THE FINE STRUCTURE OF THE WALL OF THE CONIFER TRACHEID

V. THE ORGANIZATION OF THE SECONDARY WALL IN RELATION TO THE GROWTH RATE OF THE CAMBIUM*

by

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As first observed by SANIO, the length of the tracheids in successive annual rings of conifer stems at a given height increases rapidly during the first 20–50 years of the life of the tree. After this period it was claimed by SANIO that no further length increment occurred and a constant length was attained. These observations were supported by KRIBS (1928) who found a constant final tracheid length after 40 years growth in *Pinus banksiana*, while on the other hand GERRY (1915, 1916) found the tracheid length to increase continuously in successive rings of *Pseudotsuga taxifolia*, 455 years old. Again BAILEY AND SHEPARD (1915) found no constant final length after the phase of rapid increment in tracheid length but observed that the length fluctuated about a mean. From such observations it is clear that while a considerable variation in individual trees exists, all investigators agree upon SANIO's initial observation that in any stem there is a phase of rapid length increment of the tracheids in successive annual rings during the first 20–50 years of growth, followed by a phase of less rapid length increase. These observations were given considerable significance by the investigations of BAILEY (1923) which showed that the increase in tracheid length in successive annual rings simply reflected the progressive elongation of the cambial initials from which the tracheids were differentiated. During differentiation the initials were shown to increase by 5–10% in length, so that the measurement of the tracheid length in successive annual rings provides only a rough quantitative record of the increase of the cambial initials with time.

A further extension of this work became possible when it was demonstrated by PRESTON (1934) that the organization of the cell wall in conifer tracheids could be related to cell dimensions. Before considering this point further however it is perhaps useful to consider the modern conception of cell wall organization in conifer tracheids. Following the earlier polemics as to the cell wall organization in these cells (PRESTON 1934, 1946; BAILEY AND KERR 1935; BAILEY AND BERKLEY 1942) it can now be stated that the secondary cell wall consists of three layers, corresponding (although there are other differences) to three micellar spirals of different pitch (WARDROP AND PRESTON, 1947) such that the spiral is relatively flat in the outer and innermost layers which appear bright in cross section between crossed nicols and steep in the central layer which

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appears dark between crossed nicols. For a typical tracheid the micellar orientation in these layers is illustrated in Text Fig. 1.

Now in PRESTON's original investigation (1934) it was demonstrated that the average inclination to cell length of the micelles in the early wood of any one annual ring is related to the average tracheid length by a relation of the form

$$L = K \cot \theta \qquad (1)$$

over several annual rings where θ is the angle between the average micellar direction (determined optically) and the tracheid length (see text Fig. 1). More recently it has been shown (PRESTON 1948) that the relation between the inclination θ and cell length applies to individual tracheids within an annual ring in such a way that if θ_s is the inclination for a tracheid of standard breadth, then

$$L = K_0 + K_1 \cot \theta_s \qquad (2)$$

where K_0 and K_1 are constants.

This relation applies to the central layer of the cell wall (Text Fig. 1), but it has since been shown to hold in the outer layer of the secondary wall as well (PRESTON AND WARDROP, 1949) where the inclination is different.

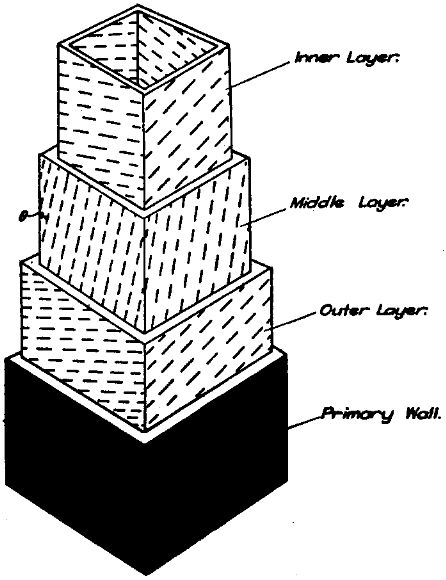


Fig. 1. Diagrammatic representation of the wall structure in a conifer tracheid. The broken lines represent the run of the preferred orientation of the molecular chains of cellulose. Differences in angular dispersion between the layers are omitted for clearness of figure

While equation (2) gives a reasonably good fit to the experimental data, however, there is, as is usual in quantitative relationships in biology, some considerable spreading of the points about the line. The micellar organization cannot therefore depend solely on cell length; and other factors, such as the influence of species differences or of environmental factors on growth, are also involved. Investigation of such factors clearly demands that a relation should be derived to express changes in tracheid length with time which describes the data at least as well as those used by PRESTON in relating tracheid length and micellar spiral angle. If such a relation could be derived then the way would be open to relate micellar spiral organization in the wall to the rate of cambial growth, an achievement which would be of considerable academic and technological importance.

