Small Angle X-ray Scattering of Woody Plants**

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Abstract——This paper deals with the measurement of small angle x-ray scattering of wood and growing bamboo. From the experiment, we obtained the following results; (1) Diffuse scattering was observed from woody plant and discrete diffraction was not observed. (2) There was no essential difference for the distribution pattern of particles in various woods and almost always diamond-shape scattering was obtained. (3) The x-ray scattering of woody plants is likely to correspond with the occurence of a system of microvoids of ir regular aixe and shape.

Introduction

Although the ultrastructure of woody plants has been extensively investigated, little is known about their x-ray scattering at small angles. The particles in a sample which are responsible for small angle x-ray scattering are small microscopic regions which have a different electron density from that of their surroundings. This scattering is produced by the particle as a whole and does not depend in any way on the internal crystal structure or amorphous state which it may possess. From this standpoint, small angle x-ray scattering would presented information for the ultrastructure of woody plant which is made up of particles and voids. Small-angle scattering from an inhomogeneous system may be of two kinds; either diffuse scattering or discrete diffraction. In cellulosic materials discrete diffraction is seldom observed but diffuse scattering is almost always observed. Statton[2] has pointed out that the scattering curves of dry fibers are likely to correspond rather with the occurrence of a system of microvoids of irregular size and shape in an otherwise nearly homogeneous cellulose matrix than with a heterogeneous structure of the latter.

Hermans and Weidinger[3] found that a very sharp diffraction maximum at low angles was caused by mild acid hydrolysis if Fortisan fiber was studied while swollen with water. If these fibers are then air-dried, the maximum disappears entirely but returns when rewet. They concluded that, after drying, the structure became so dense and the single structural elements so closely stuck together that larger aggregates of irregular size and shape are formed. Thus, it is indicated that

only swollen fibers allow valid determination of crystallite sizes. In dry fibers the material is so dense that the reciprocity law allows valid determination of void sizes only.

This paper deals with the measurement of small angle scattering of wood and bamboo. Bamboo shoots in different stage of cell wall formation are available for small angle scattering with relation to the ultrastructural changes. It is well known that the bamboo shoot grows by the internodial growth stepwisely from the first-internode to upward. Therefore every stage of the ultrastructural formation may be observed in every internode of the bamboo culm.

Materials and Methods

The wood specimens used are as follows;

    Balsa (*Ochroma* sp.) (specific gravity $\rho=0.077$)

    Kiri (*Paulownia tomentosa* STEUD) ($\rho=0.25$)

    Sugi (*Cryptomeria japonica* D. DON) ($\rho=0.56$)

    Kusunoki (*Cipnamomum camphora* SIEB) ($\rho=0.64$)

    Hoonoki (*Magnolia obovata* THUNB) ($\rho=0.54$)

    Keyaki (*Zelkowa serrata* MAKINO) ($\rho=0.60$)

    Isunoki (*Distylium racemasum* SIEB. et ZUCC.) ($\rho=0.92$)

    Shimakokutan (*Dioopyros* spp.) ($\rho=0.96$)

The bamboo samples were prepared from Moso bamboo (*Phyllostachs mitis*) which were in the growing stage. The dimension for the wood samples is $2(L)\times1.5(R)\times0.1(T)$ cm, while for the bamboo $2(L)\times0.1(R)\times1(T)$ cm. The samples of bamboo shoot were freeze-dried. The collimation employed is shown in Fig. 1 and 2 for
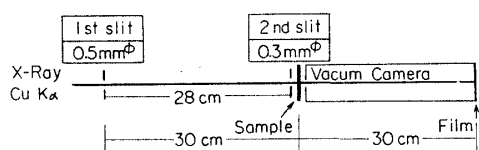

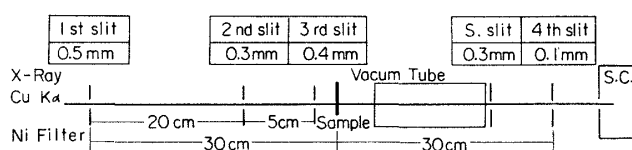Fig. 1. Pinhole collimation for the x-ray photograph of small angle.

Fig. 2. Slit collimation of small angle x-ray.

the small angle x-ray scattering. The photograph of wide angle x-ray scattering was taken by the rotation crystal camera. The rod sample is mounted with its fiber axis parallel to the surface of the film andis rotated around its longitudinal axis. For wood samples, an 0.1 cm thick bundle of fibers is placed perpendicular to the x-ray beam, which has been collimated by pinhole collimation and slit collimation. The Rigaku Denki small angle apparatus and the rotation crystal camera are attached to the Rota-flex x-ray diffraction unit (Rotating anode x-ray generator, Rigaku Denki Co., Ltd.)

A quantitative measurement of the intensity distribution of diffuse scattering

Jellinek[1], Solomon, and Fankuchen suggested resolving the curve into successive tangents and determining the slope and intercept of each tangent, which provides the size and relative number for each size.

