VARIATION OF MICROFIBRIL ANGLE WITHIN INDIVIDUAL TRACHEIDS

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ABSTRACT

Utilizing the orientation of soft-rot cavities, microfibril angles were measured in individual tracheids (pulped fibers) and thin sections of southern pine in order to determine the extent of variation. Within individual tracheids of southern pine, microfibril angles were consistent along the length of a tracheid and when measured between bordered pits. Microfibril angles were highly variable on the radial walls containing bordered pits. Microfibril angles approached 90° around bordered pits, but the angles on the walls opposite the bordered pits were consistent with the average angle along the length of the tracheid. Variation (standard deviation) was less in latewood tracheids than in earlywood tracheids. Within individual tracheids, there was no correlation between microfibril angle and tracheid width. Across an annual ring of southern pine, microfibril angle gradually decreased through the earlywood and became much smaller in the latewood.

Keywords: Cellulose, tracheids, microfibril angle, soft-rot cavities.

INTRODUCTION

In the wood cell wall, the cellulose microfibrils in the S2 layer are the most significant contributor to the mechanical properties of wood. The orientation of the microfibrils is critical, with the smallest angle to the cell longitudinal axis often providing better mechanical properties. In some models of the mechanical properties of the wood cell wall, as discussed recently (Navi 1998), the relationship between axial stiffness and microfibril angle assumed that the microfibril angle was constant within a tracheid. That microfibrils follow a helical path has been well established. However, it is unclear how constant the angle of the helix is within the S2, especially near the tips of the tracheids and in relation to cell-wall thickness and width.

For samples of softwoods with defined earlywood and latewood zones, the average microfibril angle is an average of many cells, ei-

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TABLE 1. *The average microfibril angle of earlywood and latewood tracheids of southern pine. Microfibril angles were measured beginning at one end of a tracheid, continuing along the length of each tracheid. Correlation coefficients of microfibril angle with the width of the tracheid at each point of measurement indicate no correlation of microfibril angle and location along the length of the tracheid or tracheid width.*

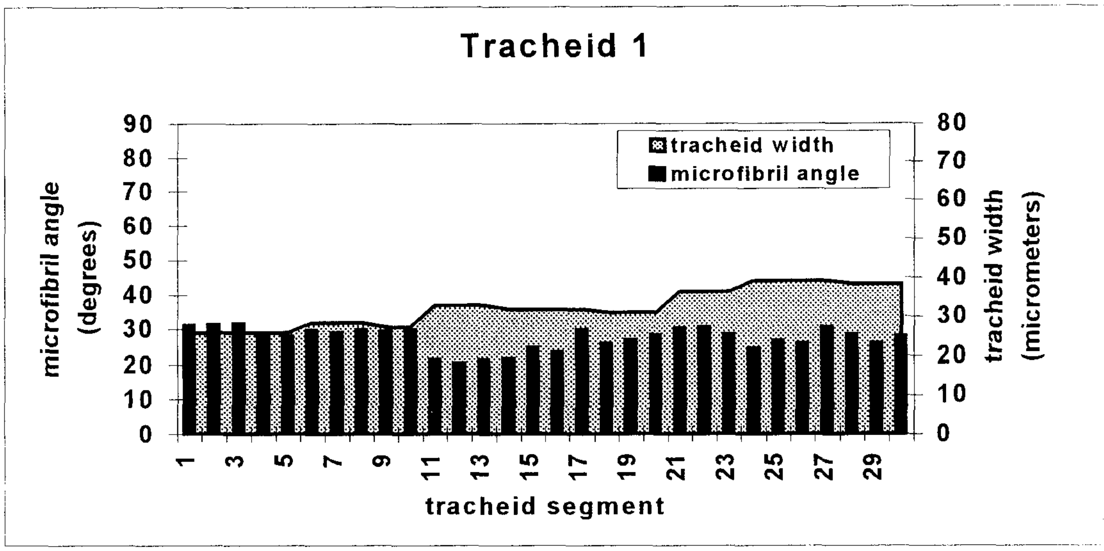
Tracheid #		Microfibril angle					Correlation tracheid width and microfibril angle
		n	Average	Minimum	Maximum	Std. dev.	
			Earlywood				
1	tangential	30	27	20	32	3.2	−0.28
2	tangential	48	26	22	32	2.3	0.22
3	radial	292	36	28	83	5.6	0.02
4	radial	42	31	26	49	5.6	−0.02
5	radial	62	48	27	89	14.4	0.06
			Earlywood excluding pits				
3	radial	282	35	28	43	2.4	−0.14
4	radial	37	29	26	36	2.6	−0.05
5	radial	25	35	27	41	4.1	0.05
			Latewood				
6		20	9	7	12	1.9	−0.14
7		28	5	2	9	1.7	−0.05
8		17	4	1	8	2.2	0.05
9		8	3	1	4	1.2	−0.28
10		7	4	1	12	3.9	−0.87

ther all earlywood, all latewood, or a mixture of both. In one growth ring, as microfibril angle changes from the first earlywood tracheid to the last latewood tracheid, it follows that the mechanical properties also change. By using a radial section, and measuring microfibril angle in individual tracheids, the variation across a ring can be measured, as opposed to using a tracheid maceration, where variability can be determined, but cannot be related to position in the annual ring.

Although recent studies have examined variability within tracheids and within growth rings (Donaldson 1998; Huang et al. 1998; Saranpää et al. 1998), one recent study used the soft-rot cavity method (Anagnost et al. 2000). Measurements of microfibril angle with the soft-rot cavity method showed a strong correlation to the X-ray diffraction and iodine methods (Anagnost et al. 2000). The growth of soft-rot cavities follows the microfibrillar orientation in a helical path, a Z-helix in the S2, around the axis of a wood tracheid. The helical path of microfibrils that occurred along the length of tracheids appeared to continue around the tips of tracheids as evidenced by the orientation of soft-rot cavities (Anagnost et al. 1999). The degree of variation within the S2 layer of individual cells was still unclear.

