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Distribution of Lignin in Normal and Tension Wood*

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Summary

The distribution of lignin in normal and tension wood of four hardwood species has been studied by examination in the electron microscope of the lignin skeletons remaining after removal of the polysaccharides with hydrofluoric acid. In normal wood fibers, the S_1 had a higher lignin concentration than the S_2 layer, which was not as highly lignified as in conifer tracheids. Vessels had a high concentration of lignin in both normal and tension wood, while the extent of lignification of the parenchyma was variable.

In tension wood fibers, the S_1 and S_2 layers were highly lignified. A thick, unlignified G-layer was often associated with an extremely thin S_2 layer with a high concentration of lignin. In both normal and tension wood, the lignin had the same orientation as the cellulose microfibrils in the different cell wall layers. The results confirm the earlier conclusion that, in the species investigated, the same amount of lignin is present in gelatinous as in normal fibers. Evidently, the lignification mechanism operates normally in the non-gelatinous layers of the fibers, as well as in the vessels and in the parenchyma of tension wood.

Zusammenfassung

Die Ligninverteilung im Normalholz und im Druckholz von vier Laubhölzern wurde untersucht. Die Ligningerüste, die nach der Entfernung der Polysaccharide durch Fluorwasserstoffsäure übrigblieben, wurden im Elektronenmikroskop beobachtet. In den Normalholzfasern hatte die S_1-eine höhere Ligninkonzentration als die S_2-Schicht, die weniger lignifiziert war als in den Koniferentracheiden. Die Gefäße hatten eine hohe Ligninkonzentration in sowohl Normal- als in Zugholz, während der Lignifizierungsgrad der Parenchymzellen variierte.

In den Zugholzfasern waren die S_1- und S_2-Schichten völlig lignifiziert. Eine dicke, unlignifizierte G-Schicht war oft mit einer außerordentlich dünnen S_2-Schicht, die eine hohe Ligninkonzentration zeigte, verbunden. Sowohl im Normal- wie auch im Zugholz besaß das Lignin dieselbe Orientierung wie die Cellulosemikrofibrillen in den verschiedenen Zellwandschichten. Die Ergebnisse bestätigen den früheren Schluß, daß in den hier untersuchten Laubhölzern in den gelatinösen und in den normalen Fasern dieselbe Ligninmenge vorliegt. Offenbar läuft der Mechanismus der Lignifizierung in den S_1- und S_2-Schichten der gelatinösen Fasern des Zugholzes normal ab.

Introduction

The distribution of lignin in wood, which is of both theoretical and practical interest, has been studied for over forty years. Most investigations have been concerned with conifers and were recently reviewed by CÔTÉ, ZABEL, and TIMELL [1966] and by MARK [1967]. During the last few years, GORING and his co-workers

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have quantitatively determined the distribution of lignin in various woods by application of ultraviolet microscopy to ultrathin section of wood. FERGUS, PROCTER, SCOTT, and GORING [1969] [SCOTT, PROCTER, FERGUS, and GORING 1969] found that in the tracheids of *Picea mariana* only 28% and 18% of the total lignin was located in the compound middle lamella of earlywood and latewood, respectively, the remainder being deposited in the secondary wall. In earlywood, the concentration of lignin was 22% (w/w) in the secondary wall, 84% at the cell corner and 49% in the remainder of the compound middle lamella. The lignin appeared to be uniformly distributed over the secondary wall, a conclusion also reached by CÔTÉ, DAY and TIMELL [1968], who used electron microscopy in examining the distribution of lignin in tracheids of *Larix laricina*.

For hardwoods, the prevalent concept until very recently has been that, at least in species of the temperate zone, most of the lignin is located in the middle lamella. In his first investigation on the subject, RITTER [1925] came to the conclusion that 73% of the lignin in *Alnus rubra* fibers is situated in this region. LANGE [1954], who was the first to apply ultraviolet microscopy for this purpose, found that in several Swedish hardwoods, the concentration of lignin in the secondary wall of the fibers was extremely low. SACHS, CLARK, and PEW [1963] and SACHS [1965] removed all polysaccharides with hydrofluoric acid and examined the residual lignin skeleton in the electron microscope. Compared to softwood tracheids, hardwood fibers from *Acer saccharum* and *Fagus sylvatica* had a less highly lignified cell wall with the exception of the S_3 layer, where the concentration of lignin was as high as in the middle lamella. Using a similar technique, FENGEL [1965] found ray cells of *Fagus sylvatica* to contain relatively little lignin in their secondary wall.

