Plant Physiology, May 1999, Vol. 120, pp. 7–10, www.plantphysiol.org © 1999 American Society of Plant Physiologists

SCIENTIFIC CORRESPONDENCE

Embolism Repair and Xylem Tension: Do We Need a Miracle?[1]

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There is widespread agreement that negative hydrostatic pressure makes water transport in the xylem intrinsically vulnerable to cavitation (Pickard, 1981; Zimmermann and Milburn, 1982; Tyree and Sperry, 1989). Xylem hydraulic conductivity is often substantially reduced by cavitation and the subsequent formation of embolized (gas-filled) conduits (Milburn, 1993). To maintain hydraulic capacity, plants must replace embolized vessels, maintain a highly redundant transport system, or repair embolized conduits. The idea that embolized vessels might be restored to their functional state is not new (Pickard, 1989) but has generally been thought to be limited to those plants and/or conditions in which the entire vascular system could be pressurized because of active solute transport by the roots (Tyree et al., 1986; Cochard et al., 1994; Fisher et al., 1997). Recent studies, however, indicate that embolism removal may be concurrent with transpiration (Salleo et al., 1996; Canny, 1997; McCully et al., 1998; Zwieniecki and Holbrook, 1998; McCully, 1999; Tyree et al., 1999), raising the question of how embolized vessels can be refilled while the majority of the water in the xylem remains under tension. Some researchers have viewed the difficulty in reconciling this conflict as evidence that the basic ideas of water transport need to be re-examined (Canny, 1997). We argue that cavitation repair and the presence of xylem tension are not mutually exclusive.

The goal of this *Scientific Correspondence* is to present a mechanism for embolism removal from the water-transport system of vascular plants. The major challenge is to propose a mechanism that is consistent with both xylem structure and known physical laws. We hypothesize that vessel embolism is a reversible phenomenon made possible by the interaction of xylem parenchyma, vessel wall chemistry, and the geometry of intervessel pits. Here we address the following questions: How does water enter an embolized conduit? How do positive pressures needed for refilling coexist with tension? How is hydraulic continuity restored?

LIVING XYLEM PARENCHYMA CELLS PROVIDE THE DRIVING FORCE FOR REFILLING

Refilling of embolized conduits requires that water enter the vessel lumen while pressurizing the gas phase until it is forced back into solution (Fig. 1). The creation of a sufficient driving gradient requires a local input of energy that may come from the activities of living cells. To support this idea, we argue that the capacity to restore losses in hydraulic conductance due to low xylem water potentials is reduced after treatments such as phloem girdling (Salleo et al., 1996; M.A. Zwieniecki and N.M. Holbrook, unpublished data) or application of $HgCl_2$ to the transpiration stream (M.A. Zwieniecki and N.M. Holbrook, unpublished data). Both treatments may affect the activity of living cells but should have minimal effects on the physical properties of water (e.g. surface tension, viscosity, and tensile strength).

Xylem vessels are generally in direct contact with numerous living cells (Core et al., 1976; Zimmermann, 1983; Lachuad and Maurousset, 1996). We hypothesize that water is released into the vessel lumen from these adjacent living cells in a manner similar to that which occurs during root exudation (Kramer and Boyer, 1995). Current understanding of water transport across cell membranes is based on the thermodynamics of irreversible processes (Dainty, 1963). Water will move from living cells to the embolized vessel if an adequate driving gradient is present. The most parsimonious mechanism for water movement into embolized conduits involves the active secretion of solutes by the living cells. Measurements of the osmotic concentration within repairing vessels, however, suggest that osmotic forces may not be adequate to explain the observed exudation (Canny, 1997; McCully et al., 1998; McCully, 1999; Tyree et al., 1999). It is possible to construct a variety of thermodynamically valid scenarios for water exudation in which the driving force appears to be

[1] This work was supported by the Andrew W. Mellon Foundation and by the U.S. Department of Agriculture (grant no. 98-35100-6081).

