INTERNATIONAL ASSOCIATION OF WOOD ANATOMISTS

Bulletin

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Editor: A. Frey-Wyssling, ETH Zürich

EDITORIAL

Besides the International Association of Wood Anatomists (IAWA), there are other institutions with interests in similar lines. In recent times one of them has disappeared and another has been newly created. The International Wood Research Society (IWRS), whose board was located in Munich, Paris, Stockholm and Zurich, has been dissolved and the International Academy of Wood Sciences has been created by Prof.Dr.F.P.Kollmann, Munich, as announced in our News Bulletin 1965/2. Since the constitution of the dissolved society did not allow the transfer of its modest capital to the new academy, this sum of about $ 450.- was given to our association. We are very grateful for this unexpected inheritance and thank the board of the dissolved society very much for its action.

The Academy of Wood Sciences has its home in Vienna (Austria). It is organized in three classes, of which the biological-anatomical class duplicates our endeavours in some respects. It will be our task to minimize undesired effects of this parallelism. The academy issues a quarterly periodical: "Wood Science and Technology", published by Springer, New York. Of course, this new journal delays for years the realization of a transformation of our News Bulletin into a printed periodical.

A possibility to broaden the scope of our News Bulletin emerged when a group of taxonomic plant anatomists under the leadership of our member Dr.Metcalfe, Kew Gardens, suggested 1) to include in the anatomical description of woody plants not only the structure of the secondary xylem but also the features of the primary stem and the leaves, such as hairiness, stomatal types etc. 2) to fuse both the Association of Wood Anatomists and the Association of the Taxonomic Anatomists and, as a consequence, 3) to publish a joint bulletin on microscopic plant structure. However, the council of the IAWA decided that such a considerable extension of our subject would lead too far away from wood anatomy and that the News Bulletin had to be continued as before, with the suggestion to drop the word "News" and to call it simply the "Bulletin of the IAWA".

One question is still open: The Association of Taxonomic Anatomists wants to prepare a glossary with definitions of all the terms used in plant anatomy. Of course this would include the terms gathered in our "Glossary of terms used in wood anatomy". As it is undesirable that those terms are redefined, I propose that the IAWA should deliberate on this copyright in the next general meeting which will occur during the International Botanical Congress, 1969, in Seattle (Wash.) and whenever possible confer it to our colleagues.

A.Frey-Wyssling
Secretary-Treasurer

A METHOD FOR THE ANALYSIS OF THE COURSE OF VESSELS IN WOOD

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Wood anatomy, to any superficial observer, appears to be a closed chapter as far as its investigation with the light microscope is concerned. The xylem anatomy of so many trees is known in sufficient detail that a species can often be recognized from a small fragment of its wood. However, the axial extent and distribution of vessels represents a gap in our knowledge of wood structure which is of concern to anyone thinking seriously about the movement of water through xylem. The investigations of VITE (1958, 1959) have revealed a great deal about the distribution of tracheids in conifers but many questions about the three-dimensional distribution of vessels in dicotyledonous trees are still unanswered.

A number of papers in the literature deal with vessel length, a question that has been approached in a number of ways. One can obtain either an indication of the longest vessels (e.g. HANDLEY, 1936), or of average length of short vessels (SCHOLANDER, 1958). However, when one thinks about the problem of how water can flow around a wound in a tree stem (where all severed vessels must be embolized under transpirational conditions) one quickly realizes that our knowledge of vessel length and vessel distribution is very fragmentary indeed.

For our work on palms and other arborescent monocotyledons we have developed various methods of analyzing vascular anatomy using microcinématography. These methods have obvious uses in other fields and we ourselves have applied them in a limited way to the study of the course of vessels in wood. The results of our first attempts are of considerable interest. The methods themselves must first be described briefly.

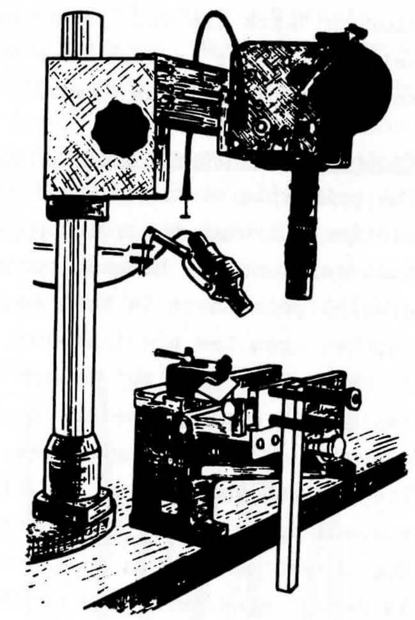
Surface photography

In principle a ciné camera is mounted above a wood specimen clamped in a sliding microtome in such a way that the surface of the specimen exposed after each transverse cut can be photographed frame by frame. Sections themselves are discarded. The resulting film permits rapid projection of sequential transverse views. Individual vessels can be seen and their positions can be followed continuously on the projection screen.

In practice it is essential that the optical axis of the camera lens be identical with the axis of the gradually advancing specimen. The cut surface

must not be displaced during sectioning. This introduces a problem if the clamp advances up an inclined plane. In addition the overall clamp advance of the microtome is normally limited to about 4 cm. This may be sufficient for certain types of investigation, such as the insertion of a lateral twig on the main axis, but for long-distance analyses it is desirable to examine much longer specimens.

We have overcome both of these problems, using a Reichert OME sliding microtome, by replacing the manufacturer's clamp with a specially constructed clamp involving rollers which permit the continuous advance of the <u>specimen</u> rather than the clamp itself. By placing the microtome at the edge of a table or bench we can section continuously pieces of "indefinite" length (Figure 1).



