

Spotlight

I Can See Clearly Now – Embolism in Leaves

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Deciphering how air enters the plant hydraulic transport tissues represents a major challenge to understanding plant drought responses. Using a non-invasive and cheap visualization technique applied to leaves, the spread of embolism is found to initiate in the midrib, increase with vein order, and is seemingly influenced by vein topology.

Vascular plants include a highly complex water transport system that is driven by the transpiring surfaces of leaves. The mechanism behind this transport system results in a negative (i.e., sub-atmospheric) pressure of xylem sap. Because of the highly cohesive property of the H₂O molecule, water can be transported under negative pressure from the soil through the entire plant system until it reaches the sites of evaporation in leaves. Although there is general agreement about the cohesion-tension theory to explain long-distance vascular transport of water, the occurrence of gas bubbles, which can expand to block conduits and disable the hydraulic transport system via embolism formation, represents a major challenge to understanding how plants will respond to more severe and long-term periods of drought in many locations worldwide [1]. The leaf vein system can be highly reticulate, and understanding how embolism spreads across vein orders has remained challenging. Thus, visualizing embolism events and how these vary across species and during drought is of crucial importance.

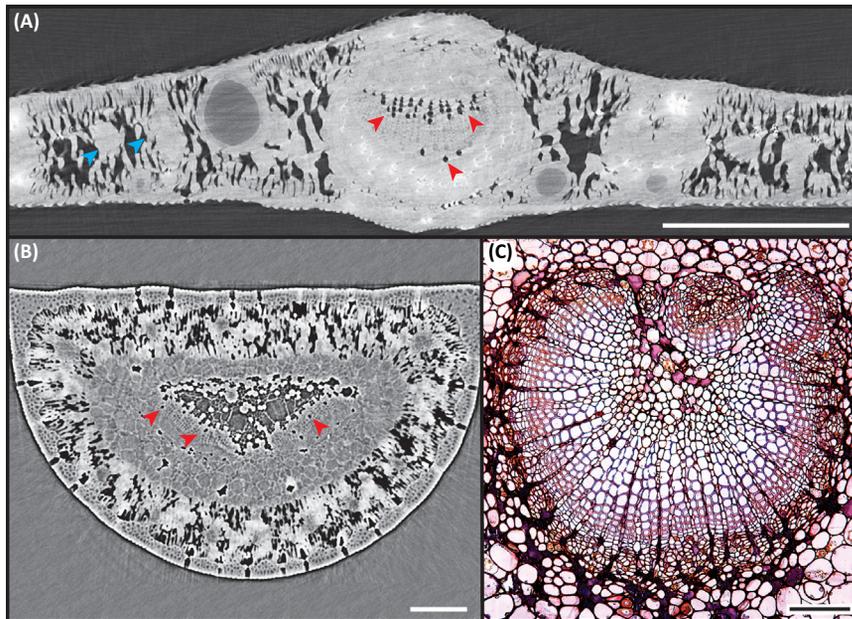
Recent findings from Brodribb *et al.* [2], based on an indirect optical technique to

quantify the amount of drought-induced embolism in leaves, highlight the potential of this non-invasive and low-cost technique to visualize xylem failure at the entire leaf level *in vivo*. The method developed by Brodribb *et al.* allows direct live visualization of putative embolism spread across distinct venation types, which is demonstrated in their paper for three fern species and two angiosperm trees. The technique is a modern, digital approach of earlier visualization techniques (see for instance references cited in [3]) and is based on stereomicroscopy, which allows non-invasive observations of embolized conduits according to differences in light transmission of veins between functional (i.e., water-filled) and air-filled conduits.

Three important findings have emerged from this new technique. First, large dehydration times are necessary (up to 70 h of shoots drying on the bench) to induce embolism in leaf veins, suggesting that leaf veins are more resistant to drought-induced embolism than was previously thought (e.g., [4]). Second, large veins are most vulnerable to embolism, while high vein orders (i.e., 3rd, 4th, and 5th vein orders) are the most resistant. Although these results contradict earlier papers using dye techniques (e.g., [5]), they are consistent with a neutron imaging study showing that embolism starts in major veins and then spreads to the minor veins under higher dehydration [6]. This second finding implies that leaves would only need to refill their major veins to become functional again. Moreover, the hydraulic vulnerability segmentation hypothesis would not apply within leaf veins because the most-distal high-order veins were not found to be most vulnerable to embolism. Hydraulic vulnerability segmentation, however, would be achieved through high vulnerability of the outside-xylem pathways in leaves [7] and/or petiole vulnerability [8]. The third important finding of the study is the role of vein topology in embolism resistance. Their results, although limited to five species, suggest that hierarchical/reticulate systems could provide an efficient

way to avoid rapid and catastrophic failure of the entire vein network. This was observed in the non-hierarchical and non-reticulate venation pattern of the fern *Adiantum capillus-veneris*, and could allow the dense and embolism-resistant minor veins to remain functional during prolonged drought, bypassing embolized conduits in major veins.