Studying the variation between tracheids, Donaldson (1998) found that microfibril angles were similar in adjacent cell walls of neighboring tracheids, with an average difference between neighboring cells of 11°, utilizing the pit aperture and polarized light methods. In that study, microfibril angle decreased from the pith outward, with a maximum angle in juvenile wood of 72°, and a minimum angle of 0° in ring 15.

Donaldson (1998) also determined that the values across a growth ring had a range of about 36°. In another study (Saranpää et al. 1998), a similar span of values (30°) was observed using measurements of macerated fibers (tracheids) of earlywood. Khalili (1999) observed that microfibril angle was less variable in latewood tracheids than in earlywood tracheids using the soft-rot cavity approach. According to Panshin and deZeeuw (1980),



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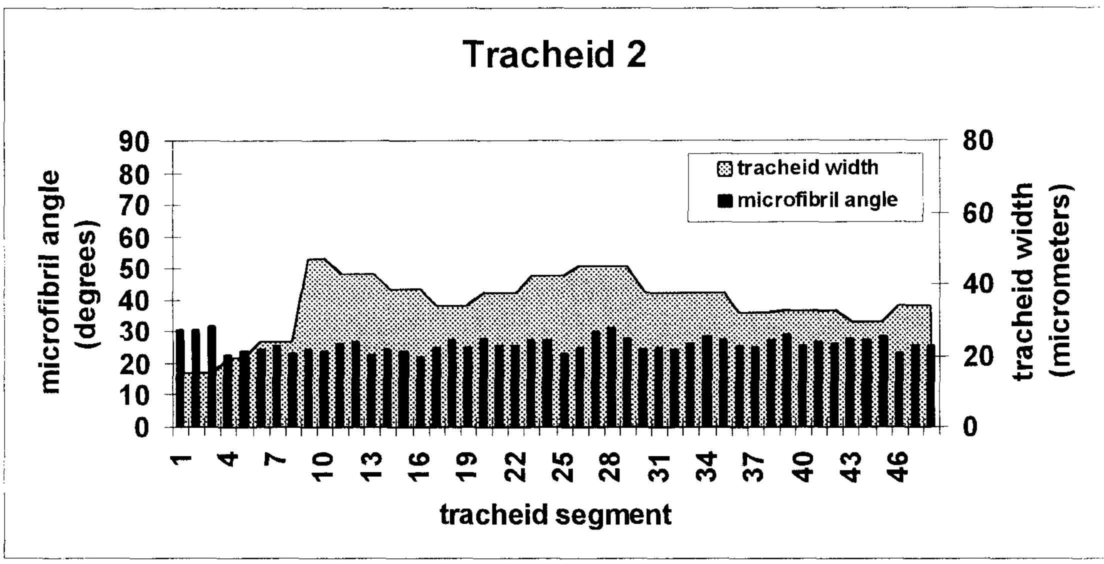

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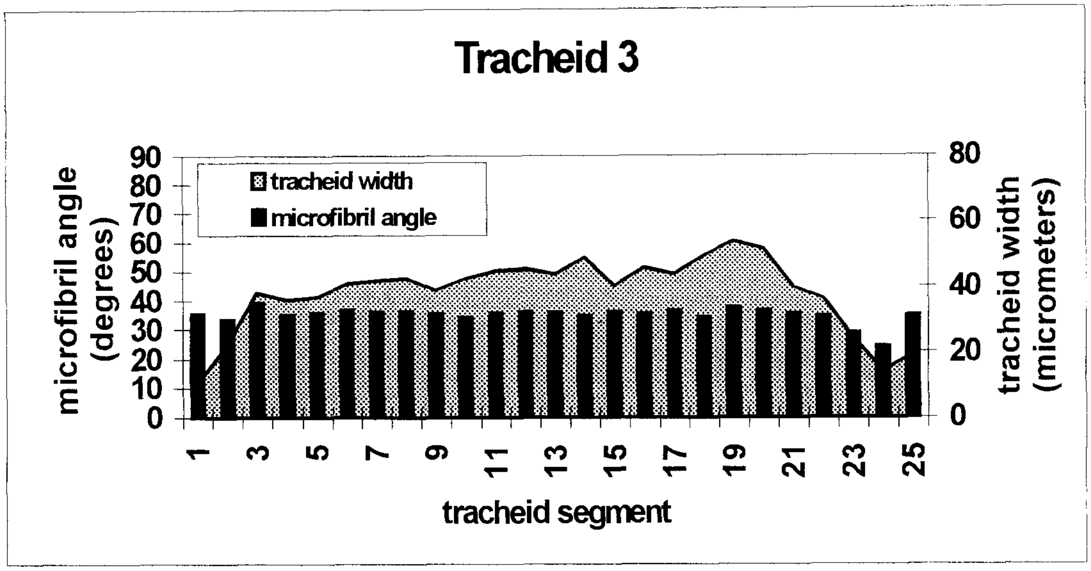
FIG. 1. Microfibril angle and tracheid width measured on the tangential walls of 2 earlywood tracheids of southern pine. A. In tracheid 1, segment 1 is very close to one end of the tracheid. Segment 30 is near the midpoint of the tracheid. B. In tracheid 2, segment 1 is near one end of the tracheid. Segment 48 is near the midpoint of the tracheid. In both A) and B), microfibril angles show little variation. The variation in microfibril angle does not appear to follow changes in tracheid width.



