

EFFECTS OF PLANTATION DENSITY ON WOOD DENSITY AND ANATOMICAL PROPERTIES OF RED PINE (*PINUS RESINOSA* AIT.)

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ABSTRACT

This study demonstrated that average ring width (or average annual radial growth rate) is a reliable parameter to quantify the effects of tree plantation density (growth suppression) on wood density and tracheid anatomical properties. The average ring width successfully correlated wood density and tracheid anatomical properties of red pines (*Pinus resinosa* Ait.) from a never-thinned experimental plantation forest with five different initial plantation densities. The results indicate that plantation density has a pronounced effect on earlywood growth, which resulted in increased latewood volumetric fraction and uniform tracheid radial and wall thickness distribution. A similar approach of average ring width correlations may be applied to study wood and tracheid properties of trees growing in the densely populated natural forests.

Keywords: Red pine, small-diameter trees, forest thinning, plantation density, pulp yield, tracheid (cell) geometry, SilviScan, maceration, radial growth, latewood content.

INTRODUCTION

In the past several decades, the economic and social environment in the United States has significantly affected forest management practices. Large areas of forest land are overpopulated with trees under suppressed-growth conditions that increase the risk of catastrophic forest fires. Selective thinning of small-diameter trees is a strategy that can reduce fuel loading and the

risks associated with these devastating fires. However, high-value, large-volume uses for these trees need to be found to mitigate the cost of expensive thinning operations. Ideally, the production of solid wood products, such as dimensional lumber or poles and posts, could generate revenues that would substantially offset thinning costs. Unfortunately, the suppressed-growth environment produces small-diameter trees with low lumber yields. Integrating the production of lumber and paper using non-saw logs and saw mill residual chips can maximize the economic value of the thinning materials.

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However, chips from these particular thinnings are very different from traditional normal wood chips, and conflicting results about the quality of the chips were reported (Myers et al. 1999, 2003; Myers 2004). The main characteristics of these materials are very narrow growth rings due primarily to growth suppression as a result of natural overpopulation (high stand density). Previous research recognized that fiber properties such as anatomical dimensions affect fiber processing and properties of end products (Seth 1990). Therefore, understanding the effect of tree growth conditions—especially stand density—on wood tracheid properties is very important to improve the value and utilization of wood chips from forest thinnings. This knowledge is essential to make thinning strategies an economically viable management tool for sustaining forest health in the United States.

In plantation forestry, stand density is managed to optimize wood yield (Yang et al. 1986) and quality and to maintain the health of the forest (Maeglin 1967; Ballard and Long 1988; Janas and Brand 1988). Recently, Turnblom and Burk (2000) and Larocque (2002) modeled the effect of stand density on red pine radial growth. Kang et al. (2004) quantified the effects of plantation density on wood density and pulp properties in jack pine plantations. They found that wood yield and fiber properties can be improved through stand density regulation. Furthermore, they found that pre-commercial thinning has a positive effect on fiber properties. Yang and Hazenberg (1994), Hatton et al. (1996), and Watson et al. (2003) studied the effect of plantation spacing on relative wood density and fiber properties in *Picea mariana*, western red cedar, and western hemlock, respectively. Watson et al. (2003) found that fiber coarseness and length were not affected by stand density for initial spacing between 0.9 by 0.9 m and 3.6 by 3.6 m. However, juvenile wood content increased when tree initial spacing was wider than 4.6 by 4.6 m.

The work of Watson et al. (2003) agrees with the early studies (Koch 1972) on the effect of environment on southern pine growth. In a comprehensive review, Koch (1972) concluded that correlation between specific gravity and any fac-

tor, such as stand density, is usually poor. Only when an environmental factor becomes limiting does it affect tracheid size, wall thickening, or both enough to show good correlation between that factor and wood specific gravity. Larson (1963), Zahner and Oliver (1962), and Zahner et al. (1964) studied the effects of growth conditions (drought or thinning) on tracheid size and earlywood and latewood features of plantation red pine, respectively. They found that growth environment affects the size of wood tracheid and the initiation of latewood.

