TRACHEID DIFFERENTIATION IN SOUTHERN PINES DURING THE DORMANT SEASON

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ABSTRACT

The differentiation of last-formed xylem tracheids of loblolly (*Pinus taeda*) and slash (*P. elliottii*) pines was followed during the overwinter dormant period in the upper Piedmont of South Carolina. Tissue samples taken from the outer portion of the stem of a poletimber-sized tree of each species in November and March were examined microscopically and tracheid transverse cell-wall thickness was measured. Cell double-wall thickness was compared between the two dormant season samples and with that of the previous year's cells of the same radial file. The comparison of cell-wall thickness indicated that the last-formed latewood cells of the annual ring continued to deposit cell-wall material through March and quite possibly into the following spring.

Keywords: Southern pine, wood formation, tracheid differentiation, dormant season.

INTRODUCTION

Past work has left some doubt and contradiction about certain aspects of cell growth and differentiation in coniferous trees (Murmanis and Sachs 1969; Murmanis 1971). In particular, research pertaining to cellular activity during the overwinter dormant period has been surprisingly scant. In this study, cambium and secondary xylem tissue of slash pine (*Pinus elliottii*) and loblolly pine (*Pinus taeda*) collected during dormancy were examined. The objectives of this study were to determine whether or not differentiation of secondary xylem (latewood tracheids) produced during an annual cycle is completed by the onset of dormancy and to investigate the possibility that differentiation of secondary xylem produced during an annual cycle is continued during the period of overwinter dormancy.

LITERATURE REVIEW

In temperate regions, cambial activity and corresponding cell formation in conifers is seasonal. Cells are formed by periclinal divisions of the cambium beginning in early March or April (Bassett 1966; Murmanis and Sachs 1969). This is followed by cell differentiation and maturation with the cambium becoming dormant in the fall. Murmanis (1971) observed that the cambium of white pine (*Pinus strobus* L.) structurally resembled dormant cells in October.

The vascular cambium of trees is generally considered by most workers to be a uniseriate layer of cells bordered by a zone of cells potentially capable of additional division (Sanio 1873; Bailey 1923; Newman 1956; Bannan 1968; Murmanis 1971). At the height of cambial activity, there are three general zones of cells found inward of the cambial layer: (1) the zone of radial enlargement, which includes xylem mother cells and daughter cells capable of further division, (2) the cytoplasmic zone, where secondary cell-wall formation occurs, and (3) the mature

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tracheid zone, where cell differentiation is completed and death of the cell occurs (Whitmore and Zahner 1966; Brown 1970).

There is little to be found in the literature related to cellular activity during overwinter dormancy. In past work, Murmanis (1971) found from six to ten undifferentiated cells adjacent to the cambium in each radial row of secondary xylem in white pine material collected during dormancy. This condition was also observed in slash and loblolly pine material collected near Clemson, South Carolina in November 1975 and 1976.

MATERIALS AND METHODS

The sample material used in this study was removed at breast height (4.5 feet) from apparently healthy slash pine (9.6 inches DBH) and loblolly pine (8.2 inches DBH) trees in an 18-year-old slash-loblolly pine plantation located on the Clemson Forest, Clemson, South Carolina. Both trees were vigorous members of the dominant crown class. Collections used in this study were made in November 1976 and March 1977.

Samples were removed from each tree using a bow saw and chisel. The March 1977 samples were taken directly above the November 1976 samples in order to minimize the effects of circumferential variation normally found in trees. Excess wood and bark were removed and the samples were cut into pieces suitable for fixing and microtome sectioning.

The material was killed and fixed in formalin-acetic acid-alcohol (10:10:80 v/v). Transverse thin sections 60 microns in thickness were made using a sliding microtome. The sections were stained using a safranin-aniline blue staining procedure and mounted on slides under cover glass with Permount for observation with a light microscope.

Three slides from each sample collection were chosen for observation and measurement. Three typical radial files of latewood cells exhibiting maximum cell diameter on each slide were used for making measurements of outside cell diameter and lumen diameter. Subsequent subtraction of lumen diameter from outside cell diameter gave cell double-wall thickness. The first five latewood cells adjacent to the vascular cambium in each radial file were measured as well as the last five cells of the latewood band produced in the previous year (1975). All measurements were made at 450 times normal magnification using a Filar micrometer eyepiece. Accuracy was estimated to be approximately ± 0.1 μm.