Accordingly in the present paper an attempt is made firstly to derive relations between tracheid length as well as stem diameter with time. Consideration is then given to the information derived from such relations as to the influence of the rate of length growth of the cambial initials upon the relation existing between micellar organization and tracheid length.

METHODS AND RESULTS

In undertaking such an investigation it is clearly necessary to collect wood samples showing as great a range as possible in growth rate. To this end samples were obtained from a variety of stations although the work reported here is concerned with data for a single species only, in three samples very different in growth rate, viz. *Pseudotsuga taxifolia* (Lamb.) Britt. Since some variation in tracheid length could be expected to occur within an annual ring, it was necessary to decide from what part of the ring to take tracheids for length measurements so that over different rings comparable tracheids could be examined. The evidence for a change in tracheid length within an annual ring is conflicting. Thus according to GERRY (1915, 1916) and WARDROP (1948), tracheids of late wood are shorter than those of early wood while the reverse of this has been observed by DADSWELL (1948). It was therefore decided to take the last formed late wood for examination in each case, as a region easily duplicated.

Measurement of tracheid length

Small pieces of late wood from successive annual rings from each specimen were selected and were macerated using the method of COHEN AND DADSWELL (1939). A suspension of the tracheids was allowed to flow over the slide covered with albumen fixative and then dried in an air oven until the tracheids adhered firmly to it. After staining for five minutes in a 1% aqueous solution of congo red the tracheids were dehydrated in absolute 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 tracheid length was subsequently measured (Table I, Fig. 2).

TABLE I

THE CHANGE IN TRACHEID LENGTH, MICELLAR SPIRAL ORIENTATION (Θ), AND STEM DIAMETER IN SPECIMENS OF *Pseudotsuga taxifolia*

Age in Years (T)	Specimen *A*			Specimen *B*			Specimen *C*		
	Tracheid Length (L) mm	Θ Degrees	Stem Diameter (D) cm	Tracheid Length (L) mm	Θ Degrees	Stem Diameter (D) cm	Tracheid Length (L) mm	Θ Degrees	Stem Diameter (D) cm
1	2.0	40	1.3	1.3	34	2.8	1.2	32	1.0
2	—	—	—	1.6	31	4.1	1.3	—	1.6
3	2.4	29	3.8	1.9	28	5.3	1.8	30	2.8
5	2.6	26	7.3	2.4	26	8.1	2.2	29	4.9
7	3.3	25	9.0	2.5	20	10.1	2.5	—	6.2
9	3.1	20	12.0	—	19	—	2.7	19	—
10	—	—	—	2.9	15	13.6	2.7	—	10.3
11	3.3	19	15.5	—	14	—	2.9	18	—
13	3.3	16	19.1	3.0	13	16.3	2.9	—	12.5
15	3.7	13	22.1	3.2	13	18.4	3.2	16	—
16	—	—	—	—	—	—	3.4	—	15.0
17	3.9	13	24.8	—	—	—	—	—	—
18	—	—	—	3.4	12	20.2	—	—	—
19	3.7	14	27.5	—	13	—	3.4	13	17.1
20	—	—	—	3.5	13	23.0	—	—	—

Measurements of Annual Ring Diameter

The average diameter of specimen each at each annual ring was determined using a travelling microscope. The data so obtained are listed in Table I.

