Water ascent in plants: do ongoing controversies have a sound basis?

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The cohesion-tension theory of the ascent of sap in plants is fundamental to the understanding of water movement in plants. According to the theory, water is pulled upwards by high tensions (low negative pressures) created in the xylem vessels and tracheids of higher plants by the evaporation of water vapour from leaves. However, much lower tensions (less negative pressures) have been found from direct measurements using a pressure probe. These do not appear to be compatible with the cohesion-tension theory. As a consequence, the validity of the cohesion-tension theory has been questioned and alternative mechanisms for sap ascent have been proposed. Recent experiments show that the conclusions drawn from the pressure probe work were premature. New direct measurements of xylem pressure support the cohesion-tension theory and the previous indirect measurements of xylem pressure.

In transpiring plants, tensions of up to 10 MPa (100 bar) or negative pressures of -10 MPa have been proposed from indirect measurements[1–5]. The problem with the higher tensions is that, under these conditions, water is in a metastable state rather than in thermodynamic equilibrium. In such a metastable state a change is ready to occur but does not occur in the absence of a 'catalysing stimulus' inside the system, such as gas seeds (bubbles) or some outside impulse that reduces the activation energy of that process. In a metastable state, water behaves like an overheated liquid and tends to cavitate. In plants, cavitation causes an interruption in the free passage of water across the vessels and a failure in the water supply to the shoot by the roots. Over the past ten years there has been a heated debate concerning the validity of the cohesion-tension theory with regard to sap ascent in the vessels of higher plants. The cohesion-tension theory has been questioned because the xylem pressure (P_x) of intact plants has been measured directly using a xylem pressure probe, [i.e. the pressure probe that is used for measuring cell turgor pressure[6,7] has been used to measure negative pressures (tensions) in xylem vessels[8–11]]. The Scholander–Hammel pressure bomb technique[12], which traditionally has been used to measure xylem pressure, has been called into question by the pressure probe results. In numerous studies[1,4,13–16], results obtained with the pressure bomb have provided support for the validity of the cohesion-tension theory. Questions arose from previous work involving the xylem pressure probe because:

- The xylem pressure probe yielded P_x values of >-0.6 MPa (relative to ambient air) or of >-0.5 MPa in terms of absolute pressure (relative to vacuum)[8–11].
- Xylem tension was independent of the transpiration rate or responses were small and slow[8,17].
- The pressure bomb technique gave lower pressures than those measured by the xylem pressure probe[8–11].

The pressure bomb[2,12] provides an indirect method of measuring negative pressure in leaf xylem. An excised leaf is enclosed in a pressure chamber except for the cut base, which protrudes through a rubber seal to the outside air (Fig. 1). The leaf is pressurized with compressed air until a balance pressure (P_b) is reached, at which point water starts to exude from the cut base. Although it is assumed that the pressure of the xylem relative to atmospheric pressure (P_x) = $-P_b$, previous direct measurements of P_x with a pressure probe have failed to corroborate this assumption.

Earlier findings that xylem pressures were high ($P_x > -0.5$ MPa) and that they were hardly affected by transpiration[8–11,17] were never corrected in later publications, although it has been shown that xylem pressure rapidly reacts to changes in the osmotic pressure of the root medium[18]. This caused some researchers to suggest that the cohesion-tension mechanism was not the major mechanism for water ascent in plants. As a consequence, there has been speculation about the different mechanisms that might be responsible, some of which are somewhat exotic[19]. Because the measurements made with the pressure probe formed the basis for the invention of new theories for long-distance water movement in plants, we concentrate here on the reliability of these measurements with respect to the cohesion-tension mechanism. The methods used to generate these results are presented in detail elsewhere[20].

