THE COHESION-TENSION MECHANISM AND THE ACQUISITION OF WATER BY PLANT ROOTS

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■ **Abstract**   The physical basis and evidence in support of the cohesion-tension theory of the ascent of sap in plants are reviewed. The focus is on the recent discussion of challenges to the cohesion-tension mechanism based on measurements with the pressure probe. Limitations of pressure probes to measure tensions (negative pressures) in intact transpiring plants are critically assessed. The possible role of the cohesion-tension mechanism during the acquisition of water and solutes by plant roots is discussed.

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INTRODUCTION

In recent years, the validity of the cohesion-tension (CT) theory of the ascent
of sap in plants has been questioned by Professor Ulrich Zimmermann and his
colleagues at the University of Würzburg, Germany (4, 59, 143–145). Since the
theory established over 100 years ago by Böhm (7) plays a key role in our under-
standing of the functioning of plants, the challenge has provoked a lively ongoing
debate (e.g. 19a, 61, 67, 91, 97, 98, 113, 117, 128). New experimental approaches
have been used to test the validity of the theory (19, 41, 73, 127, 129). Alternative
or additional mechanisms have been proposed to explain the movement of water
up to the top of the tallest trees (13, 14, 144). The topic has been reviewed recently
by Tyree (117), who concluded that the evidence in favor of the cohesion-tension
mechanism is strong, and by Tomos & Leigh (113), who discussed some aspects of
pressure probe techniques involving the measurement of negative pressure. Unlike
Tyree, Tomos & Leigh, perhaps still mindful of the intensity induced by the chal-
lenge to the cohesion-tension mechanism, avoid a clear judgment, namely a critical
assessment of the use of pressure probes to measure tensions (negative pressures).

This review provides the physical background of the CT-mechanism, which
necessitates a brief historical perspective, then discusses problems with the mech-
anism and recent evidence for and against it. The second part of the review consid-
ers the role of the cohesion-tension mechanism in the context of water acquisition
by plant roots: how plants use both variable tension in the xylem and variable
hydraulic properties of roots to regulate water uptake. Evidence that the uptake
of certain solutes and their transport across the root may be affected by the CT-
mechanism is reviewed.

ESSENTIAL FEATURES OF THE COHESION-TENSION
MECHANISM

The CT-theory relies on basic physical properties of water and on some assumptions
that may be summarized as follows:

– Water has high cohesive forces. It can be subjected to from some ten to
  several hundred MPa before columns break. When subjected to tensions,
  water is in a metastable state, i.e. pressure in xylem vessels is much
  smaller than the equilibrium water vapor pressure at the given temperature.

– Walls of vessels represent the weak part of the system. They may contain
  air or seeds of water vapor. When a critical tension is reached in the lumen

of xylem vessels, pits in vessel walls allow the passage of air through them, resulting in cavitation (embolism).

– Water in vessels of higher plants forms a continuous system from evaporating surfaces in the leaves to absorbing surfaces of the roots and into the soil (soil-plant-air-continuum; SPAC). With few exceptions, water flow within the SPAC is hydraulic in nature, and the system can be described as a network of resistors arranged in series and in parallel (123a).

– Evaporation from leaves lowers their water potential and causes water to move from the xylem to evaporating cells across leaf tissue. This reduces the pressure in the xylem, often to values well below zero (vacuum).

– Gradients in pressure (water potential) are established along transpiring plants; this causes an inflow of water from the soil into the roots and to the transpiring surfaces in the leaves.

According to these features, the cohesion-tension theory provides both a mechanism for the flow across the plant and the force driving it. The difference in water potential between the atmosphere and soil would usually be sufficient to provide enough force for even the tallest trees. However, the mechanism requires high tensions in the xylem to operate, and it is hard to envisage how these tensions could be maintained long enough. For example, it is difficult to construct a mechanical pump that would suck water from the top of a water column longer than 10 m (equivalent to 1 bar of pressure) without causing failure by cavitation. It is much easier to use positive pressures applied at the bottom. The critical question therefore is whether the tensile strength of water is sufficient to sustain states of high tension and, if so, what are the conditions required to maintain it.

TENSILE STRENGTH OF WATER, CAVITATION AVOIDANCE, AND GAS-SEEDING THEORY

Unlike the gas phase, the forces acting between molecules are high in condensed phases. Hence, liquids and solids can be subjected to high tensional forces until they fail. This may be understandable with solids such as a bar of steel, but is less intuitive for liquids. Failure by cavitation (gas embolism) is much more likely in liquids, allowing seeding caused by bubbles of water vapor or air to dominate. Liquids free of such seeds should be able to withstand very high tensions. Their tensile strength measured as the tension to be applied to cause failure is high. For a polar liquid such as water, measured values range between 5 and 140 MPa (6, 11, 80, 123, 136); the range is much smaller for nonpolar liquids such as liquid helium (1 MPa; 55). The theory of tensile properties of liquids is well developed (29, 55, 90, 112, 114). Experimental values are smaller by one to two orders of magnitude than the theoretical, because heterogenous rather than the homogenous nucleation usually dominates. During heterogenous nucleation, cavitation is initiated by gas seeds of a critical size at surfaces of vessels or by solid impurities in

the liquid, i.e. by residues of air lodged on these surfaces. Homogenous nucleation is initiated by bubbles of water vapor produced spontaneously within the liquid. This process of spontaneous formation of gas bubbles is much less probable than embolism caused by preexisting seeds. From a thermodynamic point of view, a liquid under tension is in a metastable state, i.e., it tends to proceed to a state equilibrium and to form bubbles in which the vapor assumes the equilibrium value at the given temperature, which is larger than zero (vacuum) for all liquids. For water, the equilibrium vapor pressure would be 2.3 kPa at 20°C.

THEORETICAL APPROACHES

In a simple theoretical approach, the tensile strength may be calculated from the energy stored in hydrogen bonds. Taking a value of 25 kJ/mole stored in these bonds (44), an internal (cohesion) pressure of 1400 MPa (14,000 bar) is calculated by dividing the figure given by the molar volume of water ($18 \cdot 10^{-6}$ m^3/mole). This is a high value that may be reduced by the fact that, because of thermal motion, not all of the bonds will be intact at room temperature. However, even if only 80% were intact, the resulting figure of 1,100 MPa is still high, far in excess of what a plant would need to pull water from the soil into the transpiring leaf of the tallest trees.