Ring width is an easily measurable parameter and should reflect the effects of environment on tree growth. It has been used to correlate wood density and fiber length (Dinwoodie 1965; Lindstrom 1996; Dutilleul et al. 1998). The effects of thinning on wood and tracheid properties can be effectively demonstrated from these correlations (Dutilleul et al. 1998; Mäkinen et al. 2002). In the present study, the arithmetic average ring width of red pine (*Pinus resinosa* Ait.) is used to correlate several key anatomical properties, such as diameter, wall thickness, and its distribution, wood density, and latewood content. The ring width is also used to correlate within-tree wood density profiles. These correlations then can be used to determine how growth suppression affects tracheid properties, such as geometric uniformity. The objective of the study is to use average ring-width correlations to understand the effect of plantation stand density on the anatomical properties of plantation red pine wood. One goal of our research is to use average ring-width correlations for studying the effects of suppressed-growth conditions on wood and tracheid anatomical properties of trees for fiber production from overpopulated natural forests.

EXPERIMENTAL

Raw material

The red pine logs used in this study were obtained from a 38-year-old research plantation in the Lake Superior State Forest (Michigan). The plantation stand density varied at 220, 320, 420,

620, and 820 stems per acre in five different plots to simulate growth suppression. Ten trees were harvested from each of the plantation stand density plots. A 4.9-m butt log was bucked from each tree and shipped to the USDA Forest Products Laboratory (FPL) in Madison, Wisconsin. A 61-cm section from each end of the butt log was retained for pulping studies. A 2.5-cm-thick disk was also cut from each 61-cm section (Fig. 1). Two disks from each plantation density log plot, a total of 10 disks, were selected from 100 disks produced for basic wood property analysis by SilviScan (CSIRO Forestry and Forest Products, Australia) and tracheid length measurements. One disk is from the top of the first 61-cm section; the other is from the bottom of the other 61-cm section. More specifically, the vertical heights of the disks are 0.6 and 4.3 m, respectively. These two disks are not necessarily from the same tree. The cost prohibited more disks selected for SilviScan analysis. Therefore, the 10 disks were carefully selected through visual observation to be representative of the 10 disks in each plantation plot. Table 1 lists the basic data of the 10 disks.

Sample preparation for chemical analysis and wood maceration

A Wiley mill (Thomas Scientific, Swedesboro, New Jersey) was used to produce wood meal from the mixed wood chips of each plot

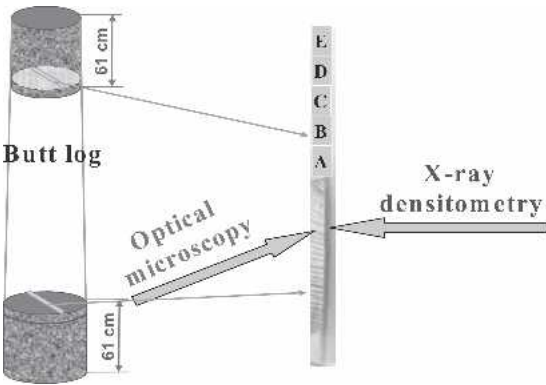


FIG. 1. A schematic diagram showing the vertical location two wood disks and the strips used SilviScan analysis.

TABLE 1. Basic data of the 10 wood disks used in this study.

Disk from plot stand					
density (stems/acre)	220	320	420	620	820
Disk from height = 0.6 m, group average ring count = 34					
Disk number	1	2	3	4	5
Diameter (mm)	226	245	200	139	169
Number of rings	33	34	36	35	33
Disk from height = 4.3 m, group average ring count = 24					
Disk number	6	7	8	9	10
Diameter (mm)	184	178	169	170	133
Number of rings	23	24	24	25	24

prior to wood chemical composition analysis. The wood chips were produced at FPL from the two 61-cm logs after hand debarking. The analysis was carried out by the FPL's Analytical Chemistry and Microscopy group.

Two adjacent strips across the entire diameter were cut from each disk. The diameter was chosen with the least differentiation in radius between the two radii from the pith to bark. One strip was sent to the Tasmanian Forest Research Center, Commonwealth Scientific and Industrial Research Organization, Division of Forestry and Forest Products, Australia, for SilviScan analysis. The other was segmented and macerated to obtain wood tracheid length.

