Limitations in the Hydraulic Pathway: Effects of Xylem Embolisms on Sap Velocity and Flow

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Abstract
     Sap flow in plants takes place in the xylem, a hydraulic system that is usually under negative pressure and in which gas and liquid phases are separated by nanoporous, fibrous pit membranes. It has long been known that this system is at risk of drawing gas nanobubbles through these membranes into the xylem sap, a process referred to as "air seeding". These bubbles then can cavitate and create embolisms. Embolized vessels and tracheids block the hydraulic pathway, reduce hydraulic conductivity, and thereby potentially reduce sap flow. Under drought stress, the number of sap-filled conduits often steadily declines with increasingly negative xylem water potential. It was long thought that removal of embolisms would be physically impossible while the system is transporting water under negative pressure. However, recent research has provided abundant evidence for seasonal and/or diurnal formation and removal of xylem embolisms in many plant species. The number of functioning conduits as well as wood water and gas content can fluctuate over a growing season and often over the course of a day. We review evidence for such changes obtained through hydraulic measurements, cryo-scanning electron microscopy, and high-resolution computed tomography. Loss and gain of functional conduits over time effectively translates into changes in the xylem conducting area. In sap flow research, conducting area is almost universally assumed to be constant over time, but this assumption turns out to be invalid for plants that form and repair embolisms. Temporal changes in the water content of other wood cells also affect the thermal conductivity of wood and therefore sap flow measurements based on heat-fluxes. Measurements of active conducting area and wood water content concurrently with sap flow measurements may be needed for accurate determination of sap flux density and volumetric flow.

INTRODUCTION

Sap flow takes place in the xylem, the hydraulic system of vascular plants. Capillary tension in cell walls of leaves creates negative pressure in the xylem, which in turn drives the flow of sap from roots to leaves. Dixon and Joly (1895) were the first to note that the xylem was well protected from intrusion of air bubbles into the sap, and they suggested that water-soaked cell walls are essentially air-tight. In fact, any air that gets into the xylem has to overcome the high surface tension of air-water interfaces in the nanopores of cell walls and fibrous pit membranes that connect xylem conduits, including vessels and tracheids (Tyree and Zimmermann, 2002). It typically takes very large pressure differentials to force air into xylem, a process known as air-seeding. Although a few plants appear to have evolved xylem pit membranes that are essentially air-tight under natural conditions and can withstand pressure differentials of 8 MPa or more (Maherali et al., 2004), most plants experience at least some entry of nanometer-sized air bubbles (nanobubbles) into the xylem when xylem pressure falls below a critical value, known as the "air seeding" pressure (Tyree and Zimmermann, 2002). Under declining pressure, these nanobubbles can cavitate and quickly expand to fill the whole conduit and form an embolism, thereby obstructing sap flow.

Embolism formation was long thought to be a catastrophic event in xylem, because refilling of conduits while the xylem was transporting sap under negative

pressure was assumed to be physically impossible (Tyree and Sperry, 1988). Positive root pressure appeared to be the only mechanism through which embolized conduits could be refilled. In its absence, embolisms were thought to lead to permanent dysfunction of conduits and tylosis formation. The realization that the hydraulic system of plants could undergo diurnal fluctuations in water content dates to Salleo et al. (1996), who were the first to conclusively show that embolized conduits in bay laurel trees could be refilled even while the xylem was under negative pressure. Since then, diurnal cycles of embolism formation and repair under tension have been found to occur in many temperate and tropical tree species, as well as in grapevine. The existence of embolism repair under tension is now firmly established (Tyree et al., 1999; Hacke and Sperry, 2003; Clearwater and Goldstein, 2005; Brodersen et al., 2010; Johnson et al., 2012), but the underlying mechanisms remain largely unknown (Holbrook and Zwieniecki, 1999; Zwieniecki and Holbrook, 2009; Nardini et al., 2011).

