COMPARING MECHANICAL PROPERTIES OF SECONDARY WALL AND CELL CORNER MIDDLE LAMELLA IN SPRUCE WOOD

by

Rupert Wimmer 1 & Barry N. Lucas 2

SUMMARY

Mechanical characterizations of the S2 layers and the cell corner middle lamella in the axial direction were investigated in spruce wood. A mechanical properties microprobe capable of measuring hardness and Young’s modulus on a spatially resolved basis similar to that of an electron beam microprobe was used. Hardness of the cell corner middle lamella was found to be almost as high as that of the secondary wall, but the Young’s modulus of the cell corner middle lamella was 50% less than that of the S2. The S2 showed constant hardness over its range of Young’s modulus, but the cell corner middle lamella exhibited a strong correlation (R² = 0.55) between hardness and the Young’s modulus. Further investigations are needed to directly combine chemical and micromechanical properties and also to investigate the mechanical effects of the high variability of cell corner middle lamella chemistry.

Key words: Spruce, Picea, hardness, Young’s modulus, secondary wall, middle lamella, mechanical property.

INTRODUCTION

High resolution mechanical property measurements of cell wall material have been done previously. Such studies have included micro-tensile tests of microtomed (usually 80 µm thick) wood sections (e.g., Wellwood et al. 1965; Kennedy & Ifju 1962; Grozdits & Ifju 1969), tensile tests on individual fibers obtained through chemical maceration (e.g., Leopold & Macintosh 1961; Schniewind et al. 1965), and a micro-compression test done perpendicular to the grain (Wilson 1964). Because the S2 layer is the thickest cell wall layer it is been suggested that it controls the strength of the entire fiber (Wardrop & Dadswell 1951; Abe et al. 1991). An analogy can be drawn between the structure of the S2 layer and that of a unidirectional fiber-reinforced composite material, in which the cellulose fibrils represent the fiber reinforcement, and the mostly amorphous hemicellulose and lignin represent the composite matrix. However, the mechanical behavior of tissues cannot be explained solely by the material properties of secondary cell walls and other factors need to be considered, one being the physical properties of the middle lamella.

1) Universität für Bodenkultur Wien, Gregor Mendel-Strasse 33, 1180 Vienna, Austria.
2) Nano Instruments, Inc., P.O. Box 14211, Knoxville, Tennessee 37014, U.S.A.
In un lignified woody tissues, the primary walls of adjoining cells are separated by a pectic layer, called the middle lamella, that essentially acts as a cementing agent. Lignin first is deposited in the primary wall at the cell corner and then in the cell corner middle lamella, tangential compound middle lamella, radial compound middle lamella, and secondary wall (Donaldson 1991). The impregnation of lignin in the cell wall and the middle lamella can alter the material properties of these structures. When lignification occurs, the distinctions between the primary cell walls of adjoining cells and the intervening middle lamella are lost, and the three layers are then referred to as the compound middle lamella. The volume fraction of the compound middle lamella in coniferous wood is c. 10–12% of the woody tissue volume (Fergus et al. 1969).

To measure the mechanical properties of the S2 and the compound middle lamella, depth-sensing indentation was applied as a testing method in this study. 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 completely embed a steel ball 0.444 inch (11.5 mm) in diameter, corresponding to a projected hemispherical surface of 1 cm², into the wood was measured. Because the steel hemisphere covers a wide range of cellular structures, the measured hardness is approximately proportional to the density of the wood or cell mass per unit volume.

The distinguishing feature of the indentations used for this investigation is their very small size. Using a mechanical properties microprobe allows one to evaluate the mechanical response of a sample with submicron spatial resolution (Oliver 1986; Oliver & Pharr 1992; Page et al. 1992) and this method has recently been used on woody tissues (Wimmer et al. 1997). The objectives of the current study were to 1) simultaneously quantify the patterns of hardness and Young’s modulus of the cell corner middle lamella (CCML) and the secondary wall (S2), and 2) look for differences and trends in these properties across tree rings of spruce.