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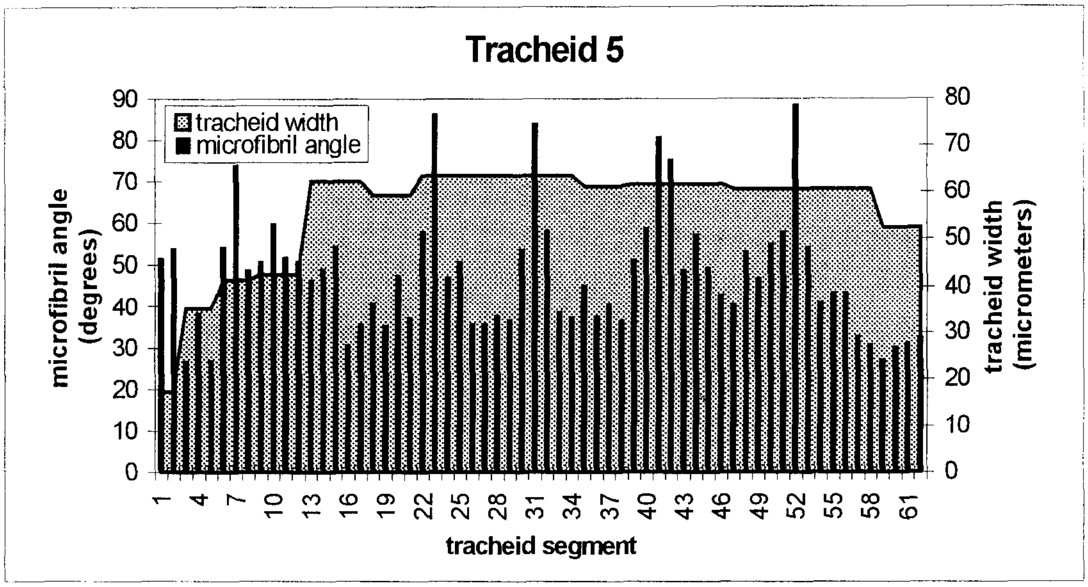

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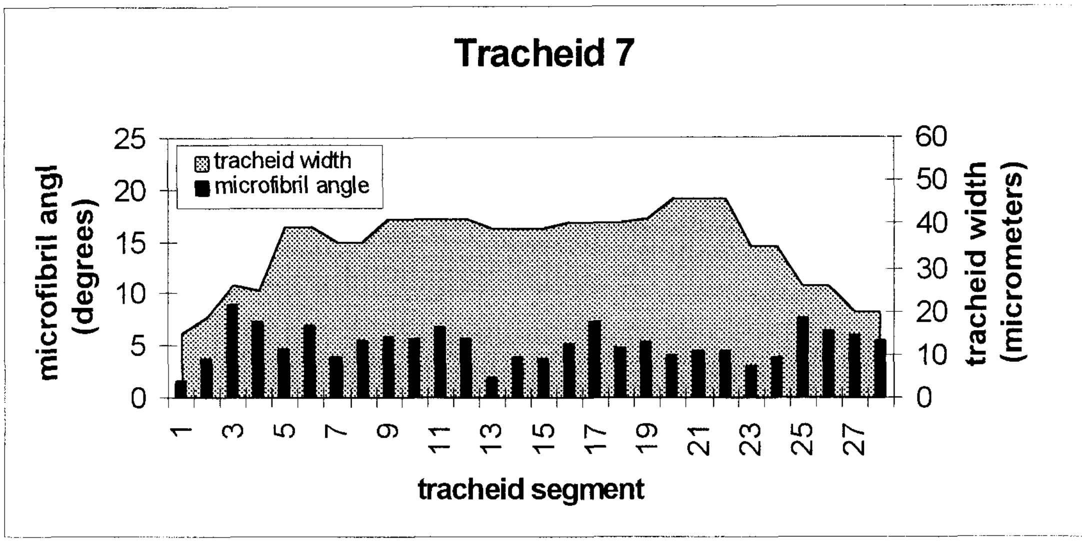
FIG. 2. Microfibril angle and tracheid width measured on the radial walls of 2 earlywood tracheids. Each bar represents the average microfibril angle for each tracheid segment. A. In tracheid 3, the average microfibril angle in each segment indicates little variability from one end (segment 1) to the other (segment 25), except in segment 3 that contains a bordered pit. In segment 3, the average angle was 55° and the maximum angle was 83° near a bordered pit. B. In tracheid 5, segment 1 is near one end of the tracheid, while segment 62 is near the midpoint of the tracheid. Tracheid 5 contains many intertracheid pits, as evidenced by the peaks containing large microfibril angles.



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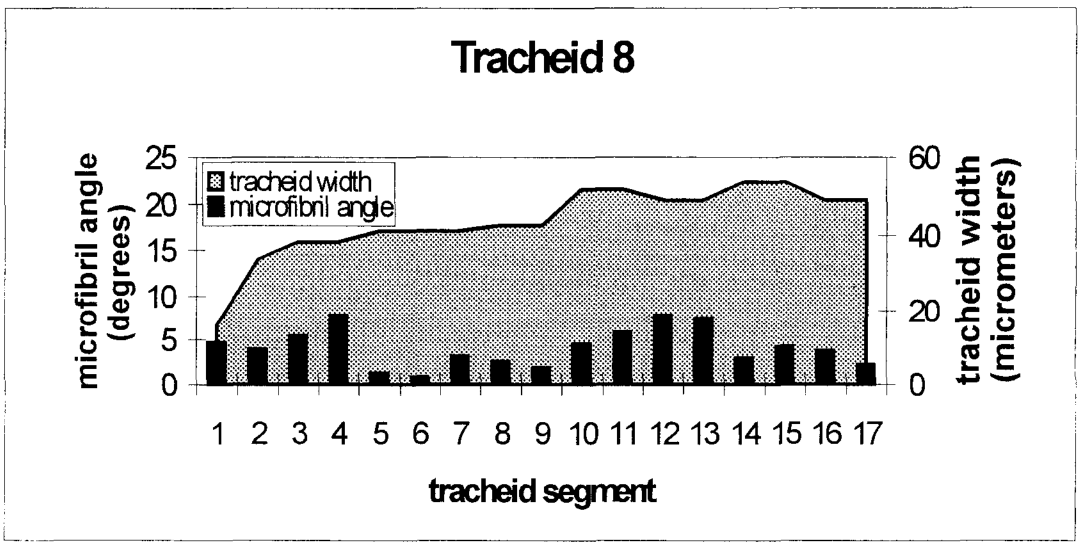