Recently, FERGUS and GORING [1968a] reported that in *Betula papyrifera*, the vessels contain a lignin based largely on guaiacyl units, whereas syringyl units predominate in the lignin present in the secondary wall of the fibers and ray parenchyma. The lignin in the middle lamella around the fibers and rays is a mixture of the two types. The same investigators [FERGUS and GORING 1968b] have also determined the distribution of lignin in the xylem of *Betula papyrifera*. Only 19% of the total lignin was present in the compound middle lamella, while 60% occurred in the secondary wall of the fibers. As much as 77% of the lignin associated with the fibers was located in the cell wall. The concentration of lignin in the latter was 16 to 19%, while the secondary wall of the vessels and rays contained 22 to 27% lignin. At the fiber cell corners, the lignin concentration in the middle lamella was 72 to 85%, but it was only 34 to 40% in the radial and tangential regions. The data reported indicate a uniform distribution of lignin within the cell walls.

Prior to this important investigation, it was generally belived, largely on the basis of the microspectrographic evidence presented by LANGE [1954], that the cell wall of hardwood fibers contained very little lignin. This wall does exhibit a quite low absorbance in ultraviolet light, but it is now clear that this is a result of the fact that the syringyl lignin present here has a lower absorbtivity than guaiacyl lignin [FERGUS and GORING 1968a].

LANGE [1954] came to the conclusion that the entire cell wall of tension wood fibers was unlignified, a view which has not been accepted by other investigators. Tension wood has indeed a lower lignin content than does corresponding normal

wood. This, however, is the result of the presence of the massive, and usually unlignified, gelatinous layer in tension wood fibers. WARDROP and DADSWELL [1948], JAYME [1951], JAYME and HARDERS-STEINHÄUSER [1950, 1953], and TIMELL [1969] have all drawn attention to the fact that the *absolute* amount of lignin present in each fiber often is approximately the same in normal and tension wood.

In many cases, the pattern of lignification of tension wood fibers has been found to vary considerably. Using staining reactions, WARDROP and DADSWELL [1955] [DADSWELL and WARDROP 1955] noted that either S_1 or S_2 could be unlignified, depending on the mode of attachment of the G-layer. SCURFIELD [1964] noted that lignification was often pronounced in the outer layers of tension wood fibers. In an electron micrographic study he also observed that lignification was initiated in the intercellular region, spreading from there into the cell wall [SCURFIELD 1967]. Recently, ROBARDS [1967] concluded that, with the exception of the G-layer, the secondary wall of tension wood fibers appeared to be normally lignified.

In a few cases, the gelatinous layer has been reported to be slightly lignified, for example in *Tristania conferta* [SCURFIELD and WARDROP 1962, 1963]. In *Quercus robur*, CASPERSON [1967] noted that, while the gelatinous layer remained unlignified in the earlywood, it was lignified in the latewood. SCURFIELD and WARDROP [1963] observed that tension wood fibers formed late in the growing season were more likely to contain a lignified G-layer than were fibers produced at an earlier stage.

In a previous investigation [TIMELL 1969] it was shown that in four North-American hardwoods, the lignin contents of normal and tension woods were the same when the comparison was made on the basis of the same content of cellulose. This means that the non-gelatinous layers, in this case the S_1 and the S_2 layers, must contain approximately the same amount of lignin in tension as in normal wood fibers. It was the objective of the present investigation to offer direct evidence for the distribution of lignin in tension wood. The approach was the same as that used previously for compression wood [CÔTÉ, TIMELL, and ZABEL 1966; CÔTÉ, DAY, and TIMELL 1968] and involved removal of all polysaccharides with hydrofluoric acid, followed by examination of the remaining lignin meshwork in the electron microscope. For comparison, normal wood was also included.

Materials and Methods

Normal and pronounced tension woods were collected from red maple (*Acer rubrum* L.), American beech (*Fagus grandifolia* Ehrl.), quaking aspen (*Populus tremuloides* Michx.), and American elm (*Ulmus americana* L.). The chemical composition [TIMELL 1969], anatomy and ultrastructure [CÔTÉ, DAY, and TIMELL 1969] of the specimens have been reported previously.

Lignin skeletons were prepared by the method of SACHS, CLARK, and PEW [1963] as modified by CÔTÉ, DAY, and TIMELL [1968]. Specimen preparation and electron microscopy were carried out according to CÔTÉ, TIMELL, and ZABEL [1966].

Results

Normal wood of aspen, beech, elm, and maple contained 20.9, 23.5, 21.7, and 25.4% Klason lignin, respectively. Corresponding figures for the tension wood were 16.8, 17.0, 20.2, and 17.5%. If the normal wood had contained the same quantity of cellulose as did the tension wood, its lignin content would have been 16.8, 17.7, 19.5, and 17.7%, respectively, values very close to those valid for the tension wood. No attempt was made to determine the small amounts of acid-soluble lignin probably also present.

