THE FINE STRUCTURE OF THE WALL OF THE CONIFER TRACHEID

IV. DIMENSIONAL RELATIONSHIPS IN THE OUTER LAYER OF THE SECONDARY WALL

by

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INTRODUCTION

It is now known (BAILEY AND VESTAL, 1937, WARDROP AND PRESTON, 1947) that the secondary cell wall of conifer tracheids is organized in a series of three coaxial micellar spirals such that the outer and inner spirals are flatter than the central micellar spiral separating them. The latter has been the subject of detailed investigation in previous papers of this series (PRESTON, 1946, 1947, 1948) particularly as regards the relation of its organization to cell dimensions. In earlier work (PRESTON, 1934) use was made of the observation by SANIO (1872), supplemented by the subsequent investigations of BAILEY (1920), that the tracheid length of successive annual rings of a conifer stem gradually increases during the first thirty to fifty years of the growth of the stem, after which it remains more or less constant. This led to the establishment of a connection between the average tracheid length in one annual ring and the cotangent of the average spiral angle of the form

$$L = K.\cot \Theta$$

where K is a constant, and Θ is the angle between the micellar winding and the longitudinal cell axis. For reasons given in the last paper in this series, these observations were recently extended to cover individual tracheids within any one annual ring, when it was found that tracheid length, L, breadth, B, and spiral angle, Θ, were related in the form

$$L = M + N\,(B^2 \operatorname{cosec}^2\Theta - 1)^{1/2}$$

This implies that for standard breadth the relation takes the form

$$L = K_0 + K\cot \Theta_{(B = 1.00)}$$

The earlier relation, in the lack of any sure evidence of the heterogeneity of the tracheid wall, was taken to apply to the whole wall thickness. It is now known that all three relations apply only to the central spiral layer.

With such a relation demonstrated for the central layer, it becomes of interest to know if a similar relation is applicable to the flatter micellar spiral of the outer and inner

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layers. If this were so, then it would mean that the entire organization of the secondary wall is conditioned by the dimensions of the cell. The present paper is concerned with an investigation, from this point of view, of the conditions in the simpler case of the outer layer. It seems reasonable, in view of the close resemblance of the birefringence in the inner and outer layers, that the inner layer will not differ essentially from the outer in this respect.

METHODS AND RESULTS

A. Measurement of micellar orientation in the outer layer

The method used previously (WARDROP AND PRESTON, 1947, 1949) for determining micellar orientation in this layer was considered too laborious and difficult to be contemplated as a routine method. Advantage was therefore taken of the fact that the simple measurement of birefringence in transverse section of conifer tracheids of increasing length (from successive annual rings) would, provided the intrinsic double refraction of the wall complex remained constant, given an indication of changing micellar orientation in these cells. Thus a decreasing birefringence of this layer in cells from successive annual rings would indicate a spiral in the layer becoming steeper with increasing cell length. It is a disadvantage of such a method that no indication is given of the absolute values of the micellar orientation in any given case, but it would indicate any trend. In point of fact, this difficulty can be surmounted to some extent, as will appear later.

The material examined consisted of a transverse disc taken from the stem of each of the following species:

Pinus radiata D. Don,
Pseudotsuga douglasii C. Carr.

Thin transverse sections of the late wood of successive annual rings were cut from each disc, and after dehydration were mounted in canada balsam. The phase difference for each ring was determined using a de Senarmont compensator (AMBRONN AND FREY, 1926) and the birefringence calculated using the section thickness, as described previously (PRESTON, 1946). An average of the values of 20 cells was taken in each case and the results are presented in Tables I and II and Figs 1 and 2.

B. Measurement of cell length

Small pieces of the late wood were selected from each annual ring, from the same blocks as were sectioned for birefringence measurements, and used for determinations of tracheid length. The specimens were macerated by delignification with chlorine water followed by warming in 0.025% sodium hydroxide (COHEN AND DADSWELL, 1939). A suspension of the tracheids so isolated was allowed to flow over a slide covered with albumen fixative which was then dried in an air oven, placed for five minutes in a 1% aqueous solution of congo red, dehydrated in alcohol, cleared in xylol, and mounted in canada balsam. The slide was then placed in a photographic enlarger and the image of the tracheids was projected at known magnification. The outlines of fifty tracheids from each specimen were then drawn on paper and the lengths of the tracheids were subsequently measured. The results are again presented in Tables I and II and in Figs 1 and 2.

