DIFFERENTIATION OF TRACHEIDS IN DEVELOPING SECONDARY XYLEM OF *TSUGA CANADENSIS* L. CARR. CHANGES IN MORPHOLOGY AND CELL-WALL STRUCTURE

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ABSTRACT

The morphology and the changes in total cell-wall mass in developing secondary xylem of two eastern hemlock trees were studied. Sixty-μm-thick tangential-longitudinal sections were microtomed sequentially from the cambium through the currently developing and the one-year-old increments. The weight and volume of these sequential sections gave data on the rate of mass production. Cross-sectional microtome sections were used to study cell-wall structures and to measure cell-wall layer areas.

Four tracheid maturation zones could be measured and described during both early- and latewood formations. The size of the cambium, cell enlargement zone, and the zone of S_1 cellulose framework formation were the same throughout the growing season. However, the size of the zone of S_2 cellulose formation changed. This zone was only one-third as wide during the formation of the thick S_2 layer in latewood tracheids as it was during the formation of the thin S_2 layer in earlywood tracheids. Despite the fact that the number of cells produced during S_2 layer formation in latewood was only one-third as many as in the earlywood zone, the rate of total mass production was more than twice as great compared to earlywood. Tracheid diameters and cell-wall layer volumes across both the currently developing and the one-year-old xylem showed that size development is complete for each layer before the appearance of the next inner layer in the tracheids. However, cell-wall layer densities continued to increase perhaps well into the second and subsequent growing seasons. Change in the relative proportion of the cell-wall layers across the growth increment was not dominated by the S_2 layer. This relative variation of the S_2 layer was the smallest of any secondary cell-wall layer across the growth increment. However, it constituted 50–70% of the total cell-wall volume.

Keywords: *Tsuga canadensis*. xylem, cambium, cell size, cell walls.

INTRODUCTION

Tracheids in the secondary xylem of conifers are derivatives of the vascular cambium. They are formed by a complex mechanism involving a series of biochemical and biophysical processes. The fully developed, mature tracheids are thick-walled cells.

The walls of differentiating coniferous tracheids are deposited by intussusceptional and appositional growth, resulting in changes in the structure of individual cells. The process of tracheid formation may be subdivided into four phases: 1)

cell division, 2) cell enlargement, 3) cellulose frame deposition, and 4) lignification.

In cell division, after the formation of the two daughter nuclei, the primary wall is first laid down. Secondary wall formation begins before enlargement is completed. It starts from the center of the cell and increases toward the cell tip (Wardrop 1964). It is believed to take place by deposition of the cellulose frame, in a sequence starting with the S_1 layer and finished by the S_3 layer. The final phase of tracheid maturation is the incrustation of lignin and lignin-like materials into the already existing cellulose framework and hemicellulose matrix.

In gymnosperms, the longitudinal system of cells consists almost entirely of tracheids. Approximately 90% by volume and 95% by weight of the secondary xylem of conifers is occupied by the tracheids (Wardrop 1964), thus a study concerned with the development and growth of conifers should concentrate on the development of these longitudinal elements.

The objectives of this study were to determine and describe quantitatively the changes in the morphology and cell-wall structure of differentiating tracheids in developing secondary xylem of eastern hemlock (*Tsuga canadensis* L. Carr.).

MATERIALS AND METHODS

Test material was obtained from two freshly cut eastern hemlock trees. The apparently healthy, 42- and 52-year-old trees were felled on June 10 and July 20, respectively. The dates of cutting were selected on the basis of cambial cell production models (Balatinecz 1966) to give a wide zone of developing early- and latewood, respectively.

Immediately after felling, a 6-inch portion of the trunk, starting from 18 inches (45.7 cm) above ground level, was put into a heavy plastic bag, and stored at -20 C until further processing. The frozen discs were cut into small blocks suitable for the preparation of tangentially microtomed specimens or into blocks suitable for microscope slide preparations.

Of the two trees, Tree No. 2 felled during latewood formation was analyzed more intensively than Tree No. 1, which provided material for the study of earlywood formation. Both trees were analyzed microscopically for the determination of the successive stages of tracheid maturation within their developing increments. Both radial and cross-sections 12–24 μm in thickness were cut on a rotary microtome from paraffin-embedded blocks. The sections were then observed under the microscope. The successive stages of cell maturation were distinguished by the aid of a polarizing microscope (Meier and Wilkie 1959; Grozdits and Ifju 1969). On the basis of the birefringency of the various cell-wall layers, five developmental zones could be distinguished. The width of each zone was measured with a filar micrometer eyepiece, and the number of cells included in each zone was counted.

In order to assess changes in total cell-wall mass in differentiating xylem, microspecific gravity measurements were made on longitudinal sections 60 μm thick cut tangentially from the cambium through the one-year-old increment of each of the two sample trees. Four blocks taken from four opposite locations of the test material of each tree were used in the microtoming. The water-logged blocks were fastened, bark facing upward, into a specially prepared rigid vise, designed

for microtoming long wood samples. The bark portion was gently removed, leaving remnants of the cambium on both the phloem and the xylem. The xylem and phloem sides were scraped off with a single-edge razor blade since it was not possible to produce large microtome sections from the soft meristematic tissue of the cambial zone. After removal of this soft tissue, sections were cut at a 60-μm target thickness through the two outermost growth increments.

In determining specific gravity of a microtomed wood specimen, difficulty arises from errors in measuring the small volumes. The surface area to volume ratio is large for these specimens. Considering this high ratio and the porous nature of wood, it is readily seen that volume measurement by either the maximum moisture content method or by the immersion method could give erroneous results. This problem was investigated by Ifju and coworkers (1965), who reported that simple measurements of sample dimensions lead to the most accurate and reliable results. Accordingly, specimen dimensions were measured as follows: length and width to the nearest 0.1 mm with the aid of a microscope stage vernier, thickness with a "Microcator" to the nearest μm.

Air-dry weights were obtained for each specimen to a meaningful limit of 10^{-6} gram. Samples adjoining each microtome specimen were used to estimate air-dry moisture content of each individual test piece. The oven-dry weights were then obtained for each specimen by correcting for the estimated moisture content. Specific gravity was calculated on the bases of oven-dry weight and green volume.

