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The Kinetics of Tracheid Development in *Tsuga canadensis* Carr. and its Relation to Tree Vigour

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ABSTRACT

Cell counts from samples taken at weekly intervals, from 14 May to 22 October 1969, in a *T. canadensis* stand in Massachusetts, U.S.A., showed that the width of the annual ring was correlated with the rate of cell production and that only the least vigorous trees (*c.* 20 tracheids year^{-1}) had a shorter growing season. The time required for completion of a cell-division cycle in the cambial zone decreased during the course of the season, from 35 to 20 days for the less vigorous trees (25–45 tracheids year^{-1}) and from 28 to 10 days for the more vigorous trees (45–100 tracheids year^{-1}). The time required for the completion of radial growth of the tracheids decreased from 18 to 9 days, with no evidence of any changes with tree vigour. The actual radial growth-rate of the tracheids was constant within the range 1·5–3 μm day^{-1}. The time required for deposition of the secondary cell wall increased from 10 to 50 days, with little evidence of any changes with tree vigour. The actual rate of deposition of cell wall material was about 0·15 μ^2 μ^{-1} day^{-1} and seemed to show little change during the course of the season. The time period required for lysis of the cytoplasm was about 4 days, with no evidence of any changes with tree vigour and little evidence of any changes during the course of the season.

INTRODUCTION

Earlier work has shown the relatively long period of time taken for the completion of radial growth in developing tracheids and for the deposition of their secondary cell walls in *Abies pectinata* D.C. (Wodzicki and Peda, 1963) and, more recently, in *Pinus sylvestris* L. (Wodzicki, 1971; Wodzicki and Zajaczkowski, 1970). Skene (1969) has shown that in *P. radiata* D. Don a cycle of cell division in the cambial zone also takes a relatively long period of time, as do the phases of radial growth and wall deposition. The time taken to complete radial growth decreases during the course of the season, whilst the time taken for deposition of the secondary cell wall increases considerably. The aim of the present work is to extend this type of observation to *Tsuga canadensis* Carr. and, more particularly, to try to find out how these time periods vary with tree vigour, vigour being defined as the width of the annual ring.

MATERIAL AND METHODS

Material was taken from a well stocked old growth stand of *Tsuga canadensis* Carr. on a moist site at Petersham, Massachusetts. The trees ranged from 0·3–3 m in girth (over bark, at breast height) and were estimated to be 50–150 years old.

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Forty-five trees, representing a wide range of vigour, were divided into three groups, trees being assigned to each group at random on the basis of an initial estimate of their vigour. Thus each group contained trees scattered throughout the whole plot and representing the whole range of vigour. At each collection one sample was taken from each tree of a group, so that during the course of the season a total of eight samples, spaced out equidistantly around the circumference, were taken from each tree at breast height. Collections were started on 14 May 1969 with the trees from group 1 and were continued at weekly intervals using trees from each group in turn (1, 2, 3, 1, 2, etc.). After the last collection (22 October 1969) there was a total of 24 collections, each with 15 samples.

The samples were removed with bark intact; they were about 5 cm long, 2 cm wide, and deep enough to include both the current and the previous season's annual ring. Portions suitable for embedding were fixed in acrolein for 24 h at 0 °C, embedded in glycol methacrylate, sectioned transversely, and stained with toluidine blue according to the methods described by Feder and O'Brien (1968).

Counts were made of the number of cells in a radial file, using an average from two radial files for each sample. Counts were made of the total number of tracheids (O) across the previous season's annual ring and the number of cells in each of five different phases of development across the current season's ring. The dividing phase (D), radial growth phase (G), and secondary wall deposition phase (W) were distinguished from one another as in Skene (1969), while the lysis phase (L) was distinguished from the secondary wall phase by the presence of the S_3 layer of the secondary cell wall and the mature phase (M) was distinguished from the lysis phase by the complete disappearance of the cytoplasm. Thus the combined lysis and the mature phases of this work correspond to the mature phase in Skene (1969). For each sample the counts were added as follows:

$S_m = M$ = total number of mature tracheids in a radial file

$S_l = M+L$ = number which have completed deposition of their secondary walls

$S_w = M+L+W$ = number which have completed their radial growth

$S_g = M+L+W+G$ = number which are no longer in the dividing phase

$S_d = M+L+W+G+D$ = total number of tracheids, mother cells and initials

Measurements of transverse tracheid dimensions were made from two samples in each vigour class using samples collected at the end of the season. The samples were chosen to have approximately the same number of tracheids in the current annual ring as the corresponding class average. Measurements were made with a filar micrometer eyepiece on tracheids from one radial file in each sample. The radial and tangential diameter measurements were 'squared off', that is, the micrometer was adjusted so that, while being as close as possible to the tracheid diameter, the product of the radial and tangential diameters was approximately equal to the actual cross-sectional area of the tracheid. Secondary wall thickness measurements (the thickness of the S_1, S_2, and S_3 layers) were made normal to the radial and tangential walls, avoiding corners and pits where thicknesses are liable to be atypical. No tracheid length measurements were made.

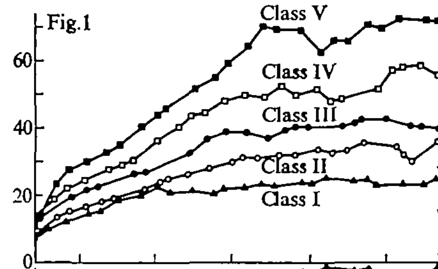
STATISTICAL TREATMENT

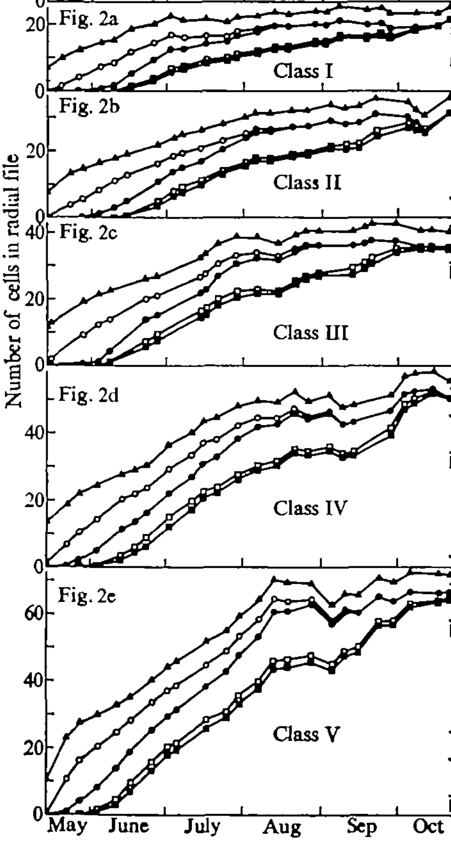
For the analysis of the counts, the samples from each collection were divided into five vigour classes, depending on the number of tracheids produced in the previous season's annual ring. The range of vigour included within any one class was adjusted so that there were approximately the same number of samples in each class when averaged over all 24 collections. The variation around the circumference of a tree was such that samples from most trees did not all come in the same class and samples from some trees occurred in all classes, from the slowest to the fastest. After the samples had been assigned to classes, within each class a three-point weighted moving average was calculated for the time of collection, for the number of tracheids produced in the previous season's annual ring, and for the numbers of cells in different phases of development, the averages being weighted according to the numbers of samples in each class at each collection. Single-point averages were also calculated for the first and last collections. The resulting averages were visualized as forming a three-dimensional graph of cell number plotted against time and vigour. After this smoothing and to form the graphs in Figs. 1 and 2, the average number of tracheids ($\bar{O}$) formed by each class during the previous season was computed (that is, the average for all 24 collections) and this average was used to adjust the smoothed cell counts in each collection by the factor $\bar{O}/O$. Thus, if, in a particular collection, the samples in a class were less vigorous than the class average for the season the counts were scaled up, and vice versa if the samples were more vigorous than the average.

