Mechanical characterisation of the developing cell wall layers of tension wood fibres by Atomic Force Microscopy

O. Arnould, M. Capron, M. Ramonda, F. Laurans, T. Alméras, G. Pilate, B. Clair

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Dear Pr Barry Gardiner,

We thank the referees for their fruitful comments that were taken into account to improve the quality of this new version of the manuscript. You will find below answers to the comments that were raised. Changes to the manuscript are highlighted in yellow in the tracked changes document whose file size is over 5MB (pdf of 8.1 MB, it can be compressed down to 5 MB) and I can't upload it on the PCI website. It can be downloaded at the following address:

https://seafile.lmgc.univ-montp2.fr/f/199e5803d86e4e168547/?dl=1.

Looking forward to hearing from you

With my best regards

Olivier Arnould

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by Barry Gardiner, 14 Mar 2022 09:36

the paper needs to give the work "a more general focus" in order to make the findings of wider interest than just those readers specialising in the specific issue of the cell wall layers of tension wood fibres. Such an expanded focus can be provided in the Introduction and Discussion as described by one of the reviewers.

> See our answer to the reviewer below.

The issue of data avialability is also very important. Normally, with open access papers the data used in the paper is made available in order to be usable for validation and confirmation by other researchers. Is it possible to make the data available with its own DOI such as using, e.g. the Dryad Digital Repository https://datadryad.org/stash or some similar system?

> All the raw AFM data and the Excel sheets corresponding to figure 6 have been deposited on the open repository website Zenodo: https://doi.org/10.5281/zenodo.6487575. This has been added in the data availability statement (lines 679-680).

Reviewed by Meriem Fournier, 13 Feb 2022 15:15

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.

> We have strongly modified the introduction accordingly (lines 51 to 71).

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.

> We have added a conclusion containing a section on this point (lines 625 to 634).

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.

> See our answer to the editor above.

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.

> As said above, we have strongly modified the introduction accordingly (lines 51 to 71).

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.

> As said above, we have added a conclusion containing a section on this point (lines 625 to 634).

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).

> We have added a section about this point in the introduction (lines 76 to 95).

A last paragraph with synthetic novelties beyond technical assessment (for instance results of fig 5) would be useful.

> As said above, we have added a conclusion containing a section on this point (lines 625 to 634).

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.

> We have added a reference and slightly modified the sentence on lines 56 to 58.

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.

> Yes we agree and we have modified the sentence accordingly to be more precise (lines 73 to 74).

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

> The introduction has been strongly edited and we have taken your comment into account.

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.

> We have slightly modified the sentence accordingly to be more accurate (lines 74 to 75).

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 ?

> Pore swelling is related to the induction of tensile stress in the crystalline microfibrils, we have slightly modified the sentence accordingly (lines 105 to 106).

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.

> Poplar micropropagated shoots (Populus tremula × Populus alba, clone INRA 717–1-B4) were acclimatized, potted in compost (3 l) and individually supplied with water and fertilizers by a drip system. After 2 months of upright growth, tension wood formation was induced at the upper side of the stem by inclining the plants at 30° from the vertical and maintaining them into this position binding them to a rigid stick. Samples were collected 22 days after tilting at 10 cm from the base of the stem. We used the clone 717-1B4 as it is a genotype that is easy to rapidly multiply in a high number of identical plants. In addition, it has been used in our lab for a number of studies related to tension wood formation. We have modified the paragraph to add these clarifications (lines 154 to 160).

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

> Yes, you are right, we switch from the word "dynamic" to "kinetic" and back again throughout the article. In fact, it seems that the term has changed over time from "dynamic" to "kinetic" nowadays, so we've replaced everything with "kinetic" accordingly.

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 ?

> We have cited only one article in the body of the text but not in Table 1 where we had cited other more recent references. We have also included all these references in the body of the text (lines 515 to 516).

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).

> We have already cited some references that show that the effect of lignification on mechanical properties is not yet obvious, especially for elastic properties. As this is not the subject of this paper and it is even more difficult when it comes to nanoindentation type measurements, we have left the paragraph as it is (lines 544 to 552).

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).

> We checked with a histochemical coloration named Richardson that makes possible an easy detection of the presence of the G-layer in tension wood fibres, proof of the occurence of tension wood in poplar. On the reverse, the absence of G-layer indicate that no tension wood is present as can be observed on the right of Fig.1. This applies in poplar and may not be the case in some other species (as described in Ghislain and Clair, 2017 or Ghislain et al., 2019). Moreover, this has been confirmed by the absence of an additional G layer in the AFM observations. We have modified the paragraph to add these clarifications (lines 186 to 190).

Reviewed by Félix Hartmann, 28 Feb 2022 09:03

I was a bit frustrated that these new results could not be used for challenging the hypothetical mechanisms of maturation stress generation reviewed in Alméras and Clair (2016). If the authors deem it useful, they could add a paragraph in the Discussion section on future measurements which should complement the present results to be able to distinguish between the existing models.

> We were also frustrated but our results, combined with immunohistochemical and gene expression analyses of specific G-layer markers, are really making progress in understanding the underlying mechanisms. A paper on these aspects is being written and will be submitted soon. In order to be able to discriminate the different hypothetical mechanisms using mechanical property measurements, it would be necessary to be able to measure at least the different elastic constants of the cell wall layers. We have added a discussion on this point and possible perspectives in the (new) conclusion (lines 636-661).

- *L220: "the thick white arrow in (b) points to a thin and softer sub-layer that corresponds to the white upper box in (b)". I don't understand in which way the arrow relates to the white box.*

> It's true it was badly written. The thick white arrow in (b) points to a thin and softer sub-layer that is more visible in Fig.4, which is an enlargement of the white upper box in Fig.2b. We have modified the legend of Fig. 2 accordingly (lines 288).

- *L186: "Derjarguin" should be corrected into "Derjaguin".*

> It has been corrected, thank you.

- *L498: "The fact that the thickness of the S2-layer decreases slightly". It should be "relative thickness".*

> Yes you're right, it has been corrected.

- *L549: "on the on the shape of the tip". Please correct the typo.*

> It has been corrected, thank you.

Missing abbreviations in the front page:

- *CML: Cell Middle Lamella*
- *CCML: Corner Cell Middle Lamella*

> They have been added, but CML actually stands for Compound Middle Lamella and CCML for Cell Corner Middle Lamella.