

Confronting Maxwell's demon: biophysics of xylem embolism repair

Maciej A. Zwieniecki¹ and N. Michele Holbrook²

¹Arnold Arboretum of Harvard University, Cambridge, MA 02138, USA

²Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA

Embolism results in a dramatic loss of xylem hydraulic transport capacity that can lead to decreased plant productivity and even death. The ability to refill embolized conduits despite the presence of tension in the xylem seems to be widespread, but how this occurs is not known. To promote discussion and future research on this topic, we describe how we believe refilling under tension might take place. Our scenario includes: (i) an osmotic role for low-molecular weight sugars; (ii) an apoplastic sugar-sensing mechanism to activate refilling; (iii) the contribution of vapor transport in both the influx of water and removal of entrapped gases; and (iv) the need for a mechanism that can synchronize reconnection to the transpiration stream through multiple bordered pits.

Xylem embolism repair

A reduction in xylem transport capacity due to cavitation represents a major challenge for plants experiencing high evaporative demand or low soil water availability. The impact of cavitation on plant productivity, however, depends on whether these gas-filled (embolized) conduits are permanently removed from the transpiration stream. Embolism repair due to root pressure has long been known to occur [1], but requires both high soil water availability and the cessation of transpiration for the production of positive pressures throughout the xylem. By contrast, the ability to refill embolized conduits while the bulk of water in the xylem remains under tension [2–4] would allow plants to restore hydraulic conductivity during periods with high demand for water transport capacity. On the surface, embolism repair under tension seems to violate the laws of thermodynamics [5,6]. Not surprisingly, the first observations of embolism repair under tension [2–4] were met with both intense interest and some skepticism [7], prompting a number of conceptual papers that sought to explain how gas-filled conduits could be refilled despite the surrounding tissues remaining at water potentials less than zero [5,6]. To date, an increasing number of studies document the existence of refilling using approaches that includes both destructive [8–13] and *in vivo* techniques [14–16], and in some cases observations of refilling have been linked to physiological treatments, such as phloem girdling [4,17], starch dynamics [17–20], and the activity of membrane transporters [14,21,22]. Nevertheless, many of the major biophysical questions concerning refilling under tension remain unanswered [23].

Understanding how embolism repair under tension occurs is critical for evaluating the costs and constraints of transporting water in a metastable state. Furthermore, without thermodynamically sound mechanistic hypotheses to guide future research efforts, the specter of Maxwell's demon [24] (see Glossary) will continue to haunt this field. In 1999, we [5] identified three issues that must be addressed if we are to understand how refilling under tension takes place: (i) the origin of the driving force that impels water into cavitating conduits, (ii) the source and pathway of water for refilling, and (iii) the physical nature of the hydraulic compartmentalization that separates refilling from functional conduits. To this, we add an urgent need for integrating such biophysical questions with the molecular approaches (i) that can connect refilling with the underlying activities of xylem parenchyma and (ii) have the power to elucidate signaling pathways involved in triggering refilling. Here, we outline a concise scenario (Figure 1) for embolism refilling that incorporates new research and ideas that have emerged over the past decade. Our goal is to provide a conceptual framework that will provoke discussion, organize existing information and provide a useful guideline for future studies of xylem refilling under tension.

Driving force for refilling

We begin with the assumption that the driving force for refilling is generated osmotically [5,6,25–27]. Although several papers have proposed “tissue pressure” [28,29]

Glossary

Cavitation: The phase change from liquid to vapor due to negative pressures. In xylem, cavitation typically results from the expansion of gas bubbles, occurring when xylem tensions exceed the inward pressure due to the curvature of the bubble's gas/water interface.

Maxwell's demon: An imaginary being invented by James Clerk Maxwell as a thought experiment to explore the second law of thermodynamics. As envisioned, the demon was capable of sorting molecules between two connected gas filled boxes such that fast molecules accumulate in one box while slow molecules accumulate in the other. The net result of the demon's actions is to create a temperature difference between the two connected boxes, thus violating the second law of thermodynamics. Also known as the sorting demon.

Phloem girdling: Process of removing of narrow band of bark and cambium from a woody plant stem, interrupting sugar transport from leaves to roots.

Pit membranes: The porous primary cell wall layer within bordered pits that separates adjacent xylem vessels. The major role of pit membranes is to prevent embolism from spreading between xylem conduits.