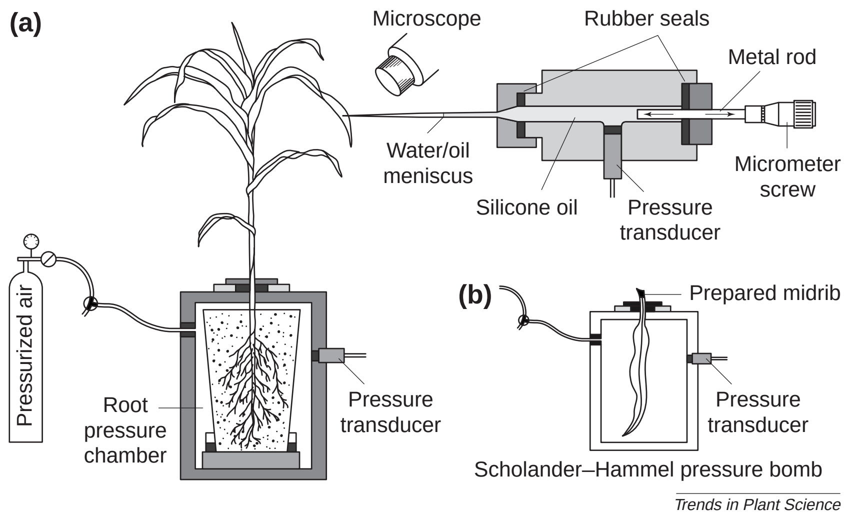

Fig. 1. (a) The root of a four-week-old maize plant was placed in a root pressure chamber so that the xylem pressure in the leaves (P_x) can be changed by altering the pneumatic pressure (P_g) in the root chamber, or by changing the illumination[20]. P_x is measured with a cell pressure probe filled with silicone oil except for the tip, which is filled with water. (b) A Scholander–Hammel pressure bomb, which measures xylem pressure in the leaf after the P_x has been measured with the pressure probe.

Direct measurement of xylem pressure in intact plants

Xylem pressure probes used by other researchers have differed from ours in that we use a cell pressure probe filled with silicone oil instead of with water[20] (Fig. 1). The pressure

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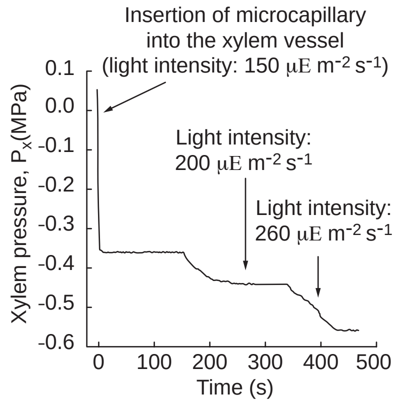

Fig. 2. Higher light intensity causes higher tension in the xylem, because increasing radiation increases the stomatal conductance to water vapour when values are <200 μE m^{-2} s^{-1}, and because higher light intensity increases the driving force on vapour diffusion (the vapour pressure difference between the leaf air spaces and ambient air). Leaf temperature, and hence the vapour pressure of water in the leaf air spaces, increases as light increases. Weighing the plant verifies that increasing light intensity causes a higher transpiration rate (data not shown)[20]. In this plant, P_x decreases from -0.36 to -0.44 MPa and then to -0.56 MPa when the light intensity was increased from 150 to 200 μE m^{-2} s^{-1} and then to 260 μE m^{-2} s^{-1}. The experiment demonstrates that xylem tension is strongly dependent on the transpiration rate and that responses are rapid, which is in agreement with the cohesion-tension theory.

probe has a capillary with a tip diameter of 5 μm. Xylem pressures were recorded down to $P_x = -1$ MPa (-10 bar). P_x was clearly dependent on the transpiration rate, which was varied by varying the light intensity (Fig. 2). We demonstrated that P_x rapidly responds to transpiration, a finding which has been questioned in earlier experiments using the pressure probe[8,17]. There is a good agreement between xylem pressure measured with the pressure probe and the balance pressure of the same leaves measured with the pressure bomb technique (Fig. 3). However, there is a slight difference between transpiring and non-transpiring leaves. This is because during transpiration, pressure gradients are built up within the leaf, which come into equilibrium when the leaf is cut for the pressure bomb measurement. Although the phenomenon is well-known[13,14], it has been ignored in many cases. It has nothing to do with the questioned large differences between the pressure bomb values and the directly measured values of P_x as postulated in the recent xylem pressure probe literature[8–11,17].

