological observations were also simultaneously done by a light microscope.

Results

ODA and NAKASONE (1978 and 1979) investigated the seasonal development of xylem in *Pinus luchuensis* at Okinawa in the subtropical zones. They described that the latewood began to form from the middle of July and typical dormancy

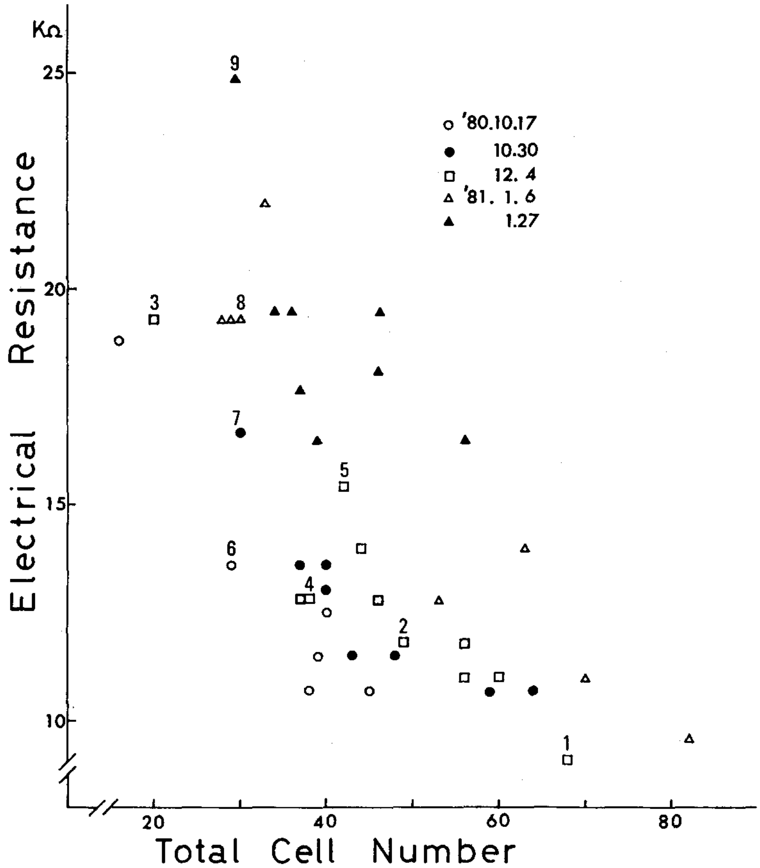

Fig. 2. Scatter diagram of electrical resistance vs. total cell number (immatured tracheids, cambial cells and phloem cells).

failed to be found in *Pinus luchuensis.* In this study, thus, electrical measurements and collections were taken during the formation of latewood.

At first, the numbers of matured tracheids which had lignified thick walls were also counted. However, any relationships were not found between the numbers and the electrical readings. Therefore, the numbers of matured tracheids were eliminated from the total cell number, which summed up the numbers of immatured tracheids, cambial cells and phloem cells. Usually it is relatively difficult to identify cambial cells. Thus, tangentially flattened cells between enlarging tracheids and phloem cells were regarded as cambial cells in this study.

In Fig. 2, the relationship between the total cell numbers and the electrical resistances is shown. The vertical and horizontal axis indicate the electrical resistance (kΩ) and the total cell number respectively. The dates of the measurements and the collections are also noted. As shown in Fig. 2, a tendancy that the total number increases with the decreasing electrical resistance seems to be found, though some scatterings are also observed. That is, it is considered that the numbers are negatively correlated with the electrical resistances. In living trees, generally, it is impossible to assume that such cells counted are completely absent and electrical resistance becomes zero. Therefore, the regression curve may be hyperbolic, though it was not calculated in this preliminary study because of fewer data and some experimental problems to be unresolved.

It appears very interesting to observe microscopically the cells in such specimens that showed definetely the negative correlationship. Photo 1 a, 1 b and 1 c are transverse sections from the specimens which are numbered 1, 2 and 3 respectively in Fig. 2. These specimens were collected from three stems on the same date. Each electrical resistance were 9.1, 11.8 and 19.3 kΩ. In particular, specimen-3 is about 10 kΩ higher than specimen-1. As shown in each photo, the states of cells in or near the cambium are considerably different each other.