Diurnal and seasonal patterns of xylem embolism formation and repair could potentially have fairly large effects on sap flow measurements. All current sap flow methods are based on the assumption that the conducting area in the wood is essentially constant over the period during which measurements are made. This assumption is used when calculating sap flow from measurements of sap flux density by multiplying the latter by the conducting area (Marshall, 1958). However, embolism formation and repair can change the conducting area substantially over the course of a day or a season; hence estimation of sap flow from sap flux density may be problematic in plants that undergo embolism formation and repair. In angiosperms, sap flow methods that are based on heat fluxes may not only be affected by variation in the water contents of vessel lumens, but also by variation in the water content of fiber lumens. Water content in fibers can vary widely over the course of a year (Utsumi et al., 1998). While fibers are not conducting water, they play an important role in heat-based sap flow measurements, because heat travels through these cells, and their thermal conductivity and specific heat capacity strongly differs between being filled with gas or water (Glass and Zelinka, 2010).

In this paper we review the evidence for temporal variations in the water and gas content of xylem conduits and fibers, discuss potential consequences for the theory and practice of sap flow measurements, and review approaches to measure conduit and wood water contents in situ.

EVIDENCE FOR TEMPORAL VARIATION IN THE WATER AND GAS CONTENT OF XYLEM CONDUITS

Hydraulic Measurements

Most evidence for temporal variation in the water and gas content of xylem conduits comes from repeated destructive hydraulic measurements. Hydraulic conductivity of xylem declines with decreasing xylem water potential, as more and more conduits fill with gas and nanobubbles are drawn under increasing pressure differentials from gas-filled conduits through pit membranes into adjacent sap-filled conduits (Sperry et al., 1988a; Tyree and Zimmermann, 2002). Each conduit that is emptied of water in turn becomes a potential source of gas bubbles for adjacent water-filled conduits. The relationship between percent loss of conductance and xylem water potential is known as a xylem vulnerability curve (Sperry et al., 1988b). Such curves have been determined for several hundred plant species (Maherali et al., 2004; Choat et al., 2012), including a number of horticultural species (Schenk, 2013). Based on these curves we know that water contents in xylem conduits normally decline and gas contents increase over time when xylem water potentials decline during a dry period.

There is now abundant evidence from hydraulic measurements that xylem embolisms can be repaired even when the xylem as a whole is under negative pressure, a process that is usually referred to as embolism repair under tension (Clearwater and Goldstein, 2005). We have found hydraulic evidence for diurnal embolism repair under tension in about half of twenty woody species tested from a variety of different

environments and plant families (Fig. 1; Schenk et al., unpublished data; Hessom et al., in review). Diurnal cycles of embolism formation and repair under tension have also been found to occur in many temperate and tropical tree species (Zwieniecki and Holbrook, 1998; Melcher et al., 2001; Bucci et al., 2003; Brodribb and Holbrook, 2004; Domec et al., 2006; Taneda and Sperry, 2008; Johnson et al., 2009), as well as in grapevine (Zufferey et al., 2011). Interestingly, there is no evidence for diurnal embolism repair in conifer wood, although it does occur in conifer needles (Johnson et al., 2012).

Cryo-SEM Studies

The earliest evidence for diurnal emptying and refilling of xylem conduits was based on the cryo-SEM method, for which plant tissue is flash-frozen in liquid nitrogen and imaged, while frozen, under scanning electron microscopy (SEM). Several cryo-SEM studies appeared to show diurnal formation and repair of xylem embolisms (Canny, 1997; McCully et al., 1998; Buchard et al., 1999). These findings initially were criticized as experimental artefacts, as it was shown that flash-freezing of xylem under tension can induce artificial embolisms (Cochard et al., 2000; Tyree and Cochard, 2003). However, in light of more recent independent confirmation of embolism repair under tension using hydraulic and imaging techniques it appears likely that conclusions from these earlier cryo-SEM studies were essentially correct, even if some of the findings may have been affected by the flash-freezing treatment.

Magnetic Resonance Imaging and High-Resolution Computed Tomography

Live imaging techniques have conclusively shown that vessels can be emptied and refilled over periods of only a few hours. Earlier magnetic resonance imaging (MRI) studies of grapevine (Holbrook et al., 2001) and cucumber (Scheenen et al., 2007) provided evidence of vessel refilling during dark hours, but these studies suffered from the relatively low resolution of the MRI technique. More recently, Brodersen at al. (2010) used High Resolution Computed Tomography (HRCT) to document refilling of grapevine vessels at very high resolution. This technique is now being used to document the patterns of embolism spread within grapevine stems during dry-down experiments (Fig. 2). This dramatic change in active conducting area induced by embolisms can be problematic for accurately estimating sap flow. Point measurements taken with sap flow sensors at one or a few locations may also not accurately reflect the active area surrounding a sensor due to the non-random distribution of embolized vessels (Fig. 2).

