Journal of Experimental Botany, Vol. 48, No. 315, pp. 1753–1765, October 1997

Journal of Experimental Botany

REVIEW ARTICLE

The Cohesion–Tension theory of sap ascent: current controversies

Melvin T. Tyree[1]

USDA Forest Service, Aiken Forestry Sciences Laboratory, PO Box 968, S. Burlington, VT 05402, USA

Received 12 February 1997; Accepted 21 May 1997

Abstract

In recent years, the Cohesion–Tension (C–T) theory of sap ascent in plants has come under question because of work published by Professor Ulrich Zimmermann and colleagues at the University of Würzburg, Germany. The purpose of this review is to (1) state the essential and testable elements of the C–T theory, (2) summarize the negative evidence for the C–T theory, and (3) review critically the positive evidence for the C–T theory and the evidence that the Scholander–Hammel pressure bomb measures xylem pressure potential (P_x) correctly, because much of the evidence for the C–T theory depends on pressure bomb data.

Much of the current evidence negates the conclusions drawn by Zimmermann from studies using the xylem pressure probe (XPP), but it is not yet clear in every instance why the XPP results disagree with those of other methods for estimating xylem pressure. There is no reason to reject the XPP as a useful new tool for studying xylem tensions in the range of 0 to −0.6 MPa. Additional research is needed to test the C–T theory with both the XPP and traditional methods.

Key words: Cohesion–Tension theory, cavitation, embolism, xylem pressure probe, pressure bomb.

Introduction

Two other reviews of the current controversy over the Cohesion–Tension (C–T) theory have been written (Canny, 1995; Milburn, 1996). The first concentrates on a number of strange and unproven ideas. The second provides an interesting, although limited, historical overview of some of the current questions. The present review is intended to put the Cohesion–Tension theory in a quantitative and biophysical context and reviews what I believe to be the strongest, quantitative evidence available for the C–T theory.

Essential elements of the C–T theory

The C–T theory was proposed 103 years ago by Dixon and Joly (1894), and some aspects of the C–T theory were put on a quantitative basis by van den Honert (1948) with the introduction of the Ohm's law analogue of sap flow in the soil–plant–atmosphere continuum.

According to the C–T theory, water ascends plants in a metastable state under tension, i.e. with xylem pressure (P_x) more negative than that of the vapour pressure of water. The driving force is generated by surface tension at the evaporating surfaces of the leaf. The tension is transmitted through a continuous water column from the leaves to the root apices and throughout all parts of the apoplast in every organ of the plant. Evaporation occurs predominantly from the cell walls of the substomatal chambers due to the much lower water potential of the water vapour in air. The evaporation creates a curvature in the water menisci of apoplastic water within the cellulosic microfibril pores of cell walls. Surface tension forces lower P_x in the liquid directly behind the menisci (the air–water interfaces). This creates a lower water potential, ψ, in adjacent regions, including adjoining cell walls and cell protoplasts. The lowering of ψ is a direct consequence of P_x being one of the two major components of water potential in plants, the other component being osmotic pressure, π:

$$\psi = P_x - \pi \quad (1)$$

The energy for the evaporation process ultimately comes from the sun, which provides the energy to overcome the latent heat of evaporation of the water molecules; i.e. the energy to break hydrogen bonds at the menisci.

[1] To whom correspondence should be addressed. Fax: +1 802 9516368. E-mail: MelTyree@AOL.COM

Water in xylem conduits is said to be in a metastable condition when P_x is below the vapour pressure of water (P'), because the continuity of the water column, once broken, will not rejoin until P_x rises to values above that of P'. Metastable conditions are maintained by the cohesion of water to water and by adhesion of water to walls of xylem conduits. Both cohesion and adhesion of water are manifestations of hydrogen bonding. Although air/water interfaces can exist anywhere along the path of water movement, the small diameter of pores in cell walls and the capillary forces produced by surface tension within such pores prevent the passage of air into conduits under normal circumstances. However, when P_x becomes sufficiently negative, air bubbles can be sucked into xylem conduits through porous walls.

The tension (negative P_x) at the evaporating surface of leaves is ultimately transferred to the roots where it lowers ψ of the roots below the ψ of the soil water. This causes water uptake from the soil to the roots and from the roots to the leaves to replace water evaporated at the surface of the leaves.

Van den Honert (1948) quantified the C–T theory in a classic paper in which he viewed the flow of water in the plant as a catenary process, where each catena element is viewed as a hydraulic conductance (analogous to an electrical conductance) across which water (analogous to electric current) flows. Thus, van den Honert proposed an Ohm's law analogue for water flow in plants. The Ohm's analogue leads to the following predictions: (1) the driving force of sap ascent is a continuous decrease in P_x in the direction of sap flow, (2) evaporative flux density from leaves (E) is proportional to negative of the pressure gradient ($-dP_x/dx$) at any point (cross-section) along the transpiration stream. Thus at any given point of a root, stem, or leaf vein,

$$-dP_x/dx = AE/K_h + (\rho g\, dh/dx) \qquad (2a)$$

where A = leaf area supplied water by a stem segment with hydraulic conductivity K_h and $\rho g\, dh/dx$ is the gravitational potential gradient where ρ = density of water, g = acceleration due to gravity and dh/dx = height gained, dh, per unit distance, dx, travelled by water in the stem segment.

In the context of stem segments of length (L) with finite pressure drops across ends of the segment,

$$\Delta P_x = LAE/K_h + \rho g \Delta h \qquad (2b)$$

Figure 1 illustrates water flow through a plant represented by a linear catena of conductance elements near the centre and a branched catena of conductance elements on the left. The number and arrangement of catena elements is dictated primarily by the spatial precision desired in the representation of water flow through a plant; a plant can be represented by anywhere from one to thousands of conductance elements.

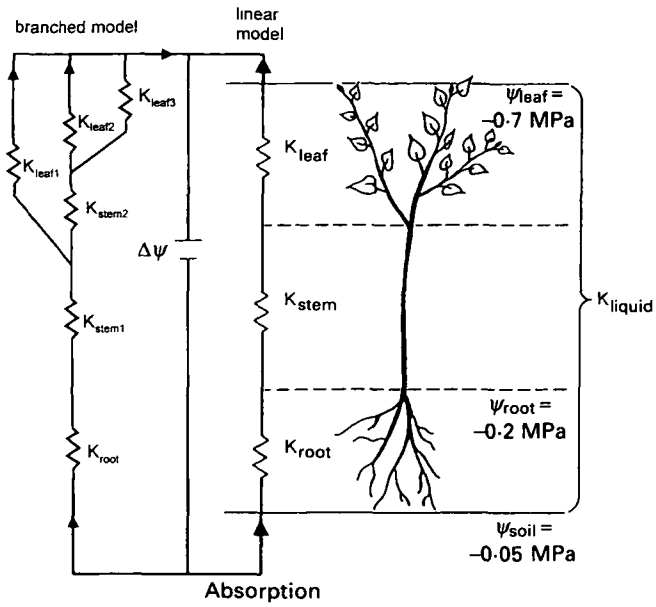

Fig. 1. The Ohm's law analogy The total conductance is seen as resultant conductance (K) of the root, stem, and leaf in series and parallel. Water flow is driven by the differences in water potential between the soil ψ_{soil} and the water potential at the evaporating surface, ψ_{evap}. On the right is the simplest Ohm's law analogy with conductances in series. On the left is a more complex conductance catena where some conductance elements are in series and some in parallel.

The Scholander–Hammel pressure bomb (Scholander et al., 1965) is one of the most frequently used tools for estimating P_x. The C–T theory does not depend on the accuracy of the pressure bomb, but much of what is known about the range of P_x tolerated by different species of plants depends on the pressure bomb. Typically, P_x can be as low as −2 MPa (in crop plants) or to −4 MPa (species in arid zones) or even −10 MPa (California chaparral species). How the pressure bomb functions is discussed later.

Negative evidence for C–T theory summarized

Using a xylem pressure probe (XPP), Balling and Zimmermann (1990) demonstrated that the Scholander–Hammel pressure bomb (Scholander *et al.*, 1965) does not measure xylem water tension correctly under many circumstances. The pressure bomb functions properly only if there is direct pressure transmission from the air in the bomb to the xylem fluid. For example, when sap efflux from the cut end of a leaf is blocked by a fluid-filled pressure transducer on a leaf at $\psi = 0$, there should be a 1:1 relationship between gas pressure in the bomb and P_x measured by the transducer. But a 1:1 relationship was not found (Balling and Zimmermann, 1990) in tobacco leaves, and this finding has been confirmed independently in *Tsuga canadensis* by C Wei and MT Tyree (unpublished results). The failure of pressure trans-

mission in the positive pressure range in *T. canadensis* is related to the elasticity of woody stems and the compression of air bubbles in embolized tracheids and does not invalidate the theoretical functioning of the pressure bomb in the normal mode of operation when $P_x < 0$.

