Time Requirement for the Tracheid Differentiation in Softwoods Measured by the Inclination Date Marking

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傾斜刺激法による針葉樹仮道管分化過程の所要時間の推定

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Résumé

Date markings by the short period inclinations of a stem were attempted on the developing xylem of 6-9 year old trees of *Cryptomeria japonica, Chamaecyparis obtusa* and *Pinus thunbergii*. The increment of tracheid numbers during the growing season and the rate of tracheid production per one day were calculated by the date marker of faint compression wood arcs corresponding to the artificial inclination of stems. At the same time, the cell number contained in the differentiating xylem was examined in trees which had some date markers in April and May and were harvested in June. Using the number of tracheids contained in their developing stages and the rate of tracheid production, the time required for a tracheid differentiation was deduced. In the case of *Cryptomeria,* the time is as follows: about 3.7 days for the radial enlarging after the cell division; 3.7 days for the S_1-deposition; 8.0 days for the S_2-deposition; 2.8 days for the S_3-deposition; 4.7 days for secondary wall lignification. The total time for the differentiation of a tracheid results in 23 days. In *Chamaecyparis* the times are 2.8, 2.8, 7.7, 2.1, and 2.6 days corresponding to the stages of radial enlargement, S_1, S_2, S_3, and secondary wall lignification, respectively. The total one is 18 days. In *Pinus* the total time was only roughly estimated about 22 days because of missing of some date markers.

要　　旨

針葉樹の仮道管が形成層帯で誕生してから，発達した二次壁を完成して成熟するまでに必要とする時間を，スギ，ヒノキ，クロマツの６－９年生の若木で測定した。すなわちこれらの樹幹を定期的に，短時間傾斜させることにより，木部にかすかな圧縮あて材のデートマーキングを記録させ，ここから算出される仮道管生産速度と，分化帯に含まれている仮道管の数を用いて分化過程に要する日数を求めた。スギにおいては，放射方向の拡大に約３．７日，S_1堆積に３．７日，S_2

堆積に8．0日，S_3堆積に2．8日，二次壁の木化に4．7日，分化過程全体に合計約23日の値を得た。ヒノキでは同様の過程に，それぞれ2.8，2.8，7.7，2.1，2.6日，合計約18日の値を得た。

クロマツについては，一部のマーキングに失敗したが，概略値として，合計約22日となり，3樹種の結果から，針葉樹仮道管の分化にはおよそ3週間を必要とすることが明かとなった。

Introduction

Tracheids occupying the major part of softwood xylem are produced from the fusiform initials in the vascular cambium and developed to the matured ones through diffentiation process. It is, however, impossible to trace the whole differentiating process of a tracheid in a living tree, because the differentiating cells are nessesary to be killed and sliced prior to the observation under microscopes. This sequence, therefore, must be deduced from a series of differentiating cells using some assumptions. Tracheids of the softwood are produced from the cambial cells by a regular way of periclinal divisions. The differentiation of these young cells is orderly performed without any disturbance of cell arrangement, in comparison with the case of hardwoods in which enormous expanding of vessel elements and also remarkable elongation of wood fibers affect severely the arrangement and differentiation of other cells. Especially in spring, the cell production in the cambial zone and the following differentiation process are also constant, so that the whole differentiating process of a tracheid could be deduced from a series of immature tracheids from the cambial zone to the mature xylem along a radial row, apart from the time passage of them. The series of differentiating tracheids, as the substitution of differentiation of a living tracheid, has offered many informations on the tracheid development.[1)~3)] Then, the developing process of tracheids is divided into several developing stages. The first one is the cambial stage (C-stage) where the multiplication of fusiform cells proceeds constantly in spring. The derivatives pushed out toward xylem-side from the C-stage begin the expanding of their cell size. In the case of softwood tracheids the expanding occurs mainly to the radial direction, so that this stage is probable to be called to the radial enlarging stage (RE-stage). The tracheids start the secondary wall thickening, namely, the deposition of S_1, S_2, and S_3 after the radial enlargement. Although the construction of microfibrillar framework has been completed during these three stages, the secondary wall is still flexible, because of the porous structure. The spaces within the framework are encrusted with lignin mainly after the S_3-stage. We call the process final stage (F-stage). In the F-stage the secondary wall of tracheids becomes very rigid. As described above, a tracheid is considered to differentiate passing through the five developing stages, namely, RE-, S_1-, S_2-, S_3-, and F-stages after the derivation from the C-stage.