Taken together, the accumulated evidence for diurnal emptying and refilling of xylem vessels is solid. Extrapolating from the limited number of species studied so far, it appears that a large number of angiosperms are able to both diurnally and seasonally form and repair embolisms (Johnson et al., 2012). There also is evidence for seasonal, but not diurnal, refilling of xylem in conifers (McCulloh et al., 2011). Based on these findings we conclude that the active sap conducting area of wood can vary widely over the day and over the season. This in turn will affect both sap velocity and flow.

EVIDENCE FOR TEMPORAL VARIATION IN THE WATER AND GAS CONTENT OF FIBRES

Gravimetric Measurements of Water and Gas Content of Wood

Seasonal variation in wood water content has been documented gravimetrically for many plant species, beginning with the work of Gibbs in the 1930s (Gibbs, 1958). Such measurements are now routinely done in fire-prone shrublands and forest to determine fire fuel hazards (Hardy and Hardy, 2007). There is surprisingly little information on the location of water and gas in the wood (Gartner et al., 2004). Depending on wood density, between 15 and 25% of water is bound in cell walls (Berry and Roderick, 2005). In angiosperms, the remaining water is located in parenchyma cells, as well as in vessel and fiber lumens (Fig. 3). Fiber lumen was found to take up between 1 and 57% (mean 14%) of wood volume in a recent study of 61 shrub species, while parenchyma cells made up

between 6 and 62% (mean 30%) of wood volume (Martínez-Cabrera et al., 2009). It is unknown how far the water in living parenchyma cells is depleted daily or seasonally, but connections via the rays to water stored in the bark probably buffers water content fluctuations in living wood cells to some degree (Sevanto et al., 2011; Steppe et al., 2012). Seasonal variation in wood water content in relation to wood anatomy was studied using four shrub species from southern California sage scrub vegetation (Fig. 3). Air content in the wood of these four species varied between 10 and 60% (mean 40%) of wood volume. In that study, *Malosma laurina*, the species with the highest proportion of parenchyma, was the one with the lowest seasonal variation in wood water content. In the other three species, wood water content varied seasonally by as much as 19 to 26% of wood volume.

Cryo-SEM Studies

Research on seasonal variation of wood water content using cryo-SEM revealed strong temporal fluctuations in the water contents of vessels and fibers in angiosperm trees (Utsumi et al., 1998), with some evidence for seasonal refilling of fibers and vessels. Tracheids of conifers showed a gradual decline in water contents over the course of a year, with no evidence for refilling (Utsumi et al., 2003).

EFFECTS OF TEMPORAL VARIATION IN WOOD WATER AND GAS CONTENTS ON SAP FLOW MEASUREMENTS

Embolism formation in vessels decreases the hydraulic conductivity of xylem and therefore reduces total sap flow unless the loss of conductivity is compensated for by an increase in the water potential differential that drives the flow. Sap velocity can theoretically increase in response to embolism formation as fewer vessels are available for sap flow. A positive correlation between embolism formation and sap velocity has indeed been observed in broad-leaved trees, but this may have been due to decreasing xylem water potentials driving both of these phenomena (Borghetti et al., 1993; Raschi et al., 1995). If large-diameter vessels embolize first, as is often the case (Tyree and Zimmermann, 2002), then the mean sap velocity should decline, because sap velocity for a given pressure differential increases in proportion to the square of the conduit diameter. Because conifer tracheids tend to be fairly uniform in diameter, one would expect that embolism formation in conifers could increase sap velocity, and the same could be true for angiosperms with uniformly-sized vessels.

