Longitudinal hardness and Young's modulus of spruce tracheid secondary walls using nanoindentation technique

R. Wimmer, B. N. Lucas, T. Y. Tsui, W. C. Oliver

Summary Using a mechanical properties microprobe, measurements of hardness and elastic modulus of tracheid walls in the longitudinal direction of spruce wood were obtained by continuously measuring force and displacement as a diamond indenter impressed a cell wall. Maximum mechanical properties were found at the edges of the walls of angular shaped tracheids. Both the hardness and elastic modulus of latewood cell walls were higher than cell walls in the earlywood. The high spatial resolution of this new concept of mechanical testing allows a direct comparison with ultrastructural and microchemical parameters of lignified cells which opens a wider area of applications for the understanding of intrinsic wood properties.

Introduction
Within each of the three secondary wall layers the microfibrils lie in helical, rather flat in S1 and S3 (some 70° to 50° to the cell length) and steeper in the central layer S2. The S2 layer is by far the thickest of all cell wall layers so that its strength controls the strength of the entire fiber (Wardrop and Dadswell 1951, Abe et al. 1991). An analogy can be drawn between the structure of the S2 layer and that of an unidirectional fiber-enforced composite material, in which the cellulosic fibrils represent the fiber enforcement, and the mostly amorphous hemicellulose and lignin, the composite matrix. According to this analogy, fibril angles of the S2 layer have a pronounced influence on the properties of a single fiber. This was demonstrated by Page et al. (1977) in their measurements of the elastic modulus.
of single spruce fibers. Salmen and de Ruvo (1985) estimated moduli of wood fibers at a given temperature and moisture content. Structural parameters of the fiber such as the amount of matrix material, the fibrillar angle of all layers and the relative thickness of these layers greatly influenced the elastic moduli of the fiber. Others have also attempted to compute stiffness from structural fiber models (Gillis 1969, Jaswon et al. 1968, Mark 1967, Treloar 1960). Even though over the years a number of these models have been employed in an attempt to quantify the theoretical strength of wood, no in-situ measurements at sufficient spatial resolution have been performed that reflect these theoretical numbers.

High spatial resolution mechanical tests, such as micro-tensile tests, have also been demonstrated e.g. by Wellwood et al. (1965), Kennedy and Ifju (1962), Grozdits and Ifju (1969) using microtomed (usually 80μm thick) wood sections. Leopold and McIntosh (1961) and others have performed tensile tests on individual wood fibers obtained through chemical maceration. A microcompression test perpendicular to the grain orientation have also been employed by Wilson (1964) across increments of several coniferous species. However, these methods suffer from certain restrictions like the strong chemical treatment during maceration or damaged, microcompressed and cut fibers during preparation with a microtome (Biblis 1970).

In this work, we have used indentation hardness as our testing concept for wood properties. According to Brinell, the impression of a steel-ball on a smooth metallic surface, not too close to the edges, delivers clear, reproducible results. At the beginning of the century, Janka (1906) proposed and developed a modified Brinell-hardness test for wood. The static force required to embed a steel hemisphere 0.444 inch (11.5 mm) in diameter, corresponding to a projected hemispherical surface of 1 cm², completely into the wood is measured. Because the steel hemisphere covers a wide range of cellular structures, hardness is approximately proportional to the density of the wood or cell mass per unit volume. This concept has made little progress for wood since the early work of G. Janka. Although there have been many studies which led to a variety of tests, there are shortcomings with all of them which led Kollmann and Coté (1968) to the statement, that “the testing of hardness of wood is a rather nebulous question”. Furthermore, the various existing hardness values are not easily comparable to one another and do not allow direct comparisons with those of other materials.

