Peer Community In Forest & Wood Sciences

"Touching the Void"

Barry Gardiner *based on peer reviews by* ***Meriem Fournier*** *and* ***Félix Hartmann***

Olivier Arnould, Marie Capron, Michel Ramonda, Françoise Laurans, Tancrède Alméras, Gilles Pilate, Bruno Clair (2022) Mechanical characterisation of the developing cell wall layers of tension wood fibres by Atomic Force Microscopy. Missing preprint_server, ver. Missing article_version, peer-reviewed and recommended by Peer Community in Forest and Wood Sciences. https://doi.org/10.1101/2021.09.23.461481

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Gardiner, B. (2022) "Touching the Void". *Peer Community in Forest and Wood Sciences*, 100007.
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Understanding the processes allowing trees to orientate their stems and branches requires an understanding of the mechanical properties of individual cells. As the cells are formed, maturation stresses are created that can lead to the reorientation of the tree. But measuring the properties within the different wood cells produced in normal wood, tension wood or compression wood requires measurements at very fine spatial resolution and the wood cells must remain in-situ so that the cell mechanical characteristics are preserved. In the article of Arnould et al (2022), measurements of the mechanical characteristics of poplar tension wood were measured in comparison to normal wood at different distances from the cambium and therefore different levels of maturation. The work required incredible care to embed the wood in resin, to cut the wood with extremely sharp microtone blades in order to minimize artefacts in the measurements, and then ultra-careful atomic force microscope (AFM) measurements across cell walls from the edge of the lumen to the middle lamella at extremely high spatial resolution. The result is a detailed picture of the kinetic development and maturation of tension wood cells in a tree. The measurements showed that the G-layer reaches close to its final stiffness long before its final thickness, and this is different from the maturation kinetics of other cell wall layers where thickening and stiffening are generally synchronous. Finally, although the G-layer in poplar tension wood fibres and in flax phloem fibres are in many ways very similar there are clear differences in the kinetics of their development and maturation. The detailed information presented in this paper can therefore help to clarify the different hypothetical mechanisms proposed to explain excess stress generation in the tension wood of trees and help move us towards a full understanding of how the "muscles" of trees work.

References:

Arnould O, Capron M, Ramonda M, Laurans F, Alméras T, Pilate G, Clair B (2022) Mechanical characterisation of the developing cell wall layers of tension wood fibres by Atomic Force Microscopy. bioRxiv, 2021.09.23.461481, ver. 4 peer-reviewed and recommended by Peer Community in Forest and Wood Science. https://doi.org/10.1101/2021.09.23.461481

Reviews

Evaluation round #2

DOI or URL of the preprint: https://www.biorxiv.org/content/10.1101/2021.09.23.461481v3
Version of the preprint: 2

Authors' reply, 07 July 2022

Dear recommender,

Thank you for your positive feedback! I have made all the corrections you requested and formatted the article for the journal using the template. I received a second email from the "editor" asking me not to post the final article on the preprint server but to send it directly to the journal, which I did.

I was not able to upload this final version in the "tracked changes document" as the size of the file is too high (7 Mo...) but you can download it in pdf here:

https://seafile.lmgc.univ-montp2.fr/f/0a729b5f8b294c49b27c/?dl=1

The final version of the article is now avalaible on line on bioRXiv and has been completely formatted:

https://www.biorxiv.org/content/10.1101/2021.09.23.461481v4

Best regards,

Olivier Arnould

Decision by Barry Gardiner, posted 27 June 2022

Revision before recommendation

The paper is very interesting and worthy of publication. But I found some small editorial mistakes and a couple of places where all was not clear. Please look at the uploaded annotated version of your paper and make any necessary changes and upload a revised version of your manuscript.

Best regards

Barry Gardiner **Download recommender's annotations**

Evaluation round #1

DOI or URL of the preprint: https://www.biorxiv.org/content/10.1101/2021.09.23.461481v1.full
Version of the preprint: 1

Authors' reply, 09 June 2022

Dear Editor,

the tracked changes document file size is over 5 MB (pdf of 8.1 MB, it cannot be compressed down to 5 MB) and I can't upload it. It can be downloaded at the following adress:

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

Best Regards,
Olivier Arnould

Download author's reply

Decision by Barry Gardiner, posted 14 March 2022

Suggested changes to "Mechanical characterisation of the developing cell wall layers of tension wood fibres by Atomic Force Microscopy"

The article has been evaluated by two reviewers with expertise in this area of wood science. They both are positive about the manuscript and recommend publication. But they have also made valuable recommendations for improvement. In particular, 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.

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?

Please attend to the reviewer comments and provide an updated version of your manuscript.

Barry Gardiner

Reviewed by Meriem Fournier, 13 February 2022

See the attached pdf

Download the review

Reviewed by Félix Hartmann, 28 February 2022

First, please note that I reviewed the following version of the preprint: https://www.biorxiv.org/content/10.1101/2021.09.23.461481v2

This preprint provides new experimental data on the differentiation of tension wood fibres. The generation of maturation stress in fibres, and especially in tension wood fibres, is essential for postural control in angiosperm trees. However, the physico-chemical changes responsible for the generation of high tensile stress in the secondary cell-wall layers are not known. In 2016, two co-authors of the present preprint published a critical review of all current hypothetical mechanisms of maturation stress generation (Alméras and Clair, 2016, https://doi.org/10.1098/rsif.2016.0550). They could reject a number of them based on their inconsistency with available data and on mechanical considerations. They concluded that new measurements at the microscopic level and new information on the timing of events were needed to distinguish between the remaining plausible models.

The present work contributes to that research programme by applying atomic force microscopy (AFM) to the measurement of tbe stiffeness of the maturing secondary cell wall. AFM is not a new tool in plant biology, but it had never been used before for investigating wood formation. The authors convincingly argue that AFM appears to be the best way to perform mechanical measurements within each cell wall layer at all stages of wood fibre differentiation.

The simultaneous measurement of the stiffening and thickening of the cell wall layers provides novel and precious insight into the kinetics of the differentiation of tension wood fibres. A striking result is that the

G-layer reaches its final stiffeness long before its final thickeness. This contrasts with the maturation kinetics of the other cell wall layers, in which thickening and stiffening are mostly synchronous. This peculiarity of the G-layer may be related to its much higher maturation stress. Moreover, the study revealed a radial stiffeness gradient across the maturing G-layer, which disappears in the the mature wood fibre.

The potential limitations of the measurement method are openly and honestly discussed. In the end, the main results appear robust.

Both the methodology and the results are original, and significantly contribute to the biology of tension wood. The manuscript is well-written and clear. I have no major issue to raise. Nevertheless, at the end of the preprint, 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.

Minor comments:

- 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.
- L186: ”Derjarguin” should be corrected into ”Derjaguin”.
- L498: ”The fact that the thickness of the S2-layer decreases slightly”. It should be ”relative thickness”.
- L549: ”on the on the shape of the tip”. Please correct the typo.

Missing abbreviations in the front page:

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