Transverse sections of normal wood from aspen, beech, and maple that had been treated with hydrofluoric acid for removal of cellulose, hemicelluloses, and pectin, are shown in Figs. 1—3. Because of their more open and looser texture, the cell wall lignins in these hardwoods were more easily disorganized than were the lignin skeletons in conifer tracheids. In all three species, the location of the former cell wall layers can be distinguished, although it must be recognized that the S_1 layer often has become detached from the compound middle lamella, and notably so at the cell corners (Fig. 1). The lignin in S_1 follows, as can be clearly seen in Fig. 3, the transverse orientation of the cellulose microfibrils in this layer, forming a fairly dense, lamellar texture, as has also been observed with softwood tracheids [FREY 1959; JAYME and FENGEL 1961 b]. The concentration of lignin in the S_1 layer is high and, based on visual examination, probably as high as in conifer tracheids. In the S_2 layer, the lignin concentration is definitely lower than in the latter, and especially so for the maple.

In Fig. 4, obtained with maple, the cell wall is terminated towards the lumen by a thin, electron-dense lamella. It is difficult to assess the exact nature of this phenomenon, which sometimes is much more pronounced, as shown in Fig. 5 for the same wood. It could represent the S_3 layer, which in that case would be highly lignified, as claimed by SACHS, CLARK, and PEW [1963] and by SACHS [1965]. The thin lamella in Fig. 4 could, however, also be the warty layer, which is known to be resistant to strong acids [FENGEL 1965]. The thicker layer in Fig. 5, which apparently is terminated by a warty layer, could be an artifact, brought about by a centripetal agglomeration of lignin fragments originally located further out in the S_2 layer. Similar phenomena have been observed with conifer tracheids [JAYME and FENGEL 1961 a, 1961 b; SACHS, CLARK, and PEW 1963; CÔTÉ, TIMELL, and ZABEL 1966]. According to CÔTÉ, DAY, and TIMELL [1968], all three layers in the secondary wall of the tracheids in *Larix laricina* are lignified to the same extent.

When tension wood was treated with hydrofluoric acid, it was noticed that the G-layer was relatively resistant to hydrolysis, despite the fact that it was not encrusted, and thus not protected, by lignin. Obviously, the highly crystalline nature of the G-layer cellulose renders it less amenable to depolymerization by the acid. Two examples of this are shown in Figs. 6 and 7. In most of the fibers, a small portion of the gelatinous layer remains in the middle of the lumen, together with a terminal lamella and/or a warty layer. In one case (Fig. 6), the G-layer has swollen and fills the entire lumen. The granular nature of the G-layer cellulose distinguishes it from the remainder of the cell wall. In Fig. 7 there is also shown part of a vessel with its warty layer. Evidently, vessel walls are as highly lignified

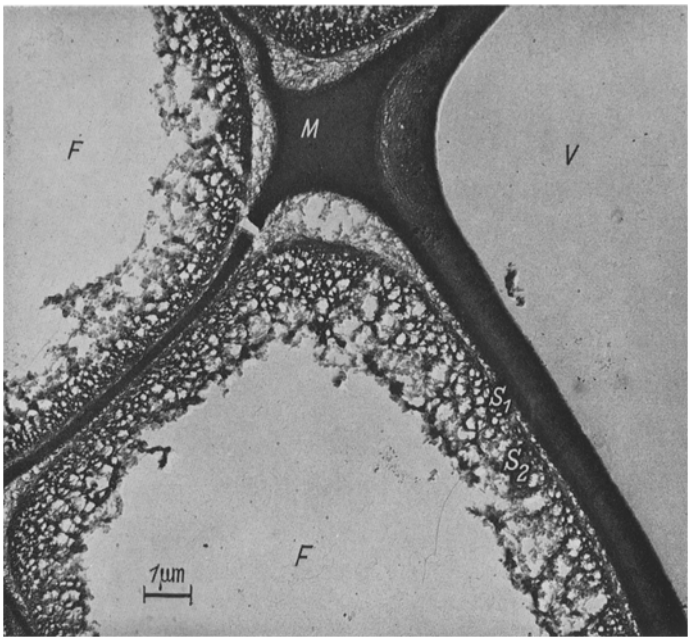

Fig. 1. Transverse section of three fibers (F) and one vessel (V) of normal aspen wood, showing the compound middle lamella (M), the S_1 and the S_2 layers. Note the higher lignin concentration in the vessel wall.

