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# SHORT TERM VARIATION IN TRACHEID DEVELOPMENT IN THE EARLY WOOD OF PICEA SITCHENSIS

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*Summary.* An examination was made of the dimensions of each individual cell along radial files of tracheids. Groups of neighbouring cells were found to have large diameters and thick walls. It is suggested that cambial development proceeds in a series of 'episodes' of enhanced activity.

## INTRODUCTION

In temperate regions, considerable differences can occur between the widths of successive growth rings. These differences have been attributed to variations in the weather from year to year (e.g. Johnson & Risser, 1973) and indeed considerable attention is devoted to developing a chronology of climate change based largely on growth ring sequences (Fritts *et al.*, 1971). However, the causal links between variation in weather and variation in the quantity and structure of the xylem which might explain these differences in ring width are not fully understood.

Direct influences of environmental factors on the production and development of secondary xylem elements have been inferred both from experiments with seedlings, and from ring sequences in climatic regions where extremes of weather tend to occur. Thus an increase in $SO_2$ concentration in the gaseous environment of trees (Liese *et al.*, 1975) or drought in forest stands (Glock & Agerter, 1962) decrease the width of growth rings and increase the proportion of late to early wood; moving seedlings from long to short days (Denne & Smith, 1971), or growing them at different temperatures (Denne, 1971), may have an effect on both tracheid production and structure. But in temperate regions, it is unusual for variations in the weather to be as extreme as those in semi-arid zones or as those frequently selected as treatment levels by

experimenters. Investigators who have taken weekly samples of the cambium from forest trees (e.g. Skene, 1972) have emphasised gradual trends in the developmental process over the whole season as the main cause of variation.

This work was conducted as part of a project (Ford, 1975) to investigate the effects of the physical environment on tree growth in a young plantation of Sitka spruce, *Picea sitchensis* (Bong.) Carr. The activity of the root and shoot meristems has been investigated as well as that of the cambium: with each of these aspects of tree growth a central problem has been to resolve the scale of environmental change which produces a measurable change in the growth rate of the meristem. The production of a growth ring involves cell division, cell expansion and wall thickening (Plate 1). To what extent are these processes influenced by changing weather patterns?