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FIG. 3. Microfibril angles and tracheid width in 2 latewood tracheids. In these tracheids the variation in microfibril angle does not follow changes in tracheid width. The smallest S2 angle was 1°, the largest 9°. For both tracheids, segment 1 is close to one end of the tracheid. For tracheid 7, segment 28 is near the opposite end of the tracheid. For tracheid 8, the segment with the highest number is near the midpoint of the tracheid.

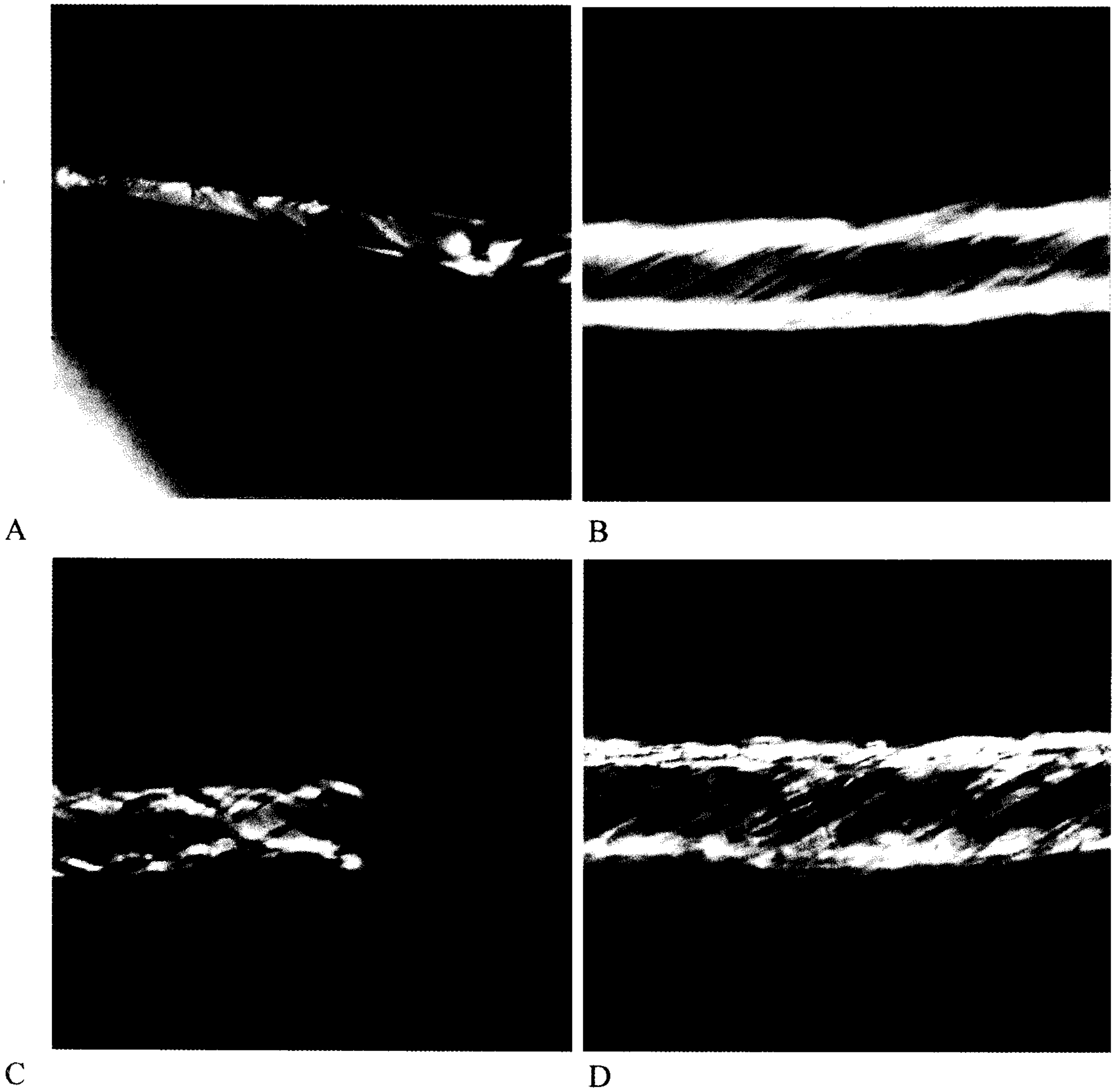


FIG. 4. Soft-rot cavities at the tips (A and C) and near the midpoints (B and D) of tracheid 1 (A and B) and tracheid 2 (C and D). In these 2 earlywood tracheids, the microfibril angles near the tips and near the midpoints are very similar. A and B) In tracheid 1, the angle near the tip is 28° and the angle at the midpoint is 26°. C and D) In tracheid 2, the angle near the tip is 31° and the angle at the midpoint is 27°.

the general pattern of microfibrillar orientation within a growth ring is a gradual decrease across the earlywood portion that continues through the latewood. Within a stem, microfibril angle generally decreases from pith to bark, varying inversely with tracheid length.

