MEASURING MAXIMUM LATEWOOD DENSITY BY IMAGE ANALYSIS AT THE CELLULAR LEVEL

Won-Kyu Park

Assistant Professor

Department of Forest Products
Chungbuk National University
Cheungju 360-763
Republic of Korea

and

Frank W. Telewski

Curator and Assistant Professor

W. J. Beal Botanical Garden
412 Olds Hall
Michigan State University
East Lansing, MI 48824

(Received January 1993)

ABSTRACT

A study was conducted to compare the precision of X-ray densitometry (XRD) and video image analysis (VIA) in measuring wood density of the last-formed latewood. The precision was determined by examining the convergence of the replicated measurements of maximum latewood density (MAXD), by XRD, and maximum percentage of cell-wall area (MAX%), by VIA. VIA was a more precise method for determining density of the last-formed tracheids than XRD. The linear relationship between MAXD and MAX% was significant at $P \leq 0.01$. The results indicate that MAX% can be used as an alternative to X-ray measurements of MAXD.

Keywords: Dendrochronology, image analysis, microdensitometry, tracheid morphology, wood density.

INTRODUCTION

The dimensions of wood cells can be reliably and quickly obtained using video image analysis (VIA) (McMillin 1982; Jagels and Dyer 1983; Telewski et al. 1983; Yanosky and Robinove 1986; Jagels and Telewski 1990). Image analysis can also be used as an alternative method to X-ray densitometry (XRD) by measuring wood density as a function of optical properties, such as intensity of reflected light, with the assumption that the optical property is proportional to wood density (Clauson and Wilson 1991).

The direct anatomical measurement of the ratio of cell-wall area to total area (CWA%) using image analysis may yield a more accurate measure of density that would be free of the optical property assumption made by Clauson and Wilson (1991). This hypothesis is based on the fact that densities of the chemical constituents (mainly cellulose, hemicellulose, and lignin) do not vary significantly within a species (Stamm and Sanders 1966; Kellogg and Wangaard 1969). Therefore, the amount of cell-wall materials within a certain volume (i.e., density of the wood) can be expressed by the CWA% measured in transverse section (Tsoumis 1964; Elliott and Brook 1967; Merkel 1984; Swain 1987; Jagels and Telewski 1990). Information derived directly from morphological measurements is more meaningful in interpreting the biological and environmental regulation of xylogenesis.

Wood and Fiber Science, 25(4), 1993, pp. 326–332

Both VIA and XRD methods possess some limitations in obtaining precise measurements. Current XRD systems have difficulty in resolving narrow rings (ca. 0.22 mm) such as those frequently found in trees exposed to severe environmental stress. Narrow growth rings can be observed in trees from semiarid sites, alpine timberline, or severely polluted sites (Bräker 1982; Cleaveland 1983; McCord 1984; Park 1990). Besides limitations in resolution, a parallax problem arising from nonparallel wood cell orientation increases in narrow growth rings. The determination of density is strongly affected by the problem. The parallax problem can be reduced if thinner sample sections are obtained for XRD (McCord 1984; Telewski et al. 1987). VIA can eliminate this problem by using microthin (ca. 15–30 μm) sections (Jagels and Telewski 1990; Park 1991).

VIA-anatomical analysis technology also has several limitations. Sample preparation is more tedious than that for XRD. VIA requires high quality surfaces or microslides of the wood specimen. Distortion or partial crushing of the cellular structure is inevitable for samples derived with an increment bore. Destaining during dehydration also causes inconsistencies in the quality of image contrast (Jagels and Telewski 1990). However, for softwoods, high quality microslides can be prepared for VIA from two 20-cm cores in one person-day (Park 1991).

Even though the advantages and limitations of both methods have been frequently documented, few attempts have been made to compare the precision of XRD and VIA methods for measuring wood density. This study will determine the precision of both methods using replicate measurements of the last-formed latewood tracheids. The density of the last-formed tracheids is measured by XRD as maximum latewood density (MAXD) and by VIA as maximum CWA% (MAX%). This study will also examine how closely MAXD and MAX% are related to similar studies of Tsoumis (1964), Merkel (1984), and Swain (1987).