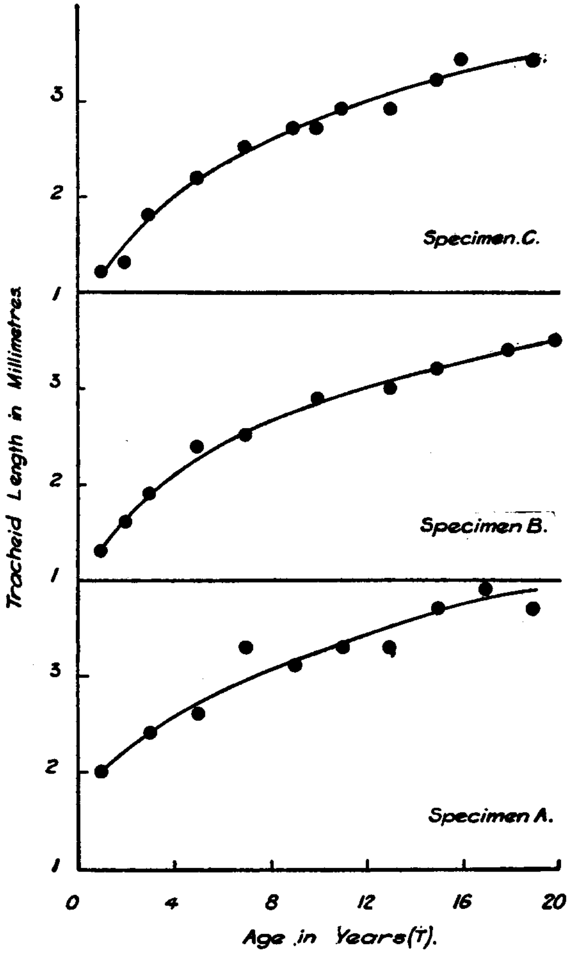


Fig. 2. Change of tracheid length with age in the three specimens of *Pseudotsuga taxifolia*

X-ray examination

For the three specimens examined the angle of inclination, Θ, of the micelles to the longtitudinal tracheid axis was determined for different annual rings (Table I). This was done by measuring the half angle of spread of the 002 diffraction arcs in the X-ray diffraction photograph of the wood from each ring examined, using CuK_{α} radiation and a specimen film distance of three centimetres. This method was chosen because the more critical methods would involve the optical examination of some 2.000 individual tracheids; so that while the angles determined by the X-ray method are approximations (being, in fact, probably overestimates), the alternatives were clearly too laborious for an exploratory study of this kind.

Treatment of data

(i) *Mathematical representation of changes of tracheid length and stem diameter with age.* In order to express quantitatively the change of tracheid length and stem diameter with age, an attempt was made to fit curves to the data presented in Table I. For this purpose an equation used originally by BACHMANN (1931) was used (see Discussion). It is of the form

$$\log G = K_0 + K_1 \log T - K (\log T)^2$$

where K_0, K_1 and K are constants and G is the magnitude of the object at time T. Over a limited range it is not always necessary to use the third term in fitting a curve to the data. The equation may then be written in the form

$$\log G = K_0 + K_1 \log T$$

For the data in Table I the curves of closest fit of the form of the latter equation were

Fig. 3. Change in stem diameter with age in the three specimens of *Pseudotsuga taxifolia*