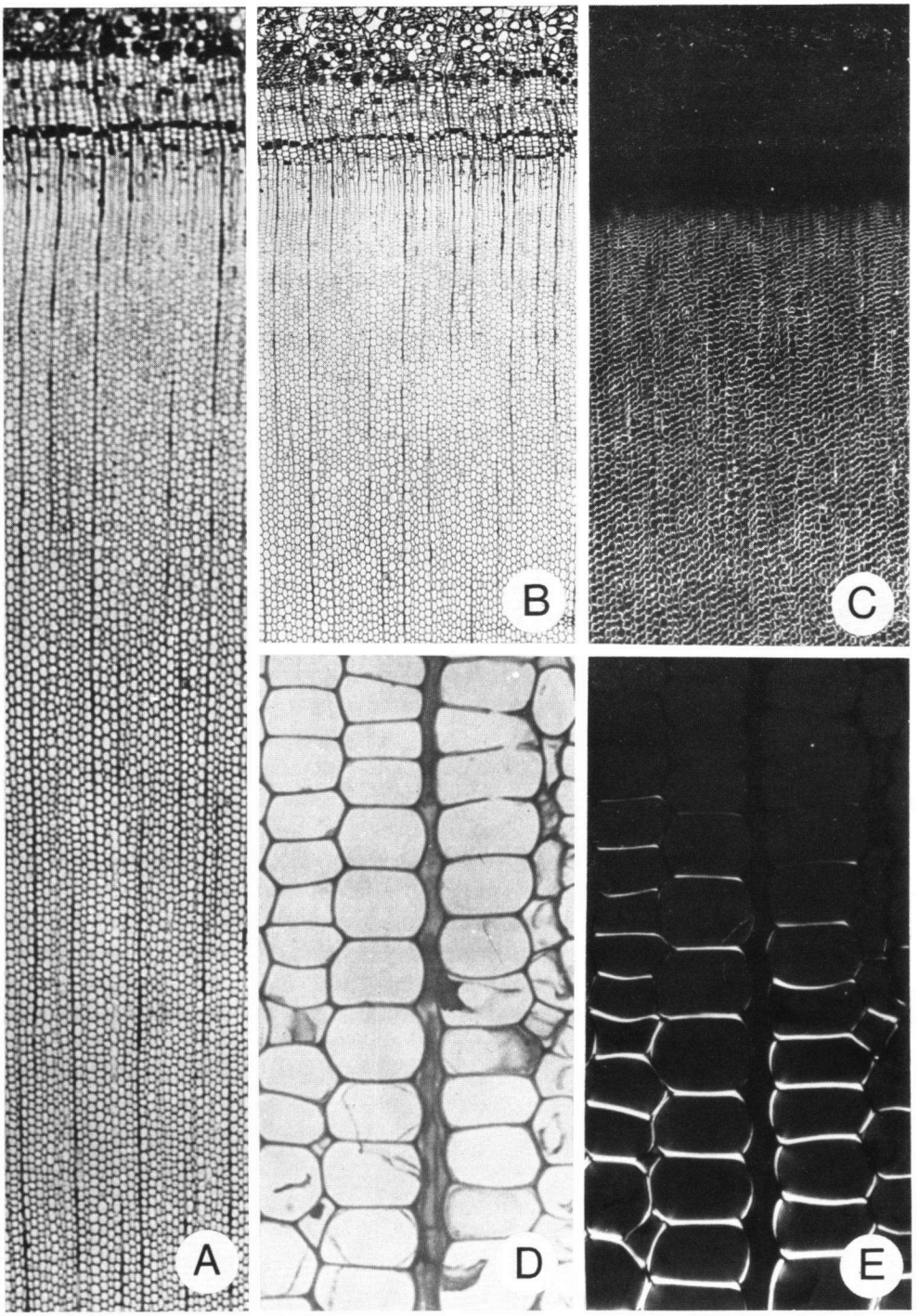
## MATERIALS

In parallel with a series of measurements of the environmental conditions, small samples were taken from a large, uniform internode of a Sitka spruce tree at 12 hourly intervals over a period of 15 days. The internode was 6 years old and in the lower part of the forest canopy. Samples were embedded in glycol methacrylate and sectioned with a glass knife. The major aim of the study was to estimate the number of cells produced during the period, and to examine the numbers undergoing expansion and wall thickening at any time. This work, and the details of the method, will be reported elsewhere. We should like to report here data demonstrating the variability of cell dimensions in single radial files of tracheids after cell wall thickening is complete, and to make some inferences about the effect of the environment on the processes of cell expansion and cell wall thickening from this variation.

The cambium selected for study was an active one. By 27 June 1974, when sampling started, mean cell number in the radial files of tracheids in the current growth ring was 117; when sampling stopped on 12 July 1974, mean cell number was 160. The cambium had therefore produced some 43 cells in 15 days. This cambium was rather more active than others on which investigations of the dynamics of cell production and development have been made. For example, Skene (1972) reported radial tracheid production of 45–100 over a complete season for vigorous trees of *Tsuga canadensis* Carr., and Denne (1974) gave rates of 0.3 tracheids/day for both seedlings and branches of *P. sitchensis*.