More worrisome is an apparent failure of pressure transmission in the negative pressure range, i.e. P_x measured with the XPP in individual vessels did not increase as expected with an increase of air pressure in the pressure bomb. Professor Zimmermann concluded that the pressure bomb overestimates tension (gives a P_x that is too negative).

He has also shown that xylem tension exceeding 0.6 MPa is rarely observed with the XPP (Zimmermann *et al.*, 1993*b*). For example, the XPP has been inserted into vessels of many species in the morning and P_x values usually are positive (on an absolute pressure scale where a vacuum is 0 MPa). As the day progresses and transpiration increases, P_x falls to -0.2 to -0.6 MPa. Then the vessel punctured by the XPP cavitates and P_x returns to positive values consistent with embolized lumens filled with water vapour and air. When many plants are probed at midday, most vessels are found to be embolized, i.e. the XPP records absolute pressures >0 MPa. Zimmermann and colleagues have proposed a number of mechanisms of sap transport in plants that are consistent with sap flow around embolized vessels, which they believe is the normal state in plants (Zimmermann *et al.*, 1993*a*, *b*). Zimmermann does not reject the C–T theory outright; he acknowledges that tension-driven water movement is occurring when he measures P_x values in the range of 0 to -0.6 MPa with the XPP, but he also postulates that other mechanisms must be at work when ψ values drop much below -0.6 MPa.

Important questions can be raised and answered by examining the current literature in the next section dealing with positive evidence for the C–T theory.

(1) Does normal water transport occur while vessels are embolized? If plants normally transpire with most vessels embolized then this condition ought to be identifiable by measuring the hydraulic conductance of stems in their native state and by quantitative tests of the Ohm's law analogue (Equations 2a, b).

(2) Do most intact xylem conduits embolize at $P_x > -0.6$ MPa or do they embolize only because the xylem wall has been damaged by insertion of the XPP? This question can be answered by looking at the mechanism of cavitation events and by using clever ways of inducing xylem tension.

(3) Is there independent evidence of the expected pressure transmission in a pressure bomb when $P_x < 0$ MPa? One way to answer this question is to use a temperature corrected stem hygrometer on woody plants.

Evidence in support of the pressure bomb and the C–T theory

The Scholander–Hammel pressure bomb

The Scholander–Hammel pressure bomb is a device used to measure the equilibrium xylem pressure, P_x. For example, a cut leaf is placed inside a pressure vessel with the petiole protruding through a rubble seal to the outside air. When compressed gas is admitted into the pressure chamber, the gas pressure is presumed to be transmitted directly to the xylem fluid raising P_x. When P_x reaches a value slightly above zero (= atmospheric pressure) then water begins to flow out of the vessels at the cut end of the petiole. The gas pressure when water first emerges is called the balance pressure, P_B. The negative of P_B is equated to P_x prior to admitting compressed gas to the chamber.

The basic hypothesis of the pressure bomb can be stated as follows: When a transpiring shoot is excised from a plant, the negative P_x prior to excision is maintained after excision in the xylem by surface tension at pit membranes in vessel walls. The actual situation is slightly more complicated because a transpiring shoot will have gradients of P_x whereas the pressure bomb measures an equilibrium P_x after the gradients of P_x have disappeared. Let us focus on how P_x changes near the point of excision, i.e. within one vessel length of the cut surface. Immediately after cutting, P_x *temporarily* becomes zero in all severed vessels. Then P_x becomes negative again as water is sucked into dehydrated cells in leaves by osmosis draining the vessels until a meniscus is re-established on pit membranes at the ends of remaining intact vessels. As water flows into the living cells their ψ becomes slightly more positive. At equilibrium two things happen: (1) Any gradients in P_x originally between the cut surface of the stem or petiole and the evaporating surface of the leaves disappears. (2) An equilibrium is established between the ψ of all living cells and the ψ of xylem fluid ($=P_x - \pi_x$). So the equilibrium P_x could be more negative than originally present at the point of cutting if there was a large pressure gradient from the cut point of the stem to the leaves, because the more dehydrated leaf cells will tend to draw water from the less dehydrated petiole or stem cells until the gradient disappears. Or the equilibrium P_x could be less negative, if there was substantial rehydration of living cells when the cut vessels were drained of water and only some of the vessels refill when the balance pressure, P_B, is established. Thus, equilibrium P_x in the xylem does not return to the P_x in the xylem at the time of cutting.

Regardless of how much the equilibrium P_x differs from the steady-state P_x in the transpiring shoot, the pressure-bomb hypothesis can be stated as follows:

When the shoot is placed in a pressure bomb with the cut end protruding though a pressure seal to the outside

air, the value of P_x can be changed by applying air pressure P_a to the entire shoot surfaces within the bomb. For every unit increase in P_a, P_x will become less negative by one unit until the balance pressure (P_B) is reached when $P_x = 0$ and water is 'balanced' on the cut end. This can be expressed as:

$$P_x = P_a - P_B \quad (3a)$$

$$P_x = \psi_x + \pi_x \quad (3b)$$

The second equality states that P_x can be expressed in terms of ψ_x (=the xylem water potential) minus π_x (= the xylem osmotic pressure). Equating Equation 3a to 3b yields:

$$\psi_x = P_a - \pi_x - P_B \quad (3c)$$

In an enclosed air space adjacent to xylem water, the water potential of the vapour phase (ψ_v) should equal ψ_x provided equilibrium of temperature and water-vapour pressure is obtained. Thus we have:

$$\psi_v = P_a - \pi_x - P_B \quad (4)$$

Equation 4 and thus the validity of the pressure bomb have been confirmed on *Thuja occidentalis* shoots over the range of 0 to −2.1 MPa using temperature-corrected stem psychrometers to measure ψ_v under rigorously documented conditions of vapour equilibrium (Dixon and Tyree, 1984). In these experiments, a large *T. occidentalis* shoot was enclosed in a pressure bomb and dehydrated to a $P_B = 2.1$ MPa. A temperature-corrected stem hygrometer was attached to the cut basal surface of the shoot outside the pressure bomb. Dixon and Tyree demonstrated that vapour equilibrium occurred with a half-time of 19 s. Thermal equilibrium never was obtained since the wood surface always was cooler than the thermocouple in the hygrometer used to sense ψ_v, but temperature stability was reached with a half-time of 27 s. A thermocouple touching the surface of the wood was used to measure the stable temperature difference between the wood surface and the sensing thermocouple. This difference could be used to make thermodynamic corrections to ψ_v. Without thermal corrections, the data resembled Fig. 2a, and Fig. 2b after correction.

These data provide strong evidence for the validity of the pressure bomb hypothesis. More recently, Holbrook *et al.* (1995) have used an elegant method to create xylem tension mechanically and further test the pressure-bomb hypothesis. Unfortunately, the experiment has caused confusion because Holbrook *et al.* (1995) did not discuss the underlying physics in sufficient detail due to space limitations in the original publication. They excised stem segments with a single leaf at the midpoint of the segment, and mounted the midpoint of the stem on the rotating axis of a motor-driven shaft (Fig. 3). The rotation of the stem segment will produce a centrifugal tension at the

