J Wood Sci (2005) 51:218–221
DOI 10.1007/s10086-004-0648-9

ORIGINAL ARTICLE

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On the detachment of the gelatinous layer in tension wood fiber

Received: December 19, 2003 / Accepted: May 7, 2004

Abstract The detachment of the gelatinous layer (G-layer), often observed on microtome cross sections, has led some authors to believe that the G-layer cannot act as the driving force of longitudinal shrinkage in tension wood. The aim of this study was to observe the detachment of the G-layer along fibers. Green wood blocks were cut transversely into two samples. One sample was kept in water and the other was oven-dried. With one face being common to both samples, the detachment of the G-layer was studied on the same fibers. Observations were performed after blocking deformation by embedding. This revealed that the detachment of the G-layer is an effect produced by the act of cutting the transverse face of the wood block to be embedded. At distances greater than 100μm from this primary surface of the sample, no detachment was observed. Drying shrinkage shows little or no effect on this detachment. The result seems to explain well why the detachment of the G-layer occurs during sectioning using conventional sliding microtomy. These observations prove the adhesion of the G-layer in massive wood and confirm the active role of the G-layer in tension wood properties.

Key words Wood cell wall · Cutting effect · Gelatinous layer (G-layer) · Growth stress · Tension wood · *Populus euramericana*

Introduction

To maintain verticality, most angiosperms are able to produce highly tensile stressed wood on the upper side of the leaning trunk. The stress asymmetry between the upper and lower sides of the trunk then permits it to bend to recover verticality.[1,2] This xylem with high-tension stress is called tension wood. It is characterized in many species by an unusual cell wall structure with a characteristic layer called the gelatinous layer (G-layer).[3] The G-layer is known to have a high cellulose content with a high degree of crystallinity[4,5] and cellulose microfibrils are oriented along the axis of the cell.[6] These differences in chemical composition and structure give tension wood some particular macroscopic properties when compared with normal wood, notably a high shrinkage.[7–11] These high macroscopic shrinkages can be explained by the properties of the G-layer itself. In spite of its structure with microfibrils axially oriented, the G-layer is subject to high shrinkage in both transverse[5] and longitudinal directions.[12] However, in order to contribute to the macroscopic behaviour, the G-layer has to have a relatively higher elastic modulus in its axial direction than the other layers of the cell and must be in tight adherence with them. With the G-layer often being observed loosely attached to the normal secondary wall (S_2) layer,[13–15] its contribution to macroscopic behavior, especially to axial shrinkage, has been put in question.[5,16] The aims of this study were to observe, after blocking deformation by embedding, the detachment of the G-layer from the S_2-layer along the fiber. Observations were made on never-dried wood and on dried wood to evaluate the influence of drying on the G-layer detachment.

Materials and methods

Plant material

Experiments were performed on poplar tension wood (*Populus euramericana* Guinier). This species is known to

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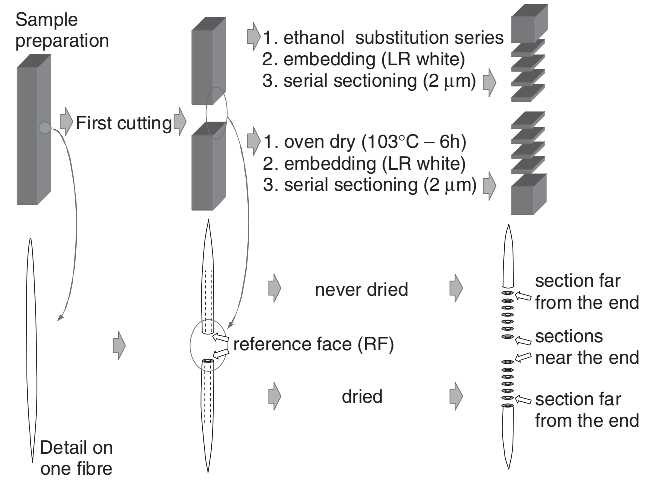

Fig. 1. Experimental protocol and terminology

have a characteristic tension wood fiber with the G-layer organized as $P + S_1 + S_2 + G$.[17] Samples were taken from the upper side of a tilted and bent young poplar tree (8 cm diameter at breast height). This tree shape, which shows the necessity and ability to restore verticality, is an indicator of the production of tension wood. Anatomical observations of samples confirmed the presence of a large number of fibers with a thick G-layer and a thin S_2-layer.