## RESULTS AND DISCUSSIONS

Fig. 1 shows radial cell diameter and radial cell wall thickness for each consecutive cell along a single radial file of tracheids. The beginning and completion points of cell expansion and cell wall thickening were identified by searching for the point of zero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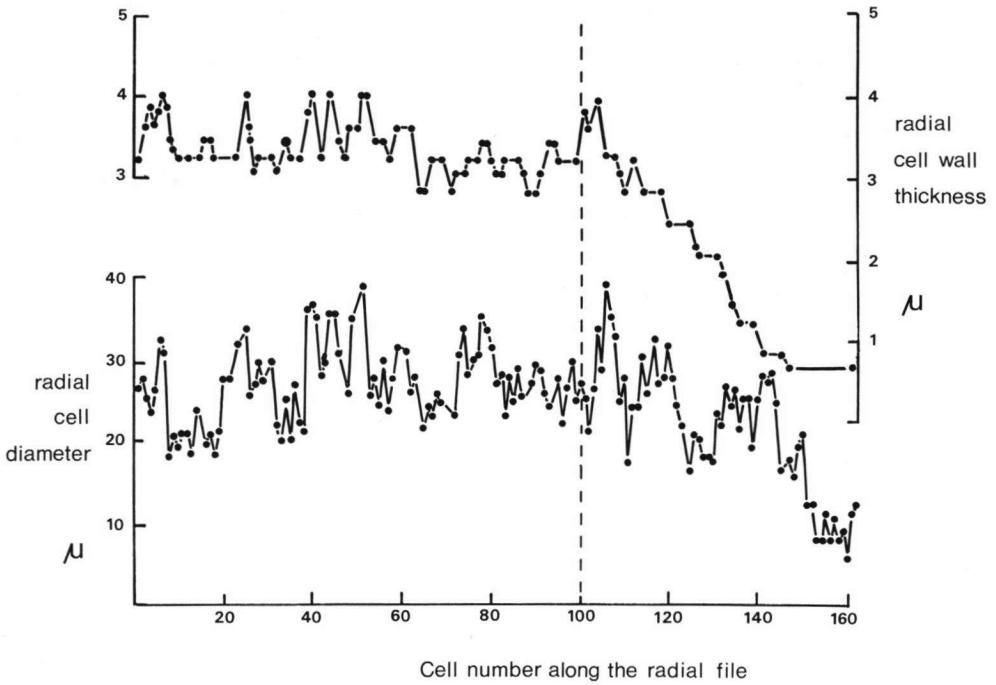


*Fig. 1.* The radial cell diameter and radial cell wall thickness for each cell along an entire file of tracheids, 12 July 1974. Cell 1 is the first cell formed in the ring.

change in values of cell diameter and cell wall thickness respectively. The beginning of both of these processes is quite easy to estimate, and Plate 1 and Fig. 1 give visual confirmation that this is so for cell wall thickening. Note also that cell wall thickening begins at the same point on neighbouring files (Plate 1C). The point of completion of both cell wall thickening and cell expansion is more difficult to estimate; but in this work, where it is the structure of fully thickened cells in the radial file that is to be examined, a conservative estimate of the end of the phase can be taken i.e. cell 100.

The simplest hypothesis that can be made about the dimensions of cells in the radial file between cell numbers 1 and 100 is that they show random variation, i.e. that the diameters or wall thicknesses of neighbouring cells are independent of each other. This hypothesis can be examined by applying a number of tests from the branch of statistics normally referred to as 'time series analysis'; see Kendall (1973) for an introduction to these techniques.

*Plate 1.*—Micrographs of glycol methacrylate embedded transverse sections of the differentiating tracheids; A, most of the growth zone for the period studied, × 36; B, the youngest tracheids, × 30; C, as B, but viewed between cross polaroids and showing clear onset of secondary wall thickening which appears to occur synchronously in adjacent radial files; D, detail from B, × 450; E, detail from C showing relatively rapid wall thickening over only 3–4 cells (i.e. about 1 day's cell production).

A first test to apply is to count the number of turning points (i.e. the 'peaks' and 'troughs') which occur in the series. If the series is random then the probability of finding a turning point in any set of three values is $\frac{2}{3}$ (Kendall, 1973). For a series of 100 cells, (n = 100), the first and last cells cannot be turning points and so must be discounted in calculating the expected number of turning points under the null hypothesis. This number, $P_1$, is given by $p_1 = \frac{2}{3}(n - 2) = \frac{2}{3}(98) = 65.3$. Since in Fig. 1 the actual number of turning points in the series of cell diameters is 57, there are fewer turning points than expected, and the series is therefore not a random one. A formula for the standard deviation of $p_1$ is given by Kendall as $\sqrt{((16n - 29)/90)}$ which in this instance is 4.17, then (actual–expected)/s.d. = 1.99 which indicates significance at $p < 0.05$. The first 100 cells of two other radial files from the same sample as that shown in Fig. 1 had 56 and 52 turning points respectively while radial files from other samples also indicated that the series of cell diameters in the file is not random.