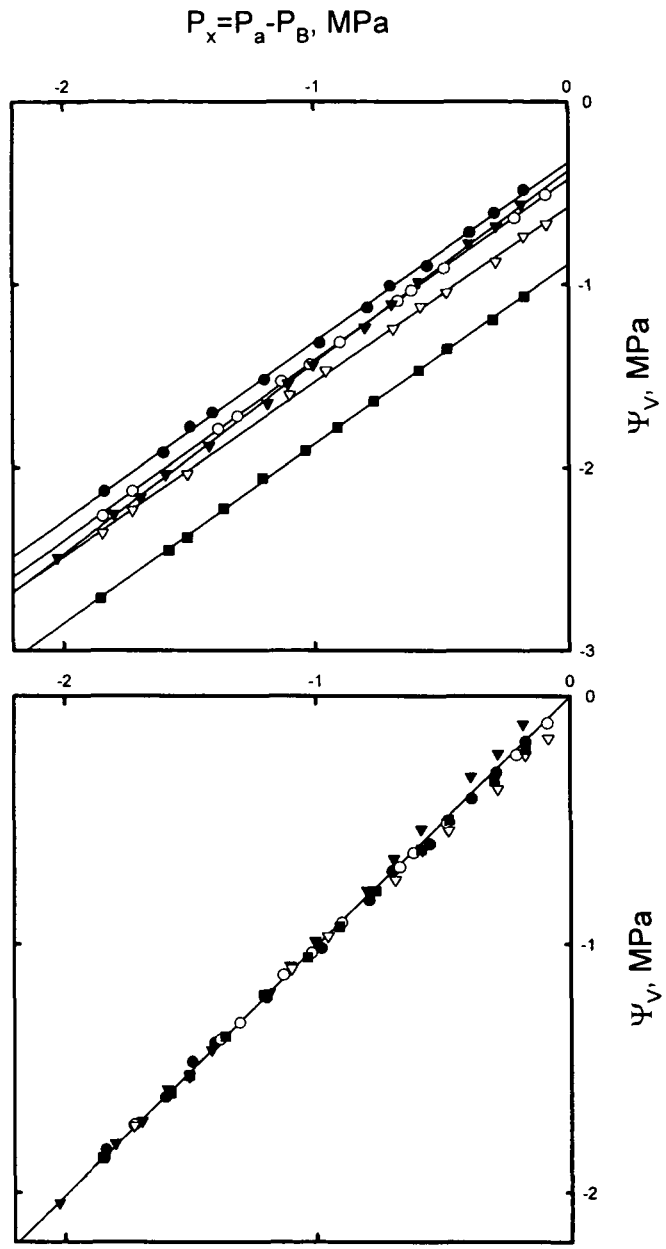

Fig. 2. Experimental demonstration of how air pressure in a pressure bomb controls the P_x in the xylem of an excised shoot of *Thuja occidentalis*. The *x*-axis is the predicted P_x based on the current air pressure (P_a = the variable) and the balance pressure (P_B = a constant in this experiment). The *y*-axis is the water potential of the vapour phase above the cut end of the shoot. A temperature-corrected stem hygrometer was attached to the cut end and the hygrometer chamber approached vapour equilibrium with a half-time of 27 s and temperature stability was reached with a half-time of 19 s. (A) The relationship before correction for the steady-state temperature difference between the cut surface of the stem and the measuring thermocouple of the hygrometer. (B) the relationship after correction for the influence of the measured temperature differences on ψ_v. The dashed line shows the 1:1 relation.

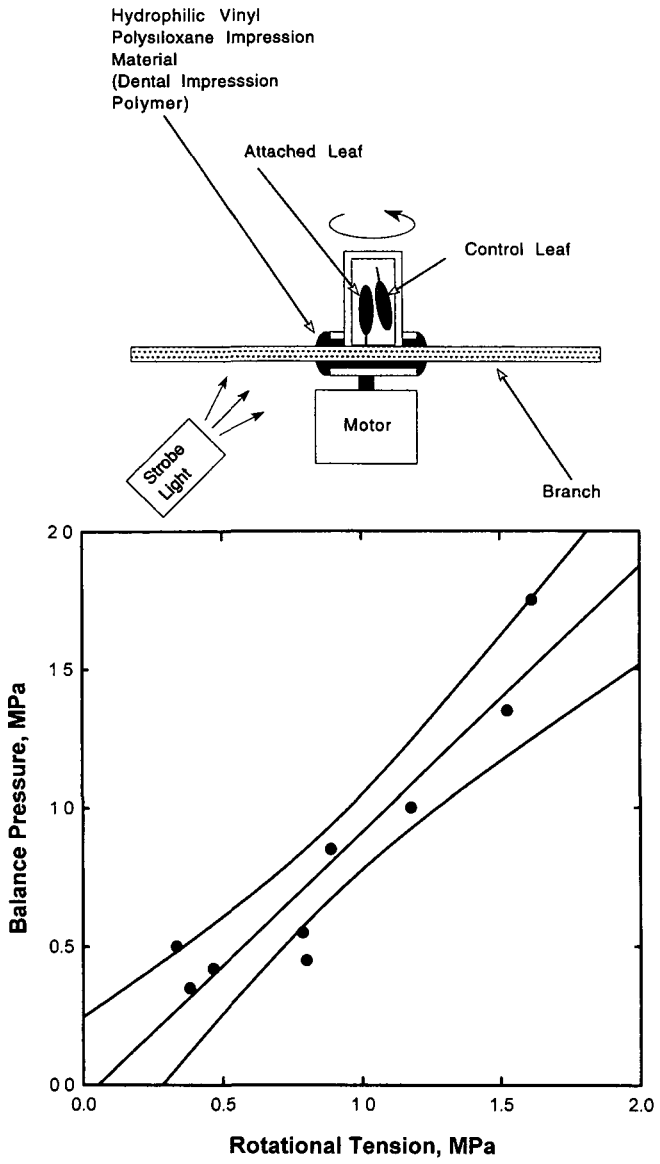

Fig. 3. The Holbrook experiment. (A) The experimental method. A stem segment was mounted on a centrifuge motor. Centrifugation of the segment induced a P_x as given in Equation 5. The strobe light was used to measure angular velocity. (B) Results show that the rotational tension was transmitted to the attached leaf; the x-axis is the calculated rotation tension $= P_x$ in Equation 5 and the y-axis is the balance pressure of the attached leaf at the end of the experiment.

midpoint of the segment which in pressure units will be equal to:

$$P_x = -0.5\sigma\omega^2 r^2 \qquad (5)$$

where σ = the fluid density, ω = the angular velocity of rotation, and r = distance between the axis of rotation and the end of the stem segment. The negative P_x at the midpoint of the segment will cause a dehydration of the living cells in the attached leaf, and the water drawn out of the leaf will travel to the ends of the rotating segment where the centrifugal forces on the water will cause it to fly off ends in a fine spray. When the motor is turned off the P_x in the midpoint will be preserved by the ψ of the dehydrated leaf cells. After centrifugation, the balance pressure of the excised leaves correlated well with the expected P_x (Fig. 3). This experiment demonstrated that xylem tensions created by centrifugation of shoots are transmitted to leaves and measured correctly with the pressure bomb. This experiment also proved that P_x values down to -1.8 MPa could be sustained without cavitation because Equation 5 correctly predicts P_x only if the water columns are continuous in the centrifuged stem. The centripetal force of the rotating mass of water molecules near the ends of the column are transmitted toward the axis of rotation by the cohesion of water. The value of P_x is most negative at the axis of rotation and rises continuously to atmospheric pressure at the ends of the stem segment. If the water columns had broken, some of the mass toward the ends would have been lost from the rotating system and P_x would have been less negative than predicted by Equation 5.

Hydraulic architecture and the Ohm's law analogue

Recent studies of the hydraulic architecture of woody plants have provided strong support for the C–T theory. The basic approach has been to obtain independent measurements of P_x (usually with a pressure bomb), E (via weight loss or gas exchange method), and K_h (with a conductivity apparatus) and then test the validity of the Ohm's law analogue, Equation 2a, b. Testing the Ohm's law analogue on a large plant (tree or liana) requires the following steps:

(1) Estimate the regression of K_h versus stem diameter over the range of diameters on the tree.
(2) Make a hydraulic map of the tree in which each branch is made up of discrete segments, the segments being delineated by nodes (branch insertion points) so that Equation 2b can be applied to each segment. Keep a record of segment length (L) and diameter so that K_h can be estimated from the regression in (1), and keep a record of leaf area attached to each segment.
(3) Measure E for representative leaves.
(4) Compute ΔP_x for each segment using Equation 2b. Then, using the map, add the ΔP_x values from the base of the tree to any shoot apex.

Benkert *et al.* (1995) measured P_x at the apex of several branches at different heights, h, in a large liana during the day when $E > 0$. They argued that the pressure gradient, dP/dh, should be $< -\rho g\, dh/dx$ (Equation 2a), i.e. P_x should decline more than -0.01 MPa m^{-1} gain in height in vertical stems where $dh/dx = 1$. Benkert *et al.* (1995) also argued that the Ohm's law analogue was incorrect

