INTERNATIONAL ASSOCIATION OF WOOD ANATOMISTS

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Editor: A. Frey-Wyssling, Secretary-Treasurer
In recent times wood anatomy has been intensively involved in the research on reaction wood (tension and compression wood). When reading these publications, I am often astonished how facts known or problems discussed since the very beginning of this century are considered as recent statements. As an example I note that, in the two symposia on wood structure edited by ZIMMERMANN (1) and COTE (2), where the reaction wood problems are treated by various authors, nobody seems to have known the fundamental observations of HARTIG (3) on tension fibres with their gelatinous layer and compression tracheids with helical splits. Another publication quoted nowhere is JACCARD’s study on the tension wood of broad-leaved trees (4), where a beautifully coloured plate shows the pink reaction of the tension fibres with chloro-zinc-iodide. Since the G-layer of tension fibres represents pure cellulose (cf. the communication of H.MEIER in this News Bulletin), it is not clear why this seemingly porous cellulose displays a similar colour with iodine as amylopectin, whilst the reagent stains amylese and the cellulose of blue but fibres violet. It is true that the two publications mentioned are not available in every library, but they ought to be consulted by anatomists specialised in reaction wood.

A study rarely cited is the first ultrastructural investigation on reaction wood by P. JACCARD and A. FREY (5). In that publication it is shown with the polarizing microscope that, in contrast to the helical texture of compression tracheids, tension fibres present an ideal fibre texture, i.e. all the ultrastructural elements run strictly parallel to the cell axis. This was found twenty years before the classical X-ray analysis of WARDROP and DADSWELL (6) substantiated that optical discovery.

To conclude this survey of neglected literature on reaction wood, I would like to refer to the endless discussions which took place around 1913 in the scientific societies in Zurich (Switzerland) on the cause of the formation of reaction wood. Whilst ENGLER (7) held that the cause was a teotropic stimulus and, therefore, called the
histologically altered xylem "geotropic" wood, JACCARD (8) defended his cause-and-effect theory of mechanical interventions by compression or tension. Both opponents produced important memoirs which have been published by the Foundation Schnyder von Wartensee in Switzerland (7, 8). The secretary-treasurer of the I.A.W.A. still has a limited number of copies of JACCARD's work, whilst that of ENGLER is out of print and, since no recent study ever mentions it, forgotten.

**Literature**

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7) ENGLER A. "Tropismen und exzentrisches Dickenwachstum der Bäume" Freischrift Schynder von Wartensee, Beer & Co, Zurich, 1918
8) JACCARD P. "Nouvelles recherches sur l'accroissement en épaisseur des arbres" Mémoire Schynder von Wartensee, Payot & Cie, Lausanne, 1919

A. Frey-Wyssling

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**THE GELATINOUS LAYER IN TENSION WOOD FIBRES OF ASPEN (POPULUS TREMULA L.)**

by Per Henrik Norberg and Hans Meier

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The formation of tension wood is induced by a gravity stimulus on the cambium. It has been found that this stimulus need only be operative for a duration of 24h (1). The cambial cell, during this time, undergoes such a physiological change, that many days later the libriform fibre derived develops an abnormal cell wall structure with a characteristic so-called gelatinous layer (G-layer).

In a recent study SACHSE (2) proposed a honeycomb texture for the G-layer of tension wood, and he supposed that hemicelluloses or pectic material were present within the combs. X-ray studies (3) on tension wood however suggest an orientation of the microfibrils in the G-layer strongly parallel to the axis of the fibres. Chemical studies on tension wood (4, 5) have not indicated the presence of abnormally large amounts of hemicelluloses or pectic substances in the G-layer.

The present paper reports on a study on isolated G-layers (the full paper is to appear in "Holzforschung"). Transverse sections about 20μ thick were taken from tension wood of *Populus tremula*. The sections were stored several days in 96% ethanol in order to shrink the G-layers, thus loosening them from the S₂-layers of the libriform fibres. The sections were then treated for five to ten minutes with ultrasonic waves in 96% ethanol. When so treated, some of the G-layers were shaken out of the sections and could be separated from the bulk by means of a sieve with 30μ mesh. The G-layer fraction also contained small amounts of cell debris, but was pure enough for studies of single G-layer cylinders with the polarizing microscope and for chemical studies. To ensure good loosening of the G-layer and to obtain a reasonably uncontaminated fraction thereof, it was important to start with fresh material.