Then, how long does a tracheid require to differentiate via the five developing stages? This is a more difficult problem to be traced than the case on the developing process of a tracheid which was mentioned above. This subject has been approached mainly by the periodical samplings of wood blocks from a same or different specimen trees.[4)~7)] The increasing curve of tracheid numbers in the current annual ring is drawn from microscopic measurement on the periodical samples, and the rate of tracheid production per one day is introduced from the curve. Using the rate and number of developing tracheids which were contained in the differentiating xylem, the time requirement for the tracheid differentiation has been calculated. Results obtained there are so fluctuating from about 2 weeks to 2 months that it is difficult to grasp the general time requirement for the tracheid differentiation. It seems to be the method of periodical samplings which cause such inconsistency. The increment of tracheids may be different in positions even in a tree, to say nothing of that between different specimen trees. Also the taking out of a wood block from a portion of living trees affects severly to growth of cambium in the wide area around the portion and results in abnormal growth of tracheids there. Therefore, many problems are remained in the results obtained by the periodical samplings. An interesting approach in which internal date markers were recorded on xylem tissue was tried by Kennedy and Farrer.[8] Repeatedly inclined seedlings of 6 months made distinct compression wood arcs on the under-side of inclination. Using the arcs as the internal date marker and the cell number contained in the developing xylem the time for differentiation was calculated. Although this approach is elegant, it is a question whether the result obtained in seedlings of only 6 months can be applied directly to ordinary big trees. The treatment of their inclinations seems to be considerably severe to the development of normal tracheids.

The observation of differentiating xylem in previous papers also seem to be not so precise in relation to the developing stages. The present authors have examined in detail the developing process of compression wood,[9][10] in addition to that of normal wood, so that the method of Kennedy and Farrer[8] on seedlings can be extended to young trees, which are more probable material than the seedlings, by very short period inclinations not to disturb the differentiation of normal wood tracheids. The time requirement will be corresponded to the more detailed developing stages, namely, RE-, S_1-, S_2-, S_3- and F-stages.

Materials and Metohds

Young trees of SUGI (*Cryptomeria japonica* D. Don; 5 specimen trees; 9 years old; about 3 m high), HINOKI (*Chamaecyparis obtusa* Endl.; 5 specimen trees; 7 years old; about 3.5 m high) and KUROMATSU (*Pinus thunbelgii* Parl.; 3 specimen trees; 6 years old; about 2.8 m high), which had been grown at the nursery of the Experimental Forests of Kyoto University, were offered to both short period inclinations for the internal date marking and long period inclinations. In the most of the former experiments, the internal date markings were started on April 12th by the 4 days inclination and followed by the 1 day treatment on April 26th, and then by the 2 day treatment from May 10th. This set of treatment, namely, 4-, 1-, and 2-day inclinations were continued to September, although a part of specimen trees were cut down on June 7th for the measurement of time requirement of the tracheid differentiation. Some examples are shown in Figs. 5-8. The inclination periods are represented on the holizontal axis of these figures. Specimen trees of the long period inclinations were leaned three times, namely, April 12th-May 12th, June 12th - July 12th and August 12th - September 12th. In all experiments the stem of specimen trees were fixed with a long splint without the bottom position and kept at 15 or 45 degrees from the vertical direction by leaning of the splint. Although the bottom position of each stem was curved, other parts were preserved straight along the leaned splint during the treatment. After the inclination the leaned stems were recovered to the original vertical position with the splint until the next treatment. After the harvesting, the specimen trees were separated into small blocks, soaked into gltaraldehyde fixative and preserved in a refrigerator. Some parts of the blocks were embedded in the epoxy resin after the post-fixation with osmium tetroxide.