The distinguishing feature of the hardness indentations with which this publication is concerned is their size. Using a mechanical properties microprobe allows one to evaluate the mechanical response of a sample with submicron spatial resolution (Oliver 1986, Oliver and Pharr 1992, Page et al. 1992). The fact that the depth resolution is extremely high (0.16 nm) means that the indenter can be used to sample very small volumes of material. While an experiment takes place, the indenter position relative to the surface is continuously monitored during the indentation and subsequently during the unloading process. With knowledge of the indenter geometry, the contact area can be determined and the nanohardness value for the applied load can be calculated (Oliver 1986). Unlike conventional hardness testers, it is not necessary any more to optically determine the area of an indentation in order to calculate the hardness.

**Material and Methods**

From a 80-year old red spruce tree (*Picea rubens* Sarg.) we took a 5 mm increment core and prepared a sequence of tree rings in a way that cross sectional areas of about 1 mm² were obtained. Samples were prepared as usually done for a
transmission electron microscopy study. To minimize leaching effects as well as possible changes in chemical composition, specimens were air dried at room temperature for several days and oven dried at 70 °C for about 20 minutes before embedding in resin according to the method of Spurr (1969). The compact structure of the woody cell wall allows no penetration with the embedding medium and thus a phase boundary exists between cell wall and the polymeride (Jayme and Fengel 1961, Fengel 1967). Under vacuum, the resin filled up the lumina and finally the resin was cured in the usual way. A surface on the embedded specimen was sectioned using a Reichert-Ultramicrotome with a diamond knife. Mechanical tests were done in pure cell wall material and the microtomed samples provided sufficient surface quality for the indentation tests. The specimens were glued on aluminum stubs using a laser technique for perpendicular alignment. Mounted specimens were fixed in the stage of the Nano Indenter II® (Nano Instruments, Inc. Knoxville, TN).

The operation principles of the computer-controlled Nano Indenter II® are described in detail elsewhere (Oliver and Pharr 1992, Willems et al. 1993). The Nano Indenter II® was enclosed in a heavy wooden cabinet, the major purpose of which is to ensure thermal stability during an experiment. The indenter itself was suspended on a pneumatic antivibration table to isolate it from building vibrations. The apparatus was located in a room stabilized at 21 °C and relative humidity was around 60% throughout the experiment. With the Nano Indenter II®, small loads in the order of 0.4 mN can be applied to a pyramidal diamond indenter, having a triangular tip at the bottom of the indenter rod, and the resulting depth displacement can be measured to 0.16 nm. A load-controlled instrument mode is used to examining hardness at shallow indentations. The system monitors the load as well as the loading rate continuously during the loading and unloading segments of the indentation procedure.

In our indentation experiments we used a test procedure determined during a previous pilot study. Each indentation experiment consisted of six segments (Fig. 1). The first segment was the approach segment with an indenter surface approach rate of 10 nm/s. Once the indenter contacted the surface it was loaded at constant rate of 2 μN/s to an indentation depth of 80 nm (A). Subsequently, a hold segment (B) of 30 seconds was inserted. This hold segment, inserted immediately after the loading segment, is not an essential part of an indentation
experiment but offers an opportunity to monitor possible creep or mechanical stabilization.

In the following unloading segment (C), 90% of the load was programmed to be removed at an unloading rate of 1.6 μN/s, after which a second hold segment of 30 seconds (D) was imposed. This hold segment, inserted after a partial unloading segment, provided the opportunity to correct for the thermal drift of the apparatus during the indentation process. Finally, a total unloading segment (E) removed the indenter from the specimen.

A remote video control system was installed to facilitate the programming of the individual indentation positions. The specimen was subsequently transferred to the indenter by means of a computer-controlled high precision X-Y stage with ±0.5 μm resolution, and an entire series of preprogrammed indentations were done in a single experimental run. No more than 15 indentations for one test run were programmed to maintain positioning resolution. After test completion, the video system was used to inspect the indentations carefully. An atomic force microscope in contact mode was also used to evaluate the surface for position and quality of the indentations (Fig. 2).