The method of statistical analysis was the "t" test for paired means at the 0.01 and 0.05 levels of significance. This method was favored because the paired "t" test has been found to be more sensitive than the standard "t" test in cases where the means can be grouped in pairs such that the variation between pairs is appreciably larger than the variation within pairs (Freeze 1967).

It should be mentioned here why cell double-wall thickness was chosen as the parameter for the degree of cell differentiation. Outside cell diameter was considered, but after a review of the literature, it was judged to have an excessive amount of variation due to various climatic factors which affect radial enlargement (Larson 1969; Balatinecz and Kennedy 1968). Secondary wall formation appears to occur at a more constant rate over time despite microclimate variations, therefore, cell double-wall thickness was selected.

RESULTS AND DISCUSSION

In the following discussion, cell 1 refers to the final cell produced by the cambium in that particular annual cycle. Subsequent cell numbers refer to cells located succeedingly inward from the cambium toward the center of the tree.

The hypothesis that the last-formed cells of 1976 were as yet not fully differentiated was tested by comparing the mean cell double-wall thickness of November 1976 latewood (Figs. 1 and 2) with that of latewood produced in the previous year, 1975 (Figs. 3 and 4). Mean cell double-wall thickness measurements are shown in Table 1. These results show that a highly significant difference exists in November 1976 between the mean double-wall thickness of the 1976 latewood and the corresponding 1975 latewood for cells 1 through 5 in both species.

To test the hypothesis that cell-wall differentiation continues during the overwinter dormant period, the cell double-wall thickness of 1976 latewood collected in November 1976 was compared to that of 1976 latewood collected in March 1977 (Figs. 5 and 6; Table 1). The results show that a significant difference in double-wall thickness exists in the November and March samples for cells 1–5 in both species.

A final comparison was made to test the hypothesis that further cell-wall differentiation might continue after March. The cell double-wall thickness of the 1976 latewood collected in March 1977 was compared with that produced in 1975 for both species (Table 1). These results show that for loblolly pine a significant difference in double-wall thickness occurred for all cells compared. For slash pine, a significant difference was found for the comparison of cells 1 through 4 and no significant difference was found for the comparison of cell 5. No comparisons were made between the two samples of 1975 latewood tracheids.

SUMMARY AND CONCLUSIONS

The results of the first set of comparisons of cell double-wall thickness indicate that a significant amount of wall formation had yet to occur for both species in

TABLE 1. *Mean double-wall thickness for last five latewood tracheids formed at breast height in 1975 and 1976 in a loblolly pine and a slash pine near Clemson, SC.*[1]

Cell no.	November 1976		March 1977	
	1976	1975	1976	1975
	.....	μm	.....	
Loblolly pine				
1	2.8a	6.3b	5.4c	8.2b
2	3.4a	9.7b	7.1c	9.2b
3	4.8a	10.9b	8.0c	10.4b
4	5.9a	13.4b	8.8c	11.8b
5	7.2a	14.2b	9.3c	11.8b
Slash pine				
1	3.7a	9.0b	5.4c	7.4b
2	3.6a	10.0b	6.9c	9.3b
3	4.9a	8.7b	7.8c	10.1b
4	4.5a	9.9b	7.7c	9.6b
5	5.3a	10.2b	8.4b	9.8b

[1] Numbers in the same row followed by the same lower case letter are not significantly different at the 0.05 level of probability according to the Student's *t*-test for significant differences. Each value represents the measurement of nine cells. No comparisons were made between the two 1975 samples.