In a more rigorous approach, the attractive forces acting in a liquid between molecules can be expressed as a change in internal energy (U) divided by the change in volume (V) of the liquid, i.e. by $(\partial U/\partial V)_T$. This term has the dimension of a pressure at constant temperature (T), and is called internal pressure. The action of the internal pressure caused by the attraction between molecules will be counteracted by the thermal pressure, which derives from the thermal motion of molecules tending to increase volume, i.e. $T(\partial P/\partial T)_V$. $(\partial P/\partial T)_V$ is called the thermal coefficient of pressure. In contrast to the energetic term, $(\partial P/\partial T)_V$ represents an entropic term ($= (\partial S/\partial V)_T$) as is easily understood. Together with the external (measurable) pressure or tension (P) acting in a liquid (such as the positive turgor pressure of cells or the negative pressure exerted on water in a vessel of a transpiring plant), the internal pressure just balances the thermal pressure, i.e.:

$$(\partial U/\partial V)_T + P = T(\partial P/\partial T)_V. \qquad 1.$$

Interestingly, physical chemists sometimes call $(\partial U/\partial V)_T$ 'cohesion pressure' of a liquid or solid, which relates to the background of the cohesion-tension theory of botanists and is based on a high $(\partial U/\partial V)_T$ of xylem solution. For water, there are tabulated values of either $(\partial P/\partial T)_V$, or of the more common coefficients of thermal expansivity, $\alpha = 1/V \cdot (\partial V/\partial T)_P$, and isothermal compressibility, $\beta = -1/V \cdot (\partial V/\partial P)_T$, which are related to the coefficient of thermal tension by $(\partial P/\partial T)_V = \alpha/\beta$ (see textbooks of physical chemistry). For liquids, $\alpha \approx 10^{-3}$ K^{-1} and $\beta \approx 10^{-3}$ MPa^{-1}. Hence, we obtain an estimate of 300 MPa

(3000 bars) for the cohesion pressure of water ($T = 300$ K). For solids, both the thermal coefficient and the compressibility are lower than for liquids which results in similar values for the coefficient of thermal tension. From a thermodynamic point of view, the cohesion-tension mechanism has a sound basis. In the presence of gas, the tensile strength of the xylem liquid would be lost. By definition, $(\partial U/\partial V)_T$ is zero for an ideal gas.

Other attempts to calculate the tensile strength of liquid water have employed the van der Waals equation, or critical tensions have been derived from the latent heat of evaporation (e.g. 112). In summary, the different theoretical approaches resulted in values for the tensile strength of water ranging between 50 and 1400 MPa. For condensed phases, it is difficult to estimate maximum tensions because there is no unique equation of state.

EXPERIMENTAL VALUES OF TENSILE STRENGTH

The Belgian chemist Donny (25) and the French physical chemist Berthelot (6) were probably the first to demonstrate experimentally tensions in liquids. Donny used a U-shaped glass tube in which one arm was sealed and the other left open or connected to a vacuum pump. When the sealed arm of the U-tube was filled with concentrated sulphuric acid, the height of the liquid column was larger than that equivalent to atmospheric pressure. Berthelot encased water in quartz tubes at elevated temperature. When the tubes were subsequently cooled, they were able to withstand tensions of up to 5 MPa (50 bar) before failing, with an audible click and with the appearance of a bubble of water vapor. Tensions within the liquid were caused by the water in the tubes contracting more than the quartz. Reynolds (77) performed experiments similar to Donny's, using mercury to fill the tubes. Later, Reynolds (78) also used U-shaped tubes that he rotated to produce centrifugal forces on the liquid, which resulted in tensions. This technique has later received greater attention in experiments by Briggs (11), and more recently by Holbrook et al (41) and Pockman et al (73). Ursprung (123) was the first to estimate the tensile strength of liquid water using a botanical object. The annulus cells of fern sporangia open at a sufficient tension within the cell sap that is usually caused by withdrawal of liquid water by evaporation. Imitating evaporation, Ursprung used highly concentrated sucrose solutions to produce a surroundings of low water potential next to the fern sporangia. He measured a maximum tensile strength of water of as large as 30 MPa (300 bar). Briggs (11) found a similar value in his famous spinning experiments with Z-shaped glass capillaries (see above).

More recently, Roedder (80) found evidence for high tensions in microscopic inclusions of water or electrolyte solution in certain naturally occurring minerals. The water in the inclusions melted at temperatures substantially higher than 0°C, even at +6.5°C in one case. Extrapolation from the melting point line indicated that this corresponded to a tension as large as 95 MPa. Zheng et al (136) used an inclusion technique to artificially prepare microfissures of water in quartz and

other minerals, which were healed at high temperature and pressure to provide a high density of the water within the inclusions. When recovered at room temperature, some of the inclusions developed tensions of up to 140 MPa at densities of the liquid water substantially smaller than 1 g/cm^3 (0.5–0.7 g/cm^3). Hence, the water was substantially stretched and tensions came close to the limit at which the compressibility should become infinite (i.e. $(\partial P/\partial V)T = 0$) and the velocity of sound zero. The theory predicts a 'spinodal line' at which this is the case (90). This line originates at the critical point and extends into the range of negative pressure. Since the spinodal line is not expected to be reached, the figure of 140 MPa of Zheng et al (136) may indicate a value of the tensile strength of water close to the ultimate limit. Maris & Balibar (55) recently performed experiments similar to those with water using liquid helium close to the absolute zero. According to the nonpolar nature of this solute, maximum tensions were only a few bar, with a maximum at 9.5 bar. Although liquid helium may be an exotic substance in plant physiology, the figure is instructive nevertheless because it indicates a lower limit of the tensile strength of liquids. The large differences between measured values of tensile strength for water are due to problems in eliminating heterogenous nucleation during the measurement.

VAPOR-SEEDING MECHANISMS (HOMOGENOUS NUCLEATION)

At a first sight, nucleation (vapor-seeding) in the bulk xylem sap may seem important, but actually it is not. On the contrary, spontaneous seeding in bulk solution (liquids) is an extremely rare event. When gas bubbles appear in a bulk solution under tension, they would need to reach a certain critical size in order to cause cavitation. Most of the very small seeds will rapidly disappear. As small bubbles grow, much more energy must be invested to overcome surface tension (surface energy) than is gained by volume expansion owing to the pressure gradient between bubble and the liquid under tension (volume work). The capillary pressure, P_{cap}, would be relevant, i.e. $P_{cap} = 2T/r$ (T = surface tension; $2r$ = diameter of void). When we look at the overall work (A) performed by a vapor bubble, we have:

$$A = 4\pi r^2 \cdot T + 4\pi/3 r^3 (P - P_w). \qquad 1.$$

Here, the terms $4\pi r^2 \cdot T$ and $4\pi/3r^3(P - P_w)$ represent the surface energy and volume work, respectively, and P and P_w represent the pressure in the solution and the water vapor pressure in the bubble. Since $P < 0$ and $|P| \gg P_w \approx 0$, the first term on the right side of Equation 1 has a positive and the other one a negative sign. With increasing bubble size, the volume work term grows faster than the surface tension term. Hence, there should be a maximum of A, where it holds that $dA/dr = 0$. At the maximum, we get from Equation 1:

$$P_{cap} = 2T/r. \qquad 2.$$

This is the equation for capillary pressure, mentioned above. Physically, it means that at maximum A, the capillary pressure just balances the forces externally exerted. When the bubble size is increased further, there is a dramatic increase in size, i.e. a cavitation. We may use the value of P_{cap} to calculate the work done by the system in the maximum, A_{max}:

$$A_{max} = \frac{16\pi \cdot T^3}{3 \cdot P^2}. \qquad 3.$$

The rate for the formation of 'successful' gas seeds (J) can be now given using the Boltzmann distribution function, i.e. by describing the rate of gas seeding in a way analogous to the Arrhenius equation used to express rates of chemical reactions as a function of temperature (29):

$$J = J_o \cdot \exp -\{(A_{max} + \Delta h_V)/kT\}. \qquad 4.$$

The heat of evaporation of water Δh_V has to be added to the overall activation energy because the growth of seeds requires evaporation of liquid water to maintain P_w at the saturation value. However, this second term is usually negligible compared with A_{max}. In the context of seeding processes as they occur in the xylem of transpiring plants, A_{max} decreases with second power of tension (P), i.e. at high tensions the probability of homogenous gas seeding should be much more pronounced than at low tensions. However, since the exponential factor is usually very small at tensions that are of interest in the xylem, homogenous nucleation rarely causes cavitation. It should be noted that the constant factor J_o in Equation 4 is proportional to the amount of liquid (water) under consideration. This reminds of the traditional view that events of cavitation should occur more often in vessel members having a bigger volume than tracheids (141). According to the very small absolute value of the exponential term this is, however, meaningless. Air-seeding through pit membranes should dominate, i.e. heterogenous nucleation.

HETEROGENOUS NUCLEATION AND EFFECTS OF GASES DISSOLVED IN XYLEM SAP

Heterogenous nucleation may occur at vessel walls due to existing gas (air) residues or in some kind of a catalytic process at these surfaces (for a classification of processes, see 117). Alternatively, air-seeding occurs across pit membrane pores. The critical pressure difference at which this occurs is again given by the capillary pressure equation, with 2r being the diameter of the pores. Since homogenous nucleation is such a rare event, most of the seeding should occur at or through surfaces such as the glass or metal surfaces of a container without pores or the xylem vessels containing pit pores. According to the size of the fissures and the gas residues contained by a glass surface, cavitation will occur when a critical size of tension is reached in the solution. From the equation for capillary pressure (Equation 2), one can calculate that a bubble diameter of 3 μm refers to

a critical tension (pressure difference) of 0.1 MPa (1 bar). Accordingly, 1 MPa (10 bar) tension refers to 0.3 μm or 300 nm, i.e. to a bubble size that is smaller than the wavelength of visible light. The highest tensions of about 10 MPa that have been indirectly measured in the xylem of transpiring plants could only tolerate gas impurities as small as 30 nm without cavitation. This is an extremely small figure. Hence, the existence of such high tensions in the xylem has been questioned.

Xylem vessel walls are different in construction from those of a glass or metal container, which will have surface microfissures even when carefully polished. Hence, cavitation is unavoidable in these containers when critical tensions are attained. Xylem walls consist of a porous net of wettable polymers (cellulose, lignin, hemicelluloses, etc). Pores (interfibrillar spaces) are of an order of 10 nm, which corresponds to 30 MPa of capillary pressure. Hence the porous hydrophilic matrix will be imbibed with water like a sponge. According to the high capillary forces (negative matric potentials), no space will be left for air-seeds of sufficient size to cause embolism. The special capacity of xylem vessels to allow for a high tensile strength is based primarily on the porous structure of its completely wettable walls rather than on the tensile strength of the bulk xylem solution, which is not the limiting factor. Since interfibrillar spaces in vessel walls are smaller than the diameters of pores in pit membranes, the latter should usually limit the tension at which air-seeding occurs. This is in accordance with experiments in which air-seeding across pit membranes has been directly demonstrated in the double-ended pressure chamber and other techniques (see below).

The fact that xylem solution usually contains dissolved gases (N_2, O_2, CO_2) should not affect its ability to withstand tensions. As for the formation of bubbles of water vapor in bulk solution, the formation of bubbles even in saturated or supersaturated solutions is a rare event. Kenrick et al (50) saturated liquids with gas at 100 atm and reduced pressure to 1 atm without effervescence. Kenrick et al (49) superheated water to 270°C under atmospheric pressure before it exploded. A superheated or supersaturated liquid should behave similarly to a liquid under tension. In the experiments, where water was superheated to 270°C, substantial internal tensions could be estimated. The supersaturation experiments indicate that air dissolved in the xylem sap should not affect its tensile strength. Therefore gases such as oxygen and nitrogen dissolved in the water taken up by plant roots or CO_2 developed by respiratory processes should pose no problem as long as they stay dissolved. An increase in temperature of xylem sap on its passage up the shoot may cause a problem when the solubility limit is reached. Freezing of xylem sap also reduces solubility, which would then cause cavitation. This phenomenon has long been known (141).

OLDER EVIDENCE IN FAVOR OF THE CT-THEORY

The Austrian botanist Böhm (7), the formulator of the CT-theory, was the first to conceive that states of tensions are involved in the ascent of sap in plants. He devised model experiments similar to those of Reynolds, but included transpiring

shoots to raise the column of mercury above atmospheric. Cavitation problems, however, seriously impeded the experiments. Nevertheless, Böhm demonstrated convincingly that cohesion-tension is the basic mechanism for the ascent of sap, even in tall trees. Böhm's experiments were continued by Dixon & Joly (24) and Askenasy (1), but broad acceptance of the idea of a cohesion-tension mechanism was slow in taking hold. Other mechanisms were favored such as tricky mechanisms of the ascent of sap in vessels in the presence of air bubbles ['Jaminsche Ketten', see (71); imbibition mechanism, see (82)]. The imbibition mechanism theory proposed that water moved up the plant by matric forces in the walls, as in a wick. Early on botanists had deduced that axial (long-distance) transport in the xylem is driven by passive mechanisms. Killing the cells around conducting vessels had no effect on the functioning of xylem (109). Thus the contribution of metabolism (vitalistic mechanisms) to the movement of water across the xylem received increasingly less attention. Recently, there have been attempts to revive these mechanisms. It has been claimed that tissue pressure (which originates from metabolic processes) supports the ascent of sap in the xylem and plays an important role during the refilling of cavitated vessels. However, these speculations have no real thermodynamic basis (13, 14; see below).