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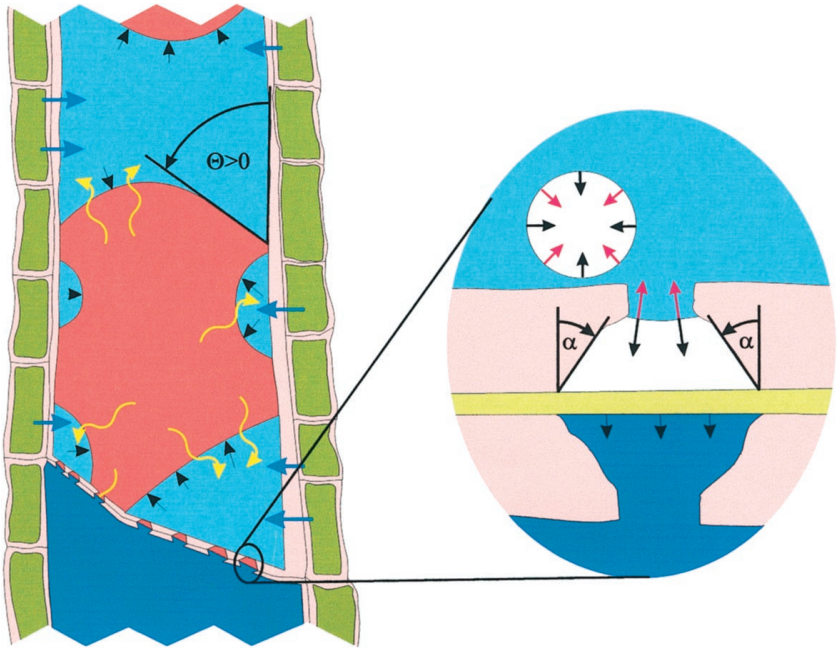

Figure 1. Hydraulic compartmentalization of vessel refilling. Left, Living cells adjacent to the embolized vessel create a driving gradient that draws water into the vessel lumen (blue arrows). Droplets are retained on the wall due to the nonzero contact angle (θ). Low permeability of the secondary wall prevents tension in adjacent vessels from being transmitted. Influx of water into the lumen compresses the gas phase (black arrows), forcing it into solution (yellow arrows). The dissolved gas then diffuses away from the refilling vessel, where it may be carried off by the transpiration stream. Right, Bordered pit geometry (inverted funnel with angle α) prevents water from entering the pit channel before the lumen is entirely filled. The upper conduit is actively refilling and the water is under positive pressure; the lower vessel is under tension. Arrows indicate the effects of hydrostatic pressure (black) and surface tension force (red) on the gas/liquid interface.

counter to the direction of net water movement. All such scenarios, however, require that the transport pathway contain a degree of complexity and/or the existence of specific properties that have not yet been empirically verified.

The identification of integral membrane proteins that act as water-selective channels in plants (Chrispeels and Maurel, 1994; Kammerloher et al., 1994; Maurel, 1997) may help to reveal possible pathways of water exudation into the vessel lumen. Recent studies indicate that these proteins are abundant in the plasma membranes of xylem parenchyma (Barrieu et al., 1998). The presence of potentially high-conductance pathways between xylem vessels and adjacent living cells suggests an important role for radial water transport. Feeding $HgCl_2$ to the xylem of a transpiring plant significantly reduces the ability of petioles to recover from the loss of hydraulic conductivity caused by water stress (M.A. Zwieniecki and N.M. Holbrook, unpublished data). Whether this effect resulted from the blocking of water channels or was a general effect of a metabolic poison (or both) is not known. Further studies of water exudation from living cells and the potential involvement of aquaporins are needed to understand exactly how water enters embolized conduits.

THE CHEMISTRY OF VESSEL WALL SURFACES ALLOWS COMPARTMENTALIZATION OF THE REPAIR PROCESS AND THUS LOCAL GENERATION OF POSITIVE PRESSURE

An essential component of any refilling mechanism is that water exuded into gas-filled vessels remain within the lumen rather than being swept away by the transpiration stream. Hydraulic isolation is also required to permit the local creation of the positive pressures required to force the gas into solution. This will occur if the secondary walls of the xylem are relatively impermeable to water and if the contact angle (θ, Fig. 1) is greater than zero. Low permeability decreases the likelihood that tensions can be transmitted through the wall; a nonzero contact angle means that water will form stable droplets on the inner surface of the vessel. Lignification occurs during the final stages of secondary wall maturation, suggesting that some lignin is localized near the inner surface. Lignin is substantially more hydrophobic than cellulose (Sarkanen and Ludvig, 1971) and effectively "waterproofs" the vessel walls (McCann, 1997). We hypothesize that lignification allows the hydraulic isolation required for refilling to occur.

Contact angles greater than zero are thought to be incompatible with the transport of water under tension because of the probability of heterogeneous bubble formation (Brennen, 1995). For contact angles less than approximately 70° this effect is thought to be small (Sadhal et al., 1997). On the other hand, nonzero contact angles increase the likelihood of gas entrapment in small crevices (Bankoff, 1958). Under tension the gas can form sites for bubble nucleation. During refilling, however, gas entrapment in bordered pits prevents premature reconnection of the liquid in the refilling vessel with an adjacent conduit.