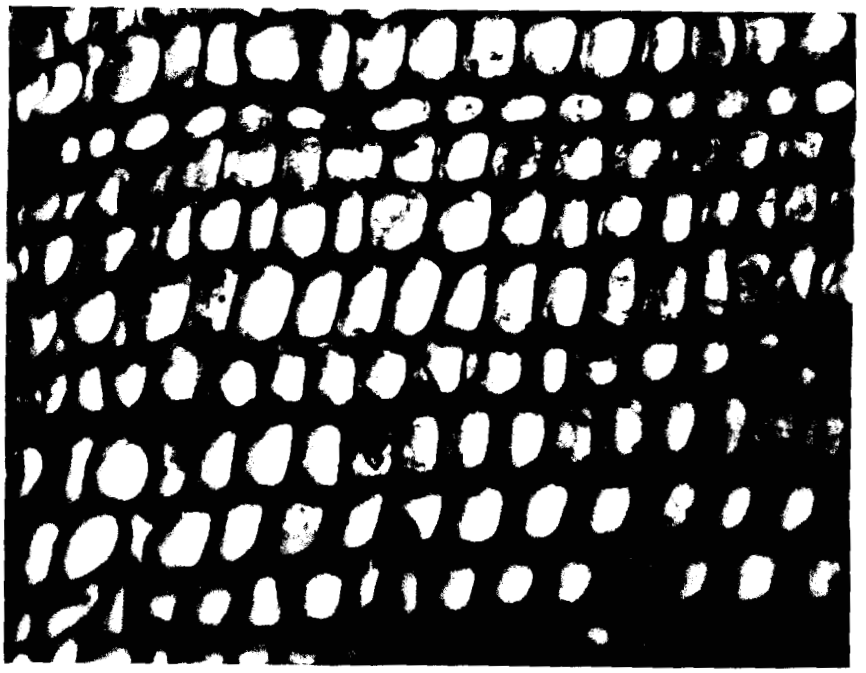

FIG. 1. Undifferentiated 1976 latewood cells collected in November 1976 from a slash pine near Clemson, SC. Cambium is at left of picture. Transverse section at 200× magnification.

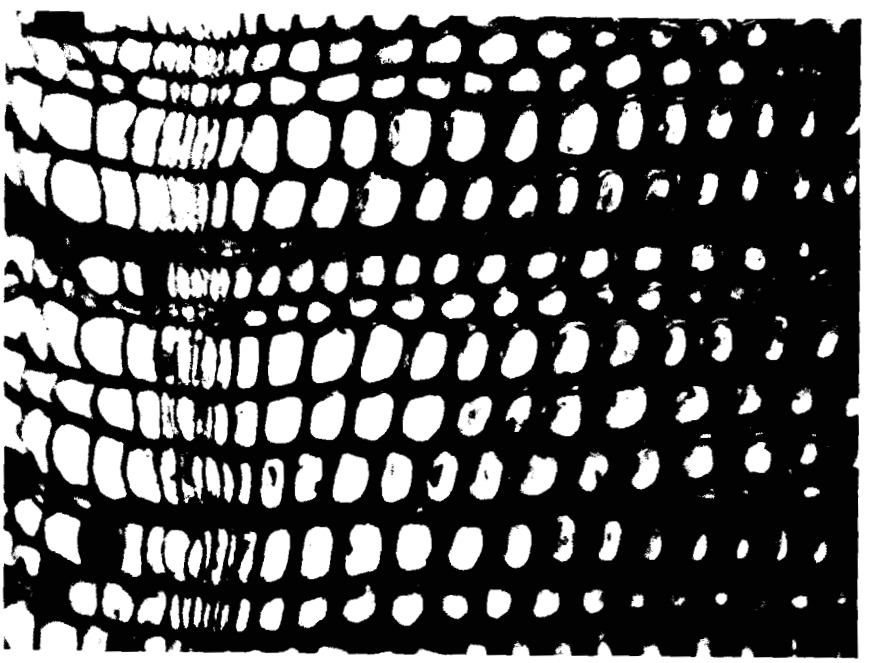

FIG. 2. Undifferentiated 1976 latewood cells collected in November 1976 from a loblolly pine near Clemson, SC. Cambium is at left of picture. Transverse section at 200× magnification.