<u>Figure 1</u>: Motion picture camera focused on the transversely planed surface of a long piece of wood. Note the special clamp on the microtome allowing unlimited advance of the specimen.

The camera itself has to be firmly supported on a solid stand, preferably with a "focusing" mount. Any single-lens reflex camera is suitable. We use a Bolex H 16 Rex with a lens of 50 or 75 mm focal length and extension rings, the camera mounted on a Wild multipurpose camera stand.

The area of wood photographed depends on the species since it has to be small enough to allow vessels to be seen clearly. An area of about 8 x 10 mm is useful for woods with wide vessels. The planed surface of the wood is brightly and uniformly illuminated by one or more stereomicroscope illuminators so that the camera lens can be stopped down to improve the depth of field. Focusing is not easy but is critical for the success of the method. The light intensity is measured with a spot exposure meter of 1 or 2^{o} coverage and the exposure computed from the lens extension.

After each cut and before each frame is exposed the newly-planed surface is flooded with water to eliminate disturbing reflections. A millimeter scale photographed on the first frame and marks throughout the length of the specimen at 1 or 5 cm intervals facilitate later quantitative analysis of the film.

Since the microtome is used as a plane the quality of the knife edge is not critical. To speed the whittling of a long sample shavings a few hundred microns thick are cut. Nevertheless knives are rapidly dulled and it is helpful to have an assistant supplying freshly-honed knives. An automatic knife-sharpener is an asset.

<u>Photography through the microscope</u>

The principle of this method is the photography of sequentially cut microtome sections through the microscope, again with a motion picture camera. Sequential sections may be made permanent or stained and mounted temporarily. The crucial point here is that each section is optically, i.e. precisely superimposed upon the previous one. Two different methods have been developed to do this, the "drawing" method and the "optical shuttle". The drawing method employs an outline sketch of one section, made with a camera lucida, to align a number of succeeding slides, fresh drawings being made as alignment is lost. The optical shuttle, on the other hand, directly superimposes the images of succeeding sections. The shuttle is the most rapid method and well suited for investigations in wood anatomy. Since these methods have been described in detail elsewhere, the reader is referred to earlier descriptions (ZIMMERMANN and TOMLINSON, 1965, 1966).

<u>Analysis of the films</u>

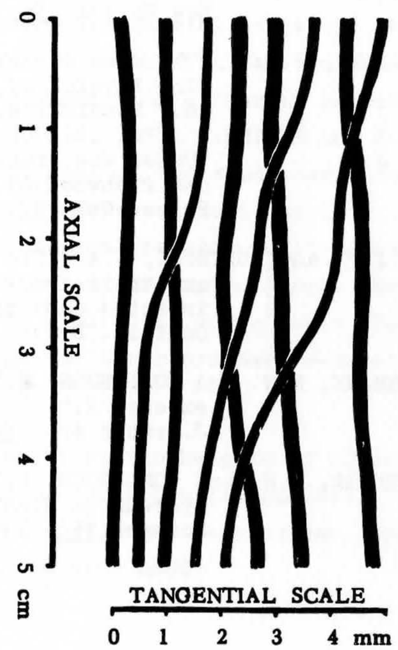
When projected with an ordinary projector, the observer "moves along" the stem and can see the changes in relative positions of vessels. Rays appear and disappear continuously. Ordinary projection may be quite illustrative for a general impression. However, the most useful analysis requires a so-called "data-analyzer". A much cheaper but considerably cruder motion picture editing machine (German: <u>Laufbildbetrachter</u>) can also be used. The data analyzer we use is basically a Kodak Analyst projector, rebuilt by L-W Photo,Inc.

(15451 Cabrito Rd., Van Nuys, California) (Model L-W 224A). It permits projection, without flicker, in either direction at any speed from 1 to 24 frames per second, as well as frame-by-frame advance. Quantitative construction of a three dimensional diagram is greatly facilitated with such a machine.

<u>The course of vessels in wood</u>

Our first attempt concerned the microscopic structure of the xylem of <u>Acer rubrum L.</u> The resulting film gives a beautiful demonstration of a vessel network (Gefässvernetzung) as described by BRAUN (1959) for <u>Populus.</u> In addition the film shows that vessels begin and end in clusters. One can see on the projection screen how individual vessels continuously move from cluster to cluster, and that individual vessels appear and disappear within a cluster <u>but never end in isolation</u>.

A motion picture sequence was shot through 15 cm lengths of <u>Quercus</u> and <u>Fraxinus</u> respectively. Projection of the film revealed a very surprising phenomenon which was particularly pronounced in <u>Fraxinus</u>. Vessels do not run parallel but describe what appears to be random, tangential deviations from their path. Individual vessels, particularly later formed (somewhat smaller) earlywood vessels, frequently are seen to cross ("jump over") their neighbours in projection. A number of vessels of <u>Fraxinus</u> were followed and their path plotted during projection on white paper. Tangential "movement" of individual vessels was found to be up to 3 mm over an axial distance of 5 cm (Figure 2).



<u>Figure 2:</u> Diagrammatic representation of vessel distribution in <u>Fraxinus americana L.</u> in tangential view. This illustration is based on tracings of 20 individual vessels over an axial distance of 5 centimeters. Note that the axial scale is foreshortened 5 times compared with the tangential scale.

