The Dynamics of "Dead Wood": Maintenance of Water Transport Through Plant Stems

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SYNOPSIS. The lack of mobility in plants is often interpreted as a sign of their passivity in the face of environmental variation. This view is perhaps most firmly entrenched with regard to water transport through the xylem in which water flows through the lumen of cells that are "dead" (*i.e.,* lack any cytoplasm or nucleus) at maturity. However, recent work demonstrates that a number of active, physiological processes may be involved in maintaining the transport capacity of this essential pathway. Here we review work relating to both embolism repair and the effect of ion concentrations on xylem hydraulic properties as examples of such dynamic processes.

INTRODUCTION

Physiological comparisons between plants and animals are complicated by fundamental differences in their basic organization and modes of acquiring resources from the environment. Plants are autotrophic and largely non-motile, while animals are heterotrophic and, to a large degree, self-propelled. Both features are tied to the fundamental cellular difference between plants and animals—the existence of a cell wall in plants and its corresponding absence in animal cells. The rigidity between plant cells resulting from their walls means not only that plants remain fixed (rooted) in one place, but also that they cannot employ mechanisms requiring moving parts, nor involve cell migration during development (Brett and Waldron, 1996). Nevertheless, plants and animals share the physiological demands associated with multicellularity allowing one to compare processes that allow each to function as integrated organisms. In this paper, we consider plants and animals in terms of physiological and behavioral mechanisms associated with hydration. Volume and osmotic regulation is, in general, a prerequisite for physiological function and both plants and animals employ a variety of mechanisms to achieve a high degree of homeostasis in the face of large fluctuations in water availability in the environment (Schmidt-Nielsen, 1990; Taiz and Zeiger, 1998). Some plants (*e.g.,* "resurrection" ferns and some bryophytes), as well as many seeds, can withstand a high degree of cellular desiccation. However, hydration is a prerequisite for photosynthesis and growth in all plants. The ultimate goal of a plant is to reach reproductive maturity and produce viable offspring, generally in the form of seeds. This can be extremely challenging to a sessile organism since its vegetative success depends directly on the surrounding environment. Thus, survival depends on a plant's ability to adapt and respond to their environment. One of the major limiting environmental constraints for terrestrial plant growth is water. Compared to animals, plants need large quantities of water to grow. We would expect then, that plants have evolved numerous mechanisms to deal with fluctuations in water availability. Because a goal of this symposium is to encourage interchange among plant and animal specialists, we emphasize the active nature of plants in maintaining tissue hydration. In particular, we focus on new work demonstrating dynamic processes operating in plant stems to maintain and enhance long-distance transport of water through the hydraulic conducting tissue of plants known as xylem.

Terrestrial plants and animals must be able to withstand the large changes in water availability and evaporative demand imposed by their environments (Campbell and Norman, 1998). Motility allows animals to adjust behaviorally to changes in the water status, both seeking out specific microsites within their environment and altering their orientation so as to reduce evaporative losses (Schmidt-Nielsen, 1990). However, similar responses are also observed in plants, despite the fact that they are not able to pick up and walk (crawl, swim, fly) away. Plants can alter their orientation to reduce their heat load and thus rates of water loss through changes in leaf orientation or by reducing their evaporative surface area by actually shedding leaves (Jones, 1992; Larcher, 1995). For example, leaf rolling occurs in many grasses in response to drought and serves to reduce rates of water loss. Wilting may also be a functional response in that a wilted leaf generally intercepts less sunlight and thus will lose less water (Chiariello *et al.*, 1987). Animals respond physiologically to changes in water availability principally through increasing the resorption of water that would otherwise be lost through their excretory system (Schmidt-Nielsen, 1990). Plants also respond physiologically to water stress, with the dominant mechanism being to reduce rates of water loss by closing their stomatal pores (Kramer and Boyer, 1995). However, the major way in which changes in water availability influence plant function relates to their water transport system.

PLANTS *VERSUS* ANIMALS: WHY ARE PLANTS SO THIRSTY?

A major difference between patterns of mass transport in plants and animals is that plants have an

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"open" or flow-through system, whereas animal circulation is largely "closed." Thus, most of the water that enters into a plant through its roots passes through the xylem and evaporates from the leaf surfaces, with only a small fraction ($\sim$1%) of this volume actually re-circulating within the plant (*i.e.,* through the plant via the sugar transport path known as the phloem). In contrast, although animals also lose water to the environment, this amount is typically quite small relative to the volume of their circulation system (Schmidt-Nielsen, 1990). As a consequence, plants require vast amounts of water compared to animals. A typical plant, such as a sunflower will use $\sim$800 times more water per day on a mass basis than a human (Raven *et al.*, 1999). The reason for this tremendous thirst is that plants are the ultimate sit-and-wait predator—they essentially open their mouths (*i.e.,* their stomata) and wait for their prey (CO_2 molecules) to diffuse in. An inevitable consequence of this foraging mode is that water diffuses out of the leaf along basically the same pathway. Because the concentration gradient for water loss to the atmosphere is much steeper than for the absorption of CO_2, the exchange rate is typically on the order of several hundred water molecules lost to the atmosphere for every CO_2 incorporated into sugars by photosynthesis (Jones, 1992).

