ESRF	**Experiment title:** Genesis of the tensile stress in cellulose of trees	**Experiment number:** SC 2238
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Scientific question and context: the basic mechanism of woody plant movements

Having a motor system is essential to terrestrial plants. It is necessary to counteract the effect of gravity and allow successful height growth, as well as to achieve adaptive reorientations. Plant movements result from changes in curvature of the stems, due to localized dimensional changes in their constitutive material, asymmetrically distributed within a stem section. In woody plants, this mechanism occurs during wood maturation. During its differentiation, wood has an irreversible tendency to shrink longitudinally, which results in the apparition of so-called "maturation stress". This can be evidenced and quantified macroscopically, however, the underlying mechanisms (how, when and why this stress appears at a sub-cellular level) are still mostly unknown. Micro-mechanical modeling predicts that some axial tension is induced in the cellulose microfibrils during maturation. However, no direct evidence of this assumption has been provided yet. Former experiments (Clair et al. 2006) at the synchrotron Spring 8 (Harima Japan) showed that a change in cellulose lattice spacing can be detected during the release of wood maturation stress.

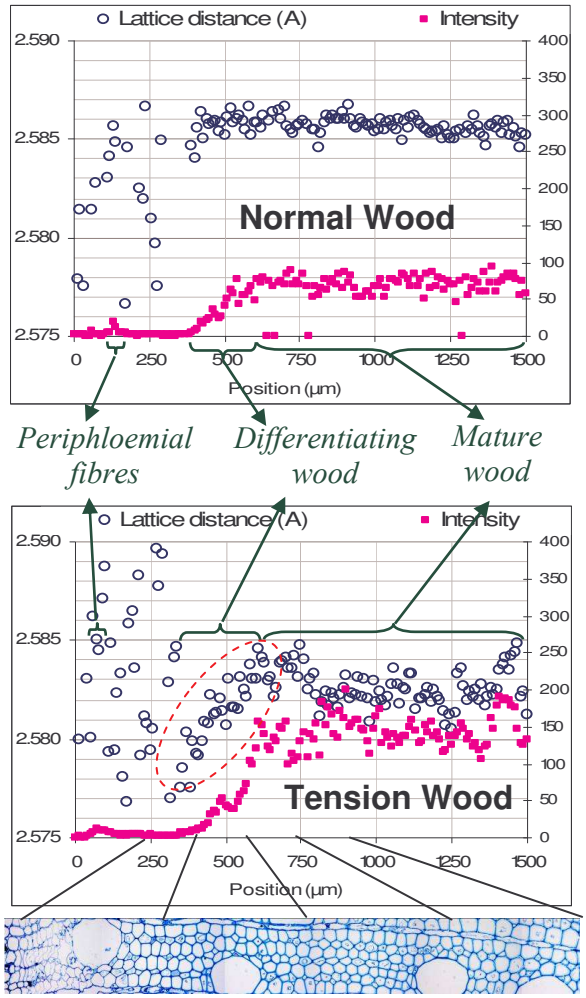
The purpose of the experiments at the ESRF's microbeam was to measure the lattice distance of cellulose within each of the cell walls from the cambium (where the cells are produced) to the mature wood in order to follow the **genesis of the tensile stress in cellulose of trees**, and then, *i.* to qualify the mechanical state of crystalline cellulose in wood at a native state (*i.e.* peripheral green wood in its mechanical environment) *ii.* to look at the changes in cellulose mechanical state along a sequence of wood differentiation and to identify at what phase of the maturation process the stress is induced and *iii.* to compare the mechanical state of cellulose and its evolution, in tension wood and normal wood.

Experimental set-up and collected data

The experimental set-up has been performed as described in the proposal. The experiment has been repeated on 4 logs of poplar (clone I-4551) previously grown tilted in a green house, to induce the production of wood with high maturation stress (tension wood) on one side of the stem. The released strains of maturation stress have been measured on the living trees at ESRF. Three logs were studied on the upper side (where tension wood was supposed to be), and one log was studied on the opposite side for control. For each log, 3 profiles were recorded, 1 mm separated from each other, with 150 successive radial positions (separated by 10µm) were shot on the transition zone between bark and mature wood.

Results: intensity and lattice distance profiles from the bark to the wood

The applied setup with the X-ray beam set perpendicular to the wood surface allows separating the signal emerging from microfibrils with low angle (typically G layer) from those with larger angle (typically S2 layer). The following figures show examples of scans with the intensity of the diffracted signal and the lattice distance (d_{004}) plotted against the abscissa in the scan (from 0 = border of the log to 1500µm = mature wood).



Intensity profiles clearly evidence the transition between non-lignified bark tissues (very low intensity) and wood (stronger intensity). The area of differentiating wood is that for which the intensity is increasing regularly, as confirmed by anatomical observations. Variations in signal intensity occurring in the wood are due to local variations in the abundance of diffracting microfibrils. In normal wood, the intensity is a bit larger on the "large microfibrils angle (MFA)" profiles than on the "small MFA" profiles, which is consistent with the relatively large mean MFA and large MFA dispersion usually reported for normal wood S2 layers. In tension wood, the intensity is much larger for low MFA, consistent with the very low MFA and high cellulose content of the G-layer. The intensity obtained for large MFA in tension wood is weak and probably originates in the contribution of the reduced S2 layers.

The d-spacing observed in the bark area is very scattered because the low intensity did not allow determining it accurately. In normal wood, the d-spacing is the same for large MFA and low MFA, and its value is constant along the abscissa, *i.e.* do not change during wood maturation. In tension wood, the d-spacing of large-angle do not exhibit any clear tendency for increasing or decreasing with abscissa. However, low-angle microfibrils of tension wood show a clear phase of increasing d-spacing in the differentiation zone (red doted circle), followed by a constant d-spacing in mature wood. These results have been observed more or less consistently on all of the 3 scans per log for the 3 logs for tension wood and 1 log for normal wood.

The observation of tension wood profiles shows that the induction of stress occurs very soon after cellulose deposition (increase in intensity and d-spacing are almost synchronous). However, the scattering of d-spacing data do not allow us estimating the precise timing of these events. Surprisingly, the absolute value of d-spacing was found systematically higher in normal wood than in tension wood. The fact that no increase in d-spacing was observed in normal wood suggests that the cellulose in native mechanical state (*i.e.* unstressed) would have a distinct d-spacing in these two kinds of wood, which could be related to a difference in crystal width already reported in the literature. However, this remains speculative because the calibration procedure used here is not optimal for comparing absolute values of d-spacing between samples.

Conclusion

The main result of this study is that we could observe a phase of increasing lattice distance in cellulose microfibrils having low MFA on the tension wood side, while such a phase could not be observed either in normal wood or in tension wood microfibrils with larger MFA. **This strongly supports the assumption that maturation stress of tension wood originates in the induction of tension in the microfibrils of the G-layer.** Moreover, the measured tension is consistent with the usual values of macroscopic maturation strain, giving further credit to this assumption. We also found that the time lag between cellulose deposition and stress induction, if there is one, is small.

To achieve an unambiguous mechanical interpretation and to progress towards the identification of the involved biochemical mechanisms, we need to evaluate with a larger precision, and for each kind of microfibril, both the absolute value of the lattice distance and the relative timing of cellulose deposition and stress induction. These advances are possible by fully exploiting the unique resolution of the ID13 microbeam, and using an experimental set-up optimized for these objectives (detailed in a new application for a beamtime).