EXPERIMENTAL METHODS

A block of *Pinus ponderosa* (5 mm × 25 mm × 20 mm, T × R × L) was cut from a disk that was collected from Bandelier National Monument, New Mexico. After extraction with alcohol/toluene (1:2 v/v; 4 h), four thin sections (360 μm) and four microthin sections (20 μm) were cut using a sliding microtome for the XRD and VIA analyses, respectively. The sample was cross-dated using the methods of dendrochronology (Stokes and Smiley 1968). It contained 40 annual growth rings (1925–1965), of which only 26 (1933–1958) were used in this study.

After air drying, thin sections were analyzed using XRD as described by McCord (1984). The sample sections were placed on X-ray film (Kodak Industrex) and were radiographed using a moving slit method. The exposed films were developed, and the optical densities of the films were determined by a Joyce-Loebl optical microdensitometer interfaced with an Apple IIe computer. Maximum latewood densities were produced using programs employing interactive graphics (McCord 1984).

The estimate of within-specimen variability was determined by scanning the radiograph of each section at three different locations. The microdensitometer slit was set at 200 μm (width in the radial direction of the specimen) and 2,000 μm (height in the tangential direction of the specimen). This setting was determined to represent the optimum size to maintain a high degree of optical resolution along the length of the scan. The slit dimensions combined with the total optical magnification factor of 5× produced an effective resolution of 40 μm by 400 μm (as area; 16,000 μm^2) (McCord 1984).

The ratios of cell-wall area to total area (MAX%) were measured using the DARWIN VIA System and Topographer III software (Telewski et al. 1983). The microslides were analyzed using transmitted Xenon light and a Nikon Optiphot microscope. Upper and lower gray level limits were chosen to distinguish the object from the background (Jagels and Telewski 1990). The program allows one to set these levels for each scan. For this study, the

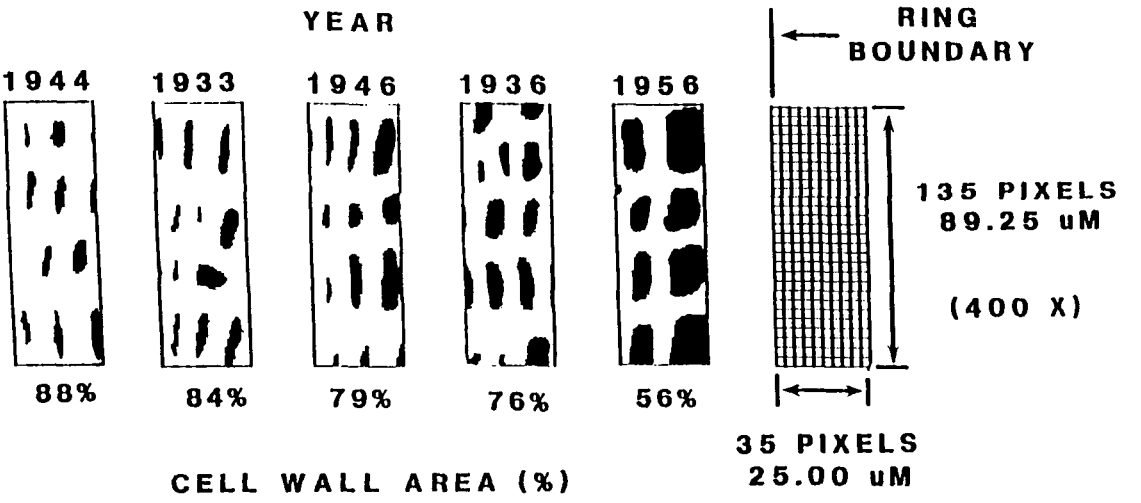

FIG. 1. Image scan window and the ratio of cell-wall area to total area for a range of selected measurements.

upper and lower level limits were fixed to separate the lumen (object) and cell wall (background) at the gray scale level of 75, out of 255 levels of gray (0 = Black, 255 = White). This level was found to be optimum for the specimen used in this study after sampling several growth rings within each growth ring series. Using a fixed gray level substantially reduced the time required for analysis. The scanning field or window was fixed to include about 3 tracheids in the radial direction and about 4 tracheids in the tangential direction (Fig. 1). The lengths of the radial and tangential sides of the scan window were 25.00 μm and 89.25 μm, respectively. Therefore, the scan field (2,231.25 μm^2) used in the analysis was about 14% of the effective window for the optical densitometer. Three arbitrarily selected locations in the last-formed latewood zone of each ring for four microsections were scanned to determine the variability in the MAX% measurements. Because the window area was fixed, the boundaries of the window were not always coincident with cell-wall boundaries (Fig. 1).