This study had two objectives. The first objective was to examine microfibril angle along the length of individual macerated fibers to determine the extent of variability along the length of each tracheid, particularly if the angle changes near the tips of tracheids. The variability was determined in both earlywood and latewood tracheids of southern pine. The relation of tracheid width to microfibril angle

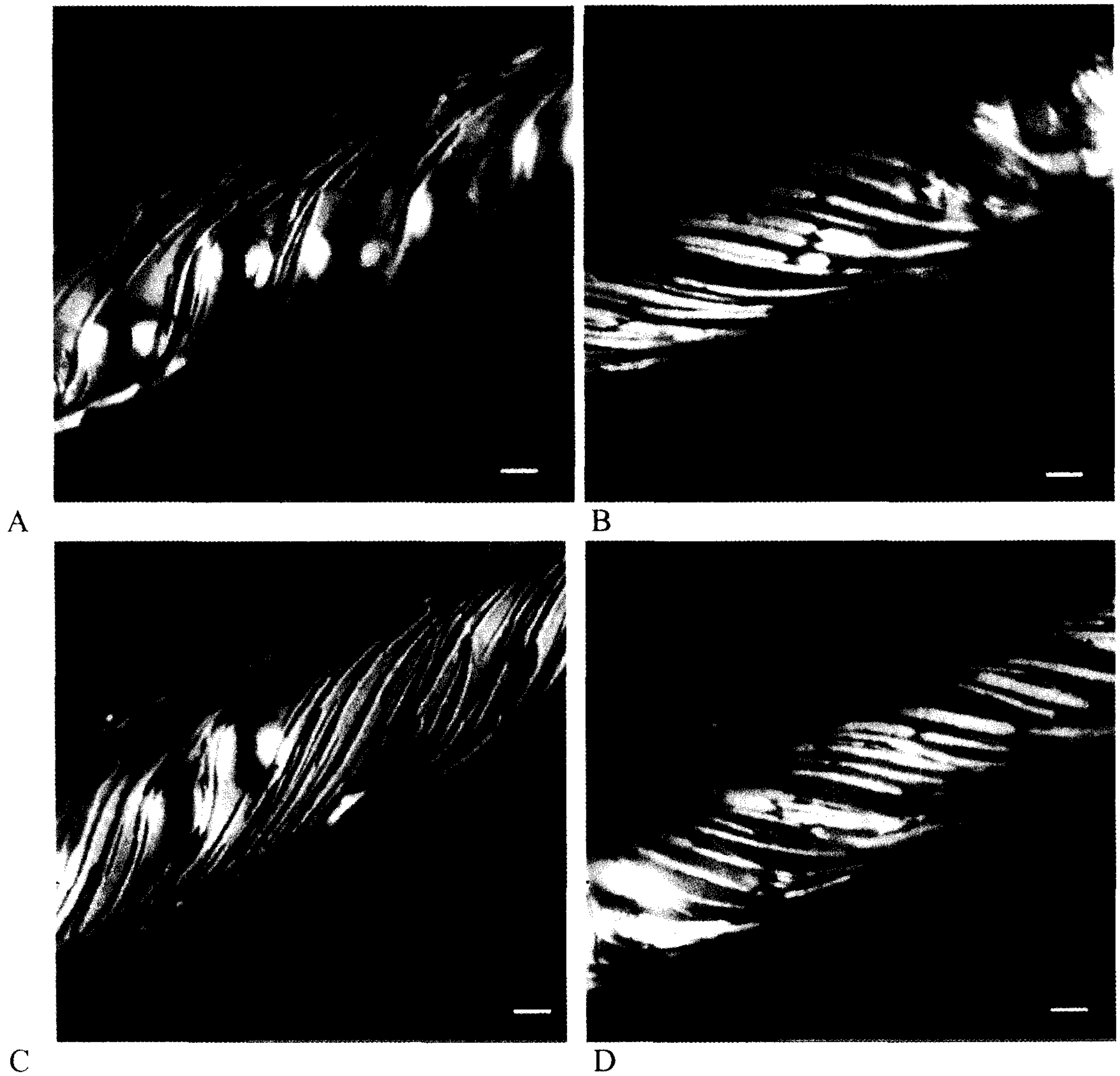


FIG. 5. Two locations on tracheid 5 showing soft-rot cavities on opposite walls. Cavities indicate large microfibril angles around intertracheid pits in the bottom wall (A) that are opposite the top wall (B). The microfibril angle of (B) is the same (36°) as that in (A) in areas away from the pits. The same is true for (C and D) with matching angles of 37°. Bar = 10 μm.

was determined and used as a measure of variability along the length, as tracheids are generally wider in the middle and narrower toward the ends. Variation of microfibril angle in individual tracheids is critical to their mechanical properties.

The second objective was to examine the change in microfibril angle in annual rings of southern pine. The average microfibril angle for the entire growth ring was compared to that of the earlywood and latewood portions of the ring.

MATERIALS AND METHODS

Microfibril angles were measured on individual tracheids (pulped fibers) and thin sections utilizing the soft-rot cavity method and image analysis (Anagnost et al. 2000).

FIG. 6. Extensive soft-rot cavity formation in an earlywood tracheid of southern pine. The arrow indicates a circular cavity in a pit border. Bar = 10 μm. From Mark (2001).

