

Observation of embolism formation and spreading in plants using synchrotron based X-ray micro computed tomography

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In vascular plants, water is transported in a metastable state below its vapour pressure. Plants are therefore vulnerable to the formation of gas embolism, which can impair the transport of water from the soil to the leaves. This embolism occurs in the hydraulic conduits especially during drought and leads to lower conductive performances that are now identified as one of the key mechanisms of drought induced forest die back¹. Several methods already exist in order to evaluate the embolism resistance of hydraulic conduits in plants. Nevertheless, they do not bring spatial information on how this embolism appears and how it spreads in the vascular network. On the other hand, for long conduits, these methods usually generate artefact measurements that are the aim of a controversy in the plant hydraulic community. HRCT is a perfect tool in order to observe the embolism in plant at the desired spatial and temporal resolutions. The goals of the experiments we carried out at ID19 (ESRF) in 2012 were: (a) How embolism spreads in the vascular system of plants when severe drought stress is imposed, and (b), Whether plants are able to dissolve gas embolism and restore the vascular transport conduits to a functional status when plants are re-watered after drought (c) To provide images that could help to fix the controversy about the behavior of long vessels trees. Tomographic scans were performed at 50 keV with 1000 or 2000 projections. Different camera setups were used according to the size of the sample and the spatial resolution. Scan time for samples was around 5 minutes. We first verified that x-ray radiations do not generate embolism in the hydraulic network. Scans were made on stems of intact leaving trees (*Oak*, *Pinus*, *Eucalyptus*, *Poplars*) that were subjected to increasing water stress. Using an LED light source suspended above the trees, transpiration could be stimulated in the plants between scans. The scans provided generally of very good quality images, with excellent contrast between gas, liquid and plant tissue volumes at a spatial resolution that allowed to visualize the hydraulic conduits. This allowed us to easily distinguish between embolised xylem conduits from water-filled ones, as well as many other anatomical features.

The images allowed the computation of the embolism rate during the artificial water stress. Results showed that long vessels species behave like the other species and that the usual experimental curves obtained by other routine methods are subjected to experimental artefacts.

High resolution images of pieces of small *pinus* allowed to follow the temporal spreading of embolism into the anatomical structure of wood. These observations bring new insights and a large field of research for the mechanistic modelling of the embolism process.

More generally, non-invasive measurements at this resolution have been difficult to obtain in the past and will help lead to breakthroughs in our understanding of plant water transport.

References

1. Choat B, Jansen S, Brodribb TJ, *et al.* Global convergence in the vulnerability of forests to drought. *Nature* **491**, 752-755 (2012)

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