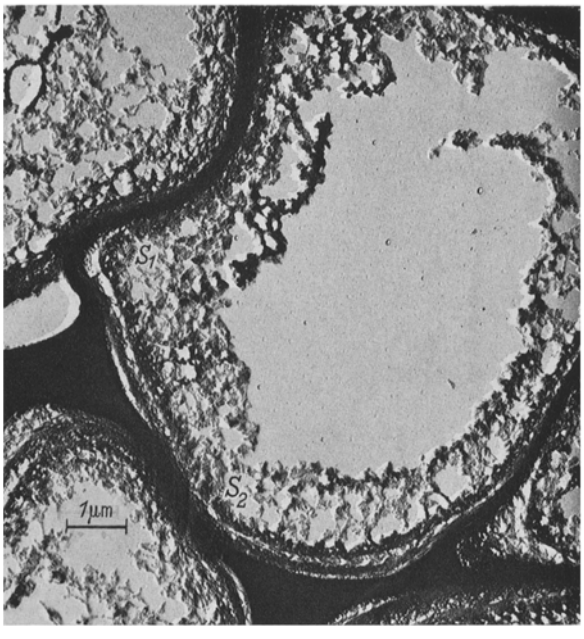

Fig. 2. Transverse section of three fibers of normal beech wood. The lignin meshwork in S_2 is partly disrupted.

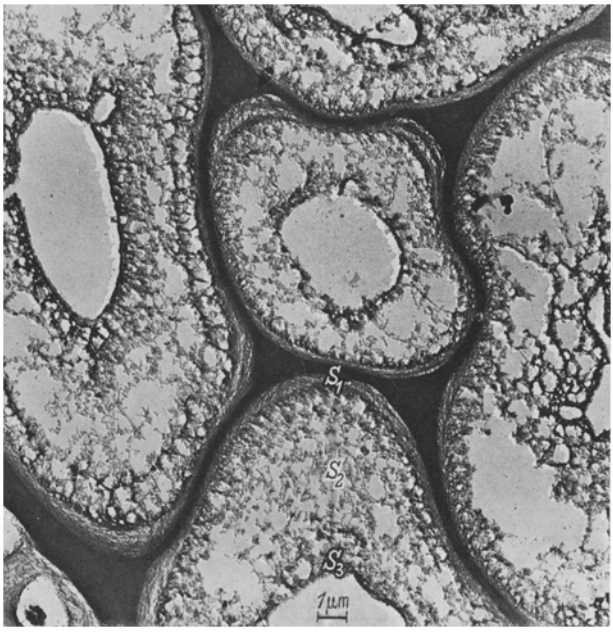

Fig. 3. Transverse section of fibers in normal maple wood. Note that the S_1 has a higher concentration of lignin than does the S_2 layer.

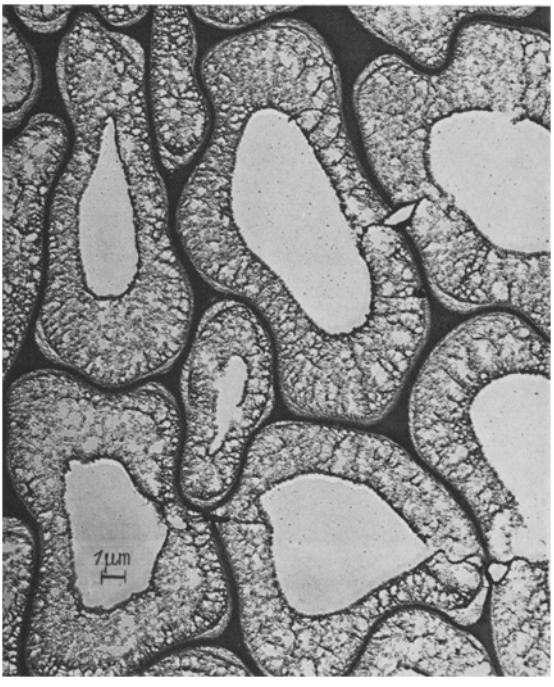

Fig. 4. Transverse section of fibers of normal maple wood, showing almost intact lignin skeletons. Note the occurrence of a thin, electron-dense terminal lamella.

