Sustained and significant negative water pressure in xylem

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DESPITE two centuries of research, the mechanism of water transport in plants is still debated[1-8]. The prevailing cohesion–tension theory[2,3], which states that water is pulled upwards by capillarity in cell-wall pores, remains vulnerable to challenge because its corollary is difficult to prove: that large negative pressures exist in xylem conduits[4-7]. Recent xylem pressure-probe and z-tube experiments suggest that cavitation limits xylem pressures to above −0.5 MPa, despite the much more negative pressures predicted by the cohesion–tension theory and measured with the standard pressure-chamber method[4,5,9,10]. Here we show, using centrifugal force to induce negative pressure between −0.5 and −3.5 MPa in intact stems, that xylem conduits remained water-filled and conductive to species-specific pressures ranging from −1.2 to below −3.5 MPa. Results were consistent when stems were air-dried or injected with air. Agreement among these techniques demonstrates that xylem can support large negative pressures, that the pressure chamber reliably measures these pressures, and that cavitation is nucleated by air entry through conduit wall pores.

Negative xylem pressures (Ψ_{px}) can exist only if cavitation, the nucleation of the phase change to vapour, does not occur. Homogeneous cavitation in water theoretically occurs between −80 and −200 MPa[11-13] and is relatively insensitive to solutes at the concentrations found in xylem sap[3]. The stability of xylem water above this pressure limit depends on the absence of heterogeneous nucleating sites where water contacts xylem walls or inclusions, and on the exclusion of air by capillary forces in cell-wall pores[3,14-18]. The xylem pressure-probe[4,9,10] and z-tube experiments[5] are ambiguous because they alter the water's environment from its native state, in the former case by the insertion of a glass capillary tube through the xylem conduit wall, and in the latter by modelling the xylem conduit as a z-shaped glass capillary tube centred on a centrifuge and rotated to generate negative pressure. The exposure of water to these foreign surfaces could have caused cavitation at a less negative Ψ_{px} than in intact xylem conduits.

We determined the minimum Ψ_{px} causing cavitation in intact xylem conduits by modifying the z-tube method[19,20] to spin stem segments rather than glass tubes as suggested previously[5]. Although conduits at segment ends were severed, segments were long enough to include entire conduits. The extent of cavitation was determined by comparing the hydraulic conductivity (k_h, xylem flow rate per pressure gradient) of the segment before and after spinning. Cavitated conduits, although initially vapour-filled, quickly fill with air as gas diffuses from surrounding air spaces[21]; these conduits are then non-conductive even at atmospheric pressure, and so reduce stem k_h[22].

Populus fremontii (Fig. 1a) showed no decrease in k_h after exposure to Ψ_{px} as low as −1.5 MPa. However, all stems were completely cavitated and were non-conductive at or below −1.6 MPa. When *Populus* stems were spun at speeds above the cavitation threshold (−1.0 MPa) for up to 3 h, there was little or no decrease in k_h (Fig. 2). Conversely, when spun below the threshold (−1.8 MPa), stems showed 100% decrease in k_h after as little as 60 s (Fig. 2). Four other species showed different cavitation thresholds of −1.4 MPa (*Salix gooddingii*; Fig. 1b), −1.9 MPa (*Acer negundo*; Fig. 1c), −3.1 MPa (*Abies lasiocarpa*; Fig. 1d), and below −3.5 MPa (*Juniperus monosperma*; Fig. 1d).
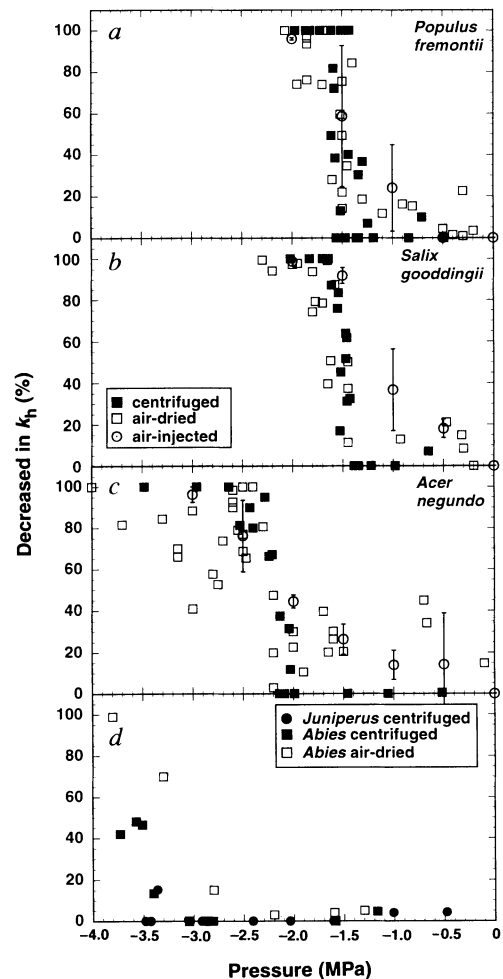