The intensity of x-ray scattering by a homogeneous assemblage of ellipsoidal particles of radius R_1 (equatorial direction) and R_2 (meridional direction) may be given by the equation 1a and 1b.

$$I(\theta) = CWR_1^3 \exp\left(-k^2 R_1^2/4\right) \qquad \text{1a}$$
$$I(\theta) = CWR_2^3 \exp\left(-k^2 R_2^2/5\right) \qquad \text{1b}$$

where $I(\theta)$ is the intensity of scattering as a function of θ, θ is half of the scattering angle, k is $(4\pi \sin\theta)/\lambda$, W is the weight of the specimen doing the scattering, and C is a constant for a given material. If there is a distribution of radius R of the particle given by $W(R)$, it can be shown that the curve of scattered radiation is then given by

$$I(\theta) = C\int_0^\infty W(R_1)R_1^3 \exp\left(-k^2 R_1^2/4\right)dR \qquad \text{2a}$$

$$I(\theta) = C\int_0^\infty W(R_2)R_2^3 \exp\left(-k^2 R_2^2/5\right)dR \qquad \text{2b}$$

A convenient plot is one of $\log I(\theta)$ against r, where r is the distance in millimeters of the scanning slit from the center of the direct beam. When photographic recording was used, r was a distance measured on the film. This logarithmic plot of equation 1a or 1b yields a straight line whose slope is a single-valued function of R and whose intercept depends, among other things, on W and R. When the assemblage contains a distribution of particle sizes, the logarithmic plot is no longer a straight line. Equations 2a and 2b, in which $I(\theta)$ is experimentally determined and $W(R)$ is the desired function, suggest the use of Fourier transforms, and undoubtedly $W(R)$ can be calculated as the Fourier transform of some amenable analytical expression (or sum of expressions) for $I(\theta)$. Jellinek *et al.* used a simple graphical method of approximating $W(R)$, not as a continuous function but rather as a set of discrete fractions. A tangent to the experimental curve is drawn at the greatest angle of scattering studied. This tangent intersects the axis of ordinates at a value K. The values corresponding to this tangent are then subtracted from the original curve and a new corrected curve not containing the contribution of this fraction is obtained. In a similar manner the next tangent of minimum slope is drawn to the new curve with its intercept K_2. The procedure is repeated until the final points yield a straight line of intercept K_n. In this way, n number of lines are obtained, with successively larger slopes, and the corresponding radii are read from an appropriate graph of slope against R on semi-log paper, and the weight fractions of particles of each of the radii can then be calculated. When each K_i is divided

by its appropriate R, the resulting values are proportional to the weight fraction of that size particle.

Results and Discussion

The complete scattering by various specimens of wood are illustrated in the photographs of Photo. 1. The time of exposure, using a 50 kV. 80 mA. rotating anode tube, was 5 hrs. The specimens were exposed parallel to the fiber axis under air-dry conditions. Almost always, the observed intensity of the small angle diffuse x-ray scattering showed a diamond-shaped diagram, with blackening extending farther out on the equator than on the meridian. But diffuse scattering from
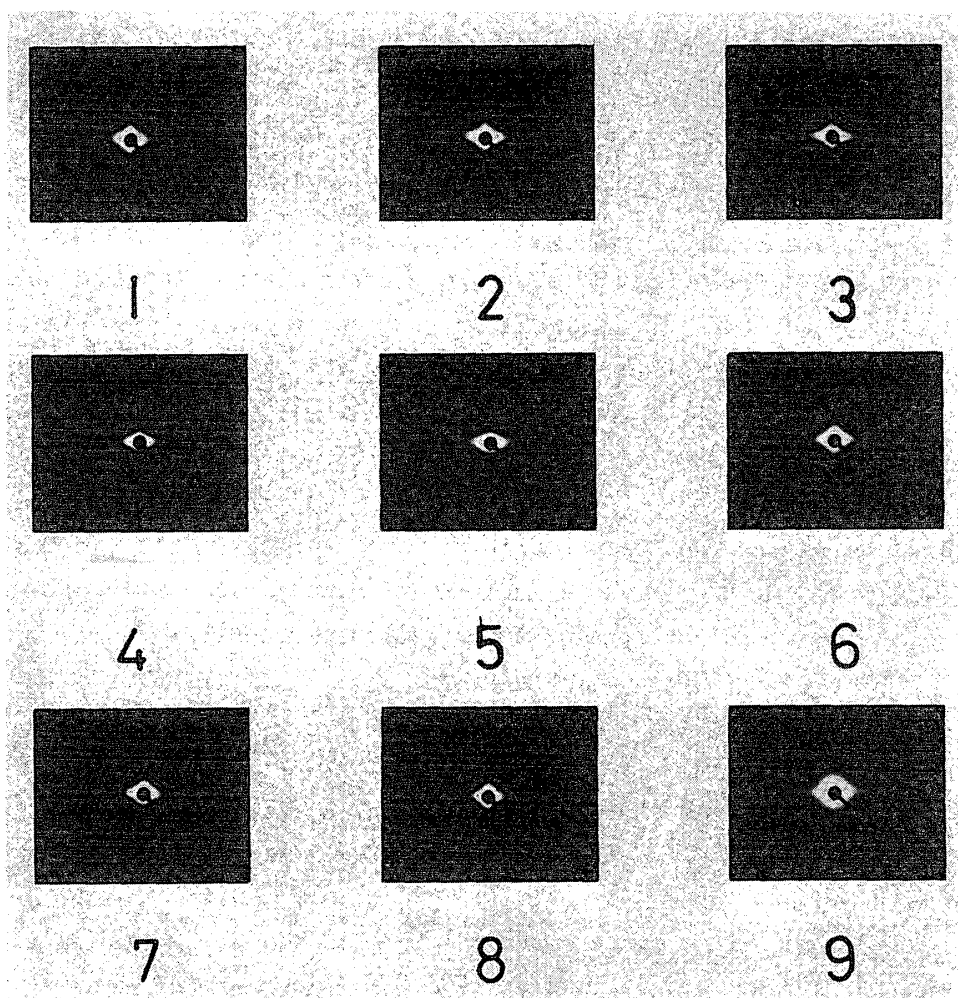