Temporal variation in wood water and gas contents, especially in vessels, tracheids, and fibers, could have a fairly large impact on all sap flow measurements that use heat flux techniques. Heat moves through xylem by conduction and convection, where conduction mainly occurs through cell walls, stagnant sap, and cytoplasm of living cells, while convection moves heat with the flow of sap (Marshall, 1958; Vandegehuchte and Steppe, 2012b). Conifer wood consists mainly of sap-conducting tracheids, typically with relatively small amounts of parenchyma, which means that only a small fraction of heat typically moves by thermal conduction. In vessel-bearing angiosperms, convection is mainly limited to sap flow in functional vessels (1 to 40% of wood volume (Gartner et al., 2004; Martínez-Cabrera et al., 2009)). A daily loss or gain of 30% of hydraulic conductivity, as shown in Figure 1, would mean a fluctuation of active water-conducting area of approximately the same magnitude. Because heat pulse methods produce estimates of sap flux density, i.e., the average volumetric sap flow per sapwood conducting area rather than actual sap velocity in vessels and because vessels normally make up only a small proportion of wood, the loss of active vessel conducting area by itself may have a relatively small impact on sap flux density measurements. However, changes in the amount of heat moved via convection would alter the proportion of heat moving via conduction, and together these changes could have a significant impact on estimated sap flux density. Moreover, differences in water contents in wood cells other than vessels would have a major effect on thermal conductivity. Water-filled wood cells have 3-17 times higher thermal conductivity than gas-filled ones (Glass and Zelinka,

2010). At high sap flow rates, effects of altered thermal conduction would be minimal, but at low flow, a large proportion of heat moves via conduction (Köstner et al., 1998; Sevanto et al., 2008), which means that temporal variation in wood water contents will have major effect on measurements of low flow rates. For these reasons, angiosperm species that show large diurnal or seasonal fluctuations in water contents of vessels and or fibers require concurrent measurements of wood water content and/or active conducting area together with heat flux measurements for accurate determination of sap flow.

IN SITU MEASUREMENTS OF WOOD WATER CONTENTS AND ACTIVE CONDUCTING AREA

A variety of methods have been used to measure wood water contents in situ, including computed tomography (Raschi et al., 1995), gamma-ray attenuation, nuclear magnetic resonance, electrical conductivity, and time-domain reflectometry (López-Bernal et al., 2012). However, most sap flow researchers will not have access to these technologies. Recently, two research teams separately developed new techniques to use sap flow sensors for measurements of wood water contents concurrently with sap flux density: López-Bernal et al. (2012) developed a method to use compensated heat pulse (CHP) sensors for both of these measurements, while Vandegehuchte and Steppe (2012a) developed a new four-needle sensor termed Sapflow+ for the same task. Both approaches will still have to be tested on species that show large temporal fluctuations in wood water contents, but these are important technical advances in sap flow technology that show a lot of promise, especially for low flow conditions.

There is no published study of in situ measurements of embolism formation and repair or the resulting temporal changes in hydraulic conductivity or active sap flow conducting area. In principle, hydraulic conductivity could be measured in situ by concurrent measurements of the xylem water potential gradient driving the flow, using stem psychrometers (Dixon and Tyree, 1984), and volumetric sap flow, using the heat balance method (Sakuratani, 1981; Baker and Van Bavel, 1987). However, stem psychrometers are highly temperature-sensitive, and it remains to be seen if they can be successfully deployed in a large variety of natural environments.

In the field, temporal variation in active conducting area could be measured by concurrent measurements of sap velocity and volumetric flow (conducting area = volumetric flow/velocity). However, sap velocity and flow measurements based on heat flux methods are affected not only by the water contents of conduits, but also by the water content of other cell types. Moreover, there is no in situ method for directly measuring sap velocity, as heat-pulse methods result in measurements of sap flux density (volume per area per time) rather than sap velocity (distance per time). A solution could be to calculate mean sap velocity by multiplying sap flux density by the conduit fraction of the sapwood (cross-sectional conduit lumen area/sapwood area). In theory, this approach could be used to calculate the active water-conducting area and its changes over time.

CONCLUSIONS

Until now, there has been little consideration of xylem embolisms as a major limitation to sap flow in the hydraulic pathway, in part due to the assumption that embolisms are catastrophic occurrences that most plants prevent at all costs. With the realization that xylem embolisms form readily and often daily in many plant species and are also readily repaired, we now know that the active sap-conducting area in the xylem can vary widely over the course of a day or a season. Moreover, water and gas content of wood can also vary temporally, affecting the thermal conductivity of wood. Future research on sap flow will have to take temporal variability in conducting area and wood water contents into account for more accurate measurements of sap flux density, sap velocity, and volumetric flow. Two essential developments towards this goal are the use of sap flow sensors to measure wood water contents concurrently with sap flow and the development of in situ methods to measure the active conducting area in the sapwood in real time. The latter approach would also allow non-destructive screening of plant species

for embolism formation and embolism repair, which would be a huge advance for research on plant drought tolerance in wild and cultivated species.