Tissue pressure: Pressure exerted by living cells on surrounding tissues that physically constrain their expansion.

Xylem embolism: Gas-filled xylem conduits that block water transport through the xylem.

Corresponding author: Zwieniecki, M.A. (mzwienie@oeb.harvard.edu).

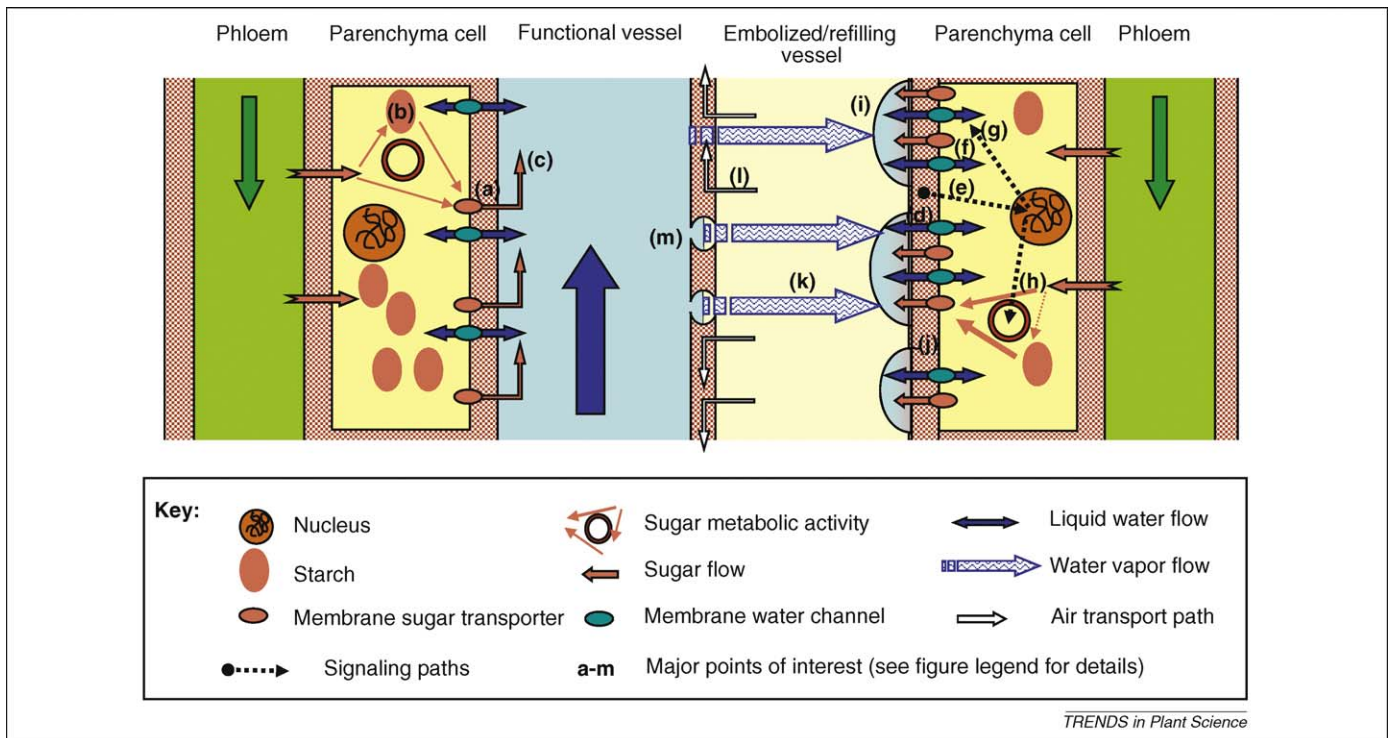


Figure 1. Embolism refilling scenario. (a) Living cells in contact with vessels release a small but steady amount of soluble carbohydrates into the xylem. (b) Starch stored in xylem parenchyma serves as a sugar capacitor. (c) These solutes are normally swept away by the transpiration stream, keeping concentrations at very low levels, (d) but accumulate in a vessel that has cavitated. (e) Sugar accumulation and the associated increase in apoplastic solute concentration triggers signaling pathways (f) for refilling that regulate sugar and (g) water membrane transport, as well as (h) sugar metabolic activity. (i) The accumulation of solutes results in water movement from xylem parenchyma cells by osmosis, forming droplets with high osmotic activity on internal vessel walls. (j) The partially non-wettable walls of xylem conduits prevent these droplets from being removed by suction from still-functional vessels. (k) Condensation of water vapor provides a second pathway by which water refills cavitated conduits, allowing adjacent conduits to provide water for refilling. (l) As the high osmotic droplets grow to fill the vessel, the embolus is removed both by forcing gas into solution and by pushing gas through small pores through the vessel walls to intercellular spaces. (m) The flared opening of the bordered pit chamber acts like a check valve until the lumen is filled, thus preventing contact with the highly wettable bordered pit membranes. Reconnection occurs once the pressure in the lumen exceeds that of the entry threshold into the bordered pit chambers; a hydrophobic layer within pit membranes might provide the needed simultaneity among multiple bordered pits.

or other non-osmotic mechanisms [25] as a driving force, a thermodynamically consistent proposal for how this could work has not been forthcoming. By contrast, osmotically driven refilling involves only components and physiological processes known to operate in ways capable of generating the necessary water inflow [19,30,31]. In 2003, Uve Hacke and John Sperry [27] proposed that refilling involves high-molecular weight solutes that act osmotically across pit membranes. This hypothesis is appealing insofar as it obviates the need for a mechanism to hydraulically isolate the refilling conduit. However, such large molecular compounds have not been observed in xylem sap. Furthermore, packing constraints limit the osmotic potential that could be generated using molecules that do not move freely across pit membranes. The maximum osmotic potential that can be generated using 10 nm diameter particles, where 10 nm represents the median pore size in pit membranes [32,33], is <5 kPa (calculated using the Van't Hoff relation: $\text{osmotic potential} = cRT$) [34].