While the method presented by Brodribb *et al.* [3] provides promising possibilities to answer fundamental questions about embolism in leaves, there remain some shortcomings where caution is warranted. First, this method should be tested on a wider range of leaf structures and anatomies: it is unknown, for example, if this method would work on thick or leathery leaves. Most importantly, the method assumes that the captured change in refractive index relates to embolism. However, this method has not been validated yet against an independent and more direct visualization approach such as X-ray microtomography (microCT), which allows direct visualization of embolized conduits and their anatomical characteristics in different vein orders (Figure 1A,B). Although X-rays are known to potentially damage DNA in living cell types, they do not affect embolism formation in stem conduits after repeated scans [9]. Validation of the optical technique with other techniques such as microCT is especially crucial if the technique is to be used to infer the hydraulic impact of embolisms on leaf hydraulic conductance [10]. For instance, the percentage of ‘embolized pixels’ that are measured would not necessarily show a 1:1 correlation with the actual percentage of embolized conduits. Hence, high conduit redundancy, especially in midribs (Figure 1C), could make it hard to accurately relate the percent of embolized pixels to actual embolized conduits. In addition, exact quantitative data of the embolized conduits will be necessary to determine their true hydraulic impact. Thus, the percentage of ‘embolized pixels’ reported by this method could overestimate the true hydraulic impact on



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Figure 1. Leaf Cross-Sections from X-Ray Computed Microtomography (microCT) and Light Microscopy. MicroCT cross-sections provide direct visualization of embolized conduits (red arrows) in the leaf midrib of *Eucalyptus* sp. (A) and a needle of *Pinus pinaster* (B) *in vivo*. Note, no embolized conduits are present in the minor veins (blue arrows) in (A). Light microscopy of a cross-section of *Viburnum rhytidophyllum* (C) reveals the high number of xylem conduits in the leaf midrib, and the wide range of their conduit dimensions. A high percentage of embolized midrib conduits would probably be necessary to induce significant hydraulic decline given the redundancy of conduits. Scale bars, 500 μm in A and B, 100 μm in C.

leaf hydraulic conductance. By validating this technique against microCT, modeling, and/or detailed anatomical observations to support the occurrence of embolism, this technique has the potential to be a useful and cheap tool to investigate the impact of drought on leaves.

One of the most promising outcomes of this technique concerns the production of spatiotemporal maps of embolism formation in entire leaves, which opens up possibilities to investigate hydraulic aspects of a large diversity of vein topologies. It has been hypothesized, for instance, that a brochidodromous vein pattern (i.e., with the secondary veins not terminating at the leaf margins, but joining together in a series of loops or arches) would enable higher redundancy and safety against damage: if a loop is damaged, water could still flow to the entire leaf area, whereas a leaf with craspedodromous venation

(i.e., with secondary veins terminating at the leaf margin) would become disconnected from the main water source [11].

The findings from Brodribb *et al.* [3] also provide evidence for air-seeding as the main mechanism for the spread of embolism. Although not discussed, the images and movies presented appear to show that there is also evidence for ‘novel’ formation of embolism in conduits that are not connected to the embolized ones, which supports similar observations on progressive embolism spread in wood based on microCT [9]. These observations indicate that these conduits become embolized by a mechanism other than air-seeding.

Finally, because major veins were found to be more vulnerable to embolism, Brodribb *et al.* [3] hypothesized this could be due to larger conduit sizes present in these

lower-order veins. Whether or not embolism resistance is related to conduit size and the nature of the tracheary element (tracheid vs vessel element) remains unclear and deserves more detailed anatomical observations. Detailed anatomy will also be necessary to understand conduit connectivity in vein nodes, which may function as a hydraulic bottleneck. To understand why major veins are more vulnerable to embolism, special attention should be paid to the ultrastructure and chemistry of air-seeding pores between cellulose microfibrils in interconduit pit membranes, which are known to play a major role in drought-induced embolism [1,12]. In fact, almost nothing is known about interconduit pit membranes in leaves with respect to their physical and chemical properties, although it is assumed that these pit membranes are similar to those in secondary xylem of the stem.

In conclusion, if used in combination with other techniques, this method provides a promising and cheap means to better understand water transport mechanism in plants and hydraulic failure. This could lead to potential applications in the field of biomimetics such as solar-driven, nano- and microfluidic devices.

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