The physiological implication of this structure is obvious: water must spread tangentially during its ascent in the stem. Extrapolated, 3 mm spread over 5 cm corresponds to 30 cm spread over an axial distance of 5 meters. The spreading in Acer and Populus is even more extensive than in Fraxinus. This obviously must be of considerable physiological significance in the distribution of water on its way from roots to leaves. Clearly, such vessel distributions also raise some very interesting developmental questions.

The above method has been restricted to a study of the vertical distribution of wood elements. However, it also suggests itself for use in the analysis of changes in the radial direction, particularly the length and distribution of rays.

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STATISTICAL ANALYSIS OF THE WOOD STRUCTURE OF BEECH (FAGUS SILVATICA L.)

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Introduction

In wood science the study of wood structure provides both the biologist and the technologist with basic information on the material. For the wood anatomist, the microscopic wood structure is the very medium for identification of unknown species as well as for the study of comparable features in relation to the botanical location of the species or the influence of exogenous factors on the growth structure. Beside these facts the wood biologist may be interested in wood structure as a chronological sign of differentiation in the cambium. The cell production in the meristem and especially the mechanism of cellular determination are still marked as fields with many gaps of knowledge, in spite of the very important efforts which have already been made. This is due especially to the complex nature of the meristematic tissue itself and the diversity of both exogenous and endogenous factors that modify its metabolism. As the wood tissue is the most accurate result of tissue differentiation in the meristem, an effective analysis of these results, e.g. a statistically planned analysis of the tissue, should give us a highly significant method and a quantitative measure for cambial activity. This has been proved by M.W.BANNAN (1950) in his investigations on the cambial activity of coniferous trees, and is used by others describing different aspects of the woody tissue (for beech wood vide: H.SCHULZ, 1957; W.KNIGGE and H.SCHULZ, 1961; J.RAK, 1964; and D.FENGEL, 1966). The goal of the study here presented is to describe a method which is suitable to measure structural details of plant tissues in relation to special physiological behaviour. At first, a normal and undisturbed old tree was investigated; in further work, the method of the statistical tissue analysis shall be applied to young beech plants with modified transpiration or other changes of their ordinary metabolism.

Programme of investigation

In one beech, 60 years old disks have been cut at the heights of 20 cm, 120 cm, 220 cm, 320 cm, 420 cm, 520 cm and 620 cm above ground level. In each of these disks four equal positions (I - IV) have been chosen, differing in 90° from each other. From this material we have used:

Figure 1

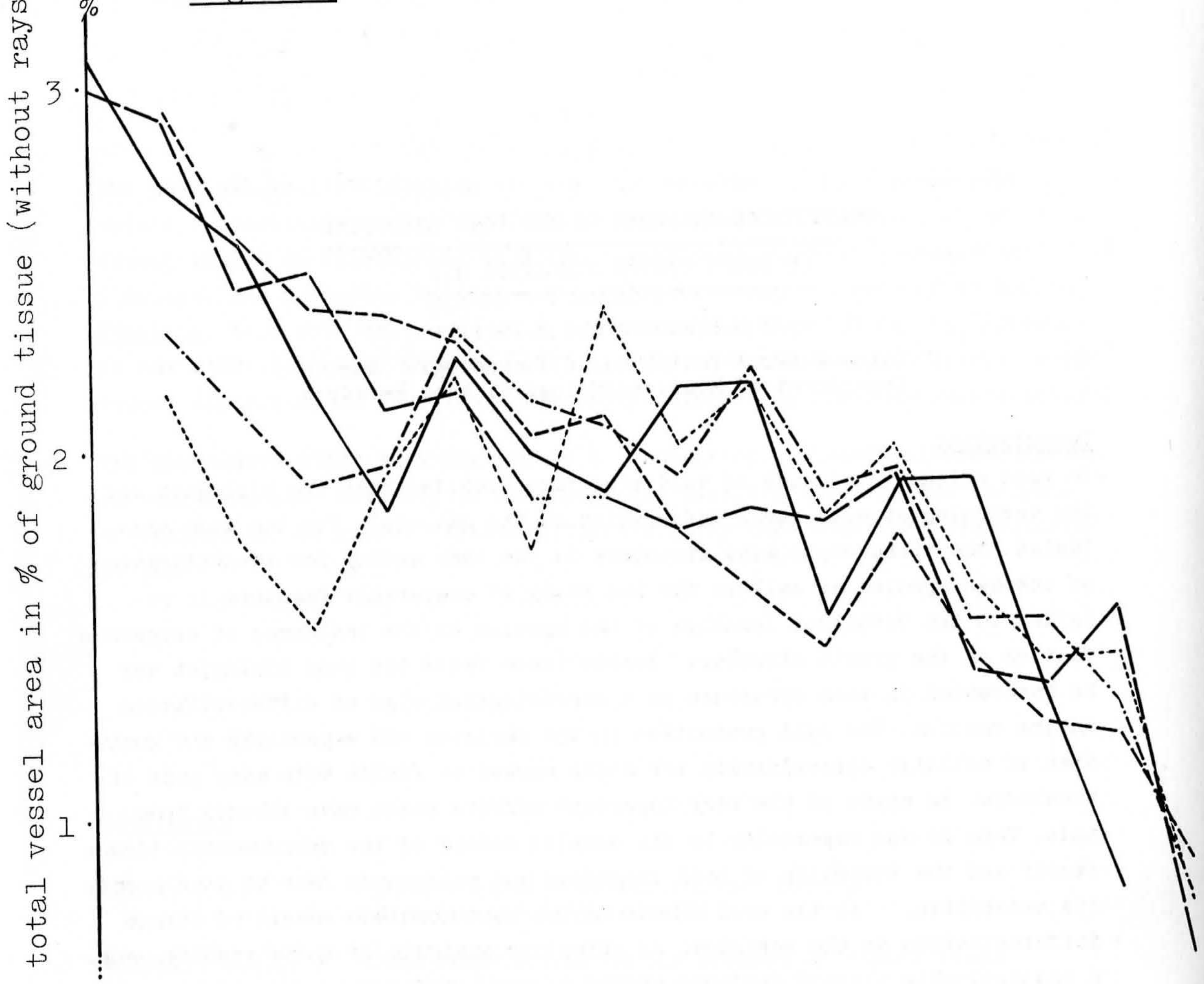
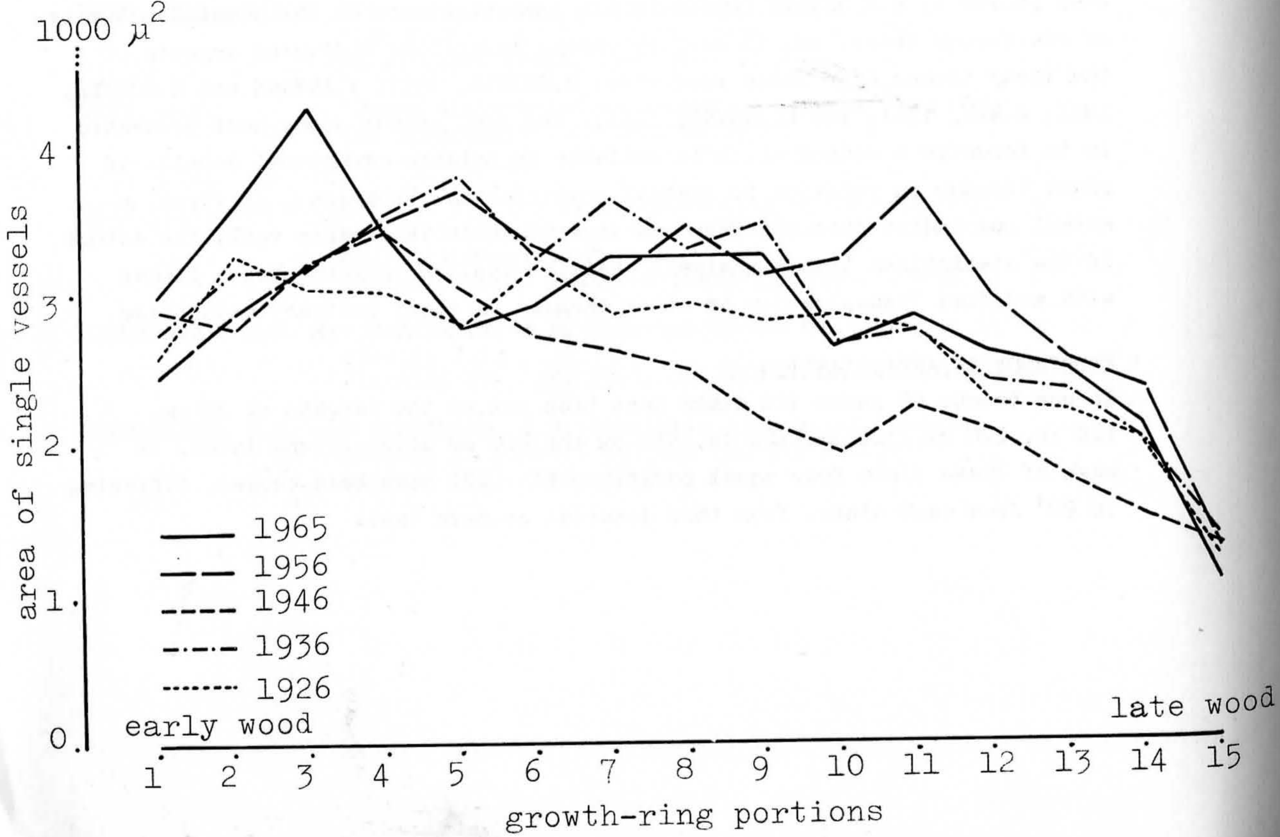


Figure 2



i) in the disk 420 cm and the positions I-IV the three outermost growth-rings adjacent to the cambium to prove whether one ring, or the mean value out of three rings, should be considered;
ii) in the disks 20/220/420 and 620 cm and the positions I-IV the growth-ring 1965 (adjacent to the cambium) to prove the influence of stem height on the analysis;
iii) in the disk 520 cm and the position I the growth-rings 1918 (adjacent to the pith), 1926, 1936, 1946, 1956 and 1965.

As descriptive elements for the analysis of the wood structure we have measured:
i) the number of vessels per tissue unit
ii) the total area of the vessels per tissue unit
iii) the total area of the fibre ground-tissue per tissue unit
iv) the main axis of vessels in the radial and tangential directions
v) the number of parenchyme cells per tissue unit.

For this purpose each growth-ring was divided radially into 15 equal parts from the early to the late wood; in the tangential direction all measurements represent a constant growth-ring portion of 0,15 mm. As we have not included ray-tissue, these 0,15 mm ring portions have been placed into the whole section individually, in order to avoid the broad rays of beech which would have changed the measurements completely. The material was sectioned against the grain. These sections were projected on white paper in order to trace the structural details of interest. Afterwards, these paper rolls were conditioned to a special humidity content. The drawings were then cut and the area of the different elements was calculated from the weight of the paper portions.

