MORPHOMETRIC ANALYSIS APPLIED TO WOOD STRUCTURE. I. CROSS-SECTIONAL CELL SHAPE AND AREA CHANGE IN RED SPRUCE

Richard Jagels
Assistant Professor
College of Forest Resources, Forest Products Laboratory
University of Maine, Orono, ME 04469

and

Mary V. Dyer
Research Technician
College of Forest Resources, Forest Products Laboratory
University of Maine, Orono, ME 04469

(Received June 1982)

ABSTRACT

A new method for assessing seasonal change in cross-sectional tracheid shape in conifers is described and tested with red spruce. An equation (called circularity index) was used to reference cell shape to a circle with equal circumference. Cell lumen boundaries were measured on a rear projection digitizer coupled to a computer. Circularity indices and cell lumen areas were determined, and curves of these parameters were plotted across growth rings. Circularity index curves proved to be a sensitive measure of true latewood, and cell lumen area curves detected variation in transition latewood. Cell-wall area changes that were related to site differences correlated with earlier specific gravity determinations. Cell lumen area curves provided an explanation for nonsignificant specific gravity differences in spruce wood following nitrogen fertilization.

Keywords: Circularity index, cell lumen area, image analyzer, nitrogen fertilization, cell-wall area, red spruce.

INTRODUCTION

The measurement of parameters such as tracheid length, cell-wall thickness, and fibril angle generates data that correlate with certain wood properties, the usefulness of which has been well documented. However, one kind of measurement that has been difficult to assess in a quantitative way is cell shape. Consequently, researchers have used descriptive terms to denote such shape change as the progressive radial transformation of tracheid cross sections across a growth ring (i.e., cells become more rectangular or flattened), or used indirect measures of shape change (Mork 1928). With the development of sophisticated computers and digital, automated image analyzers, it is now possible to quantify cell shape and analyze and compare shape changes either spatially or temporally (Fisher 1972; Gahm 1972).

The applicability of automated or semi-automated image analysis to wood structure research has recently been demonstrated (Jagels et al. 1982; McMillan 1982; Quirk 1981). These systems greatly reduce the time required for the measurement of classical wood parameters. However, the greatest value of these automated systems may reside in their ability to rapidly process complex, interactive data, which will enable us to devise analyses beyond those traditionally performed.

Wood and Fiber Science, 15(4), 1983, pp. 376–386

The wood of red spruce (*Picea rubens* Sarg.), like that of other softwoods displaying a gradual transition from earlywood to latewood, poses special problems in the attempt to analyze seasonal variations in wood quality. Mork (1928) presented a method to assess the boundary between earlywood and latewood, but as Larson (1969) has pointed out, this method was not designed for gradual transition species. Staining techniques have been utilized to differentiate earlywood from latewood (Haasemann 1963; Wiksten 1954), but these have limitations and provide only a rough estimate of the boundary.

The formation of latewood involves two independent events: cell-wall thickening and the cessation of radial enlargement of tracheids (Larson 1969). That the two events are independent can be demonstrated by the anomaly of transition latewood, which exhibits only one of the two characteristics. Unfortunately, Mork's index (Mork 1928) permits some transition latewood to qualify as true latewood (Larson 1969).

Two cross-sectional morphometric parameters that can differentiate between earlywood and latewood are cell shape and cell area. If one postulates that cell-wall thickening is uniform around the cell—and data support this contention (Smith 1967)—then cell shape can be measured either at the lumen boundary or at the middle lamella boundary. The lumen boundary is generally easier to define visually.

A simple way of quantifying shape is to reference the observed shape to a known regular geometric shape. An unhindered biological cell with symmetrical internal forces evenly distributed over a bounding membrane of uniform thickness will assume a spherical shape, and a plane section through that sphere will be a circle. For a given circumference, a circle encloses the greatest area. If an observed cell cross section is enclosed by a shape other than a circle, its area will be less than the area enclosed by a circle with the same circumference. The ratio of the two areas will be a measure of the degree of deviation from circularity, or what can be termed circularity index. The formula derived from this relationship is

$$\text{C.I.} = 4\pi\frac{A}{C^2} \tag{1}$$

where C.I. = circularity index, A = area, and C = circumference. By definition, C.I. for any circle equals 1.0 and for any other shape is less than 1.0. A plot of C.I., cell by cell, across a growth ring should provide some measure of the initiation of latewood since a rectangular shape has a lower C.I. than a square shape (C.I. for perfect square = 0.785; C.I. for a rectangle with sides in a ratio of 1:2 = 0.698). A more thorough review of C.I. for quantifying shape in medical histology was presented by Biedenbach et al. (1975).