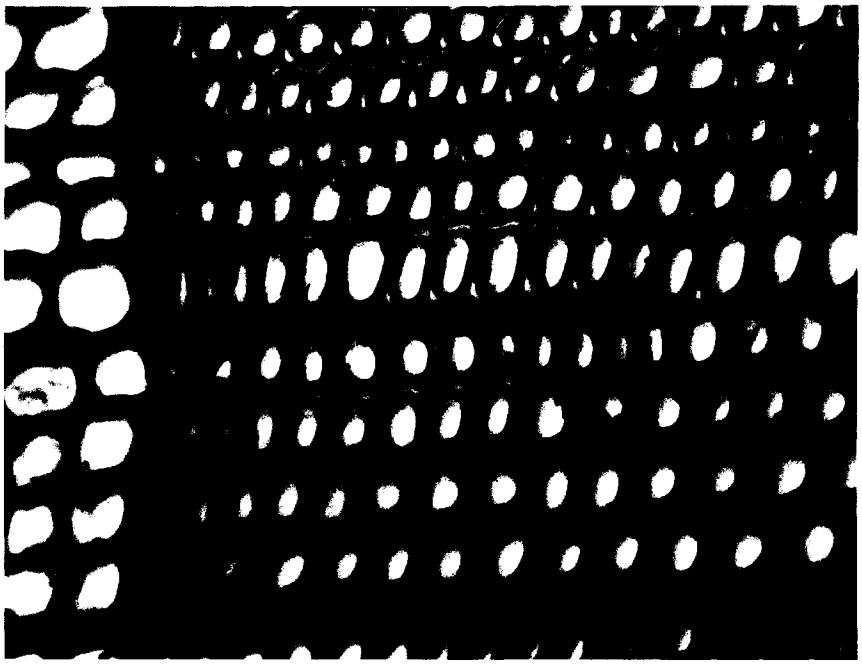

FIG. 3. Fully differentiated 1975 latewood cells collected in November 1976 from a slash pine near Clemson, SC. 1976 earlywood is at left of picture. Transverse section at 200× magnification.

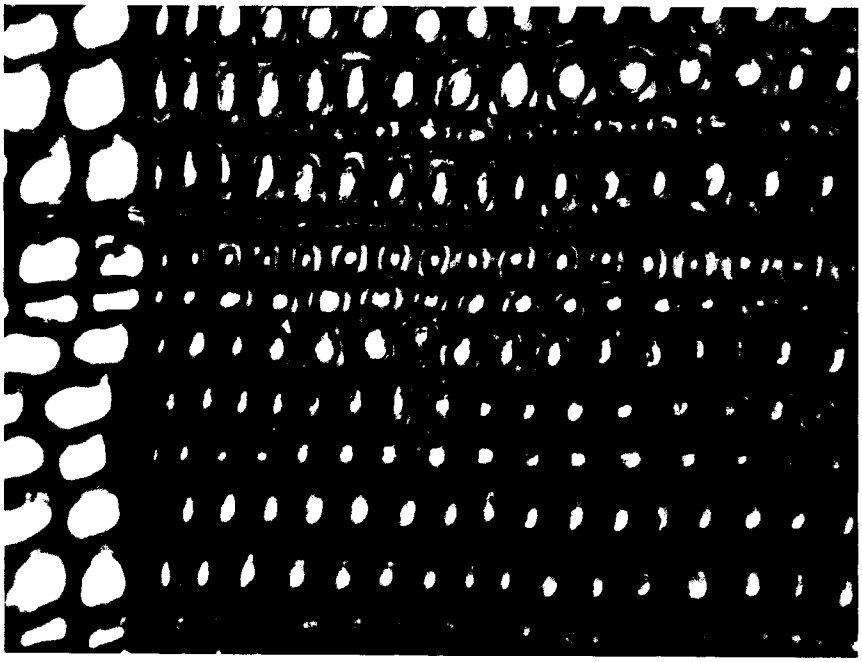

FIG. 4. Fully differentiated 1975 latewood cells collected in November 1976 from a loblolly pine near Clemson, SC. 1976 earlywood is at left of picture. Transverse section at 200× magnification.