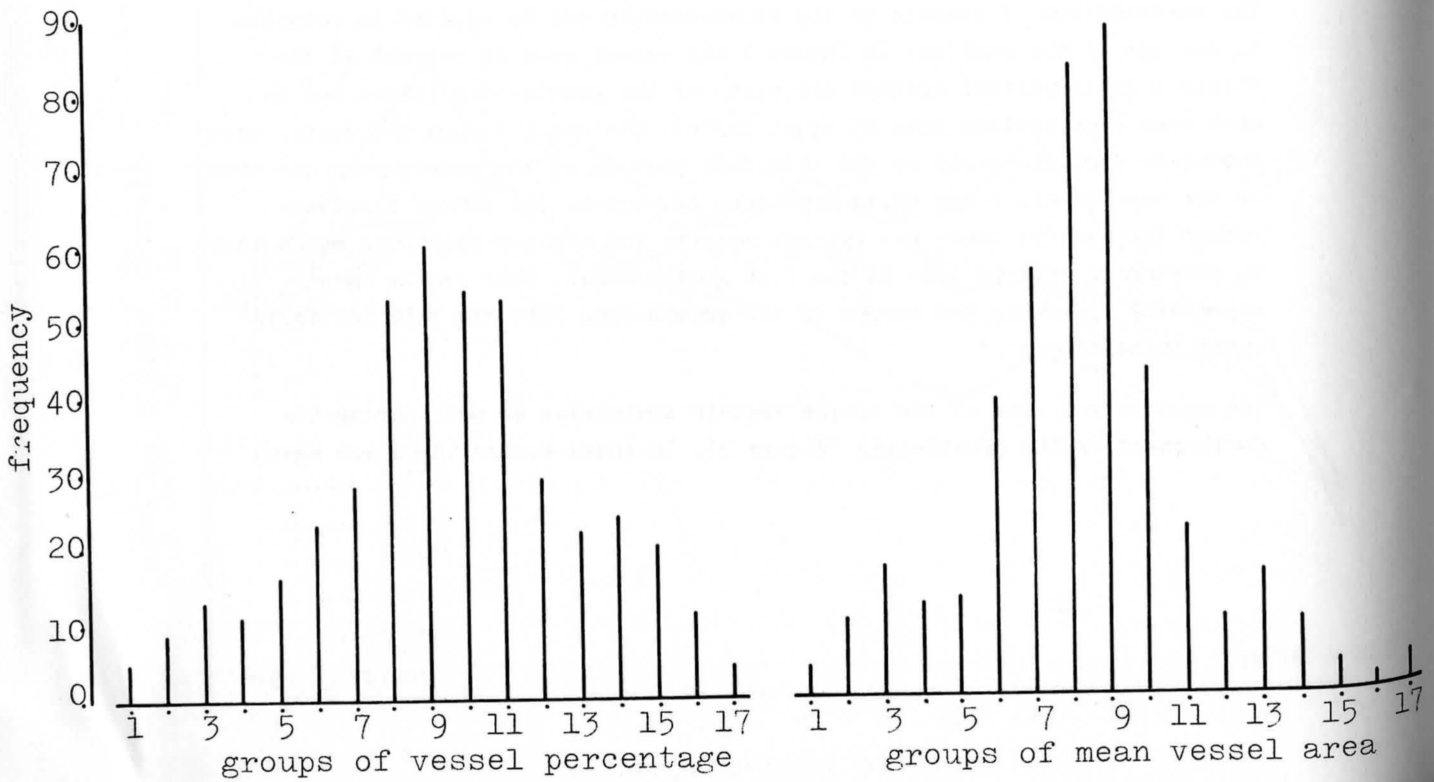
Results

The distribution of vessels in the cross-section can be studied in relation to the age of the cambium. In figure 1 the vessel area in percent of the tissue unit is plotted against the width of the growth-ring (which has in each case been divided into 15 equal parts). Obviously enough the vessel area decreases from the early to the late wood portion of the growth-ring and this is the case in all rings which have been inspected. All curves fluctuate rather frequently: there are typical maximum and minimum positions which seem to correspond more or less in the five growth-rings. This can be shown especially by moving the curves of the growth-ring 1965 and 1956 leftwards until coincidence.

The mean vessel area of the single vessels diminishes as well during the development of the growth-ring (figure 2). In these curves there are again

Figure 3

Groups of vessel percentage %		Frequency	Groups of mean vessel area μ^2		Frequency
1	0 -0,50	5	1	- 600	4
2	0,51-0,70	9	2	601- 960	10
3	0,71-0,90	13	3	961-1320	17
4	0,91-1,10	11	4	1321-1680	12
5	1,11-1,30	16	5	1681-2040	13
6	1,31-1,50	23	6	2041-2400	39
7	1,51-1,70	28	7	2401-2760	56
8	1,71-1,90	53	8	2761-3120	83
9	1,91-2,10	60	9	3121-3580	88
10	2,11-2,30	57	10	3581-3940	43
11	2,31-2,50	53	11	3941-4300	22
12	2,51-2,70	29	12	4301-4660	10
13	2,71-2,90	22	13	4661-5020	16
14	2,91-3,10	24	14	5021-5380	10
15	3,11-3,30	20	15	5381-5740	4
16	3,31-3,50	11	16	5741-6100	2
17	3,51-	4	17	6101-	4



various fluctuations which sometimes show corresponding tendencies.

Another way of interpretation of the various measurements is the analysis of the frequency distributions. This has been done for the above mentioned values. In both cases the frequency curves seem to be divisible into three binominal curves as shown in figure 3.