As candidates for refilling, low-molecular weight osmotica require a mechanism to achieve hydraulic isolation between embolized and functional vessels. One hypothesis is that air trapped within bordered pits and maintained by the convex curvature of air-water interfaces in the pit channel isolates refilling conduits from adjacent sources of apoplastic water [5,35,36]. With such an isolating mechanism in place, osmotica used in refilling can be arbitrarily small. Because ionic osmotica (e.g. K^+) [8,37] have not been

observed using X-ray elemental analysis in concentrations sufficient to generate the needed osmotic pressures, our scenario focuses on low-molecular weight sugars as the principle osmoticum driving refilling [19]. Sugar concentrations in xylem sap have been little studied, but their involvement in refilling is consistent with observed dynamics of starch content in stems that undergo embolism-refilling cycles [17–20]. Furthermore, the amount of starch stored within stems corresponds well to what would be needed for refilling. For example, to create an osmotic concentration equivalent to -1.0 megapascal [MPa] throughout the xylem of *Populus trichocarpa*, a species that exhibits significant refilling under tension, requires ~ 1 mg of starch per gram of fresh stem weight, a value within the range observed in *P. trichocarpa* stems (unpublished data). This calculation assumes that the starch is fully converted into hexose sugars and that the Van't Hoff relationship holds.

One aspect of refilling under tension which is not in question is that it is an energy requiring process that requires the involvement of living cells. Consistent with this is the observation that treatments which alter the activity of plasma membrane proton pumps have marked effects on embolism repair [4,6,20]. Phloem girdling also leads to a marked decrease in refilling [16,17], an observation that has been interpreted in terms of the potential role of the phloem in supplying energy, osmotica and/or water needed for refilling [4–6]. More recent studies, in

which both circumferential (girdling) and vertical cuts block refilling, have been interpreted as supporting a role for tissue pressure [17,23,38]. An alternative perspective is that damage to the phloem might result in a systemic wound response that prevents the upregulation [17] of sugar secretion required for refilling rather than the severing of an essential supply line.

Mechanisms of embolism detection

Embolism formation is a discrete event, raising the question of how repair processes are activated. One possibility is that embolism repair is set in motion by physical attributes associated with cavitation [16,17]. Tests of the hypothesis that vessel wall vibrations trigger embolism repair show a significant effect on starch depolymerization in vascular-associated cells of water stressed stems in response to ultrasonic stimulation [18]. This hypothesis deserves further examination, including the refinement of experimental methods to better match both the duration and intensity of the mechanical perturbations resulting from cavitation. However, the fact that cavitation is a transient event suggests to us that other mechanisms should also be explored. In our opinion, a sensing mechanism that relies on a sustained signal that can be amplified following cavitation is likely to provide a more robust mechanism for launching the physiological responses of xylem parenchyma.

