Negative Xylem Pressures in Plants: A Test of the Balancing Pressure Technique

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Xylem tension was experimentally imposed by centrifugal force to assess the stability of negative pressures within the xylem and the estimation of those pressures with a pressure chamber. Balancing pressure measurements of leaves attached to a spinning branch at the axis of rotation closely agreed with tensions calculated from the rotational velocity. This agreement demonstrates that the xylem is capable of sustaining large negative pressures and directly validates the balancing pressure technique.

The cohesion theory of sap ascent states that water is pulled through the plant by a tensile force generated by the evaporation of water from leaf surfaces (*1*). A corollary is that a continuous column of water exists between soil and leaves, whose continuity under tension is maintained by the strong attraction between water molecules (*2*, *3*). In energetic terms the cohesion theory is attractive in its simplicity; it allows plants to harness solar energy directly for water uptake in the form of evaporation. Negative pressures (below vacuum), however, would have to exist within the xylem to overcome matric and osmotic forces in the soil as well as the gravitational and frictional energy losses that occur during transport (*4*).

The cohesion theory was first proposed 100 years ago (*5*) but became widely accepted only after a method was developed for measuring xylem pressure, the results of which indicated the existence of tensions sufficient to move water from soil to leaves (*6*, *7*). According to the cohesion theory, when the tension in the xylem is abruptly relieved by cutting, water is drawn from the xylem into living cells by osmosis until equilibrium is achieved between the surface tension of the new air-water interfaces within the xylem and the cell water potentials. Measurement of xylem tension involves pressurizing the gas phase surrounding the leaf until water is forced from the relatively elastic-walled living cells back into the rigid xylem elements. The balancing pressure is reached when the distribution of water within the leaf or twig is restored to its condition immediately before cutting (as indicated by the appearance of water at the cut surface). At this point the externally applied pressure is considered to be equal in magnitude to the tension previously existing in the xylem. This method has been successfully compared with psychrometric and cell pressure probe determinations of leaf water status (*7*, *8*). None of these techniques, however, provides a direct measurement of xylem pressure, leaving open the criticism that the pressure chamber technique has never been explicitly verified with a model system in which known tensions could be experimentally generated (*9*, *10*).

Efforts to use a modified pressure probe to directly measure xylem pressures have led to the suggestion that both the cohesion theory and the balancing pressure method need to be reexamined (*9*, *11*). Data collected with the modified pressure probe suggest that xylem pressures are frequently between 0 and +0.1 MPa and only rarely fall below −0.1 to −0.3 MPa (*9*, *11*, *12*); measurements of midday xylem pressure by the balancing pressure method are typically an order of magnitude more negative (*1*). Substantial disagreement between the results from the two techniques persists even when measurements are made on the same plant (*12*). Proponents of the xylem pressure probe argue that the balancing pressure method overestimates the tension in the xylem because air-filled spaces in the leaf interfere with the propagation of pressure across the tissue and because energy must be expended in compressing air spaces (*9*).

We used rotational motion to create known tensions in the xylem to experimentally test the pressure chamber technique. When an object undergoes simple circular motion, its inertia is experienced, in the object's reference frame, as an outward (centrifugal) force. The maximum tension (T) in the spinning water column is calculated by integrating the formula for centrifugal force over the length of the tube, resulting in $T = 0.5\sigma\omega^2 R^2$, where σ is the density of the fluid, ω is the angular velocity, and R is the distance from the axis of rotation to the end of the water column (*3*). The experiment entailed spinning a branch about its midpoint at a constant angular velocity (Fig. 1). Each branch had a single leaf attached at the axis of rotation, the xylem of which experienced tensions equal to the maximum value calculated from the rotational velocity and branch length (*13*). A second leaf, not attached to the branch, was simultaneously spun in the same apparatus as a control.

The balancing pressure of the attached leaf closely agreed with the calculated rotational tension (Fig. 2). Balancing pressures of the control leaves varied between 0.2 and 0.4 MPa (0.29 ± 0.08 MPa, mean ± SD) and showed no relation with the calculated rotational tension ($T = 0.626$). All balancing pressures before spinning were less than 0.05 MPa, indicating that the branches were equally hydrated at the start of the experiment.

The transmission of tensions induced within the spinning branch to the attached

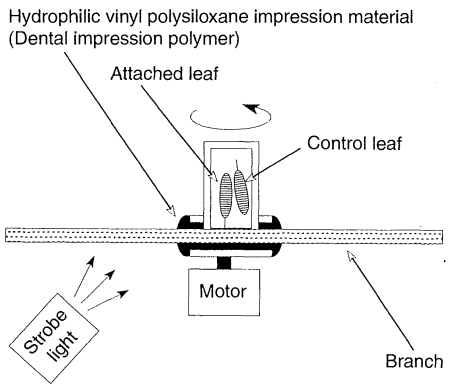

Fig. 1. Experimental apparatus used to impose xylem tensions. The unattached leaf experienced the same temperature and rotation as the attached leaf and thus served as a control.

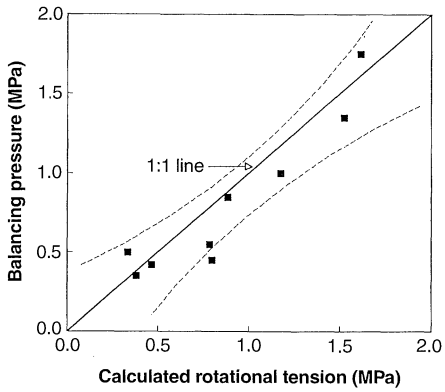

Fig. 2. Relation between xylem tension as determined by balancing pressure measurements of the leaf that was attached to the branch during spinning and the calculated rotational tension in the branch. Each point represents a different branch; dashed lines indicate the 95% confidence interval. The linear regression [balance pressure (MPa) = 0.96 × calculated tension (MPa) − 0.05; coefficient of determination, r^2, is 0.88] was highly significant ($P < 0.001$). The slope and y intercept of this relation were not significantly different ($P > 0.5$) from 1.0 and 0.0, respectively.