Cell area can be classified as: (1) total cell area (CAT), which is equal to the total area within the middle lamella boundary; (2) cell-wall area (CAW), which is equal to the area circumscribed by the middle lamella and limited, to the interior, by the cell lumen boundary; and (3) cell lumen area (CAL), which is equal to the area enclosed within the cell lumen boundary (Fig. 1).

A decrease in CAL signals either an increase in CAW, a decrease in CAT (cell-wall thickness remaining constant), or the concurrence of both (the possibility of both increasing is not likely in a radial file sequence for a coniferous wood). The

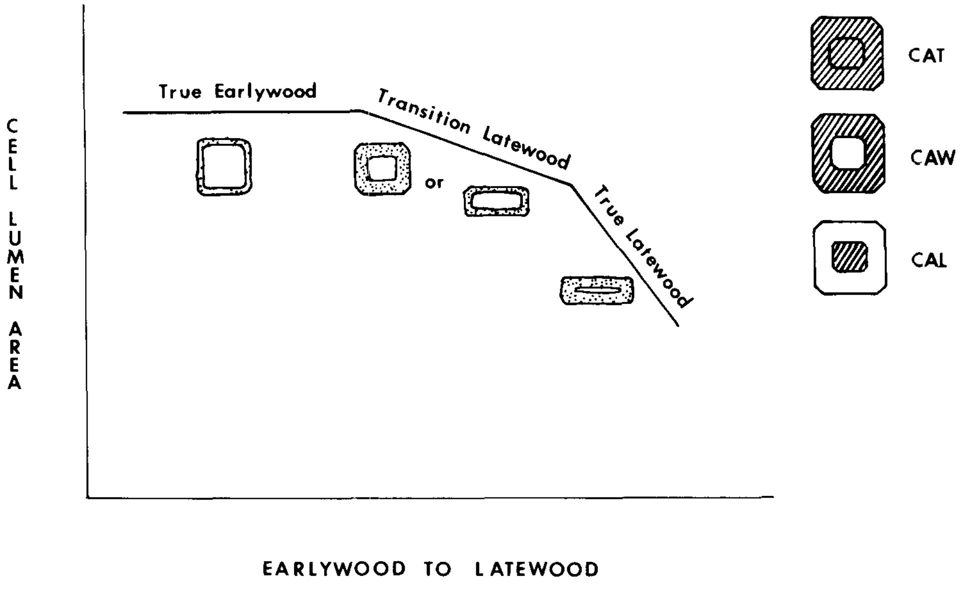

FIG. 1. A hypothetical cell lumen area curve across a growth ring showing theoretical slopes associated with true earlywood, transition latewood, and true latewood. Total cell area (CAT), cell-wall area (CAW), and cell lumen area (CAL) are depicted to the right.

independent increase of CAW or decrease of CAT each separately denotes transition latewood, while a synchronous increase of CAW and decrease of CAT signify the formation of true latewood. Therefore, the curve of CAL, plotted cell by cell across a radial file, should decline at a steeper angle at the beginning of true latewood than of transition latewood. A hypothetical curve illustrating these points is drawn in Fig. 1. The portion of the curve that is horizontal represents true earlywood where shape and cell-wall thickness do not change. Transition latewood, where either shape or cell-wall thickness, but not both, is changing is depicted by the next portion of the curve where the slope is not steep. The final portion of the curve depicts true latewood, where both shape and wall thickness have changed and the slope is steeper. The shapes of the actual CAL curves (Figs. 4–6, 8) are referenced to this hypothetical curve.

An analysis of both the C.I. curve and CAL curve for a radial file of cells across a growth ring should provide information concerning the boundary between earlywood and latewood as well as the presence or absence of transition latewood. The C.I. curve is unitless and, therefore, is strictly a measure of shape change, while the CAL curve provides additional quantitative information. Tracing the cell lumen boundary on a computer-coupled graphic digitizer provides the input data necessary to calculate both C.I. and CAL.