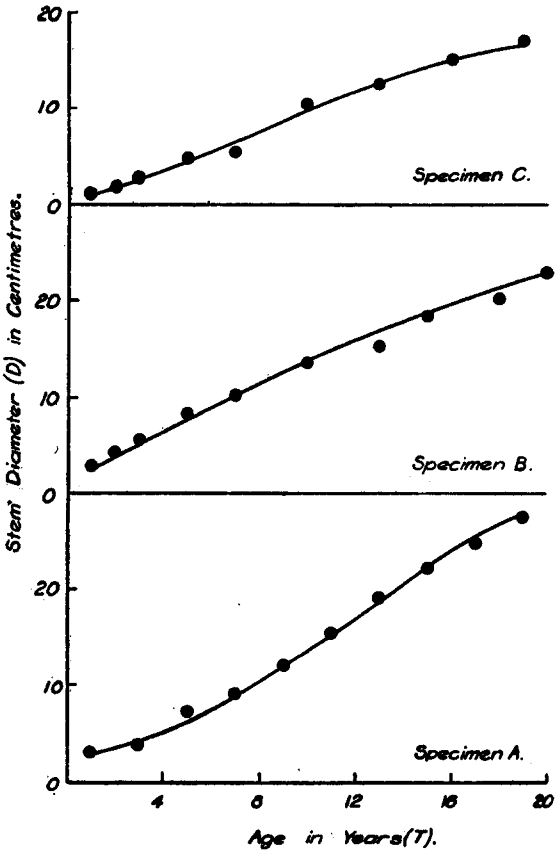
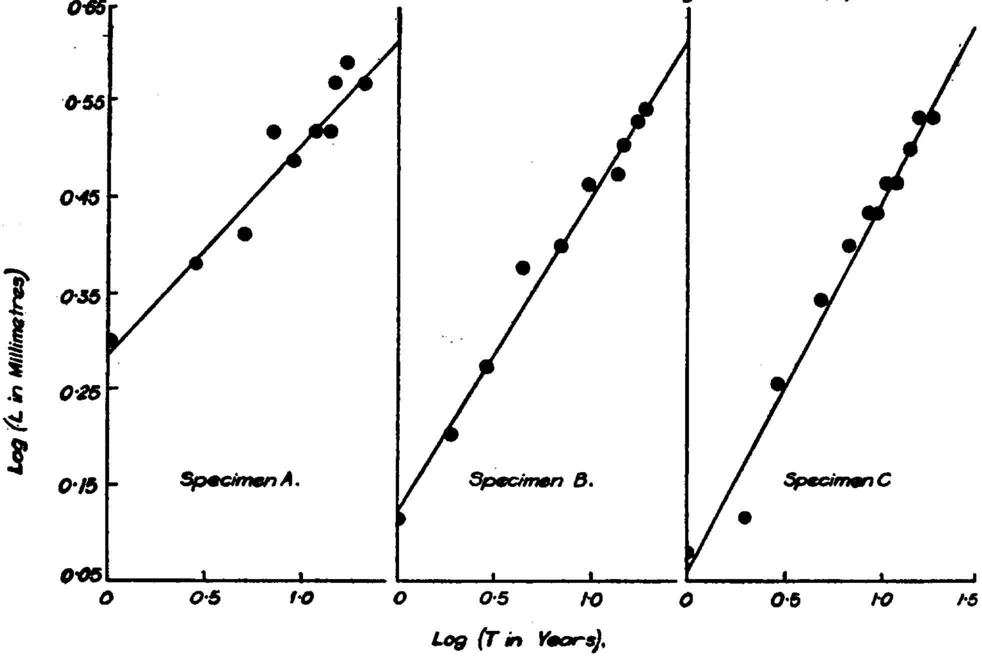


Fig. 4. Relation between log (tracheid length) and log (age) in the three specimens of *Pseudotsuga taxifolia*



calculated using the method of least squares. The equations for the curves so obtained were as follows:

Specimen A

$$\log L = 0.287 + 0.227 \log T \tag{3}$$
$$\log D = 0.113 + 0.988 \log T + 0.044 (\log T)^2 \tag{4}$$

Specimen B

$$\log L = 0.118 + 0.332 \log T \tag{5}$$
$$\log D = 0.437 + 0.594 \log T + 0.089 (\log T)^2 \tag{6}$$

Specimen C

$$\log L = 0.052 + 0.379 \log T \tag{7}$$
$$\log D = 0.026 + 0.974 \log T + 0.025 (\log T)^2 \tag{8}$$

where L is in mm, D is in cm, and T is in years. The data represented by equations 3–8 illustrated in Figs 3 and 4.

(ii) *Relation to length of the micellar spiral organization of the central layer of* the secondary wall. For the data in Table I an attempt was made to fit a relation of the form

$$L = K_0 + K \cot \Theta$$

using the method of least squares. The relations so obtained between L and cot Θ for the three specimens examined were as follows:

Specimen A

$$L = 1.645 + 0.515 \cot \Theta \tag{9}$$

Specimen B

$$L = 0.774 + 0.597 \cot \Theta \tag{10}$$

Specimen C

$$L = 0.567 + 0.713 \cot \Theta \tag{11}$$

Equations 9–11 are plotted in Fig. 5.

