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)

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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

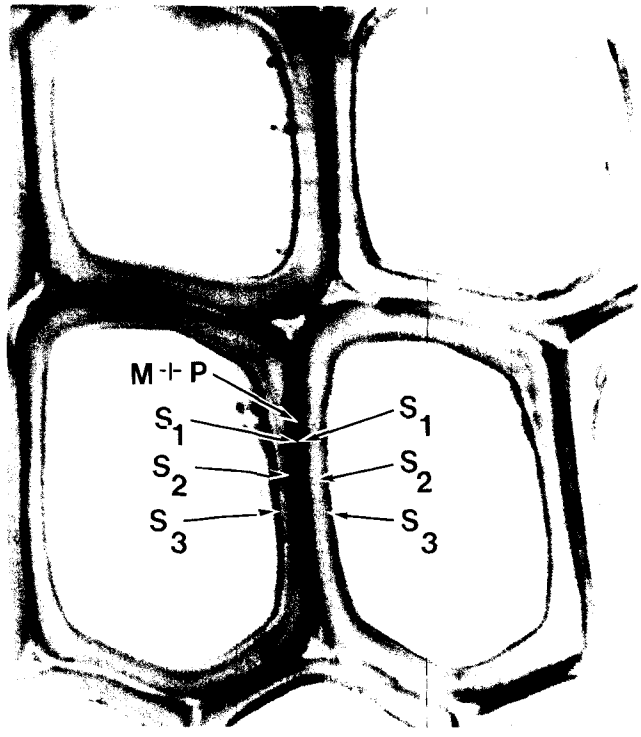

FIG. 1. A sample from the photomicrographs of tracheids used to measure cell dimensions and cell-wall layer sizes (×1,025).

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).