C. *X-ray Examination*

While the X-ray diagram of conifer wood gives no indication of the micellar orientation in the outer layers of the constituent tracheids, change in orientation of the central layer can be followed by this means. It was thought desirable, as a complement to the optical determinations on the outer layer, to examine also by an X-ray method the organization of the corresponding central layer. For this purpose, X-ray diagrams were taken of tangential sections about ½ mm thick from corresponding regions of the specimens. The spread of the equatorial arcs was determined visually and the spiral angle calculated (see ASTBURY, PRESTON, AND RANGANATHAN, 1949) as a rough check that the relation between orientation in the central layer and cell dimensions was, in the present material, of the type already established for other species. The results are presented in Tables I and II, Figs 1 and 2.

D. *Treatment of data*

1. *Calculation of relative micellar orientation from birefringence measurements.* As already stated, the values of the birefringence in Tables I and II cannot be used to calculate the exact values of the micellar orientation in the outer layer of the secondary wall, unless the maximum birefringence of this layer is determined in some way such as that described in a previous paper (WARDROP AND PRESTON, 1947). There are, however, two ways in which a rough approximation can be reached. Either it can be assumed that the intrinsic birefringence already determined for *Picea* applies also to the present material, or the assumption may be made that the maximum birefringence observed in transverse section in the present investigation corresponds to transverse orientation. Since in point of fact the maximum birefringence observed here is actually somewhat greater than the intrinsic birefringence determined for *Picea*, the former method is inapplicable. A maximum birefringence of 0.013 in the case of *Pseudotsuga* (Table I) and of 0.025 with *Pinus* (Table II) were therefore taken as representing transverse orientation. Since the micelles are almost certainly never entirely transverse in these cases, the values obtained for the inclination of the micelles to the longitudinal axis thus determined represent maximum values, so that the inclination is actually steeper than that calculated. Nevertheless such data would indicate the trend of the change in micellar orientation in the outer layer with increasing cell length and does give a clearer mental picture of the point at issue.

The calculation of the relative values of Θ can be carried out as follows. If n_γ and n_α are taken as the major and minor axes of the index ellipsoid of cellulose so that the maximum birefringence is $n_\gamma - n_\alpha$, and $n_{\gamma'} - n_\alpha$ is the birefringence of the outer layer in transverse section when the micelles are inclined at an angle Θ to the longitudinal cell axis, then it follows that

$$\frac{(n_{\gamma'})^2\cos^2\Theta}{(n_\alpha)^2} + \frac{(n_{\gamma'})^2(1-\cos^2\Theta)}{(n_\gamma)^2} = 1$$

whence,

$$\cos\Theta = \frac{n_\alpha}{n_{\gamma'}}\left\{\frac{(n_\gamma - n_{\gamma'})(n_\gamma + n_{\gamma'})}{(n_\gamma - n_\alpha)(n_\gamma + n_\alpha)}\right\}^{1/2}$$

2. *The relation of micellar orientation and cell length.* It will be clear from Tables I and II that the birefringences in transverse section do, in fact, decrease markedly as tracheid length increases. This makes it reasonable to determine whether the data can

TABLE I

ORIENTATION AND CELL LENGTH IN THE SECONDARY CELL WALL LAYERS OF *Pseudotsuga douglasii*

Annual Ring	Tracheid Length* (mm)	Birefringence of outer layer in Transverse section**	Relative spiral angle outer layer (degrees)	Spiral Angle Central (degrees)
1	2.0	0.006(?)	43	40
3	2.4	0.013	90	29
5	2.6	0.008	52	26
7	3.3	0.009	56	25
9	3.1	0.007	47	20
11	3.3	0.007	47	19
13	3.3	0.007	47	16
15	3.7	0.004	34	13
17	3.9	0.005	39	13
19	3.7	0.006	43	14