In 1911, Renner (76) provided the first quantitative data on the tensions present in shoots of transpiring plants. To measure transpiration, he used a capillary filled with water (potometer) that he attached to an excised transpiring twig. Using a clamp, he was able to partially interrupt the transport along the xylem, thus increasing the axial hydraulic resistance and tension. However, the high forces exerted in the vessels by transpiration made it difficult to completely interupt the flow. More important, Renner compared the suction force created by the twig with that of a vacuum pump and found that the tensions created by the twig were larger by a factor of up to 10 to 20 than that provided by the pump. He concluded that the pressures created by transpiration within the xylem must have been substantially smaller than vacuum. These experiments provided incontrovertible evidence that the tensions in the xylem were sufficient to move water, even through tall trees, and helped to resolve the controversies surrounding the mechanisms for the ascent of sap in plants (23). It was agreed that the ascent of water was a passive process ultimately driven by the evaporation of water from leaves, i.e. by the water potential difference between soil and the dry air, and that, in the absence of transpiration as the force driving water flow across the soil-plant-air-continuum, root pressure would serve as the auxiliary engine. Root exudation, however, is dependent on metabolic energy to create an osmotic gradient between soil solution and xylem.

THE SCHOLANDER-HAMMEL PRESSURE BOMB

Current support for the CT-mechanism comes mainly from indirect measurement of xylem pressure (water potential) using the pressure chamber re-invented by Scholander et al in 1965 (84, 119, 120). The pressure chamber or Scholander-Hammel

bomb is a compensation technique used widely to measure the water potential of shoots or parts thereof. When a transpiring shoot is excised from a plant, the tension (negative pessure) in the xylem is released to atmospheric causing an uptake of water into the leaf and stem cells. The original status of completely filled functioning vessels is then restored by applying air pressure to the sample in the chamber. When the vessels are refilled with xylem solution, the compensating or balancing pressure (P_b) is equal to the pressure in the xylem, as it was before excision, i.e. $P_x = -P_b$. Since the osmotic pressure of the xylem sap (π_x) is usually small in transpiring plants, $-P_b$ would also be a good measure of the water potential of the shoot, twig, or leaf under investigation. Note that the Scholander-Hammel bomb is based on osmotic (thermodynamic) equilibrium. Gradients within the sample prior to excision are leveled out. These gradients could be due either to a drop in water potential across the transpiring leaf (between stomata and xylem vessels) or to gradients within a shoot, a potentially important problem in rapidly transpiring leaves. By definition, the technique averages these gradients (5, 115). Consequently, there may be differences between the P_x measured directly in an intact plant and the average P_x measured with the pressure chamber (117, 127, 129), a factor sometimes overlooked when questioning the validity of the Scholander-Hammel bomb in measuring xylem pressure (4, 59). Nevertheless, averaged xylem pressures (water potentials) as measured with the Scholander-Hammel bomb have their physiological and ecological significance in that they represent a quantitative measure of the water status of plant samples and a measure of the force driving water between different parts of plant, e.g. between the root and the shoot.

Recently, Holbrook et al (41) combined the spinning technique of Reynolds (78) with the pressure chamber technique. They used excised stem segments with a single leaf attached at the midpoint of the segment, which they then rotated by a motor-driven shaft. Rotation caused a centrifugal tension at the midpoint that was sensed by the attached leaf. The P_x at the midpoint was calculated from the segment length and angular velocity of rotation and this value was then compared with the P_b measured in the leaf with a pressure chamber after centrifugation to check the validity of the measurements with the bomb. A 1:1 relationship was found between the two values for P_x values down to -1.8 MPa.

Using the Scholander-Hammel bomb, P_x values of as low as -10 MPa have been measured in water-stressed shoots (e.g. 52). According to theory this requires xylem solution that is quite free of air or water vapor seeds. Since capillary pressure also governs the seeding of air across vessel walls, pit pore diameters should also have been low. The porosity of pit-membranes rather than the volume of vessel members is critical in preventing dysfunction of vessels and, hence, withstanding drought stress (92). The genetics of pit morphology and porosity are hypothesized to have been under high selective pressure for plants that are adapted to arid habitats (117).

The mechanism of air-seeding across pit pores has been tested in double-ended pressure chambers (19). In this elegant technique, a shoot or branch sectioned at

both ends is bent around in a large pressure chamber so that the cut ends protrude into the atmosphere. With a capillary attached to one end, water could be passed continually through the segment under positive pressure from a water column and the flow rate measured by weighing at the other side. When gas (air) pressure is applied to the section in the bomb, the flow will decrease substantially to the point where the pressure difference between xylem (which is at atmospheric pressure) and bomb pressure exceeds the minimal value to cause air-seeding across pit pores. Vulnerability curves obtained in this technique indicate that air-seeding across pit pores is the mechansim driving most of the cavitation events (91). In a modification of this experiment (41), Pockman et al (73) also used centrifugation to cause cavitation in cut segments of shoots that were subjected to different tensional stresses in the xylem. Cavitation was then followed by measuring the axial hydraulic resistance of stem segments. As in the double-ended pressure chamber, air-seeding across pit pores was induced at pressure differences of a few MPa between atmosphere and xylem lumen.

RECENT EVIDENCE AGAINST THE CT-THEORY

For over 100 years, the validity of the cohesion-tension theory has occasionally been called into question [see Sachs (82); Pfeffer (71)]. More recently, Greenidge (34), Plumb & Bridgman (72), and Canny (13, 14) have questioned whether the tissue surrounding vessels contributes to the axial flow or if osmotic mechanisms play a significant role in transpiring plants. Given the large volume of water passing through transpiring plants, mechanisms based entirely on osmotic pressure provided by roots would cause enormous problems in getting rid of the osmotic solutes (e.g. nutrient salts) in the shoot as the water evaporates. Such elimination would only be possible by recycling via the phloem, resulting in a rapid turnaround of solutes at high metabolic costs.

Over the past decade, Zimmermann and his co-workers have been collecting evidence against the cohesion-tension mechanism. The Würzburg group used the cell pressure probe, earlier used to measure turgor of individual cells, to assess xylem pressure in intact transpiring plants (45, 95, 108, 113). The equipment, then called a 'xylem pressure probe' (4), was filled with water instead of low-viscosity silicone oil. The tip of the probe was carefully introduced into the xylem and its actual location was identified by the change in pressure, which tended to become negative (smaller than vacuum) as the xylem was punctured. These were the first direct measurements of xylem pressure in intact transpiring plants. They showed that xylem pressure was less negative (the tension smaller) than expected from measurements taken in the pressure chamber. The Scholander-Hammel bomb had not, therefore, been correctly measuring xylem pressure, P_x (water potential). In absolute terms, tensions of only a few tenths of a MPa (only a few bars) were found. In many cases, only subatmospheric pressures were recorded. However, as precision was gained, P_x values became more negative, with a minimum close to -1 MPa.