Wide sections of 30-40 μm thick containing overall transverse section of a stem (see Figs. 1A and 3) were taken at the various points from the top of the stem to the bottom, and mounted in canada balsm. Succeedingly, thin section of 2 μm thick and ultrathin sections of about 0.1 μm thick were taken from the embedded specimen block by Sorvall JB-4 semiultramicrotome and Porter MT-1 ultramicrotome attached with a glass knife or a diamond knife. The thin sections were put on a glass slide and stained with safranin[10] and observed by ordinary light (Figs 2 and 4) and polarization microscopes. The ultrathin sections were stained with uranyl-acetate or shadowed by Pt-Pd alloy, and examined by a transmission electron microscope (JEM-7).

Results and Discussion

First of all, the effect of periodical inclination for the internal date marking was traced on the current annual ring, in addtion to the examination of influences of the artificial treatments on the cambium. The periodical marks could be detected as dark colored bands, namely, feeble compression wood arcs. These arcs were the most noticeable at one side on the overall transverse section of a stem where the cambium was forced to be lower-side during the inclination, and spread to both lateral-sides (Figs. 1A and 3). As the width of arcs at the lower-side was affected by the period of inclination, so each marker could be identified without confusion with the next date marking. For instance, a set of inclinations of 4 days (April 12th) , 1 day (April 26th) and 2 days (May 10th) was expressed by a series of wide-, narrow- and medium-sized arcs, respectively (Fig. 1B). Then, the occurrence of arcs was surveyed along the stem axis from the top of a tree to the bottom. As the result, the arcs were wide and clear near the top and became weaker to the bottom. This implies the upper position of a stem is more sensitive to the stimulus of an inclination. The limit where the arc of one day inclination could be surely detected was about 2 m from the top in SUGI and 2.5 m in HINOKI. However, the compression wood arc was weaker in KUROMATSU, so that the one day arcs were often missed at the lower position than 1 m from the top (Fig. 6).

On the other hand, the production of tracheids seemed to be activated by the artificial inclination. For instance, when tracheid numbers of the current annual ring in a tree of HINOKI which was leaned for 1 month intervals in early spring (4/12-5/12), in transition from spring to summer (6/12-7/12) and in late summer (8/12-9/12) were compared between the lower-side of inclination having the center of compression wood arcs and two lateral-sides where the arcs became very weak and the original condition of cell production may be preserved well (Fig.3) , the tracheid number of the lower-side at the 1.5 m position was about 2 times to those of the lateral-sides. But, examined more in detail, the numbers to the outer edge of the first arc were not so different between at the lower-side and at the lateral-sides, whereas the numbers to the second or third arcs at the lower-side were much larger than those at the lateral-sides. These results imply the activation of cambium caused by the artificial inclination is noticeable especially in summer. The influence of the inclination decreased from the top to the bottom. In the case of the short period inclinations (Fig. 1A and B) the influence was reduced remarkably, and it became negligible at the about 1.5 m from the top where the compression wood arcs were very narrow, although the inclination only in summer may cause a slight increase of cell production. It is desirable, of course, for this study that the steady date markings are recorded without artificial influence on xylem tissue of the position as bottom as possible where the tissue is older than that of the top position. Considering these points, the stem of 1.5-2.3 m position from the top where the stems have annual rings over 5 years were mainly used for the examination of the time requirement for tracheid differentiation. But in KUROMATSU, younger stems were necessary to be used on account of the weakness of compression wood arcs.