* Average of 50 measurements ** Average of 20 measurements

TABLE II

ORIENTATION AND CELL LENGTH IN THE SECONDARY CELL WALL LAYERS OF *Pinus radiata*

Annual Ring	Tracheid Length* (mm)	Birefringence of outer layer in Transverse section**	Relative spiral angle outer layer (degrees)	Spiral Angle Central (degrees)
1	1.7	0.022	71	38
2	—	0.025	—	—
3	2.0	0.025	90	42
4	—	0.024	—	—
6	2.4	0.024	79	38
8	2.9	0.023	74	26
10	2.9	0.020	64	20
12	3.2	0.020	64	27
15	3.2	0.018	58	20

* Average of 50 measurements ** Average of 20 measurements

be fitted to a relation between micellar orientation and cell length of the type described above for the central layer. This was done by using the relative values of Θ for the outer layer and determining the line of closest fit for the data of L and cot Θ. This procedure was also applied to the values of L and the actual values of Θ for the central layer, for which the relation $L = K_0 + K. \cot \Theta$ was originally developed. In both cases the spiral clearly becomes steeper the longer the tracheid. With the outer layer the spread of the observations is greater than in the central layer but whether this difference is due to experimental error or to other factors cannot be decided. The line of closest fit was found by the method of least squares (YULE AND KENDALL, 1937) and the resulting equations for the data in Tables I and II, and presented in Figs 3 and 4 are as follows:

Pseudotsuga douglasii

Central Layer . . . . . . $L = 1.60 + 0.52 \cot \Theta$

Outer Layer . . . . $L = 2.34 + 0.86 \cot \Theta$

Pinus radiata

Central Layer . . . . . . $L = 0.57 + 1.09 \cot \Theta$

Outer Layer . . . . $L = 1.48 + 3.28 \cot \Theta$

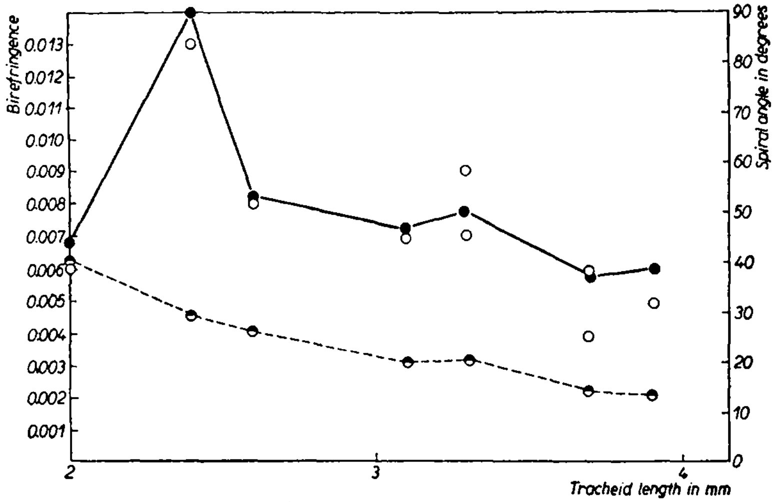

Fig. 1. The relation between birefringence and relative spiral angle in the outer layer, and the observed spiral angle in the central layer, for *Pseudotsuga*. ○ Birefringence in transverse section of outer layer; ● Relative spiral angle in outer layer; ⊖ Spiral angle in central layer. Note that the birefringences are plotted individually, while for simplicity of drawing the angles calculated for a tracheid length where two angles have been determined are the averages of these angles.