Even more remarkable was the finding that xylem pressure was not or was hardly affected by transpiration (4, 142). The finding of low tensions and the lack of dependence on transpiration led to the conclusion that the CT-mechanism of the ascent of sap in plants was not correct, or at the very least, it was not the dominating mechanism hitherto proposed. Other mechanisms such as the osmotic uptake of water into the xylem (i.e. root and stem pressures) or capillarity were more likely alternatives (144). Since problems with cavitations make direct measurement of tensions difficult experimentally, the finding that tensions measured with a probe tended to become bigger (pressure was more negative) may indicate greater skill in measuring tensions, which, in turn, made the authors more willing to accept the CT-theory, at least as an additive mechanism. Throughout, Zimmermann and co-workers barely discuss the actual position of the pressure probe in the tissue and the criteria whereby they ensured its location. Largely, it was the reaction in pressure that convinced them that the probe was in the lumen of functioning vessels. Under these conditions, they obtained less negative xylem pressures with the probe than with the Scholander-Hammel bomb. However, in a recent paper, a 1:1 relationship was demonstrated between xylem pressures measured with the pressure probe and the pressure chamber for corn and sugar cane under conditions of low transpiration (see above; 59). This relationship is in line with other observations and may indicate a change forthcoming in the debate (145). The conclusions from the pressure probe experiments by Zimmermann's group remain: (*a*) Cohesion-tension is not the dominating mechanism driving water across the SPAC, and (*b*) the Scholander-Hammel bomb incorrectly measures xylem tension.

This challenge to a key theory in plant water relations has had repercussions for other researchers in the field. The apparent difficulty of the CT-theory in explaining how water under high tension is sustained for long periods of time caused widespread reevaluation of the validity of the CT-mechanism. The problems with direct measurements underscore the fact that the xylem is a vulnerable pipe. The controversy over direct measurements of xylem pressure and the mechanism of refilling cavitated vessels remains very much alive (13, 14, 121, 146).

RECENT EVIDENCE FROM PRESSURE PROBE EXPERIMENTS IN FAVOR OF THE CT-MECHANISM

Direct measurements of xylem P_x with pressure probes may appear to be straightforward, with no further tests or critical assessments needed to check their validity, but this is not so. Earlier measurements in my lab in Bayreuth have shown that the pressure probe or similar devices may be used to measure tensions in small compartments such as in the root xylem or artificial osmotic cells (39, 97, 102, 137). While making these measurements, it became clear that the equipment was limited in its application to ranges of tensions smaller than $\sim$0.8 MPa. These ranges, however, are smaller than those of interest within the context of the CT-theory.

In 1997/98, the problem was reanalyzed in collaboration with Dr. Tyree and his student Chungfang Wei from the University of Vermont, using intact maize plants (127, 129). When properly secured, functioning vessels in the midribs of the leaves were accessible to puncturing with the probe. To improve the rate of puncturing, the pots with the roots of the plants were encased in big pressure chambers to apply pneumatic pressure to the root, thereby increasing P_x (lowering tension). By applying pulses of pressure to the roots, it was possible to follow rates of propagation of pressure (change in water potential) within the xylem from roots to shoots. According to the CT-theory and to the fact that root hydraulic conductance was high, pressure changes should rapidly propagate along the xylem when the pressure probe was in a functioning xylem vessel. If this condition did not obtain, measurements were disregarded. Hence, the proper position of the tip of the pressure probe could be tested rigorously.

When the tip of the probe was not located in a functioning vessel, there was a delay in the response, although the pressure was negative. When the tip was positioned outside the vessels, this may have resulted in a drop in pressure and in a response smaller than 1:1 between pressure applied to the root and pressure measured with the pressure probe. When pneumatic pressures were applied to the root, it was possible to verify whether the system was functioning properly. The tests ensured that during measurement, the tips of pressure probes remained open to allow for good hydraulic contact between the xylem vessel and the pressure transducer of the probe. With these precautions, P_x was measured in a range down to -1 MPa (-10 bars). However, the tendency of the system to cavitate increased with mounting tension (129). This result accords with earlier findings with artificial osmotic cells (102). In agreement with the CT-mechanism, xylem pressure immediately decreased when light intensity was increased, which caused a simultaneous increase in the measured rate of transpiration. In the early experiments from the Zimmermann lab, this response was missing or was hardly detectable (4, 142). The papers from the Zimmermann lab gave no indication how the functioning of the system was tested other than the occurrence of a negative pressure. In our experiments, it was shown that step changes in the applied pneumatic pressure caused a 1:1 response in P_x (see above). However, as P_x exceeded atmospheric pressure, the response in P_x became much smaller than 1:1. Under these conditions, guttation occurred at the leaf margins, which indicated that the drop in response was attributable to leakage out of the xylem. This explanation is reasonable in terms of shoot hydraulics and is in line with the CT-mechanism.

In separate experiments, xylem pressure, P_x, was first measured at a given rate of transpiration. The leaf tip in which the measurements had been performed was then harvested and P_x determined with the aid of a pressure chamber. Both results were in good agreement, i.e. there was a 1:1 agreement between $|P_x|$ and P_b. P_x of leaves of transpiring plants was slighty smaller, on average, than that of nontranspiring plants. However, as transpiration rates were small during the experiments, gradients within leaves should have been also small. The results obtained with the Scholander-Hammel bomb and with the cell pressure

probe were therefore similar, at least in a range of xylem pressures of down to −1 MPa.

Most important was the fact that tensions were measurable only up to 1 MPa (10 bars) despite care to avoid cavitations caused by vibration, shaking of fixed leaves, etc. The pressure probe itself or the set-up with the probe in a vessel could well have been the reason for the limitation. We therefore tested the tensile strength of the probes in a series of experiments where tips of pressure probes completely filled with silicone oil were closed with glue. In a Berthelot-type experiment, tensions were set up by cooling the capillaries of probes with dry ice. The maximum tensile strength was about 1.6 to 1.8 MPa, which was much smaller than that of liquids in the absence of air-seeds at the walls (see above), but larger than the critical value of 1 MPa found during the experiments when the tip was in xylem vessels. The tensile strength of the probe may be regarded as low. However, a tensile strength of 1.6 to 1.8 MPa would be equivalent to seed diameters of $2r =$ 190 to 170 nm, which is a fairly small value (wavelength of visible light: 400–800 nm). Because internal surfaces in the probes are of differing materials (perspex, glass, silicone rubber, silicon), heterogenous seeding may occur at lower tensions than in the classical experiments that used only quartz tubes (6, 112). Tensile strength of 1.6 to 1.8 MPa is currently the limit for pressure probes. With the tip in a vessel, this limit was reduced further, perhaps because puncturing created porous by-passes in the wall next to the glass tip. Detailed tests of the tensile strength of pressure probes filled either with oil or with water indicated no substantial differences in their ability to sustain tensions (129). Indeed, oil-filled probes (used in Bayreuth) had a somewhat higher tensile strength than water-filled probes (used in Würzburg).