Maceration

Each strip was evenly divided into five blocks: A (pith end), B, C, D, and E (bark end), along the radial direction (Fig. 1). An approximately 2-mm-thick (radial direction) slice was cut from each block and then further reduced to matchstick size. A total of about 0.33 g was obtained from each slice. Maceration of the matchstick-sized wood samples was based on the method of Brisson, Gardner, and Peterson (Yeung 1998). The volumetric composition of macerating solution was one part hydrogen peroxide (30% reagent solution), 4 parts deionized water, and 5 parts pure (100%) glacial acetic acid. All chemicals were from commercial sources. Approximately 35 mL of macerating solution was added into a 40-mL vial to macer-

ate approximately 0.33 g of wood sample. The vial was then capped and placed in an oven at temperature of 60°C. After 7 days, the sample appeared as a white-translucent material and the maceration solution became clear. The vial was removed from the oven and allowed to cool to room temperature. The macerated sample was gently removed with a forceps and placed in a clean vial. The fiber sample was mixed with deionized water for several minutes to separate any fiber bundles into individual fibers. The fiber sample was then analyzed by a Kajaani FS-100 fiber analyzer (Metso Automation, Helsinki, Finland) to determine the length distribution.

SilviScan analysis

SilviScan, a commercially available instrument for wood analysis, combines X-ray diffraction densitometry with optical microscopy (Fig. 1) to determine wood density and tracheid diameters (Evans 1994; Evans et al. 1995). The tracheid wall thickness is calculated from the measured density and tracheid diameters by assuming an isotropic wall thickness. The SilviScan-II was used to analyze the samples and has a spatial resolution of 50 μm . The identification of earlywood and latewood was accomplished by using a procedure based on density variation in the SilviScan data analysis software.

RESULTS AND DISCUSSION

Wood chemical analysis

Table 2 lists the chemical compositions of wood from various plantation density plots (the

sample for 220 stems/acre was lost during sample preparation). Ring width was not used to correlate the chemical compositions because the data were obtained using the mixture of chips from many logs in each plantation plot. The Klason lignin was reduced by 1.5%, while the glucan and xylan content increased by 2% when the plantation density increased from 320 to 820 stems/acre. Because earlywood has a higher specific surface area than latewood, an increase in latewood volumetric fraction reduces the total tracheid surface area. Furthermore, lignin content is proportional to tracheid surface area in general. Therefore, the low lignin content of trees from a high stand density plot implies that a high latewood volumetric fraction can result as stand density increases. The decrease in lignin content and the increase in glucan and xylan content have a positive effect in chemical pulping in terms of pulp yield as demonstrated in our companion study (Zhu and Myers 2006).

Average ring width

The average ring width is simply the arithmetic mean of the annual ring widths measured by SilviScan analysis. This can be referred to as the average annual radial growth rate of the tree. We will use the terms of average ring width and average annual growth rate interchangeably throughout this article. The growth suppression is nonexistent in early years for the plantation densities studied in this work. Growth suppression may become important only in the mature wood. Therefore, the data from the first 10 years were arbitrarily excluded in calculating the average ring width. We found that the annual ring

TABLE 2. *Chemical composition of trees from five different plantation density plots.*

Sample	K. Lignin	AS Lignin	Arabinan	Galactan	Rhamnan	Glucan	Xylan	Mannan	Total Carbon	Total Yield
320	29.59	0.90	1.63	2.13	0.15	40.94	5.93	11.22	62.0	92.5
420	28.55	0.81	1.69	2.02	0.09	42.10	6.19	11.68	63.8	93.1
620	28.43	0.83	1.71	1.92	0.12	42.32	6.43	11.26	63.8	93.0
820	28.09	0.82	1.70	2.05	0.12	42.18	6.60	11.26	63.9	92.7
Std	0.25	0.04	0.02	0.07	0.02	0.41	0.09	0.16	0.6	0.5
Rstd	0.80	5.80	1.60	2.90	15.90	1.00	1.40	1.40	0.9	0.6
<i>n</i>	84	21	91	91	86	91	91	91	91	82

width decreased significantly at year 10 from the peak at about year 6 for all the disks studied. However, the average ring width of the entire disk was used in correlating the yearly averaged tangential growth and length-weighted mean fiber length for reasons to be discussed later.

Wood physical properties

The correlation between average ring width and plantation density was first examined. For the five plots investigated, the average ring width decreased as the plantation density increased (Fig. 2). However, there was significant deviation from linearity for the upper disks. These results indicate that the average ring width is a reliable parameter to represent tree growth conditions (plantation density in this study). Next, a correlation between average ring width and average wood density was made.