Root chamber experiments: test of the model

When the pneumatic pressure applied to the root is increased stepwise, there is a nearly 1:1 relationship between the pressure applied and the response in P_x when $P_x<0$ (Fig. 4), and a much lower slope when $P_x>0$. When xylem pressure exceeds atmospheric pressure, water droplets are exuded from the leaf margins and the stem, a phenomenon known as 'guttation'. The lower slope during guttation is caused by the low resistance of the water leakage from xylem vessels through guttation pathways: about 90% through stomates and 10% through hydathodes (stomata-like pores by which small vessels terminate – data not shown). The direct measurements in the extended range of P_x are in line with an electrical analogue of water flow in plants[2,21]. Kirchhoff's rules and Ohm's law apply. We have modeled the water flow on the hydraulic architecture of maize plants (Fig. 5). The driving force is the difference between the water potential of the soil (ψ_{soil}) + the applied pneumatic pressure (P_g) and the pressure at the evaporative surface. The model prediction matches the results in Fig. 4. The finding that the water flow across the plant can be viewed as a catenary process[21] provides further evidence for the validity of the cohesion-tension theory[2]. These results add to other recent evidence that favours the cohesion-tension theory using indirect measurements of xylem pressure. In these, a centrifugation technique rather than the Scholander–Hammel bomb was used to verify the existence of high xylem tensions in transpiring plants[22,23].

Limitations of the xylem pressure probe

Compared with earlier measurements[11,17,18], the range of P_x values measured with the pressure probe has almost doubled from -0.5 MPa to nearly -1.0 MPa. Experimentally, this is difficult to achieve because any gas-seeding (which would cause cavitation) has to be carefully excluded. Liquids under tension are not easy to handle. Our experience indicates that experimental difficulties (i.e. the handling of negative pressures) play a key role during direct measurement. Few measurements have been reported to verify the range over which the xylem pressure probe can measure negative pressure without cavitation. Our results indicate that using silicone oil in the probe chamber might cope with these negative pressures better than water[20]. This might be because silicone oil is more adhesive to the Perspex surfaces in the pressure probe, and this effect might override the effect of the higher surface tension of water compared with that of silicone oil. To measure the tensile strength of pressure probes, the tip of the pipette of an oil-filled probe is sealed and the capillary is locally cooled (Fig. 6). Negative pressures are generated in the system because the volume reduction of oil is bigger than that of glass. Negative pressure cannot be created with the aid of the movable rod in the probe (Fig. 1) because the movement of that piston immediately causes cavitations[24]. The system filled with silicone oil can withstand negative pressure to -1.6 MPa before cavitation (Fig. 6). With water, a maximum value of about -0.7 MPa is obtained[20]. At maximum values, cavitation occurs spontaneously after a short period of time. However, at pressures just below the maximum, the system can be maintained for several hours. This suggests that cavitations are caused by gas seeds already present on the surfaces. It is known that the formation of new seeds of critical size, either in bulk solution or on surfaces is a rare event[25–27]. Assuming that cavitation is caused by gas seeds in the system, the critical tension at which cavitation occurs can be calculated from the capillary pressure equation, [i.e. $P_{cap} = 2\tau/r$, where τ is the surface tension of the oil ($\tau = 2.5 \times 10^{-2}$ N m^{-1} at 20°C) and 2r the diameter of the bubbles]. When the pressure difference between the interior of the gas bubble (which is largely identical to the oil vapour pressure) and the liquid exceeds P_{cap}, cavitation occurs. A critical pressure of -1.6 MPa (oil-filled probe) means that the maximum bubble size (2r) is 63 nm. The maximum pressure of -0.7 MPa (water-filled probe;

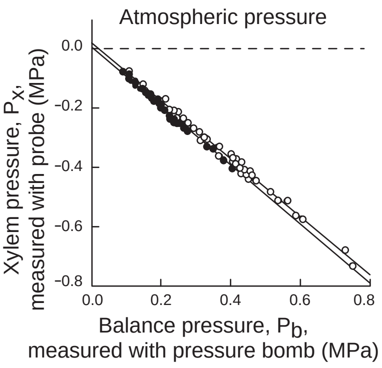

Fig. 3. Comparison between xylem pressures (P_x) measured with the pressure probe and the balance pressures measured with a pressure bomb (P_b). There is good agreement as expected from the pressure-bomb theory. Xylem pressures measured with the pressure bomb are somewhat smaller for transpiring leaves (unfilled circles) compared with non-transpiring leaves (filled circles). The effect is due to the fact that in a transpiring leaf, water potential gradients develop that cause a more negative overall leaf water potential and xylem pressure potential during equilibration in the Scholander–Hammel pressure bomb[13,14].