These facts which have been described in the above-mentioned diagrams must be regarded together with a biological parameter such as growth-ring-width. For this purpose we have classified the various measurements of ring width into four groups: 1-2 mm, 2-3 mm, 3-4 mm and over 4 mm, and noted the results of vessel areas correspondingly. Thus, curves are obtained as shown in figure 4. It seems that there is a tendency to form a higher percentage of vessel area in small rings than in broader ones. This is especially obvious in the first portion of the growth-rings. The integration of all 15 ring portions shows the main tendency even more clearly: within the four growth-ring groups in the smallestrings a percentage of vessel area of 33,2 % is measured, 32,6 % in the second group, 30,2 % in the third and 27,2 % only in the group of the broadest rings.

Discussion

In the statistical analysis of beechwood we have dealt with vessels, fibres and longitudinal parenchyma cells only, in order to avoid irregularities due to incidental variations within the ray tissue. Thus, the amount of vessel area has always been related to the ground-tissue (fibres) and the longitudinal parenchyma. The division of all growth-rings into 15 equal portions gives different values of absolute width of each portion according to the differences in ring width but makes it possible to compare relative growth-ring areas. The first comparison of the four positions I to IV within the same growth-ring, where position I represents the growth direction north of the stem, position II east, position III south and position IV west, does not show significant differences. As it is demonstrated in figure 5, there is another interesting fact of much greater importance than the various fluctuations in the relative amount of vessel area: the correspondence of maxima and minima of the four curves. The same phenomenon can be discovered in measurements of one position only but at different heights, or at the same height but in different radial distances from the cambium (e.g.figures 1 and 6); it seems that during differentiation the cambium produces - after a period of high vessel-area formation - a smaller amount of conductive tissue. As it can be demonstrated in figure 6 in a comparison of vessel-area