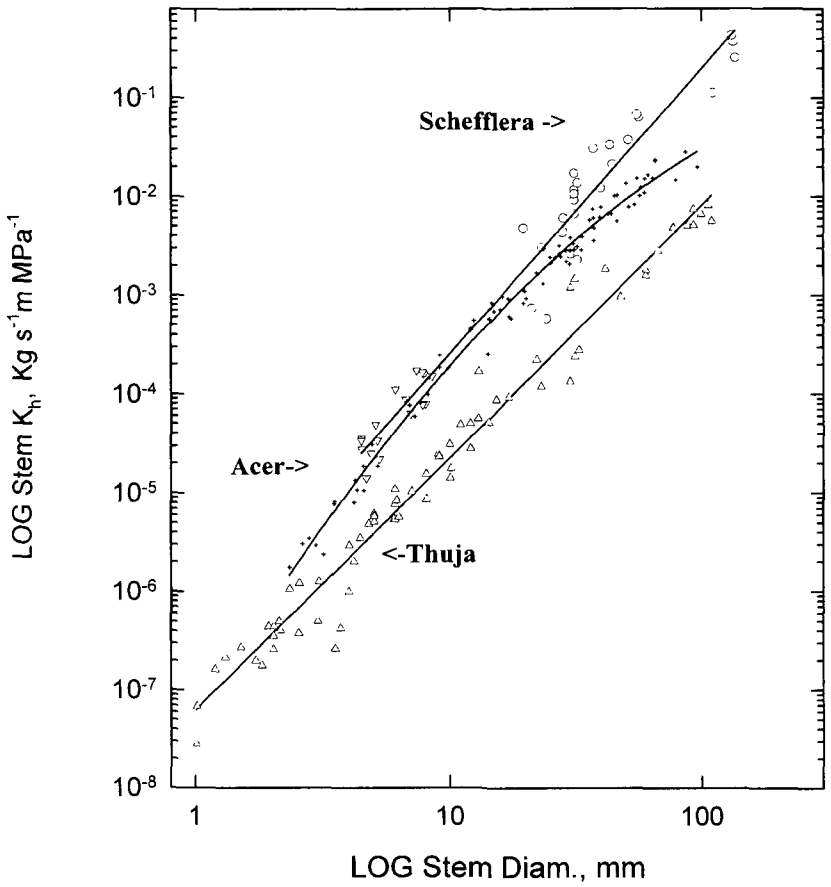

Fig. 4. Hydraulic conductivity of stems per unit pressure gradient, K_h, plotted versus diameter of the stem (excluding bark, x-axis). Species represented are *Thuja occidentalis*, *Acer saccarum*, and *Schefflera morototoni*. Both axes are logarithmic Adapted from Tyree *et al.* (1991).

because the pressure gradient was $> -\rho g\ dh/dx$. They pointed to similar examples of P_x (measured with a pressure bomb) versus height in tall trees failing to satisfy Equation 2a. The implication of this argument would appear to be that water transport must be driven by a force in addition to dP/dx. It will be seen later that such reasoning is invalid because Equation 2a correctly predicts dP/dx along the *pathway* of water transport, i.e. along the axis of stems. The error made by Benkert *et al.* (1995) is in using Equation 2a to predict the pressure gradient between apices at different heights with no knowledge of K_h along the pathway of water flow. It is erroneous to assume dP/dh at shoot apices can be predicted by dP/dx without knowing the functional dependence of K_h versus x.

Zimmermann would predict that the left side of Equation 2a should be larger than first term of the right side (AE/K_h). He suggested that xylem conduits usually are embolized when P_x is more negative than -0.6 MPa. So K_h should be lower at times of maximum E. Values of K_h are measured in a conductivity apparatus under positive P_x, so few conduits will be embolized and hence K_h should be maximal (reducing the estimated AE/K_h). Zimmermann also stated that the pressure bomb underestimates P_x, i.e. gives values that are too negative (inflating $-dP/dx$). But studies have confirmed equality in Equation 2a, suggesting that both measures of K_h and of P_x (measured with the pressure bomb) are correct (Tyree, 1988; Ewers *et al.*, 1989).

An examination of case studies will prove the points just raised. Figure 4 shows K_h versus stem diameter for a gymnosperm, a temperate angiosperm, and a tropical angiosperm. K_h varies over several orders of magnitude for a given species because large-diameter stems can transport more water and hence are more conductive than small-diameter stems. The species variation is even more remarkable; e.g. a *Schefflera* stem can be as much as 100 times more conductive than a *Thuja* stem of the same diameter.

A parameter of more interest is leaf specific conductance, $K_L = K_h/A$. This parameter is useful because dP_x/dx in a stem segment is inversely proportional to K_L at a given E if gravity is ignored in Equation 2a:

$$-dP_x/dx = E/K_L \qquad (6)$$

Ranges of values of K_L versus stem diameter are shown

in Fig. 5. Again, K_L can differ by up to two orders of magnitude between species for stems at a given diameter. Also, K_L increases as a function of diameter, so $-dP_x/dx$ is steepest in the smallest diameter branches. In some cases $-dP_x/dx$ can be as much as 1 MPa m^{-1} as in *Thuja* where $K_L = 10^{-5}$ kg s^{-1} m^{-1} MPa^{-1} for stem segments 2–4 mm in diameter and $E = 10^{-5}$ kg s^{-1} m^{-2} at noon. The consequence of this pattern of K_L versus diameter can be seen in P_x profiles predicted by the Ohm's law analogue and the hydraulic map of representative trees. Figure 6 shows plots of P_x versus path length, x, from the base of the tree to representative branch apices. The P_x profiles were calculated using values of E at midday, K_L, and the hydraulic maps.

Looking at the pattern of P_x at branch apices in the *Thuja* profile, it can be seen that there is no consistent pattern in declining P_x with height up the tree. The apices mark with '*' are at heights 4.1 and 7.5 m in the hydraulic map, but have P_x values of -1.0 and -0.9 MPa, respectively, giving a $dP_x/dh = +0.071$ MPa m^{-1} rather than the required value of -0.01 MPa m^{-1} needed to lift water against the force of gravity. Tyree (1988) computed the mean P_x of all small-diameter branches versus h in *Thuja* and concluded that there is no consistent decline of P_x

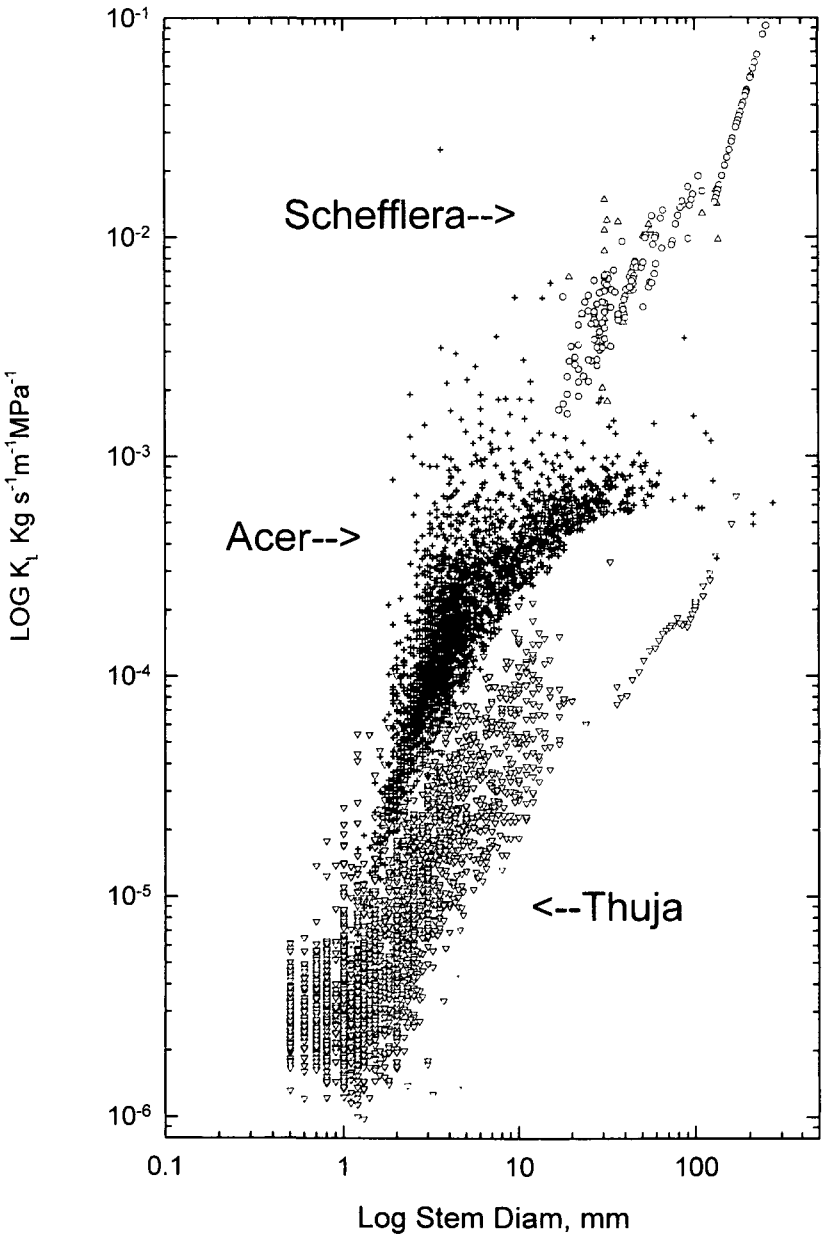

Fig. 5. Leaf specific hydraulic conductance $K_L = K_h/A$, where A = leaf area apical of the stem segment on which K_h was measured plotted versus diameter of the stem (excluding bark). Species are as in Fig. 5; both axes are logarithmic. Adapted from Tyree *et al.* (1991).