Sample preparation

Samples were maintained in water as soon as they were taken from the tree. Wood sticks (4 mm in longitudinal direction, $1 \times 1\,mm^2$ in cross section) were longitudinally cut by splitting to guarantee a good axial direction. To avoid shrinkage, the samples were kept in a drop of water during the preparation. They were then cut in the middle of axial direction, perpendicular to the fiber, with a new razor blade (Feather S35 type) to obtain two samples. Both samples were perfectly symmetrical and the effect of the tool on both faces was considered as identical. One sample was oven-dried (105°C, 6 h) and the other kept in water (Fig. 1). With one face being common to both samples, it was possible to recognize fibers on both samples and then compare the effect of drying on the same fiber. This common face was called the reference face (RF). Wet samples were dehydrated with an ethanol series and embedded in LR White resin (two exchanges of resin/ethanol mixture for 1 h, followed by two exchanges in pure resin for 1 h and kept overnight at room temperature). Dried samples were directly embedded in LR White resin after being removed from the oven (two exchanges under vacuum in pure resin for 1 h and kept overnight at room temperature). Serial cross-sectioning (2 μm thickness) was performed with a glass knife. Sectioning of oven-dried samples was more difficult and the flatness of the sections was often more irregular. This may be because the penetration of the resin into dry samples without the ethanol series is less efficient. Sections were stained with toluidine blue mixed with azure II, mounted on glass slides, and observed under an optical microscope. Images were obtained with a digital camera and measurements were taken with image-analysis software.

After polymerization of the resin, all deformations of the tissue are supposed to be blocked, and then sectioning does not alter the shape and size of the cell wall layers (compression deformations inevitably produced by the cutting effort, perpendicular to the cutting direction, were not considered because they do not affect the interpretation of results in this article). This method allows the observations of the cells from the RF to inside the sample, conserving the morphology as it was before embedding. Thus, all the deformations observed in the cell shapes are the results from cutting the RF and drying shrinkage before embedding. As proof that G-layer detachment had occurred before embedding, the presence of resin between the G-layer and the S_2-layer can be observed (continuity of knife trace in resin between G-layer and S_2-layer) in Fig. 2. The shape of the cell wall layers (notably G-layers) was followed from the cutting end, along the fibers. Detachment of the G-layer was taken into account as far as was visible under a microscope (magnification 630×).

In this report, "never-dried wood" refers to resin-embedded wood without oven-drying. However, the consequences of dehydration by ethanol series needed for embedding are not well known. Notably, a partial shrinkage could occur as suggested by Ishimaru and Sakai.[18]

Results

G-layer detachment in never-dried wood

As is frequently observed on thin transverse sections, the poplar samples studied showed some fibers in which the G-layer was partially detached from the S_2-layer. This phenomenon was clearly visible at the end of the sample (near RF), but gradually disappeared at sites farther removed. Figure 2 shows the same group of cells observed at six distances from the RF (10, 18, 28, 50, 70, and 150 μm). Twenty-five fibers in which the G-layers were detached on the surface of the sample were followed for up to 300 μm from the RF. It appears that at 40 μm from the RF, only half of the fibers still showed a detached G-layer. At 100 μm from the RF, the 25 fibers observed showed no further delamination between the G-layer and S_2-layer. Continuing observation up to 300 μm showed no detached G-layer.

Effect of drying on G-layer detachment

In the oven-dried part of the sample, fibers presenting G-layer detachment also showed detachment in the nondried part of the sample (Fig. 3). The same number of fibers showed detachment of the G-layer. Similar depth (slightly more) was needed to recover adherence between the G-layer and S_2-layer. Like in the never-dried sample, no

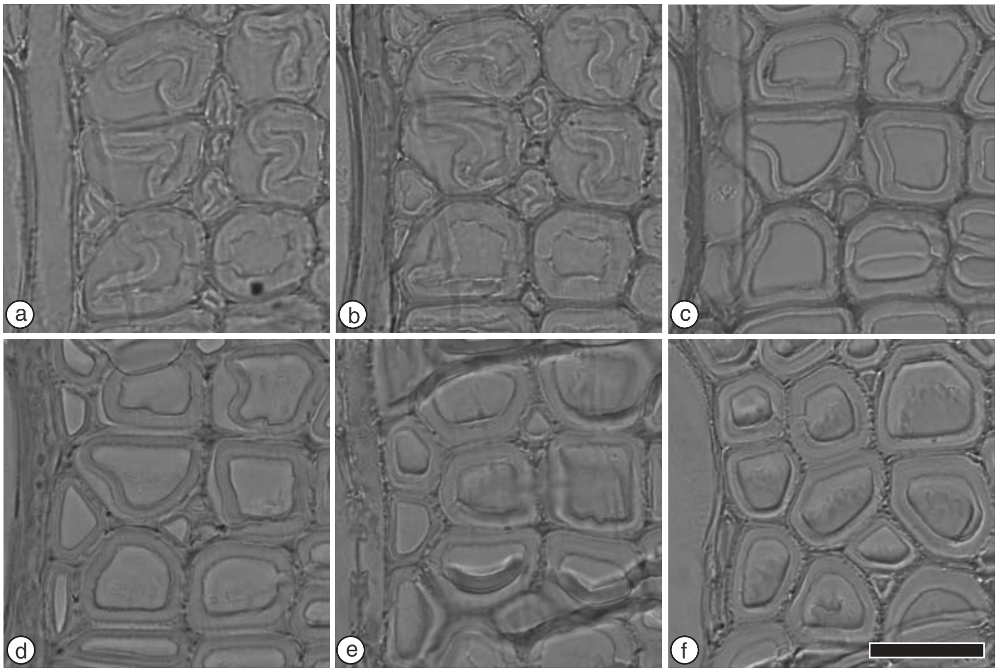

Fig. 2a–f. Transverse sections of never-dried poplar tension wood. Observation of detachment of the G-layer from S_2-layer versus distance (D) to the reference face (cutting surface). **a** $D = 10\mu$m, **b** $D = 18\mu$m, **c** $D = 28\mu$m, **d** $D = 50\mu$m, **e** $D = 70\mu$m, **f** $D = 150\mu$m. *Bar* 20μm