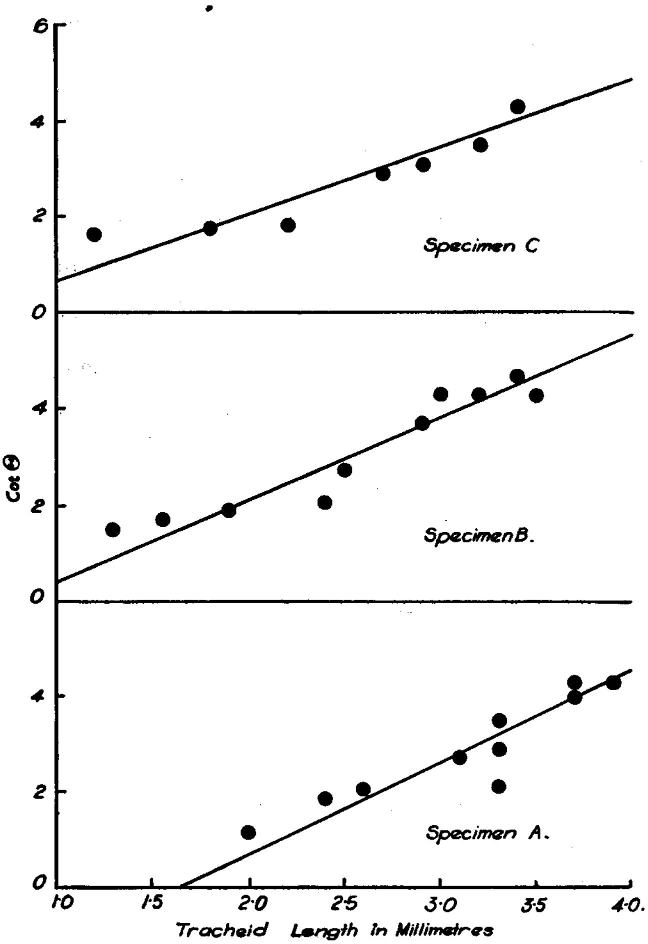


Fig. 5. The relation between tracheid length and cot Θ in the three specimens of *Pseudotsuga taxifolia*

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DISCUSSION

An inspection of Figs 3 and 4 shows that the observed values for the increase in tracheid length and of stem diameter with time can be represented by a relation of the general form proposed by

BACHMANN. Undoubtedly equations of different kinds could be used to describe the data and, indeed, preliminary attempts to use the autocatalytic curve (ROBERTSON, 1922) were more or less successful. However it was demonstrated for the writers by Mr E. J. WILLIAMS of the Section of Mathematical Statistics, C.S.I.R.O., Melbourne, that this relation was not likely to represent the data more satisfactorily than the simpler equation of BACHMANN which was finally used. Also, as GRAY (1929) has pointed out, no biological significance can be attributed to the exact form taken by a growth equation and its chief function is to facilitate analysis of the curve of growth. Thus from this point of view as well, it would seem immaterial which relation was chosen to represent the data.

Now in equations 9, 10 and 11 representing the change of cot Θ with L it will be observed that considerable variation in both of the constants occurs suggesting that the relation between L and cot Θ is not constant within a species. This being so, it is possible that environmental influences may be operative in governing tracheid length and micellar spiral organization.

One possible way in which environmental influences may be manifest is in a change of the rate of growth of the cambial initials which would influence the tracheid length and might conceivably also be a factor in governing micellar organization. In order to consider this view further it was thought desirable to be able to study both the rate of change of tracheid length with time, $\frac{dL}{dT}$, and the rate of change of micellar spiral angle with tracheid length, $\frac{d\Theta}{dL}$. However, the form of the equations does not permit of the ready observation of $\frac{dL}{dT}$ and $\frac{d\Theta}{dL}$ although by differentiation the values can be calculated. It is to be appreciated, however, that equations 3, 5, 7, 9, 10 and 11 represent the curves most nearly fitting the data so that the values of $\frac{dL}{dT}$ or $\frac{d\Theta}{dL}$ obtained by differentiating them will not necessarily apply exactly to the actual observed values. Such an analysis, however, should indicate the way in which the length increment varies with time, and also the change in spiral angle (Θ) with length (L).

Consider an equation of the form

$$\log L = k_0 + k_1 \log T$$

then differentiating with respect to T

$$\frac{dL}{dT} = \frac{k_1 L}{T} \tag{12}$$

By substituting values of T in equations 3, 5 and 7 corresponding values of L are obtained which, upon substitution in equation 12, give the values of $\frac{dL}{dT}$ at any time T. In this way values of $\frac{dL}{dT}$ were obtained for each of the specimens used, and are listed in Table II and are plotted in Fig. 6.