MATERIALS AND METHODS

Red spruce was the species chosen for this initial test of our equipment and parameters. The wood is generally classified as a gradual transition softwood species (Panshin and deZeeuw 1980). Samples were taken from trees being used to evaluate the effects of nitrogen fertilization on poorly drained ("wet") and well-drained ("dry") sites in Maine. Four breast-height discs were utilized representing plots identified by Minerowicz (1981) as: wet area, fertilized; wet area, control; dry area, fertilized; and dry area, control. Two samples, approximately ½-inch wide in the tangential direction, located on opposite sides of the tree, and including all growth rings from the year of fertilizer application outward to the bark, were cut from each disc, avoiding compression wood. Cross sections, 20 μm thick, were cut on a sliding microtome, stained with chlorazol black E, and mounted on 1 in. × 3 in. microscope slides. Photomicrographs were taken at a magnification of 32×, using a Zeiss Photomicroscope and high contrast Kodak Technical Pan Film 2415. The 35-mm negatives were inserted into cardboard slide mounts for rear projection on a Talos 660-RP digitizer interfaced with an IBM Model 3031 (Jagels et al. 1982). For each growth ring, the middle lamella perimeters of radial files of tracheids were traced using a radio-controlled cursor; and each lumen within that perimeter was traced. These measurements were made on the growth rings coinciding with the year of fertilization and the four years following fertilization. Computer programs were executed using the Cartesian coordinates generated during measurement to calculate circularity indices and cell lumen areas. The C.I. and CAL curves were plotted using a CALCOMP plotting system; these curves were photocopied onto 8½ in. × 11 in. transparency sheets so that various combinations of curves could be superimposed for analysis.

OBSERVATIONS

The C.I. and CAL curves were obtained from a limited sample of four trees. With this sample size, rigorous statistical tests were not performed. The observations in this study, therefore, provide only preliminary clues to possible physiological and resulting anatomical events. As such, they serve as a screening device and suggest phenomena that need to be tested in greater depth.

Circularity index

The C.I.'s were plotted along radial files of cells from first-formed earlywood tracheids to last-formed latewood tracheids. For most growth rings the plot showed a break in the curve near the end of the growth ring, changing from a zero or slightly positive slope to a strongly negative slope (Fig. 2). In all cases this point of slope change corresponds well with the approximate beginning of true latewood as defined by Larson (1969). Circularity index for a perfect square is 0.785, but tracheids have rounded corners and, therefore, earlywood tracheids have C.I.'s that range mostly between 0.8 and 0.9. Occasional dips in the earlywood portion of the curve identify cells that are flattened along the radial axis, possibly a result of pseudotransverse divisions (Fig. 2). A change in C.I. signals a change in shape, but does not indicate the direction of that shape change. For this reason it is important to select radial files of cells that are distant from rays since cells near rays often become flattened along the radial axis. Mork's index (Mork 1928) has been calculated and marked on the C.I. curves in Figs. 2 and 3. In all cases it occurs earlier in the growth ring than the break in the C.I. curve, but follows no consistent relationship with C.I. declivity.

For most growth rings, the slope of the C.I. curve turns steeply negative once the shift in slope occurs (Fig. 2). However, for a few growth rings the shift to a

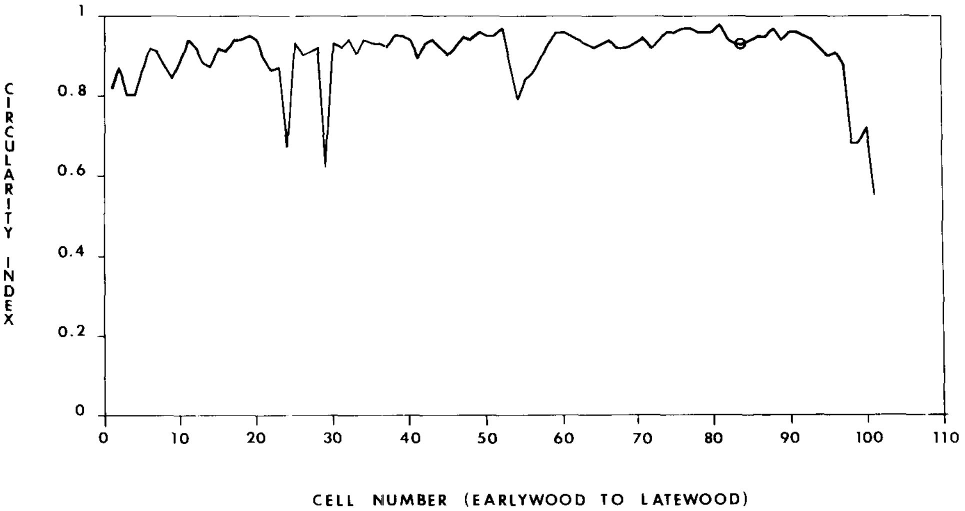

FIG. 2. Circularity index curve of a dry area, fertilized growth ring demonstrating abrupt slope change at the beginning of true latewood. The earlywood-latewood boundary as defined by Mork is marked by a circle.

negative slope is gradual and the declivity is less steep (Fig. 3). A closer look at these rings revealed, as expected, transition latewood with shape change but no wall thickening.