The coefficient of variation (CV) for each annual measurement was calculated from three replicated measurements for each of the 26 rings in a specimen to obtain within-specimen variabilities of the measurements MAXD and MAX%. The CV is derived by dividing the standard deviation by the mean of the annual observations. Since variability is often correlated with the range of the measurements, the CV enables a comparison of the variations among large values and the variations among small values. The between-specimen variability was also determined by the coefficient of variation (CV) derived from the four specimens. Each annual observation for a specimen is derived from the average of three replicated measurements.

The yearly coefficients of variation for the two analytical measurements were compared by the Wilcoxon-Mann-Whitney (WMW) rank sum test using the SAS program (SAS Inc. 1985). To examine possible differences in the measurement variability due to relative narrowness of rings, the WMW rank sum test was also separately applied to two groups of narrow and wide rings. Eighteen rings that are 0.4 mm or wider were designated as the wide group and eight rings that are smaller than 0.4 mm were designated as the narrow group. To examine the relationship between density and cell-wall area percentage, a linear regression analysis was performed to derive the R-square and linear equation.

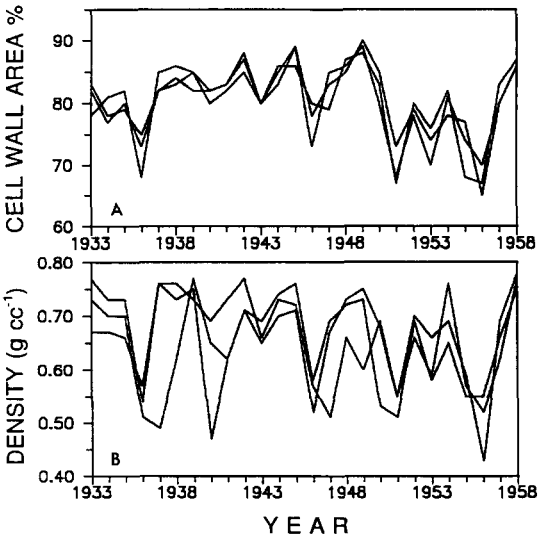

FIG. 2. Three replicated within-specimen measurements of: A. maximum cell-wall area % measured by VIA (MAX%) and B. maximum latewood density measured by XRD (MAXD).

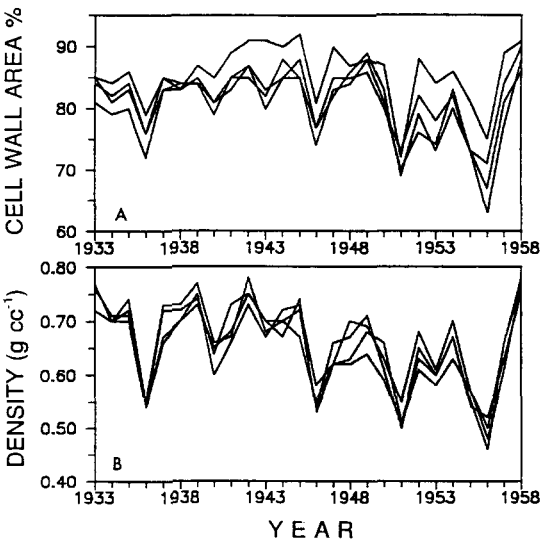

FIG. 3. Measurements of: A. maximum cell-wall area % measured by VIA (MAX%) and B. maximum latewood density measured by XRD (MAXD), of four specimens.

RESULTS

Figure 2 illustrates the within-specimen variabilities of the XRD and VIA measurements. The MAXD measurements apparently showed larger variability among three observations than the MAX% measurements. The between-specimen variabilities are shown in Fig. 3. The between-specimen variability in the MAX% measurements (Fig. 3A) is larger than the within-specimen variability (Fig. 2A), but is reversed for the MAXD measurements (Figs. 2B and 3). This tendency could be more clearly shown in the following analysis.

The results for the WMW rank sum test are given in Fig. 4. The overall within-specimen variability of MAX% was smaller (CV = 2.8%) than that of MAXD (CV = 6.4%). The difference was more obvious in the analysis of the wide-ring group. The between-specimen variability of both measurements was not significantly different in the analyses of all groups. In general, the between-specimen variabilities were greater in the narrow ring group than in the wide group. Figure 5 is a plot of MAXD vs. MAX%. The R-square for the linear regression was 0.780 ($P < 0.01$).