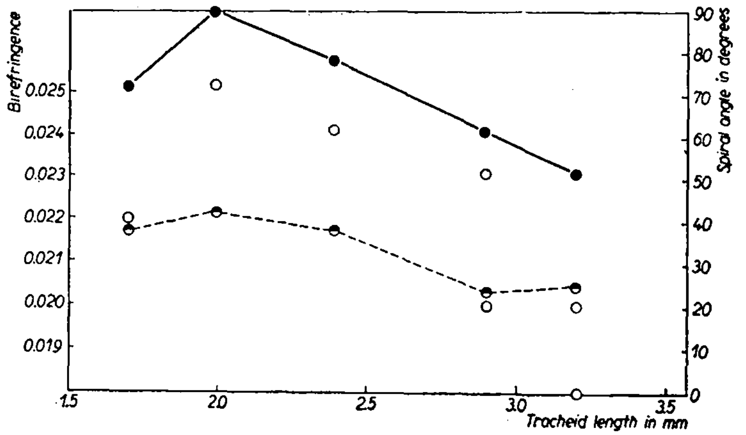

Fig. 2. Data as in Fig. 1 for *Pinus*

DISCUSSION

From the above data it is clear that the observed changes in birefringence with increase in cell length are consistent with the view that the spiral micellar orientation in the outer layer, like that in the central layer, becomes steeper as cell length increases. It can thus be said that the entire organization of the secondary wall layer is governed by the length of the cell.

These results also possess considerable interest in relation to the work of BAILEY AND VESTAL (1937), upon the cell wall organization of conifer tracheids. These investi-

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gators have claimed that conifer tracheids can be grouped, according to the organization of their cell wall, into four types such that the micelles are:

1. in the central layer parallel to the major cell axis and in the outer layer perpendicular to it;
2. in the central layer parallel to the major cell axis and in the outer layer possess a spiral orientation;
3. in the central layer spirally orientated with respect to the major cell axis and in the outer layer perpendicular to it, and
4. in both the central and outer layers possess a spiral orientation with respect to the major axis of the cell.

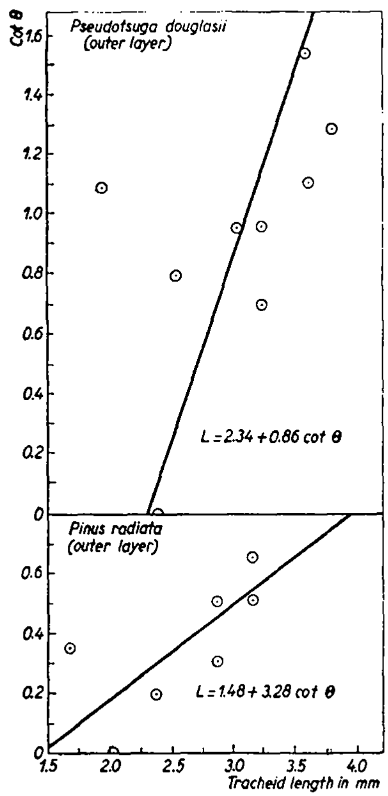

Fig. 3. The relation between tracheid length and cot Θ for *Pseudotsuga*, outer layer and *Pinus*, outer layer

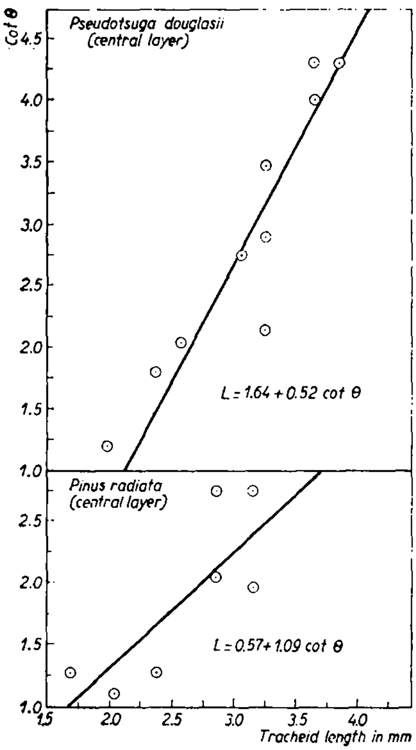

Fig. 4. The relation between tracheid length and cot Θ for the central layer of *Pseudotsuga* and *Pinus*

These conclusions were based upon the observed direction of growth of iodine crystals in swollen cell walls and it is not, in any case, clear what they mean in terms of the structure of the untreated wall. The point which should be made here, however, is that the above groups (2), (4) and (3) simply correspond to the expected organization of tracheids from outer, intermediate and inner annual rings respectively between which all grades of organization may be found. Thus the "types" described by these workers are but variations of the general pattern of micellar organization in conifer tracheids.