When C.I. curves for opposing radii of a single growth ring are compared, one often finds more cells produced on one side of the tree than on the other (Fig.

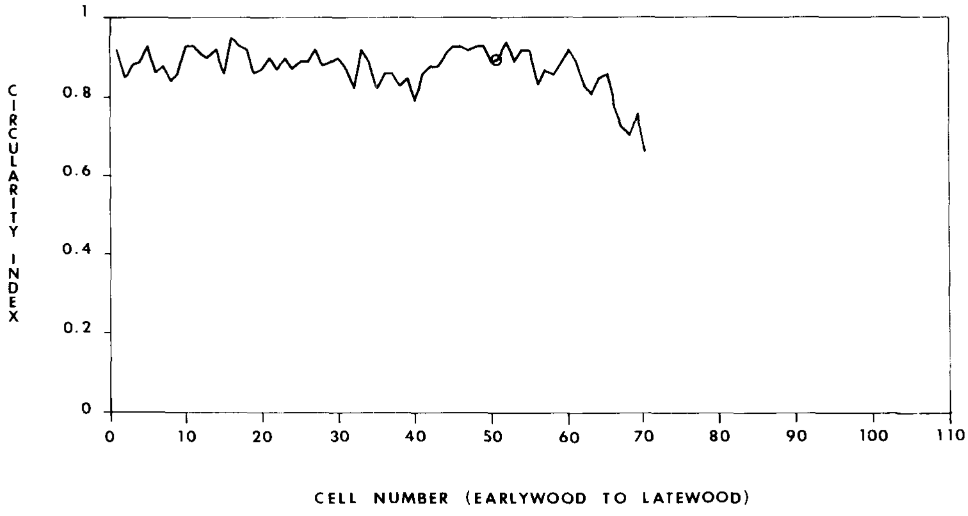

FIG. 3. Circularity index curve of a dry area, fertilized growth ring showing a gradual slope change at the beginning of transition latewood. The earlywood-latewood boundary as defined by Mork is marked by a circle.

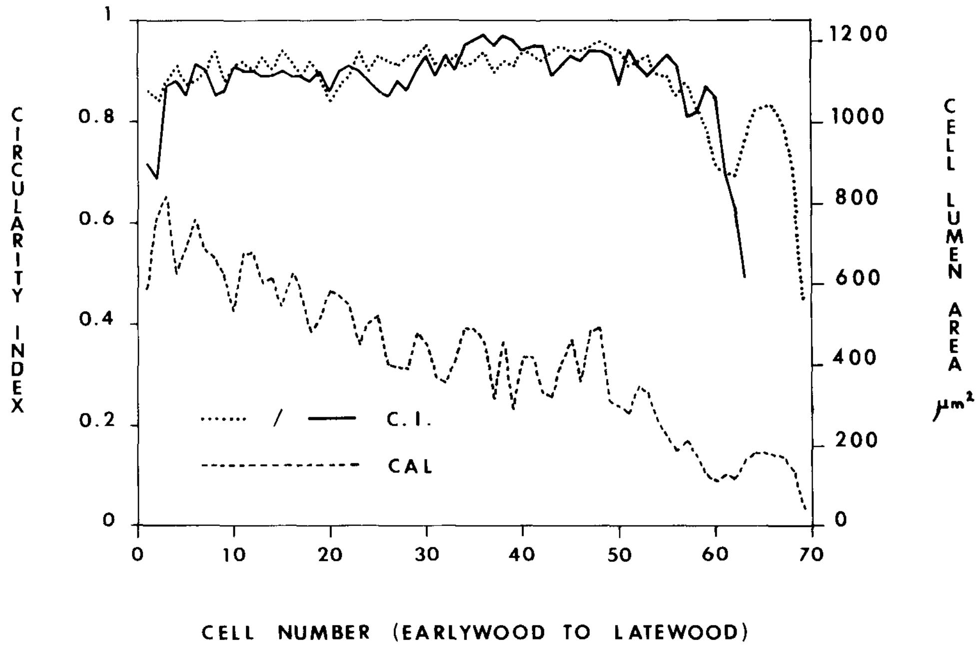

FIG. 4. Circularity index curves for opposing radii of a dry area, fertilized growth ring. The curve for the longer radius shows that the added cells may mark an incipient false growth ring. CAL curve for one radius is also plotted for reference.