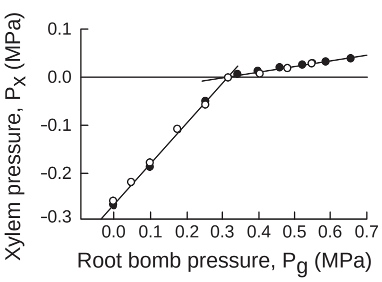

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Fig. 4. The response of xylem pressure (P_x) to pneumatic pressure applied to the root (P_g) is linear. Filled circles indicate decreasing P_g, unfilled circles indicate increasing P_g. When P_x is negative (below atmospheric), the slope of this response is close to unity (slope = 0.850; $r^2 = 0.997$). When P_x is positive (above atmospheric), the slope is significantly smaller than unity. This is because of guttation (the exudation of water droplets from the leaf margins and the stem, which occurs when the xylem pressure exceeds atmospheric pressure), when the hydraulic resistance of this pathway is much lower than that of the root and other tissues where most of the drop in pressure occurs.

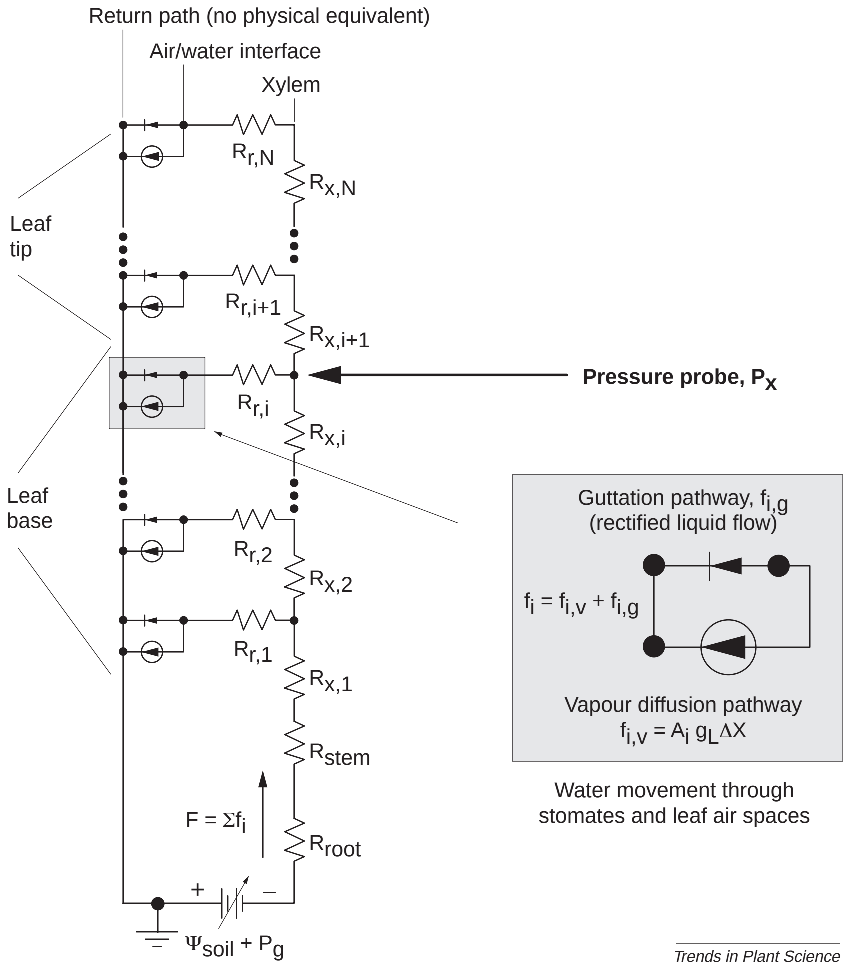

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Fig. 5. Electrical analogue of sap flow in maize plants. P_x and P_g are the pressures of the probe and root chamber, respectively. The discrete components of the electrical analogue consist of resistors, rectifiers and constant current sources for vapour diffusion. R represents the hydraulic resistances of the plant parts. Rectifiers represent the pathway of guttation ($f_{i,g}$) (guttation is the exudation of water droplets from the leaf margins and the stem, and occurs when the xylem pressure exceeds atmospheric pressure). The vapour diffusion rate ($f_{i,v}$), is controlled by leaf vapour conductance (g_L), leaf area (A_i), and the vapour concentration difference between the leaf and the air (ΔX). R_i values were measured using a high-pressure flowmeter[30], and a computer program was written to solve the steady-state water flow through each discrete component in the model (data not shown). The model predictions agree with Fig. 4.