Succeedingly, the compression wood arcs were examined more precisely on the thin sections. The long period inclination induced the typical compression wood arc accompanying with two types of transitional zones: one was the zone from normal wood to compression wood, another was from compression wood to normal wood. It has been proved that stimuli of inclination and recovery affect directly to all differentiating tracheids and form the transition zones of two types.[8)10)11)] In the case of short period inclinations, however, an immature tracheid must be affected during its differentiation by two times of stimuli induced by the inclination and also the recovery, so that deveolping process of the tracheid is changed first from the normal wood type to the compression wood type, and next recovered to the normal one. When the stimulus of a short period inclination is added to a series of differentiating tracheids, some characters of compression wood must be recorded to each tracheid, even though the characters are different according to the period of inclination and to the developing stage to which each tracheid belongs at the time of inclination.

In Fig. 4 some characters of compression wood recorded in mature tracheids are represented. On the relatively wide arcs (see Fig. 4A), intercellular space, thicker and highly lignified S_2, a faint lignin-rich layer between S_1 and S_2 were sculptured in the tracheids from the outer part of arcs to the inner one, while the faint lignin-rich layer could be detected at one or two rows of tracheids on a very narrow arc (see Fig. 4B). In the ultimately feeble arc the lignin-rich layer could be detected only at corner region of a tracheid. Then, this character was chosen for the date marker. A tracheid having only the faint lignin-rich layer between S_1 and S_2 is considered to be the tracheid which belonged to the S_1-depositing stage at the inclination, because the stimulus of inclination affects immediately developing tracheids, as already mentioned.

Based on these observations the increasing of tracheid number was traced on the sections of specimen trees from April to September using the date markers (Figs. 5A and 6). The rates of tracheid production per one day(R), calculated from the increment curve, were maximum in May and decreased gradually to July, although it increased sometimes slightly in August or September (Figs. 5B and6).

If the specimen trees are harvested in June after the several date markings, they are expected to bring a reliable curve of the rate of tracheid production because of their relatively constant production of early wood tracheids (Figs. 5B and 6). Therefore, these trees were offered to the measurement of the time requirement for the tracheid differentiation. That is, the tracheid numbers(N) from the annual ring boundary of the preceding year to each maker of the treatments of April 12th, April 26th and May 10th were measured. In addition to these numbers, the number to the cell just depositing S_1 in the differentiating xylem, which can be considered to correspond to the maker cells in the compression wood arcs as mentioned above, was counted. Some examples of tracheid number to the marker cell in arcs and also to the S_1-depositing cell at the harvesting point, June 7th, are represented in Figs. 7 and 8. The rate of tracheid production per one day(R) was taken from the differential calculus of the numbers. The values of 0.7 in Fig. 7 and 2.0 in Fig. 8 imply the rate at the harvesting point, June 7th. It is interesting to note that the reciprocal number of them (1/R) results in the time difference from the C-stage between neighboring tracheid along a radial row. Therefore, the time requirement for a tracheid to move to its neighboring position in a radial row toward the mature-side results in 1/R days, so that the time(t) required for passing through a particular developing stage can be obtained by multiplication of 1/R by the cell number(n) contained in the developing stage, namely, $t=n/R$.

For this deduction the tracheid number(n) contained in developing stages at the point of harvesting must be examined. They were obtained from the specimen trees harvested on June 7th in each species by a combined technique of various microscopies on serial sections. For instance, the number in the RE-stage was judged from the cell size on radial direction. Those of S_1-, S_2- and S_3- stages were detected under a polarization and a trasmission electron microscope. In relation to the F-stage the result of some previous papers[1)~3) 9) 10)] were also referred for the measurement of the number in addition to the staining effect of safranin and the decreasing of cytoplasm during the lignin deposition.