Again, for an equation of the form

$$L = K_0 + K_1 \cot \Theta$$

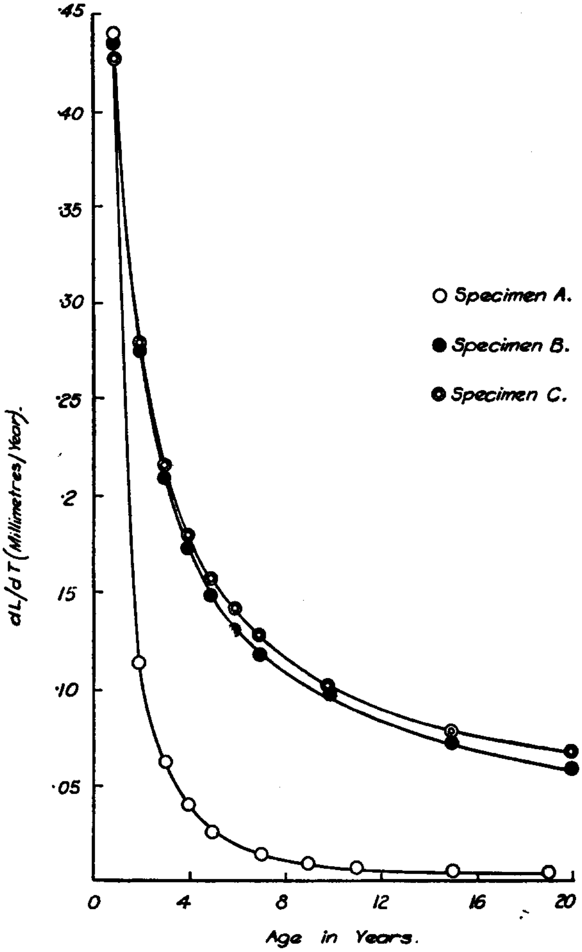

Fig. 6. Rate of change of tracheid length with time in the three specimens of *Pseudotsuga taxifolia*

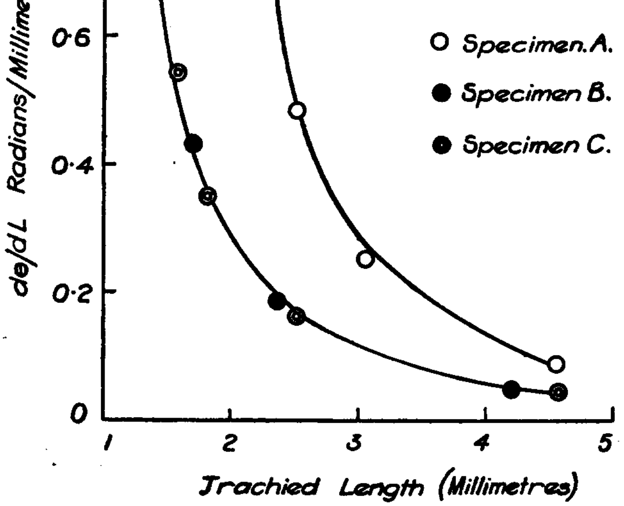

Fig. 7. Rate of change of micellar spiral angle with tracheid length in the three specimens of *Pseudotsuga taxifolia*

(equations 9–11 above), differentiating with respect to Θ gives

$$\frac{d\Theta}{dL} = -\frac{1}{K_1 \operatorname{cosec}^2\Theta} \tag{13}$$

From equation 13, by substituting values of Θ, $\frac{d\Theta}{dL}$ can be calculated for each of the three specimens employed and the corresponding values of L can be obtained by substitution of the appropriate values of Θ in equations 9, 10 and 11 respectively. These values of $\frac{d\Theta}{dL}$ are plotted against L in Fig. 7.