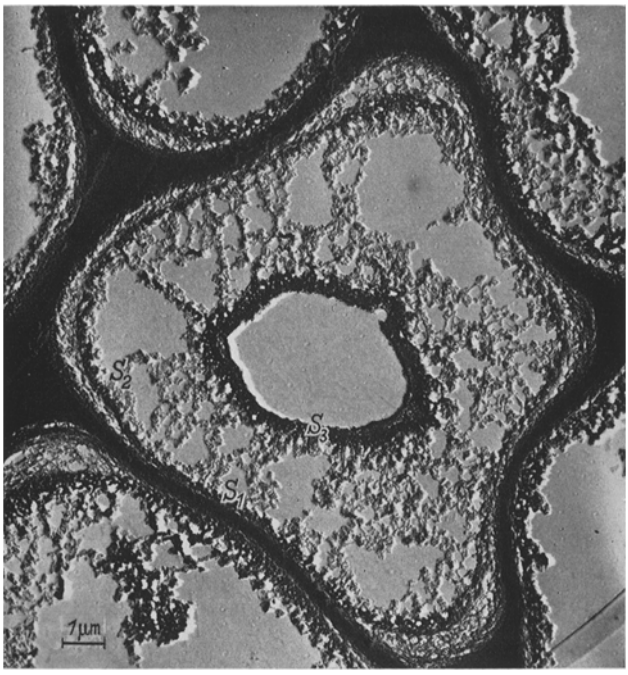

Fig. 5. Transverse section of fibers in normal maple wood, showing anapparent, high concentration of lignin adjacent to the lumen.

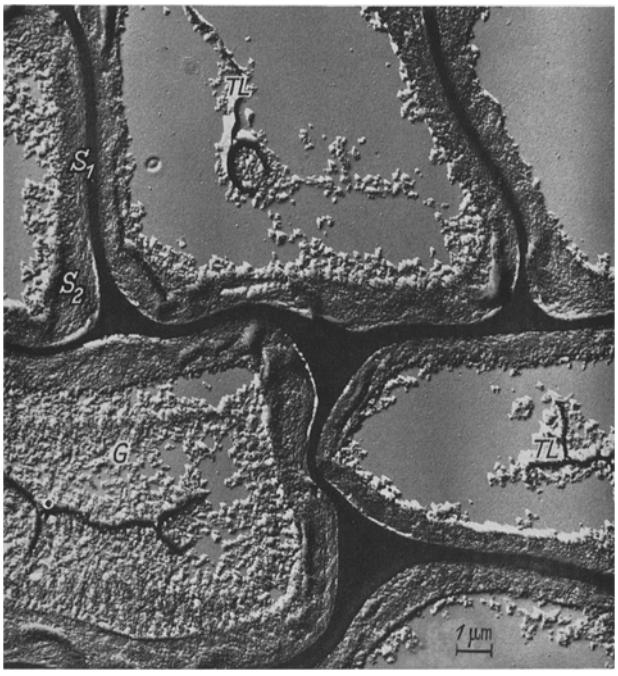

Fig. 6. Transverse section of beech tension wood fibers during removal of the polysaccharides. Note the presence of a terminal lamella at the center of the remaining, dispersed G-layers.

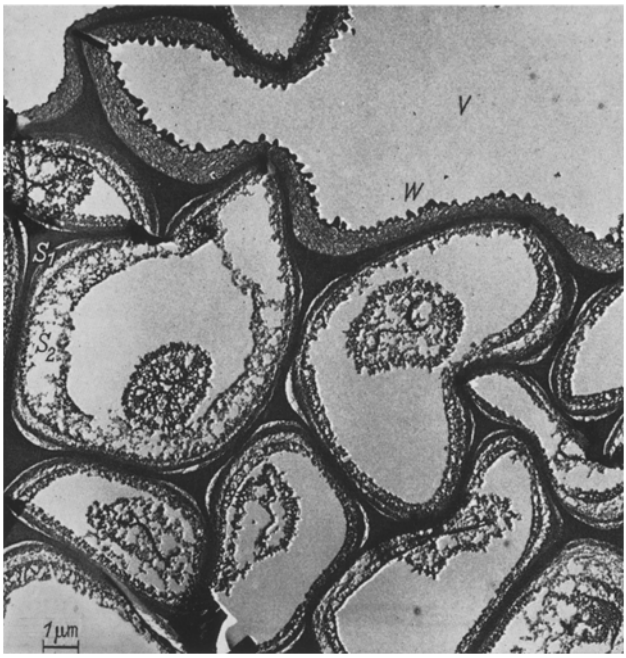

Fig. 7. Transverse section of beech tension wood fibers containing only small fragments of unhydrolyzed G-layers. The vessel wall is highly lignified and has a warty layer (W).