Chemical sensing could provide such a mechanism for detecting cavitation. We propose that a continual, albeit small, amount of sugar “leaks” from xylem parenchyma cells. In functional vessels, secreted sugars will be carried away with the transpirational stream maintaining a low concentration of sugar in parenchyma walls. Upon cavitation, apoplastic sugar concentrations will increase, rising quickly to levels much higher than would occur even if transpiration ceased but the vessel remained water-filled, providing a detectable signal strictly associated with embolism. Although extracellular membrane sugar sensors have not been reported in plants, they exist in other organisms [39]. A membrane-based sensing mechanism could provide the information necessary for a cell to alter its metabolic activity to produce the higher rates of sugar secretion needed to account for observed rates of refilling.

A model for gas removal

Whether naturally formed or experimentally induced, embolism can be removed within a relatively short time, sometimes less than an hour [20], although in some studies embolism repair requires up to 12 h [40]. Even when embolism is artificially induced using external pressurization to more than 2 MPa, this pressure can dissipate and give way to water in a very short time [4]. This suggests that much of this air is pushed out of the vessel in the gas phase, as numerical simulations of embolism repair involving gas being forced into solution and diffusing through wet walls cannot account for the observed rates of refilling [35,41,42]. Thus, we propose that vessel walls have inter-linked gas-filled channels that permit the movement of gases. These passages must be of sufficiently small diameter that they do not seed cavitation, yet sufficiently numerous to support the needed permeability. In addition,

they must be at least partially hydrophobic [43] if they are to remain gas-filled. We have previously shown that the interior xylem walls are partially wettable [43] (contact angle $\sim 50^\circ$), and scanning electron microscope images show that water can “bead” up on the interior vessel surfaces [44]. The surface properties of xylem secondary walls are hypothesized to maintain an air-gap within bordered pits, thus isolating hydraulically a refilling conduit from adjacent, functional ones [35]. They might also contribute to the ability of gases to be removed directly through conduit walls by preventing water from spreading over the interior surface of the vessel and thus prevent gas in the xylem lumen from exiting via gas-filled pores in the xylem walls.

Previous studies have described pressure thresholds for refilling based on the requirements for gas dissolution [40,42]. If dissolution is the primary pathway for gas removal, then the pressure thresholds predicted for both water vapor and air-filled conduits will certainly hold. However, the possibility that gases might exit through conduit walls might allow embolism repair to take place at lower pressures than predicted for the dissolution of air, depending on the permeability of conduit walls. At the upper end, the geometry and surface properties of the bordered pits is thought to place a limit on how much pressure could be sustained without prematurely making contact with adjacent conduits [5,43].

Water vapor transport: a new pathway providing inflow of water

The accumulation of high osmotic activity water within refilling vessels can only occur if water is not pulled away to adjacent conduits along the hydraulic pressure gradient. Although mechanisms to block water flow through the relatively large channels leading into bordered pits have been proposed [5,43], it is possible that small, water-filled pores exist within vessel walls. Estimates of wall hydraulic permeability from previously embolized vessels are reported to be $\sim 2.26 \times 10^{-7} \text{ m MPa}^{-1} \text{ s}^{-1}$ [41]. Thus, the refilling rate per 1 mm of vessel length and 50 μm in diameter with 1.0 MPa hydrostatic difference across the wall must exceed $4.75 \times 10^{-5} \text{ mm}^3 \text{ s}^{-1}$. It is widely assumed that the water required for refilling is provided by the phloem via parenchyma cells and that water channels and their activity play a key role [18,19,22]. However, whether xylem parenchyma are capable of supplying water for refilling at the required rate has not been addressed. Assuming cell membrane hydraulic conductivity between 2.6×10^{-8} and $5.1 \times 10^{-6} \text{ m MPa}^{-1} \text{ s}^{-1}$, as reported for maize parenchyma cells [45], and living cells in contact with vessels over $\sim 25\%$ of wall surface, the osmotic pressure gradient across the membrane would have to be between ~ 40 and ~ 0.2 MPa just to balance the estimated leak across the vessel perimeter. For the higher membrane permeability coefficients, these values appear feasible. However, the fact that water would have to flow through other living cells and potentially cross multiple membranes after being supplied by phloem makes it unlikely that a sufficiently large osmotic gradient can be generated for fast refilling to occur. This calls into question the assumption that water is supplied only via living cells.