Fig. 1) shows a portion of a section with most of the G-layers shaken out. Fig. 2) shows the fraction of isolated G-layers.
Measurements of the birefringence of isolated G-layers

The G-layer displays a fibre texture, in contrast to the helical texture of wood fibres (14), so that its indices of refraction can easily be measured. The birefringence of G-layer cylinders, isolated by ultrasonics from transverse sections, was determined by direct measurements of the refractive indices $n_E$ and $n_{nw}$ by the immersion method ($n_E$: polarised light vibrating parallel to the fibre axis; $n_{nw}$: polarised light vibrating perpendicular to the fibre axis). As immersion liquids, mixtures of cinnamon oil and amyl alcohol were used, the refractive indices of which were measured in a half-globe refractometer according to Abbé using sodium light ($\lambda = 586 \text{ nm}$). The G-layer cylinders which had been stored in ethanol were air dried on a microscope slide before immersion. Mean values of about twenty measurements are given in Table 1) plus, for comparison, the corresponding values for ramie fibres.

Table 1: Refraction indices and birefringence.

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<th>$n_E$</th>
<th>$n_{nw}$</th>
<th>$n_E - n_{nw}$</th>
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<tr>
<td>G-layer of Populus tremula</td>
<td>1.586</td>
<td>1.522</td>
<td>0.064</td>
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<tr>
<td>Ramie fibre (6)</td>
<td>1.600</td>
<td>1.532</td>
<td>0.068</td>
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The values for ramie fibres are only slightly higher than those for the G-layers, suggesting that the cellulose microfibrils run parallel with the axis of the fibres. A honeycomb texture as put forward by SACHSE (2) is therefore not substantiated.

Fig. 1) Transverse section of tension wood after ultrasonic treatment. In a few cells the G-layer (G) is still present, in most of them, however, it has been shaken out. x 1 600.

Fig. 2) G-layer fraction as obtained by ultrasonic treatment of transverse sections of tension wood and by sieving off the G-layers. x 650.

Fig. 3) Electron micrograph of part of a G-layer. Specimen shadowed with Pt/carbon. The microfibrillar texture shows parallel orientation which, however, has been somewhat distorted by ultrasonic treatment. x 42 000.
Electron microscopic study of the G-layer

During the past few years several authors have investigated tension wood with the electron microscope, either by the replica technique, or by the viewing of ultrathin sections (2,3,7,8). As a common feature of these studies it might be noted that, in transverse sections of methacrylate-embedded material, the G-layer always appeared as a very porous, obviously heavily swollen structure. As cited by Cote and Day (9), this has been interpreted as an indication of relatively weak lateral bonding and a lack of incrusting substances in the G-layer. This interpretation has been strongly confirmed throughout our work.

For our electron microscopic investigation, the G-layer fraction was treated with ultrasonics in about 30% ethanol to obtain at least some disintegration of the G-layers, without dissolution of incrusting substances possibly present. The suspension was applied to Formvar films and shadowed with platinum/carbon as described earlier (10). Figs. 3) and 4) show portions of a partially disintegrated G-layer cylinder. In spite of the fact that the specimens shown have undergone no chemical treatment at all, only bands of laterally aggregated microfibrils are visible. No trace of incrusting material, e.g. hemicelluloses or pectin, can be observed as in the case of normal holocelluloses when identically prepared (10). Figs. 3) and 4) give the impression, almost, of electronmicrographs of alpha celluloses. The microfibrillar bands are well aligned where the texture has not been too much distorted by the ultrasonic treatment.

Chemical investigation of the G-layer

a) Search for pectic substances

The "gelatinous" form of the G-layer might suggest the presence of pectic substances. Untreated tension-wood sections were therefore histochemically studied for the localisation of pectins. Two methods were used, ruthenium red coloration and the hydroxylamine-ferric chloride reaction as described in the literature (11, 12). These methods were applied to methacrylate-embedded G-layer cylinders (

Fig. 4) As Fig. 3), but even more distorted and fibrillated by ultrasonic treatment. x 42 000.

Fig. 5) G-layer fraction in water. x 950.

Fig. 6) The same sample as in Fig. 5) but dried on the slide. Identical G-layer cylinders are marked with a cross. Note the remarkable transverse shrinkage. No longitudinal shrinkage can be observed. x 950.
by JENSEN (11). Both methods showed conclusively that the G-layer was free from pectic material. Not even a pectin-rich inner border (cf. CASPERSON, 12) could be observed. However, both methods indicated the presence of pectic substances in the S₀-layers of tension wood fibres, especially in the late wood. It seems probable that the middle lamellae and primary walls also contain pectine, the detection of which, however, might be masked by the presence of lignin.