The cross-sections prepared for the determination of xylem maturation zones in Tree No. 2 were also used for cell size and cell-wall layer measurements. Such measurements were not performed on the differentiating tissue of Tree No. 1 felled during earlywood formation. The cell walls of developing earlywood tracheids were too thin to allow accurate measurements of their thickness and layered structure. For Tree No. 2 the tangential double wall thickness, and radial and tangential diameters of each tracheid in two replicate radial rows from each of the four blocks were determined. A filar micrometer eyepiece was used for these measurements. The radial rows of tracheids were started at the cambium and followed through the developing and one-year-old increments.

The area occupied by cell walls and those of the individual cell-wall layers were measured on cross-sections for Tree No. 2 by the method described by McIntosh (1965). Micrographs were prepared at a total magnification of 1,025×. From the prints, cell-wall layer thickness on all four walls was measured with a standard photo-interpreter. The middle lamella and primary wall were measured together, and the measurement then was divided by two. The external and internal perimeters for each cell-wall layer were measured by a map-reader.

The cell-wall layer area was calculated as follows: the average radial wall thickness weighted by its total length plus the average tangential wall thickness weighted by its total length was multiplied by the average of the external and internal perimeters of the particular cell-wall layer. The use of cell-wall layer perimeters instead of cell diameters assured that the cell corners were included only once in the larger areas. A sample from the photomicrographs of tracheids used to measure cell-wall and cell-wall layer dimensions is shown in Fig. 1.

From the cell-wall layer areas and from the tangential and radial diameters, the following were calculated for each cell: 1) relative area of each layer based on the area of total cell cross-section; 2) relative area of each cell-wall layer based
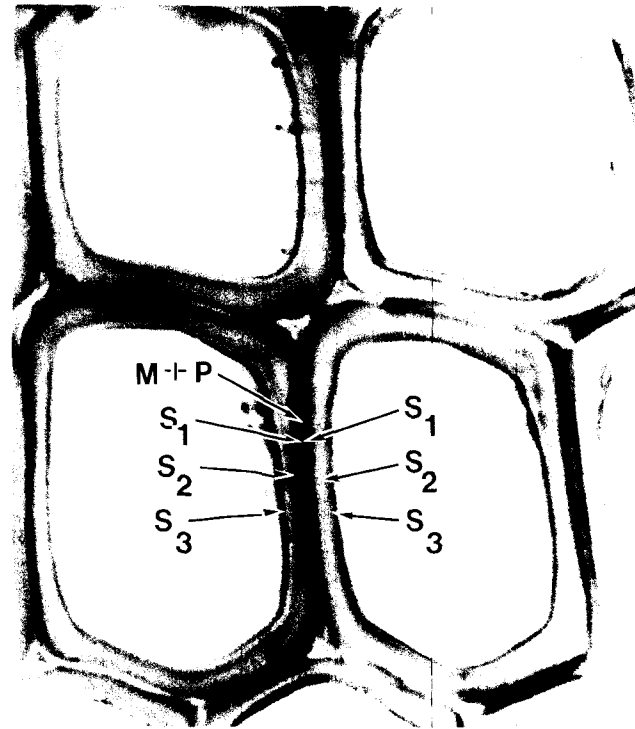
FIG. 1. A sample from the photomicrographs of tracheids used to measure cell dimensions and cell-wall layer sizes (×1,025).

on the total cell wall area. The above parameters were calculated for four representative radial rows of tracheids from both the current and one-year-old annual increments from four opposite locations of the test material.

RESULTS AND DISCUSSION

Xylem maturation zones

The number of cells in the stage of differentiation is a function of the rate of entry from the cambial zone, the duration of cell enlargement, and duration of cell-wall thickening (Wilson and Howard 1968). The duration of cell-wall thickening further depends on the rate of cell-wall formation and the final wall thickness attained (Wodzicki and Peda 1963). If one accepts the above sequence in differentiation and observes the optical properties of the cell-wall layers in the secondary xylem, the zones and stages of cell-wall formation may be established from microscope slides of the developing xylem (Grozdits and Ifju 1969).

Table 1 is a summary of the measurements of the five zones of development in differentiating earlywood and latewood. The mean radial width, termed as "zone width" of the five distinct zones and the average number of tracheids in each zone are compiled in Table 1 for the two sample trees. The cambium had a width equivalent to the width of two cambial zone cells. In addition, the cambial zone included three phloem and five xylem cells in the tree developing earlywood, and three phloem and only four xylem cells in the tree developing latewood.

TABLE 1.   *Summary of microscopic measurements of xylem maturation zones of two trees producing earlywood and latewood, respectively.*

Type of differentiating xylem	Average	Cambium		(M + P)		(M + P) + S$_1$		(M + P) + S$_1$ + S$_2$		(M + P) + S$_1$ + S$_2$ + S$_3$	
		No.* of cells	Zone width (μm)	No. of cells	Zone width (μm)	No. of cells	Zone width (μm)	No. of cells	Zone width (μm)	No. of cells	Zone width (μm)
Earlywood	Simple	2	51	5	132	4	140	17	610	18	679
(Tree No. 1)	Cumulative	2	51	7	183	11	323	28	933	46	1,612
Latewood	Simple	2	27	4	61	4	106	6	195	36	1,638
(Tree No. 2)	Cumulative	2	27	6	88	10	194	16	389	52	2,027

* Estimated no. of cells.

These cells had thin walls consisting of the middle lamella and primary wall (M ± P) only. Both of these cell-wall layers are optically isotropic; therefore they showed no birefringence from either radial or cross-sectional view (part of Fig. 2a and b).

The next four cells from the cambium inward in both sample trees showed definite birefringence both on cross-sections and radial sections. These cells were depositing the carbohydrate framework of the first layer (S$_1$) of the secondary wall. Their birefringence arose from the highly oriented cellulose microfibrils in S$_1$ (Fig. 2a and b).

Formation of the S$_2$ layer started in the 10th and 9th cell from the cambium in Tree No. 1 and No. 2, respectively. Its presence could be detected on cross-sections only by the aid of cellulose-strands (Fig. 2c). Due to the steep microfibril orientation, the S$_2$ layer had no birefringence on cross-sections, but on radial sections it showed definite birefringence. Its angle of maximum extinction was different from that of the S$_1$; therefore cells containing only (M + P + S$_1$) layers were easily separated from cells having (M + P + S$_1$ + S$_2$) layers (Fig. 2d).