Although both animals and plants rely on liquid transport for the internal distribution of a variety of materials, there are fundamental differences in the nature of how this mass transport occurs. Animals generally rely on a contractile pump that propels fluids along gradients in positive pressure, while in plants, water moves along gradients in negative pressures. A typical tree might expend more than 400 liters per day, accomplishing this feat of mass transfer with a hydraulic system that operates without any moving parts. The driving force for water movement through plants arises as a direct outcome of the evaporation of water from the leaves (Zimmermann, 1983). In essence, water is pulled through the plant by a tensile force generated by the evaporation of water from leaf surfaces (Holbrook *et al.*, 1995). Transport of water under tension requires the existence of a continuous column of water between soil and leaves whose continuity under tension is maintained by the strong attraction between water molecules (Pickard, 1981). Instead of using their hard-won CO_2 as a source of metabolic energy to drive a pump, plants employ a structural solution that enables them to distribute water to their highly branched system utilizing energy supplied directly by the sun (Hales, 1727). This mechanism for water transport, first described in the 19th century, is known as the "cohesion" theory. An added benefit of evaporative water loss from leaf surfaces is that it provides a cooling mechanism that prevents leaf overheating and maintains leaf temperatures within reasonable bounds (Larcher, 1995).

One consequence of employing solar energy to drive water flow in plants is that the hydrostatic pressure in the xylem varies widely during the day. At night when transpiration is at a minimum, the tensions will be low; during the day the tensions in the xylem must be much greater (and thus the absolute pressures more negative) to pull water from the soil at the rates needed to satisfy the evaporative losses through the stomata (Passioura, 1982). To put these changes in perspective, diurnal fluctuations in the chemical potential of the water in the xylem are on the same order as if the leaf cells were transferred each day between a freshwater and a marine (seawater) environment. Animals that move between such dramatic osmotic environments (eurohaline animals) do so by actively regulating their ionic balance (Schmidt-Nielsen, 1990). In contrast, leaf cells maintain volume and osmotic concentrations in the face of large fluctuations in the chemical potential of their surrounding solution because of their strong, but elastic cell walls (Kramer and Boyer, 1995). Specifically, changes in the potential of the water surrounding the living leaf cells are, to a large extent, balanced by changes in turgor pressure. This is another example of how plants utilize "structural" solutions to the problem of maintaining cellular homeostasis and hydration despite large diurnal fluctuations in water availability.

INTRINSIC CHALLENGES TO WATER TRANSPORT: CAVITATION

The major problem that plants face in transporting water from the soil to leaves is that of cavitation (Tyree and Sperry, 1989; Pockman *et al.*, 1995). Substantial forces are required to overcome the potential losses due to gravity and friction, as well as the matrix forces within the soil, resulting in hydrostatic pressures well below zero (Passioura, 1982). Typical daytime pressures within the water column in the stem of a tree are on the order of -15 atmospheres, with much more negative values occurring in plants of arid regions. It may seem surprising that this essential supply line operates routinely at pressures much lower than we can generate in the laboratory without going to great effort (Briggs, 1950). Indeed, doubts regarding the stability of such tensions within the xylem have generated much controversy regarding the feasibility of the mechanism (Zimmermann *et al.*, 1994). However, the existence of tensions within the xylem has been confirmed by both indirect and, more recently, direct measurements (Melcher *et al.*, 1998). What allows this metastable state to persist is the existence of an energy barrier (Pickard, 1981). Cavitation requires the expansion of a gas:liquid interface (Brennen, 1995). Because of the very large surface tension of water, highly curved interfaces require a large amount of energy to expand. This is where the structure of the transport conduits plays a major role is reducing cavitation. Cavitation is thought to occur primarily by air being sucked into the vessels, providing a bubble that nucleates the phase change (Zimmermann, 1983). This process is called "air seeding." Plants resist air seeding by constructing the walls of their xylem vessels in such a way that any air:water interface is kept very small

(nm), thus reducing the probability of gas expansion due to the high curvature.