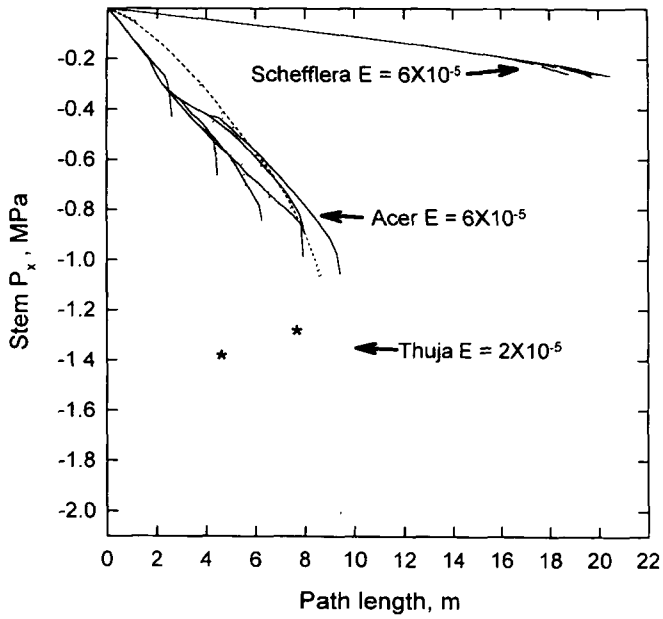

Fig. 6. Profiles of xylem pressure potential, P_x, versus distance water must travel from the base of the tree to a stem apex. All values include the gravitational potential gradient required to lift water up the tree. The curves were calculated from the hydraulic maps and the representative midday evaporative flux densities (E) indicated for each species in the graph. Adapted from Tyree *et al.* (1991).

versus h (Tyree, 1988; Fig. 3). Nevertheless, P_x declined with increasing distance along every pathway such that dP_x/dx always was < -0.01 MPa m^{-1} in all stem segments. Tyree (1988; Fig. 5) also found that the Ohm's law analogue combined with diurnal measurements of E provided good predictors of actual diurnal changes in P_x measured with a pressure bomb. The agreement between measurement and theory suggests that three conditions were met simultaneously: (1) the pressure bomb provides a correct estimate of P_x; (2) the method of measuring K_h yields valid results; and (3) the Ohm's law analogue is correct.

Other studies have confirmed the correctness of the Ohm's law analogue. Ewers *et al.* (1989) studied the hydraulic architecture of a large woody vine (>20 m long) growing along the ground to avoid the effects of gravity on P_x. They measured K_L, E, and constructed hydraulic maps. They used the Ohm's law analogue to compare predicted values of $-dP_x/dx$ with values measured on bagged leaves using a pressure bomb. The predicted gradients (0.083 ± 0.033 MPa m^{-1}) agreed with the gradients measured during midday with a pressure chamber (0.076 ± 0.016 MPa m^{-1}). Measured and predicted gradients also agreed when $E = 0$. Tsuda and Tyree (unpublished results) compared the predicted drop in P_x from soil to leaf (based on gravimetric measures of E and whole shoot and root hydraulic conductances) to values of P_x measured with a pressure bomb. They found good agreement in young *Acer saccharinum* plants (Fig. 7).

The stark contrast between these examples and Zimmermann's predictions should be noted. In every case cited, K_L, was measured with positive pressures, so according to Zimmermann, all K_L values would be overestimates of the values that apply during midday when he believes vessels are embolized. Zimmermann also suggested that the pressure bomb underestimates P_x. Hence, the Ohm's law analogue (Equation 2b) should be violated if he is correct. Since Equation 2b is quantitatively correct, Zimmerman's observations must not represent the normal status of plants, and the pressure bomb must be presumed more reliable than suggested by his studies.

The mechanism of cavitation and the tensions that seed cavitations

Finally, recent studies on the mechanism of cavitation in xylem conduits have confirmed that cavitations are air-seeded. The air-seeding hypothesis provides strong evidence for the existence of large negative P_x values prior to cavitation events.

A cavitation event in xylem conduits ultimately results in dysfunction. A cavitation occurs when a void of sufficient radius forms in water under tension. The void is gas filled (water vapour and some air) and is inherently

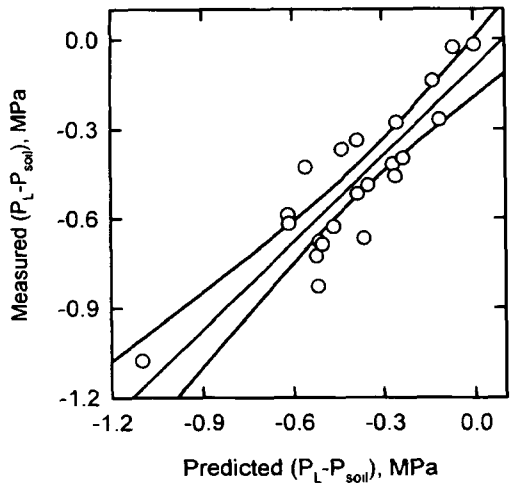

Fig. 7. Computed versus measured $\Delta P_x = P_L - P_{soil}$ for *Acer saccarinum* plants growing in pots. Each point represents a different plant. Evaporative flux density (E) was estimated from weight of water lost from the potted plants and leaf area measured at the end of the experiment. E was varied by controlling light intensity. Once a steady-state E was reached, the balance pressure of a transpiring leaf was estimated (P_L). Soil-water potential (P_{soil}) was estimated by the balance pressure of nontranspiring plants. So the y-axis is the measured pressure drop from soil to leaf. At the end of the experiment, the entire plant was harvested and the hydraulic conductance of the root (K_R), the stems (K_S) and the leaves (K_L) was estimated with a high pressure flowmeter. The predicted ΔP_x on the x-axis was computed by applying Equation 2b to each conductance element (root, stem, and leaf). Unpublished data of Makoto Tsuda and Melvin T Tyree.

unstable, i.e. surface-tension forces will make it collapse spontaneously unless the water is under sufficient tension (negative pressure) to make it expand. A digression follows to explain why this is true.

The chemical force driving the collapse is the energy stored in hydrogen bonds, the intermolecular force between adjacent water molecules. In ice, water is bound to adjacent water molecules by 4 hydrogen bonds. In the liquid state, each water molecule is bound by an average of 3.8 hydrogen bonds at room temperature. In the liquid state, hydrogen bonds are forming and breaking all the time permitting more motion of molecules than in ice (Slatyer, 1968). However, when an interface between water and air is formed, some of those hydrogen bonds are broken and the water molecules at the surface are at a higher energy state because of the broken bonds. The force (N = Newtons) exerted at the interface as hydrogen bonds break and reform can be expressed in pressure units (Pa), because pressure is dimensionally equal to energy (J = Joules) per unit volume of molecules, i.e. $J\,m^{-3} = Nm\,m^{-3} = N\,m^{-2} = Pa$. Stable voids in water tend to form spheres because spheres have the least surface area per unit volume; and thus, a spherical void has the minimum number of broken hydrogen bonds per unit volume of void. The pressure tending to make a void collapse is given by $2\tau/r$, where r is the radius of the spherical void and τ is the surface tension of water (= 0.072 Pa m at 25 °C).

For a void to be stable, its collapse pressure ($2\tau/r$) must be balanced by a pressure difference across its surface or meniscus = $P_v - P_w$, where P_w is the absolute pressure (= P_x + the atmospheric pressure) of the water and P_v is the absolute pressure of the void.

$$P_v - P_w = 2\tau/r \qquad (7)$$

P_v always is above absolute zero pressure (= perfect vacuum) since the void usually is filled with water vapour and some air. Relatively stable voids are common in daily life, e.g. the air bubbles that form in a cold glass of water freshly drawn from a tap. An entrapped air bubble is temporarily stable in a glass of water because P_w is a relatively constant 0.1 MPa and P_v is determined by the ideal gas law, $P_v = nRT/V$, where n = the number of moles of air in the bubble, R = gas constant, T = absolute temperature, and V = the volume of the bubble. So the tendency of the void to collapse ($2\tau/r$) makes V decrease which causes P_v to increase according to the ideal gas law because P_v is inversely proportional to V. The rise in P_v provides the restoring force across the meniscus needed for stability. But an air bubble in a glass of water is stable only temporarily because according to Henry's law, the solubility of a gas in water increases with the pressure of the gas. So the increased pressure exerted by $2\tau/r$ makes the gas in the bubble more soluble in water and it slowly collapses as the air dissolves, i.e. as n decreases.