It is of interest to note in Table 1 that the total number of tracheids undergoing S$_2$ formation was appreciably greater in Tree No. 1 developing earlywood than in Tree No. 2 producing latewood. This might be surprising in light of the well-known fact that latewood tracheids have very thick S$_2$ layers as compared to earlywood cells. One would thus expect that latewood S$_2$ layer development should take a longer time than formation of a thinner S$_2$ layer in earlywood tracheids. However, the number of cells at a certain stage of differentiation is not so much the function of time but rather the function of the number of cells produced by the cambial initials within a certain period of time. Apparently, a significantly greater number of cells is produced in a given period of time when earlywood is produced than during latewood formation. Thus, there must be a greater number of tracheids present at the same stage of development in earlywood than in latewood.

The S$_3$ layer of the secondary wall could be identified on cross-sections. It appeared as an inner bright circle under polarized light (Fig. 2f). Although the S$_3$ layer showed a definite contrast with almost all common stains, staining was not used as a tool to identify the presence of the S$_3$ layer in the differentiating tracheids. The cell wall to cytoplasm interfaces are always dense because of organelles (Kutscha and Gray 1972). They also stain easily and show an impression similar to that of the S$_3$ layer (Fig. 2e).
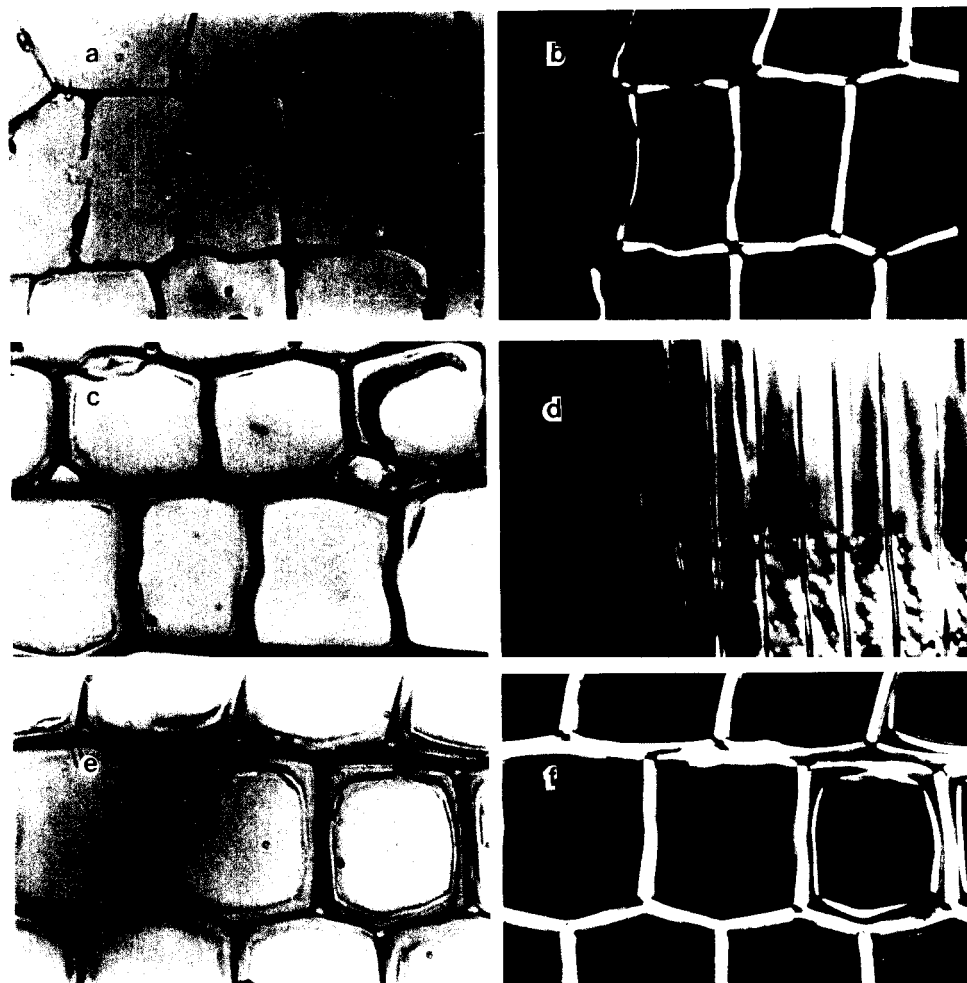
FIG. 2. Micrographs of tracheids at various maturation stages. a and b, first appearance of S₁ layer and its progressive deposition; c, beginning of S₂ layer deposition; d, on radial sections the maximum birefringence of the S₂ layer makes it distinct from that of the S₁ layers; e and f, the S₃ layer appears as an inside ring. a, c and e were photographed under ordinary light, b, d and f were photographed in polarized light (a and b ×500, c ×560, d ×180, e and f ×620).

From microscopic observations, four xylem maturation zones were positively defined. These were: cambial zone, zone of cell enlargement, S_1 cellulose frame deposition (M + P + S_1), and S_2 cellulose frame deposition (M + P + S_1 + S_2). Although the presence of an S_3 layer could be readily determined, the radial width of this zone of deposition could not be ascertained. However, its presence could be detected in the 29th and 17th tracheid in Tree No. 1 and No. 2, respectively.