Photo. 1.　Small angle x-ray scattering of wood.
　　　　Experimental condition: Point foucus was used. The time of exposure, using a 50 kV, 80 mA, rotating anode tube was 5 hrs.
　　　　1. Balusa, 2. Kiri, 3. Hoonoki, 4. Sugi, early wood. 5. Sugi, late wood, 6. Kusunoki, 7. Keyaki, 8. Isunoki, 9. Shimakokutan.
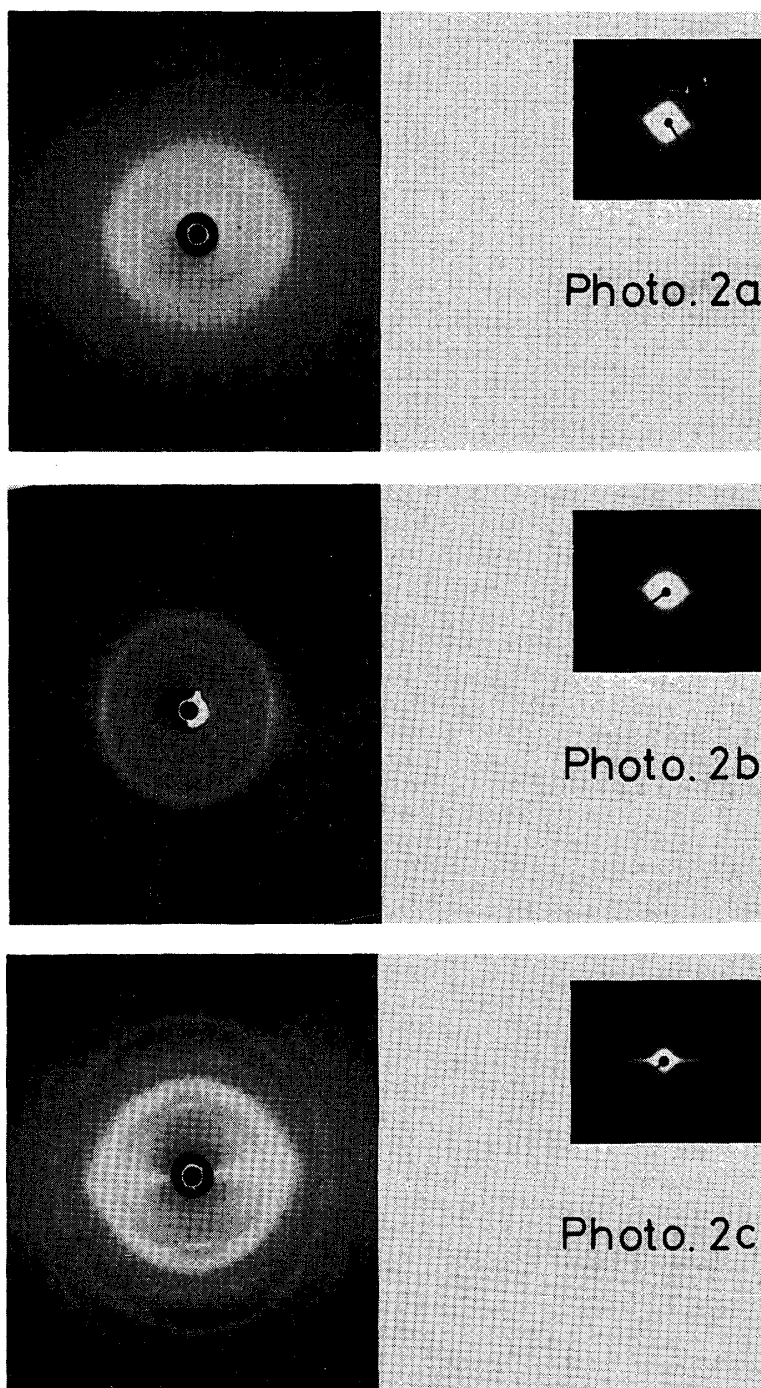
Photo. 2a~2c. Small angle x-ray scattering (reight) and wide angle x-ray diffraction (left).
Experimental condition of small angle: Point foucus was used. The time
of exposure, using a 60 kV, 100 mA rotating anode tube was 48 hrs. The
specimens were prepeared from freezed dry and were exposed parallel to
the fiber axies in the air-dry condition.
Experimental condition of wide angle diffraction: The time of exposure,
using a 40 kV, 60 mA rotating anode tube was 1 hr. Photo. 2a : Edible
prouts. Photo. 2b : 2nd internode of the 349 cm height bamboo shoot.
Photo. 2c : matured bamboo.