MATERIAL AND METHODS

From a 80-year-old red spruce tree (*Picea rubens* Sarg.) a 5 mm increment core was taken and a sequence of five tree rings prepared so 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 then 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 of the embedding medium and thus a phase boundary exists between the cell wall and the polymeride (Jayme & Fengel 1961; Fengel 1967). Under vacuum, the resin filled the lumina and then 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 of pure cell wall material and of the cell corner middle lamella. 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 & Pharr 1992; Willems et al. 1993; Wimmer et al. 1997).
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®, loads on the order of a few μN can be applied to a pyramidal diamond indenter, with a resolution better than 50 nN, and the resulting depth displacement can be measured to 0.16 nm. A load-controlled instrument mode is used in examining hardness at shallow depths. The system controls the load as well as the loading rate continuously during the loading and unloading segments of the indentation procedure. 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; Wimmer et al. 1997). Unlike conventional hardness testers, it is not necessary to optically determine the area of an indentation in order to calculate the hardness (Fig. 1a).

Because the radial and tangential oriented compound middle lamella were too narrow (<0.5 μm), the indentation tests were done solely on cell corner compound middle lamella (CCML) which provided surfaces of approximately 2 × 2 μm (Fig. 1b). Two sets of measurements were made. In the first set 5 tree ring samples were prepared and subsequently measured with the Nano Indenter II®. Each indentation was checked carefully using reflected light microscopy and for more detailed analysis an atomic force microscope in contact mode (Park Scientific Instruments®) was used. In total 267 single measurements were obtained. All these measurements were done solely in latewood because their tracheid walls were wide enough for locating the indentations. The second set of measurements characterized properties along a radial file of tracheids. In total, properties of 12 consecutive tracheids, and the adjacent CCML, were measured over a distance of 250 μm. Measurements started with the last latewood tracheid and proceeded toward the earlywood.

RESULTS

Dataset 1

Figures 2a and b are box-and-whisker plots showing Young’s modulus and hardness of radial and tangential S2-walls, CCML and also Spurr’s resin as a control. In this data set (267 measurements), the average Young’s modulus of tangentially and radially oriented tracheid walls was 16 GPa and the hardness was 0.30 GPa. Differences between radial and tangential cell walls were not significant. Both hardness and Young’s modulus of the CCML were lower than the S2, but to a different degree. The S2 layer displayed an elastic modulus nearly twice that of CCML but the S2 was only 10% harder than CCML.

Dataset 2

The second data set comprised measurements made across a 250 μm transect along a radial file of tracheids in a single tree ring (Table 1). The Young’s modulus of S2 walls in this second dataset was nearly three times as high as the CCML, and the hard-
ness of the $S_2$ walls was approximately 20% more than the CCML. Coefficients of variation were considerably higher for CCML than for $S_2$ walls. Figures 3a and b depict the mechanical trends for CCML and $S_2$ layers for the measured distance from latewood towards earlywood.
Three out of 76 CCML hardness values were above those of the $S_2$ walls, which could be due to scatter. The hardness of the very last latewood tracheids in the growth ring is low, but hardness increases significantly in the subsequent 1–2 tracheids. In contrast, CCML values do not show this difference. The lowest values for Young’s
modulus and hardness of both the $S_2$ and the CCML occurred at the distance of 220 μm. Figure 4 shows a scatter plot presenting hardness values plotted against their corresponding Young’s modulus for CCML and $S_2$. Different clusters are observed for the $S_2$ and CCML. Hardness and Young’s modulus of CCML are highly correlated to each another ($R^2 = 0.55$, $p < 0.001$), but no such correlations occur for the $S_2$ ($R^2 = 0.09$, n.s.).

