Letters

Vessel content debate revisited

About two centuries ago, the majority of physiologists thought that xylem vessels were filled with gas, like trachea in insects. Others thought that they were filled with water and nutrients and conducted sap like veins conduct blood in animals. Archille Richard[1], like many of his contemporaries, was convinced by a simple experiment: he cut a plant segment, dissected it in water and observed many air bubbles trapped in the vessel lumens.

The debate was closed nearly a century later, with the acceptance of the cohesion–tension theory[2]. Henry Dixon and John Joly[2] suggested that sap was not pushed from below by the roots but pulled from above by the transpiring leaves. Because of the pull, tensions (negative pressures) develop in the xylem conduits, but because of cohesion between water molecules, the water columns do not break. When a cut is made in the xylem, the sap pressure in the severed vessels is released to zero and their content rapidly migrates to places where the pressure is still negative. The capillary pressures that develop in the air–water meniscus at the cut end of the vessels are much too weak to counter-balance the pressure in the sap. This is what happened to Richard's samples: they were probably cut from a transpiring plant and air entered the initially water-filled lumens upon excision. Richard's observations were careful and can easily be reproduced, but his conclusions were wrong because his samples were corrupted by the presence of negative pressures in the vessels.

A century later, vessel content is being debated again. The majority of physiologists think that they are filled with water. Others, such as Martin Canny[3] and co-workers, think they are filled with air and, partially, water. Canny used liquid nitrogen to freeze xylem segments intact on transpiring plants and observed, with a cryo-scanning electron microscope (cryo-SEM), many air bubbles trapped in the vessel lumens. Using a cryo-SEM we have reproduced Canny's observations using walnut (*Juglans regia*) petioles[4]. We observed that up to 30% of the vessels were apparently partially air-filled during the day while the negative pressure was –0.7 MPa in the xylem. If we assume that the capillary pressures that develop at the air–water interface of the bubbles are counter-balanced by the sap pressure, then we would conclude that this pressure could not be more than a few kPa below zero. If correct, these observations will have profound consequences because they will negate the occurrence of large negative pressures in the sap and, therefore, they will invalidate the cohesion–tension theory.

But, on the stage of our cryo-SEM, have we been repeating Richard's error? What if our samples were vitiated by the presence of negative pressures in the vessels when frozen with liquid nitrogen? To test this hypothesis, we cut walnut petioles under water, recut small segments while still under water and only then did we freeze them with liquid nitrogen. In doing so, we released the xylem pressure to zero and stopped sap flow through the petiole. When samples were prepared in this way, the content of the vessels remained obstinately filled with sap throughout the day. We had to eliminate the possibility that hypothetically air-filled lumens were rapidly refilling by the time the samples were immersed in water (for ~30 s). We placed a petiole on the rotor of a centrifuge and used the centrifugal force to generate increasingly low pressures in the xylem[5,6]. The design of the centrifuge enabled us to measure the hydraulic conductance of the sample while it was spinning. If the vessels were becoming air-filled at high pressures (about –0.4 MPa) then we would have expected the conductance to decrease sharply in the first stage of the experiment. This was not the case. The conductance decreased significantly only when the negative pressure became less than –1.4 MPa, a value that corresponds to the point of embolism induction as determined by the standard hydraulic approach[7] and the pressure chamber[8] (Fig. 1).

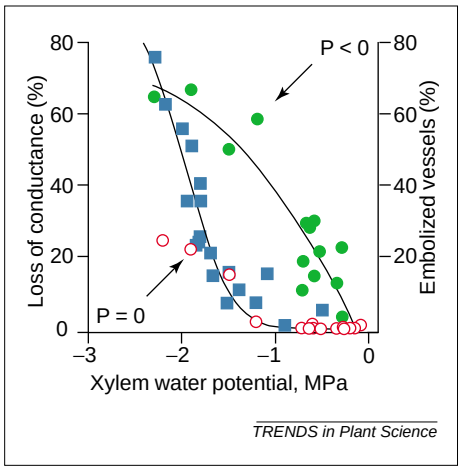

Fig. 1. Proportion of vessels filled with air (embolized) in the xylem of walnut (*Juglans regia*) petioles. Petioles were either frozen with liquid nitrogen while the xylem was still under negative pressure (filled green circle) or shortly after the xylem pressure was returned to 0 MPa (unfilled red circle). The proportion of vessels containing air in their lumens was counted on a frozen cross section in a cryo-scanning electron microscope. The occurrence of negative pressures upon freezing increased the presence of air in the vessels considerably. When pressures were released to zero before freezing, the observations agreed with the change in petiole hydraulic conductance (filled blue square). *Adapted from Ref. 4.*

We have concluded from our experiments that the observation of samples frozen intact on a transpiring plant was vitiated by the occurrence of negative pressures in the sap. These artifacts are probably because the freezing process using liquid nitrogen is relatively slow (up to 10 s in our study, which is long enough for cavitation to occur and for water to migrate out of the vessel lumens). 'Direct observations' should not always be considered as factual: xylem vessels are probably filled with sap at a negative pressure when plants are transpiring, even though our eyes might disagree.

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