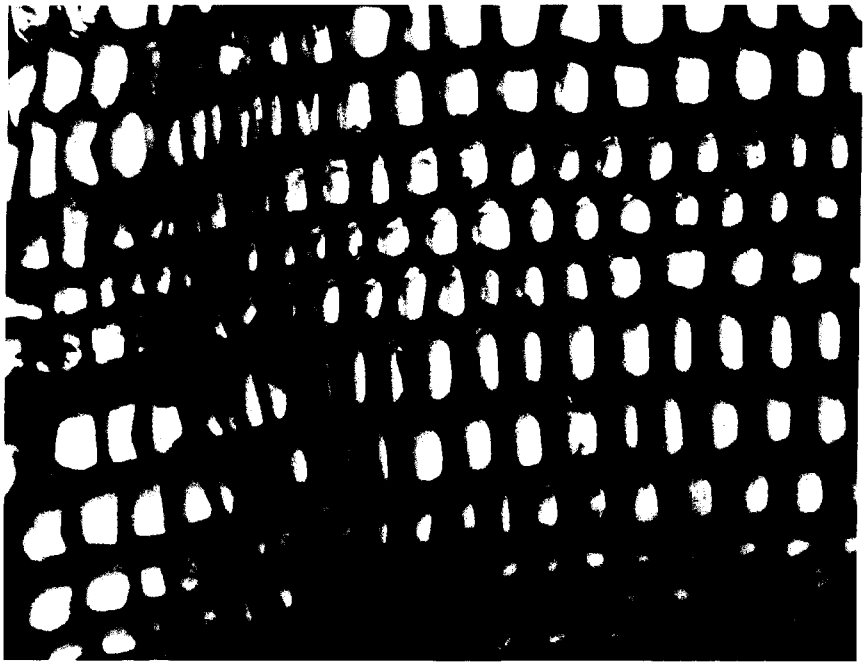

FIG. 5. Undifferentiated 1976 latewood collected in March 1977 from a slash pine near Clemson, SC. Cambium is at left of picture. Transverse section at 200× magnification.

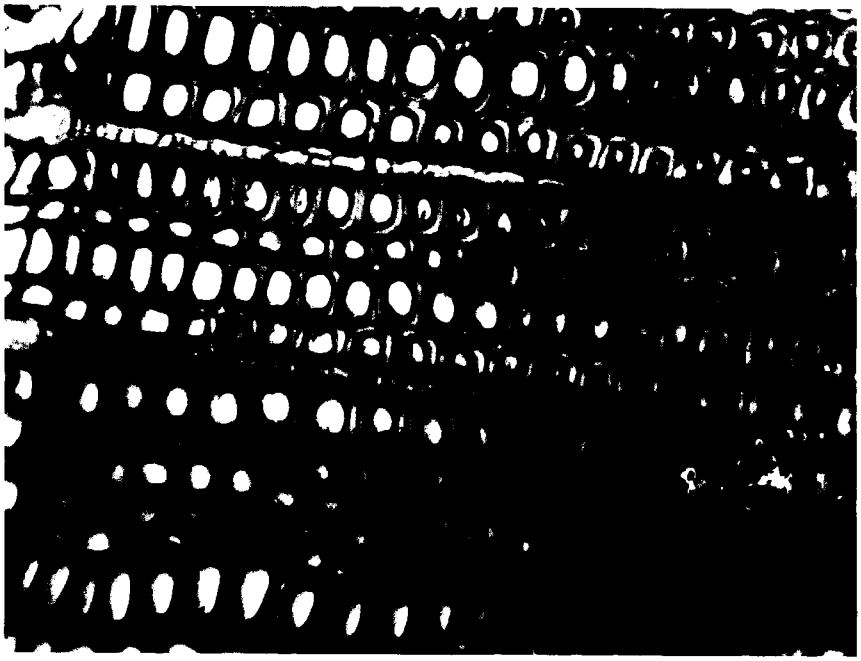

FIG. 6. Undifferentiated 1976 latewood collected in March 1977 from a loblolly pine near Clemson, SC. Cambium is at left of picture. Transverse section at 200× magnification.

the last five 1976 latewood cells collected in November 1976. Thus, one can conclude that differentiation, i.e., secondary wall formation, of secondary xylem produced in the late stages of an annual cycle is not completed by the onset of overwinter dormancy in slash and loblolly pine.

The results of the second set of comparisons show that a significant amount of secondary cell-wall formation had occurred in the 1976 latewood cells from the time of the first collection in November 1976 to the time of the second collection in March 1977 in both slash and loblolly pine. Therefore, one can conclude that differentiation (secondary wall formation) of latewood tracheids produced during the late stages of an annual cycle continues during the overwinter dormant period in these species of pine, at least through March of the following year.

The final set of comparisons shows that with one exception, a significant amount of secondary cell-wall formation (as compared to the previous year's latewood cells) had yet to occur in the five last-formed 1976 latewood cells collected in March 1977 for both species. Thus, one can conclude that secondary wall formation of latewood tracheids of loblolly and slash pines during the late stages of an annual cycle continues into the following spring.

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