The wood density was calculated by integrating the SilviScan-measured radial density profile over the entire disk; it is the wood disk cross-sectional area weighted mean of the SilviScan radial density data. This calculation assumes disk axisymmetry. Linear regression analysis indicates that the wood density increases as average ring width decreases (Eq. (1), Table 3).

To study the effects of growth suppression through plantation density on local wood den-

sity, we compared yearly averaged wood densities of both the earlywood and latewood band of the two disks with very different average ring widths. The results (Fig. 3) clearly show that the latewood density for the disk with small average ring width was lower than the latewood density of the disk with large average ring width, especially in the last 10 years. These results were seen despite the bulk wood density increasing as average ring width decreased (Eq. (1), Table 3). The opposite was observed for earlywood but with much less difference. The cross-sectional area weighted mean densities of earlywood and latewood for all 10 disks were calculated using the yearly average wood densities as shown in Fig. 3. The calculated mean wood densities were correlated to average ring width. The results (Eq. (2a) and (2b) in Table 3) indicate that earlywood density increased (negative slope) while latewood density and average ring width decreased. This indicates that the difference in wood density between earlywood and latewood becomes less distinct, or wood density becomes more uniform as a result of growth suppression under high plantation density.

Latewood or earlywood volumetric fraction can also be calculated using the SilviScan data. Latewood volumetric fraction increased as average ring width decreased (Fig. 4), which results in increased latewood mass fraction despite a decrease in latewood density. Larson (1963) and Zahner et al. (1964) suggested that the shift from earlywood to latewood in red pine can take place as a result of developing water stress, slowing crown growth, and declining auxin levels. Zahner (1964) and Zahner and Oliver (1962) suggested that growth suppression caused early formation of flattened (latewood) tracheid. Therefore, the increase in latewood volumetric fraction at high stand density can be attributed to growth suppression in mature wood years. The latewood mass fractions for the 10 disks were calculated and found to correlate well to the average ring width of the disk (Eq. (3), Table 3).

Tracheid diameters

SilviScan analysis reveals that the tracheid radial diameter varies significantly (as much as

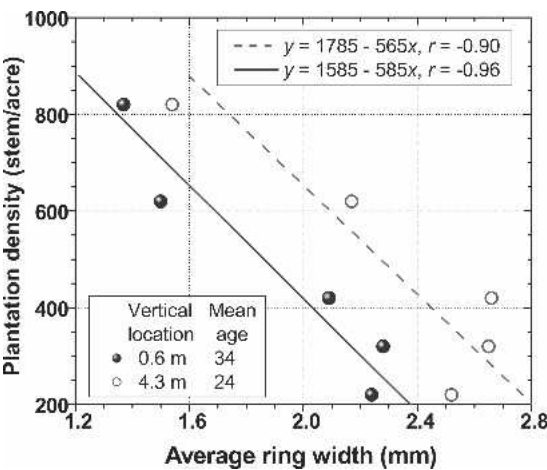


FIG. 2. Correlation between plantation density and tree average annual radial growth rate (average ring width).

TABLE 3. Linear regression results showing the correlations between various parameters (dependent variable, y) and average ring width (independent variable, x , or $\ln(x)$) for Eqs. 8a and 8b) in mm.

Equation label	Dependent variable y (=)	Wood band	Slope $a(x \text{ or } \ln(x))$	Intercept (+) b	Correlation coefficient, r
1	Wood density (kg/m^3)		-14.7	460	-0.30
2a	Cross-sectional area weighted-wood density (kg/m^3)	Earlywood	-15.8	403	-0.52
2b		Latewood	47.5	588	0.45
3	Latewood mass fraction		-0.453	0.439	-0.59
4a	Tracheid number-weighted mean radial tracheid diameter (m)	Earlywood	3.36	28.40	0.72
4b		Latewood	1.37	22.23	0.62
5a	Yearly average tracheid tangential growth (m/year)	Earlywood	0.101	0	0.93
5b		Latewood	0.082	0	0.86
6a	Length-weighted tracheid length (mm)	A (Pith, Fig. 1)	0.175	0.45	0.52
6b		C (Fig. 1)	0.190	0.91	0.40
6c		E (Bark, Fig. 1)	0.183	2.15	0.36
7a	Mean and standard deviation of tracheid wall thickness distribution		0.41	1.76	0.99
7b			0.451	0	0.99
8a	Slopes of the correlations between local wood density vs local earlywood and latewood band width	Earlywood	25.8	-74	0.69
8b		Latewood	17.2	-102	0.32