The time requirements for the developing stages of a tracheid obtained by this method were very similar between specimen trees of SUGI and also between those of HINOKI. The averaged time requirements in SUGI are as follows: 3.7 days for the RE-stage; 3.7 days for the S_1-stage; 8.0 days for the S_2-stage; 2.8 days for the S_3-stage; 4.7 days for the F-stage. As the result, tracheids were shown to need 23 days for their whole differentiation. On the other hand, in HINOKI they needed 2.8 days for the RE-stage, 2.8 days for the S_1-stage, 7.7 days for the S_2-stage, 2.1 days for the S_3-stage and 2.6 days for the F-stage, and the total time of differentiation is 18 days. The time requirement for the C-stage is difficult to be deduced by this method, as the periclinal cell division is still repeated in the stage. In KUROMATSU the precise date marking failed regretably, because of missing of one day inclination. Although the result obtained in KUROMATSU should be considered only a reference, the total time for differentiation is about 22 days. Judging from these results, the roughly estimated time requirement for whole differentiation of a softwood tracheid seems to be about 3 weeks, the period of which is shorter than that of some previous papers calculated by periodical samplings. This value is very usuful to discuss the various metabolisms in differentiatlng xylem. It is, however, a future problem whether this time requirement can be directly applied to the tracheids during the summer wood formation. Other internal date markings such as the pinning method[12)13)] and the electric stimulation[14)] are expected to bring new data on the summer wood formation, if the precise date marking becomes possible on the particular tracheid without any artificial influence caused by the treatments.

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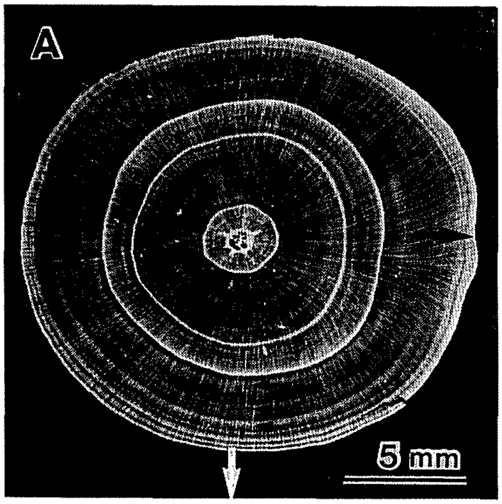

Fig. 1A Overall view of a stem section at 1.0 m from the top in a tree of SUGI which was treated by short period inclnations. Many narrow arcs of compression wood (for instance, small arrows) are recorded in the current annual ring at the lower-side of inclination (white arrow) and are spreading to both lateral-sides (brack arrows). The direction of all figures are represented similarly to this photograph.

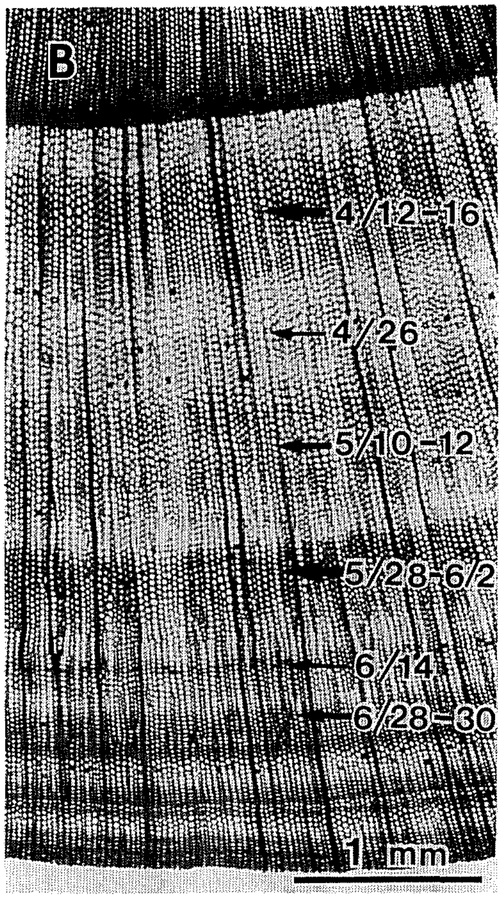

Fig. 1B Enlarged view of the lower-side of the fig. A. Thick-, thin- and medium-sized arrows show the 4-, 1- and 2-day inclinations, respectively.