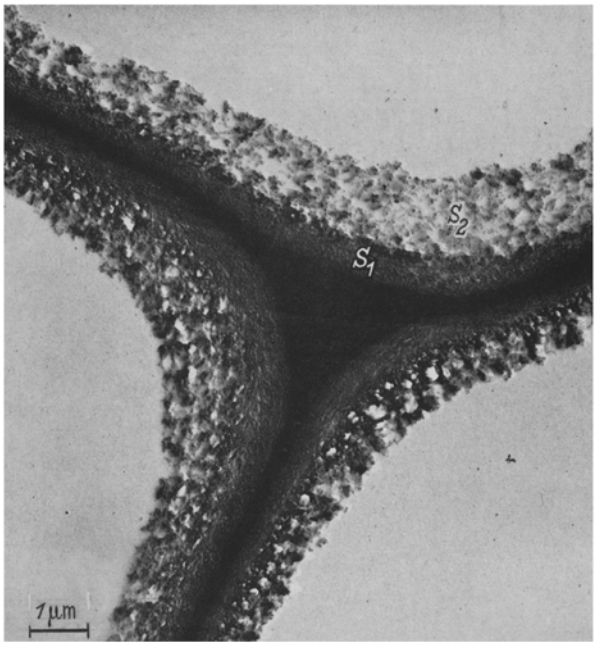

Fig. 8. Transverse section of aspen tension wood fibers.

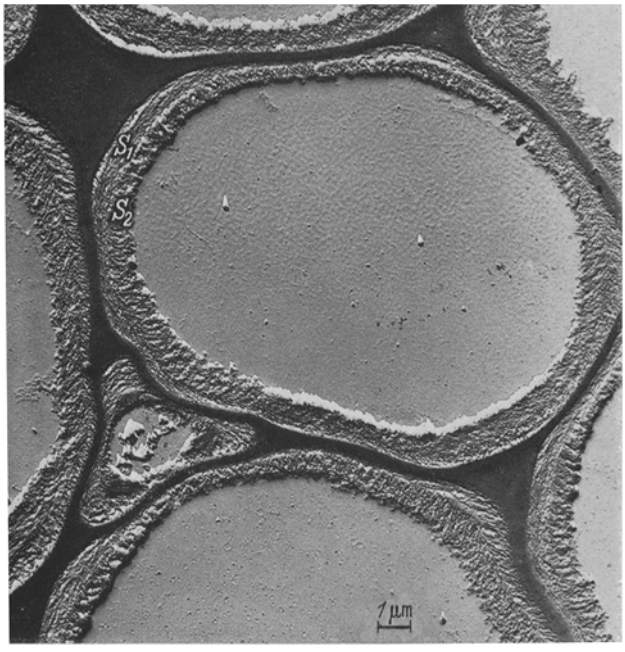

Fig. 9. Transverse section of maple tension wood fibers.

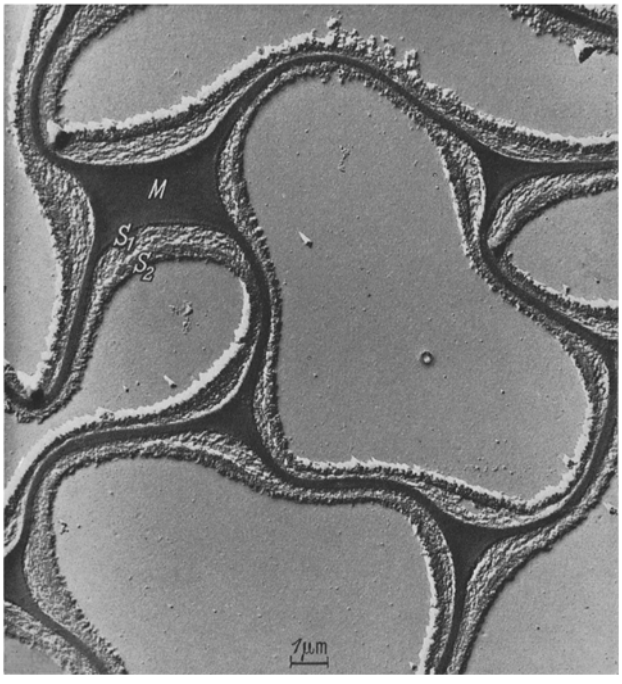

Fig. 10. Transverse section of maple tension wood fibers. Note that S_2 is thinner than S_1.

in tension as in normal wood (Fig. 1), confirming the earlier observation of SCURFIELD and WARDROP [1963].

The distribution of lignin in tension wood fibers of beech, aspen, and maple are shown in Figs. 7—10. The S_1 layer has a high concentration of lignin, the texture of which reflects the largely transverse orientation of the microfibrils (Figs. 8 and 9). The extent of lignification of the S_2 layer varies, as can be seen from Figs. 7 and 8. In beech, narrow S_2 layers have a higher concentration of lignin than do wider ones. When the S_1 and S_2 layers are of equal width, as in the maple fiber shown in Fig. 9, the two layers seem to be lignified to the same entent. Fig. 10 shows some maple fibers with unusually thin S_2 layers. In this case, the S_2 appears to have a denser lignin meshwork than does the somewhat thicker S_1 layer.