$\tau = 7.3 \times 10^{-2}$ N m^{-1} at 20°C) correlates with a bubble of 420 nm [i.e. the bubble size that causes cavitation is much smaller than or similar to the wavelength of visible light (400–800 nm)]. Nevertheless, gas seeds are much too small to be visible. Thus, if the pressure probe is used to measure pressures of -12 MPa (the lowest value estimated from pressure bomb data[5]), the maximum size of the air bubbles that could exist in the probe would be <8 nm.

Conclusions

It appears that air-seeding limits the application of the probe for the direct measurement of plant xylem under high tension. This important point has been overlooked throughout the recent discussion about the validity of the cohesion-tension theory. Unfortunately, limitations of the technique have led to claims that the cohesion-tension theory is invalid.

Because of the higher surface tension of water compared with silicone oil, filling the pressure probe with water might reduce the tendency for gas seeding. However, this is not true. It appears that the adhesion of liquids to internal surfaces and the presence of gas seeds on these surfaces play an important role, as has been demonstrated in other experimental systems[15,25–27]. This limits the application of the pressure probe to high tension ranges. Our results demonstrate that the conclusions drawn from recent pressure probe experiments that indicate that the cohesion-tension theory is invalid or is, at least, not the major mechanism for sap ascent, has no experimental basis. The technique cannot be used in the range of pressures (tensions) that are required. At present, the cell pressure probe can be used to measure xylem pressures down to -1 MPa (-10 bars). Within this range of pressures, the results obtained are in agreement with the cohesion-tension theory. Similar results have been obtained with the Scholander–Hammel bomb (indirect measurement of xylem pressure) and the pressure probe. Other limitations might explain why previous pressure probe results indicated a lower limit of -0.5 MPa (-5 bars). In some studies only subatmospheric pressures have been reported[8,17]. The most probable limitation is air seeding through a leak at the point of probe insertion into the xylem, but additional limitations might occur when working in the field or in a greenhouse rather than under laboratory conditions. Technical improvements to the pressure probe technique are necessary: such as the proper exclusion of gas seeding in the equipment, which largely originate from air (gas) seeding through hydrophobic cracks (fissures) in the surfaces within the probe or from hydrophobic adhesion failure of seeds at these surfaces[25–29]. Sufficient suppression of these effects is required. One possibility would be a drastic reduction of the surface area within the probe (i.e. reducing the size of the probe). Another would be the use of 'clean' materials (which tend to have no gas seeds at the surfaces) and the creation of a strong interaction (adhesion) with the liquid used (water, silicone oil or another liquid). However, these requirements are difficult to realise.

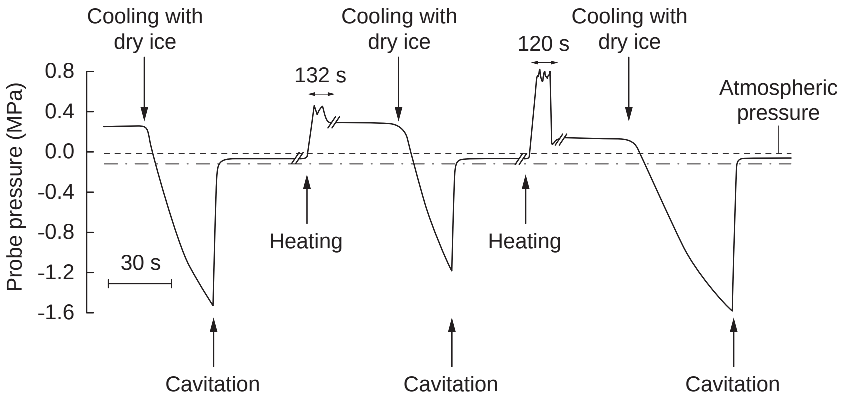

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Fig. 6. Measurement of pressure probe tensile strength. Probes and micropipettes (Fig. 1) are filled with silicone oil and sealed with glue. Negative pressures are created by cooling the probe capillary from the outside using dry ice (arrows). The example demonstrates that pressure probes can withstand negative pressures of down to −1.6 MPa (−16 bars). Upon cavitation (bubble formation) the pressure jumps to a value above vacuum (i.e. to a value close to the vapour pressure of the silicone oil as predicted). Data of the critical tension (tensile strength) varies somewhat between different probes and fillings, and appears to depend upon impurities and other materials within the probes that can cause gas-seeding. This experiment demonstrates that cavitation can be reversed by pressurizing the probe for a short period of time (heating)[29].

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