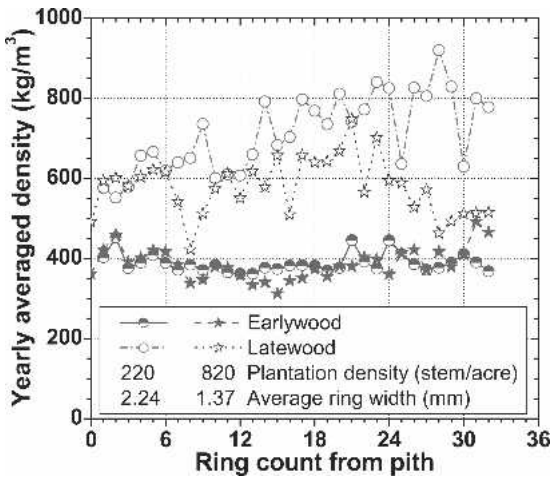


FIG. 3. Earlywood and latewood density for two disks with different average ring widths.

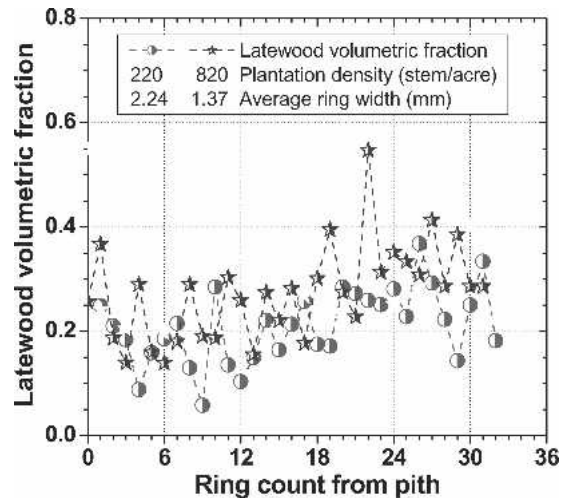


FIG. 4. Latewood volumetric fraction for two disks with different average ring widths.

100% for a given year) between earlywood and latewood (Fig. 5). The yearly average earlywood radial diameter increased with age almost linearly in the first 10 years of the juvenile wood and then remained a constant in the mature wood for a given tree. The yearly average radial diameter of earlywood has a stronger dependence on average annual radial growth rate (average ring width) than that of latewood (Fig. 5). Because the yearly average tracheid radial diameters are

close to a constant except for the first 10 years, the mean radial diameter of the tracheids across the entire diameter can be a good representation of the radial size of the tracheid. The tracheid number-weighted mean radial diameter of each disk was calculated using the SilviScan-measured radial diameter and cell population profiles, excluding the first 10 years. We found that both the earlywood and latewood mean

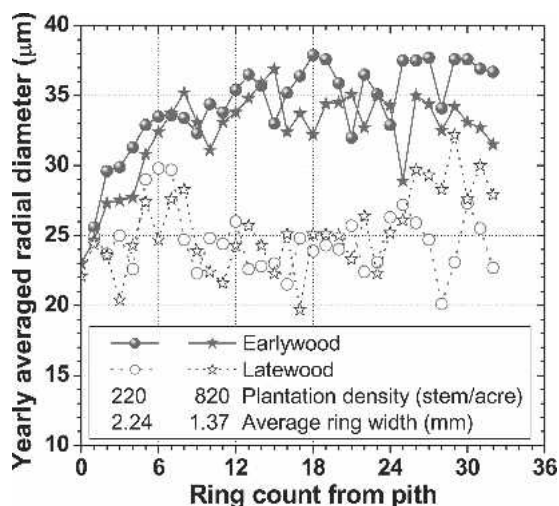


FIG. 5. SilviScan-measured yearly average tracheid radial diameter profiles for two disks with different average ring widths.

tracheid radial diameter decreased as average ring width decreased, which agrees with that observed under drought conditions by Larson (1663). The linear dependence of the mean tracheid radial diameter of earlywood on average annual radial growth (average ring width) is stronger than that of latewood (Eq. (4a) and (4b) in Table 3). As a result, trees with slow annual radial growth have a more uniform distribution of radial-cell diameter.