Shimakokutan showed a different shape. Under air-dry conditions, all specimens did not show discrete diffraction, but only diffuse scattering. Photo. 1 shows that this scattering does not depend in any way on the apparent specific gravity of wood, but depends upon the specific gravity of cell wall. Photo.1 also shows that the particles in the cell wall are ellipsoidal particles having a long axis parallel to the fiber axis. In order to clear the relationship of the scattering pattern and ultrastructure, we used a bamboo which was in the growing stage and compared small angle interference with wide angle diffraction for each growing stage. The results with bamboo are shown in Photo. 2a-2c. In the amorphous halo of the wide angle diffraction pattern of edibld sprout, a few microcrystallines of cellulose fiber are recognized at random orientation. When the orientation of cellulose microfibrils advanced with the maturation of bamboo and the sickle-saped interference of (002) arc on the equator appeared clearly, the small angle x-ray scattering pattern exchanged from diamond-shape to discus shape. With matured bamboo, the x-ray fiber diagram was a complete one and the small angle x-ray scattering was a similar pattern to that of Shimakokutan, extending farther out on the equator. These photographs show that if the sample causes a large decrease in the amount of diffuse scattering there is an accompanying increase in density and vice versa. So we may conclude as follows, deducing from the formation of the cell wall; When we consider a two phase system, that is substance and void, there are a large amount of voids in the structure of edible sprout because the small angle x-ray pattern showed a large amount of diffuse scattering (Photo. 2a), and as growth proceeded the voids were filled up so the amount of diffuse scattering decreases from the edible stage to maturity (Photo. 2b and 2c).

For a valid measure of the sizes of the inhomogeneities we employed Jellinek[1], Solomon, and Fankuchen's method to wood and bamboo samples. The quantitative measurements of the intensity distribution of diffuse scattering are not easy to obtain, as pointed out previously. However, it may be useful to obtain a parameter on a relative basis. Examples are shown in figures 3~5 for wood samples and in figures 6~8 for bamboo which is in the growing stage. This "quantitative" information is of the same nature as is qualitatively obtained by describing the diamond shape of the diffuse scattering in Photo. 1, so we used the scattering function of ellipsoids for calculating equations 1a and 1b. Figure 3 shows log intensity plotted against the square of scattering angle for Shimakokutan, Hoonoki and Balsa, using the slit collimation system from $2\theta = 3°$ to 0.2° along the equator. Such a curve would result if inhomogeneities of size were present, as they most surely are. An analysis of these curves can be made to determine a relative distribution of sizes by resolving the curve into successive tangents and determining the size and relative number of
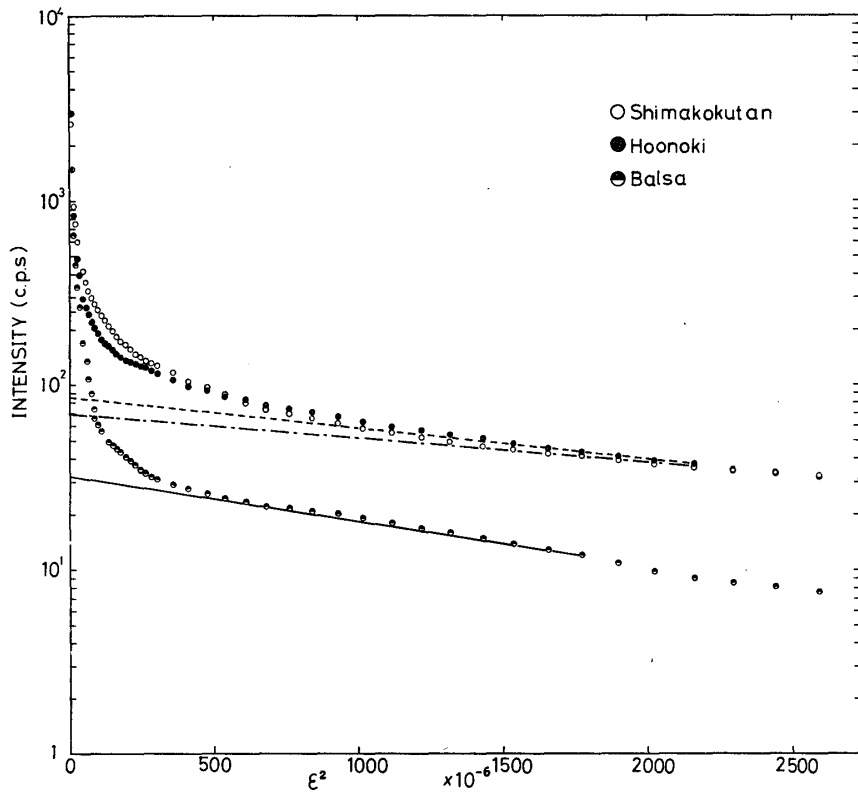
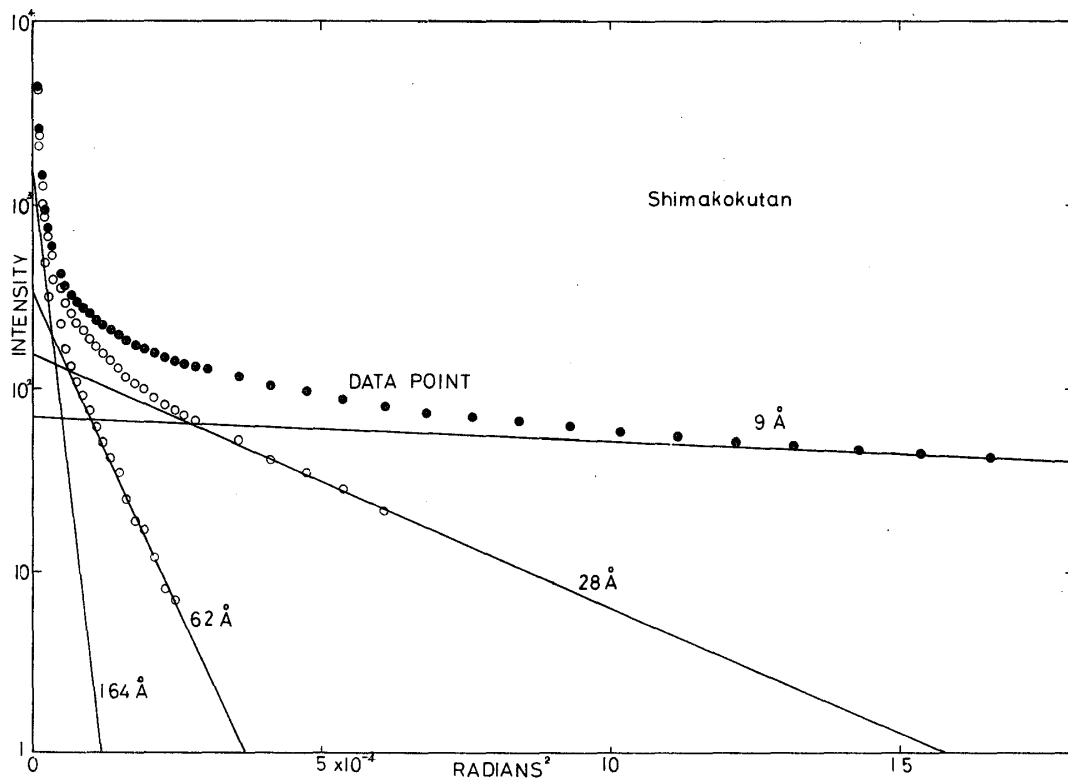Fig. 3. Log intensity plotted against square of scattering angle of wood.

