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# PRECAUTIONS FOR THE STRUCTURAL ANALYSIS OF THE GELATINOUS LAYER IN TENSION WOOD

by

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## SUMMARY

The gelatinous layer (G-layer) of tension wood fibres in hardwood contributes to the mechanical function of the living tree and has significant consequences on properties of solid wood. Its size, shape and structure observed by optical or electron microscopy exhibits characteristic anatomical features. However, we found that sectioning of non-embedded wood samples results in an uncontrolled swelling of the G-layer. In order to assess this artefact, the shape and thickness of the G-layer was monitored by serial sections from an embedded wood sample, from its trimmed transverse face to that located several hundreds of micrometers deep. The results revealed that the initial cutting before embedding produced a border effect responsible for the swollen aspect, which is similar to sections from non-embedded material. After a conventional embedding technique was applied, a section of at least 30 micrometers deep in the trimming surface is required to observe an un-swollen G-layer.



***Key-words:*** artefact, fibre wall, gelatinous layer (G-layer), tension wood


## INTRODUCTION

The study of the structure of tension wood fibres is of considerable academic and practical interest, both for biologists interested by the stimuli producing them and for material scientists studying their influence on wood properties. Tension wood is a peculiar wood produced in the upper side of the trunk or branches of angiosperms with a high tensile stress generated during the maturation of the cells. The dissymmetry of stress between upper face (high tensile stress) and the opposite face (low tensile stress) induces an active generation of bending moments, which permits to maintain the vertical orientation of the main trunk or a predetermined angle of the branches (Wardrop 1964; Fisher & Stevenson 1981; Archer 1986; Fournier et al. 1994). Although tension wood is essential for tree survival, it is generally considered to be a defect by wood users. It exhibits unusual physical and mechanical behaviour such as a high longitudinal shrinkage during drying and a high stiffness in longitudinal direction (Clarke 1937; Chow 1946; Grzeskowiak et al. 1996; Jourez et al. 2001; Clair et al. 2003a).

Both biomechanics and material scientists need to study in detail the formation and mechanical aspects of tension wood. Tension wood exhibits important changes of the cell-wall structure compared to normal wood (Onaka 1949). Normal wood fibres are made of a middle lamella, a thin primary wall and a thicker secondary wall. The secondary wall is composed of 3 sub-layers, called $S_1$, $S_2$ and $S_3$. In many species such as beech, poplar, oak or chestnut, tension wood contains fibres with a special morphology and chemical composition due to the development of the so-called gelatinous layer (G-layer), which replaces the $S_3$ layer and partly or wholly the $S_2$ layer (Onaka 1949; Saiki & Ono 1971). The G-layer is known to have a high cellulose content with a high degree of crystallinity (Norberg & Meier 1966; Côté et al. 1969) and to contain microfibrils orientated along the axis of the cell (Fujita et al. 1974; Chaffey 2000). These differences in structure and composition of the G-

layer led scientists to study its contribution to tree mechanics as well as to wood properties.

Visualisation techniques of complex biological tissues such as tension wood are a prerequisite to understand their formation and function. Light microscopic observations are the most common technique using thin sections (10 to 20 µm), which are generally produced by sectioning fresh wood samples with a conventional sliding microtome (Washusen & Evans 2001; Clair et al. 2003a). When high resolution optical microscopy or observations with transmission electron microscopy are required, resin-embedding techniques are applied and sectioning is performed with glass or diamond knives resulting in thin or ultra-thin sections (2 to 0.08 µm) (Sugiyama et al. 1986; Yoshida et al. 2002). These observations provide not only anatomical information, but also permit measuring the dimension and surface area of the G-layer in transverse sections, which are important parameters of mechanical models aiming at evaluating the contribution of the G-layer to the macroscopic behaviour.

However, due to the high internal stress in tension wood, precise measurements of these parameters are not straightforward as the microscopic structure of the G-layer is likely to be altered during specimen preparation for microscopy. Therefore, this study investigates the dimensional and organisational deformation of the G-layer induced by the sample preparation process and aims to propose a procedure avoiding artefacts and misinterpretations.