In specimen-1 which indicated the lowest resistance, thin walled cells are abundantly observed at the lower half in Photo 1 a. They are immatured tracheids (I), and their walls are not sufficiently lighified. Protoplasms are almost usually found in their lumina. Tangentially flattened and thinnest walled cells are cambial cells (C). Their walls appear moistened, so that the cambial cells seem to be active. Upward adjacent to the cambial cells, many phloem cells (P), for instance sieve cells or phloem parenchyma cells, are located. On an average, 36 immatured tracheids, 7 cambial cells and 25 phloem cells were existed in one radial file of cells (68 cells in all).

On the other hand, in specimen-3 which indicated highest resistance (Photo 1 c), the states of cells observed are considerably different from Photo 1 a. Only fewer cambial cells are observed. Their appearances are similar to so-called dormant cambial cells, which are extremely flattened and have thick walls. Immatured tracheids are also very fewer, and their walls are relatively thick, as if in the temperate zones typical dormancy begins soon. Phloem cells are also fewer. On an average, 5 immatured tracheids, 4 cambial cells and 11 phloem cells were located (20 cells in all).

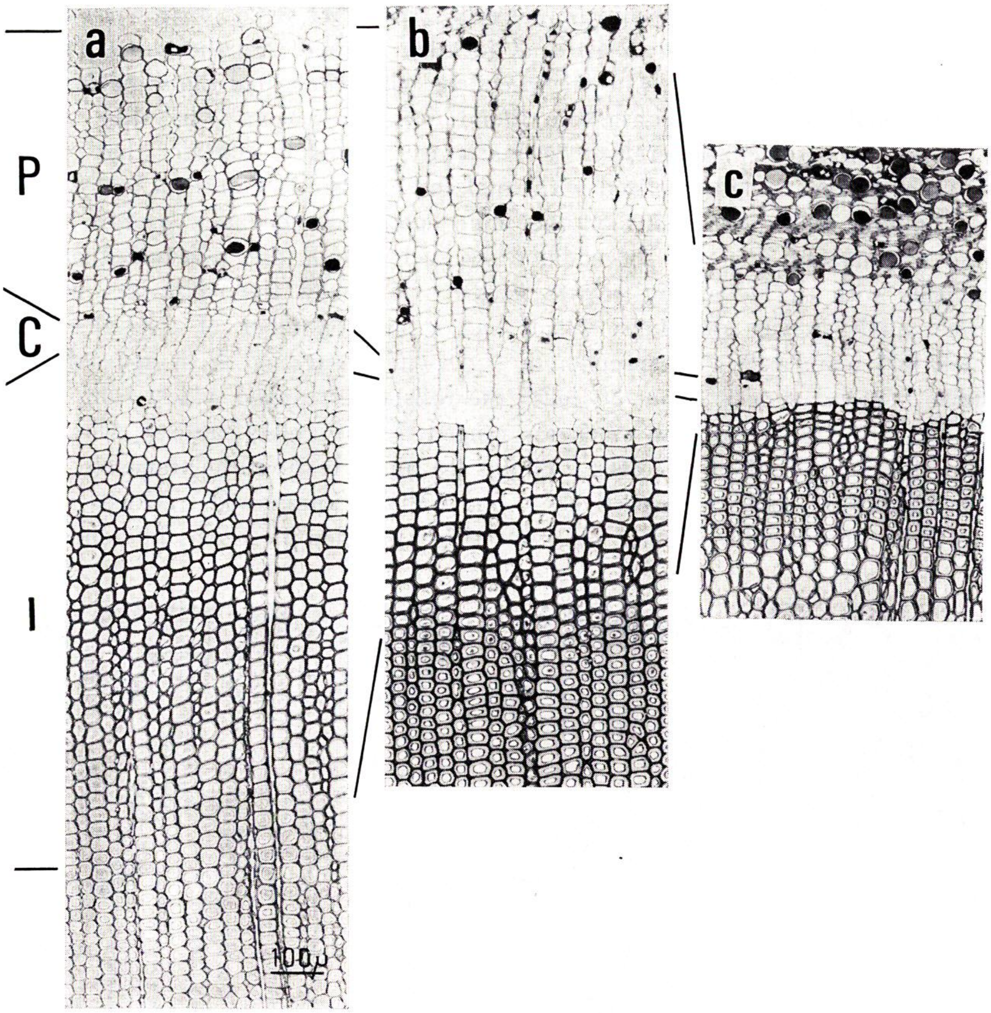

Photo 1. Transverse sections from the specimens in Fig. 2. a, b and c correspond to specimen-1, -2 and -3, respectively. P; Phloem cells, C: Cambial cells, I: Immatured tracheids.