TABLE 2. *Microfibril angles of earlywood tracheid 3. In 25 segments of this tracheid, the orientation of 292 soft-rot cavities was measured. The average, standard deviation, and range of values indicate that similar variability occurred along the length of the tracheid. Segment 3 contains a bordered pit.*

Segment	Average	St. dev.	Minimum	Maximum	Range	n
1	35	2.4	33	33	6	6
2	34	3.6	30	41	12	12
3	55	16.8	43	83	40	10
4	39	2.8	33	43	10	20
5	35	1.6	32	38	6	19
6	35	2.3	31	39	9	22
7	37	1.8	32	40	7	17
8	36	1.8	32	38	6	9
9	36	2.5	34	41	7	11
10	35	2.6	29	38	9	11
11	34	0.8	33	34	2	7
12	35	2.2	32	40	8	15
13	36	2.0	32	39	6	11
14	36	3.8	30	40	10	8
15	35	2.2	31	38	7	11
16	36	2.0	31	39	7	14
17	35	1.9	32	39	7	11
18	36	2.2	32	41	10	17
19	34	1.9	31	37	5	9
20	38	1.7	33	40	6	12
21	36	2.1	33	39	6	11
22	35	2.2	32	39	7	13
23	34	0.2	34	35	0	4
24	31	3.6	27	37	10	5
25	35	3.3	29	38	9	7

Microfibril angle measurements in individual tracheids

Southern pine (*Pinus* sp.) blocks that were previously exposed to soft-rot fungi were macerated in a mixture of hydrogen peroxide (50%) and glacial acetic acid (50%). Microfibril angles were measured along the length of 5 earlywood tracheids and 5 latewood tracheids. Of the 5 earlywood tracheids, 2 were measured on the tangential wall, and 3 were measured on the radial wall. Microfibril angles were measured in segments of each tracheid and the results for each segment presented graphically. In tracheid 3, from 4 to 22 cavities were measured in each of 25 tracheid segments, for a total of 292 measurements. For all other tracheids, one cavity was measured per segment. In order to examine the influence of bordered pits on microfibril angle, measurements were reexamined ignoring cavities around intertracheid pits and ray-tracheid pit crossings.

Tracheid width measurements

The width of each tracheid was measured at the point of each microfibril angle measurement. The relationship between tracheid width and microfibril angle was described with a correlation coefficient.

Microfibril angle measurements across annual growth rings

Radial sections (20μm) of pine were sliced from wood blocks previously exposed to a soft-rot fungus. The unstained sections were examined with differential interference contrast light microscopy. Starting with the first earlywood tracheid and ending with the last

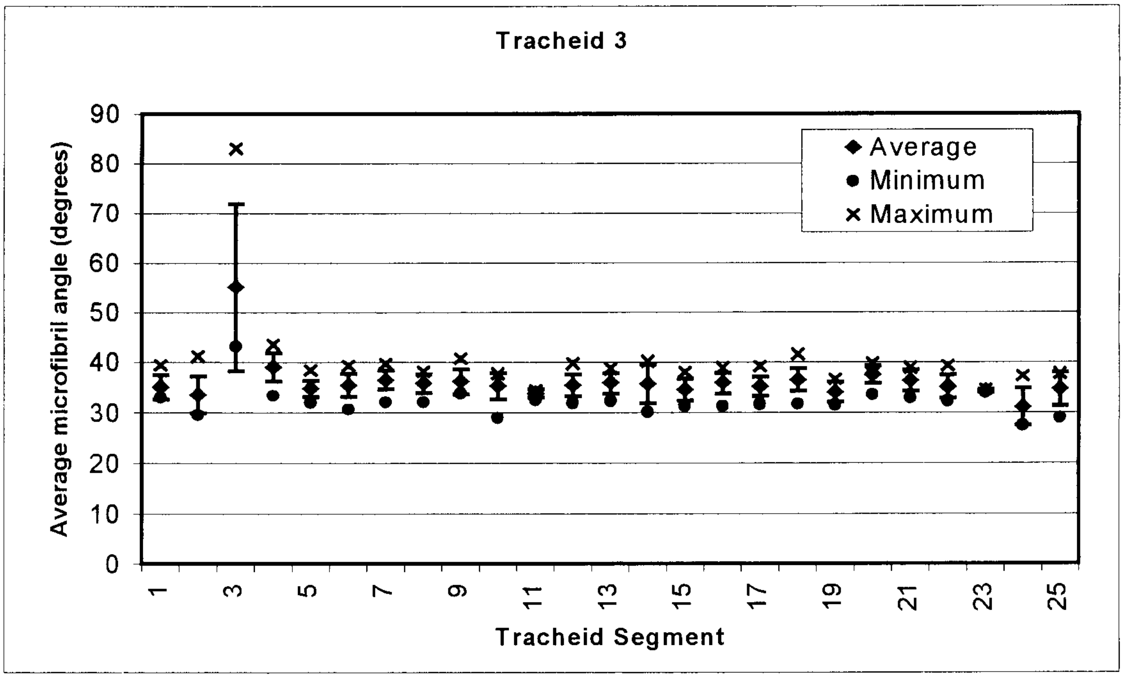


FIG. 7. The variation of microfibril angle in one tracheid of southern pine. The average, minimum, and maximum values of microfibril angle for each segment indicate very little variation along the length of the tracheid. The variability is consistent along the length of the tracheid, except in segment 3 that contains a bordered pit. A total of 292 cavities were measured.

latewood tracheid, the microfibril angles were measured in as many tracheids as possible. Tracheids were omitted that did not contain cavities.