Air bubbles are rarely stable in xylem conduits because transpiration can draw P_w to values <0. As P_w falls towards zero the air bubble expands according to the ideal gas law, but V can never grow larger than the volume of the conduit, so P_v can never fall low enough to allow $P_v - P_w$ to balance $2\tau/r$ without a decline in P_w. Once the bubble has expanded to fill the lumen, the conduit is dysfunctional and no longer capable of transporting water. Fortunately for the plant, a dynamic balance at the meniscus in cell walls ultimately is achieved. This stability will be discussed first in the context of a vessel and its pit membranes.

As the air bubble is drawn up to the surface of the pit membrane in vessel cell walls, the pores in the pit membrane break the meniscus into may small menisci at the opening of each pore. As the meniscus is drawn through the pores, the radius of curvature of the meniscus, r_m, falls toward the radius of the pores, r_p. Again, as long as r_m exceeds r_p, the necessary conditions for stability are achieved, i.e.

$$P_v - P_w = 2\tau/r_m \qquad (8)$$

Usually, a dysfunctional conduit eventually will fill with air at atmospheric pressure (as demanded by Henry's law), so P_v eventually approaches 0.1 MPa as gas diffuses through water to the lumen and comes out of solution. When P_v equals 0.1 MPa, the conduit is said to be fully embolized. As P_w rises and falls as dictated by the demands of transpiration, r_m adjusts at the pit-membrane pores to achieve stability. When the conduit is fully embolized, both sides of Equation 8 can be expressed in terms of xylem pressure potential,

$$P_x \cong -(P_v - P_w) = -2\tau/r_m \qquad (9)$$

The minimum P_x that can be balanced by the meniscus is given when r_m equals the radius of the largest pit-membrane pore bordering the embolized conduit. If the largest pore is 0.1 or 0.05 μm, the minimum stable P_x is -1.44 or -2.88 MPa, respectively. So the porosity of the pit-membrane is critical to preventing dysfunction of vessels adjacent to embolized vessels (Sperry and Tyree, 1988). When P_x falls below the critical value, the air bubble is sucked into an adjacent vessel, seeding a new cavitation.

Consequently, the genetics that determines pit morphology and pit-membrane porosity must be under strong selective pressure. A safe pit-membrane will be one with very narrow pores and one thick enough and thus strong enough to sustain substantial pressure differences without rupturing.

The situation for tracheids of conifers is different because air movement from an embolized tracheid to an adjacent tracheid is prevented by the sealing (aspiration) of the torus against the overarching border of the pit. In most cases, the porosity of the margo that supports the

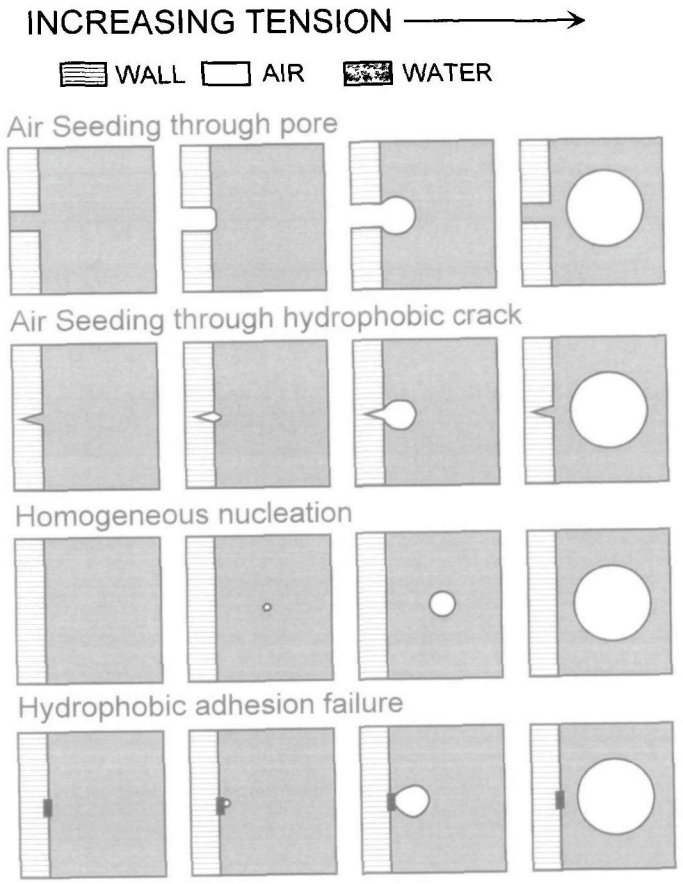

Fig. 8. Four possible mechanisms of cavitation induction. (1) Air-seeding through a pore occurs when the pressure differential across the meniscus is enough to allow the meniscus to overcome surface tension and pass through the pore. (2) Air-seeding through a hydrophobic crack occurs when a stable air bubble resides at the base of a hydrophobic crack in the wall of a xylem conduit. When the P_x becomes negative enough the bubble is sucked out of the crack. (3) Homogeneous nucleation involves the spontaneous generation of a void in a fluid. It is a random process requiring thermal motion of the water molecules. The hydrogen bonds at a specific locus are broken when all water molecules randomly move away from any locus at the same instant with sufficient energy to break all hydrogen bonds between water molecules. As the tension in the water increases the hydrogen bonds are stretched and weakened so the energy needed to break the bonds decreases making a homogeneous nucleation more likely. (4) Hydrophobic adhesion failure is similar to homogeneous nucleation except that hydrogen bonds are broken between water and a hydrophobic patch in the wall where the energy of binding between water and wall is reduced.

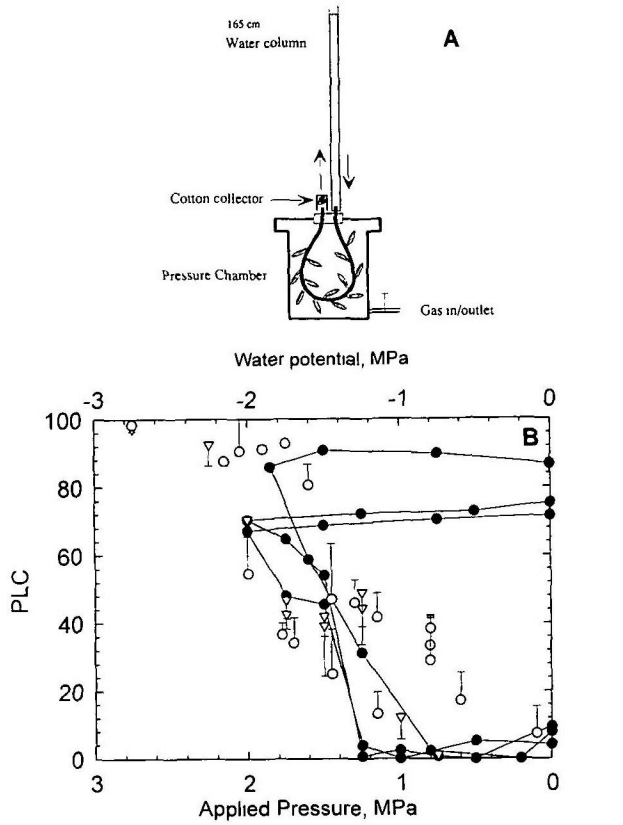

Fig. 9. The first experimental test of the air-seeding hypothesis. (A) The experimental apparatus. A willow branch is bent around in a large pressure bomb so that both cut surfaces are outside the bomb. Water continually passes through the stem segment under positive pressure from a water column to a cotton collector. Flow rate is estimated by measuring the weight change of the cotton collector over known time intervals. (B) The results are shown as a vulnerability curve where the *y*-axis is the per cent loss of hydraulic conductivity (*PLC*) and the *x*-axis is the negative P_x or the air pressure in the bomb needed to cause the plotted *PLC*. Open symbols are for *PLC* induced by negative P_x and closed symbols are induced by positive pressure applied in the pressure bomb. See text for more details.

torus is too large to prevent meniscus passage at pressure differences exceeding 0.1 MPa (Sperry and Tyree, 1990). But the margo is elastic, so a pressure difference of only 0.03 MPa is sufficient to deflect the torus into the sealed position. Air bubbles pass between tracheids when the pressure difference becomes large enough to rip out the torus from its sealed position (Sperry and Tyree, 1990).

A biophysical understanding now exists of the stability of emboli and how they can be sucked into a water-filled conduit from a neighbouring embolized conduit. Plants always will have some embolized conduits to seed embolism into other conduits. Embolisms are the natural consequence of foliar abscission, herbivory, wind damage, and other mechanical fates that might befall a plant. It is now appropriate to question whether all emboli are seeded from adjacent conduits or whether another mechanism occurs in some or most of the cases.