4). Cell number is reasonably well correlated with ring width among all trees (coefficient of variation = 6.8%). If C.I. curves for opposing radii of the same growth ring are plotted together, one sometimes sees the relationship shown in Fig. 4. Both curves show a nearly simultaneous entry into latewood, but on the longer radius the curve suggests that the added cells had begun to revert to earlywood (curve shifts to positive slope), then changed back to latewood—a phenomenon that might be interpreted as an incipient false growth ring.

Cell lumen area

Curves for cell lumen area demonstrate that the production of transition latewood often begins early in the growing season. That is, either cell-wall thickening or cessation of cell enlargement often commences soon after the cambium initiates activity. This preliminary assessment indicates that cell-wall thickening precedes cessation of cell enlargement, but this aspect needs further analysis.

On the basis of the reasoning presented in the introduction to this paper, one would expect that the slope of the CAL curve would shift and become more steeply negative when true latewood commences; this can be seen in Fig. 5. In some cases, however, the shift from transition latewood to true latewood is more prolonged and the slope change is less apparent (Fig. 4). For some growth rings, CAL increases for the first few cells and then decreases (Fig. 6). This may be an important indicator of either late summer growth factors of the previous year or early spring factors of the current year.

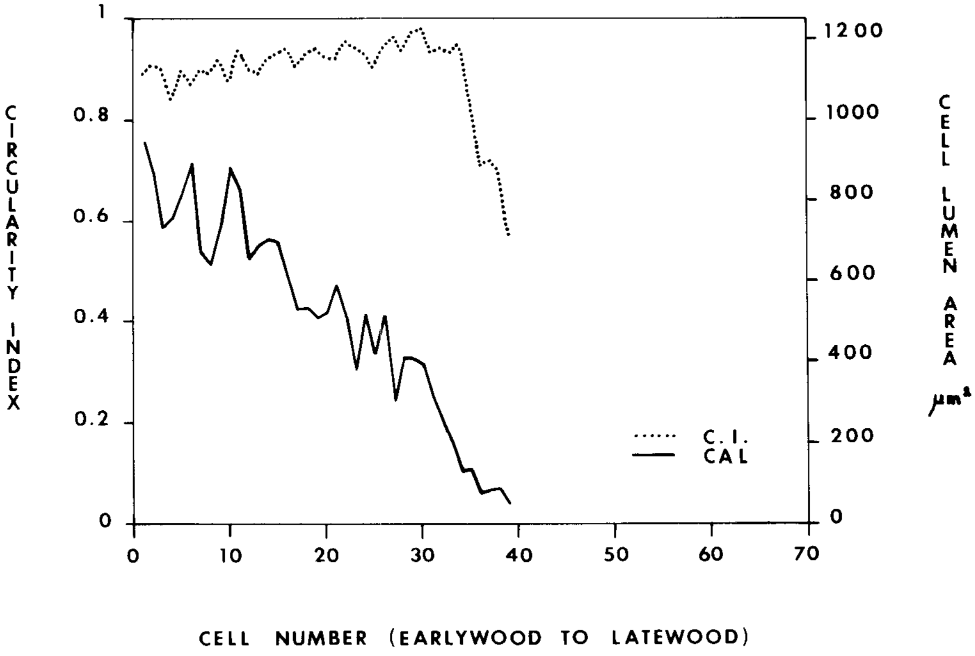

FIG. 5. C.I. and CAL curves of a dry area, fertilized growth ring. CAL curve approaches hypothetical curve (Fig. 1).