The same test can be applied to the series of cell wall thickness although, in this case, a restriction must be placed on the value of $n$ used to calculate the expected probability. The limit of accuracy imposed by the degree of magnification and the scale of the graticule eyepiece used to make the measurements, required values of wall thickness to be rounded to units of 0.56 $\mu$m. In a number of instances in Fig. 1 neighbouring cells were given the same value of wall thickness, and so it cannot be judged whether or not they formed turning points which were just not resolved due to the rounding errors. The series was therefore condensed so that neighbouring cells with the same value were counted as one. This reduced the effective series length to 57, so that the expected number of turning points under the null hypothesis was 38; the actual number was 28 (s.d. 3.13), and the series can be judged not random with $p < 0.002$.

To judge that these series are not random by the turning point test indicates that if cells are increasing in diameter from neighbour to neighbour along the file, the probability is that they will continue to increase. Similarly, once the series shows a decrease, the probability is that this will continue. And the same can be said for wall thickness. The implication is that these two processes of cell development do vary as cells are produced, but that this variation is of such a duration as to effect more than one cell of the radial file. The next question is to ask: 'What is the scale of the variation?'. Are just small groups of cells, say 2 or 3 affected or does the variation come in larger blocks?

One way to examine this is to draw the graph of a moving average. A new series, $y_n$, is formed from the original series by a rule of the following type:

$$y_n = \tfrac{1}{3}(x_{n-1} + x_n + x_{n+1})$$

this is a simple three point moving average; 3 m.a. Note that there can be no moving average term centered on the end points of the original series. The rule can be extended by including additional terms on either side of $x_n$ in a balanced way, and increasing the