There is no doubt that cavitation does occur in plants. One can detect ultrasonic emissions that occur when a water column breaks and one can measure the resulting decrease in conducting capacity due to the formation of air embolism (Milburn, 1993; Tyree et al., 1986). Cavitation will occur if the xylem is mechanically damaged, if the tension in the water become too large, or may result from freezing due to the low solubility of gases in ice (Sperry, 1995). The loss of transport capacity in one xylem vessel will increase the tension in adjacent vessels, which can lead to a "runaway-cavitation" situation unless the stomata are able to close in time (Tyree and Sperry, 1988). However, stomatal closure has the cost of reduced CO_2 uptake. The ability to refill a cavitated vessel would allow plants to regain their original transport capacity, without the delays and costs of having to construct additional wood.

PHYSIOLOGICAL RESPONSES TO COUNTER CAVITATION: EMBOLISM REPAIR

The question of whether embolized vessels can be refilled such that they can once again transport water under tension is currently a matter of some discussion. The answer to this is certainly "yes" for plants that are able to generate root pressure (Sperry et al., 1987; Fisher et al., 1997). Root pressure results from the active loading of ions into the xylem by root tissues and results in the entire vascular system being pressurized (Kramer and Boyer, 1995). Refilling of embolized vessels by root pressure is important in herbaceous plants. For example, xylem vessels in corn that are embolized during the day may become refilled over night due to root pressure. In grapevines, vessels embolized due to winter freezing become refilled in the spring as a result of root pressure. However, root pressures occur only when the soil is moist and transpirational water loss is very low. Thus, this method of repairing cavitated conduits will not work either when the plant is experiencing drought or during the day when transpiration is occurring—exactly those conditions when one might think that loss of hydraulic capacity due to cavitation would be most severe.

Up until a few years ago, the question of whether embolized vessels can be refilled during periods of active transpiration (i.e., when there is tension in the xylem) would have been greeted with a resounding "No." Because positive pressures are required to force gas back into solution, it is difficult to conceive of how positive pressures could be locally generated within a "sea" of tension. However, beginning in 1996 empirical evidence suggesting that refilling of embolized conduits was occurring despite the existence of negative pressures has steadily accumulated (Salleo et al., 1996; Zwieniecki and Holbrook, 1998; Melcher et al., 2001). How strong is this evidence? Because the traditional techniques used to assess embolism repair require destructive sampling, for a long time the evidence was indirect. Furthermore, the use of highly processed sampling techniques such as cryo-SEM to visualize air- versus water-filled conduits has recently been questioned and further studies are needed to establish the validity of this technique (Cochard et al., 2000). However, measurements of flow through excised branch segments that show increases in hydraulic conductivity (as well as a corresponding decrease in the % loss of conductivity) have been difficult to explain without invoking a mechanism that returns embolized conduits to their functional state (Holbrook and Zwieniecki, 1999). Recently, direct evidence for embolism repair through the use of magnetic resonance imaging to directly image and follow, over time, the status of individual xylem conduits in vivo shows great promise for resolving some of the uncertainty surrounding the question of embolism repair (Holbrook et al., 2002).

The major difficulty with the idea that embolized conduits can be refilled despite the existence of tension in the xylem is the absence of a comprehensive mechanism to explain how this could occur. Any description of how repair takes place must explain: 1) how water is forced into a gas-filled conduit against an apparent gradient in chemical potential; 2) how the positive pressures needed for gas dissolution are locally contained despite the existence of tension in adjacent conduits; and, 3) how a refilled vessel makes a stable transition from positive to negative pressures and thus resumes its role in water transport (Holbrook and Zwieniecki, 1999). Below, we consider these three requirements in turn.

The first step in refilling involves the movement of water into the gas-filled conduit. If the water in adjacent vessels remains under tension, water will not flow spontaneously into this gas-filled space (i.e., the gradient will be in the wrong direction). Although the driving force for water movement into gas-filled conduits is not known, we can imagine that it will involve the generation of an osmotic gradient as a result of the active pumping of solutes into the embolized vessel by living xylem parenchyma cells. In this way, refilling is analogous to how root pressures are generated. Xylem vessels are typically in contact with many living cells (Core et al., 1976; Lachuad and Maurousset, 1996). Studies in which the living cells within the stem are killed eliminates the ability of stems to recover midday losses in xylem conductance (Zwieniecki et al., 2000), consistent with the involvement of these living cells in embolism repair. Although further studies are needed to confirm this, we note that this is the first demonstration of a direct role for living cells within the wood in water transport through the xylem.