Variation in tracheid dimensions in developing xylem

In addition to measurements of xylem maturation zones, radial cell diameters, tangential double wall thicknesses, and cell-wall layer areas were also determined
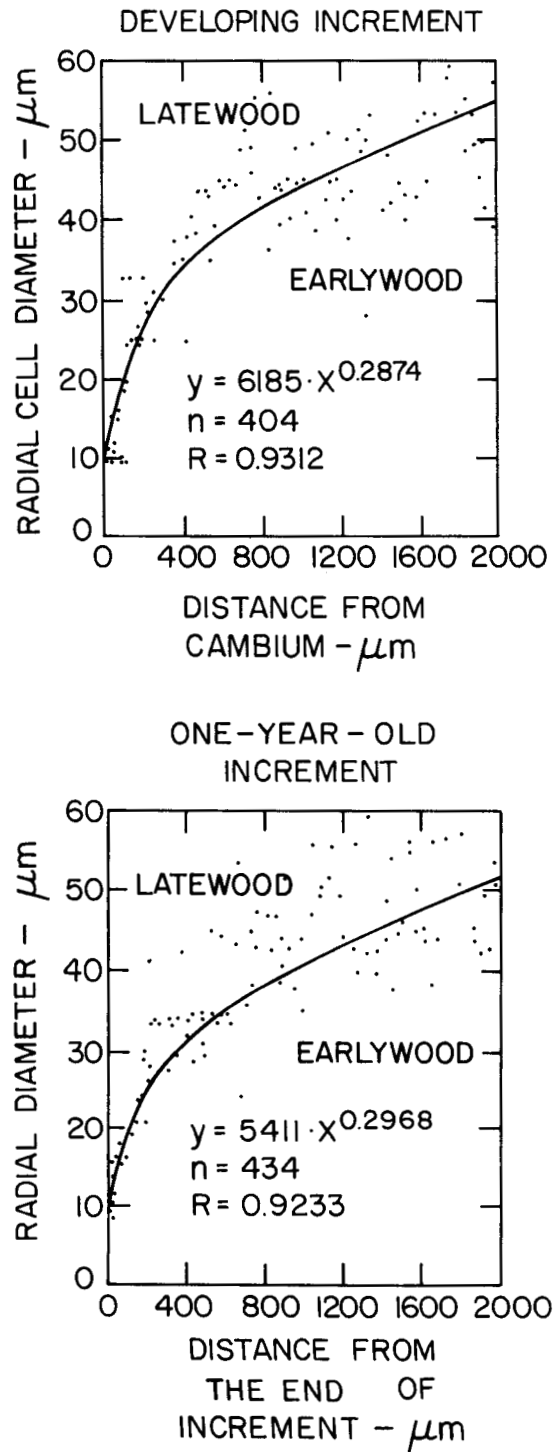
DEVELOPING INCREMENT

LATEWOOD

EARLYWOOD

$$y = 6185 \cdot X^{0.2874}$$
$$n = 404$$
$$R = 0.9312$$

RADIAL CELL DIAMETER – μm

DISTANCE FROM
CAMBIUM – μm

ONE-YEAR-OLD INCREMENT

LATEWOOD

EARLYWOOD

$$y = 5411 \cdot X^{0.2968}$$
$$n = 434$$
$$R = 0.9233$$

RADIAL DIAMETER – μm

DISTANCE FROM
THE END   OF
INCREMENT – μm

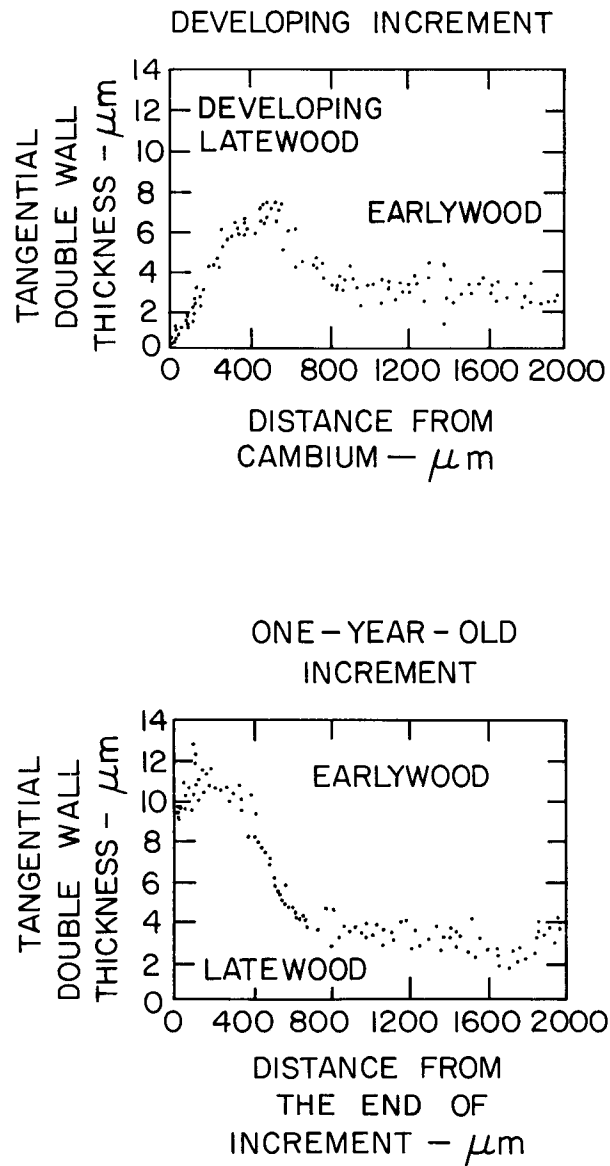FIG. 3. Intra-increment variation of radial diameter of tracheids in developing latewood and in the one-year-old increment of eastern hemlock, Tree No. 2.

DEVELOPING INCREMENT

ONE - YEAR - OLD
INCREMENT

Fig. 4.   Intra-increment variation of tangential double cell-wall thickness in developing latewood and in the one-year-old increment of eastern hemlock, Tree No. 2.

for the developing and one-year increments of Tree No. 2. Variations in radial cell diameters and tangential double wall thicknesses in both the developing and one-year-old increments are shown on Figs. 3 and 4, respectively. Radial cell diameters in both increments showed the same type of variation ($y = ax^b$) and they were almost identical in terms of coefficients of the equations fitted to the data.