Oliver and Pharr (1992) demonstrated that the solutions for an indenter in the shape of a parabola of revolution can be used to obtain accurate results for indentation load-displacement data obtained with a Berkovich indenter. Referring to Fig. 3, at any time during loading, the total displacement \( h \) is written as

\[
h = h_c + h_s
\]

where \( h_c \) is the vertical distance along which contact is made (contact depth) and \( h_s \) is the displacement of the surface at the perimeter of the contact. Upon un-
loading, the elastic displacements are recovered, and when the indenter is fully withdrawn, the final depth of the residual hardness impression is $h_f$.

The three key parameters obtained during the experiment are peak load ($P_{\text{max}}$), the depth at peak load ($h$), and the initial unloading contact stiffness ($S$). It should be noted that the contact stiffness is measured only at the peak load, and no restrictions are placed on the unloading data being linear during any portion of the unloading. The analysis begins with the equation:

$$E_r = \frac{\sqrt{\pi} S}{2 \sqrt{A}}$$

which relates to reduced modulus, $E_r$, to the contact area, $A$, and the measured stiffness, $S$. This relationship is based on the work of Bulychev et al. 1976, Bulychev and Alekhin 1987, and holds for any indenter that can be described as a body of revolution of a smooth function and is thus not limited to a specific geometry (Pharr et al. 1992). Measurement of the initial unloading slope can thus be used to determine the reduced modulus, $E_r$, if the contact area at peak load can be measured independently. The area of contact at peak load was determined using the geometry of the Berkovich indenter (Fig. 4) and the depth of contact, $h_c$.

The reduced modulus, $E_r$, accounts for the effect of elastic deformation of the indenter through the assumption that a similar elastic process occurs in the indenter as well as in the sample. The resultant definition of $E_r$ adapted from Stillwell and Tabor (1961) is therefore:

$$E_r = \left(\frac{1 - v_s^2}{E_s} + \frac{1 - v_i^2}{E_i}\right)^{-1}$$

where $v_s(i)$ is Poisson’s ratio and $E_s(i)$ is Young’s modulus of the sample (indenter). Thus, the determination of the Young’s modulus by nanoindentation is based upon the elastic behavior of the material that might possibly be slightly plastically deformed. We used longitudinal Poisson’s ratios for densified wood (Staypak), which is a material made by compressing wood under high temperature and pressure, which has a final density nearly as high as the value cited for the cell-wall substance (up to 1400 kg/m$^3$, Mark 1967). It gives the only direct
Fig. 4. Geometry of the Berkovich indenter as used in the experiments

Measurement available for cell wall Poisson's ratios (Table 1). Finally, the hardness (H), the mean pressure the wood material will support under load (P_max), is defined as:

\[ H = \frac{P_{\text{max}}}{A} \]

where A is projected area of contact at peak load (Fig. 3 and 4). It should be noted that the hardness measured using this definition may be different from that obtained from conventional definitions in which the area is determined by direct measurement of the size of the residual hardness impression. The reason for the difference is, that a portion of the contact area under load may not be plastically deformed, and as a result, the contact area measured by observation of the residual hardness impression is less than that at peak load.

Parts of the measured data were subjected to an analysis of variance that used a general linear-model procedure (GLM) of the SAS statistical software package (SAS Institute, Cary, NC). We used Tukey's multiple range test because it controls the maximum experimentwise error rate and can handle unequal cell sizes. Level of significance for comparisons among the means was 5%.

<table>
<thead>
<tr>
<th>Species</th>
<th>( v_{LR} )</th>
<th>( v_{LT} )</th>
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<tbody>
<tr>
<td>Engleman spruce</td>
<td>0.44</td>
<td>0.50</td>
</tr>
<tr>
<td>Norway spruce</td>
<td>0.38</td>
<td>0.51</td>
</tr>
<tr>
<td>Sitka spruce</td>
<td>0.37</td>
<td>0.47</td>
</tr>
<tr>
<td>Staypak</td>
<td>0.397</td>
<td>0.447</td>
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Results

Mechanical properties of different cell wall locations
Tests were employed on cross cut radial and tangential cell walls as well as on the
cell wall edges of the angular shaped tracheids from six tree rings within the same
tree. The means and standard deviations of the nanoindentation values for
hardness and Young's modulus are presented in Table 2. Tukey's multiple range
test showed significant higher hardness and Young's modulus values for the edge
wall location than for the tangential cell wall (p < 0.05). There was no significant
difference in hardness and Young's modulus between the radial and tangential
cell wall.