We propose a new pathway by which water enters refilling vessels: vapor transport from adjacent vessels and fibers. Including vapor driven water transport in the refilling model does not eliminate a physiological role of xylem parenchyma cells but it reduces the burden placed on the radial transport of water from phloem to xylem and thus helps account for observed rates of refilling. Kinetic analyses of water condensation due to energy imbalance in soil crevices [46] show that rate of condensation with a constant pressure gradient around 1.0 MPa is in the range of $\sim 5 \times 10^{-4} \text{ mm}^3 \text{ s}^{-1}$. At this rate, the time required for refilling 1 mm in length of a 50- μm diameter vessel would be only ~ 4 s. Actual rates of water inflow due to vapor transport and condensation are likely to be somewhat lower as differences in chemical potential between water-filled conduits and the refilling conduits could be as much as an order of magnitude less (i.e. 0.1 MPa), and morphological and thermal consideration of embolized vessels might further reduce the condensation rate. Nevertheless, increasing the time estimates even twofold still suggests that the evaporation–condensation process cannot be omitted in the physical understanding of refilling. This pathway for water entry into refilling conduits would allow water for refilling to be drawn directly from functional vessels or nonfunctional reservoirs of water in wood fibers [47,48] bypassing any membrane limitations, as well as the need for yet further levels of biological control.

Synchronized hydraulic reconnection across multiple bordered pits

The valve-like activity of the bordered pit structure [5,39,41,43] is thought to be an important component of the refilling process, but the problem of simultaneous reconnection among hundreds of bordered pits remains a significant unknown. Bordered pit membranes are designed to facilitate water flow, thus potentially endangering the refilling process if contact between adjacent vessels is made prematurely. The solution might lie in the structure and chemical heterogeneity of bordered pit membranes themselves. Recent analysis of pit membrane structure demonstrates substantial variation between their properties, including thickness (10 s to 100 s of nm), density (fully opaque to thin sieve-like membranes) and the presence of different density layers [49]. These properties might impact hydraulic permeability, stretching [32], surface properties, and spatial and temporal aspects of membrane penetration by water with consequent effects on the required level of simultaneity to assure functional refilling under tension.

Future research directions

Significant progress has been made towards the goal of understanding of refilling embolized vessels in transpiring plants. This new knowledge allows us to formulate this comprehensive perspective that addresses all aspects of refilling, a process that sits squarely on the interface between physics and biology. However, the formulation of this scenario will represent a major step forward, only if it stimulates new research into the many unknowns identified here. In particular, we anticipate that progress would

be made from exploring physiological and physical elements of the refilling process, including:

- controls on sugar metabolism in stems of plants pre-, during and post-stress;
- signaling pathways associated with refilling;
- theoretical and experimental tests of micro-environmental properties in refilling vessels;
- physicochemical properties of bordered pit membranes.

The self-healing of xylem embolism represents a critical component of how plants respond to resource limitations. We believe that the future research in this area will illuminate the remaining areas of uncertainty and, by doing so, eliminate a role for a Maxwellian demon in the refilling of xylem embolism.

Acknowledgements

We thank Matthew Gilbert for helpful comments. This work was supported by grants from the National Science Foundation (IOS-0919729), the Air Force Office of Scientific Research (FA9550-09-1-0188) and the Andrew W. Mellon Foundation.