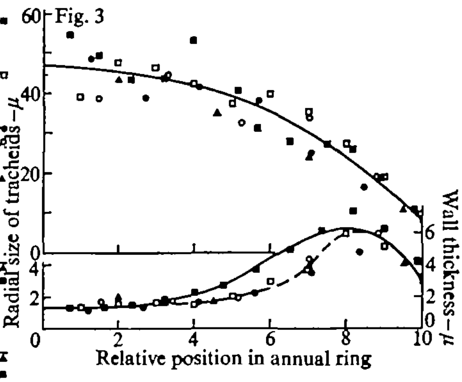
RESULTS

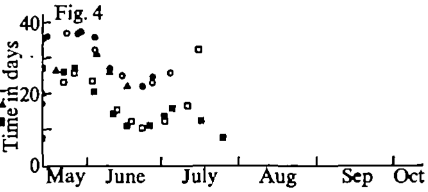
By the time of the first collection (14 May) cambial activity was well under way and in the more vigorous classes radial growth of the tracheids had started. Cambial activity is unlikely to have started more than a month earlier, i.e. at the first time the daily minimum temperature was over freezing-point for more than 2 days in succession and the daily maximum over 16 °C, and it could have started as little as 2 weeks earlier. The initial rates of increase in total cell number (Fig. 1) were similar in the least vigorous trees, classes I and II, and the greater amount of growth of the trees in class II was clearly due to the same initial growth-rate being maintained over a longer period of time. In classes II–V differences in vigour are due to differences in rate of increase in cell number, differences in length of the growing season being very slight. In classes II–V growth in cell number continued at a more or less steady rate until early August, at which time there seemed to be a rather abrupt decrease in cambial activity. There is some evidence of a resurgence of activity 3 weeks later in classes IV and V, but this made relatively little contribution to the final cell number. In class I cambial activity slowed down much sooner, early July, but activity seemed to continue at a very slow rate until early August.

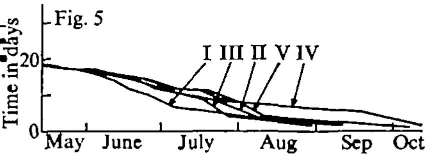
This pattern of growth in total cell number is broadly similar for all stages of tracheid

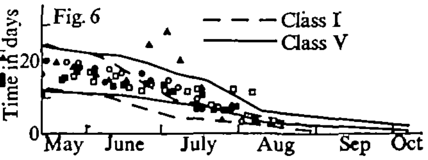












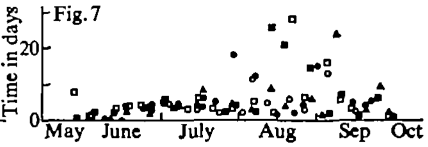


FIG. 1. Total cell number (S_d) plotted against time for each class.

FIG. 2. Cell counts, for each phase of development, plotted against time.

■ S_m, □ S_l, ● S_w, ○ S_g, ▲ S_d

FIG. 3. Radial size of tracheids and thickness of the tangential secondary wall plotted against relative position in the annual ring for each class. A symbol is plotted for every fifth tracheid (see Fig. 1 for symbols). Dashed line gives wall thickness for classes I–IV, solid line for class V.

FIG. 4. Generation time of cambial zone plotted against time. See Fig. 1 for symbols.

FIG. 5. Calculated time periods for completion of radial growth, calculated from Figs. 2 and 3 assuming a constant radial growth rate of 2 μm day^{-1}.

FIG. 6. Observed time periods required for the completion of radial growth of the tracheids plotted against time. The curves represent calculated times, assuming constant radial growth rates of 1·5 and 3 μm day^{-1} for classes I and V. See Fig. 1 for symbols.

FIG. 7. Observed time periods required for the lysis of the cytoplasm plotted against time. See Fig. 1 for symbols.

development (Figs. 2*a*–*e*). The initial times at which cell number begins to increase occur later in the season, depending on the time required for completion of the preceding phases of development, but the marked decreases in growth-rate in early July (class I) and early August (classes II–V) seemed to occur at about the same time, suggesting that whatever inhibits cambial activity at these times also inhibits the later phases of development. This factor is unlikely to have been a shortage of water since there was a total of 20 cm of rain during the last week of July and the first week of August, and temperatures were in the range 15° daily minimum to 27° daily maximum.