The variation of tracheid yearly average tangential diameter between earlywood and latewood was only about 20%. The yearly average tracheid tangential diameter increases linearly with tree age (Fig. 6). The intercepts of the linear relationships are a constant for both earlywood and latewood of 23.1 μm with a relative standard deviation of only 2.1% for all wood disks studied. Because the linear tangential growth relation was observed from year 1, annual ring widths from the entire disk were used in calculating the average ring width to correlate the average annual growth of tracheid tangential diameter. For the five disks with height equaling 0.6 m and an average of 34 rings, the slope of the linear relationship increased as average ring width increased for both earlywood (Eq. (5a) in Table 3) and latewood (Eq. (5b)). A wide aver-

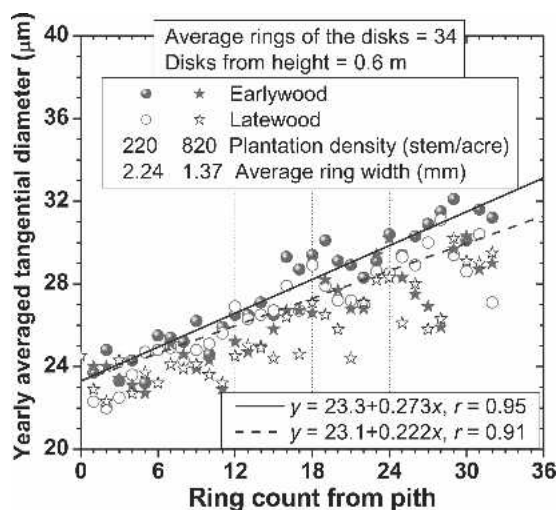


FIG. 6. SilviScan-measured yearly average tracheid tangential diameter profiles for two disks with different average ring widths.

age ring width resulting from low stand density in plantations was also associated with great tracheid tangential diameter. This clearly suggests that the tree radial growth induced the growth in the tracheid tangential diameter. Further analysis of the data from all 10 disks reveals that the average annual growth of tracheid tangential diameter of earlywood and latewood is linearly correlated with a zero intercept and a proportionality constant of 0.82 with $r = 0.96$.

Fiber length

Recall that each of the 10 wood strips used in maceration was evenly divided into five sections with A, B, C, D, and E, representing wood blocks from pith to bark (Fig. 1). Comparisons were made among wood blocks of equivalent radial locations in various trees of different diameters (but not the same radial distance because of the variation in disk diameter). The comparisons were made among wood blocks of about the same average age, which is important because tracheid length is proportional to wood age. The average ages of blocks A, C, and E are 2, 8.5, and 22.5, respectively. Linear regressions of the three data sets for blocks A, C, and E indicate that the mean fiber length increases lin-

early with disk average ring width (over the entire disk) for all wood blocks at different radii independent of tree height (Eqs. (6a), (6b), and (6c) in Table 3). Furthermore, the slopes of the fitted lines of the three data sets are approximately equal to 0.18. The data scattering for a specific section is due to the tracheid length variations from tree to tree and the variations in average age of the blocks. The standard deviations of the average age of the blocks for sections A, C, and E are 0.46 (22.5%), 1.1 (12.9%), and 3.4 (15%), respectively. Unfortunately, earlywood and latewood were not separated before maceration because of difficulties in cutting. Therefore, the effect of plantation density on tracheid length of earlywood and latewood cannot be separately quantified.

Figure 7 shows the fiber number distribution of fiber length in sections A, C, and E from two bottom disks with different average ring widths (220 and 620 stems/acre). Because of the variations in mean fiber length from tree to tree, the two disks were selected so that the mean lengths of the three sections (A, C, E) all approximated the regression (Eqs. (6a), (6b), and (6c)). The results (Fig. 7) indicate that the fiber-length distribution of a wood block with a higher average ring width contains more long fiber than a similar sample from a high-density plantation plot.

