ESRF	**Experiment title:** Visualizing tree strategies to face the risk of freeze-thaw-induced embolism under changing climate	**Experiment number**: LS-2760
Beamline: ID19 (C06)	**Date of experiment**: from: 29.5.2018 to: 30.5.2018	**Date of report**: 22.8.2018
Shifts: 3	**Local contact(s)**: Elodie Boller and Lukas Helfen	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Lintunen, Anna*; Institute for Atmospheric and Earth System Research, University of Helsinki

Salmon, Yann*; Institute for Atmospheric and Earth System Research, University of Helsinki

Suhonen, Heikki*; Department of Physics, University of Helsinki

Report:

Winter embolism influences tree survival and growth through formation of gas bubbles in xylem conduits: gases are not soluble in ice, and the bubbles may expand and fill the conduits with air during thawing [1, 2]. The fate of gas bubbles during thawing, i.e. whether they collapse or expand to embolize xylem conduits, is dependent on their size and on the water potential of the surrounding xylem sap [3]. The bubbles formed during freezing are assumed to occur in the middle of the conduit lumen, and their size is hypothesized to correlate positively with conduit diameter [2, 3, 4], but these assumptions are only theoretical and have not been directly measured. It has also been suggested that cavitation occur at the moving ice front during the freezing process, but the conduits become filled with air (i.e. embolized) during thawing [5]. The freezing process in trees remains poorly understood, partly because bubbles in frozen xylem have been visualized before only few times using a normal microscope [1] or a (cryo-)scanning electron microscope [6, 7], and even in those cases only few bubbles were measured in the microscopic wood sections.

We used x-ray phase micro-tomography to study the size and frequency of gas bubbles in frozen xylem of angiosperm trees, and their connection to xylem and bark anatomy, water status of the tree and its ice nucleation temperature. Nine 1-m-long branches of *Betula pendula* and three branches of *Fraxinus excelsior* were sampled from mature trees in May and immediately recut under water in Turku, Finland. The branches were saturated in water for 48 hours covered with a black plastic bag.

The saturated branches were then frozen with three different treatments. The *Betula* branches were divided in three subsets, control (fully hydrated), bench-dried (partially embolized to test the effect of branch water potential on bubble formation) and branches coated with Vaselin (to test if a barrier to air diffusion out of the branch during the freezing process would impact the bubble formation, Table 1). The water potential of the branches was measured with a pressure chamber. The cut end of each branch was sealed with self-amalgamating tape and placed to the test chamber vertically in a dish. A T-type thermocouple was attached on the surface of each branch to detect the freezing exotherms (i.e. heat released during phase transition). The temperature was first dropped from room temperature to +5C, kept there for 15 minutes, decreased to -15C in 1 h 45 minutes. After freezing the branches, they were moved to -20C freezer and cut there to 8-cm-long samples with diameter of ~5 mm. Each sample was sealed in a plastic tube and stored in the freezer for a week. After this, the samples were shipped in dry ice to the ESRF in Grenoble, France. In ESRF, the samples were stored at -80C for one week before the synchrotron measurements.

In ESFR, the sample holder consisted of a custom-made cup of expanded polystyrene with a lid to isolate the sample from room temperature. The sample holder was glued on a metal plate that could be screwed to the sample plate of the X-ray facility. On the bottom and on the upper part of the cup, a layer of dry ice was inserted to keep the sample frozen during the whole experiment. The upper layer of dry ice was separated with a mounted cardboard plate so that there was a part in the middle where the X-rays could transmit through without encountering dry ice. Inside the cup, there was a hole with the depth of approximately 10 mm on the bottom and in the lid, where a tube with a sample could be firmly mounted. The tube was made of cryo-durable plastic. On both ends of the tube, custom-made plastic plugs were attached with the size and shape exactly corresponding to the holes made to the bottom and the lid of the cup and the inner diameter of the tube.

Table 1. Branch sample characteristics. Water potential for the control branches was -0.23 (SD ± 0.05) MPa, and for the bench-dried branches -0.57 (SD ± 0.05) MPa.

No	Species	Treatment	Ice nucl. T	No of high-res scans I	No of high-res scans II
1	*Betula pendula*	*Control*	-0.7	3	1
2	*Betula pendula*	*Control*	-1.0	3	2
3	*Betula pendula*	*Control*	-1.8	3	2
4	*Betula pendula*	*Vaselin*	-1.6	3	2
5	*Betula pendula*	*Vaselin*	-0.8	3	2
6	*Betula pendula*	*Vaselin*	-1.1	3	2
7	*Betula pendula*	*Bench-dried*	-2.4	3	2
8	*Betula pendula*	*Bench-dried*	-2.5	3	2
9	*Betula pendula*	*Bench-dried*	-2.4	3	2*
10	*Fraxinus excelsior*	*Species*	-3.7	2	3
11	*Fraxinus excelsior*	*Species*	-5.9	2	3
12	*Fraxinus excelsior*	*Species*	-5.8	2	3

* Additional Z-scans from one of the spots to cover longer field of view.