## MATERIAL AND METHODS

### *Plant material*

Experiments were performed on poplar (*Populus euramericana* Guinier) tension wood. This species is known to have a characteristic tension wood with a G-layer and to produce a high longitudinal tensile stress. The tree was chosen according to its tilted state and its capacity to restore verticality. Wood samples were taken from the upper side of the trunk and the presence of tension wood with a G-layer was confirmed by anatomical observations, showing a large amount of fibres with a G-layer and a thin $S_2$ layer. Samples were maintained in water as soon as they were taken out from the tree to prevent shrinkage due to drying effects. No further chemical fixation was performed.

### *Samples preparation*

Wooden sticks (1 cm in longitudinal direction, 1 x 1 mm in transverse section) were trimmed off by splitting to guarantee a good axial direction. They were then cut manually with a brand new razor blade to produce a clear transverse surface and to obtain cubes of about 0.8 $mm^2$ in size. In order to observe the transverse surface, great care was taken to enhance the quality of the sections. Compression forces due to the penetration of the blade were minimised by the use of new and perfectly sharp blades and appropriate leading angles. A final surface (hereafter denoted as FS) was performed manually to provide the best control of the section quality. In order to avoid shrinkage, the samples were covered with a drop of water during preparation. The small size of the block was chosen to make the resin-embedding process easier.

*Optical observations*

Samples were dehydrated with ethanol series and embedded in LR White resin. After polymerisation, all the deformations of the tissue may become blocked. Although it is known that the sectioning induces compression along the cutting direction, it only causes homogeneous deformation conserving the shape of the cell walls prior to embedding. Thus, any deformation observed in the cell shape can be considered as a result of preparing the FS.

A series of transverse sections (2 µm in thickness) was cut, stained with a mixture of Toluidine blue and Azure II, mounted in water on glass slides and observed under an optical microscope. Images were obtained with a digital camera and measurements were conducted with software for image analysis.

## RESULTS

*Transverse sections*

Transverse sections observed under the optical microscope allow us to follow the change in lumen shape and G-layer thickness along the fibres (Fig. 1). Near the cutting surface (first trimming face with a brand new razor blade), the G-layers appear as wavy structures with very irregular lumens. The more remote sections were made from the surface, the smoother and more regular the lumina become. About 20 µm from the surface, their contour is totally smooth and does not change with additional depth. A concomitant change of the G-layer thickness is observed; it is thicker near the cutting surface and becomes thinner, stabilising after about 20 µm. The thickness of 50 G-layers is measured in 7 sections (2, 8, 14, 24, 30, 42 and 150 µm deep) to evaluate changes along fibres. Thickness measurements are made from the same cells of each section. Observation of the swelling is illustrated in Fig. 2 by the presentation of mean values and standard deviations of the G-layer thickness versus the distance from the sectioning edge. Taking as reference the value observed at 150 µm from the edge, the G-layer exhibits an increase of the relative thickness of about 60% (Fig. 2).

*Longitudinal sections*

Observations of longitudinal sections (Fig. 3) confirm the swelling of the G-layer near the surface as observed in transverse sections. However, precise measurements of the swelling were not feasible because of difficulties to cut longitudinal sections through the middle of a fibre.

## DISCUSSION

*Explanation of the artefact observed*

This study shows that during cross sectioning, some major changes occur in the G-layer thickness and the transverse shape near the surface. A possible explanation could come from the specificity of the G-layer and the tension wood. The G-layer is characteristic of tension wood with a very high tensile stress. Therefore, it is most likely that the G-layer itself plays an important role in tensile growth stress, having a much higher tensile stress than other parts of the cell wall (Clair et al. 2003b). During the first sections through a fibre wall, there will be a redistribution of local

axial stresses on the new cut surface, which may lead to some local delamination between the G-layer and other wall layers near the cut end of the fibre. Moreover, the G-layer is known to be essentially composed of cellulose, with microfibrils highly orientated along the fibre direction. The cell wall organisation and the absence of lignin gives G-layers a very weak transverse rigidity, which permits some transverse deformation of the layer. Furthermore, unlike normal wood cells in which the $S_3$ layer protects the lumen size and shape by a framework made of many tilted microfibrils, the lumen of fibres with a G-layer is not protected by a $S_3$ layer.

