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Observation of ice formation and freeze induced embolism within trees

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Aim of the Proposal Summary (L2243):

Using high resolution x-ray microtomography in ID19 beamline, we aimed to localize ice formation within living plants and freeze/thaw-induced embolism in xylem conduits. We focused on two major goals: i) embolism observation and localization as well as propagation of embolism, and ii) ice propagation and anatomical structure. During a 72 hours shift (from 27th November until 29th November 2013), Different experiments were performed depending on resolution (medium ~6µm per pixel or high ~1.5µm per pixel) and time scale of picture acquisition (static or dynamic, freezing and thawing):

1) Visualisation of freeze/thaw induced embolism on stem segments and branches.

Temperature treatments were performed in freeze boxes we designed specially for these experiments. The freeze boxes were installed outside of the beamline and samples were conditioned for several temperature cycles (from 5°C to -20°C) and observed as fast as possible. These experiments were successful, as we were able to observe vessels, which were previously full before temperature treatment and frozen, empty on thawing (Fig.1). Interspecific variability was tested: three woody species exhibiting contrasted anatomical structures (*Betula pendula*, *Juglans regia* and *Pinus sylvestris*) were used, and results obtained on excised branches confirmed on complete plants (Fig.2). These results allow to prove that embolism occur only during thawing and not during freezing process. This is the end of a controversy we will publish as soon as possible.

2) Dynamic visualisation of embolisation in a living stem.

This experiment was more risky as we needed to freeze and thaw during the x-ray beam exposure. We had to develop and adapt a specific set-up for this experiment using a cryo-stream (Fig.3). We also intended to perform acoustic measurement during the scans. This required the building of a lead-made x-ray protection for the acoustic sensor that was installed on the sample. This experiment was the first time that acoustic and in situ pictures were recorded in real time on the same sample (Fig.4). This was successful and the complete dynamic of freezing and thawing was acquired in *B. pendula* and *F. sylvatica*.

3) High resolution dynamic freezing.

We expected to observe air bubbles and discriminate ice from liquid sap. This experiment was even more risky. Despite the set-up we developed and the time window we kept for this experiment, we were not able to acquire satisfying pictures, due to technical problems with the rotation plate on the beam line.

Conclusion

Our understanding of fundamental processes involved during plants freezing was improved on the way embolism occurs. For the first time we prove by images that embolism occurs during thawing. These findings are especially relevant to understand frost stress, future species distribution in natura, and the potential effect of climate change on plants. Results will be published in high-impact scientific papers.

However, further investigations would be required in order to understand the physical mechanisms. These experiments would need to improve the sample environment in order to accurately control the sample temperature and proceed with high resolution acquisition.

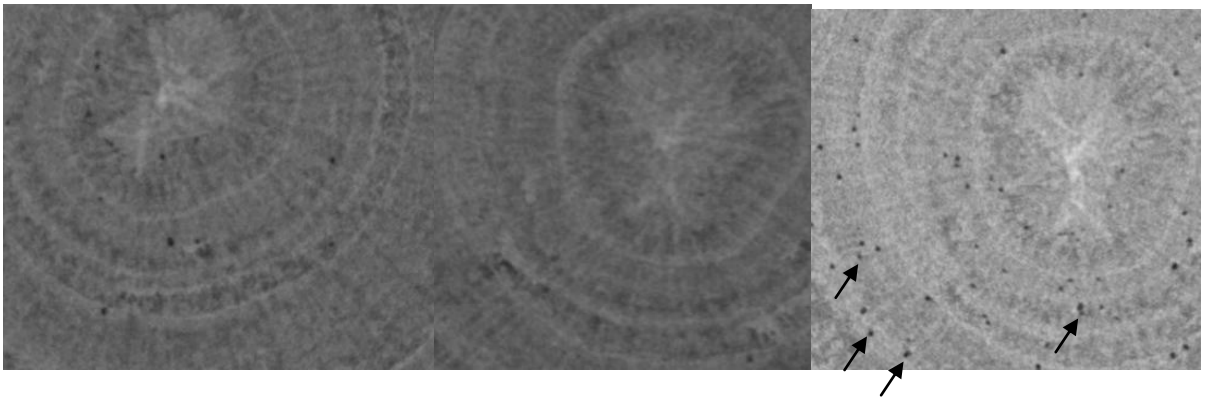

Fig.1 Cross section of Betula pendula sample before freezing (left), frozen (middle) and thawed (right). Arrows indicate vessels which were full on the previous pictures.

Fig.2 Freeze box designed for freezing a complete tree without freezing the root system. Samples (here Pinus sylvestris) were then quickly transported for image acquisition.