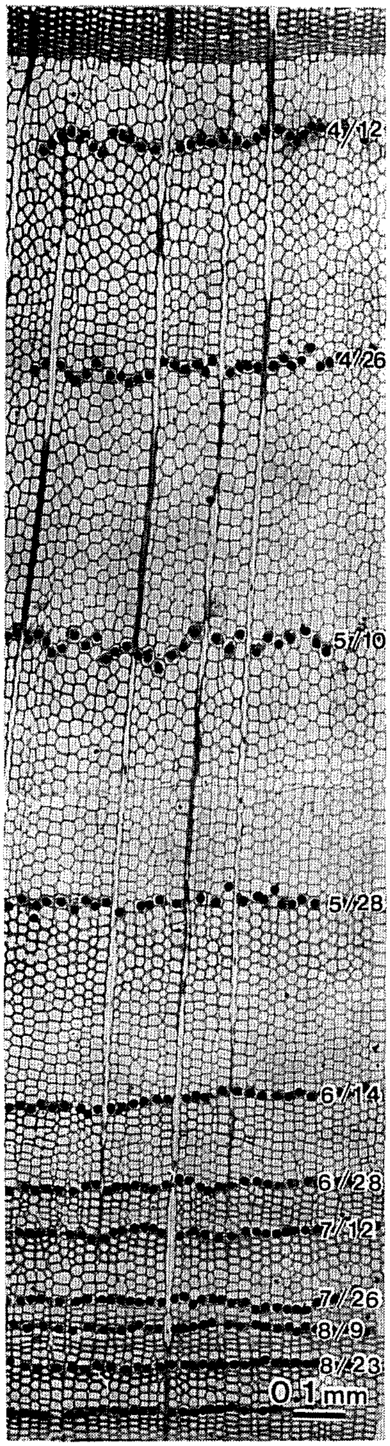

Fig. 2 More enlarged photograph of the same position of fig. 1 **B** which was obtained on a thin section stained with safranin. The dotted tracheids are marker cells corresponding to each inclination (see Fig. 4)

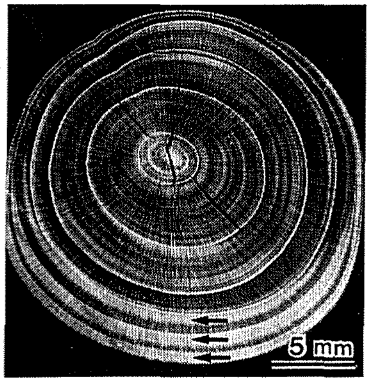

Fig. 3 Overall view of a stem section at 1.5 m from the top in a tree of HINOKI which was treatd three times by one month inclination. The current annual ring has three compression wood arcs at the lower-side of inclination (arrows).

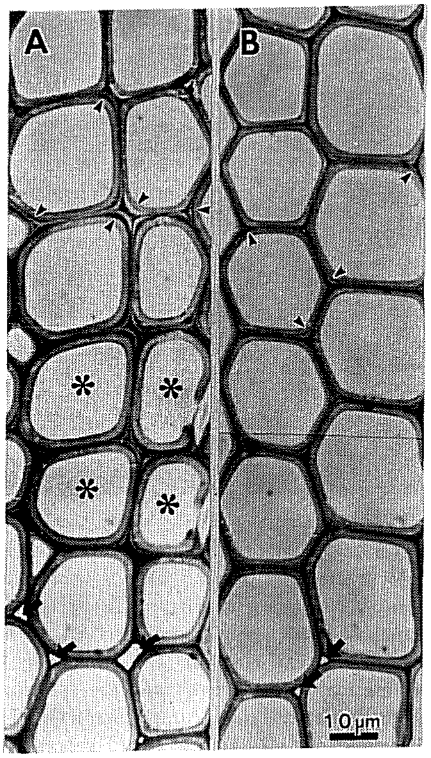

Fig. 4 Characters of tracheids contained in a compression wood arc of 2-day inclination (**A**) and 1-day inclination (**B**) in SUGI. The arc of **A** is composed of some tracheids having various characters of compression wood, namely," intercellular space (thick arrows)", "thick and highly-lignified secondary wall (asterisks)", and "faint lignin-rich layer between S_1 and S_2 (arrow heads)". These characters are weaker in **B**. Cells having the faint lignin-rich layer at the cell corner (see arrow heads) were used as the marker cell.