TABLE II

THE RATE OF CHANGE OF TRACHEID LENGTH (L) WITH TIME (T) IN SUCCESSIVE ANNUAL RINGS OF *Pseudotsuga taxifolia*

Time (T) (Years)	Specimen A	Specimen B	Specimen C
	Growth Rate dL/dT (mm/year)	Growth Late dL/dT (mm/year)	Growth Rate dL/dT (mm/year)
1	0.440	0.436	0.428
2	0.129	0.275	0.279
3	0.063	0.210	0.216
4	0.038	0.173	0.180
5	0.025	0.149	0.157
6	—	0.132	0.141
7	0.014	0.119	0.128
9	0.009	—	—
10	—	0.094	0.102
11	0.006	—	—
15	0.005	0.071	0.079
19	0.004	—	—
20	—	0.060	0.067

Now from the data of Table II and Fig. 6 it is not immediately apparent how the rate of change in tracheid length $\frac{dL}{dT}$ influences the rate of change of micellar spiral orientation with length. This can, however, be seen from the following argument.

Consider the value of L corresponding to any time T for specimens A, B and C Fig. 2. It can be seen that the lengths of B and of C, *i.e.* L_B and L_C are nearly equal and that the length of A, *i.e.* L_A is greater than either of these. Also it is apparent from Fig. 6, using a similar nomenclature, that $\frac{dL}{(dT)_B}$ and $\frac{dL}{(dT)_C}$ are both greater than $\frac{dL}{(dT)_A}$ at any time T. Referring to Fig. 7 it will be seen that, for any time T at the corresponding lengths L_A for specimen A, L_B for specimen B and L_C for specimen C

$$\frac{(d\Theta)}{(dL)_A} \text{ is greater than either } \frac{(d\Theta)}{(dL)_B} \text{ or } \frac{(d\Theta)}{(dL)_C}.$$

At any time, however, since $\frac{(dL)}{(dT)_B}$ and $\frac{(dL)}{(dT)_C}$ are greater than $\frac{(dL)}{(dT)_A}$, that is A is elongating more slowly, a further unit increment in L at these rates would be achieved in a longer time by A than by B or C. Hence it appears that the change in Θ per unit change in length is greater in the case of A than of B or C, but that change in unit length is achieved more slowly in the case of A than in the case of B and C. Now Θ is the angle between the major tracheid axis and the tangent to the micellar spiral and decreases with increasing length; thus, for a unit increment in length the micellar spiral steepens more in the specimen of lower growth rate, A, than for the specimens B and C with a higher growth rate. Thus for these specimens it appears that in tracheids which have elongated more rapidly the micellar spiral tends to remain flatter than in tracheids which have elongated more slowly. It seems, therefore, that while the micellar spiral orientation is governed by the cell length as shown by PRESTON (1934, 1948), it is also influenced secondarily by the rate at which this length has been attained. Now while this conclusion is based only upon somewhat scanty experimental evidence, and so perhaps cannot be regarded as adequately proved, it does provide a basis for some most interesting speculations and would appear to offer an explanation for some observations hitherto unexplained.

TABLE III

THE RATE OF CHANGE OF THE ANGLE OF MICELLAR SPIRAL ORIENTATION (Θ) WITH TRACHEID LENGTH (L) IN *Pseudotsuga taxifolia*

Specimen A		Specimen B		Specimen C	
L in mm	$d\Theta/dL$ radians/mm	L in mm	$d\Theta/dL$ radians/mm	L in mm	$d\Theta/dL$ radians/mm
2.25	0.802	1.40	0.692	1.57	0.545
2.52	0.486	1.70	0.419	1.82	0.351
3.05	0.227	2.38	0.196	2.52	0.164
4.55	0.089	4.20	0.051	4.58	0.042

Thus, while in general the micellar spiral becomes steeper with increasing tracheid length, this is not always the case especially in the innermost annual rings. Thus it has been observed (PRESTON, 1934, PRESTON AND WARDROP, 1949) that while the tracheid length increases continuously at any level from inner to outer annual rings, the spiral angle frequently passes through a maximum, usually in the second ring. This may now be associated with the fact that if the growth rate obtained from the observed data is plotted against time it is found to increase for the first two to three years and then to decrease. Thus, if it is assumed as concluded above that the micellar spiral orientation is governed not only by cell length but also by the rate at which that length was attained, and that the micellar spiral remains flatter in cells which have extended more rapidly, then it may be that in these first few annual rings the influence of growth rate on micellar orientation is, as it were, outweighing the normally dominating influence of cell length.