![Figure 4. Hardness–Young’s modulus scatterplot showing relationships for cell corner middle lamella and $S_2$.](#)
DISCUSSION

Because the mechanical properties microprobe allowed measurements on a spatially resolved basis that was smaller than the width of single cell walls, the effects of cell wall proportions and wood density on mechanical properties were not being directly measured as in other wood property studies (e.g. Wellwood et al. 1965). In this study, the mechanical measurements of S2 walls and the CCML reflect the ultrastructural and chemical variability present in wood. The S2 layer and CCML differ chemically and structurally, thus knowledge of the factors controlling the biochemical and biomechanical processes occurring during cell wall formation are important.

Cell corners of sprucewood are highly lignified (e.g., Downes et al. 1991; Wimmer & McLaughlin 1996), as is the CCML (Fergus et al. 1969). Because no data were found for direct mechanical CCML measurements, results that refer to the mechanical behavior of isolated lignin are discussed. Cousins et al. (1975) obtained Young’s modulus of dioxane lignin from a continuous indentation test. The lignin was chemically dissolved using dioxane and precipitated to a thick syrup in a thin stream and dried. The dry lignin powder resulted in a rod and appeared to be hard and brittle. The Young’s modulus of these lignin samples was 3.3 GPa with a coefficient of variation of 12%. Young’s modulus for CCML was twice the dioxane lignin elasticity. A few points might be worthy of discussion. First, isolating lignin using dioxane introduces the problem that all methods of isolation have, namely, these methods either fundamentally or at least partially change the native lignin structure (Fengel & Wegener 1989). Further, during the lignin isolation process extractives and other chemical components are removed or, at least, have changed their molecular structure. Cousins (1976) has compared different lignins and found periodate lignin to be the one that undergoes the fewest changes in either physical or chemical structure during isolation. This type of lignin had the highest moduli but was also most sensitive to moisture changes. The present work was done at the equilibrium moisture of 10.9% which gave mechanical values 10% under those expected at 0% moisture (see Wimmer et al. 1997 for further discussion). The periodate lignin (Cousins 1976) would give 4 GPa at 10.9% equilibrium moisture content.

Meier (1961) analyzed spruce and pine fibers at different stages of maturation and found that the middle lamella/primary wall of cambial cells was rich in pectic acids, arabinose and galactose. Therefore, it is likely that a change of these chemical components could alter the mechanical properties of the CCML. Pectin plays an important role during the lignification of wood cells, involving the cation Ca2+. Studies have demonstrated that the cambium has an especially high Ca2+ concentration (Wardrop 1976). Pectin is a good chelator of Ca2+, building strong ‘egg in the box’ structures that can be formed between polygalacturonic acids and Ca2+ (Grant et al. 1973). Westermark et al. (1986) found Ca2+ to be high in unlined tissues at high lignin concentrations indicating that pectin may act as a selective binder for Ca2+ in the cambial cells. Pectin degrades or will be removed prior to the lignification apparently releasing Ca2+ ions that are used in lignification. The released and positively charged Ca2+ ions bind to negatively charged groups of lignin and this could affect the mechanical properties of the CCML.
From cell wall fracture investigations it is known that fractures occur predominantly between the middle lamella and S\textsubscript{1} layers or between the S\textsubscript{1} and S\textsubscript{2} layers (Donaldson 1995). The rapid change from lignin-rich to lignin-poor lamellae as observed within the S\textsubscript{1} layer (Maurer & Fengel 1991) favors cell wall fracture. Mark (1967) demonstrated that wood fracture is rarely initiated within the compound middle lamella. It is more likely that the S\textsubscript{1} layer is the first to undergo mechanical failure, usually because of shearing. Mark (1967) showed that the shear stresses in the S\textsubscript{1} and S\textsubscript{2} layer are typically opposite in direction, as might be anticipated from the difference in their microfibrillar orientation angles. This agrees with finding high CCML hardness which is an indicator for high mechanical strength.

The mechanical measurements along a 250 μm radial transect showed the CCML to have a higher variability than the S\textsubscript{2}. The high variability of the CCML could be explained by the high variability of its chemistry. In a recent study, Tirumalai et al. (1996) used Raman microprobe spectroscopy to study the concentration of lignocellulosics in the CCML of black spruce. They found that the relative concentration of lignin and cellulose varies considerably, partially because of lignin deficient regions. Similar results were found in birch (Daniel et al. 1991).