Photo 1 b is the transverse section of specimen-2, which indicated middle resistance between those of specimen-1 and -3. In comparision with specimen-1, immatured tracheids become tangentially more flattened and have thicker walls. Judging from the appearances of the cambial cells, their activities seem to begin to be reduced gradually. However, the states of cambial cells appear more similar to those in specimen-1 than specimen-3. On an average, there found 20 immatured tracheids, 6 cambial cells and 23 phloem cells (49 cells in all).

In these specimens, the states of cells were densely correlated with the electrical resistances. However, some exceptions were also found. For instance, in specimen -4 and -5 in Fig. 2, the electrical resistance of specimen-4 (12.5 kΩ) was lower than specimen-5 (15.4 kΩ). Nevertheless, the number of cells in specimen-4 (38 cells) was also fewer than specimen-5 (42 cells). In other words, both the resistance and the number of specimen-5 were all together higher. Furthermore, the numbers were often different even in the specimens which indicated the same resistance.

Although some exceptions were found and the reasons why they occurred were not still resolved, generally it seems to be allowed to consider that electrical resistance is negatively correlated with total cell number in *Pinus luchuensis* stems. And limiting to the specimens on the same date, such tendancy appears furthermore emphasized (Fig. 2). In addition to such trend, the resistances increased gradually with the advancing growth period. Especially, the resistances increased more rapidly in the specimens which had a small number of cells than those which had many cells. In specimen-6, -7, -8 and -9 in Fig. 2, in spite of almost similar number of cells (about 30 cells), the resistances ranged widely from 13.6 kΩ to 24.9 kΩ. And immatured tracheids ranged 6 to 9 cells; cambial cells, 5 to 6 cells; phloem cells, 16 to 17 cells. Photos 2 a and 2 b show the transvere sections from specimen-6 and -9, respectively. The walls of immatured tracheids in specimen-9 are considerably thicker than those in specimen-6. Such difference seems