DISCUSSION

Large within-specimen variability in the MAXD measurements can be partially explained by errors induced by the densitometer. The optical densitometer used in this study does not compensate for the angle of ring boundaries. Some modified densitometers can facilitate this compensation (Lenz et al. 1976).

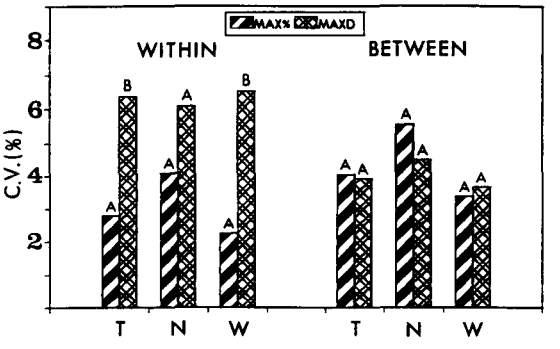

FIG. 4. Within- and between-specimen measurement variabilities determined by the coefficient of variation for maximum cell-wall area % measured by VIA (MAX%) and maximum latewood density measured by XRD (MAXD). Same capital letters between two bars represent insignificant ($P < 0.05$) differences (CV: coefficient of variation; T: total rings; N: narrow rings; W: wide rings).

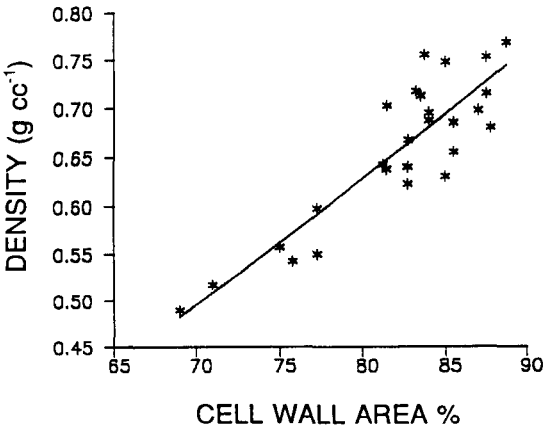

FIG. 5. Relationship between maximum latewood density (MAXD) and maximum cell-wall area % (MAX%) ($Y = 1.32X - 0.43$).

Large uncertainty involved in determining the ring boundary (latewood-earlywood) between adjacent rings also increases the variability. The technique employed in this study relies on arbitrary judgments of the actual ring boundary in density profiles (McCord 1984). This result suggests that densitometry may not provide a reliable estimate of MAXD unless the ring angle and boundary can be compensated for (McCord 1984; Schweingruber 1988). Some densitometric systems can minimize this problem by adjusting the scan direction by rotating the optical image of the X-ray film. Another factor is the slit size for the MAXD measurements. The resolution of rings, specifically narrow rings, decreases with increased slit size. Even though the scanning angle is well adjusted, the density scan corresponding to maximum latewood density can hardly exclude some undesirable cells such as resin ducts and adjacent earlywood cells.

Because of the relatively small scan area for VIA (14% of that used in densitometry), each scan represents only a small locality of the sample. On the other hand, integration of more cells in each densitometric measurement can be more representative of the sample if the boundary is well adjusted. Therefore, the sampling error associated with biological heterogeneity would be greater in the VIA measurements than in the XRD measurements.

VIA produced the less variable measurement within a specimen. It indicates that systematic errors associated with the optical densitometer used in this study may play a greater role in determining the precision of the measurements than the sampling errors. The superiority of VIA over XRD diminished in between-specimen measurements. This probably results from the inconsistent quality of microsections. The microsections for this analysis may show large differences because they were made during the early stage of microtechnique development described by Park (1991).