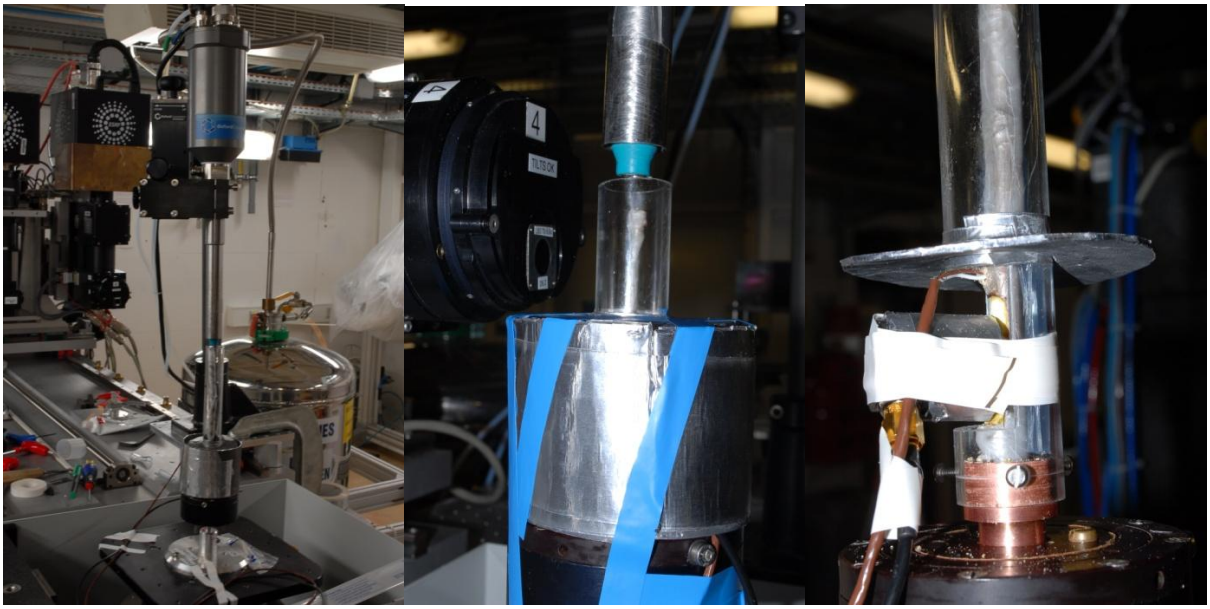

Fig.3 Experimental set-up designed for real time acquisition of tomography and acoustic emission. The acoustic sensor is protected by a lead shield. From left to right: overview of the set-up with the cryo stream on the top cooling down the piece of wood inside a Plexiglas tube and acoustic sensor protected by a lead shell; zoom on the lower part with and without the lead shell.

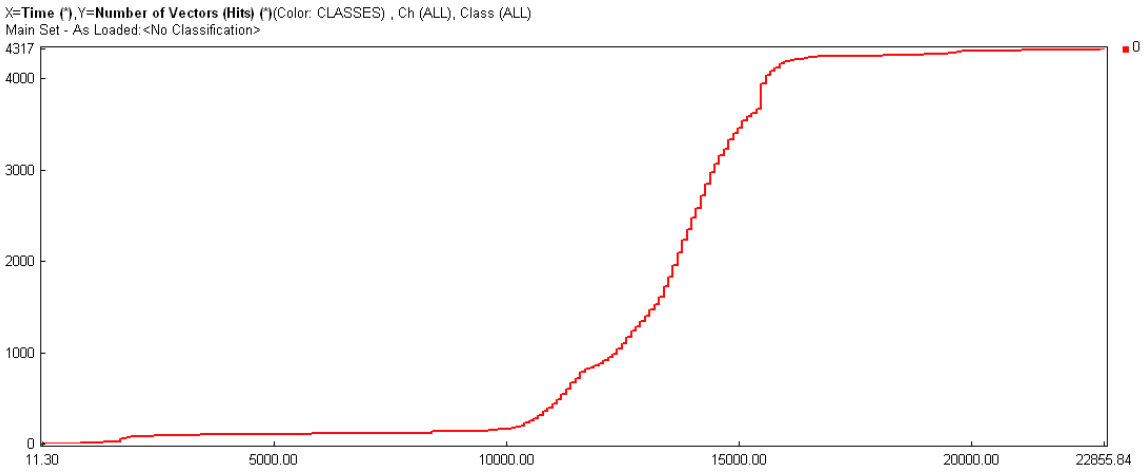

Fig.4 Cumulated acoustic emissions recorded on above set up. Acoustic emissions started after sample started to freeze, whereas vessels were still full. Embolism was then only observable on thawing.