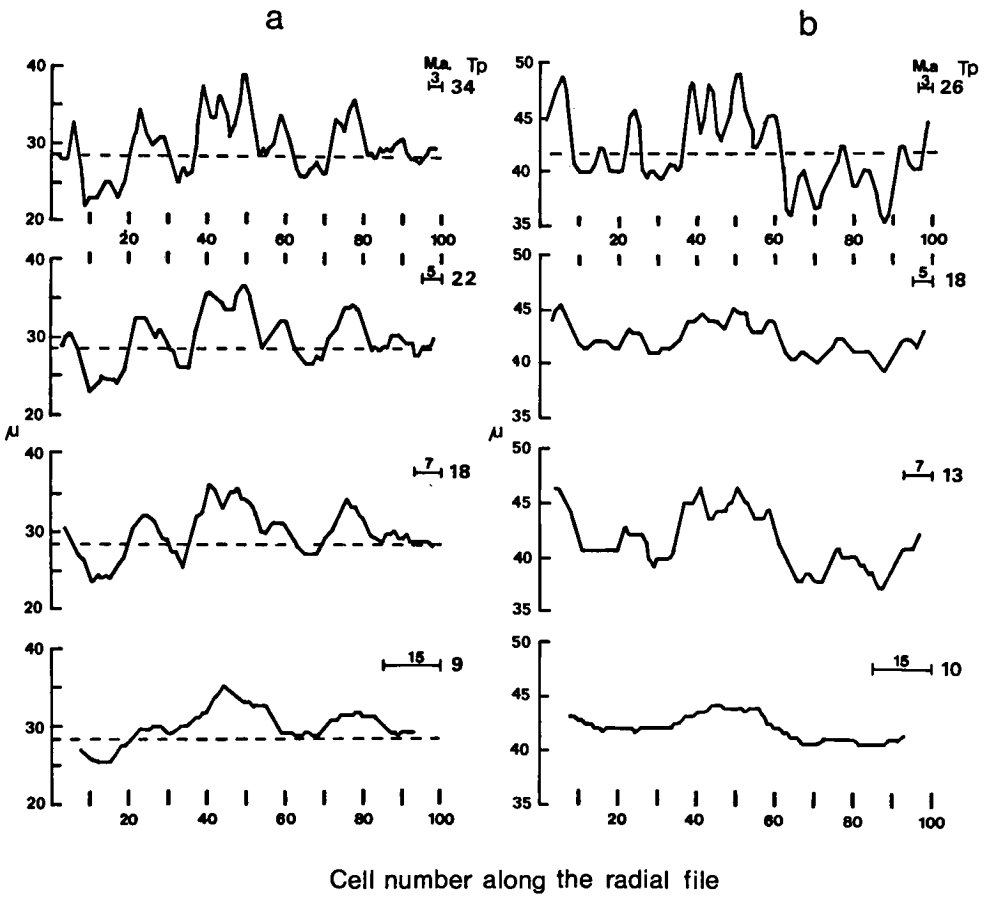


*Fig. 2.* Moving averages, m.a., of increasing order for the series of (a) radial cell diameter and (b) radial cell wall thickness; cells 1–100 shown in Fig. 1. Turning points, Tp, in the original series were 57 and 28 respectively.

denominator accordingly (Fig. 2). As the order of the moving average is increased the graph becomes smoother, but certain peaks and troughs remain whilst others disappear. A visual inspection of the graphs of the series suggests that there is a high frequency variation, present in the 3 m.a. curve which is superimposed on a lower frequency variation which persists up to the 7 m.a. curve. It is of interest to note that there is only a small reduction in the number of turning points when moving from 5 m.a. to 7 m.a.

Visual impressions are important in interpreting series such as these but the different scales of variation need to be quantified, and this can be done by calculating the autocorrelation function of the series. For the series $x_1, x_2, x_3, x_4, \ldots x_n$ a correlation coefficient can be calculated between each value and its nearest neighbour

i.e. the normal calculation for a set of bivariate values but using the constructed series of the $n - 1$ pairs $(x_1, x_2), (x_2, x_3), (x_3, x_4), \ldots (x_{n-1}, x_n)$. A second correlation coefficient can be calculated by pairing each value with its second neighbour, the $n - 2$ pairs $(x_1, x_3), (x_2, x_4), (x_3, x_5), \ldots (x_{n-2}, x_n)$, and a third coefficient by pairing each value with its third neighbour and so on. The order of neighbour with which the pairing is made, $k$, is frequently referred to as the 'lag'. Before calculating the series of correlation coefficients, the mean is subtracted from each member of the series and the formula for the correlation, $r$, at lag $k$ is:

$$r_k = \frac{\frac{1}{n-k}\sum_{i=1}^{n-k}(x_i - \bar{x})(x_{i+k} - \bar{x})}{\frac{1}{n}\sum_{i=1}^{n}(x_i - \bar{x})^2}$$

The denominator in the equation is the variance of the series and when $k = 0$ (i.e. at zero lag) then $r = 1$. The values of $r_k$, $r_{-k}$ are symmetrical about $r_0$, and it is convention to consider only positive values of $k$. The first 20 values of the autocorrelation function for the series of cell diameters and cell wall thickness are graphed in Fig. 3.