A frozen sample, cut to a about of 40 mm length, was placed inside the tube before the tube was mounted to the sample holder. The branch sample was kept in position inside the tube using play-dough at both ends. The play-dough was soft when the sample was pressed in, and got rigid when frozen thus keeping the branch firmly and preventing movements of the sample during the measurements. All preparations were made in a cool box above the dry ice ensuring that the sample stayed frozen during the preparations.

We used the beamline ID19 with pink beam of 35 keV for the X-ray measurements. First, a low-resolution scan with the whole cross-sectional view was taken of each sample (pixel size of 3.61 µm) to measure the total area of ice versus gas filled conduits. Based on this figure, spots for the high-resolutions scans (pixel size 0.36 µm) were selected. The field of view for the high-resolution images was 0.7 mm^2. From each sample, we first took scans from the center to the edge of the sample to cover the possible radial variability, and then we selected additional spots for additional high-resolution scans to account for the heterogeneity in the sample (Table 1).

We were able to visualize bubbles and gas pockets in vessels and fibres in the xylem of both studied species. Preliminary analysis shows that gas pockets are formed in vessels already during freezing, i.e. gas volumes of no defined shape and larger than a bubble, but that has not embolised the entire vessel, are visible (Fig. 1). Gas bubbles are visible in frozen fibers and they can occur either as individual bubbles or series of bubbles (Fig. 1). Both of these findings are new and important to improve our understanding of winter embolism formation. More detailed analysis are ongoing.

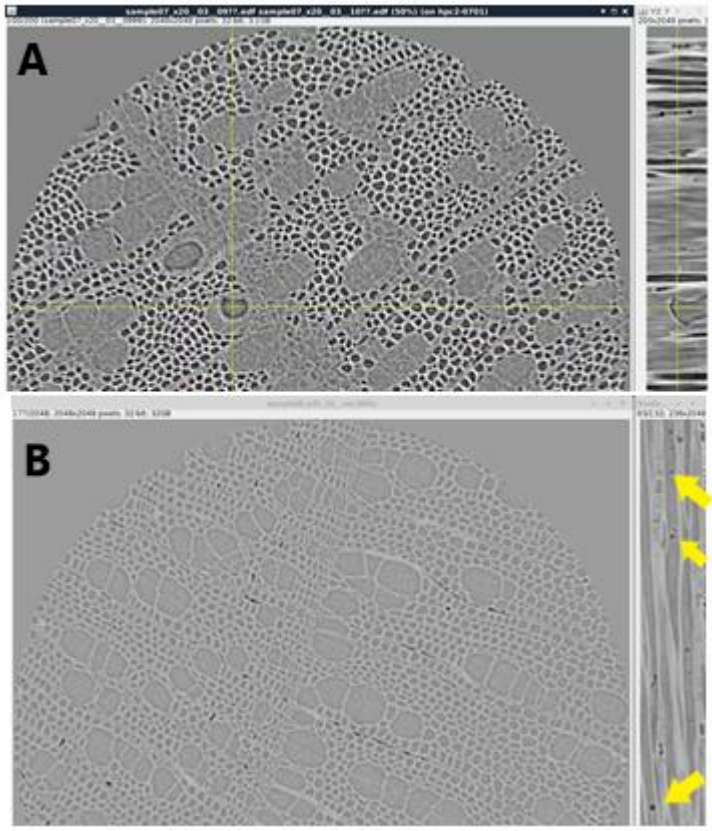

Fig. 1. Visualization of a gas pocket in a vessel (yellow cross in the upper pic) and bubbles in fibres (yellow arrows in the lower pic) of *Betula pendula*.

References:
[1] Sucoff, E. 1969. Physiol Plant 22: 424-431.
[2] Sperry, J.S. and Sullivan, J.E.M. 1992. Plant Physiol 100: 605-613
[3] Pittermann, J. and Sperry, J.S. 2006. Plant Physiol 140: 374-382
[4] Pittermann, J. and Sperry, J.S. 2003. Tree Physiol 23: 907-914
[5] Charra-Vaskou, K. et al. 2015. J Exp Bot, 67: 739-750
[6] Robson, D.J. et al. 1988. J Exp Bot 39: 1617-1621
[7] Utsumi, Y. et al. 1999. Plant Physiol 121: 897–904