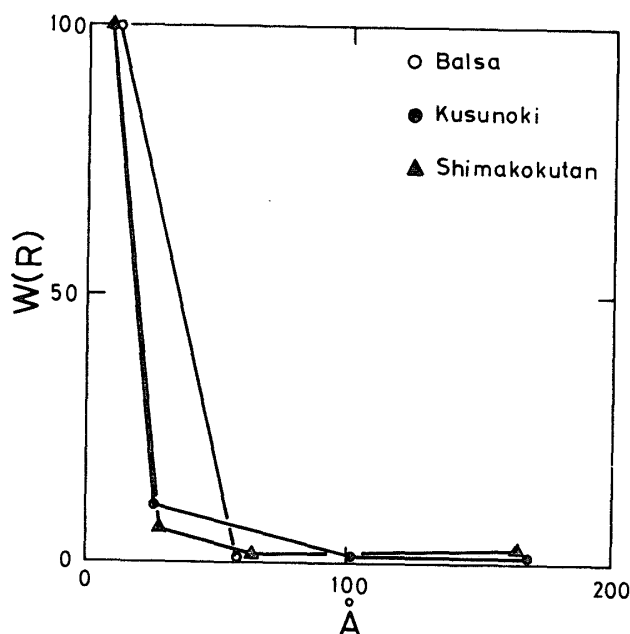Fig. 4. Analysis of equatorial diffuse scattering of Shimakokutan.