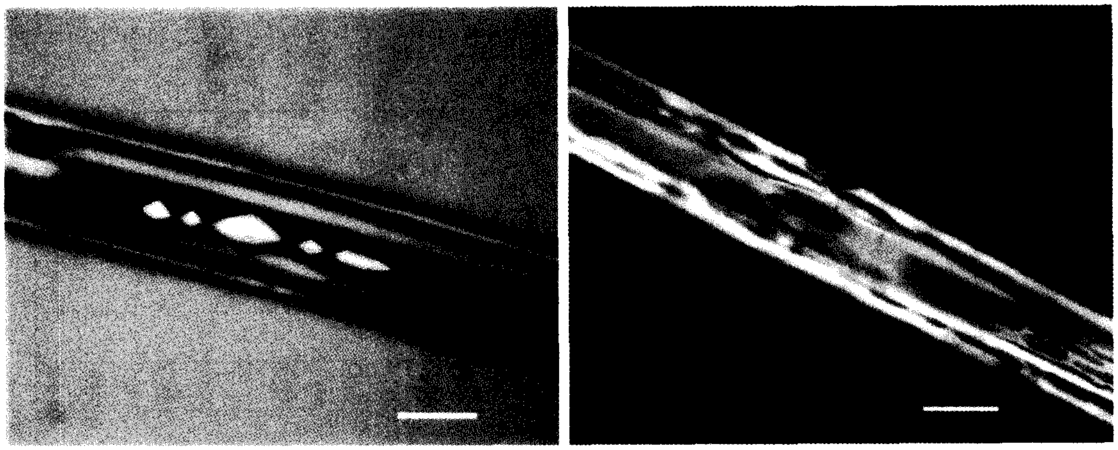
RESULTS AND DISCUSSION

Variation of microfibril angle in individual tracheids

Within individual tracheids, microfibril angle exhibited little variability from end to end (Table 1) (Figs. 1–4). For individual tracheids of southern pine, the average microfibril angles observed in earlywood tracheids were 26° to 48°, while those of latewood tracheids were 3° to 9° (Table 1). In latewood tracheids, the variation was generally less (lower standard deviation) than for earlywood tracheids. Standard deviation was utilized as a measure of variability within latewood and earlywood zones; percent variation was not critical. In individual earlywood tracheids, the minimum to maximum values for microfibril angle were 10° to 14° apart, excluding those measured near bordered pits. The range increased to 62°

TABLE 3. *Microfibril angle in two annual rings of southern pine, from 2 trees. The numbers in parentheses are standard deviations.*

	Microfibril angle					
	Entire annual ring					
Annual Ring	Mean	Min	Max	n	Earlywood Mean (std. dev.)	Latewood Mean (std. dev.)
A	19 (13.1)	1	41	60	27 (9.8)	6 (3.6)
B	18 (7.2)	5	31	66	22 (4.9)	8 (2.7)



A B

FIG. 8. Soft-rot cavities in latewood tracheids of southern pine that indicate very steep microfibril angles. A) Microfibril angle of 2° was measured utilizing the chain of diamond-shaped cavities. B) A microfibril angle of 2° was measured in the small cavity lying in the center of the tracheid.

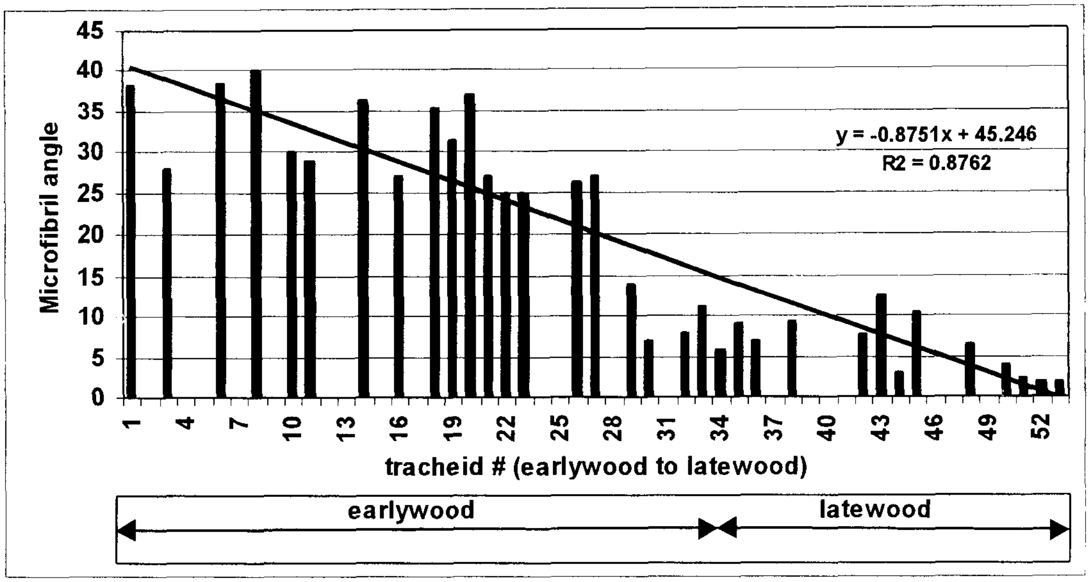
when considering the microfibril angle near pits. In individual latewood tracheids, the span from minimum to maximum was 3° to 11°. In tracheid 10, a higher degree of variability was observed.

For both earlywood and latewood tracheids, the microfibril angles near the tips of the tracheids were similar to those near the midpoint of the tracheid (Figs. 1–3). In tracheid 1, the angle near the tip was 28° (Fig. 4A), while the angle in the middle was 26° (Fig. 4B). In another earlywood tracheid (tracheid 2), the angle near the tip was 31°, while near the midpoint it was 27° (Fig. 4, C and D).

FIG. 9. Soft-rot cavities growing in a helical orientation in the tip of a southern pine tracheid. From Anagnost et al. (1999).

Microfibril angles on the radial wall of earlywood tracheids 3, 4, and 5, exhibited very little variation in microfibril angle in the regions between pits (Table 1). In tracheid 5, the microfibril angles measured on the opposite wall from the wall containing intertracheid pits were consistent with the average for the entire tracheid, ignoring those cavities around the pits (Fig. 5). Microfibril angles on the radial wall of earlywood tracheids 3 and 5 were the most variable in the regions closest to intertracheid pits (Fig. 2). This was especially evident in tracheid 5, where the microfibril angle approached 90° around intertracheid pits. Circular cavities (arrow) formed in pit borders, with angles of 90° at the point that is perpendicular to the cell axis (Fig. 6).