Changes in tangential cell-wall thickness across the developing increment il-
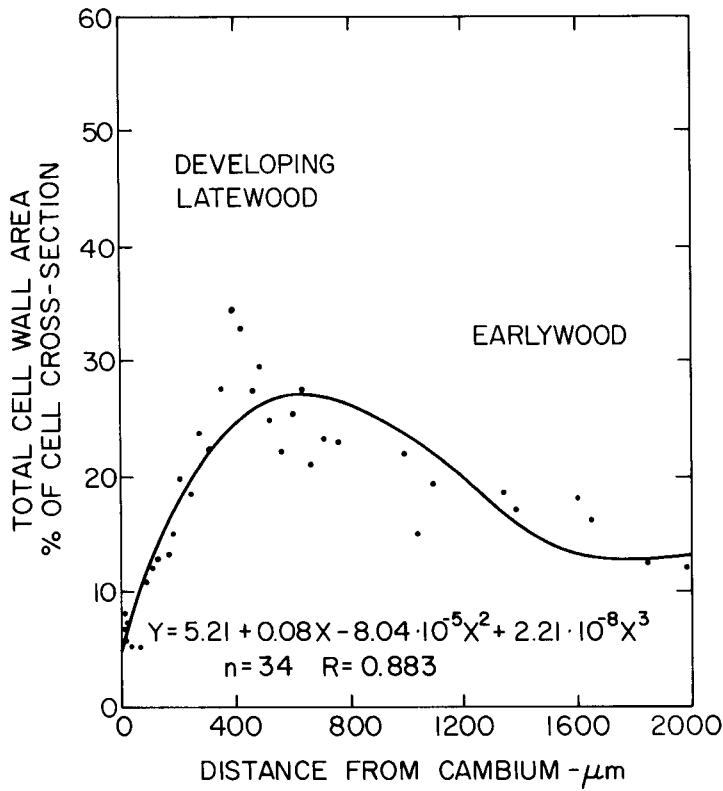
FIG. 5.   Changes in cell-wall cross-section during latewood formation in eastern hemlock, Tree No. 2.
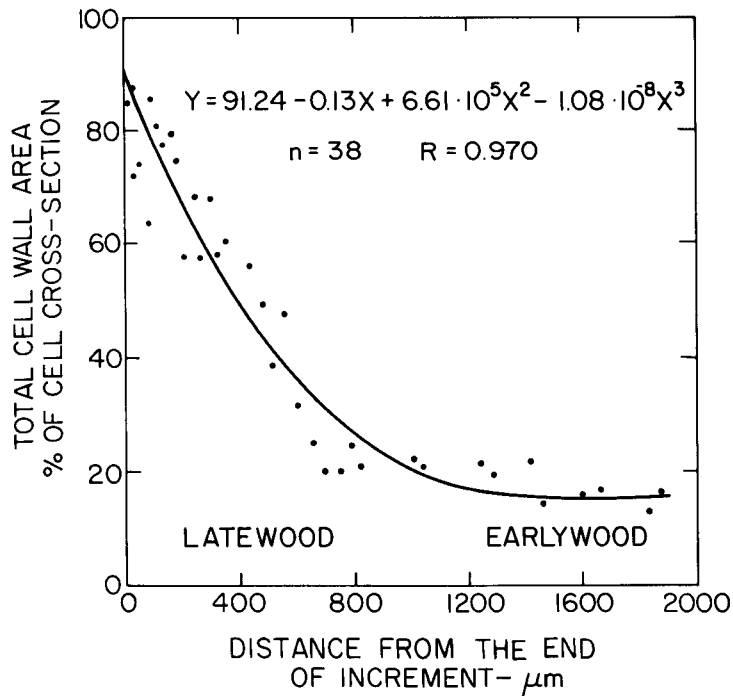

FIG. 6.   Changes in cell-wall cross-sectional area within the one-year-old xylem of eastern hemlock, Tree No. 2.
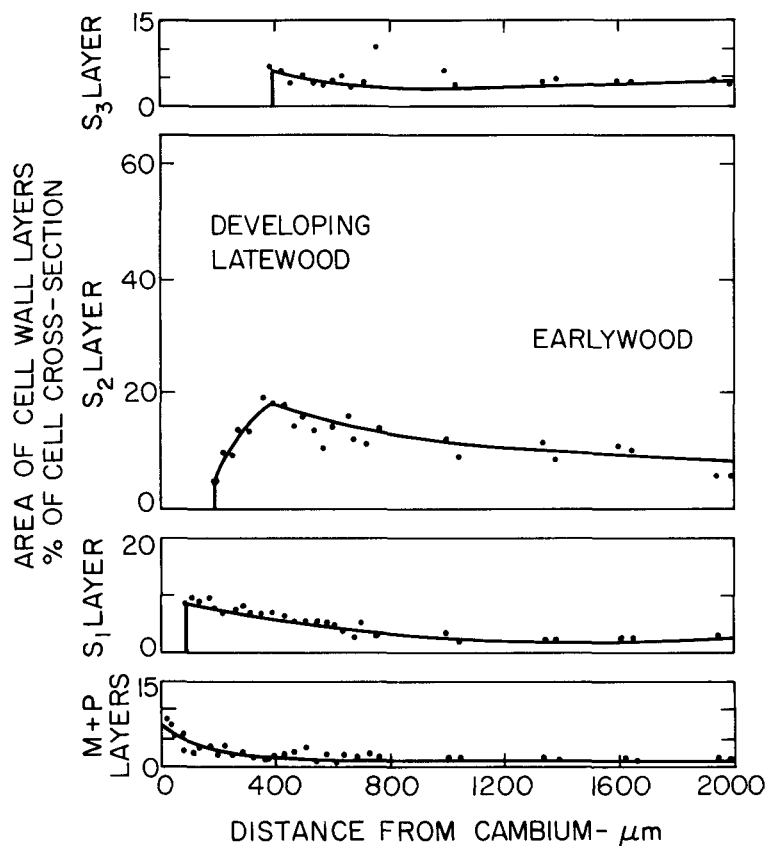
FIG. 7. The appearance and distribution of cell-wall layer cross-sectional area in developing late-wood of eastern hemlock, Tree No. 2. Visually fitted curves.

lustrate well the rate of cell-wall deposition from the cambium to the thick-walled summer wood tracheids. The one-year-old increment had a maximum tangential wall thickness just before the end of the ring (Fig. 4).

Measurements of radial diameters and cell-wall thicknesses and their variations across growth increments have been reported by only a few authors. Murray and Thomas (1961) showed diameter and tangential wall thickness variations within annual increments of four species: western hemlock, western red cedar, Douglas-fir, and southern pine. Fergus and coworkers (1969) found similar variations in black spruce, and they suggested that morphological details may be of considerable interest as they may represent general characteristics of coniferous woods.

Changes in the ratio of total cell-wall area to total cell cross-section area across the differentiating xylem and the one-year-old xylem are shown in Figs. 5 and 6. In the cambial zone, the cell wall constituted only 5 to 7% of the total cell area. As the cells matured into tracheids with distinct secondary walls, the cell-wall area increased to 30% of the total cell area (Fig. 5). In the one-year-old increment, this variation went from 17% in the earlywood to 90% in the latewood (Fig. 6).