References

- 1 Slatyer, R.O. (ed.) (1967) *Plant-Water Relationships*, Academic Press
- 2 Salleo, S. and Lo Gullo, M. (1989) Xylem cavitation in nodes and internodes of *Vitis vinifera* L. plants subjected to water stress limits of restoration of water conduction in cavitated xylem conduits. In *Structural and Functional Responses to Environmental Stresses: Water Shortage* (Kreeb, K. et al., eds), pp. 33–42, SPB Academic Publishing
- 3 Salleo, S. and Lo Gullo, M. (1993) Drought resistance strategies and vulnerability to cavitation of some Mediterranean sclerophyllous trees. In *Water Transport in Plants under Climatic Stress* (Borghetti, M. et al., eds), pp. 99–113, Cambridge University Press
- 4 Salleo, S. et al. (1996) Xylem recovery from cavitation-induced embolism in young plants of *Laurus nobilis* – a possible mechanism. *New Phytol.* 132, 47–56
- 5 Holbrook, N.M. and Zwieniecki, M.A. (1999) Xylem refilling under tension. Do we need a miracle? *Plant Physiol.* 120, 7–10
- 6 Tyree, M.T. et al. (1999) Refilling of embolized vessels in young stems of Laurel. Do we need a new paradigm? *Plant Physiol.* 120, 11–21
- 7 Tyree, M.T. and Yang, S. (1992) Hydraulic conductivity recovery versus water pressure in xylem of *Acer saccharum*. *Plant Physiol.* 100, 669–676
- 8 Canny, M.J. (1997) Vessel contents during transpiration – embolism and refilling. *Am. J. Bot.* 84, 1223–1230
- 9 Edwards, W. et al. (1994) Reversing cavitation in tracheids of *Pinus sylvestris* L. under negative water potentials. *Plant Cell Environ.* 17, 389–397
- 10 Hacke, U.G. et al. (2001) Cavitation fatigue. Embolism and refilling cycles can weaken the cavitation resistance of xylem. *Plant Physiol.* 125, 779–786
- 11 McCully, M.E. et al. (1998) Daily embolism and refilling of xylem vessels in the roots of field-grown maize. *New Phytol.* 138, 327–342
- 12 Zwieniecki, M.A. and Holbrook, N.M. (1998) Diurnal variation in xylem hydraulic conductivity in white ash (*Fraxinus americana* L.), red maple (*Acer rubrum* L.) and red spruce (*Picea rubens* Sarg.). *Plant Cell Environ.* 21, 1173–1180
- 13 Zwieniecki, M.A. et al. (2000) Dynamic changes in petiole specific conductivity in red maple (*Acer rubrum* L.), tulip tree (*Liriodendron tulipifera* L.) and northern fox grape (*Vitis labrusca* L.). *Plant Cell Environ.* 23, 407–414
- 14 Kaldenhoff, R. et al. (2008) Aquaporins and plant water balance. *Plant Cell Environ.* 31, 658–666
- 15 Holbrook, N.M. et al. (2001) *In vivo* observation of cavitation and embolism repair using magnetic resonance imaging. *Plant Physiol.* 126, 27–31
- 16 Scheenen, T. et al. (2007) Intact plant magnetic resonance imaging to study dynamics in long-distance sap flow and flow-conducting surface area. *Plant Physiol.* 144, 1157–1165