In tracheid 3, a total of 292 soft-rot cavities were measured in 25 segments from one tip (segment 1) of the tracheid to the other (segment 25) (Fig. 2). The average angle was 36°, and the averages for each segment ranged from 31° to 39°, except for the 55° average angle in segment 3 that contained a bordered pit (Fig. 7, Table 2). The range of values within each segment was very similar and was at



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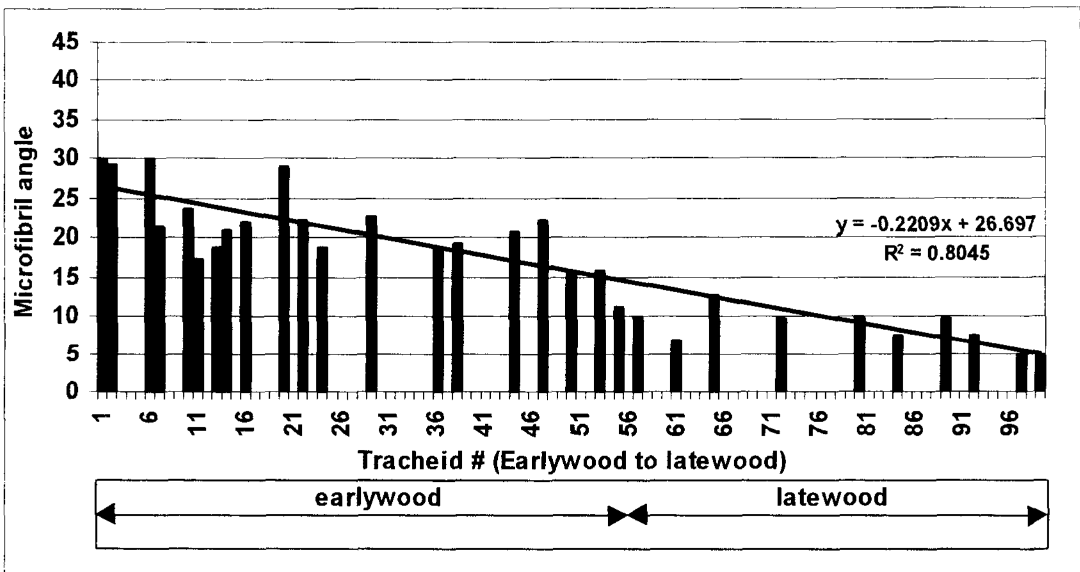

B.

FIG. 10. Microfibril angle measurements in two annual rings. The bars represent the average microfibril angle for each tracheid. A. This annual ring contains 53 tracheids with the first 33 being earlywood. A total of 60 soft-rot cavities were measured in 33 tracheids. The microfibril angle appears to decrease gradually across the annual ring. B. In this annual ring that is 99 cells wide, the first 56 tracheids are earlywood followed by 43 latewood tracheids. A total of 66 cavities were measured in 30 tracheids. The microfibril angle decreases gradually, with no abrupt change in the earlywood to latewood transition. The trendline and r^2 value of 0.80 indicate a relationship of position in the ring, or age, to a decrease in microfibril angle.

the most 12° (Fig. 7, Table 2). The extent of variation (standard deviation) within each segment was consistent with that of the entire tracheid (Tables 1 and 2).

The smallest angle observed was 1° in latewood tracheids 8, 9, and 10. Microfibril angles of less than 10° were found in latewood tracheids, in both individual tracheids and in thin sections. Cavities of 1° to 5° were not uncommon (Figs. 3 and 8); however, no angles of 0° were observed.

Correlation of tracheid width and microfibril angle

For most individual tracheids, there was no correlation between width and microfibril angle as evidenced by correlation coefficients that approached 0 (Table 1). Microfibril angles remained consistent despite variations of as much as 35 μm for tracheid widths. In tracheid 3, in which 292 cavities were measured, the correlation between tracheid width and microfibril angle was 0.02. This indicates that not only is microfibril angle independent of tracheid width, but it is independent of position along the length of a tracheid.

Microfibril angle at the tips of tracheids

The microfibril angles in latewood tracheids were generally consistent along the tracheid length. Very few soft-rot cavities were observed near the tips of latewood tracheids, even those that contained many cavities along the length. The narrowness of the tip combined with the thickness of the latewood cell wall and inherent birefringence of the cell wall increases the difficulty of observing cavities at the tips of latewood tracheids. A continuous chain of cavities on both the top and bottom wall was nearly impossible to observe. In contrast, at the tips of earlywood tracheids, it is much easier to visualize cavities, which typically form a helical orientation around tracheid tips (Fig. 9).

Microfibril angles across annual growth rings

Microfibril angles were measured in one annual ring of mature wood from two trees. In annual ring A, which was 53 cells wide, it was possible to measure the orientation of 60 soft-rot cavities in 33 of the tracheids. Annual ring B was 99 cells wide. In 30 of these tracheids, 66 measurements of soft-rot cavity orientation were obtained. The microfibril angle gradually decreased from the first earlywood cell to the last latewood cell of an annual ring, with maximum angles of 41° and 31° and minimum angles of 1° and 5°, respectively (Table 3; Fig. 10). The variability (standard deviation) was considerably greater in the earlywood than the latewood with results similar to other reports (Donaldson 1998; Saranpää et al. 1998; Khalili 1999).

CONCLUSIONS

The microfibril angle within individual tracheids showed little variability along the length of the tracheid, with variation being less in latewood tracheids than in earlywood tracheids. On the radial wall of samples containing pits, the microfibril angle between bordered pits was consistent along the length of the tracheid. However, the microfibril angle was highly variable on the radial walls of earlywood tracheids considering the microfibril angles in the regions containing bordered pits. Within individual tracheids there was no correlation between microfibril angle and tracheid width.

Within growth rings of southern pine, the variability of microfibril angle within the earlywood cells was greater than that of the latewood cells. The microfibril angle decreased gradually going across a growth ring. The largest angles observed approach 90°, and were located on the radial walls of earlywood tracheids around the borders of intertracheid pits. The smallest microfibril angles were approximately 1°, observed in latewood tracheids.

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