Development of cell-wall layers in differentiating latewood tracheids

The area occupied by individual cell-wall layers was measured on micrographs (Fig. 1) using the method described by McIntosh (1965). The measurements were
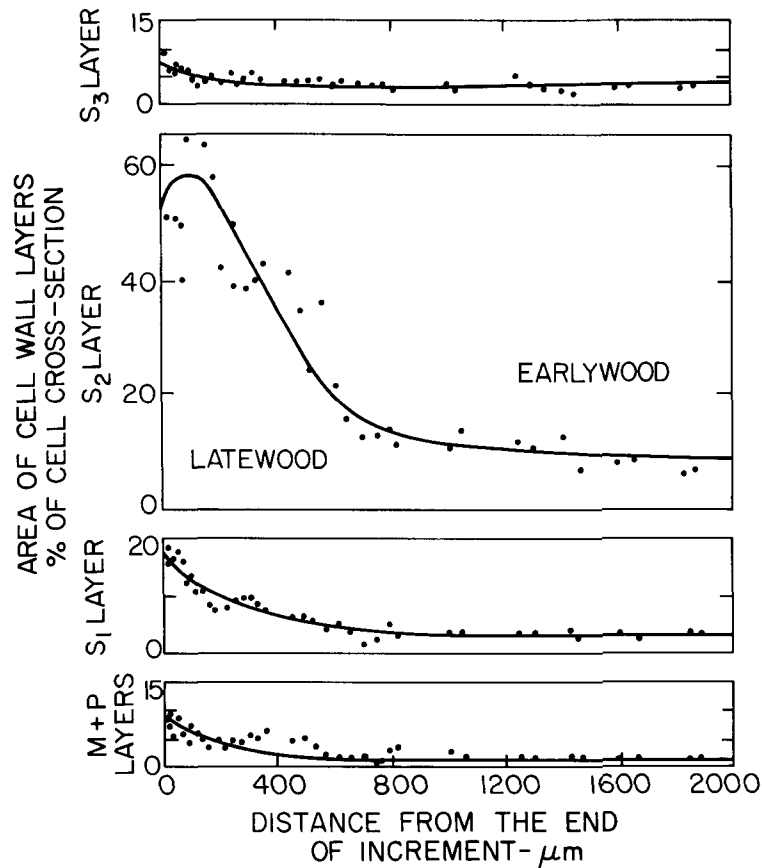
FIG. 8.   Intra-increment variation of cell-wall layer cross-sectional area in the one-year-old increment of eastern hemlock, Tree No. 2. Visually fitted curves.

made on representative radial rows of tracheids from the cambium through the one-year-old increment of Tree No. 2. The actual area of each cell-wall layer was calculated as a percentage of the total cell area and as a percentage of the total cell-wall area.

Percent cell-wall layer areas are shown in Figs. 7 and 8. In the differentiating xylem (Fig. 7), the successive appearance of the cell-wall layers is evident. In the one-year-old increment (Fig. 8), each cell-wall layer area increases to some degree towards the latewood. The (M + P) layer, the S_1 and the S_3 layers show smaller increases, probably a result of decreasing cell diameters at the end of the increment. When the thickness of these layers remains unchanged across the increment and the diameter of the cells substantially decreases (Fig. 3), the relative area occupied by such layers increases.

The S_2 layer shows a larger increase towards the latewood than do the other three layers. Its thickness increases substantially from earlywood to latewood, which is the reason for the large increase of the area it occupies within latewood cells.

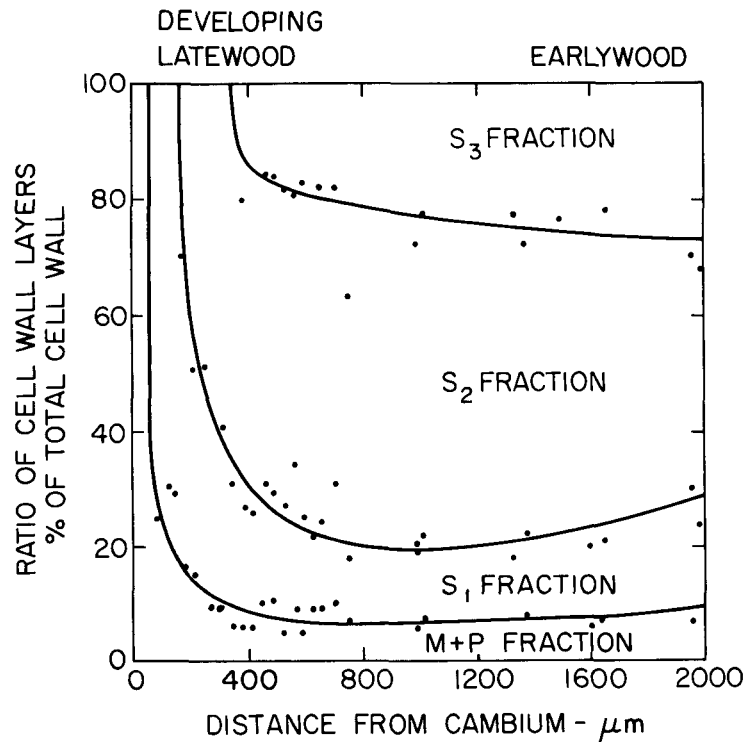The importance of the S_2 layer in total cell morphology is evident from both

FIG. 9.   Changes in the proportions of cell-wall layers in developing latewood of eastern hemlock, Tree No. 2. Visually fitted curves.

the cell-wall thickness and the area measurements. In Fig. 8 the curve representing relative S_2 layer area is quite similar to the curve of the total cell area in Fig. 6. The variation in total tangential cell-wall thickness across the annual increments (Fig. 4) is almost identical to the variation of S_2 layer area in Figs. 7 and 8.

There has been increasing scientific interest in cell wall to lumen area ratios and in related basic cell measurements. The reason for this lies partly in the increasing awareness among timber users that most of the technical properties of wood are determined not so much by bulk density but to a considerable extent by the basic anatomy of the wood at either the cellular or ultrastructural level (Dinwoodie 1969). In addition, detailed studies of wood require these basic cell measurements. For example Fergus et al. (1969), Fengel (1969), Meier and Wilkie (1959), and Lange and Kjaer (1957) all used cell-wall areas in their studies of the anatomy of various species of wood.