Figure 4

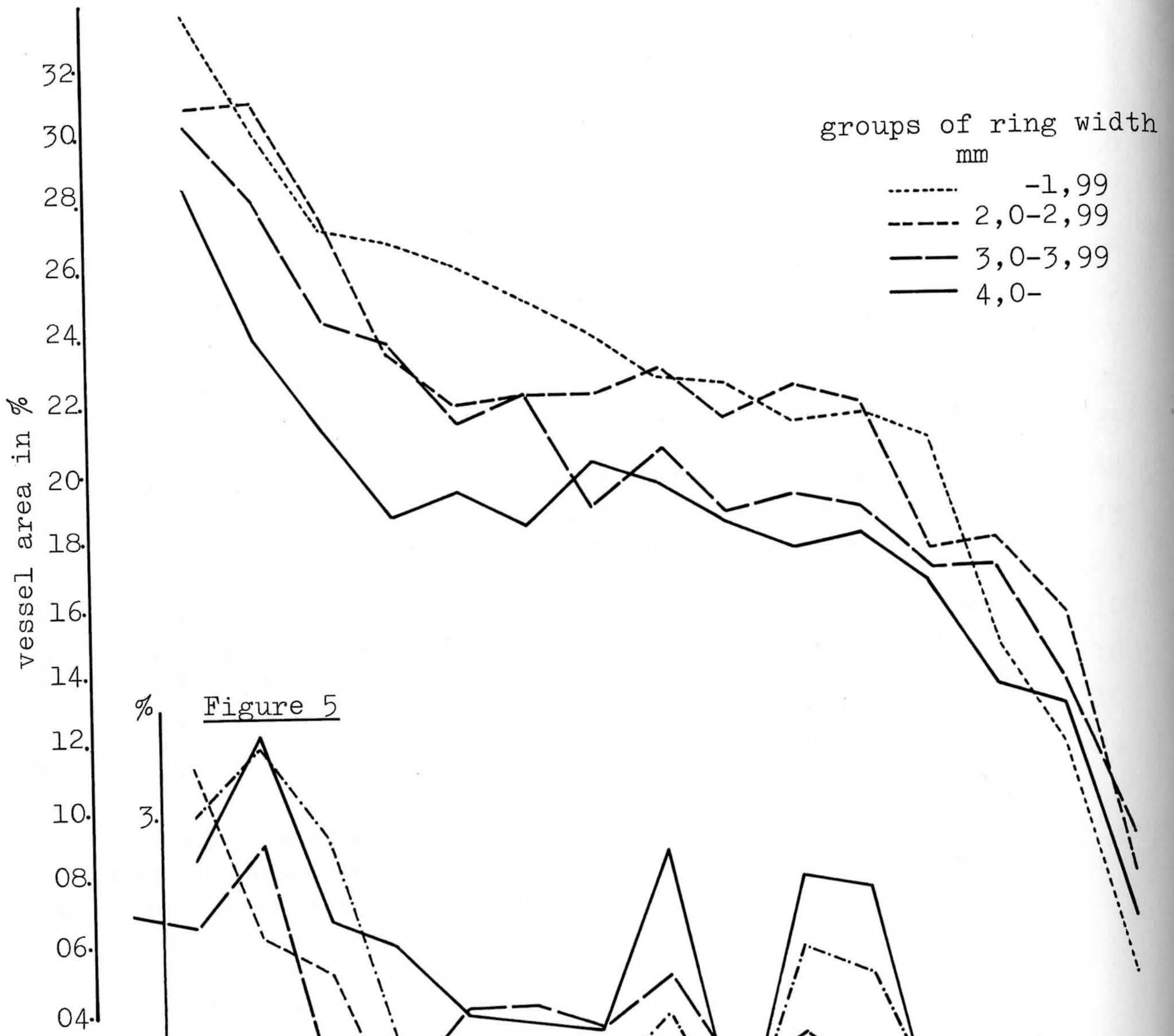
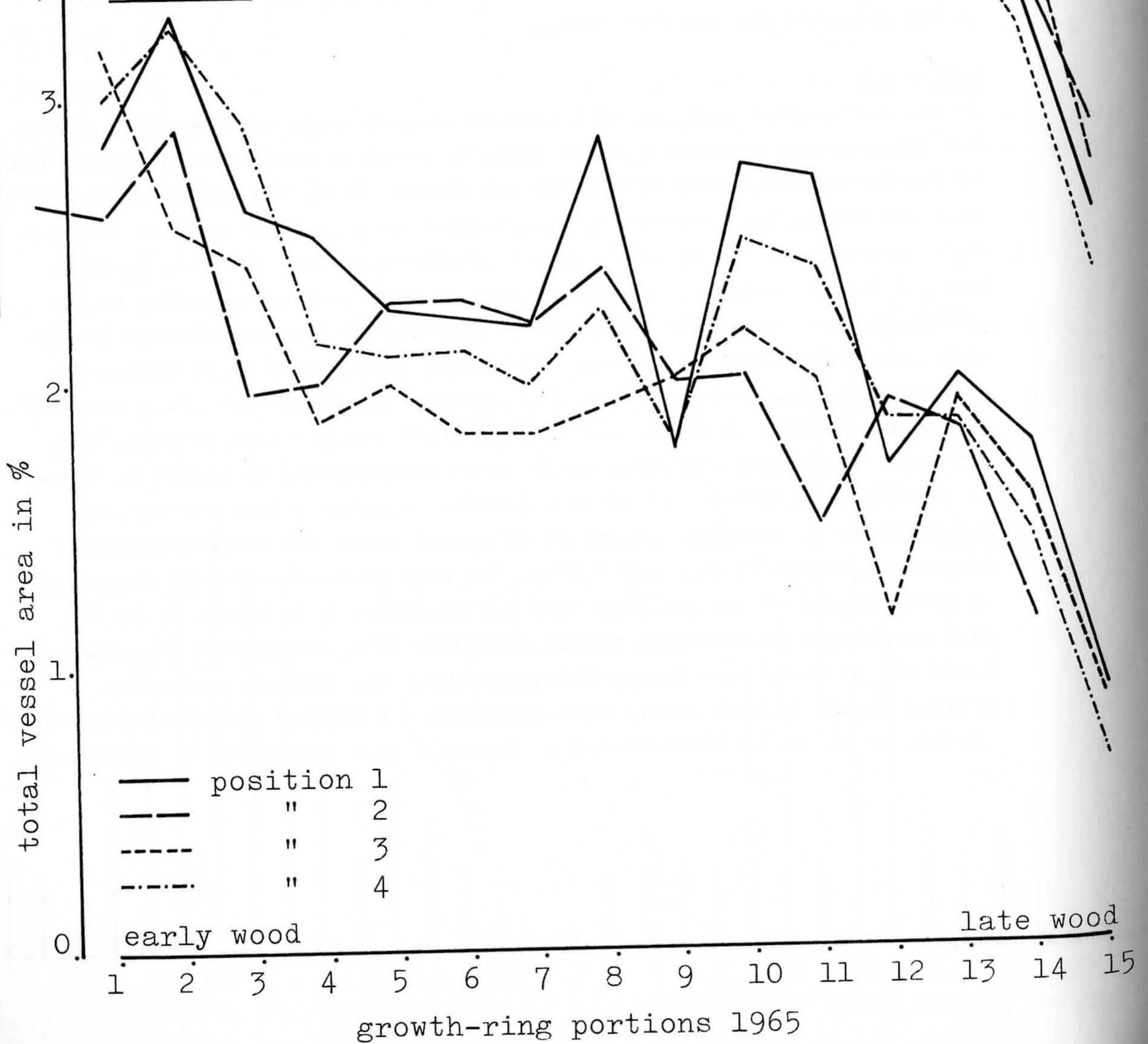


Figure 5



formation with the production of longitudinal parenchyma, a peak in the vessel curve corresponds more or less regularly to a minimum in the parenchyma curve. Thus, an internal change between vessel and parenchyma production must occur during differentiation. It is conceivable that exogenous factors such as water deficiency or alterations in the transpiration habitus may influence this relationship. It is therefore intended to use the method of statistical analysis of structural elements in relation to various physiological modifications of the plants.