FIG. 1 Decrease in hydraulic conductivity (k_h) in xylem against xylem pressure (Ψ_{px}) generated by centrifuging (filled symbols) and air-drying (open symbols) stems of a, *Populus fremontii*, b, *Salix gooddingii*, c, *Acer negundo* and d, *Abies lasiocarpa*. Centrifuge data only are shown for *Juniperus monosperma* (d). Decrease of k_h from air injection of xylem (a–c, open circles) is shown against the negative of the injection pressure for comparison. All species were from Pima County, Arizona, except *A. lasiocarpa* from the Wasatch Mountains of Utah.

METHODS. In centrifuge experiments, stems were re-cut underwater to a length (26–40 cm) several times longer than all or most of the conduits in each species (W.T.P. and J.S.S., unpublished observations). The bent ends that centre the water column in z-tubes[20] were unnecessary because of capillarity at inter-conduit pits. Initial k_h of each segment was measured using a method described previously[30]. The stem was centred on the rotor of a centrifuge (Sorvall, GLC-1) and spun to generate a pressure (P); $P = 0.5\sigma\omega^2 r^2$, where σ is the density of water, ω is the angular velocity (rad s^{-1}) and r is half the total stem length (cm)[19,20]. Angular velocity was determined using the centrifuge tachometer. After 4 min (2 min for *A. lasiocarpa*) centrifugation, stems were left in damp paper towels for 20–60 min to allow air to diffuse into vapour-filled conduits. Final k_h was measured after removing 2–5 cm from each end under water to eliminate blockage by drained, severed conduits. Cavitation was expressed as the percentage decrease of final k_h from its initial value. The centrifuge was too slow to completely cavitate *Abies* or *Juniperus*. In air-drying experiments, decrease in k_h was calculated from native and flushed k_h[30], and xylem pressure was measured with the pressure chamber. Air-injection experiments were performed as described previously[25].

From these data we conclude that negative Ψ_{px} can be sustained for extended periods with little or no cavitation unless Ψ_{px} falls below a defined, species-specific threshold. The higher cavitation pressures seen in pressure-probe[4,9,10] and z-tube[5,20] studies appear to be a property of technique rather than of xylem.

We obtained the same relationship between Ψ_{px} and k_h when we air-dried stems and measured Ψ_{px} with the pressure chamber[23] (Fig. 1; compare results for air-drying and centrifugation). The greater scatter in the air-dried as opposed to centrifuged results (especially *A. negundo*) was probably in part a result of pressure-chamber error as stems reached 100% cavitation[8]. However, the general agreement between the methods indicates that the pressure chamber can measure negative Ψ_{px} when cavitation is not extensive.