REFILLING MECHANISMS

Usually, it is assumed that refilling of cavitated vessels occurs when transpiration is switched off. Under these conditions, root and stem pressures play a predominant role. However, there are observations that refilling can occur even during the day, i.e. when xylem sap is still under tension (13, 14). Refilling has been followed by cryo-scanning electron microscopy just counting the number of cavitated vessels and measuring the water potential of the shoot during the day. It has been proposed that the tissue pressure generated by xylem parenchyma may cause refilling (and may also support the ascent of sap; 13). It is thought that the production of osmotic solutes (such as sugars derived from the degradation of starch) creates a high turgor in xylem parenchyma cells, which then causes some squeezing out of water from the tissue protoplasts into vessel lumina (14). Others have speculated that water would be driven into cavitated vessels by capillary forces (41a, 146). This, in turn, would increase the volume of droplets sitting at the vessel walls at atmospheric pressure or nearly so. A positive pressure would be created in the liquid phase that would compress the air and eventually remove it from the vessel.

For this latter model to work, it has been claimed that vessel walls and pits would be lined up with hydrophobic material to reduce its permeability to water to close to zero. Hence, vessels would be hydraulically isolated from surrounding tissue and would be just filled via the pits. Both types of models are highly speculative and, in fact, violate basic physical (thermodynamic) principles. They ignore the fact that vessel walls are quite permeable to water (e.g. 70a). The models postulate (in the absence of an active water transport) an uphill movement of water from regions of a low (tissue) to those of a high water potential (xylem lumen), which is not possible. Recently, it has been demonstrated in a model experiment that the observation of cavitated vessels by cryo-scanning electron microscopy may be prone to artifacts (18a, 19a). It was shown that cavitations may occur artifactually during the freezing of samples, even when attempts are made to perform this very rapidly. Furthermore, the frequency at which cavitations occur, may increase when vessels are under tension (see also 54a). When this turns out to be true, the discussion about the filling of cavitated vessels under conditions where other vessels are under high tension and the water potential of surrounding tissue is low, would have to be reconsidered. Test experiments are missing to show that artifacts can be excluded. However, it remains true that mechanisms that are evidently impossible (because they violate basic principles) can *a priori* be excluded (121).

ROLE OF COHESION-TENSION DURING WATER ACQUISITION AND TRANSPORT BY ROOTS

It has been proposed that the cohesion-tension mechanism causes variability and even adjustment in the hydraulic resistance of the xylem [resistance to long-distance transport of water (92, 117, 118, 122)]. Transpiration under conditions of water shortage should result in high tensions in the xylem that, in turn, may cause vessel dysfunction. When critical tensions are reached, air-seeding will occur across pit membrane pores. Failure of vessels and increased axial resistance would result in an increased tension in remaining intact vessels, which should then also cavitate in a vicious cycle (runaway cavitation). Eventually, reduction of water supply to the shoot would cause negative shoot-water potentials and a closure of stomata. To operate as a feedback system this would require an effective mechanism(s) of refilling of vessels when tensions are switched off during the night (see above). According to the diameter of pit pores, different species may regulate the axial hydraulic resistance at different set points (117).

Regulation of water flow across the SPAC by reversible xylem dysfunction would be a means by which plants could operate under conditions of low water potential and severe drought stress. To function properly it requires that (*a*) stomata closure is induced at even lower water potentials and that (*b*) the water supply by the root is not sufficient to balance the water status of the shoot. In the past, there has been a strong emphasis on the former process, i.e. on the output function [regulation of stomatal conductance (21, 47, 87)]. Little attention has been paid

to the input function, i.e. to the regulation of water acquisition by roots and how this would be affected by xylem tension. The input function is as important for the water balance as are water losses. The reason for the lack in our knowledge of the input function is that, unlike the shoot, the hydraulic architecture of roots in the soil is much more difficult to assess. Comprehensive understanding of root hydraulics involves the measurement of water relations at the cell and tissue/organ level in addition to the xylem in a developing structure. Root morphology and anatomy must be taken into account, which vary in reponse to growing conditions. Furthermore, there are interactions between solute (nutrient) relations and the water, i.e. there are osmotic processes besides the purely hydraulic movement of water as is usually discussed within the framework of the CT-mechanism. Roots are not just 'hydraulic machines'. Osmotic and even active solute transport processes complicate the analysis of root water. They are most obvious in the phenomenon of root pressure, which relates to active uptake of solutes and plays a role during the water supply of shoots under conditions of low transpiration. In the following, hydraulic properties of plant roots will be reviewed in order to work out possible mechanisms of an adjustment or even a regulation of water uptake according to the needs of the shoot. There will be a focus on the role of cohesion mechanisms during these processes. Most of the results will refer to excised roots (individual roots and root systems), where most of the data have been collected.

VARIABLE ROOT HYDRAULIC RESISTANCE

Root hydraulic resistance (inverse of the root hydraulic conductance) is often adjusted by the size of the root system, i.e. a certain root:shoot ratio is maintained by root growth to supply the shoot with water and nutrients and to allow for mechanical stability of the shoot. Furthermore, water flow is adjusted or is even regulated by a variable hydraulic conductivity of roots at a given size and root system structure (anatomy). Depending on the species and conditions, changes can be up to three orders of magnitude. Since water uptake of a given root is proportional to its surface area, root hydraulic conductivity (root Lp_r) should be referred to unit surface area in m^2 (if known) and to unit pressure (osmotic or hydrostatic) driving the flow from the soil solution to the root xylem. Hence, root Lp_r would have the dimensions of $m^3 \cdot m^{-2} \cdot s^{-1} \cdot MPa^{-1}$ or $m \cdot s^{-1} \cdot MPa^{-1}$. That roots increase their hydraulic conductivity according to demands from the shoot, i.e. in response to tensions developing in root xylem, has long been known (8–10, 12, 20, 27, 54, 66, 81, 94, 98, 103, 107, 126).

The radial rather than the axial component of water transport limits water uptake by roots (30, 31, 43, 63, 107). Near the root tip, where xylem vessels are not yet fully developed and offer considerable resistance, both radial and axial components have to be taken into account (31, 60). Variable root hydraulic resistance has been explained in terms of different models. Brewig (10) assumed an adjustment of the permeability of root cell membranes. The development of the root's

endodermis clearly also plays an important role. In the endodermis, the development of apoplastic barriers (Casparian bands, suberin lamellae, and secondary wall thickening) are thought to interrupt the apoplastic path that forces water to cross membranes. In mature roots, the endodermis is thought to be the major limiting barrier for water uptake at least in mature roots. The exodermis (hypodermis) also plays a role (64, 65, 68, 69, 79, 107, 110, 140); the exodermis undergoes a development similar to that of the endodermis, albeit with a time lag. Under stress conditions, the existence of an exodermis may cause a substantial reduction in the hydraulic conductivity of roots (22, 79, 93, 140). Another component could be plasmodesmata, which may act in a valve-like fashion to allow water uptake under favorable conditions (66, 134).