S\textsubscript{2} layers have a significantly higher Young’s modulus than the CCML, but are not particularly harder than the CCML. Maximum Young’s modulus did not exceed 27 GPa and the mean is less than theoretically calculated values for S\textsubscript{2}, which vary between 28 GPa for earlywood and 35 GPa for latewood (Cave 1968). The final 1 or 2 latewood tracheids in a tree ring are usually very narrow radially. The mechanical properties, particularly hardness, of these final tracheids were significantly lower than those of other tracheids. A change in cell wall chemistry likely explains this finding.

Fukazawa and Imagawa (1981) showed in a UV-microscopic study with Abies that cells in the terminal zone of the latewood have a distinctive high lignin content. Wilson and Wellwood (1965) and also Wu and Wilson (1967) found that in conifers earlywood lignin content was consistently 2 to 3% higher than latewood lignin content. Conversely, the per cent cellulose content is higher in latewood (De Zeeuw 1965; Fengel 1969) and there are longer cellulose chains, better packing and higher crystallinity (Lee 1961). These characteristics explain the mechanically stronger latewood (Wimmer et al. 1997) with the exception of the very final (1–2) latewood tracheids which have a different chemistry.

A discussion of the trends in Figure 4 leads to the question of positional dependencies of the indentations within CCML and S\textsubscript{2}. Measurements in CCML, if done very close to S\textsubscript{2}, could be affected by the S\textsubscript{2} mechanical properties and vice versa. However, if this were the case, the Young’s modulus should be affected first due to the longer range of the elastic strain fields during an indentation. Therefore, the S\textsubscript{2} Young’s modulus should be affected much more by position within S\textsubscript{2} than is hardness, the latter remaining nearly constant. As far as the strong correlation between hardness and Young’s modulus of CCML is concerned, it is possible that there is not a positional effect within the CCML, but rather that the high chemical variability due to lignin deficient regions is the main cause for this phenomenon. Due to the variability in chemistry and, consequently, packing density, hardness and Young’s modulus are affected
in the same way resulting in a strong correlation. On the other hand, in the S₂ the amount of cellulose is more or less constant over the measured distance of 250 μm and therefore packing density has not affected hardness which stays equal along a large range of Young’s moduli. But the orientation of the cellulose in the S₂ (microfibrillar angle) might have been altered to some extent influencing the Young’s modulus considerably as found by Cowdrey and Preston (1966).

CONCLUSIONS

For a better understanding of the mechanical behavior of wood it is essential to have basic information on the property values of single wood components such as the S₂ and the CCML. The mechanical properties microprobe used in this investigation was capable of measuring hardness and Young’s modulus on a spatially resolved basis similar to that of an electron beam microprobe. The CCML was found to be mechanically strong with hardness values almost as high as the secondary walls. As a hypothesis, in the lignin enriched middle lamella the positively charged Ca²⁺ ions could bind to the negatively charged groups at the lignin, providing additional strength to the CCML.

Although we have measured mechanical properties from S₂ and CCML solely, it has to be reemphasized that in solid wood the individual cells are not isolated and they rarely operate in isolation from neighboring cell walls. Due to the high variability of the chemistry in the CCML as well as cellulose orientation in the S₂, further investigations are needed that link chemical and micromechanical properties at a high spatial resolution.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the assistance of Drs. W.C. Oliver and T.Y. Tsui for their collaborative role. Helpful ideas and suggestions were provided by Drs. B. Gardner and B. Hinterstoisser. Thanks are due to Prof. E.A. Wheeler for intensive editing, and to two anonymous reviewers. Research was conducted while the senior author was a Visiting Scientist at the Oak Ridge National Laboratory, Oak Ridge, TN 37831 partly with joint funding by the Austrian Science Foundation (Schrödinger Scholarship J799-BIO) and by the U.S. Environmental Protection Agency under Interagency agreement with the U.S. Department of Energy.

REFERENCES