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leaf indicates that at least some of the xylem elements are capable of sustaining pressures below −1.5 MPa. This contradicts measurements made with the xylem pressure probe in which cavitation generally occurred at much higher pressures (>−0.4 MPa) (*9*, *11*, *12*). It is also at odds with predictions of the stability of water in the xylem based on measurements of cavitation thresholds in artificially constructed water columns subjected to centrifugal force (*14*). In the latter study, the mean cavitation threshold for distilled water in a glass tube was −0.26 MPa; stability below −1.0 MPa only occurred when stringent standards governing the purity of the water and cleanliness of all surfaces were observed. On the basis of these experimental results, Smith (*14*) concluded that xylem pressures more negative than −1 MPa are highly improbable. An alternative explanation consistent with the data presented here is that glass tubes are an inappropriate model system for assessing the stability of water under tension in the xylem.

The implications for water transport mechanisms proposed on the basis of xylem pressure probe measurements versus the balancing pressure method are profound (*15*). The balancing pressure technique indicates that hydrostatic gradients in the xylem are adequate to explain observed rates of water movement. The much smaller tensions measured by the xylem pressure probe require the existence of an additional, unknown mechanism for water transport in plants. Agreement between the balancing pressure and experimentally generated tension in the xylem provides empirical validation of the ability of the balancing pressure technique to measure negative xylem pressures, supporting the cohesion theory as the primary mechanism for water transport in higher plants.

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13. Branches of *Cercis occidentalis* were cut from the plant, recut under water to remove potential air blockage of the xylem, and left to hydrate with their cut base in water. The three leaves closest to the center of the branch were covered in plastic to prevent water loss during the period of hydration. The other leaves were removed. After 15 min the outer two of the remaining leaves were cut off, and a balancing pressure measurement was made on one of them. The remaining attached leaf plus the other excised leaf were then carefully inserted into a 2-cm diameter aluminum tube, which could be bolted to a branch holder fitted to the shaft of a motor. The branch was then fixed in place with a quick-setting (2 min) dental epoxy. The branch plus leaves was then spun at a constant speed for 15 min, which was estimated to be approximately three times the characteristic time constant for the leaf and branch to come into equilibrium. Rotational frequency was determined with a strobe light. The motor was then stopped and the leaves quickly removed from the chamber (<15 s), wrapped in plastic to prevent water loss, and balancing pressure measurements were made on both leaves. The spun branches were between 30 and 100 cm in length; angular velocities varied between 50 and 400 radians/s.
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Inhibitors of HIV Nucleocapsid Protein Zinc Fingers as Candidates for the Treatment of AIDS

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Strategies for the treatment of human immunodeficiency virus–type 1 (HIV-1) infection must contend with the obstacle of drug resistance. HIV-1 nucleocapsid protein zinc fingers are prime antiviral targets because they are mutationally intolerant and are required both for acute infection and virion assembly. Nontoxic disulfide-substituted benzamides were identified that attack the zinc fingers, inactivate cell-free virions, inhibit acute and chronic infections, and exhibit broad antiretroviral activity. The compounds were highly synergistic with other antiviral agents, and resistant mutants have not been detected. Zinc finger–reactive compounds may offer an anti-HIV strategy that restricts drug-resistance development.

Successful therapeutic management of HIV-1 infection and the associated acquired immunodeficiency syndrome (AIDS) may be achieved by antiviral strategies targeted to retroviral features that are highly conserved and thus mutationally intolerant. Sequence analysis of retroviral components has revealed a highly conserved structural motif, termed the retroviral-type zinc finger, that is arranged in a peptide segment Cys-X_2-Cys-X_4-His-X_4-Cys (CCHC; X, any amino acid) and coordinated to zinc (*1*, *2*). The chelating residues (3 Cys, 1 His) and the spacing of the zinc finger array are absolutely conserved among all known lentiretroviruses and oncornoretroviruses, and mutations in the zinc-chelating residues result in noninfectious virus (*3*). Two such CCHC-type zinc fingers are contained within the HIV-1 p7 nucleocapsid (p7NC) protein, a maturational product of the $Pr55^{gag}$ and $Pr160^{gag\text{-}pol}$ precursor polyproteins. Within the precursor polyproteins the fingers function in packaging of viral genomic RNA into progeny virions, whereas the same zinc fingers of the processed p7NC function in an early phase of retroviral infection (*3*, *4*).

The nucleophilic CCHC zinc finger donates electrons to the C-nitroso group of 3-nitrosobenzamide and certain other electrophilic groups (*5*), resulting in modification of the zinc-coordinating cysteine thiolates, ejection of zinc from the array, and inactivation of HIV-1 infectivity. Hence, electrophilic disulfide-substituted benzamides (DIBAs) discovered as active against HIV-1 by the National Cancer Institute's drug screening program were identified as potential zinc finger–reactive compounds. Molecular structures of five of the DIBA-type compounds are shown in Fig. 1. DIBA-1 and DIBA-2 are closely related congeners differing by only a single acetyl group, and DIBA-3 is a low molecular weight derivative of DIBA-1. DIBA-4 is a congener of DIBA-1 in which the *p*-aminophenyl sulfonamide moiety has been replaced with a DL-isoleucine residue, and DIBA-5 is a para-para positional isomer of DIBA-1 in which the spatial relation be-