PATHWAYS FOR WATER AND SOLUTES

The hydraulic and osmotic properties of roots are related to their structure and anatomy, critical data when interpreting root transport. Depending on the species, growth conditions, and developmental state, there are large differences in roots. Roots do not behave like ideal osmometers (62, 96, 98, 105–107), as shown by measuring root reflection coefficients (σ_{sr}) that were smaller than unity, although in some cases values of close to unity have been reported (28, 33). Deviation in the behavior of roots from that of ideal osmometers (in analogy to cells) is caused by some or partial by-pass of water and solutes (nutrients, test solutes) in the apoplast even in the presence of Casparian bands and suberin lamellae in the endo- and exodermis, although these structures impede solute movement depending on the polarity and charge of the latter. Owing to the existence of apoplastic barriers, roots are not leaky, but tend to have permeability coefficients of solutes (nutrient salts) similar to those of cells (96, 140). In addition to the apoplast, there are two more parallel pathways in the root cylinder: the transcellular and the symplastic path. The transcellular path is across cell membranes and is dominated by water channels (aquaporins), whereas the symplastic path is mediated by plasmodesmata. To date, the transcellular and the symplastic pathways have not been separated experimentally, so they are summarized as a cell-to-cell path. However, evidence suggests that the transcellular component is dominating cell-to-cell Lp.

COMPOSITE TRANSPORT MODEL OF ROOT

According to root structure, there will be both hydraulic and osmotic water flow across roots. The relative contribution of the two components varies depending on conditions. The simple view describing water movement across roots in terms of osmotic processes in which root membranes play the dominant role [osmometer model of root (53)] does not hold as does the view that roots can be treated as simple

and constant hydraulic resistors within the SPAC. Both views do not account for adjustments of root Lp_r and low root σ_{sr}. Because there are no membranes along the apoplast, hydraulic flow dominates along this path. Flow across the apoplast is through a porous medium exhibiting no selective properties, i.e. the reflection coefficient of the apoplast will be zero or close to it. Consequently, gradients in osmotic pressure will cause little or no water flow, unlike the cell-to-cell path. Here, osmotic gradients have to be considered besides the hydrostatic ones. Unlike walls, membranes have selective properties; their reflection coefficient is close to unity. Because there are parallel pathways of different selectivity in roots (as in other plant tissue), the intensity by which disparate components of water potential act on the two pathways is different. Responses of roots differ depending on whether there is a difference in either hydrostatic pressure (such as tensions in root xylem during transpiration) or osmotic pressure between xylem sap and soil solution, which causes root pressure in the absence of transpiration. Differences should disappear in the presence of apoplastic barriers that completely interrupt the apoplastic water flow or in the presence of a very high permeability of cell membranes for water. Both extremes, a dominating apoplastic transport in the presence of hydrostatic gradients and a dominating cell-to-cell transport, are found in plant roots (96, 98, 100, 107).

Work with root and cell pressure probes and with other techniques has provided a composite transport model of the root. The composite root structure is comparable to that of membranes, which exhibit arrays of differential permeability and selectivity and form the basis of a composite transport model (48, 101) that has been adapted to roots. The composite transport model of the root explains (*a*) differences between osmotic and hydraulic water flow and the variabilty of root Lp_r, (*b*) low reflection coefficients, and (*c*) differences in root hydraulics among species.

Differences between hydraulic and osmotic water flow can be considerable: by an order of magnitude in herbaceous species and up to three orders of magnitude in woody species (96, 104, 107). Differences are expected from the composite transport model. In the absence of hydrostatic pressure gradients such as the tension in the root xylem, the apoplastic path should be inefficient because of its low reflection coefficient. Therefore, only the cell-to-cell component is left for water movement, which has a much higher resistance. Hence, when switching from hydrostatic to nonhydrostatic water flow, there should be a change in the resistance as is observed when measuring nonlinear pressure-flow curves of roots (20, 27, 66, 81, 104). The finding explains the unresolved problem of variable hydraulic resistance in terms of a switch between transport models (apoplastic versus cell-to-cell), which is related to the intensity of transpiration and the consequent existence of tensions in the root xylem.

Low reflection coefficients of roots are a consequence of apoplastic by-passes in arrays where Casparian bands are not yet developed, or even of some by-pass flow across the Casparian band itself. The latter conclusion derives from a comparison of root Lp_r and cell Lp, in experiments in which the endodermis was punctured and from chemical analyses of Casparian bands (42a, 70, 85, 86, 99, 100, 105–107,

133, 139). In the presence of two parallel pathways with different reflection coefficients or passive selectivity, reflection coefficients of individual pathways contribute to the overall reflection coefficient according to their hydraulic conductance. In the root, the reflection coefficient of the cell-to-cell pathway will be close to unity and that of the apoplast close to zero. Hence, the overall value should be somewhere in between (as was indeed found). It should depend on the efficiency by which apoplastic barriers interrupt water flow. The model predicts that at low overall hydraulic conductivity, effects of by-passes should be relatively high and root reflection coefficients low, as found for woody species. On the other hand, when artificial by-passes are created, root σ_{sr} decreases, also as expected. Both the absolute values of root σ_{sr} and the differences found between species support the model.

PHYSIOLOGICAL CONSEQUENCES OF COMPOSITE TRANSPORT AND ROLE OF COHESION TENSION

The most obvious consequence of the model is that it provides a means of increasing root Lp_r as transpiration develops and demands for water from the shoot increase. In terms of the model, this is mediated by an increase in the hydraulic component in the presence of increased tensions in the root xylem. Tensions cause a switch from osmotic cell-to-cell transport to hydraulic apoplastic transport. On the other hand, root Lp_r will be low at low rates of transpiration, i.e. during the night and during periods of water stress. Under these conditions, roots will be protected from excessive water loss to the soil by their low root Lp_r. Thus, the composite transport model provides an optimization of the water balance under different conditions, simply by a physical adjustment. This has been termed a coarse regulation of the water balance (96, 98, 100, 107), whereas a fine regulation is obtained by the activity of water channels (see below). As a result of composite transport, roots exhibit a high and adjustable water permeability in the presence of a low solute permeability. These unique transport properties are optimal for roots. They could be hardly achieved by a semipermeable endodermis in which the apoplastic path is completely blocked off (classical model of endodermis). In the context of the cohesion mechanism, tensions in the root xylem caused by a demand for water from the shoot increase root Lp_r, which is useful.

The traditional osmometer model fails to explain the variable Lp_r and low reflection coefficients. The Fiscus model (27) explains the variability in terms of a dilution of xylem sap, i.e. a decrease in the osmotic driving force in the xylem. However, detailed quantitative studies have shown that this cannot fully explain the measured effects (140). Since there are, for technical reasons, no direct measurements of the contribution of plasmodesmata (symplastic transport component), the quantitative contribution of plasmodesmata is not known although it has been suggested to play a role (66, 134). However, since water channels contribute to most of the root cell Lp, the role of plasmodesmata may be much smaller than thought originally (116, 135).