Transverse microtome sections of tension wood were then treated with pectinase, the solution was sucked off, concentrated, and chromatographed. A main spot of galacturonic acid and faint spots of galactose, glucose, mannose and xylose were detected on the chromatogram. Enzymatic hydrolysis and dissolution of pectic substances from the S₂-layer readily explains the easy loosening of the G-layer from S₂ after a pectinase treatment.

b) Chemical analysis of the G-layer fraction

By quantitative paper chromatography of a hydrolysate of the G-layer fraction (according to the method of SAEMAN, 13) it could be shown that the G-layer consists of very pure glucan which is most likely pure cellulose (Table 2). It can of course be argued that beside the cellulose another, perhaps amorphous glucan, is present in the G-layer. However, as our electron micrographs gave no indication of the presence of a large amorphous phase, and as chemical studies on tension wood of Fagus silvatica (5) further revealed no extractable glucan, it seems probable that the G-layer is pure cellulose. The analysis data of hydrolysates from tension wood and normal wood in the same stem of Populus tremula are also given in Table 2.

The longitudinal shrinkage of tension wood

In a recent article, SACHSE (6) reviews and further extends the many hypotheses on the extraordinary longitudinal shrinkage of tension wood during drying. He believes that a honeycomb texture of the G-layer together with the presence within the combs of hemicelluloses or pectic material is responsible for a shortening of the G-layer and, at the same time, for the longitudinal shrinkage of tension wood. SACHSE’s honeycomb texture can, however, be rejected on the basis of our results (Table 1), since the birefringence of a G-layer with such a texture should be very low. Furthermore, our chemical analyses of the G-layer revealed only trace amounts of hemicelluloses or of pectic material.

It was therefore decided to investigate whether or not the G-layers really shrink longitudinally. A suspension of G-layer cylinders from our G-layer fraction was photographed first in water, than air dried and rephotographed (Figs. 5 and 6). The G-layer cylinders showed a marked transverse shrinkage of 35 to 25% but extremely slight, if any, longitudinal shrinkage. The large longitudinal shrinkage of tension wood can therefore not be attributed to the G-layer, but must be effected by the S₁- and/or S₀-layers. Either (i) the helix of the cellulosic microfibrils in these layers or in one of these layers is flatter than in normal libriform fibres; or (ii) the flat helix of the S₁- combined with the thin S₀-layers and the gelatinous layers permits longitudinal shrinkage of the whole fibre with considerably more ease than in normal libriform fibres. In the case of the normal fibre, longitudinal shrinkage is prevented by the presence of a thick and stiff S₀-layer with a steep microfibrillar helix.

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<th>Table 2: Sugar analyses</th>
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<td>Weight (mg)</td>
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<td>Tension wood (transverse sections)</td>
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<td>G-layer fraction (shaken out from transverse sect.)</td>
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<tr>
<td>Residue (after shaking out most of the G-layers from the transverse sections)</td>
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<td>Normal wood from the same stem</td>
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When thin transverse sections of tension wood are viewed in the polarising microscope, not only the S₁-layer, but also the thin S₀-layer exhibit strong double refraction indicating, here too, a relatively flat microfibrillar helix. It therefore seems probable that the S₁- and the S₀-layers are the source of the longitudinal shrinkage.

The extremely high transverse shrinkage of the G-layer cylinders indicates a very porous texture. Many large, longitudinal capillaries between the cellulosic microfibrils must be present. In the fresh state these capillaries are filled with water which gives rise to the characteristic "gelatinous" aspect of the G-layer. How the protoplast creates such an abnormal cell-wall layer is a problem for future investigation.
Whether the higher longitudinal shrinkage of tension wood compared with normal wood is the main cause for the reerection of oblique stems is still open to discussion (cf. Sachsse, 6). It can, however, readily be imagined that, during maturation of the wood, the $S_1$- and $S_2$-layers slowly lose water and that the tension-wood fibres re-erect the stem by their longitudinal shrinkage.