<table>
<thead>
<tr>
<th>Table 2. Longitudinal hardness and Young's modulus (GPa) at different tracheid wall locations (S.D. standard deviation, n number of measurements) for red spruce</th>
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<tbody>
<tr>
<td>Cell wall location</td>
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<tr>
<td>--------------------</td>
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<tr>
<td>Radial wall</td>
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<tr>
<td>Tangential wall</td>
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<td>Wall edge</td>
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Mechanical properties of earlywood and latewood cell walls
With this test series on six tree rings we were only able to complete measurements
on seven earlywood cell walls that showed reliable results (based on visual post-
indentation evaluation) while in latewood 70 measurements were obtained. Mean
values for hardness and Young's modulus were higher for latewood cells but
Tukey's range test was not significant at the 5% level. Therefore an additional
nanoindentation test series for a single tree ring was performed along a radial
tracheid file starting with the last cell in latewood and finishing on the first
earlywood tracheid. We tested exclusively on tangential walls and used Mork's
criterion (Denne 1989) to classify the results in latewood, transition- and early-
wood (Table 3). Latewood tracheid cell walls in the longitudinal direction ac-
cording to this result were harder and had more higher elastic modulus than
earlywood tracheid cell walls.

<table>
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<tr>
<th>Table 3. Longitudinal hardness and Young's modulus (GPa) of earlywood-, transitionwood- and latewood-tracheids measured along a radial tracheid line (S.D. standard deviation, CV% coefficient of variation, n number of measurements)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Earlywood</td>
</tr>
<tr>
<td>Hardness</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>min</td>
</tr>
<tr>
<td>max</td>
</tr>
<tr>
<td>SD</td>
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<tr>
<td>CV%</td>
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<tr>
<td>n</td>
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</table>
Mechanical properties and relative tracheid wall thickness
On 75 measured latewood tracheids out of six tree rings we evaluated the relative position of the indentation across the cell walls. Plotted data of mechanical properties against their relative wall position showed maxima in the middle of the secondary wall with a slight drift toward the middle lamella side (Fig. 5).

Discussion
We measured a mean longitudinal hardness for pure cell wall of 0.3 GPa with maximum values of 0.38 GPa and a Young's modulus of 14.6 GPa with maximum values up to 30 GPa. Cave (1968) did a mathematical stress analysis and compared results with measured tensile strength as a function of mean microfibril angle related to the cell wall material present in the wood sample. According to his findings Young's modulus of dry pure samples varies from 28 GPa in earlywood to 35 GPa in latewood. Gibson and Ashby (1988) used 35 GPa as a Young's modulus for pure cell wall. Suzuki (1969) in his study also utilized the relationship between fibrillar angles and Young's modulus and presented Young's moduli for S2 wall layer in earlywood of 16 GPa and in latewood of 28 GPa for Cryptomeria japonica wood samples. Our maximum values are in the range of these numbers although the average Young's modulus was lower than reported in the literature. Young's moduli for wood fibers from the literature are thoroughly based on the analogy of helically wound fibril-reinforced composite rubes assuming that other factors are not relevant (Cave 1968). Our in-situ measurements reflect also the variability of lignin and cellulose, cellulose crystallinity, cellulose chain length and other parameters. Moisture, because of its effect on mechanical properties of wood was an important factor in our experiments too. The sample surface interacted with room climate conditions. Our experiments were per-
formed under a controlled climate of 21 °C and 60% rel. humidity. Equilibrium moisture content of wood for this climate is 10.9% (Kollmann and Coté 1968). According to ASTM D 2555-73 for red spruce grown in the United States the ratio between dry and green Young's modulus is 1.28 (Bodig and Jayne 1982). Therefore, Young's modulus of the specimen measured could have easily been lowered by 10%. Furthermore, the embedding procedure used (Spurr's resin) could have caused minor chemical modification effects and changes of intrinsic molecular structure. But based on previous studies (Erickson and Rees 1940, Saka et al. 1982, Saka and Goring 1983) there is little evidence that lignin or other chemical components in the specimen are altered for such reasons.