Four mechanisms for the nucleation of cavitations in plants have been proposed. These are illustrated in Fig. 8, which shows for each mechanism the sequence of events that might occur as P_x declines in the lumen of a conduit. See Zimmermann (1983), Tyree *et al.* (1994), and Pickard (1981) for a detailed discussion of the four mechanisms. Other air-seeding mechanisms have been proposed that apply to SCUBA divers (Yount, 1989). Such mechanisms also might occur in plants when gas solubility decreases in xylem water as it warms, but little is known about the importance of this mechanism in plants. This study is

concerned only about which mechanism occurs most frequently in plants.

Experiments can discriminate between the air-seeding mechanism and the other three in Fig. 8 and Yount (1989). All mechanisms predict cavitation when xylem fluid is under tension, but the air-seeding mechanism predicts that air can be blown into vessels while the fluid is under positive pressure. The air-seeding mechanism requires only a pressure differential ($P_a - P_w$), where P_a is the air pressure outside and P_w is the fluid pressure inside (Fig. 9a). It makes no difference if P_a is 0.1 and P_w is -3.0 or if P_a is 3.1 and P_w is 0.1. Experiments have shown that the same vulnerability curve results whether P_w is reduced by air dehydration or P_a is increased in a pressure bomb (Cochard *et al.*, 1992). A vulnerability curve is a plot of per cent loss hydraulic conductivity (*PLC*) versus the P_x required to cause the *PLC* by cavitation events. The results of this experiment are shown in Fig. 9b (see also Jarbeau *et al.*, 1995).

Willow stem segments with leaves were enclosed in a pressure chamber with cut ends protruding into the open air. Water was passed continually through the xylem under positive pressure. While stem conductance was being monitored, the gas pressure, P_a, in the pressure chamber was increased gradually. Initially, the hydraulic conductance of the stem segment did not decrease until a critical pressure of 1 MPa was applied. (Each solid circle in Fig. 9b represents the application of pressure for 30–40 min.) When P_a was increased gradually beyond the critical value the stem conductance began to fall (increased *PLC*). When P_a was gradually decreased, the *PLC* stopped decreasing. The vulnerability curve from this experiment was identical to that found for similar branches dehydrated in the air.

This is the strongest evidence presented to date that the air-seeding mechanism explains how cavitations occur, though there is other circumstantial evidence (Crombie *et al.*, 1985; Sperry and Tyree, 1988). The air-seeding hypothesis can be viewed as providing strong evidence for the existence of large negative P_x values prior to cavitation events. The aspiration of the meniscus into a cavitating vessel is driven by a pressure *difference* = $P_a - P_w$. If the pressure difference is, say, 8 MPa when $P_w = 0.1$ and $P_a = 8.1$ MPa, it follows that P_w must equal -8.1 MPa ($P_x = -8.0$) when $P_a = 0.1$ MPa. Sperry *et al.* (1996) compared the vulnerability curves of numerous species measured by the application of positive air pressure versus curves measured by bench-top dehydration where P_x values were measured by the pressure bomb method. A 1 : 1 agreement was found for $-P_x$ or P_a from 1.0 to 9.5 MPa (Fig. 10). For this agreement to exist, two things have to be true: (1) The air-seeding hypothesis must be the mechanism of cavitation and (2) the pressure bomb must measure values of P_x correctly. For readers who still may doubt the accuracy of the pressure bomb, after all the arguments in this paper, one other elegant

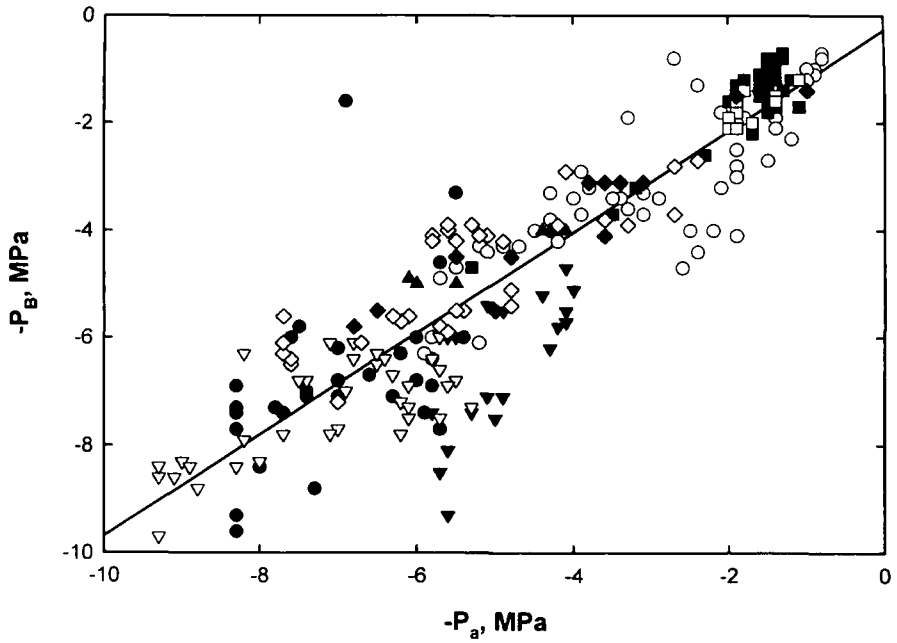

Fig. 10. Vulnerability curves were generated in two ways. (1) Cavitations induced by negative P_x in which the pressure bomb was used to estimate $P_x = -P_B$ measured on leaves attached to the dehydrated stems. (2) Cavitations induced by positive pressure, P_a, in an experiment like that in Fig. 9. The vulnerability curves were examined in the range of 40–60 *PLC* and for each *PLC* value corresponding values of $-P_a$ and $-P_B$ were recorded and plotted on the *x*- and *y*-axis, respectively. These data were compiled from vulnerability-curve data on 12 species. (modified from Sperry *et al.*, 1996). The slope is not significantly different from 1.0 (Student's *t* test, $t_s = 0.301$, $P > 0.5$) indicating that negative pressures are measured correctly by the pressure bomb and that cavitations occurred by air-seeding.

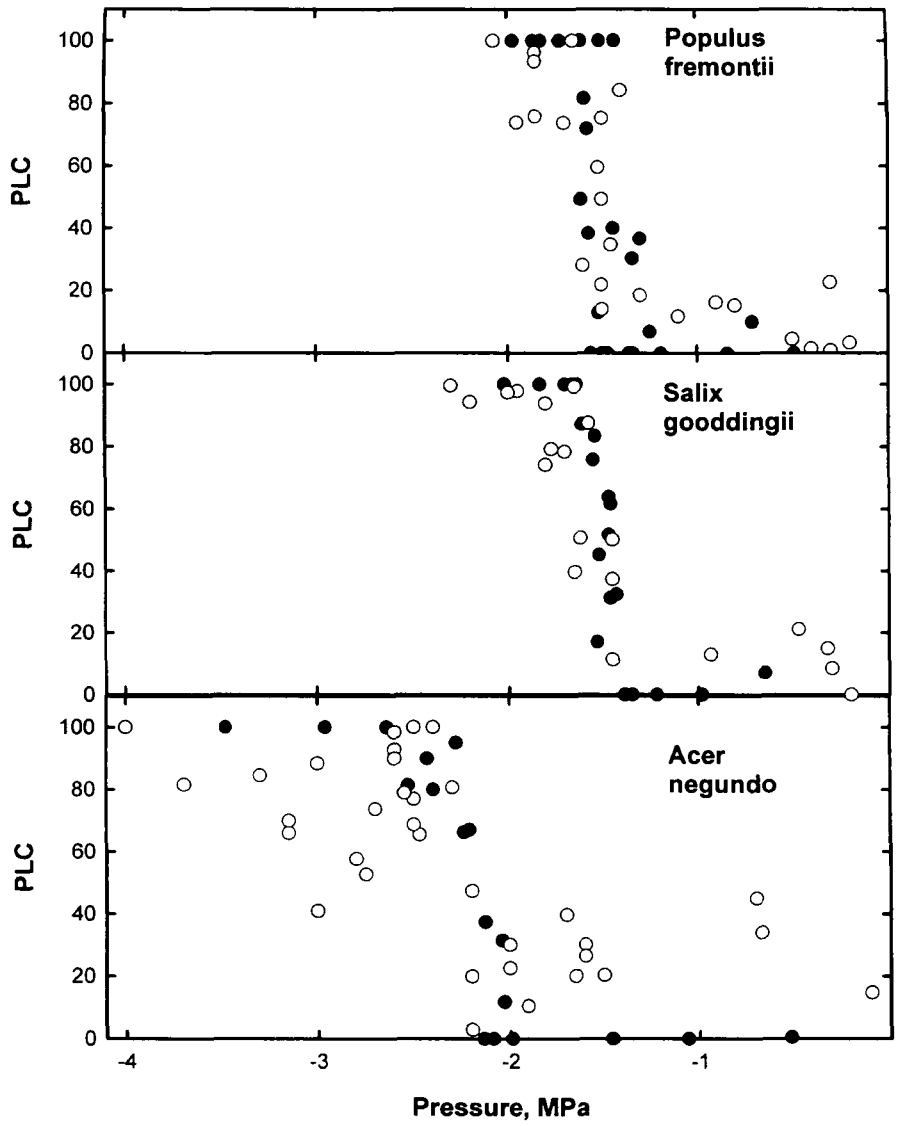

Fig. 11. Comparison of vulnerability curves obtained by different methods. The *y*-axis is per cent loss hydraulic conductivity (*PLC*) induced by a 'pressure' on the *x*-axis: solid symbols, negative pressure induced by centrifugation; open symbols, negative pressure induced by air-dehydration and measured on excised leaves with a pressure bomb; dotted symbols, *PLC* induced by positive pressure as in Fig. 9.