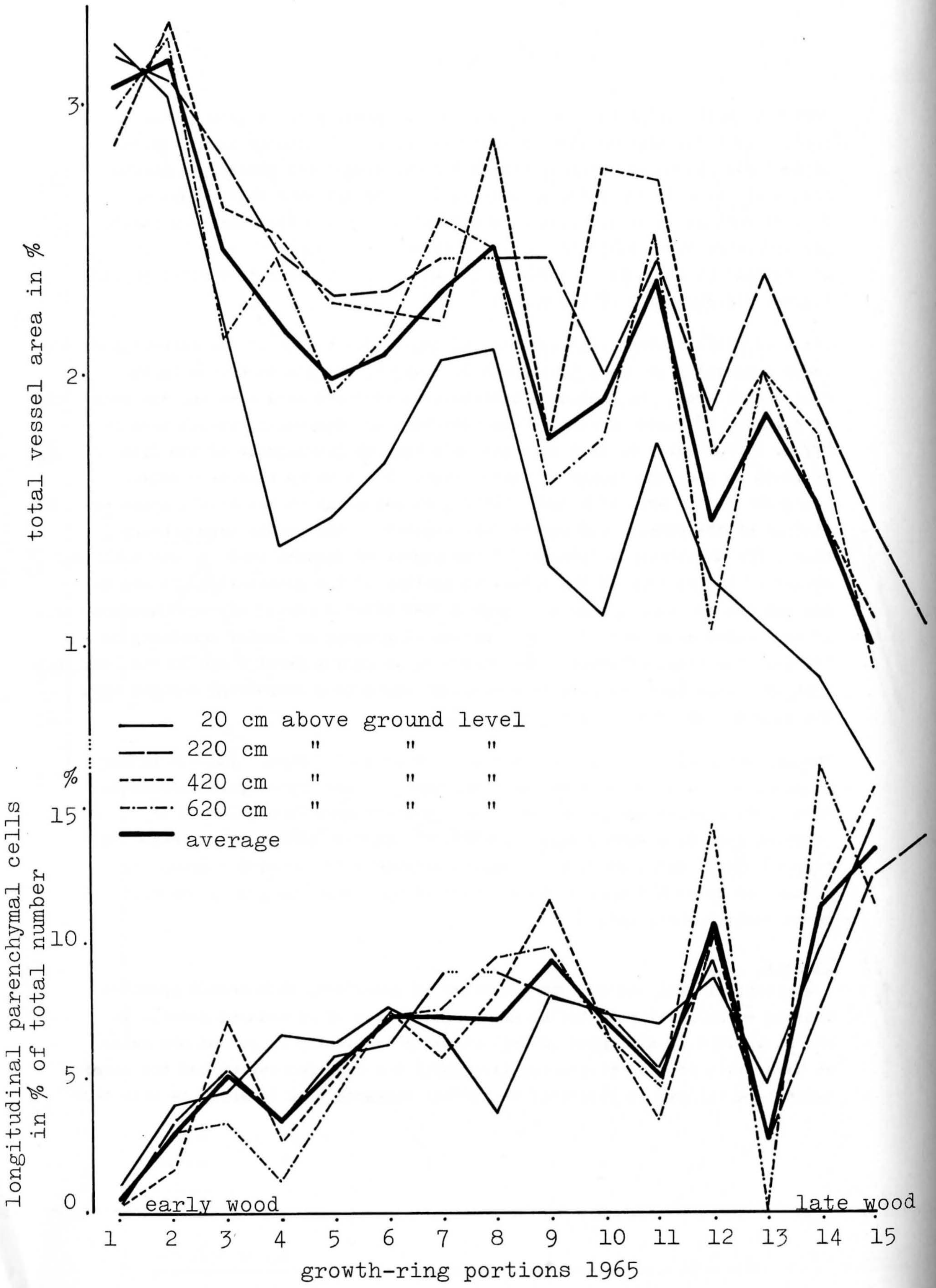
There is still another qualification of the wooden tissue of the investigated beech stem which is shown in figures 2, 3 and 4, where alterations in the mean vessel area, the frequency distribution of the vessel area and the relative amount of vessel areas are demonstrated. The decreasing vessel area in figure 2 from early to late wood may be a typical development of the size of elements as e.g. the change of fibre length. These facts have been known, since the famous work of K.SANIO (1872), as dependent on the growth characteristics of the cambium and can be incorporated in the growth laws already known. The simultaneous decrease of the number of vessels leads to the smaller amount of vessel area in the late-wood portion of the growth-ring. It may be pointed out in the diagrams of figure 4 that after a period of rapid decrease of the vessel area there follows a period of greater or lesser constancy to 11/15 of the ring, afterwards the curves again show a great slope in the last portions. This last change from a constant value to a decreasing one may mark the true change from early to late wood.

The frequency of the mean area of single vessels in figure 3 shows a rather singular distribution diagram. It seems that, in the investigated material, three vessel sizes are dominant: vessels with an area from 961-1320 u^2 (representing late wood), from 3120-3580 u^2 and from 4660-5020 u^2 (representing the first early wood) with special reference to the middle group. It remains to be proved whether this distribution can be changed by means of physiological modifications.

Summary

A statistical analysis of wood structure is described. This method provides results which facilitate the accurate description of structural detail. It can be applied to all types of cell elements or tissues in phloem and xylem. We have taken into consideration especially the vascular system and the longitudinal parenchyma in beechwood as further research work is going on with this

Figure 6



plant material. The statistical analysis of plant tissues will be of value for studies of the differentiation mechanism of the cambium as well as for the description of the structure itself.

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BOOK REVIEW

GOERKE, Heinz: "Carl von Linné, Arzt, Naturforscher, Systematiker"
Wissenschaftliche Verlagsgesellschaft m.b.H.
Stuttgart, 1966 (Band 31, Serie "Grosse Naturforscher")
pp 232, 28 Abb.

In the series "Grosse Naturforscher" the 'Wissenschaftliche Verlagsgesellschaft m.b.H., Stuttgart', has published a biography on Carl von Linné which gives an excellent picture of the famous scientist's life and work. The biographer, basing his work on studies and biographies about Linné written in Swedish, gives a rounded portrait not only of Linné's scientific activity but also of his personality and character. He succeeds in stressing Linné's merits with botanics in a very objective way without going off into heroics.

The fluency of his style and the well applied citations of passages of letters written by Linné will fascinate the specialist as well as the amateur. An extensive bibliography completes the handy and carefully outfitted volume.

L.Meier