The measured hardness of the cell walls is around 0.3 GPa which is in the same range as hardness for pure Aluminium. Ylinen (1943) has given an empirical formula to estimate Brinell-hardness ($H_B$) for wood:

$$H_B = (\beta \rho_0 + \alpha)10^{-4}[\text{GPa}]$$

For axial (end)-hardness Ylinen (1943) gave constants for $\beta = 1263.3$ and $\alpha = -213.3$ and using the cell wall density of $\rho_0 = 1.5\, \text{g/cm}^3$ a hardness of 0.17 GPa is calculated. Kollmann and Coté (1968) reported a strong relationship between maximum crushing strength and hardness. Janka (1906), evaluating the results of hardness tests of 280 wood species, found an empirical relationship between hardness ($H_J$) and crushing strength ($\sigma_{cB}$):

$$H_J = (2\sigma_{cB} - 0.05)[\text{GPa}]$$

Within a tracheid we clearly found maximum mechanical properties at the edges of angular shaped tracheid cell walls. No reference was found that would reflect this finding. It is likely that in normally angular shaped spruce tracheids local packing densities in the laminar structure in $S2$ is higher at the edges than in the middle of radial and tangential cell walls. A mechanistic explanation would be that the cell walls suffered from a pre-stress treatment at the edges during the process of cell wall expansion and thickening which would have resulted also in locally higher mechanical properties.

Pure cell walls in latewood of spruce have higher mechanical properties than in earlywood for several reasons. Degree of crystallinity of cellulose is higher in latewood than in earlywood (Lee 1961). The cellulose microfibrils exhibit smaller (steeper) angles in latewood than in earlywood when measured in the same tracheid row (Preston 1934). Wu and Wilson (1967) found a general pattern of variation in lignin content with maximum amounts in earlywood and minimum in the latewood. The reported lignin differences average only a little over 2 percent and range from 1 to 5 percent. Inversely, cellulose has its maximum in latewood and its minimum in earlywood (Hale and Clermont 1963). These variations within the growth ring explain the higher values of hardness and Young's modulus for latewood.

The numbers for hardness and modulus of elasticity against their relative positions within a cell wall (Fig. 5) implies two interpretations. The shift downward the middle lamella indicates the change in chemical composition thereof (Saka et al. 1982). The smoother shift downwards $S3$ also reflects the influence that may come from the resin present in the cell lumina. Resin hardness was about half of the hardness measured for cell walls (0.127 GPa) and modulus of elasticity was 1/6 of the cell wall elasticity (3.4 GPa).
Generally, the linear dimension of the indent is roughly 7 times the indentation depth. The plastically deformed zone can be considered to be contained within a hemispherical cap below the indenter that has a diameter equal to the diameter of the indent which was approximately 1 μm in our experiments (Fig. 2). But the elastic strain field on the other hand is going to extend further into the surrounding material with approximately up to 10 times the diameter of the indent. Because adhesion between the innermost cell wall layer and the resin at the phase boundary is usually not very good the resin effect should be small even when the measurement was done closely to the phase boundary between cell wall and resin. The high coefficients of variation (Table. 2) of the mechanical properties measured in earlywood cell walls (only 4–8 μm in thickness) are evidences for the influence of surrounding material on the measurements.

Conclusions

In conclusion, the nanoindentation technique provides a new insight for high spatial resolution mechanical properties of wood. For the first time, mechanical properties on single tracheid cell walls in earlywood and latewood were measured in-situ. Mechanical properties vary not only within a tree ring, but significant differences were also found within a single tracheid. Further investigations are required to optimize preparation of samples and various test conditions.

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