Again, since the micellar spiral orientation remains flatter in cells which have elongated rapidly than in more slowly elongating cells, it might be supposed that if the rate of cell elongation were sufficiently great then no change in micellar spiral orientation would occur irrespective of the extent to which the cell has extended in length. Now this argument applies to secondary walls which have been laid down after cell extension has ceased, but it is a striking coincidence that in primary cell walls no change in micellar spiral orientation occurs irrespective of the degree of cell extension. It may be that here is a pointer which may eventually render it possible, from the point of view of cell wall organization, to consider cell wall formation in primary walls accompanied by extension growth and cell wall deposition in secondary walls simply as two aspects of one governing principle of cell wall formation: in the case of primary walls under conditions permitting dimensional changes to occur and in the case of secondary walls under conditions when dimensional changes are not possible. Now many properties of timber, such as tensile strength, compressive strength and swelling properties, most probably depend upon the micellar organization of the tracheids. Thus tracheids with steeper micellar spirals would be expected to possess a greater tensile strength than tracheids with a flatter micellar spiral as has been observed in the case of cotton fibres (MEREDITH, 1946). If, then, the rate of extension of the initials from which the tracheids arise plays a part in governing the micellar orientation of the tracheids then it is conceivable that the known dependence of the properties of timber on growth rate may find an explanation in terms of the foregoing discussion.

While the above argument would suggest that the rate at which a given cambial initial extends in the growing stem may influence the orientation of the secondary wall subsequently formed, it would at present be unwise to regard growth rate as a causative factor since both growth rate and orientation may depend on some third factor at present undefined, such as the metabolic state of the cambial initials during differentiation. One further point may, however, be considered. It might be argued from a consideration of equations 3–8 that since both tracheid length (L) and stem diameter (D) are related to the time of growth T, it should be possible to develop a relation between tracheid length and stem diameter and in view of the above considerations ultimately to relate micellar organization to the rate of diameter growth. However it has been pointed out (WILLIAMS 1948) that a relation between D and L can be tested by determining how much better a regression of L on both D and T together fits the data than a regression of L on T alone, since a relation between D and L would consist of two parts; one which is due to the fact that both L and D depend on T and the other which would depend on a direct relationship (if any) between L and D for a given value of T. Upon examination of this point it was found (WILLIAMS, 1948) that for the present data in no case was the relation D and L significant. It was thus concluded that at least for the present results the development of a relation between D and L was unjustified and further experimental work is required before this point can be definitely decided.

SUMMARY

The tracheid length, and angle of micellar orientation in tracheids from successive annual rings in three specimens of *Pseudotsuga taxifolia* has been measured. For the data obtained the mathematical relation developed by PRESTON between spiral angle and tracheid length has been applied, and further relations between tracheid length and age have been derived. From a consideration of these relations it has been concluded that the micellar spiral organization of the secondary wall in conifer tracheids is dependent upon the tracheid length in a way which is governed by the rate of extension of the cambial initial from which the tracheids are formed.

RÉSUMÉ

Nous avons mesuré la longueur du trachéide et l'angle d'orientation micellaire dans des trachéides d'anneaux successifs de trois spécimens de *Pseudotsuga taxifolia*. Nous avons appliqué aux résultats obtenus la relation mathématique entre l'angle de la spirale et la longueur du trachéide, développée par **Preston** et nous en avons déduit d'autres relations entre la longueur du trachéide et l'âge. Nous pouvons conclure de ces relations que l'organisation de la spirale micellaire de la paroi secondaire des trachéides de conifères dépend de la longueur des trachéides et en particulier de la vitesse d'extension de la cellule du cambium à partir de laquelle le trachéide se développe.

ZUSAMMENFASSUNG

Die Länge der Tracheiden und der Winkel der Mizellenspirale in den Tracheiden von auf einander folgenden Jahresringen in drei Proben von *Pseudotsuga taxifolia* wurden bestimmt. Der mathematische Ausdruck von **Preston**, der die Abhängigkeit des Spiralwinkels von der Tracheidlänge wiedergibt, wurde auf die Versuchsergebnisse angewendet und weitere Gesetzmässigkeiten zwischen Tracheidlänge und Alter wurden aufgedeckt. Hieraus geht hervor, dass die Abhängigkeit des Aufbaus der Sekundärwand der Konifertracheiden von der Tracheidlänge durch die Ausdehnungsgeschwindigkeit der Cambialzellen beherrscht wird, aus denen sich die Tracheiden entwickeln.

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