Heartwood formation can be traced as an aspect of aging in a living tree. Due to the necrobiosis of the tissues differentiated by the cambium, the aging processes are of a very complex nature. On some occasions they occur very rapidly, for instance in the axial conducting tissue and the mechanical tissue (exceptions are known in a number of undershrubs where living fibres can be observed; cf. A. Fahm and W. Arno, 1962). According to the diagram in fig. 1 (H.H. Bosshard, 1965), the mechanical and conducting tissue undergo qualitative aging i.e. a loss of protoplasm which changes the living cell into a mere shell. This loss of protoplasm which can be termed necrobiosis occurs in the storage tissue as well, but there the whole process is subject to a more or less pronounced retardation. We therefore qualify each heartwood formation as a change of the status of the living storage tissue by necrobiotic alterations into the status of a skeleton tissue. In this connection there arise a number of questions, for instance whether these skeleton tissues may be called "dead" (they are still incorporated in a living organism). Furthermore one should have the possibility to decide with a high degree of accuracy whether a tissue or a single cell is "dead". Finally one may be interested in a classification which covers the different known forms and types of heartwood formation. - It is the aim of these notes to give some impulse for a subsequent discussion of the different points in question.

Nomenclature
First of all it may be helpful to fix the nomenclature, i.e. to give the definition of our interpretation of different terms describing heartwood formation. It has been common practice to distinguish between sapwood trees ($\textit{Alnus incana}$), ripewood trees ($\textit{Abies alba}$), and trees with regular formation of heartwood (e.g. $\textit{Pinus}$ species) or irregular formation of heartwood (e.g. $\textit{Fagus silvatica}$). H. K. Boeslender/ B. Thunberg/R. Thunberg/H. B. Daweley/R. W. Millis). This terminology is contestable because it is based mainly on macroscopically visible characteristics and takes too little account of the cytology
of the aging storage tissue. In young trees, the sapwood zone commonly ex­
tends from the cambium to the pith; in older trees it usually comprises only
a number of peripheral rings. Exceptions are found in the species previously
referred to as "sapwood trees" which possess wood of the sapwood-type also
in the vicinity of the pith. Too little is as yet known of the actual changes
in the living tissue of these types in the vicinity of the pith, but we have
good evidence of that the cytological aging of cells (AFREY-WYSSLING and
H.H. BOSSHARD, 1959) at advanced tree ages is basically the same as in woods
forming heartwood, with the exception that it begins later. Apart from these
so-called sapwood trees, the transformation into heartwood usually begins
regularly, but at least three modifications can be observed:

(1) Woods having light heartwood (cf. ripewood trees). They clearly reveal
necrobiosis of the storage cells without building large quantities of pigmen­
ted heartwood substances. Occasionally, however, coloured heartwood substances
are found in the individual storage cells, so that it must be assumed that
their precursors are formed in the cambium of these woods as well, but obvi­
ously remain unpigmented.

(2) Woods with obligatory-coloured heartwood (cf. trees with regular heart­
wood formation). In this group, which may also be known as the oak type,
pigmented heartwood substances are invariably formed in the storage tissue
and are generally capable of penetrating into the cell walls of all tissue
units.

(3) Woods with facultative coloured heartwood (cf. trees with irregular
heartwood). This group may be designated as the ash type. The brown heart­
wood of an ash need not be present in all samples and need not affect the
entire heartwood portion. In addition, the pigmented heartwood substances
are commonly retained as wall coating or droplike inclusions in the cells
of the storage tissue. In this case, the cell walls are not impregnated
either in the storage tissue or in the rest of the xylem (H.H. BOSSHARD, 1955).

I would suggest that we discuss this nomenclature problem in extenso in or­
der to come to conclusions and to recommend well-described and well-based
terms for future use.

Vitality of the storage tissue
The parenchymatic tissue exercises a storage function. It can be shown from
similar processes in the leaves of the plant that storage is a very active
biochemical process depending on a fully active cell. The parenchymatic tissue
in the living tree therefore shows, especially in the outer part of the stem,
high vitality. With the transformation of outer sapwood into inner sapwood,
outer and inner heartwood, there occurs a gradual change in this vitality.
The observations of these changes are possible only with the help of adequate
methods defining the vitality of cells. We have therefore concentrated our
work on this point and obtained the following results:

Form and size of cell nuclei as an indication for cell vitality: Cells in the
neighbourhood of the cambium doubtless show the highest activity. Their nuclei
have the largest volume and surface. It is possible to calculate the degree
of slenderness, the surface and the volumes of cell nuclei in trees, beginning
in the cambial zone and following the radial direction into the stem. The find­
ings in these measurements can be summarized as follows:

- The form and dimensions of the cell nuclei are a very valuable indication
for tracing the activity of a parenchyma cell. Cells with high activity and
high vitality possess large nuclei. The cambium cells themselves have virtually
round nuclei (the degree of slenderness is almost 1), the cells of the cambial
zone possess oval nuclei with a high degree of slenderness whereas, in the
inner part of the sapwood, the nuclei in the parenchyma cells again become
more and more rounded. These latter nuclei are smaller than the round ones in
the cambium. That means that the form alone is not significant in this case.
It must be regarded together with the size. It is conceivable that the bio­
chemical processes in cells of high activity must be governed by large nuclei.
It has been shown that the surfaces of cell nuclei are not of a uniform struc­
ture, but possess porous fields which are quite probably involved in the ex­
change of cell nucleus substances with the surrounding protoplasm.

- Measurements of cell nuclei shapes and dimensions have to be correlated
with measurements of the cells themselves, due to the increase of the nuclear
mass with the development of the cell.

Dimensions of nucleoli as an indication for the vitality of a cell: As a further
indication of cell vitality, the dependence of the diameter of nucleoli on the
age of a cell can be measured; as a result, it is clearly visible that cells
in the neighbourhood of the cambium possess large nucleoli, whereas cells
further away from this zone have small nucleoli only. An interesting fact to
be observed is an increase of the diameter of these nucleoli in the very
transition zone between sapwood and heartwood. This finding confirms a statement by M.M. CHATTAWAY in 1952, indicating that, in the zone of intermediate wood between sapwood and heartwood, the cells display a high activity for the last time. According to our experience, the observation of the cell nuclei is of greater significance than the measurements of dimensions and shapes of the nuclei for the description of cell vitality.

Migration of nuclei as an indication of cell vitality: During the investigations on the cytology of ray cells, we have collected material in different seasons. Strangely enough, we could find a migration of nuclei changing from a central position in the cell to a marginal position. Migrations like that are only known of cells which are still capable of division, or of alterations of the cell walls. Correlations of the portion of migrating nuclei with the age of sapwood reveal that, in the neighbourhood of the cambium (a tissue of high activity), the portion of migrating nuclei is greater than near the intermediate zone. - Again, this feature is of great significance in describing the vitality of tissue and should be used much more.

Literature

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therefore be advisable to recommend the organization of small ad-hoc symposia in Europe or America to discuss impressions of the Melbourne meeting and to develop interest in the next I.U.F.R.O. conference to take place in Munich in 1967.

3. At present the Sub-Group covers the fields of Spiral Grain, Fibre Characteristics, Heartwood Formation, and Specific Gravity. It is recommended that consideration be given to adding a committee dealing with Descriptive Wood Anatomy. The interest in anatomical work is world-wide and becoming more and more important owing to the fact that new timber resources will have to be explored. This committee should co-operate closely with the I.A.W.A.

4. The present conference has shown that interest in both the Microscopic Characteristics Sub-Group and the Macroscopic Characteristics Sub-Group of the Wood Quality Group is extensive, and that each covers a wide field of research. Therefore I feel it be of advantage if the two Sub-Groups were reconstituted as two separate Groups.

We recommend the incorporation in this Sub-Group, which in the meantime has become a full working group of Section 41, of a committee dealing with Descriptive Wood Anatomy (paragr. 3 of the report). The discussions have shown that there is a considerable demand for more information on wood anatomy. Therefore we feel it might be a real progress if wood anatomy could be handled in this connection in close co-operation with our association of wood scientists. This will never involve competition between I.U.F.R.O. Section 41 and I.A.W.A.; there will rather be strong evidence for activating the pure and applied wood anatomy and to strengthen the interest of all wood scientists in this field. We therefore hope that our members agree with this recommendation to the chairman of I.U.F.R.O., Section 41.

In addition to the lectures, different field trips were organised, e.g. in afforestations of Pinus radiata and Eucalyptus species. Furthermore, it was possible to watch demonstrations in the laboratories of the Division and to visit different industrial plants.

This symposium was most interesting and valuable. We are greatly indebted to the organising committee and to the staff of the Division for all the help and friendship they have shown us. Once again we have been confirmed in the opinion that symposia with a small number of attendants are more active and efficient than big conferences.

H.H. Boeshaar