Fig. 5. Weight distribution of particles of equatorial diffuse scattering of Wood. Experimenial condition used is slit collimation $(2\theta=3°-0.2°)$.

each size from the slope and intercept of each tangent. From figure 3 we obtained the weight distribution of particles using the graphical method of analysis above mentioned. Figure 4 showes an example of this graphical method of analysis for Shimakokutan and figure 5 showes weight distribution of particles for three specimens of wood. The distribution of particles has a maximum at the average of 10 Å and then droppes abruptly. A vafiety of sizes of particles from minute particles of 10 Å to larger particles of about 200 Å were observed from the samples which have a variable specific gravity. The average size of minute particles from eight samples is 10 Å. This is in agreement with the minute value showed by Statton. But larger particle size is larger than Statton's value. This difference is due to the measurement range of the experimental condition used. Figure 6 to 8 show the analysis of equatorial diffuse scattering of Moso bamboo in the growing stage and the weight distribution of the particle sizes. The weight distribution of particle sizes of each sample at various growing stages showed that the distribution of large particles decreased gradually from the lower part of an internode in which the cell walls were in the differenciated stage to the upperpart, in which the cell walls were almost matured. The degree of maturation falls away from the upper part of a internode to the lower part on the internodial growing stage. From these results we have deduced that as the cell wall formation on the growing stage of bamboo is advanced from juvenile to mature, so the distribution of larger particle sizes is decreased and the density of cell wall is increased. It may be concluded from these facts that the particles corresponding with the small angle x-ray scattering are voids and that the
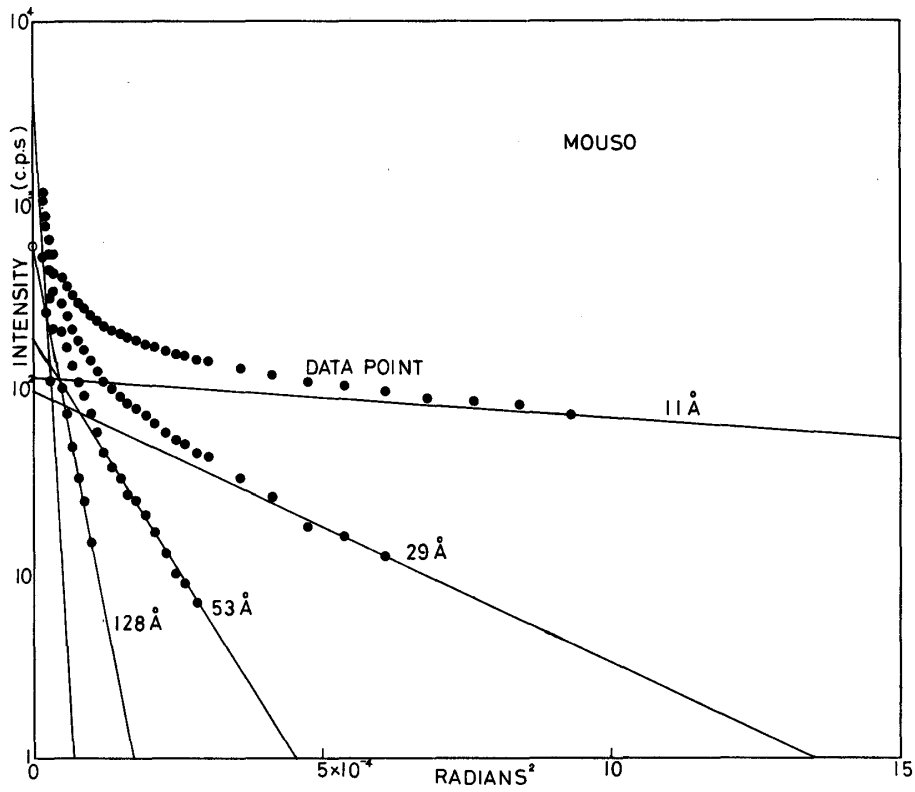
Fig. 6. Analysis of equatorial diffuse scattering of Mouse bamboo (mature stage),
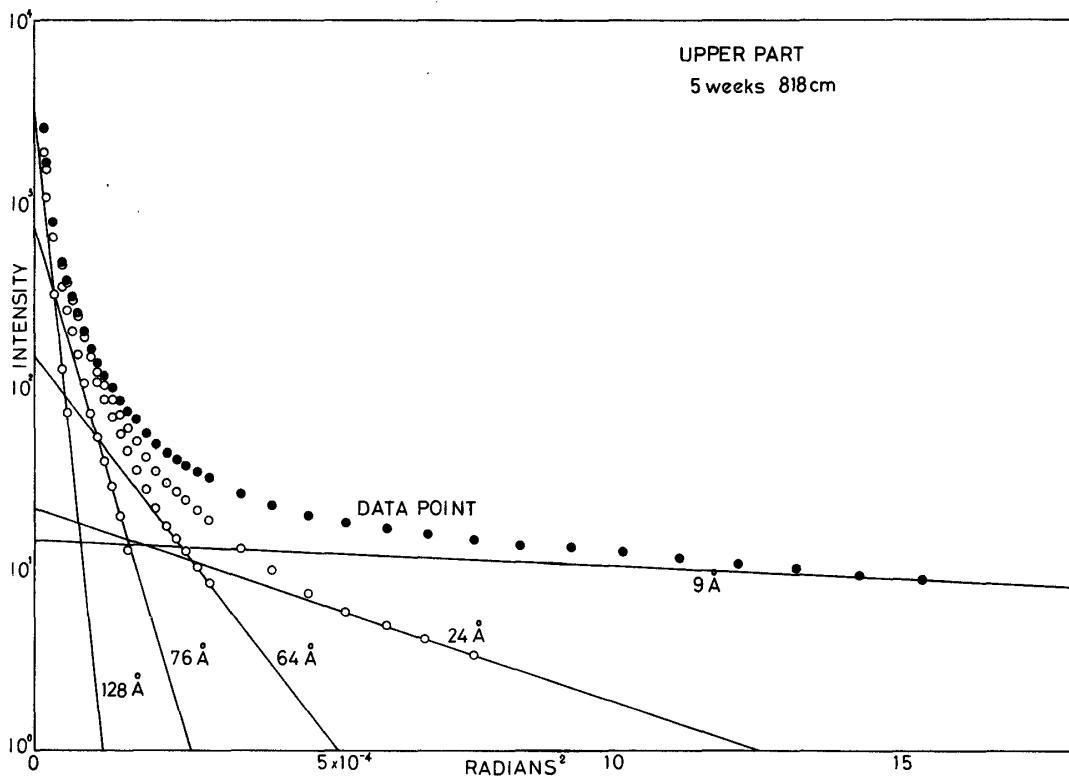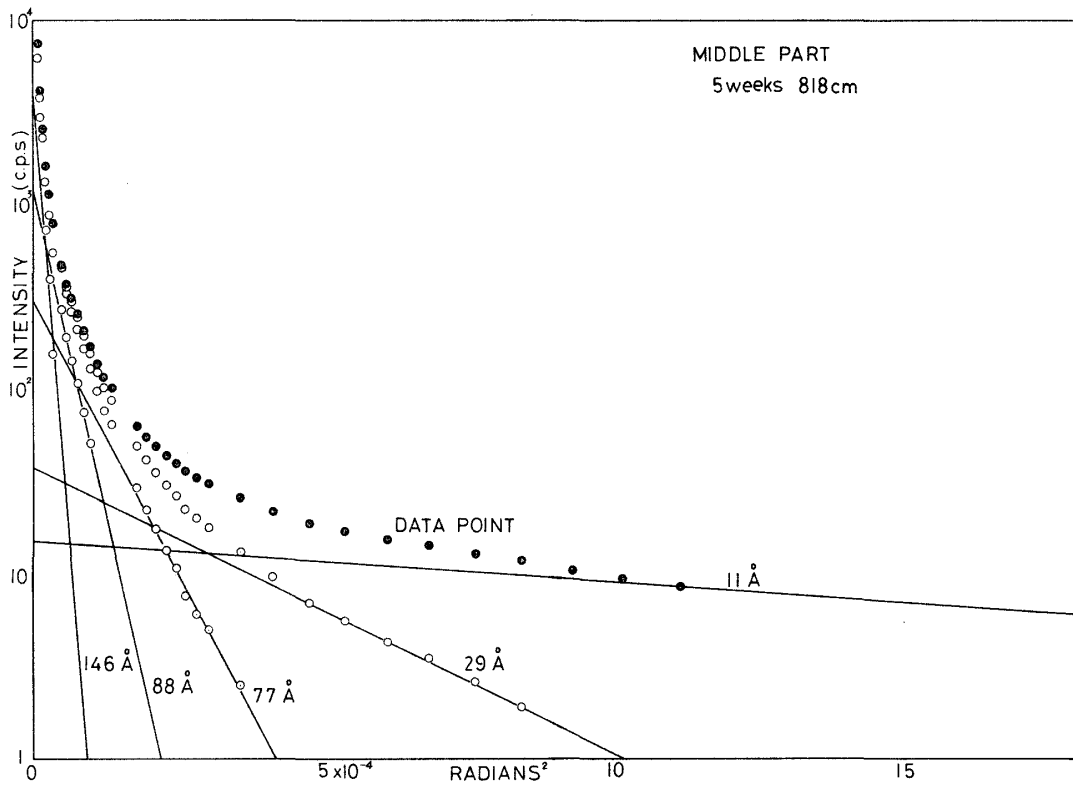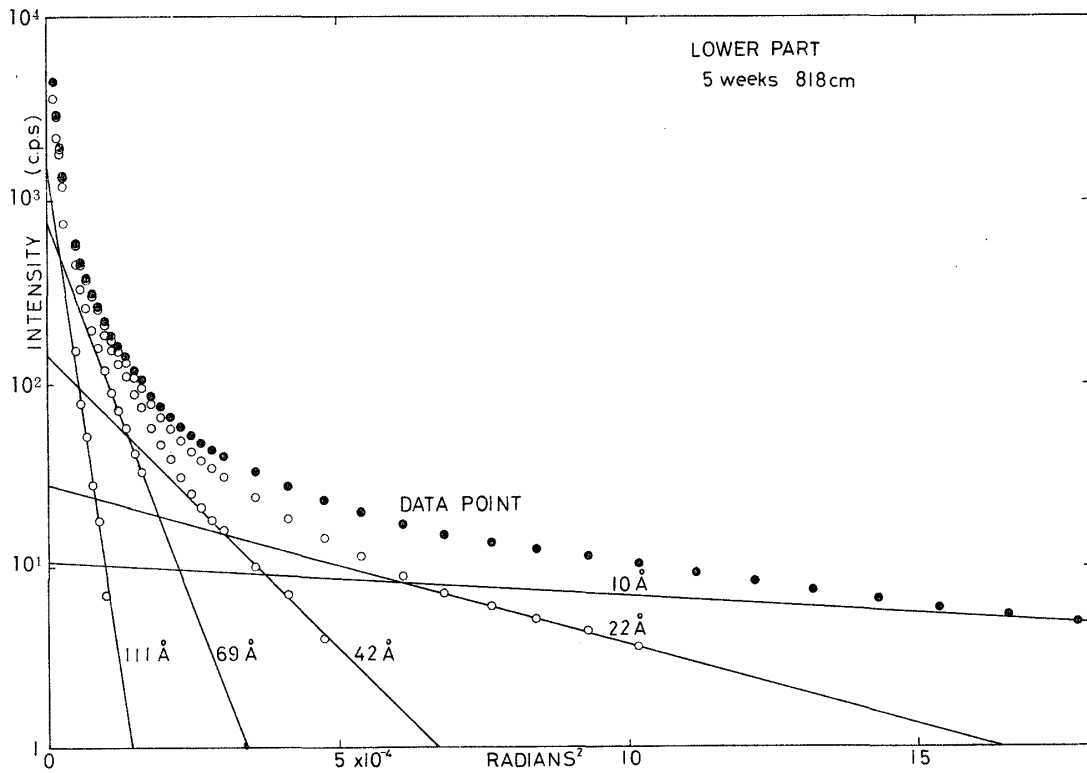Slit collimation system A was used.