These results raised the questions of what nucleated cavitation, and why it occurred at different pressures in different species. The 'air-seeding' hypothesis[24] states that xylem cavitation is nucleated by air sucked through wall pores when the pressure difference across the air–water meniscus in the pore exceeds that which can be sustained by capillarity. The air-seeding pressure difference will be the same whether the air is pulled in by negative Ψ_{px} as in an intact plant, or pushed in by elevated air pressure. We tested this prediction by measuring the k_h of stems inserted through a steel 'pressure sleeve' as a function of air pressure inside the sleeve[25]. In support of the hypothesis, the air pressure causing a decrease in k_h in air-injected stems was equal and opposite to the negative Ψ_{px} causing cavitation in air-dried or centrifuged stems (Fig. 1; compare results for air-injected, air-dried, and centrifuged).

Our results join the already considerable evidence supporting the existence of substantial negative pressures in xylem and the validity of the pressure-chamber method for estimating them[8]. By using the same approach of spinning stems, a 1 : 1 correspondence has been demonstrated[26] between centrifugally induced pressure (to −1.6 MPa) and pressure-chamber measurements of leaves attached at the centre of rotation. The pressure chamber was also consistent with Ψ_{px} predicted from pressure-probe measurements of cell turgor (where cavitation is irrelevant) and estimations of osmotic potential of cell sap[27]. Our evidence for the air-seeding mechanism also corroborates previous studies[8], and demonstrates that other potential causes of xylem cavitation, such as nucleation at the wall–water interface[3]. are relatively unimportant. Air-seeding will occur through the largest pores in the conduit wall, these being located in the inter-conduit pit membranes[24]. These thin, porous areas in conduit walls allow inter-conduit passage of water but not a gas–water meniscus; this minimizes air-blockage of xylem when conduits are damaged. The pore diameters in pit membranes range from less than 0.05 to over 0.4 μm (as opposed to <0.01 μm in cell wall proper), depending on species and location in the plant, and their predicted air-seeding pressure has been shown to correspond with the cavitation pressure in several species[28,29]. Although the occurrence and mechanism of xylem cavitation is reasonably well documented, its greater significance for determining the drought tolerance, habitat preference and regulation of water use of a species is a matter of continuing study. □

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Dorsal cell fate specified by chick *Lmx1* during vertebrate limb development

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THE positional cues that govern the fate of cells along the dorsoventral axis of the developing vertebrate limb are established in the mesoderm before outgrowth of limb buds. In *Drosophila*, a LIM/-homeodomain gene, *apterous*, expressed in the dorsal compartment of the wing disc, specifies dorsal cell fate[1,2]. Here we report the isolation of a vertebrate LIM-homeodomain containing gene, Chick *Lmx1* (*C-Lmx1*). Transcripts for *C-Lmx1* are detected in the presumptive dorsal limb mesoderm and are restricted thereafter to the dorsal mesoderm of the developing chick bud. *C-Lmx1* expression is regulated by the overlying ectoderm where *Wnt7a* messenger RNA is localized[3]. *Wnt7a*, required for normal development of the dorsoventral axis in mouse limbs[4], can induce ectopic expression of *C-Lmx1* in ventral mesoderm. Misexpression of *C-Lmx1* during limb outgrowth causes ventral to dorsal transformations of limb mesoderm. We propose that *C-Lmx1* specifies dorsal cell fate during chick limb development.

Screening of a chick limb complementary DNA library with a mouse LIM/homeodomain probe produced several LIM/homeodomain clones including *C-Lmx1*. This cDNA encodes a protein that contains two LIM domains linked to a homeobox

FIG. 2 Decrease in k_h in centrifuged stems of *Populus fremontii* as a function of time at pressures above (filled circles, −1.0 MPa) and below (open circles, −1.8 MPa) the cavitation threshold of −1.2 MPa from Fig. 1a. Note the different time scales (filled circles, minutes; open circles, seconds). Limited cavitation at the longer spinning times above the threshold may have developed from lowering of negative pressure by evaporation during spinning.