In Fig. 3 radial tracheid diameters and the thickness of their tangential secondary walls are plotted against relative distance across the annual ring for each class. There is a point for every fifth tracheid along a radial file and, except for the first and last points of each file, each point represents the average of 10 tracheids, five from each of the two samples. Tangential tracheid diameter and the thickness of the radial secondary walls are not shown. Tangential diameter was essentially constant across the annual ring and showed no consistent differences between classes (average tangential diameter was 36 μm). The thickness of the radial secondary walls averaged 1·25 times that of the tangential walls and showed the same seasonal pattern. Actual dimensions were similar in all classes and there were no differences in the proportion of different-sized tracheids in the annual ring. The only difference was the clear indication of thicker-walled tracheids in the middle third of the annual ring in class V. This difference was based on only two samples but further results from three more samples (not shown) showed a similar difference.

THE PERIOD OF TIME TAKEN FOR TRACHEID DEVELOPMENT

The time periods for tracheid development were calculated in the manner described by Skene (1969) and the results are summarized in Figs. 4–9. The calculations were limited to the first 90 per cent of the annual ring to avoid the extreme scatter introduced by the slow rate of increase in tracheid number and relatively large sampling errors at the end of the season. In Fig. 4 the generation times for the cambial zone are plotted against time for each vigour class; the generation time is the time taken for the total number of cells in the annual ring, S_a, to increase in number by an amount equal to the number of cells in the cambial zone. There was a decrease in generation time (increase in rate of cell division) over the period late May to late June with a subsequent increase in generation time. This later increase can be attributed to the decrease in growth-rate in early August which has an early influence on the estimated generation time because of the method of calculation. Classes I, II, and V showed a relatively short generation time for the first collection, but the significance of this is doubtful in view of the small number of samples involved. It is evident from Fig. 4 that the trees in classes IV and V had a shorter generation time than the less vigorous trees. This decrease in generation time with increase in tree vigour is shown more clearly by an alternative method of calculation in which straight regression lines were fitted to the adjusted, but unsmoothed, cell counts S_a and S_g, using the period

14 May–2 July for class I and the period 14 May–13 August for classes II–V. The generation times were calculated as follows:

$$P = \frac{S_d - S_g}{b_d}$$

where P is the generation time in days, b_d is the slope of the regression line for total cell number against time, and $S_d - S_g$ is the mean number of cells in the cambial zone (estimated from the regression lines). Confidence limits on P were calculated using a modification of standard inverse regression methods according to the following formula:

$$\frac{P}{1-k_\alpha} \pm \frac{k_\alpha P^2 + (2/n)T^2 k_\alpha (1-k_\alpha)}{1-k_\alpha}$$

where

$$k_\alpha = \left(\frac{s^2}{T^2 b_d^2}\right) F_\alpha$$

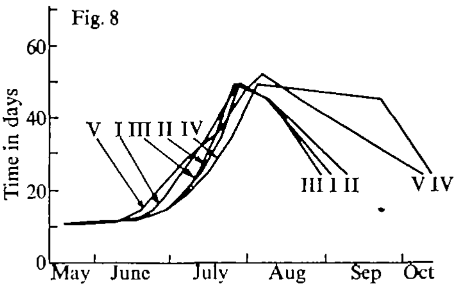
and where T^2 is the corrected sum of squares for the independent variable, s^2 is the pooled estimate of variance about the S_d and S_g regression lines, n is the number of pairs of observations used to calculate the regression lines, and F_α is the F value on 1 and $2(n-2)$ degrees of freedom for the selected significance level α. The results are summarized in Table 1 which shows a decrease in generation time with increase in tree vigour for all classes.

TABLE 1. *Generation times for the cambial zone calculated from linear regressions*

Vigour class	I	II	III	IV	V
Generation time in days	26	25	23	19	14
5% confidence limits	18, 39	21, 30	15, 32	15, 23	10, 19

The time periods required for the completion of radial growth of a tracheid are plotted against time in Fig. 6. There are no clear differences between classes and the time periods are about 18 days in late May, decreasing to about 9 days by the beginning of July. The lines in Figs. 5 and 6 show time periods calculated from the radial diameters of the cells of the cambial zone (data not shown) and of the mature tracheids (Fig. 3) assuming that the tracheids have constant radial growth-rates, namely 2 μm day^{-1} in Fig. 5 and 1·5 and 3 μm day^{-1} in Fig. 6. In Fig. 5 the lines are quite similar except for the steep decline of class I due to the early completion of most of its annual ring and for the long tails of classes IV and V due to their late resurgence of cambial activity. If this late resurgence of activity is considered to be a sampling error, then classes II–V would be similar throughout the season. In Fig. 6 the lines shown are for classes I and V and, for the most part, the observed time periods seem to be consistent with an almost constant tracheid radial growth-rate lying between 1·5 and 3 μm day^{-1}. The most marked exception to this is with class I in late June and early July because of the reduced rate of development of the annual ring at that time (Fig. 2*a*).