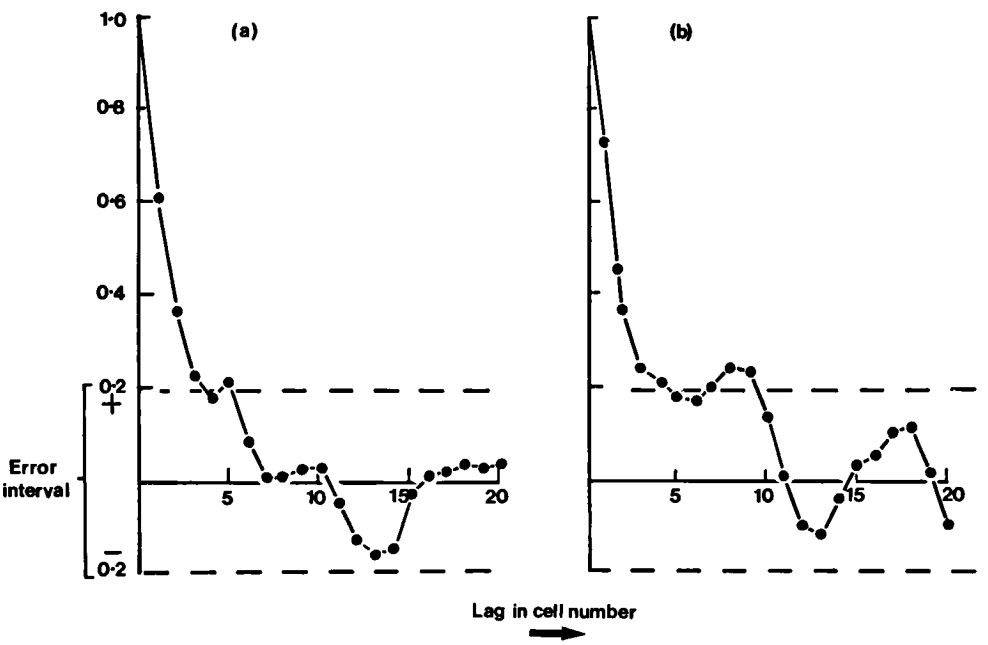


*Fig. 3.* The first 20 terms of the autocorrelation function for (a) radial cell diameter, (b) radial cell wall thickness, cells 1–100 of the radial file shown in Fig. 1. Error intervals are drawn at $p = 0.05$. Standard deviation of the series is $1/\sqrt{n}$.

The autocorrelation functions illustrate that neighbouring cells are of similar diameter and have similar wall thickness; the first three neighbours have a significant correlation for cell diameter and the first four for cell wall thickness. However, in both instances, more distant neighbours along the radial file are significantly positively correlated: number 5 for cell diameter, and numbers 7, 8, and 9 for cell wall thickness; although the values of $r_k$ are subsequently not significant, they do give further indication of some regular variation in the series. The autocorrelation functions of other radial files have a similar appearance to those in Fig. 3; the first three or four neighbouring cells are always positively correlated, and there is a gap in significance followed by a second peak of significant positive correlation, though the precise location of this varies between lag 5 and 10.

The interpretation of the autocorrelation functions can be linked to that of the moving averages analysis. The high frequency variation apparent in the 3 m.a. curve is related to the significant terms for the close neighbours in the autocorrelation function, and the lower frequency variation which is apparent in the 5 and 7 m.a. graphs to that of the second peak in significance.

This structure of variation in the series leads to a hypothesis for cell development in the radial file which incorporates two ideas. Firstly, that when cell development varies more than one cell at a time is affected; secondly, that cell development fluctuates in an 'episodic' manner. To place an environmental interpretation on this episodic development, the cambium can be considered to experience the environment as a series of 'shocks', some favourable and perhaps some not; but whenever a 'shock' is received a number of cells are influenced, hence the strong positive correlation for the first few lags of the autocorrelation function. However, it can be seen from the 5 and 7 m.a. graphs that, although there is distinct evidence of a low frequency variation, the lengths of the peaks and troughs are not constant i.e. maxima can lie between 8 and 15 cells apart. Thus the effect of the 'shocks' stands up clearly in the autocorrelation function but the correlation between 'shocks' themselves is less clear. This is what one might expect, since variation in the weather is 'episodic' rather than periodic. However, the response to a new 'episode' tends to be clear cut. If we assume that the 100 cells were formed at a rate of 3/day then the 5 and 7 m.a. graphs of cell diameter suggest that there were 5 or 6 surges of development in some 33 days.

An interesting feature of variation in the radial file of tracheids is that cell diameter and cell wall thickness have similar autocorrelation functions. To examine how these parameters relate to each other, the cross-covariance function between the series, $c_k$, must be calculated. In this case there are two series, $x$ (cell diameter) and $y$ (cell wall thickness) and a similar set of pairings to that required to calculate the autocorrelation function is made, but this time between the two series i.e. for $c_0$ $(x_1, y_1)$, $(x_2, y_2)$, ... $(x_n, y_n)$ and for $c_1$ $(x_1, y_2)$, $(x_2, y_3)$, ... $(x_{n-1}, y_n)$. However, unlike the autocorrelation function, values of the cross-covariance function at $-k$ are not equal to those at $+k$; the series for $c_1$ $(x_1, y_2)$, ... $(x_{n-1}, y_n)$ is not equal to the series for