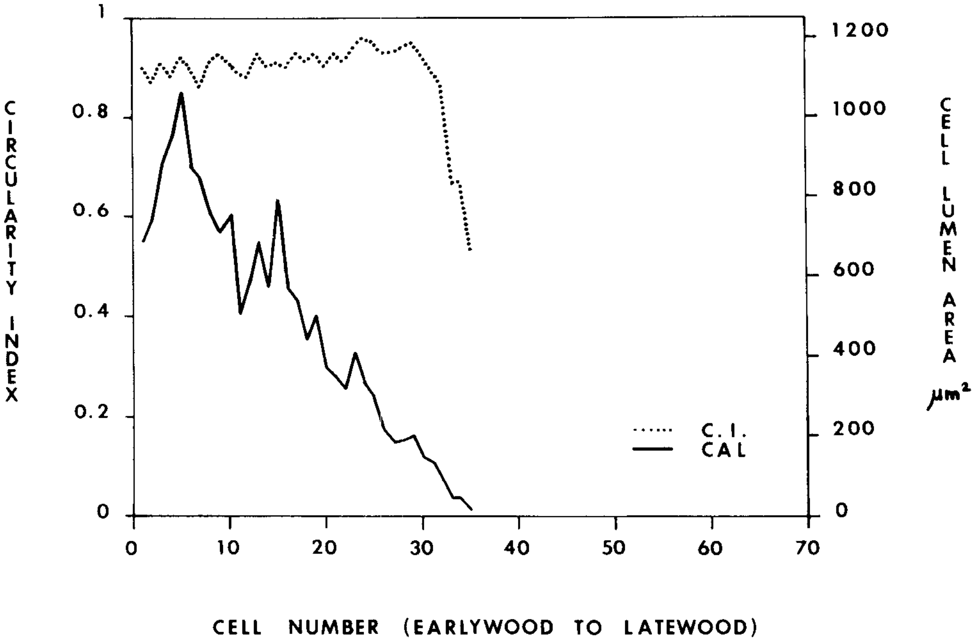

FIG. 6. C.I. and CAL curves of a dry area, fertilized growth ring. The steeply positive slope at the beginning of the CAL curve may be an important indicator of late summer or early spring growth factors.

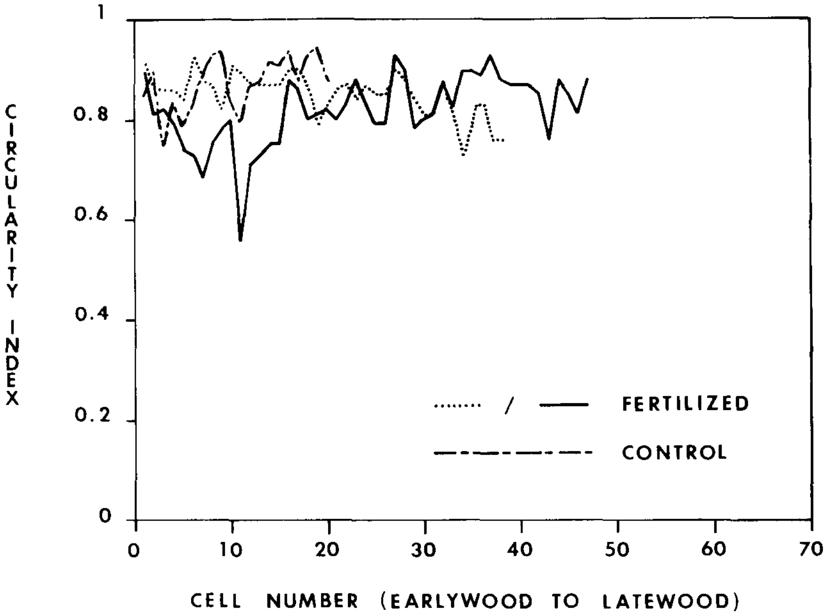

FIG. 7. C.I. curves of three wet area growth rings lacking true latewood.

Effect of site on C.I. and CAL

Of twenty growth rings analyzed, three rings lacked a true latewood as defined both by Larson's (1969) criteria and C.I. curves (Fig. 7). All were from wet site trees; one was from a control tree and two were from a fertilized tree. All three were wide growth rings within their respective series. This suggests that on wet sites when external factors favor increased growth, some internal factor may limit latewood production. Further investigation is required.

On dry sites true earlywood (as shown by CAL curves) persists for a very short period, or in some cases no horizontal portion can be observed on the curves, while on wet sites true earlywood persists for a longer period. These differences can be seen by comparing Figs. 5 and 8.