The time periods required for the deposition of the tracheid secondary walls rose from about 10 days in late May to a maximum of about 50 days at the end of July. The results are summarized in Fig. 9 which shows the considerable scatter with no clear differences between classes. The lines in Fig. 8 show the time periods calculated on the assumption of a constant rate of cell wall deposition (per unit of cell wall), using the data from Fig. 3 and the growth curves from Fig. 2. The lines in Fig. 8 were calculated from the cross-sectional area of the secondary wall of the mature tracheid and the perimeter of the tracheid at the mid-point of the thickness of its secondary wall, given a deposition rate of 0·15 $\mu m^2\ \mu m^{-1}\ day^{-1}$. In late June/early July the calculated periods for class I tended to be longer than for classes II–IV because the trees in class I completed a greater proportion of their annual ring early in the season, while the calculated periods for class V tended to be longer because of the greater thickness of the cell walls in the middle third of the annual ring (Fig. 3). The relatively long 'tails' for classes IV and V are due to the late resurgence of growth. In Fig. 9 lines calculated for classes III and V, given deposition rates of 0·1 and 0·2 $\mu m^2\ \mu m^{-1}\ day^{-1}$, are superimposed on the observed periods and the clear correspondence between the points and the curves suggests that there is a more or less constant rate of cell wall deposition within the range 0·1–0·2 $\mu m^2\ \mu m^{-1}\ day^{-1}$. The only evidence of any differences between classes occurs in the middle of the growing season when there is a tendency for the class I trees to have a slow rate of wall deposition (0·1 $\mu m^2\ \mu m^{-1}\ day^{-1}$) and for the class V trees to have a high rate of (0·2 $\mu m^2\ \mu m^{-1}\ day^{-1}$). The scatter, however, is such that the differences are unlikely to be significant and much more data would be required to substantiate the point.



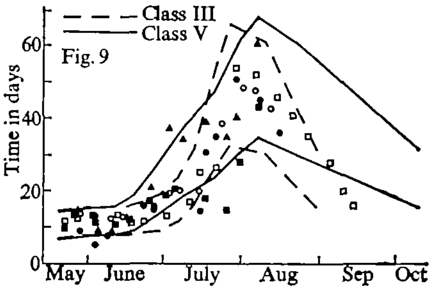


FIG. 8. Calculated time periods for completion of secondary wall deposition, calculated from Figs. 2 and 3 assuming a constant deposition rate of 0·15 $\mu m^2\ \mu m^{-1}\ day^{-1}$.

FIG. 9. Observed time periods required for the completion of secondary wall deposition plotted against time. The curves represent calculated times, assuming constant deposition rates of 0·1 and 0·2 $\mu m^2\ \mu m^{-1}$ day^{-1}, for classes III and V. See Fig. 1 for symbols.

The time required for lysis of the cytoplasm was fairly short, averaging about 4 days. The results are summarized in Fig. 7. There was no difference between classes nor any pronounced seasonal changes, except for a few long periods towards the end of the season corresponding to a time of very slow general development. Since the time required for lysis was estimated from the first appearance of the S_3 layer of the secondary wall to the complete disappearance of the cytoplasm, the short time period suggests that lignification must be almost complete by the time the S_3 layer is laid down and that within a cell cellulose and lignin deposition occur concurrently. It does not necessarily mean, however, that deposition occurs concurrently within any particular layer of the cell wall.