As the composite transport model proposes substantial apoplastic transport of water even in the endo- and exodermis, a considerable drag of solutes such as nutrient salts might also be suggested. However, this is not the case. When apoplastic dyes such as PTS (trisodium 3-hydroxy-5,8,10-pyrenetrisulfonate) are added to the root medium, they are found only in small quantities in the root xylem. Less than 1% of the concentration offered in the root medium has been found in the root xylem of different species, suggesting that PTS is filtered off at the exodermis and endodermis (35, 89, 140). These results compared with the quantitative data available for the overall and cell-to-cell passage of water might also suggest substantial differences between water and ionic and polar dyes used to trace water movement. However, for rice roots it has been proposed that sodium passes apoplastically in much higher quantities than is usually the case for dyes (131, 132).

From experiments on roots of *Arabidopsis thaliana*, it has recently been proposed that most of the calcium would arrive at the shoot after passing across the root apoplastically (130). There was no competition between Ca^{2+}, Ba^{2+}, and Sr^{2+} added to the mechanism. The conclusions are problematic in view of the fact that Ca^{2+} should move slowly across the apoplast even when there are no apoplastic barriers. Calcium is usually thought to travel with the transpiration stream in the root cortical apoplast up to the endodermis where the Casparian band prevents further movement. An uptake into the endodermal symplast is required (17, 56), using either Ca^{2+}-ATPases or Ca^{2+}-channels in the presence of a favorable electrochemical potential gradient (26). The thesis of a substantial apoplastic transport of Ca^{2+} was derived from results showing that transpiration linearly increased calcium uptake by the shoot even in the presence of Ba^{2+} and Sr^{2+} (130). Whether Ca^{2+} transport by membrane proteins in the plasmamembrane of endodermal cells is sufficient to allow for the high calcium requirements of the shoot is undetermined. High rates of transcellular calcium flux may also present a problem because of the risk of compromising intracellular Ca^{2+} levels required during $[Ca^{2+}]_{cyt}$ signals. The finding of an apoplastic transport of Ca^{2+} across the entire root cylinder is intriguing, but is at variance to other results which showed that there was no close coupling between Ca^{2+}-uptake and water uptake or transpiration (1a, 25a). It differs from results obtained with the root pressure probe during the past two decades (42a, 98, 100, 106). These results indicate that roots behave like osmometers although not as perfect ones. Most of the salts are effectively filtered off at the endo- and exodermis. However, in rice, the contribution of apoplastic transport to the overall uptake of NaCl was much larger than that given for the apoplastic tracer PTS (131, 132). The physicochemical basis of the differences in the selective properties of apoplastic barriers in the root (which may vary during root development) are not yet understood nor is the fine structure of apoplastic barriers known in sufficient detail (42a).

For the stress hormone abscisic acid (ABA), an apoplastic transport across the root cylinder affected by transpiration (cohesion tension) has been proposed in numerous studies with seedlings of maize, sunflower, and aspen (31, 32, 42, 42a, 124, 125). The results indicate a considerable by-pass flow of ABA when added in

nM up to 100 μM concentrations to the root medium. About 10% of the externally applied concentration of ABA was found in the root xylem. The apoplastic flow of ABA compensated, or even overcompensated, for the dilution by water flow in the xylem. Hence, the ABA signal (xylem concentration) was strongly affected by apoplastic ABA flow. In corn roots, the existence of an exodermis reduced both the flow of water and ABA, but did not change the xylem concentration much. In corn, ABA also specifically affected water channel activity (42). The results show that overall water flow (transpiration), which is usually thought to be regulated with the aid of the stress hormone ABA at the level of stomata, may interact with the ABA signal from the root in some kind of feedback loop. On the other hand, ABA may increase water uptake via water channel activity in the root under nontranspiring conditions, thus inducing water uptake along the cell-to-cell path (42).

ROLE OF COHESION TENSION ON WATER CHANNELS IN ROOTS

Water channels are transmembrane proteins with a molecular weight of about 30 kDa and six transmembrane helices spanning the membrane, thus forming a pore of an internal diameter just sufficient to allow the passage of water in a single file (16, 51, 57, 83, 116). Four of these functional units are arranged in the membrane to form a stable tetramer. Water channels can be reversibly affected by mercurials that bind to SH groups of cysteine (58, 88, 101, 111). The activity of water channels is also affected by high salinity, solute concentration, temperature, heavy metals, oxidative stress, and by the deprivation of nutrients to the roots (15, 36, 36a, 38, 74, 75, 101). In some species, there is a diurnal rhythm in the hydraulic properties (root Lp_r) that correlates with a rhythm in the level of m-RNA encoding for putative aquaporins (18, 37). Whether tension in the xylem or pressure gradients across a root membrane such as at the endodermis could affect the open/closed state of aquaporins is more difficult to answer than whether xylem tensions affect apoplastic water flow. Water potential in the root apoplast is suggested to affect water channel activity (46). In roots, high salinity causes changes in both root Lp_r and root cell Lp, but more extensively in the latter parameter (2, 3). This may be interpreted as an effect of ion concentration or of dehydration of cell membranes and shrinkage of membrane pores caused by tensions within water channels, as once proposed for ion channels (138).

CONCLUSIONS

Older and more recent evidence provides strong support for the cohesion-tension theory of the ascent of sap in plants. The CT-mechanism has a solid physical basis. Direct measurements of xylem pressure in transpiring plants have shown

that responses of P_x to changes in transpiration or to pressure applied to the root are as predicted by the cohesion-tension theory. The disagreement found in earlier measurements with the probe performed by Zimmermann and co-workers is most likely due to experimental limitations and artifacts that have not been checked for properly. At present, the limit for the use of pressure probes in the xylem of transpiring plants is at a P_x of about -1 MPa (-10 bar), which is less than the highest tensions of up to 10 MPa (100 bars) claimed to exist in the xylem. Within these limits, measurements of xylem pressure conducted with the probe agreed with those conducted with the Scholander-Hammel bomb. To extend the range of the pressure probe, the technique needs to be further refined.

The cohesion-tension mechanism plays a role during the acquisition of water by plant roots. Tensions provide a strong gradient in hydrostatic pressure that favors the apoplastic component of water uptake in relation to the cell-to-cell component. This results in nonlinear pressure-flow relations (increase of root hydraulic conductivity with increasing xylem tension) and in a coarse regulation of water uptake by plants in accord with the composite transport model of the root. According to the model, tensions in the xylem set up by transpiration cause a switch from cell-to-cell to apoplastic water transport, which increases root hydraulic conductivity and the ability to take up water even at low soil water potential. On the other hand, when transpiration is switched off at night or during water stress, hydraulic resistance would be high, thus minimizing the loss of water to the dry soil. Water channels would provide a fine regulation of water uptake in the presence of effective apoplastic barriers (roots grown under unfavorable conditions). Water channel (aquaporin) activity in plasma membranes of roots is under metabolic control and is affected by many external parameters. It is not yet clear whether water channel activity is also affected by pressure gradients set up across the root cylinder in response to high tensions in the xylem.

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