In Vivo Study of Embolism Formation in Lianas using Cold Neutron Radiography

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Motivation

According to the cohesion-tension theory, water transport in plants is driven by transpiration at the leaves.
While transpiration pulls the water through the plant the pressure inside the xylem (the water conducting tissue) falls below vapour saturation pressure or may even become negative (i.e. the water is under tension). This thermodynamically metastable state favours the (unlimited) growth of micro gas bubbles, leading to embolism. As a result, the affected xylem vessels are blocked and lose their capacity for water transport. There is, however, theoretical and experimental evidence that embolised xylem vessels can be repaired (for a possible refill mechanism consult [4], [5], [6]).

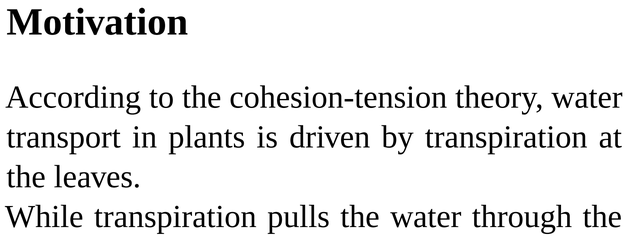

Figure 1: The liana *Parthenocissus quinquefolia* (Boston Ivy, Dreilappige Jungfernrebe, Wilder Wein).

Lianas are especially vulnerable to embolism because of their special hydraulic architecture: Their slender and flexible stems have extremely wide xylem vessels (up to 150 µm) allowing for high transport capacities and small stem diameters. Embolism prevention and repair plays a major role in their water management [3].

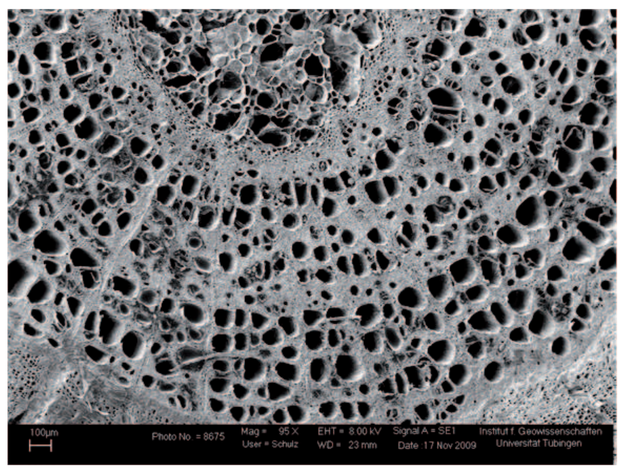

Figure 2: Scanning electron microscope image of the xylem of the liana *Parthenocissus tricuspidata* showing its wide xylem vessels.

References

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Methods

Due to a lack of adequate examination methods most studies about occurrence and repair of plant vessel embolism employ indirect examination techniques which often produce ambiguous data [1], [2], [4].
Cold neutron imaging allows for direct observation of embolism formation and potential vessel refilling. In a series of experiments performed at the cold neutron radiography and tomography (CONRAD) beamline of the Helmholtz-Zentrum Berlin potted lianas and excised sprouts were subjected to water stress in order to provoke embolism events. Consecutive neutron radiographs revealed changes in the water status of the plant and made the direct detection of embolized xylem vessels possible. D_2O as a contrast agent allowed the visualization of water ascent in the liana stem.

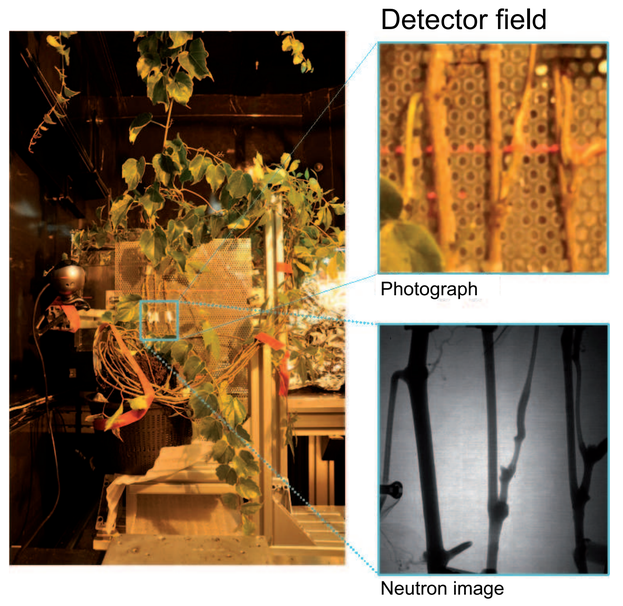


Figure 3: Experimental setup with magnified detector field views.

Results

Visualisiation of Water Ascent

Using heavy water (D_2O) as contrast agent the water ascent inside the xylem *in vivo* can be visualised *in vivo* via neutron radiography (see Fig. 4).