The relationship between cell-wall area % and density ($r = 0.7803$) was weaker than those reported in other studies. Diaz-Váz et al. (1975) obtained a correlation coefficient between relative cell-wall area and X-ray density of 0.9725 for *Pseudotsuga menziesii* and 0.9578 for *Pinus contorta.* A strong linear relationship between cellular dimensions and wood density ($r = 0.9846$) was reported for *Pinus sylvestris* by Merkel (1984). Swain (1987) also reported a strong linear relationship between cell-wall area percentage determined by VIA and X-ray density ($r = 0.99$) for *Pinus sylvestris,* using a relatively large VIA scan area of 57 μm $\times$ 264 μm. The reduced value of R-square in this study may be the result of the decreased scanning area used in the VIA. However, the relationship is still strong enough that cell-wall area percentage can be considered representative of the X-ray density if the image scan area is held constant.

The cell-wall area percentage and X-ray density can be compared to theoretical calculations for true density based on the calculated density of cell-wall substances. Haygreen and Bowyer (1989) report the density of cell-wall substances to be about 1.5 g cc^{-1}. This density is an ideal physical value for a lignified cellulosic cell wall that is completely nonporous. If there is no void within the cell wall, we can calculate the density as a function of the total volume of cell wall in a wood sample:

$$\text{density} = (\text{cell wall volume \%}/100) \times 1.5 \text{ g cc}^{-1} \quad (1)$$

Therefore, if a sample of wood was composed of 50% cell-wall material and 50% lumen or air space, the theoretical density would be 0.75 g cc^{-1}. However, the data presented in Fig. 5 indicate a large underestimation of density if cell-wall area percentage were substituted for cell-wall volume percentage in Eq. 1, if the assumption is made that area can be substituted for volume. For example, a cell-wall area of 80% correlates to an X-ray density of 0.64 g cc^{-1}. If 80% cell-wall area is substituted into the equation, the theoretical density should be 1.2 g cc^{-1}.

Some discrepancy in the density can be accounted for by minute cavities, capillaries, and irregularities in the cell walls; however, this should not exceed 5% of the cell wall (Haygreen and Bowyer 1989). Another source of error could be the assumption that area can be considered an infinitely thin volume and substituted into Eq. 1. As mentioned earlier, the problems of transmitted light through a wood sample are complicated by the alignment of the tracheids to the transverse surface being scanned (parallax). Error due to parallax would result in an artificially high measure of cell-wall area, which is consistent with the observed data in Fig. 5. Ideally, to remove this bias, the surface of the wood should be directly scanned, eliminating any possible parallax.

As said earlier, some error will arise in the X-ray densitometry methodology. The source of error, again, is a problem in parallax. If the growth ring boundary is not perfectly aligned to the X-ray source in the tangential plane during radiography, and not perfectly aligned to the optical scan beam on the transverse plane while scanning the radiograph, there will be an averaging of density with adjacent earlywood of the following growth ring. This averaging will result in a lower than predicted value for the latewood density of last-formed tracheids and a higher than predicted value for first-formed earlywood density.

CONCLUSIONS

VIA was a more precise method for determining density of the last-formed tracheids as a relative quantity (MAX%) than X-ray densitometry. The lower precision in X-ray densitometric measurements probably resulted from systematic errors associated with the optical densitometer used in this study, mainly due to the scanning aperture not being parallel to the ring boundaries as observed on the transverse face. These errors can be reduced by attaching a compensation prism on the optical densitometer to rotate the light beam (Lenz et al. 1976) or a photosensor rotatable and dividable to ignore irregularities on samples (such as the dendro 2003, Walesch Electronic). During the VIA, angles in the transverse ring boundaries are effectively compensated for by rotation of the sample on the microscope stage or by rotation of the video camera. The significant linear relationship between MAX% and MAXD indicates that MAX% measured by VIA can be used as an alternative to X-ray measurements of wood density. VIA has an ability to measure cell dimensions as well as density. In terms of dendrochronological studies, the value of MAX% could be used directly in climatic reconstructions without having to convert to a wood density value.

REFERENCES

BRÄKER, O. U. 1982. Alpine Europe. Pages 135–137 *in* M. K. Hughes, P. M. Kelly, J. R. Pilcher. and V. C. LaMarche, Jr., eds. Climate from tree rings. Cambridge Univ. Press, Cambridge, U.K.

CLAUSON, M. L., AND J. B. WILSON. 1991. Comparison of video and X-ray for scanning wood density. Forest Prod. J. 41:58–62.

CLEAVELAND, M. K. 1983. X-ray densitometric measurement of climatic influence on the intra-annual characteristics of southwestern semiarid conifer tree-rings. Ph.D. dissertation, University of Arizona, Tucson, AZ. 177 pp.