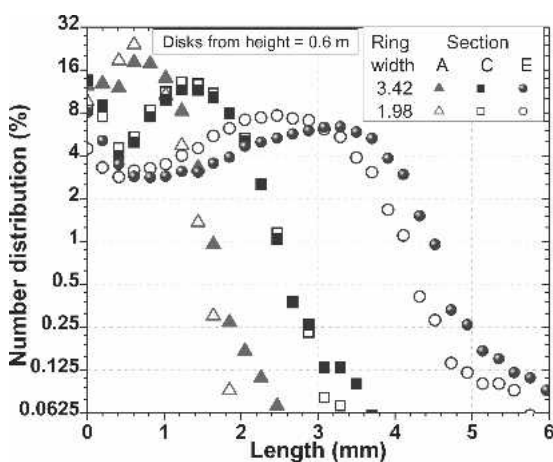


FIG. 7. Comparisons of fiber-length distributions of three sections of two disks with two different average ring widths.

Tracheid wall thickness

In previous studies, we discovered that a tree grown under suppressed-growth conditions tends to produce tracheids with uniform wall thickness distribution (Klungness et al. 2006; Vahey et al. 2007). Figure 8 shows the SilviScan cell-wall thickness profiles for two disks with different ring widths. The results indicate that tracheid wall thickness of a disk from a high plantation density (820 stems/acre, an average ring width of 1.37 mm) is thinner in general than the wall thickness of a disk from a low-density plot (220 stems/acre, average ring width 2.24 mm). This effect is much more pronounced on latewood than on earlywood, especially in the last 10 years. As a result, the tracheid wall thickness for the disk with a narrower average ring width was more uniform. This uniformity can be illustrated by the wall-thickness probability-density distribution (Fig. 9). A bimodal distribution was observed for the disk from 220 stems/acre plot with a very narrow and strong first peak corresponding to the earlywood and a broad second peak representing the latewood. The earlywood and latewood are indistinguishable for the disk from 820 stems/acre plot from the wall-thickness probability-distribution. Furthermore, the distribution is fairly constant.

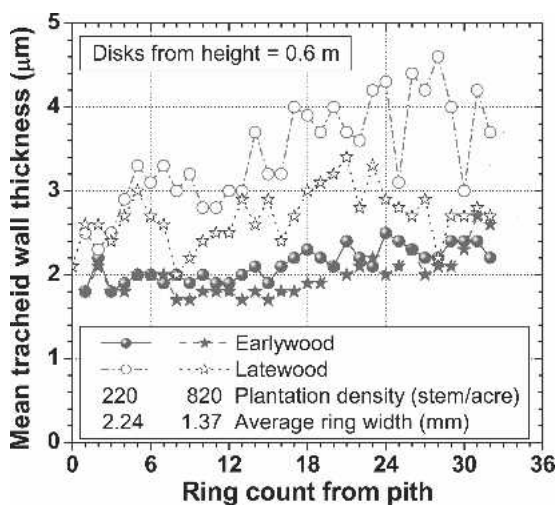


FIG. 8. SilviScan-measured yearly average tracheid wall thickness profiles for two disks with different average ring widths.

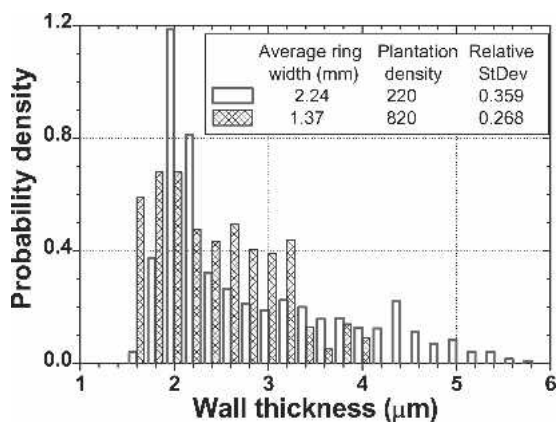


FIG. 9. Probability distribution density function of yearly average tracheid wall thickness for two disks with different average ring widths.

We calculated the mean and the standard deviation of tracheid wall thickness from the probability distribution density functions for the five bottom disks. We found that both the mean (Eq. 7a, Table 3) and the standard deviation (Eq. 7b) wall thickness decreased linearly as average ring width decreased. A small standard deviation indicates small variability in wall thickness, or more uniform distribution. This suggests that growth suppression may produce tracheids with relatively uniform thin walls, which confirms our discovery (Klungness et al. 2006).