THE FINE STRUCTURE OF INTERVESSEL PITS MINIMIZES GAS VOLUME SO THAT HYDRAULIC CONTINUITY CAN BE RE-ESTABLISHED

The compartmentalization of refilling, and thus the creation of positive pressures sufficient to dissolve all of the gas within the conduit lumen, is strongly dependent on the geometry and surface properties of the connections (pits) between vessels. Pits are narrow channels through the thick secondary cell walls of the vessel elements (Zimmermann, 1983). At the center of each pit is the relatively thin pit "membrane, " which is formed from the primary cell wall and consists of a dense network of hydrophilic cellulose polymers. Let us consider two adjacent vessels, i.e. one that is functional (i.e. under tension) and the other that is undergoing refilling. The functional side will be water-filled right up to the pit membrane, where its stability results from the surface tension of the air-water interfaces in the pores of the pit membrane. Characteristics that increase this stability include small pore radii and small contact angles (hydrophilic composition) of the pit membrane. On the embolized side of the pit membrane, the channel through the secondary cell wall must be gas-filled to prevent the tension from being transmitted while the lumen of the vessel is refilling. Characteristics that help maintain a gas-filled bordered pit include a small entry radius (relative to the size of the vessel lumen) and less-wettable channel walls. The pressure in the liquid phase must be sufficient to dissolve all of the gas within the conduit lumen but not so great as to exceed the air entry threshold of the air-water interfaces that connect the embolized conduit to adjacent, functioning vessels. The absolute pressure needed to force the gas back into solution, however, may be relatively small, because dissolution is facilitated by diffusion into adjacent cells and the "stirring" afforded by the movement of the transpiration stream in nearby vessels (Tyree and Yang, 1992).

Stable restoration of hydraulic continuity requires that the volume of undissolved gas be minimized at the time the tension is re-established. We believe that the geometry of bordered pits plays a critical role in allowing the gas within the vessel lumen to be forced into solution prior to the liquid phase making contact with an adjacent vessel. Specifically, if the sum of the angle of the pit chamber (α, Fig. 1) and the contact angle is greater than 90°, then water that is pushed into the bordered pits will form a convex gas liquid interface. Because it takes less pressure to expand a large convex gas/water interface than a small one (Laplace's law), water exuded into the vessel will tend to fill the lumen rather than the bordered pits. As water droplets connect across the center of the vessel, their interfaces will coalesce to form concave surfaces. Differences in concavity ensure that the pressure on the gas within the lumen exceeds that of the gas within the bordered pits. In a concave interface, the surface tension force acts to increase the pressure on the gas phase, whereas in a convex interface the energy to minimize interfacial area reduces the pressure on the gas phase. Thus, although the volume of the pits is extremely small compared with the vessel lumen, the gas within the lumen will dissolve first. The situation is further stabilized because the curvature of the gas/water interface within the bordered pit increases as gas is forced into solution, whereas in the lumen the curvature decreases.

The tension existing in adjacent vessels will be transmitted to the refilling conduit as soon as the advancing water contacts the hydrophilic pit membrane. For this transmission to be stable, the radii of the gas spaces within the pit channel must be less than the critical value for bubble expansion at that tension. We hypothesize that both the wall chemistry (contact angle) and the geometry of the bordered pit contribute to this stabilization. Specifically, the volume of gas remaining at the time the meniscus touches the pit membrane will be minimized if the sum of the contact angle and the angle of the walls of the pit chamber equals approximately 90°.

SIGNIFICANCE

We believe that the ability to repair cavitated vessels has profound implications for understanding the structure and physiology of vascular plants. The reversibility of embolisms leads to an understanding of measured hydraulic conductivity as a dynamic balance between the processes of damage and repair. We believe that substantial scientific investment into the study of cavitation repair is required before we can fully understand the diversity of the developmental and physiological responses of plants to water availability. At this time, new techniques and investigations into cavitation repair at a fundamental level are required if we are to resolve the temporal dynamics of vessel refilling and to understand the mechanism of embolism removal.

ACKNOWLEDGMENTS

We thank M.J. Burns, T.S. Feild, W.F. Pickard, H.A. Stone, and M.V. Thompson for helpful comments.

Received November 9, 1998; accepted February 5, 1999.

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