DISCUSSION

The time periods reported here for radial growth and secondary wall deposition are not only similar in magnitude to those reported for ***Pinus radiata*** by Skene (1969) and for *P. sylvestris* by Wodzicki (1971), but show similar seasonal trends: a decreasing time for radial growth and an increasing time for cell wall deposition. The actual rates of radial cell growth were 1·5–3 μm day^{-1} in the present study compared with 1–4 μm day^{-1} found by Wodzicki. The rates of cell wall deposition in the present study were about 0·15 $\mu^2\,\mu^{-1}$ day^{-1} and similar to that for *P. radiata* earlywood and latewood (0·15 and 0·12 $\mu^2\,\mu^{-1}$ day^{-1}, respectively, calculated from unpublished data from the study by Skene (1969)). Wodzicki showed rates of increase in cell wall thickness varying from 0·1 to 0·3 μm day^{-1}, increasing from a low rate in late May to a maximum in early July and with a steady marked decline for the rest of the season. While the present data are not precise enough to allow a detailed comparison of these trends in deposition rate, they do not suggest such a marked decrease in deposition rate during the later part of the season as that described by Wodzicki. The apparent difference in deposition rate in the two investigations may be due largely to the methods of calculation. A constant rate of increase in cell wall thickness implies a decreasing rate of deposition of cell wall material because, as the wall becomes thicker, the internal surface area of the cell becomes smaller. The present method of calculation makes some allowance for this by basing the calculated rate on the tracheid perimeter at the mid-point of the cell wall. With thin-walled cells the difference between the two methods is trifling; for tracheids of square cross-section where walls are 10 per cent of the width, the present method would give a rate of 0·108 $\mu^2\,\mu^{-1}$ day^{-1} while Wodzicki's would give 0·1 μm day^{-1}. As wall thickness increases, however, the difference become large; for walls 50 per cent of tracheid width the present method would give 0·175 μm^2 μm^{-1} day^{-1} while Wodzicki's would give 0·1 μ day^{-1}.

There was a large amount of scatter in the generation times reported for *P. radiata* by Skene (1969), but they are of a similar magnitude to those reported here. The generation times of the more vigorous trees seem to be rather shorter than those of *P. radiata* whose large annual increments are clearly due to the much longer growing season.

Gregory and Wilson (1968) showed that in *Picea glauca* (Moench) Voss growth-rate was correlated with tree vigour (as measured by the thickness of the annual ring) and that for a given vigour trees in Alaska had a higher growth-rate than those in Massachusetts. This higher growth-rate was associated with a higher mitotic index but within each region, however, mitotic index was essentially constant for trees of different vigour. They conclude that the Alaskan trees had a higher rate of cell division than those in Massachusetts but that within each geographical area the growth-rate is determined by the number of cells in the cambial zone, the rate of cell division being constant. This is in contrast to the results reported here in which, although the number of cells in the cambial zone is correlated with growth-rate, both within the season and between classes, there also seem to be quite substantial differences in generation time. With the two most vigorous classes a greater proportion of the higher growth-rate can be accounted for by the shorter generation time (approximately half that of the less vigorous classes) than by the greater number of cells (20–30 per cent more than the less vigorous classes).

There is no reason why species should not differ in this way, but two points should be borne in mind. Firstly, both methods (the mitotic index method used by Gregory and Wilson and the growth-rate method used here) depend directly on the number of cells believed to be in the cambial zone. No unequivocal method of defining the width of the cambial zone exists, so it is necessary to do so from the general appearance of the cells and this may bias the results if their appearance changes with growth-rate or time. Secondly, the mitotic index is the time spent by a cell in mitosis as a proportion of the total mitotic cycle and is not a measure of the rate of cell division (Clowes, 1961). In particular, if, within a geographical region, the duration of both mitosis and interphase are influenced by the same factors in such a way that both tend to vary together, then the mitotic index would be constant over quite a wide range of cell division rates. In their paper Gregory and Wilson show the rate of tracheid production and the number of cells in the cambial zone for trees of different vigour. Calculation from these data confirms their view that, for trees of a given vigour, those in Alaska have a shorter generation time than those in New England but, within each region however, trees with a greater vigour tend to have a shorter generation time as in the present study. It is interesting to note that in New England the decrease in generation time and increase in number of cells in the cambial zone of *P. glauca* made approximately equal contributions to the increase in vigour, but that in Alaska the major contribution was made by the increase in number of cambial zone cells.

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