Within-tree correlation of wood density with ring width

Figure 10 shows the within-tree correlation of local wood density and local band width of earlywood and latewood of two disks (220 and 620 stems/acre) with different average ring widths. We used the logarithm of local earlywood or latewood band width instead of the linear ring width (sum of earlywood and latewood) to correlate to wood density. The differences in wood density and growth between earlywood and latewood are significant and warrant separate examination as evidenced by Fig. 10. Also, the relation between local wood density and local wood band width is nonlinear, confirming the findings of Dutilleul et al. (1998) that the depen-

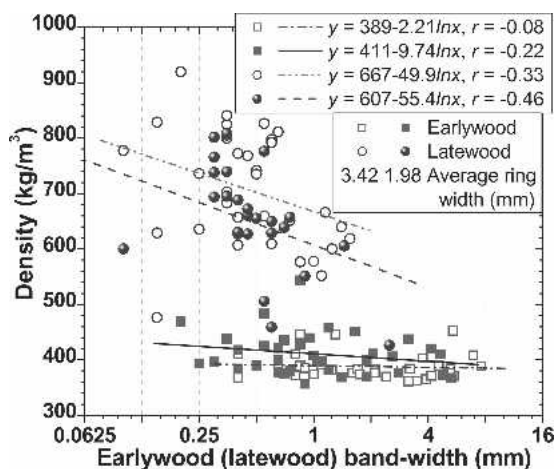


FIG. 10. Within tree correlations between SilviScan-measured yearly average earlywood and latewood density and earlywood and latewood band width, respectively.

dence of density on ring width is stronger for slow- than for fast-growth trees. These results indicate that the dependence of density on band width is more pronounced for latewood (great negative slope) than for earlywood. Therefore, the findings of Dutilleul et al. (1998) based on tree-growth category can be applied to earlywood (representing fast growth) and latewood (slow growth). The slopes of the correlation between local wood density and local wood-band width can also be correlated to averaged ring width (Eqs. (8a) and (8b) in Table 3). The slopes for both earlywood and latewood increase linearly (less negative) as average ring width (annual radial growth rate) increases. However, the slopes are negative in general for the 10 disks studied except for data obtained from earlywood, especially disks from the top (fast growth). This indicates that wood density can increase or at least not be affected very negatively by radial growth (ring width) in fast-growth trees (such as juvenile wood and earlywood). This argument is important to consider in culturing high-density, fast-growing trees and deserves further validation in the future.

CONCLUSIONS

The effects of plantation stand density (growth suppression) on tree growth, wood den-

sity, and tracheid properties of red pine were examined in this study. We found that trees grown in a high-density plot produced logs with a low average ring width (or average annual radial growth rate). Average ring width appeared to be a reliable parameter to correlate the effects of tree growing conditions (stand density in this study) on wood density and tracheid anatomical properties. This approach of average ring width correlations may be applied to study wood and tracheid properties of trees in a natural forest, such as those trees under suppressed-growth conditions in the U.S. National Forests.

Tracheid mean radial diameter, the growth rate of yearly average tracheid tangential diameter, and the mean fiber length all decrease as average ring width decreases. Increasing stand density negatively affects the growth of tracheid radial and tangential diameters and fiber length. In other words, a high-stand density (associated with low average annual radial growth rate or low average ring width) produces trees small in diameter and tracheid dimensions (radial, tangential, and length). The growth suppression (high-stand density in this study) may cause early shift from earlywood to latewood and result in reduced tracheid diameters, uniform tracheid wall thickness distribution, and high volumetric latewood fraction, confirming our early findings (Klungness et al. 2006). In trees from a natural forest, suppressed-growth conditions produced trees with a more uniform distribution of tracheid wall thickness than trees under normal growth. The high latewood volumetric fraction in logs from a high-stand density also explains the low lignin and high glucan and xylan content from chemical composition analysis. The variation in chemical composition explains the difference in chemical pulp yield observed in our companion study (Zhu and Myers 2006).

Within-tree correlations between wood density and wood (earlywood or latewood) band-width indicate that wood density can increase or not be affected very negatively by radial growth (ring width) in fast-growth trees, such as juvenile wood and earlywood. This argument has significant practical importance in culturing fast-

growing, high-density trees, and therefore needs further validation in future studies.

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