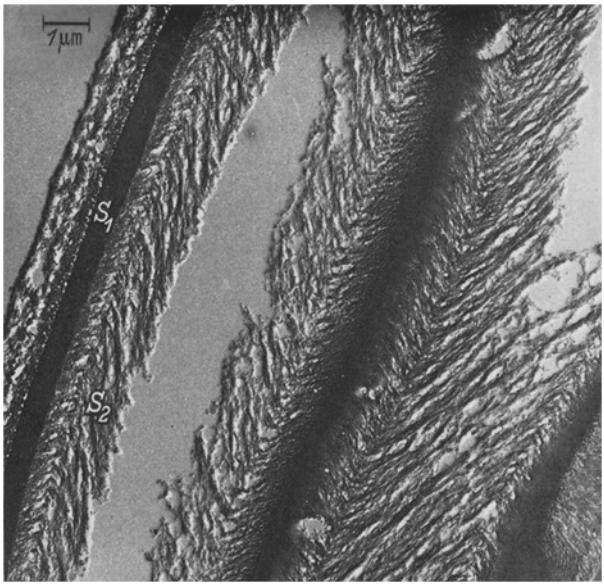

Fig. 11. Oblique, tangential section of beech tension wood fibers. Note the different orientation of the lignin in the S_1 and S_2 layers.

Tangential sections of beech and elm tension wood treated with hydrofluoric acid are shown in Figs. 11 and 12. In both cases, the lignin clearly mirrors the different orientation of the cellulose microfibrils in S_1 and S_2. Both layers appear to be as highly lignified as in conifer tracheids.

Transverse sections of radial parenchyma in aspen and beech tension wood are shown in Figs. 13 and 14. The cell wall of the aspen parenchyma has a much lower and the wall of the beech parenchyma a much higher concentration of lignin than do the fiber walls. In *Fagus sylvatica*, ray cells have been reported to be only slightly lignified [FENGEL 1965], whereas in *Betula papyrifera* they contain more lignin than the fibers [FERGUS and GORING 1968b]. Evidently, hardwood parenchyma have a variable lignin content, depending on factors which remain to be determined.

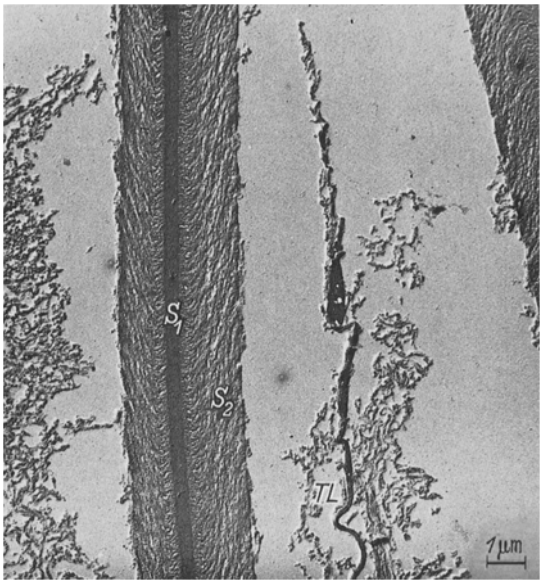

Fig. 12. Tangential section of elm tension wood fibers, containing remnants of the G-layer.

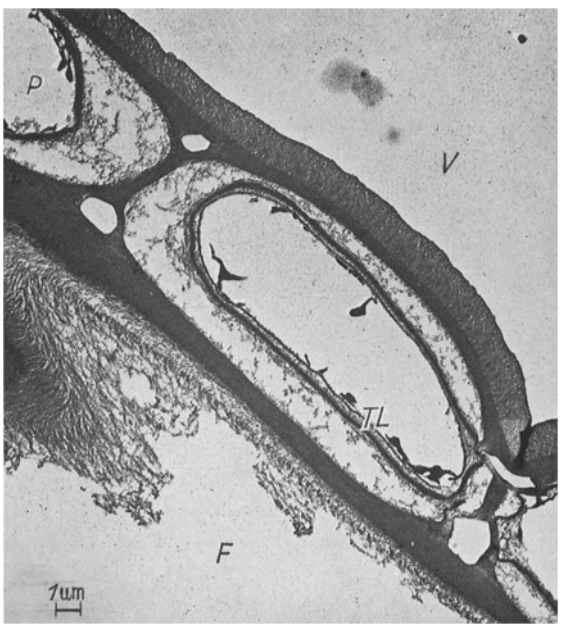

Fig. 13. Tangential section of aspen tension wood. The secondary wall of the two parenchyma (P) cells contain very little lignin and possessess a terminal lamella (TL) and a warty layer.