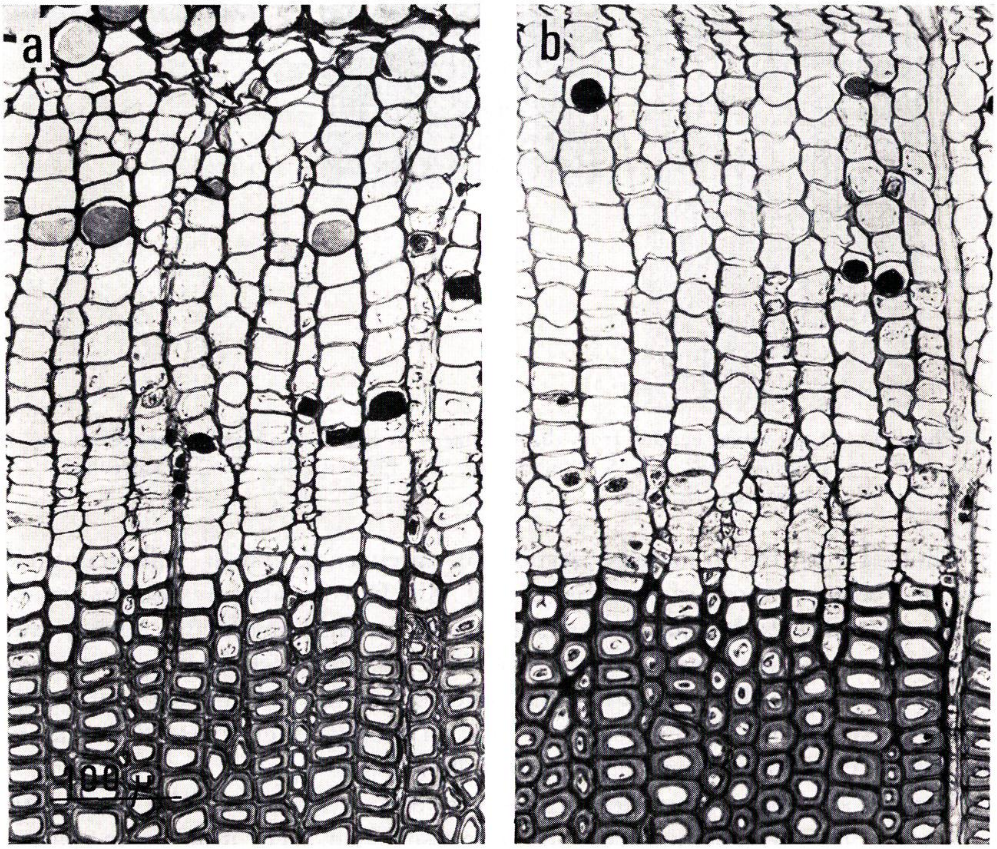

Photo 2. Transverse sections from the specimen-6 and -9 in Fig. 2. a and b correspond to the specimen-6 and -9, respectively.

to be derived from the seasonal changes in rate of the wall formation of differentiating tracheids. It has been known that electrical resistances of tree stems are seasonally variable (GLERUM 1973, NEWBANKS and TATTAR 1977, DAVIS et al. 1979). Thus, seasonal changes in rate of wall formation may be one of the reasons for the seasonal variation of electrical resistance.

As mentioned above, the numbers of cells are summed up each numbers of immatured tracheids, cambial cells and phloem cells. In regard to xylem formation, the number of immatured tracheids are most noticeable and significant. Therefore, if the number of them in stem can be nondestructively estimated, it is very meaningful and interesting. From such view point, the relationship between the electrical resistance and the number of only immatured tracheids was tried to be examined (Fig. 3). As well as in the case of total cell number, negative correlationship seems to be suggested to some extent.

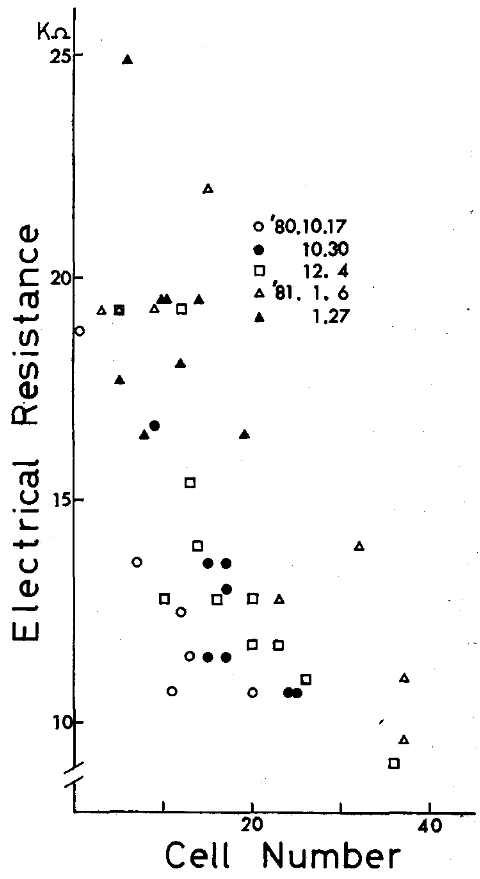

Fig. 3. Scatter diagram of electrical resistance vs. cell number of only immatured tracheids.

Discussion

As a result of the electrical measurements of *Pinus luchuensis* stems, the electrical resistances showed a tendancy to decrease with the increasing numbers of cells, which summed up the numbers of immatured tracheids, cambial cells and phloem cells. In addition to the total number, such trend was also found between the resistances and only the numbers of immatured tracheids. In particular, the latter relationship may be significant and interesting, because immatured tracheids are directly contributory to xylem formation.