To fill the need for knowledge of the amount and distribution of cell-wall material in xylem growth zones, several methods have been developed. Indirect methods like β-ray absorption, microphotometric measurements, and x-ray/microdensitometry or direct methods of simple measurements of cross-sections (Elliott and Brook 1967; Smith 1965; Ladell 1959) are described in the literature. All of these give average cell dimensions or detailed descriptions of individual cells. There is a need for a method of obtaining reliable and practical measurements of
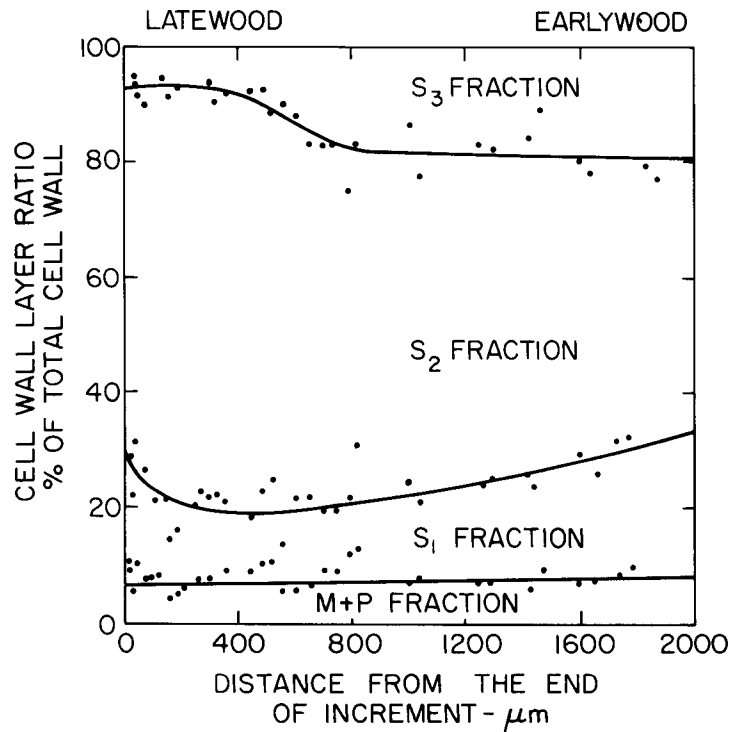
Fig. 10. Intra-increment variations of cell-wall layer composition in one-year-old increment of eastern hemlock, Tree No. 2. Visually fitted curves.

cell dimensions. Perhaps it is one of the basic tools missing for describing and understanding the physical, chemical, and mechanical behavior of wood at the cellular level.

In order to describe the properties of cell-wall layers from data measured on cells having more than one cell-wall layer, it is necessary to know the area of the individual layers and their relative proportions. Proportional composition of cell wall as to its layers is easily calculated as percentages of the total wall area. This fractional composition of cell walls is shown in Figs. 9 and 10, respectively, for the currently developing and one-year-old increments. In the zone of tracheid differentiation (Fig. 9), ratios of cell-wall layers change drastically with the formation of each new layer. However, as the cell-wall thickening is completed at approximately 400 μm from the cambium in Tree No. 2, cell-wall layer ratios follow the same trend as those across a mature increment (Fig. 10).

Changes in cell-wall layer ratios across the one-year-old increment are shown in Fig. 10. There appears to be little if any change in the (M + P) cell-wall fraction. The other three layers show considerable variations. The S_1 layer constitutes 15 to 20% of the total cell-wall area. The S_3 layer changes from 8% to 20%. Both of these layers have larger fractions in the earlywood than in the latewood zone. The S_2 layer shows the smallest relative variation of the three secondary layers. It is at maximum in latewood constituting 70% and minimum in earlywood constituting 50% of the total cell-wall area.
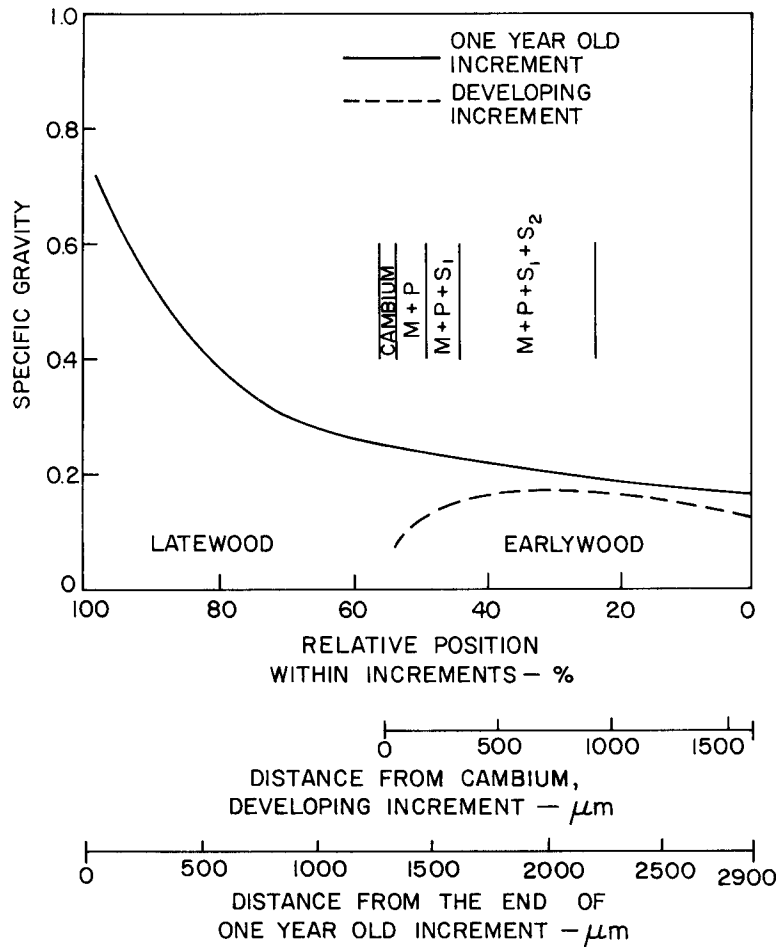
FIG. 11. Variation of specific gravity in developing earlywood and in one-year-old growth increment of eastern hemlock, Tree No. 1.