experiment can be presented to show that xylem conduits can sustain substantial tensions without cavitation. Tension can be controlled independently and induced in stems by centrifugation (Holbrook *et al.*, 1995), so it follows that centrifugation can be used to induce cavitations and measurable loss of hydraulic conductivity. It has been shown that vulnerability curves produced by centrifugation are the same as those produced by bench-top dehydration (Pockman *et al.*, 1995, Alder *et al.*, 1997) (Fig. 11).

Conclusions

The Scholander–Hammel pressure bomb seems to measure P_x correctly. This has been confirmed by two direct and two indirect pieces of evidence. The pressure bomb agrees with xylem tension measured directly with a temperature-corrected thermocouple hygrometer and with tensions created by centrifugation. The pressure bomb is validated indirectly because it has been used to confirm two hypotheses: (1) the air-seeding hypothesis and (2) the hypothesis that water flow can be treated as an Ohm's law analogue. Both hypotheses make quantitative predictions about P_x and the pressure bomb measurements have been used to confirm both hypotheses. The following must be true for the confirmation to occur: (1) the hypothesis must be true and (2) the pressure bomb must measure P_x correctly. More experiments of the type described above must be repeated to confirm the accuracy of the pressure bomb with other plant species. This

study's tentative conclusion is that the XPP must be incorrect if it does not agree with the pressure bomb. The XPP does not measure $P_x < -0.6$ MPa in plants; the most likely explanation is that the XPP induces embolisms by air seeding when $P < -0.6$ MPa. It is less clear why the XPP disagrees with the pressure bomb in direct comparisons made by Balling and Zimmermann (1990), so this work should be repeated in other laboratories and with additional species. There is no reason to reject the XPP outright as a useful method to measure xylem-fluid pressure in the range of 0 to -0.6 MPa. Further advances in XPP technology may even extend the useful range to more negative pressures.

Studies of xylem dysfunction due to cavitation provides the best evidence that water is transported in plants in a metastable state as proposed by the C–T theory. Much of what is now known about xylem anatomy and evolution is best explained in terms of the function of xylem structures in the avoidance of cavitations and readers may consult Tyree *et al.* (1994) for details. Recent studies of the Ohm's law analogue for sap flow in plants have provided strong quantitative support for the C–T theory.

Acknowledgements

I thank the following for their critical review of an earlier draft of this manuscript: Peter Becker, Stephen Davis, Frederick Meinzer, John Sperry, and David Yount.

References

Alder NN, Pockman WT, Sperry JS, Nuismer S. 1997. Use of centrifugal force in the study of xylem cavitation. *Plant, Cell and Environment* (in press).

Balling A, Zimmermann U. 1990. Comparative measurements of the xylem pressure of *Nicotiana* plants by means of the pressure bomb and pressure probe. *Planta* **182,** 325–38.

Benkert R, Zhu JJ, Zimmermann G, Türk R, Bentrup FW, Zimmermann U. 1995. Long-term xylem pressure measurements in the liana *Tetrastigma voinierianum* by means of the xylem pressure probe. *Planta* **196,** 804–13.

Canny M. 1995. A new theory for the ascent of sap: cohesion supported by tissue pressure. *Annals of Botany* **75,** 343–57.

Cochard H, Cruiziat P, Tyree MT. 1992. Use of positive pressures to establish vulnerability curves: further support for the air-seeding hypothesis and possible problems for pressure-volume analysis. *Plant Physiology* **100,** 205–9.

Crombie DS, Hipkins MF, Milburn JA.1985. Gas penetration of pit membranes in the xylem of Rhododendron as the cause of acoustically detectable sap cavitation. *Australian Journal Plant Physiology* **12,** 445–53.

Dixon HH, Joly J. 1894. On the ascent of sap. *Philosophical Transactions of the Royal Society London, Series B* **186,** 563–76.

Dixon MA, Tyree MT. 1984. A new temperature corrected stem hygrometer and its calibration against the pressure bomb. *Plant, Cell and Environment* **7,** 693–7.

Ewers FW, Fisher JB, Chiu ST. 1989. Water transport in the liana *Bauhinia fassoglensis* (Fabaceae). *Plant Physiology* **91,** 1625–31.

Holbrook NM, Burns MJ, Field CB. 1995. Negative xylem pressures in plants: A test of the balancing pressure technique. *Science* **270,** 1193–4.

Jarbeau JA, Ewers FW, Davis SD. 1995. The mechanism of water stress-induced embolism in two species of chaparral shrubs. *Plant, Cell and Environment* **126,** 695–705.

Milburn JA. 1996. Sap ascent in vascular plants: challenges to the cohesion theory ignore the significance of immature xylem and the recycling of Münch water. **78,** 399–407.

Pickard WF. 1981. The ascent of sap in plants. *Progress in biophysics and molecular biology* **37,** 181–229.

Pockman WT, Sperry JS, O'Leary JW. 1995. Sustained and significant negative water pressure in xylem. *Nature* **378,** 715–16.

Scholander PF, Hammel HT, Bradstreet EA, Hemmingsen EA. 1965. Sap pressure in vascular plants. *Science* **148,** 339–46.

Slatyer RO. 1968. *Plant water relationships.* London, New York: Academic Press.

Sperry JS, Saliendra NZ, Pockman WT, Cochard H, Cruiziat P, Davis SD, Ewers FW, Tyree MT. 1996. New evidence for large negative xylem pressures and their measurement by the pressure chamber method. *Plant, Cell and Environment* **19,** 427–36.

Sperry JS, Tyree MT. 1988. Mechanism of water stress-induced xylem embolism. *Plant Physiology* **88,** 581–7.

Sperry JS, Tyree MT. 1990. Water-stress-induced xylem embolism in three species of conifers. *Plant, Cell and Environment* **13,** 427–36.

Tyree MT. 1988. A dynamic model for water flow in a single tree: evidence that models must account for hydraulic architecture. *Tree Physiology* **4,** 195–217.

Tyree MT, Davis SD, Cochard H. 1994. Biophysical perspectives of xylem evolution: Is there a tradeoff of hydraulic efficiency for vulnerability to dysfunction. *IAWA Bulletin* **15,** 335–60.

Tyree MT, Snyderman DA, Wilmot TR, Machado J-L. 1991. Water relations and hydraulic architecture of a tropical tree (*Schefflera morototoni*). *Plant Physiology* **96,** 1105–13.

Yount DE. 1989. Growth of bubbles from nuclei. In: Brubakk AO, Hemmingsen BB, Sundnes G, eds. *Supersaturation and bubble formation in fluids and organisms.* Trondheim: Tapir Publishers. 131–63.

van den Honert TH. 1948. Water transport in plants as a catenary process. *Discussions of the Faraday Society* **3,** 146–53.

Zimmermann MH. 1983. *Xylem structure and the ascent of sap.* Berlin: Springer-Verlag.

Zimmermann U, Haase A, Langbein D, Meinzer F. 1993*a*. Mechanism of long-distance water transport in plants: a re-examination of some paradigms in the light of new evidence. *Philosophical Transactions of the Royal Society of London* **341,** 19–31.

Zimmermann U, Benkert R, Schneider J, Rygol J, Zhu JJ, Zimmermann G. 1993*b*. Xylem pressure and transport in higher plants and tall trees. In: Smith JAC, Griffiths H, eds. *Water deficits: plant responses from cell to community.* Oxford: BIOS Science Publishers, 87–108.