To generate positive pressures within an embolized conduit it must be hydraulically compartmentalized to allow generation of local positive pressures. Thus, we focus our attention on the periphery of the refilling conduit—specifically those points where contact is made with adjacent conduits. The thick, heavily lignified xylem walls contain numerous pores or "pits"

that enable living cells to access the xylem fluid (intercellular pits), as well as pits that interconnect adjacent xylem conduits (intervessel pits). Intervessel pits consist of a straight-walled channel connected to a chamber. At the center of this chamber is a partition formed from a primary cell wall and commonly referred to as the ''pit membrane.'' We believe that the geometry of these intervessel pits provides a structural opportunity for the hydraulic compartmentation necessary for refilling without subsequently impeding water flow once the vessel has been refilled (Zwieniecki and Holbrook, 2000). Specifically, the flared opening into the pit chamber could allow the pit to function as a valve through the formation of a convex meniscus. The actual curvature of this meniscus depends both upon the surface energy between water and the interior surface of the vessel, as well as the actual geometry of the pit chamber. If the sum of the contact angle and the flared opening into the pit chamber are greater than 90°, then the curvature of the meniscus when water reaches the pit chamber will resist further expansion. Based on direct measurements of both contact angle and pit chamber geometry, we estimate the positive pressures that can be balanced by the forces arising from surface tension of this curved interface to be on the order of 1 to 3 atmospheres (Zwieniecki and Holbrook, 2000). Microcapillary measurements of water flow through individual xylem vessels that had been previously air-filled are consistent with the existence of a pressure threshold needed to push water into bordered pits (Zwieniecki et al., 2001a). Calculations of the rate at which the gas within a pressurized conduit would be forced into solution indicates that pressures of one to three atmospheres is sufficient to allow refilling to occur within a period of hours, consistent with suggestions that repair takes place during the day.

The final stage in refilling, reconnection to adjacent conduits, is difficult to fathom given our current understanding of the structure of the pit membrane. Because xylem conduits are interconnected in many places (Zimmermann, 1983), the hydraulic contact must be established at all of the pits almost simultaneously to prevent the vessel from re-cavitating. Thus, refilling will be stable only if there exists a mechanism that could synchronize the reconnection through these many pits. For such synchronization to occur, a mechanism must exist to delay or somehow coordinate the pressure transmission through pit membranes. Without such a mechanism, it is hard to understand how repair could occur except under conditions in which the tensions in adjacent conduits were substantially (or completely) eliminated. Thus, a great deal of work is needed to understand the contribution of embolism repair to the dynamic maintenance of water transport capacity in plants. In particular, empirical studies that better resolve the occurrence and conditions present during repair are needed, as well as detailed structural studies of the pit membrane.

IONIC EFFECTS ON XYLEM HYRAULIC RESISTANCE

The traditional view of the xylem as a series of inert tubes considers the xylem to have only two states: a vessel can be water-filled and thus able to transport water with a fixed resistance or it can be embolized and thus unable to transport water under tension. However, the hydraulic resistance of the xylem is not fixed, but instead varies in response to the ionic concentration of the transported fluid (Zwieniecki et al., 2001b; van Ieperen et al., 2000; Zimmermann, 1983). The mechanism underlying these changes in resistance appear to result from the swelling/deswelling of hydrogels located in bordered pit membranes (Zwieniecki et al., 2001b). With increasing concentrations of ions, these hydrogels are hypothesized to shrink, increasing the porosity of the pit membrane and thus decreasing its resistance to water flow. These changes are both reversible and repeatable, suggesting that plants could actively modulate their xylem resistance by altering the ionic concentration of the fluid in the xylem.

The functional significance of ion-mediated changes in xylem resistance remains to be explored. However, the effect of ions on xylem hydraulic properties occurs in all species so far examined, suggesting that it may play an important role (Zwieniecki et al., 2001b). In particular, the ability to enhance flow through the xylem by increasing the ionic concentration of the xylem sap may allow plants to compensate for losses in conductive capacity due to cavitation. It could also allow plants to effect short-term, dynamic changes in the hydraulic architecture of the xylem and thus to prioritize water delivery to those leaves currently experiencing the most favorable conditions for photosynthesis. Such a mode of autonomous regulation could be appropriate for fine-tuning water transport through a highly branched system. Such active redistribution would be analogous to changes in blood flow patterns in vertebrates resulting from constriction of capillaries. Further studies focusing on in vivo changes in xylem sap ion concentration and xylem properties are needed to understand the significance of this newly recognized mechanism. What is clear, however, is that plants have the ability to rapidly modify their hydraulic properties, opening the door to understanding how they may utilize this to enhance their capacity to optimize water delivery to leaves.

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