Cell lumen areas for the first-formed earlywood of dry site growth rings were larger than for wet site growth rings. This difference was greatest for control trees. An examination of percent cell-wall area for the growth rings confirmed that the difference reflected real cell-wall volume changes. Percent cell-wall area for the dry site control growth rings was 48.17, while for wet site control rings the value was 65.33. This agrees with specific gravity differences noted by Minerowicz (1981) who found higher specific gravities for wet site than dry site trees.

Effects of nitrogen fertilization on C.I. and CAL

Radial growth is enhanced on wet and dry sites following nitrogen fertilization but is greater on dry sites; the maximum response, however, occurs sooner on wet sites than on dry sites (Minerowicz 1981). On both wet and dry sites, the C.I. curves indicate that the increased wood production precedes true latewood formation. A look at the CAL curves shows that, in fact, this is mostly transition

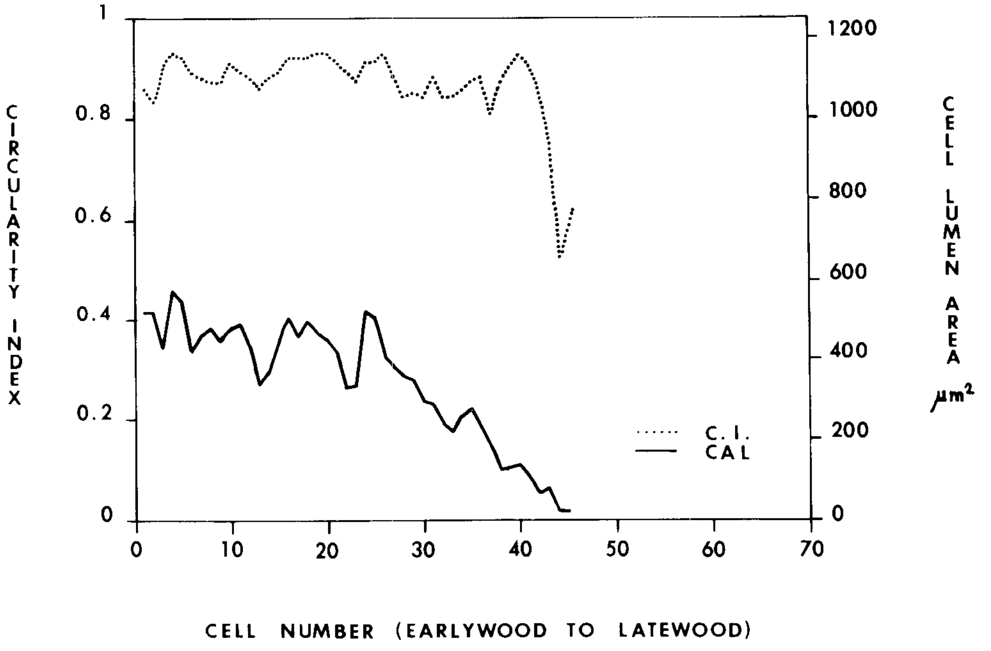

FIG. 8. C.I. and CAL curves of a wet area, fertilized growth ring. The true earlywood portion of the CAL curve is evidenced by a nearly zero slope. (Compare with Figs. 1 and 5.)

latewood (Figs. 4–6, 8). The lower percentage of latewood is offset by the increased amount of transition latewood so that percent cell-wall areas for the year of fertilization (50.3%—dry site; 56.8%—wet site) show the same relationship as that found at the year of maximum response (49.0%—dry site; 60.5%—wet site).

DISCUSSION

The morphometric analysis of tracheid cross-sectional shape, using circularity index, has been tested with a gradual transition conifer. Circularity index has proven to be a useful means for assessing cell shape change across a growth ring and is a reproducible indicator of true or functional latewood in red spruce as observed anatomically. The C.I. also appears to be a sensitive indicator of false growth rings or even minor cambial perturbations that result in cell shape change.

Morphometric analysis across a growth ring, by providing a sensitive, cell by cell, record of cambial activity through growth ring curves, obviates the necessity for artificial classifications of the growth ring. The terms *earlywood* and *latewood* clearly have different biological connotations in a ring porous hardwood versus a gradual transition softwood. But the categories may not even be comparable between abrupt transition and gradual transition softwoods. Direct plots of growth ring curves based on morphometric analysis provide the opportunity for direct comparison among different species for interpretive analysis.