- 17 Bucci, S.J. *et al.* (2003) Dynamic changes in hydraulic conductivity in petioles of two savanna tree species: factors and mechanisms contributing to the refilling of embolized vessels. *Plant Cell Environ.* 26, 1633–1645
- 18 Salleo, S. *et al.* (2008) Vessel wall vibrations: trigger for embolism repair? *Funct. Plant Biol.* 35, 289–297
- 19 Salleo, S. *et al.* (2006) Phloem as a possible major determinant of rapid cavitation reversal in stems of *Laurus nobilis* (laurel). *Funct. Plant Biol.* 33, 1063–1074
- 20 Salleo, S. *et al.* (2004) New evidence for a role of vessel-associated cells and phloem in the rapid xylem refilling of cavitating stems of *Laurus nobilis* L. *Plant Cell Environ.* 27, 1065–1076
- 21 Sakr, S. *et al.* (2003) Plasma membrane aquaporins are involved in winter embolism recovery in walnut tree. *Plant Physiol.* 133, 630–641
- 22 Nardini, A. *et al.* (2008) Vein recovery from embolism occurs under negative pressure in leaves of sunflower (*Helianthus annuus*). *Physiol. Plant.* 133, 755–764
- 23 Clearwater, M. and Goldstein, G. (2005) Embolism repair and long distance transport. In *Vascular Transport in Plants* (Holbrook, N.M. and Zwieniecki, M.A., eds), pp. 201–220, Elsevier
- 24 von Baeyer, H. (ed.) (1998) *Maxwell's Demon*, Random House
- 25 Sauter, J. *et al.* (1996) Interrelationships between ultrastructure, sugar levels, and frost hardiness of ray parenchyma cells during frost acclimation and deacclimation in poplar (*Populus×canadensis* Moench 'robusta') wood. *J. Plant Physiol.* 194, 451–461
- 26 Ameglio, T. *et al.* (2001) Winter stem xylem pressure in walnut trees: effects of carbohydrates, cooling and freezing. *Tree Physiol.* 21, 387–394
- 27 Hacke, U.G. and Sperry, J.S. (2003) Limits to xylem refilling under negative pressure in *Laurus nobilis* and *Acer negundo*. *Plant Cell Environ.* 26, 303–311
- 28 Canny, M. *et al.* (2001) The cohesion theory debate continues. *Trends Plant Sci.* 6, 454–455
- 29 Canny, M.J. (1998) Applications of the compensating pressure theory of water transport. *Am. J. Bot.* 85, 897–909
- 30 Westhoff, M. *et al.* (2008) The mechanisms of refilling of xylem conduits and bleeding of tall birch during spring. *Plant Biol.* 10, 604–623
- 31 Ameglio, T. *et al.* (2004) Temperature effects on xylem sap osmolarity in walnut trees: evidence for a vitalistic model of winter embolism repair. *Tree Physiol.* 24, 785–793
- 32 Choat, B. *et al.* (2004) Changes in pit membrane porosity due to deflection and stretching: the role of vested pits. *J. Exp. Bot.* 55, 1569–1575
- 33 Chatelet, D. *et al.* (2006) Xylem structure and connectivity in grapevine (*Vitis vinifera*) shoots provides a passive mechanism for the spread of bacteria in grape plants. *Ann. Bot.* 98, 483–494
- 34 Nobel, P.S. (ed.) (1991) *Physicochemical and Environmental Plant Physiology*, Academic Press
- 35 Vesala, T. *et al.* (2003) Refilling of a hydraulically isolated embolized xylem vessel: model calculations. *Ann. Bot.* 91, 419–428
- 36 Konrad, W. and Roth-Nebelsick, A. (2003) The dynamics of gas bubbles in conduits of vascular plants and implications for embolism repair. *J. Theor. Biol.* 224, 43–61
- 37 Canny, M. *et al.* (2001) Cryo-scanning electron microscopy observations of vessel content during transpiration in walnut petioles. Facts or artefacts? *Plant Physiol. Biochem.* 39, 555–563
- 38 Domec, J. *et al.* (2006) Diurnal and seasonal variation in root xylem embolism in neotropical savanna woody species: impact on stomatal control of plant water status. *Plant Cell Environ.* 29, 26–35
- 39 Rolland, F. *et al.* (2000) Glucose-induced cAMP signalling in yeast requires both a G-protein coupled receptor system for extracellular glucose detection and a separable hexose kinase-dependent sensing process. *Mol. Microbiol.* 38, 348–358
- 40 Holttta, T. *et al.* (2006) Refilling of embolised conduits as a consequence of 'Munch water' circulation. *Funct. Plant Biol.* 33, 949–959
- 41 Zwieniecki, M.A. *et al.* (2001) Hydraulic properties of individual xylem vessels of *Fraxinus americana*. *J. Exp. Bot.* 52, 1–8
- 42 Yang, S. and Tyree, M.T. (1992) A theoretical model of hydraulic conductivity recovery from embolism with comparison to experimental data on *Acer saccharum*. *Plant Cell Environ.* 15, 633–643
- 43 Zwieniecki, M.A. and Holbrook, N.M. (2000) Bordered pit structure and vessel wall surface properties – implications for embolism repair. *Plant Physiol.* 123, 1015–1020
- 44 Canny, M.J. and Huang, C.X. (1993) What is in the intercellular spaces of roots? Evidence from the cryo-analytical-scanning electron microscope. *Physiol. Plant.* 87, 561–568
- 45 Kim, Y. and Steudle, E. (2007) Light and turgor affect the water permeability (aquaporins) of parenchyma cells in the midrib of leaves of *Zea mays*. *J. Exp. Bot.* 58, 4119–4129
- 46 Philip, J. (1964) Kinetics of capillary condensation in wedge-shaped pores. *J. Chem. Phys.* 41, 911–916
- 47 Jacobsen, A. *et al.* (2005) Do xylem fibers affect vessel cavitation resistance? *Plant Physiol.* 139, 546–556
- 48 Melcher, P. *et al.* (2001) Water relations of coastal and estuarine *Rhizophora mangle*: xylem pressure potential and dynamics of embolism formation and repair. *Oecologia* 126, 182–192
- 49 Jansen, S. *et al.* (2009) Morphological variation of intervessel pit membranes and implications to xylem function in angiosperms. *Am. J. Bot.* 96, 409–419