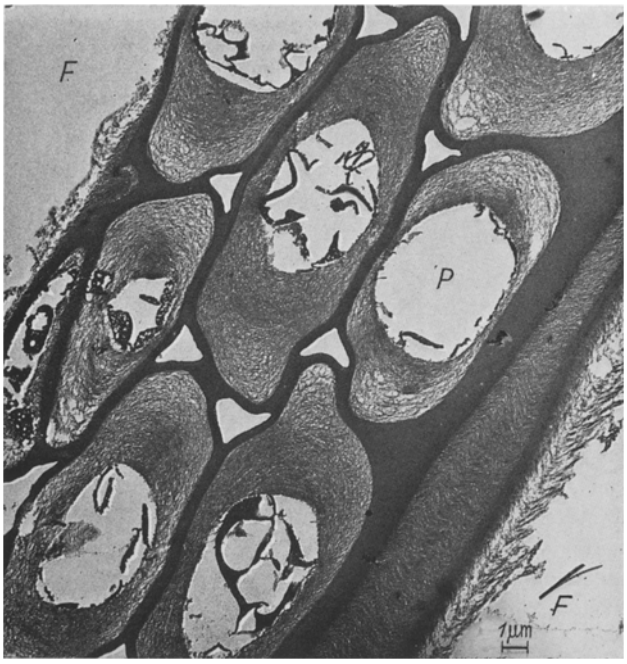

Fig. 14. Tangential section of beech tension wood. The wall of the parenchyma cells is highly lignified Remnants of a terminal lamella and cytoplasmic debris are present in the lumina.

Discussion

The method used here for studying the distribution of lignin in wood, while affording high resolution, also has certain drawbacks. Especially with hardwoods, swelling often occurs in the course of acid hydrolysis, resulting in distorted lignin skeletons, which can fill the entire lumen. Lignin is not a fully polymerized product, and treatment with any acid causes further condensation, whose effect on the texture of the lignin remains uncertain. Guaiacyl lignin should theoretically be able to condense more than syringyl lignin. Does this mean that the present method overestimates the amount of guaiacyl lignin ? GORING and his co-workers (unpublished) are at present investigating the distribution of lignin in both compression and tension wood. The results of these studies will be of great interest.

The present evidence confirms that in both normal and tension woods, vessel walls are highly lignified, and probably to the same extent as are tracheid walls in conifers. Parenchyma walls seem to have a variable lignin content. In normal wood, the S_1 layer has a relatively high lignin concentration. The S_2 layer is less highly lignified than S_1. Our results confirm those of SACHS, CLARK, and PEW [1963], who found that in comparison with conifer tracheids, hardwood fibers have a looser, more open lignin meshwork in their secondary wall. FERGUS and GORING [1968 b] found that the secondary wall in birch fibers had a lignin concentration which was 80% of that in spruce tracheids. The present results would seem to indicate a somewhat lower concentration for the species studied here, and certainly in maple wood. Whether or not the S_3 is more highly lignified than the S_1 or S_2 layers, cannot be decided on the basis of the evidence presented here.

Vessels and rays appear to be normally lignified in tension wood. It is clear that the non-gelatinous layers in the cell wall of the four species investigated are highly lignified, as had been concluded earlier on the basis of chemical evidence [TIMELL 1969]. The S_1 layer probably has a lignin concentration which is slightly higher than in normal wood. The S_2 layer is sometimes less lignified than S_1 but often it apparently has the same or an even higher lignin concentration than the latter. Compared to normal wood, the S_2 layer in tension wood fibers almost invariably is more highly lignified. It is notable that the more narrow the S_2 layer, the higher seems to be its concentration of lignin. This means that in fibers with an unusually wide G-layer, the narrow S_2 layer tends to have a higher than normal lignin concentration. With other species, DADSWELL and WARDROP [1955] observed that the greater the development of the G-layer, the less was the degree of lignification of the remainder of the cell wall. Obviously, if normal and gelatinous fibers contain the same amount of lignin, the more narrow S_2 layer in the latter fibers must be more highly lignified than the former.

Tension wood lignin is very similar to that present in corresponding normal wood [BLAND 1958, 1961; BLAND and SCURFIELD 1964], and lignification apparently proceeds in the same way [SCURFIELD 1967]. These facts, in conjunction with the highly lignified nature of the non-gelatinous cell wall layers, indicate that the lignification mechanism operates normally in these layers, as well as in the vessels and parenchyma. The factors that prevent formation of lignin in the gelatinous layer are not known at present. Very likely, however, they are of hormonal nature.

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