Circularity index, in addition to providing information pertaining to cambial activity, should also be of value in assessing other shape changes in wood. For instance, C.I. might be used to assess the limits of compression wood on a cross section. A C.I. value limit, above that achieved in the first-formed earlywood tracheids, could be set on an automatic image analyzer and compression wood areas would be automatically plotted. Circularity index may also have potential in assessing pit aperture shape changes, such as those associated with drying defects or biological degradation.

By repeated measurements of the same radial files of cells on the Talos 660-RP digitizer, and more recently on a Zeiss Videoplan image analyzer, we have demonstrated the consistency of the measurements with different modes or operators. For future measurements, we have chosen to use the Zeiss Videoplan since it is a video interactive system, allowing us to immediately detect errors.

Although C.I. curves can detect transition latewood that is characterized by a shape change (Fig. 3), in general, they are a much less sensitive measure of transition latewood than CAL curves. The unitless nature of C.I. provides for a very sensitive test for true latewood, but the area-sensitive CAL curve provides more data on transition latewood.

Perhaps the most significant finding from the analysis of CAL curves was that transition latewood is initiated quite soon after cambial activity begins in red spruce, and this initiation occurs earlier in trees on dry sites than trees from wet sites. The transition latewood zone occupies an increasing percentage of the growth increment while true latewood percentage declines as ring width increases (compare Fig. 5 to Fig. 4). Calculated cell-wall area percentages (50.3% for Fig. 5 ring and 49.0% for Fig. 4 ring) suggest that the reduced percentage of true latewood is counterbalanced by the increase in transition latewood so that no change in percent cell-wall area occurs. These data may also explain the findings of Shepard and Shottafer (1979), who determined specific gravity values of breast-height samples of red spruce wood and found no significant differences following fertilization. Minerowicz (1981), however, found a 6.9% reduction in specific gravity, averaged over all trees, following fertilization. The present study is limited by sample size, while the two previous studies may be biased by uncontrolled factors such as compression wood.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Thomas Brann, Louis Morin, Suzanne Worcester, and William Phillips for guidance in computer processing and Douglas Gardner and Cynthia Muir for assistance with data collection.

REFERENCES

Biedenbach, M. A., R. W. Beuerman, and A. C. Brown. 1975. Graphic-digitizer analysis of axon spectra in ethmoidal and lingual branches of the trigeminal nerve. Cell Tissue Res. 157: 341–352.

Fisher, C. 1972. Current capabilities and limitations of available stereological techniques IV. Automatic image analysis for the stereologist. J. Microscopy 95H:385–392.

Gahm, J. 1972. Current capabilities and limitations of available stereological techniques I. Stereological measurements with lines, circles, and structural standards. J. Microscopy 95H:368–373.

Haasemann, M. 1963. Bestimmung des Spätholzanteils bei Fichten- und Kiefernholz mit Hilfe des Auflichtmikroskopes. Holztechnologie 4:277–280.

Jagels, R., D. J. Gardner, and T. J. Brann. 1982. Improved techniques for handling and staining wood fibers for digitizer assisted measurement. Wood Sci. 14(4):165–167.

LARSON, P. R. 1969. Wood formation and the concept of wood quality. Yale University, School of Forestry, Bulletin No. 74.

MCMILLAN, C. W. 1982. Application of automatic image analysis to wood science. Wood Sci. 14(3): 97–105.

MINEROWICZ, E. A. 1981. The influence of selected site factors on wood quality of spruce fertilized with nitrogen. M.S. thesis, University of Maine, Orono.

MORK, E. 1928. Die Qualität des Fichtenholzes unter besonder Rücksihtnahme auf Schleif- und Papierholz. Papier Fabrikant 26:741–747.

PANSHIN, A. J., AND C. DEZEEUW. 1980. Textbook of wood technology, 4th ed. McGraw-Hill, New York.

QUIRK, J. T. 1981. Semiautomated recording of wood cell dimensions. Forest Sci. 27(2):336–338.

SHEPARD, R. K., JR., AND J. E. SHOTTAFER. 1979. Effects of fertilization on specific gravity and growth rate of red spruce (*Picea rubens* Sarg.). Wood Sci. 12(2):100–102.

SMITH, D. M. 1967. Microscopic methods for determining cross-sectional cell dimensions. U.S. For. Serv. Res. Paper, FPL 79.

WIKSTEN, A. 1954. Method for measuring springwood and summerwood in annual rings III. Differentiation of spring and summerwood through staining. Meddel Fran Statens Skogsforsoksonstalt 34:476–488. Translation No. 19, Fac. For., University of British Columbia, Vancouver.