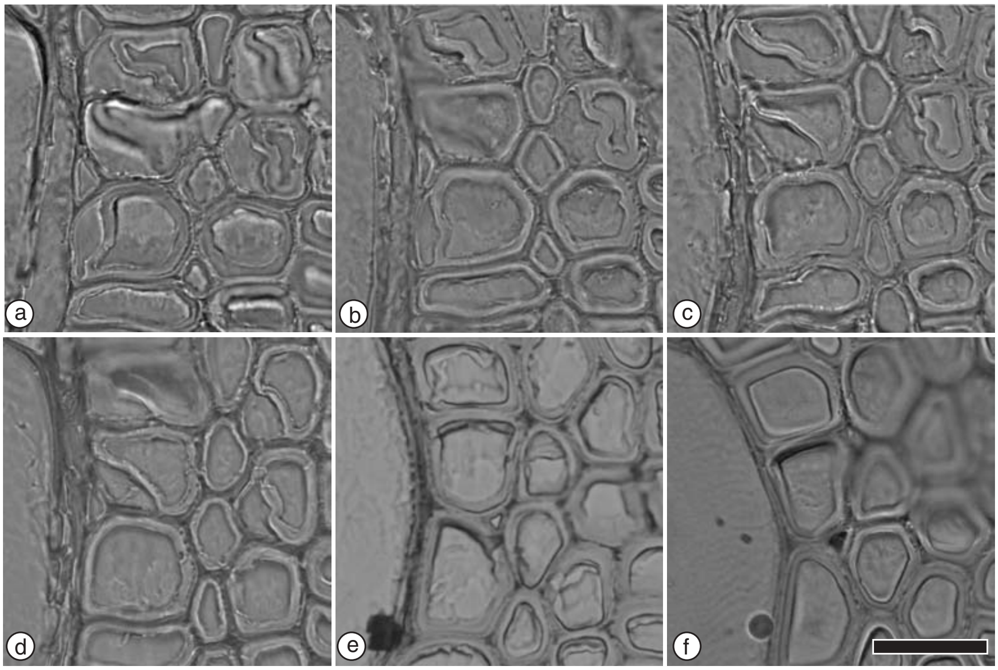

Fig. 3a–f. Transverse section of dried poplar tension wood. Distance (D) to the reference face: **a** $D = 10\mu$m, **b** $D = 16\mu$m, **c** $D = 34\mu$m, **d** $D = 50\mu$m, **e** $D = 96\mu$m, **f** $D = 150\mu$m. Observed cells are the same as in Fig. 2. *Bar* 20μm

detachment of the G-layer was observed at distances greater than 100μm from the end.

Discussion

The observations noted in this study show that detachment of the G-layer, which is often observed, is a border effect. In our observations, this affected only the first 100μm near the RF. Therefore, detachment of the G-layer is foreseeable when using conventional sliding microtoming, because the thickness of sections is usually around 10–20μm. Observations made on the dried wood suggest that G-layer detachment shows little or no dependence on drying shrinkage.

Our observations agree well with the observations made by Okumura et al.[19] They followed the thickness variation of the G-layer all along tension wood fibers on embedded samples; however, no detachment was observed on the electron micrographs they presented. This is likely because in order to observe total length of the targeted fibers, the sections were cut far enough from the border of the sample. In these conditions, detachment would not be observed.

As reported by some authors,[20] observation near the end of the sample (Figs. 2, 3) shows that the largest deformations of detached G-layer are always oriented in the same direction. Action of the tool (razor blade) on the G-layer seems to be the trigger of the detachment. However, layers other than the G-layer have never been reported to be subject to detachment during sectioning. The reasons for the specificity of the G-layer will have to be considered to explain its detachment from the S_2-layer. Some works are in progress to determine if the high tensile stress, which can be expected in the G-layer,[21] could be the trigger of this detachment.

Thus, in tension wood fiber, the G-layer is always in adherence to the S_2-layer in massive wood. The adherence is strong enough not to be significantly altered by the high transverse and longitudinal shrinkage of the G-layer. These observations prove the contribution of the G-layer to the mechanical and physical properties of tension wood. Because the G-layer shrinks during drying,[12] the present study reinforces the idea that the G-layer is the driving force of macroscopic longitudinal shrinkage of tension wood.

Acknowledgments The study was supported by a Grant-in-Aid for Scientific Research from the Japanese Society for the Promotion of Science (nos. 14656069, 14360099, 14002805). CB is a recipient of a JSPS Fellowship.

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