Variations of the S_2 fraction seem rather small, especially when compared to other dimensional changes of the S_2 layer. This might suggest that the density of wood and its variation across the annual increment may not be influenced solely by the thickness of the S_2 layer, but rather by the diameter of the cells in the test specimen. Data are needed to compare these cell-wall layer area distributions across the annual increments to those of other species.

Total mass production as indicated by variation in density

Variation in specific gravity across the zones of differentiating xylem was used to assess the total mass produced through each of the tracheid maturation zones in the two sample trees developing earlywood and latewood, respectively. The data were based on oven-dry weight and green volume. Volume of each specimen was measured except for the cambial scrapings for which it was calculated from estimated width and length of the scraped area and from the thickness of the
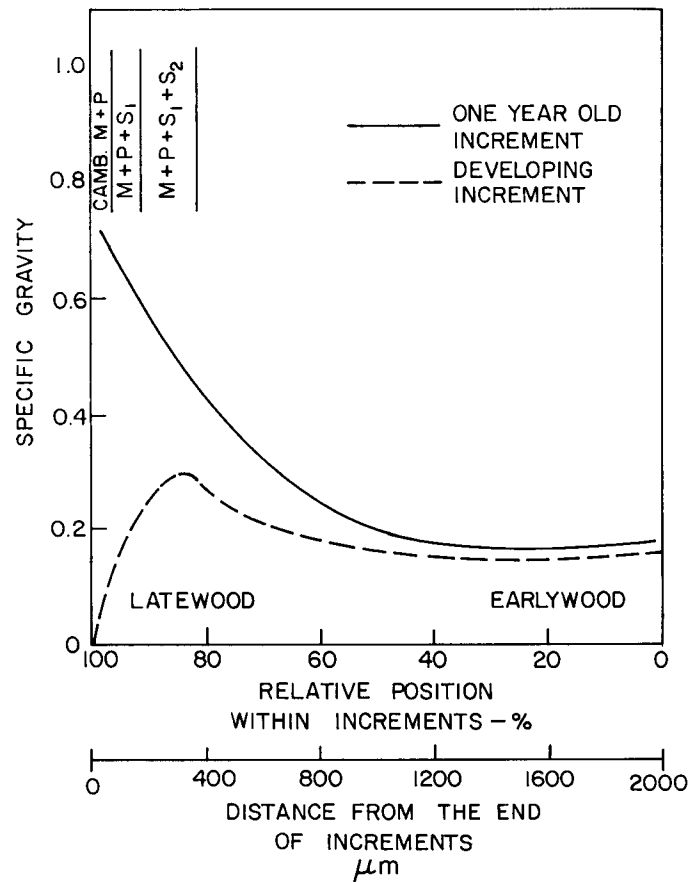
Fig. 12. Variation of specific gravity in developing latewood and in one-year-old growth increment of eastern hemlock, Tree No. 2.

cambial zone measured under the microscope. Results of these determinations are shown in Figs. 11 and 12 for the developing xylems and for the one-year-old increments of the two trees respectively.

The rate of cell-wall material deposition is rapid through the formation of S_1 and S_2 layers of the secondary wall. It reaches maximum approximately 700 μm in Tree No. 1 and 360 μm in Tree No. 2 (Table 1). These, however, are not the points where the tracheids have fully developed. The reason for decreasing specific gravity after reaching the maximum in the developing increment may be found from the trend of mass distribution in the one-year-old rings. Intra-increment variation of specific gravity in eastern hemlock is such that it is stable or gradually increasing in the earlywood zone; then it increases rapidly as the latewood formation begins. The trend and magnitude of specific gravity variations are the same in the earlywood bands of the increments, but in the latewood portion the developing xylem lags behind the one-year-old increment. After the maximum point, the rate of mass production in the developing xylem is slower than the rate of specific gravity increase in the one-year-old increment.

The superimposed specific gravity curves of the current and one-year-old in-

crements designate the end of cell-wall production at those points where the two density curves diverge. However, caution must be taken, since it is known that annual increments vary in width from year to year. The variation is mainly in the width of the earlywood zone (DeZeeuw 1965); therefore the inflection point could be due to a wider band of springwood formation in the current year, the phenomenon most likely to exist between two consecutive increments. The average radial span of the developing xylem in Tree No. 1 was 1,612 μm, while that for Tree No. 2 was 2,027 μm. In the case of Tree No. 2, the cambial zone of the current annual increment, as was shown previously in this study, was still producing new tracheids yet the current ring was already as wide as the one-year-old ring.

Aside from the differences in radial width, intra-increment specific gravities between neighboring rings may vary in magnitude throughout the entire width of the rings (Ifju et al. 1965). Also, another reason for caution in directly superimposing the developing and one-year-old annual increments for determining the end of tracheid maturation is that it is quite conceivable for some lignin to deposit after formation of the completed cellulose framework of the cell walls. This is more pronounced during latewood formation, since lignification of the thick S_2 layer can involve approximately 70% of the tracheid lignin content (Lange and Kjaer 1957).

CONCLUSIONS

From the results of this study on the changes in cell-wall morphology during tracheid development in coniferous xylem the following conclusions may be drawn:

1. Based on the optical properties of cellulose and the use of a polarizing microscope, four stages of tracheid cell-wall development can be measured. The number of cells and the width of cambium, (M + P) and (M + P + S_1) developmental zones are about the same during both early- and latewood formations, but the number of cells and consequently the width of (M + P + S_1 + S_2) or the zone of S_2 cell-wall layer development is much wider during earlywood formation in contrast to latewood formation.

2. During latewood formation, tangential double cell-wall thickness variation illustrates the rate of cell-wall depositions. Variation in specific gravity and in tangential double wall thickness were similar; this suggests this morphological detail may be of interest in respect to general characteristics of coniferous woods.

3. Cell-wall layer ratios across one-year-old growth increments show that while the S_2 layer is the bulk volume of the tracheids, it varied the least in relative volumes from earlywood to latewood. On the other hand, the relative volumes of S_1 and S_3 layers varied the most; their relative volumes more than doubled from latewood to earlywood zones. The (M + P) volumes were the most stable across the growth increments.

4. Total mass production during earlywood and latewood formation as indicated by variations in specific gravity shows that the newly or currently formed wood lags behind total weight of the one-year-old wood.

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