As for type (1) above there is no evidence in the present data that such an organization exists although it was almost realized in the wood fibres of *Nothofagus cunnighamii* (WARDROP AND PRESTON, 1947). It is to be suggested that perhaps type (1) above may represent a tracheid of type (4) which was distorted in a manner such that the micellar spiral of the outer layer became flatter and the central spiral steeper. In any case, because of the technique employed the recognition of this type of organization is, of necessity, suspect.

The fact that the micellar orientation of the outer layer of the secondary wall becomes steeper with increasing cell length also bears upon the problem as to why this layer does not contribute any detectable pattern to the X-ray diagram (PRESTON, 1946). Thus if the inclination in the outer layer is, say, only 45°, the contribution of this layer would appear as a "tail" to the equatorial arcs from the central layer particularly since the micelles of the outer layer would seem to possess some considerable angular dispersion (WARDROP AND PRESTON, 1947, 1949) and would have their contribution to the X-ray diagram further reduced by diffuse scattering from lignin and other amorphous materials present in this layer. Furthermore the appearance of meridional arcs arising from micelles oriented transversely to the major cell axis would be expected to be found only in cells of which the micelles of the central layer were present in a relatively flat spiral. Now, as PRESTON (1946) has shown, a spurious meridional arc arises in the X-ray diagram of the central layer when the micellar spiral is relatively flat, so it would seem that the very conditions under which transversely oriented micelles of the outer layer could be detected by X-rays are also the conditions under which spurious meridional arcs arise, at almost the same position on the X-rays diagram.

It may perhaps further be pointed out that if the spirals of the outer and inner layers of the secondary walls discussed here have the same sign, as they were shown to have in *Picea* sp. (WARDROP AND PRESTON, 1947) then the inter-layer relationship resembles that already demonstrated in *Cladophora* (ASTBURY AND PRESTON, 1940) and in some vessels (PRESTON, 1939) in the sense that as the spiral micellar organization changes the angle between the spirals tends to remain constant. It may be significant that this is in contrast to the primary-secondary wall relationship in the only case which has yet been studied (PRESTON, 1947), where the orientation in the primary wall is apparently quite independent of that in the central layer of the secondary wall.

In conclusion, therefore, the demonstration presented in this paper that the micellar organization in the outer layer of the secondary wall, as well as that in the central layer, is dependent upon cell dimensions, further emphasizes the distinction between primary and secondary walls, since in the former no such relation exists.

SUMMARY

A study of the optical properties of the outer layer of the secondary wall of tracheids of *Pseudotsuga douglasii* C. Carr and of *Pinus radiata* D. Don (*Pinus insignis*) shows that the micellar organization of the cellulose component is dependent upon tracheid length. This has been interpreted as a steepening of the micellar spiral with increasing length, and it has been shown that the relation between cell length and the spiral angle is of the same kind as that previously demonstrated in the central layer. Thus the organization of the whole secondary wall is shown to be dependent upon cell length.

RÉSUMÉ

L'étude des propriétés optiques de la couche externe de la paroi secondaire des trachéides de *Pseudotsuga douglasii* C. Carr et de *Pinus radiata* D. Don (*Pinus insignis*) montre que l'organisation

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micellaire du constituant cellulosique dépend de la longueur du trachéide. Ceci est probablement dû à ce que la spirale micellaire s'étire lorsque la longueur du trachéide s'accroît; il a été montré que la relation entre la longueur de la cellule et l'angle de la spirale est analogue à celle qui existe dans le cas de la couche centrale. Ainsi l'organisation de toute la paroi secondaire dépend de la longueur de la cellule.

ZUSAMMENFASSUNG

Die Untersuchung der optischen Eigenschaften der äusseren Schichte der Sekundärwand von Tracheiden von *Pseudotsuga douglasii* C. Carr und *Pinus radiata* D. Don (*Pinus insignis*) zeigt, dass der mizellare Aufbau des Zelluloseanteils von der Länge der Tracheiden abhängt. Dies wurde durch ein Steilerwerden der Mizellenspirale mit wachsender Länge der Tracheiden erklärt. Es wurde gezeigt, dass das Verhältnis zwischen der Zellänge und dem Spiralwinkel dem früher für die Mittelschichte bewiesenen ähnelt. So hängt also der ganze Aufbau der Sekundärwand von der Zellänge ab.

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