The explanation of this artefact by the interaction between the tool and the sample is unlikely, as it is a negligible effect. Transverse compression during crosscutting (in the direction of the blade movement) cannot be avoided during the cutting process itself. However, our observations show that the deformations that might be induced by sectioning are fully recovered and do not affect the final result. Otherwise, the cell shape would be influenced by the cutting direction, which is not the case. The observations do not suggest any preferential direction of swelling. Furthermore, the deformations observed do not affect all parts of the outside cell wall, but are restricted to the G-layer. Thus, this border effect cannot be explained by the tool-material interaction alone.

*Perspectives to studies on the G-layer*

Our results clearly demonstrate that the use of transverse cross sections for anatomical observations of tension wood containing a G-layer can easily be mislead by artefacts. Most standard methods for sectioning wood samples do not include embedding, but perform sections of softened samples after boiling in water. Thus, on a 10 to 20 µm thick section, a G-layer is always observed in the transversally swollen condition. The distance to the border of embedded samples is generally not taken into account while sectioning with a microtome. Measurements of the G-layer thickness in this condition will over-estimate the G-layer thickness of the cell wall compared to the state *in vivo*. Moreover, the wavy shape of the G-layer, supposed to be characteristic of this layer, is an artefact as based on our observations. Both an increase of the thickness and wavy structure prove that a certain change occurs in the G-layer organisation. Cellulose molecules certainly would be less ordered in a swollen condition than in a native state with a loss of the perfectly parallel arrangement of microfibrils together with an increase of the inter-microfibrillar space. Sections of 30 µm thick prepared by Norberg and Meier (1966) according to conventional sectioning were followed by an ultrasonic treatment to extract G-layer tubes from the sections. They reported that the estimated birefringence of cellulose in the G-layer tube was slightly smaller than those of ramie fibres. This could indicate that the ultrastructure of cellulose, particularly the cellulose orientation, can be somehow disordered by the cutting artefact.

To avoid the end effect due to cutting, the use of classical microtomy has to be avoided. Sectioning after embedding, taking into account the distance of the sectioning area to the border is a good solution. Use of confocal microscopy, which permits the optical slice at monitored depth from the cutting edge, would be another possibility. Only sections that are cut at least 30 µm from the end surface have to be examined to ensure that this artefact is avoided.