$c_{-1}$ $(x_2, y_1)$, ... $(x_n, y_{n-1})$. The formula for the terms of the cross-covariance function is;

$$c_{-k} = \frac{1}{n-k} \sum_{i=1}^{n-k} (x_{i+k} - \bar{x})(y_i - \bar{y}) \qquad k = 0, 1, 2, \ldots, n$$

$$c_k = \frac{1}{n-k} \sum_{i=1}^{n-k} (x_i - \bar{x})(y_{i+k} - \bar{y}) \qquad k = 0, 1, 2, \ldots, n$$

The cross-covariance function for cell diameter and cell wall thickness along the radial file is shown in Fig. 4. It illustrates that there is a general correlation between the two parameters: large cells tend to have thick walls and *vice versa*, and this is confirmed by lining up with a ruler the peaks of cell diameter and cell wall thickness in the raw series (Fig. 1). However, the highest values of the cross-covariance functions are at

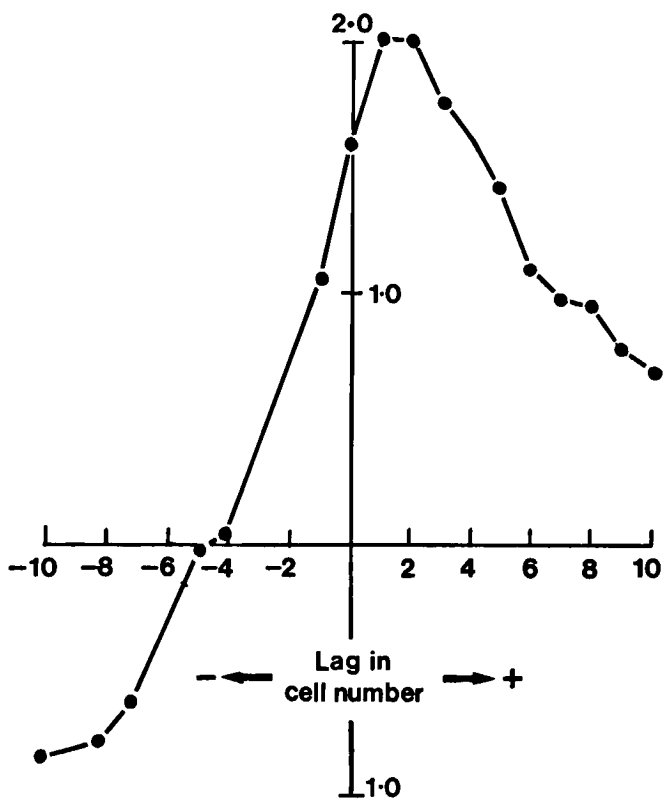


*Fig. 4.* Cross-covariance function cell diameter: cell wall thickness, cells 1–100 along the radial file shown in Fig. 1. Cell diameter is correlated with the cell wall thickness of previously formed cells where the lag is shown as negative and with that of later formed cells where the lag is positive.

lags 1 and 2 i.e. moving along a radial file from the first formed earlywood towards the cambium the maxima and minima in cell diameter tend to preceed those of cell wall thickness by 1 or 2 cells. This result must be interpreted with care, since it could be caused not just by a simple shift of one series relative to another, but also by differences in the rate of increase or decrease in the two quantities.

From the point of view of cambial physiology, a most intriguing feature of the analysis is that variation in radial tracheid diameter is closely related to variation in cell wall thickness. The two developmental processes are physically separated in the growth ring, there is a lag in time as a cell passes through one stage to the next and yet the results are correlated. This suggests that the environment which influences wall thickening is that which exists early in the life of the tracheid and before the process of thickening has started, i.e. that cells move out of the process of expansion with the course of their wall thickening process largely determined.

The application of some techniques of time series analysis to the sequences of cell dimensions along a radial file illustrates that the considerable variation which exists does have a coherent structure. This is compatible with the hypothesis of there being environmental influences on cell development which could act as a series of stimulating or retarding shocks affecting a chain of developmental processes. An interesting possibility is that these techniques could be used to 'finger print' the variation which exists within specific growth rings and so assist in the aligning of neighbouring series of ring widths which is necessary in the development of ring width chronologies.

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