General comments

The fist attempt of mapping cell wall stiffening in maturing wood using the now well known but complex and meticulous Atomic Force Microscopy)

Authors are one of the most experienced research group on these techniques applied to wood.

Many technical difficulties occur when applying AFM to wood, due to its living and hygroscopic nature as well as its cellular and heterogeneous structure. Here techniques are meticuluously described, potential artefacts due to preparation are carefully limited and discussed.

One main weakness : the paper is presented with very narrow objectives and it seems made only for very specialized researchers on maturation stresses and wood « muscle » biomechanics. However, on one hand wood formation processes interest a wider community (stiffening studied though indentation module could provide general information of the wood maturation processes and kinetics, not just for mechanical stress studies) see for instance papers of C.K. Rathgeber. On the other hand, an accurate mapping of wood stiffness variation at the cell wall scale interests all researchers in wood mechanics as a cellular material. So I suggest to improve the introduction by some more general considerations about why a better knowledge of cell wall changes, with space (as wood is an heterogeneous cellular material) and time (to understand maturation processes and lignification) is an important and not completely solved question, and how the technique developed here on G layer and tension wood could bring new informations to these questions. I suggest also to finish the discussion by a more general paragraph about the main novelties of the paper for the communities of wood sciences who study wood formation and wood mechanics, beyond tension wood studies.

About data availability : I am not sure that *« The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request* » corresponds to the current standards on open science. I let the editor make recommendations probably you must give more information about how data are organized and stored for a public access.

Introduction

The paper has chosen to present the objectives of the research from the point of view of elucidating the mechanisms of high tensile stress in G layer in tension wood. It is consistent and well written (just see details below). However, as said before, a more general focus could improve the impact of the paper.

Material and methods

OK for me just see details below. But I am not a specialist of these techniques.

Results and discussion

OK, nice and clear figures. Results and images are impressive. Puzzling questions as the increase of the indentation modulus in the embedding resin. Very innovative ones as « stiffening kinetics are faster in the G layer ».

Deep discussion with relevant cited litterature. However it is difficult for a reader non specialist of techniques to find his way and understand what is important for cell wall formation or wood mechanics studies beyond the assessment of the techniques. The comparison with blast or flax fibers is also very interesting but it should be introduced by some more general considerations (why such comparisons ? what are the main known differences between these tissues according to cell wall structure and biosynthesis). A last paragraph with synthetic novelties beyond technical assessment (for instance results of fig 5) would be useful.

Detailed comments :

Line 55-56 : First about "named maturation stress" : in the litterature, these autostresses are often called "growth stress" especially in Archer 1986. So a reference about maturation stress (papers that defined maturation stress and used the term first) would be useful. Then about "maturation stress is high" : in solid mechanics, stresses are a tensor, with several components and spatial changes, each component of normal stresses can be tensile or compressive. So "high" is vague, and in compression wood which is a reaction wood, maturation stress is not high but the sign changes from tension to compression. Lastly, reaction wood is not really produced by the tree in response to mechanical disturbance (see for instance current research about flexure wood) and the complexity of stilmuli that provoke the differentiation of reaction wood is not the matter of this paper. So I sugest to change slightly the paragraph

Line 62 : Mechanical stress is not generated only in G layer and high tensile stress can be also generated without any G layer,. However it is true that in some species as poplar, G layer is indeed associated to high tensible stress. Be more accurate.

Line 73 Is pore swelling associated to the induction of tensile stress in crystalline microfibrills or related to stress transmission of stresses from the microfibrils to the matrix ?

Line 124 This hybrid poplar plant was grown in controlled greenhouse conditions for two months (INRAE, Orléans, France). What does the reference « INRAE Orléans France » mean ? It it just a location (not really relevant) or a reference to specific greenhouse conditions of growth and tilting. Was the plant just be tilted allowing a righting process (but usually far from the base) or maintained in tilted position that insure the formation of tension wood during the whole period (and not just in the base). Is there any reason of the choice of hybrid tremula x alba or more accuratly of clone 717-1-B4 ? As it is not the matter of the paper to make choices about the plant material and growing techniques, I suggest to cite a paper from INRA Orleans where the material and techniques are described.

Line 196 : Dynamics or kinematics ? Is dynamics the proper term to define observed spatial changes associated to maturation process ?

Line 427 and table 1 : As you said, your values are logically lower than usual published ones for mature wood as observed samples, wood is both very juvenile and not fully mature (and also a high MFA in S2 layer of tension wood is also often reported). However you cite only one paper (Eder et al 2013), not really recent. What does it means ? the techniques although well known are not easy to apply to wood analysis ?

454 : « The effect of lignification on the mechanical properties of the cell wall is not yet well understood », interesting sentence as many non specialists believe it is well known that lingification is responsible for stiffness. Must be vulgarized (but it is not the matter of this paper).

Fig 1 legend : a detail but how can you be sure that wood produced before tilting is normal wood. As tension wood can be differentiated in not tilted trees. (but we are sure that tilting will stimulate tension wood formation).