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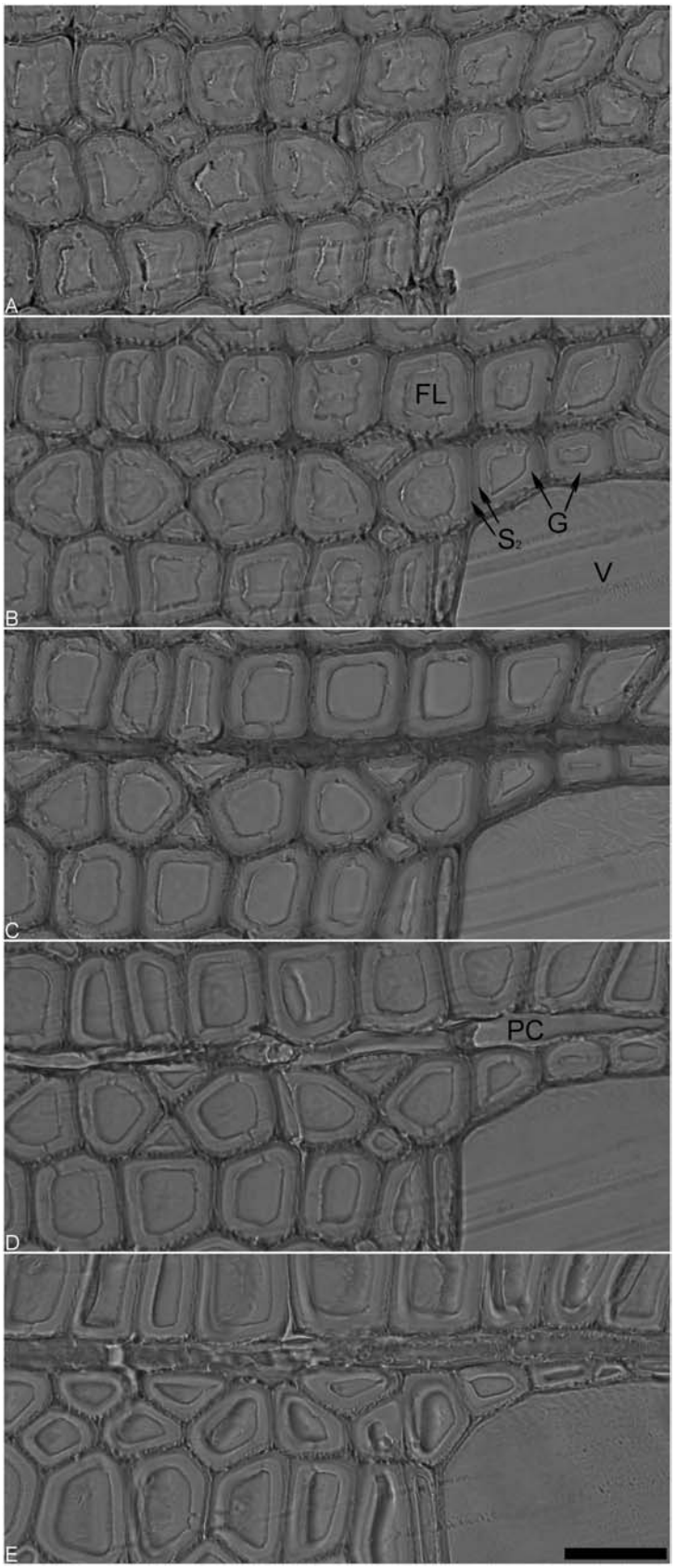


Fig. 1. Transverse sections of poplar tension wood illustrating changes in the shape and thickness of the G-layer in relation to the distance (D) to the cutting surface -- A: D=2 µm -- B: D=14 µm -- C: D=24 µm -- D: D=42 µm -- E: D=150 µm. -- FL=fibre lumen, G=G layer, $S_2$=$S_2$ layer, V=vessel, PC = parenchyma cell. -- Scale bar = 20 µm.

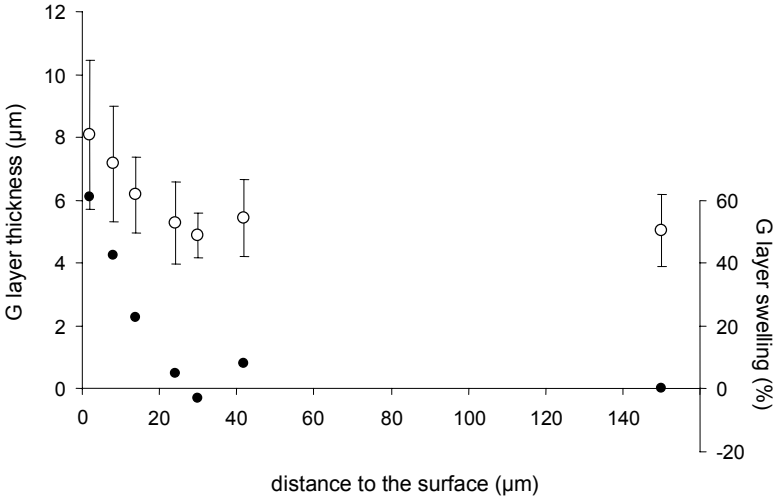


Fig. 2: G-layer mean thickness (empty circle) and standard deviation versus distance to the cutting surface (made after 50 measurements) and the corresponding swelling (full circle) taking measurements at 150 µm as reference.

Fig. 3: Longitudinal section of poplar tension wood fibres showing an increase in the G-layer thickness near the cutting surface. LDW = Lignified Double wall ($S_2$+$S_1$+P+intercellular layer+P+$S_1$+$S_2$), G = G-layer. Scale bar = 10 µm.