Fig. 7a.

Fig. 7b.

Fig. 7c.

Fig. 7a~7c. Analysis of equatorial diffuse scattering of Bamboo shoot on the 5 weeks growing stage. 818 cm height, 30th internode. Upperpart (Fig. 7a), middle part (Fig. 7b) and lower part (Fig. 7c).
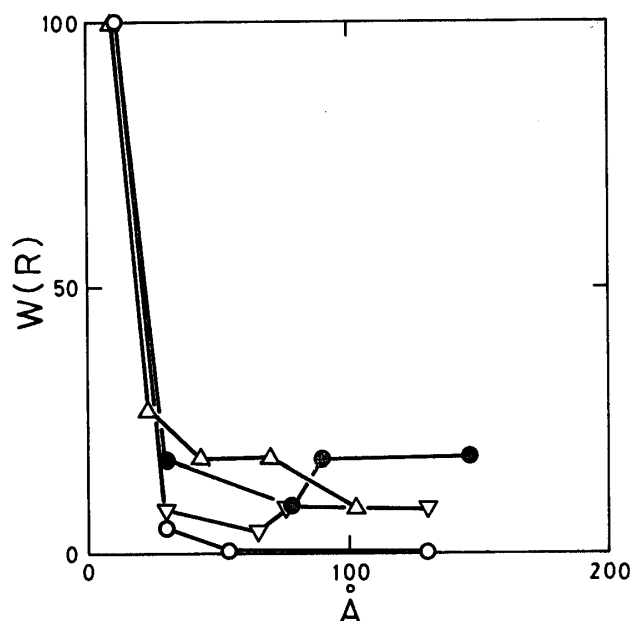
Fig. 8. Weight distribution of particles of equatorial diffuse scattering in bamboo
and bamboo shoot. ○ mature, ● lowerpart, △ middle part, ▽ upperpart.
818 cm height bamboo short was used.

cell wall has many voids in its first stage of cell wall formation and during the process of maturation these voids are filled up with the cell wall substance.

The micellar theory of Frey-Wyssling pictures the type of structure which is indicated by all of Statton's data. This structure pictures a continuous network of dense matter which is pierced by elongated voids. He based this structure on much evidence from many experiments. The Frey-Wyssling's picture shows a variety of sizes of voids from minute holes of 10 Å to larger spaces of 100 Å. This is in agreement with the type of scattering curve as shown in figure 4. A spectrum of particles sizes was observed from wood samples ranging from 10 to 200 Å. Similar determinations of pore or void sizes have been made on viscose yarns and these voids rang from 20 to 280 Å. Sawabe *et al.* have investigated the void structure in dry cell walls of various wood samples by nitrogen adsorption measurements. They show that void diameters for all woods are found to be in the range of 20～300 Å and most voids are of the range of 25～50 A in diameter. They show that an essential difference of void size distribution does not exist among the wood samples used. We have observed from figure 5 and figure 8 that there is no essential difference for the distribution pattern of particles in various woods as well as and matured bamboo.

Conclusion

We obtained the following conclusions from experiments with the small angle

x-ray scattering of woody plants.

1) Under air-dry conditions, woody plants did not show discrete diffraction but only diffuse scattering.

2) As the observed intensity of the small angle diffuse x-ray scattering showed a diamond-shaped diagram with blackening extending farther out on the equator than on the meridian, it may be concluded that the particles are ellipsoidal particles extending farther out on the meridian than on the equator.

3) As the cell wall formation during growth advances from juvenile to mature, the distribution of larger particles decreases.

4) The minute range of particles is almost always about 10 Å.

5) It may be concluded from our experiments that the scattering curves of woody plants are probably due to the occurence of a system of microvoids of irregular size and shape.

References

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