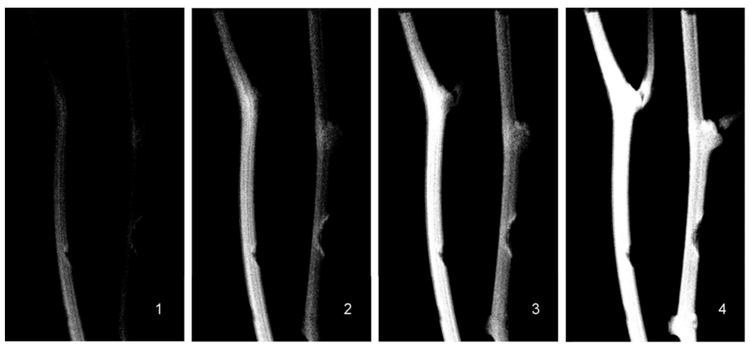


Figure 4: Consecutively (left to right) taken neutron images showing D_2O-uptake and -ascent in excised liana sprouts. Dark grey xylem areas indicate H_2O, light grey areas indicate D_2O.

Detection of Embolism Formation

Water stress leads to a decrease of water potential inside the xylem vessels of the liana. If the potential falls below a plant specific pressure threshold bubbles inside the vessels may emerge and lead to embolism. The formation of embolism is shown in Fig. 5: In a normalised image, embolised vessels appear as a bright fiber-like structure parallel to the stem axis (see Fig. 5(b)).

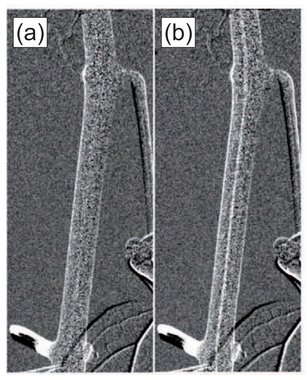
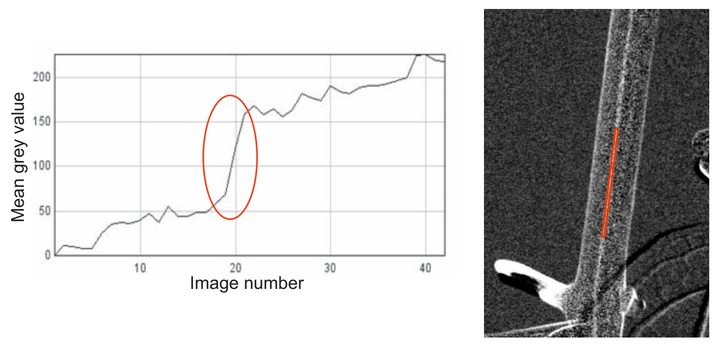


Figure 5: Normalized neutron radiographs of *Parthenocissus tricuspidata*. (a) Sprout before water stress occurs. (b) The same sprout after exposure to water stress. The bright structure parallel to the stem axis represents an embolised vessel.

Quantification of water loss

Applying appropriate image processing, contrast variations between consecutively taken radiographs (or within one radiograph) can be quantitatively interpreted in terms of water loss of a xylem vessel. In Fig. 6(a), the red labeled area shows an increase of about 100 grey values which translates to a reduction of the water layer thickness of roughly 100 µm of the red bordered xylem section of Fig. 6(b). This is in agreement with the typical dimension of a xylem vessel.



(a) (b)

Figure 6: (a) Development of the mean grey value of consecutively taken neutron radiographs during the experiment. The embolism event is identified by the steep increase of grey values in the images 18 through 21. (b) The grey values depicted in (a) correspond to the red bordered xylem section.

Neutron tomography

Neutron tomography facilitates the visualisation of the plant water distribution and its temporal variation. It requires, however, much longer exposure times than radiography if the same spatial resolution is aspired. This necessity may obfuscate time-dependent processes.

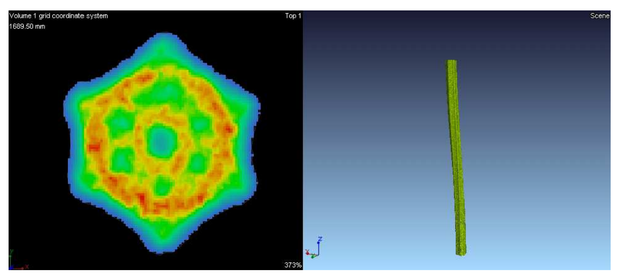


Figure 7: Tomographic picture of *Clematis vitalba*. The colours blue and green indicate a high, red and yellow a low cell water content.

Conclusion

Neutron radiography has proven to be a valuable tool for directly studying the water transport in plants. Using D_2O as contrast agent enables tracking of water ascent inside the xylem *in vivo*. Taking advantage of the excellent sensitivity of CONRAD, the water status of xylem can be monitored. In this way embolisms of individual xylem vessels can be detected immediately. Cold neutron radiography appears also as a promising approach to study the still poorly understood mechanisms of vessel refilling (embolism repair).