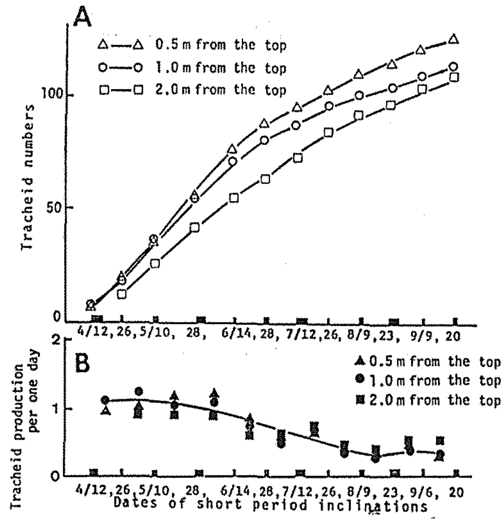

Fig. 5 The number of tracheids produced from April to September at three positions from the top in a tree of SUGI(A), and the tracheid production per one day (B). Long-, short- and medium-sized lines on the holizontal axes show the period of inclinations, namely, 4-, 1- and 2- days, respectively.

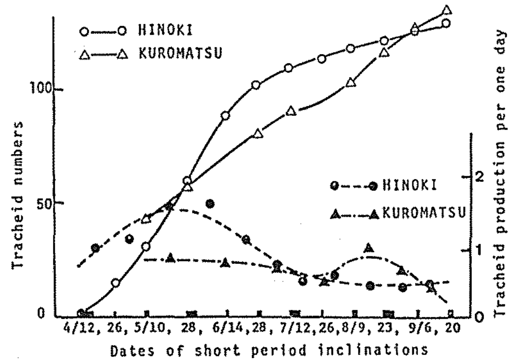

Fig. 6 The number of tracheids (solid lines) produced and the tracheid production per one day (dotted lines) at 1.5 m from the top in a tree of HINOKI and at 1.3 m in a tree of KUROMATSU.

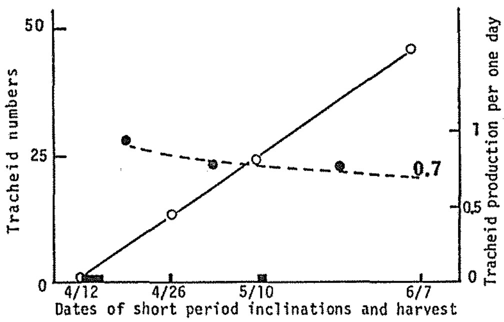

Fig. 7 The number of tracheids (solid line) produced at 1.7 m from the top in a tree of HINOKI and the tracheid production per one day (dotted line). On June 6th when the tree was harvested, tracheid production per one day is 0.7.

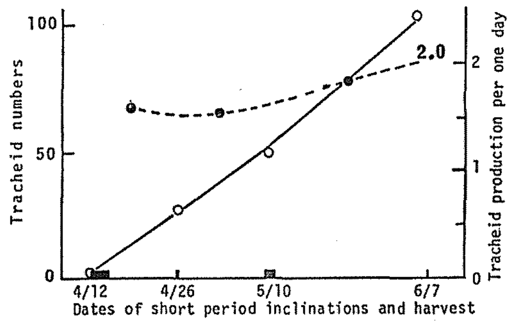

Fig. 8 A similar diagram of fig. 7 at 2.3 m from the top in another tree of HINOKI. On June 6th, tracheid production per one day is 2.0.