It has been described that electrical current flows easily through cambial region or outermost wood (WARGO and SKUTT 1975), wood-bark interface (SHORTLE et al. 1977) or phloem along with associated cork cambium and vascular cambium (CARTER and BLANCHARD 1978). The cells in such tissues are mainly composed of aqueous solutions where the flow is accomplised by a movement of ions (FENSON 1966, TATTAR 1974). And also SHORTLE et al. (1977) mentioned that electrical resistance depends strongly upon the concentration of mobile cations, especially potassium. In *Pinus luchuensis* stems examined, therefore, it also seems to be able to be presumed that most of the externally applied current flows chiefly through the liquid-rich tissues such as immatured tracheids, cambial cells and phloem cells.

Electrical current is directly related to the conducting path area (FENSON 1966) or the cross-sectional area (LEVENGOOD 1973). Based on this relationship, thus, it appears possible to estimate the states of the cells in or near the cambium in forest trees by electrical measurements. Practically, ZHURAVLEVA (1972), WARGO and SKUTT (1975), SMITH et al. (1976) and SHORTLE et al. (1977) measured electrical resistances of forest trees and mentioned that the poorer, slow-growing trees had higher resistance than the fast-growing trees. However, they did not examined the reasons for the difference in resistance from the morphological view of the states of cells in tissues through which electrical current flows. Therefore, it seems to be very significant to elucidate the relationshop between the electrical resistance and the states of cells in stems. Because, the states of cells, especially the number of cells, in stems can be nondestructively estimate, if such electrical method as employed in this study is fully established.

However, some experimental problems remain still to be unresolved. Although LEVENGOOD (1973) described that electrical current variations are not simple function of changing temperature, in general, it has been well known that increase in temperature results in the decrease of electrical resistance (FENSON 1966, GLERUM 1969, TATTAR and BLANCHARD 1976, NEWBANKS and TATTAR 1977, SHORTLE et al. 1977, DIXSON et al. 1978). And seasonal variation of electrical resistance is also reported by GLERUM (1973), NEWBANKS and TATTAR (1977) and DAVIS et al. (1979). In this study, in fact, the electrical resistance increased with the advancing growth period, in spite of the absence of typical dormancy such as in the temperate zones (ODA and NAKASONE 1978, 1979). Therefore, it is important that electrical readings should be taken only on warmer days during the growing seasons, when the temperature is reasonably constant, and only electrical resistance measurements on any one day should be compared as described by SHORTLE et al. (1977). In our additional studies, more measurements and collections should be taken on each one day throughout one growth period. And another experimental conditions, for instance shorter duration of electrical application, shorter distance between electrodes, lower voltage, should be examined.

Conclusion

The possibility that electrical measurements of tree stems may be able to estimate nondestructively the states of cells in the tissues through electrical currents flow is suggested to some extent in this preliminary study. Although the availability or efficiency of this method is apparent, however, some problems remain still to be unresolved. When this method is theoritically and experimentally proved and confirmed in additional studies, it is expected to contribute extensively to the elucidation of xylem formation in forest trees.

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要　　約

リュウキュウマツ (*Pinus luchuensis*) 樹幹の各部位の電気抵抗値と，その部位中の形成層付近の細胞の状況，特にその細胞数とを比較，検討した。

電気抵抗は樹幹上に 5 cm 間隔で十分に深く差し込んだ電極に約 150 ボルトの電圧を 5 秒間かけ，その間の最高電流値から算出した (Fig. 1)。測定直後に電極の中間部より試料を打ち抜き，光顕用切片を作った。横断面切片上で，未成熟仮道管，形成層細胞，師部細胞の数を測定した。

その結果，細胞数が増加するにつれ，抵抗値は減少して行った。すなわち，両者は負の相関関係にあることが示された。また，未成熟仮道管の数だけと，抵抗値との間にも同様の関係が示された (Fig. 2, 3, Photo 1 a, b, c)。

抵抗値には季節的な変動があるなど (Photo 2 a, b)，この測定法にはまだ幾つかの未解決な問題点が残っている。しかしながら，この方法が十分に確立されれば，非破壊的に樹幹内部の分化帯の状態を推定することが可能になり，木部形成経過の解明に大きく貢献することが期待できる。