DIAZ-VÁZ, J. E., R. ECHOLS, AND W. KNIGGE. 1975. Vergleichende Undersuchung der Schwankungen von Trachеidendimensionen und rïntgenoptisch ermittelter Rohdichte innerhalb des Jahrings. Forstwissenschaftliches Centralblatt 94:161–175.

ELLIOTT, G. K., AND S. E. G. BROOK. 1967. Micropho-

tometric technique for growth-ring analysis. J. Institute Wood Sci. 18:24–43.

HAYGREEN, J. G., AND J. L. BOWYER. 1989. Forest products and wood science, 2nd ed. Iowa State University Press, Ames, IA. P. 196.

JAGELS, R., AND M. V. DYER. 1983. Morphometric analysis applied to wood structure. I. Cross-sectional cell shape and area change in red spruce. Wood Fiber Sci. 15:376–386.

———, AND F. W. TELEWSKI. 1990. Image analysis. Pages 76–93 *in* L. Kairiukstis and E. Cook, eds. Method of dendrochronology: Applications in the environmental sciences. Kluwer Academic Pub., Dordrecht, The Netherlands.

KELLOGG, R. M., AND F. F. WANGAARD. 1969. Variation of the cell-wall density of wood. Wood Fiber 1:180–204.

LENZ, V. O., E. SCHÄR, AND F. H. SCHWEINGRUBER. 1976. Methodische Probleme bei der radiographiisch-densitometrischen Bestimmung der Dichte und der Jahrringbreiten von Holz. Holzforschung 30:114–123.

MCCORD, V. A. S. 1984. A new computerized X-ray densitometric system for tree-ring analysis. M.S. thesis, Univ. of Arizona, Tucson, AZ. 150 pp.

MCMILLIN, C. W. 1982. Application of automatic image analysis to wood science. Wood Sci. 14:97–105.

MERKEL, H. 1984. Über den Witterungseinfluß auf die Jahrringstruktur der Gemeine Kiefer (*Pinus silvestris* L.): Ein Beitrag zur dendroklimatologie der Nadelholzer, Ph.D. dissertation, Albert-Ludwigs-Universität zu Freiburg i Br. 135 pp.

PARK, W.-K. 1990. Development of anatomical tree-ring chronologies from southern Arizona conifers using image analysis. Ph.D. dissertation, University of Arizona, Tucson, AZ. 234 pp.

———. 1991. Development of the microtechniques for the image analysis of tree rings. Research Reports of Agricultural Sci. of Chungbuk National Univ., Korea 9:16–21.

SAS INSTITUTE INC. 1985. SAS/STAT guide for personal computers, version 6. SAS Institute Inc., Cary, NC. 378 pp.

SCHWEINGRUBER, F. H. 1988. Tree rings: Basics and applications of dendrochronology. D. Reidel Pub. Co., Dordrecht, Holland. 276 pp.

STAMM, A. J., AND H. T. SANDERS. 1966. Specific gravity of the wood substance of loblolly pine as affected by chemical composition. Tappi J. 49:397–400.

STOKES, M. A., AND T. L. SMILEY. 1968. An introduction to tree-ring dating. Univ. of Chicago Press, Chicago, IL. 73 pp.

SWAIN, C. P. 1987. Dendroclimatology of *Pinus sylvestris* L. in the British Isles. Ph.D. dissertation, Liverpool Polytechnic, Liverpool, UK. 190 pp.

TELEWSKI, F. W., J. M. BURNS, AND L. ULAN. 1987. A thin-section technique for X-ray densitometric analysis of narrow tree-ring series. Pages 651–657 *in* G. C. Jacoby and J. Hornbeck, eds. Proceedings of the international symposium on ecological aspects of tree-ring analysis. August 17–21, 1986, Marymount College, Tarrytown, NY.

———, A. H. WAKEFIELD, AND M. J. JAFFE. 1983. Computer-assisted image analysis of tissues of ethel-treated *Pinus taeda* seedlings. Plant Physiol. 72:177–181.

TSOUMIS, G. 1964. Microscopic measurement of the amount of cell wall substance in wood and its relationship to specific gravity. Tappi J. 47:675–677.

YANOSKY, T. M., AND C. J. ROBINOVE. 1986. Digital image measurement of the area and anatomical structure of tree rings. Canadian J. Botany 64:2896–2902.