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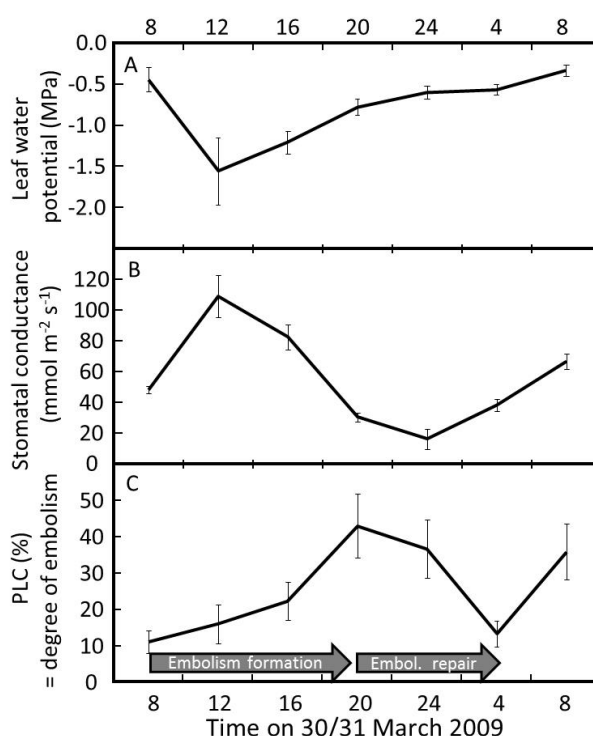
Fig. 1. Water relations and diurnal embolism formation and repair in *Encelia farinosa* (*Asteraceae*) shrubs, grown outdoors in 1.2 m tall pots on the California State University Fullerton campus. Shown are (A) leaf water potentials, (B) stomatal conductance, and (C) percent loss of hydraulic conductance (PLC) in stems (=% embolism). Methods are described in Hessom et al. (in review). The periods of embolism formation and repair under tension are indicated by arrows. All error bars are standard errors.
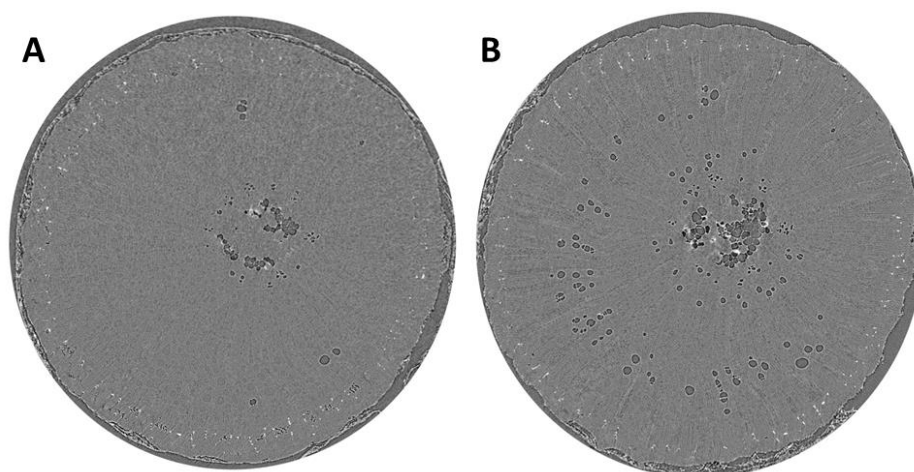

Fig. 2. Transverse images of a grapevine stem captured with high resolution computed tomography (HRCT; see details of the method in Brodersen et al. (2010, 2011)) during a dry-down experiment. A: Image on the left represents the sample under well watered conditions (Ψ_{stem} -0.6 MPa) and revealed little to no cavitation, B: while the image of the right represents that same stem after significant water stress (Ψ_{stem}=-2.2 MPa).
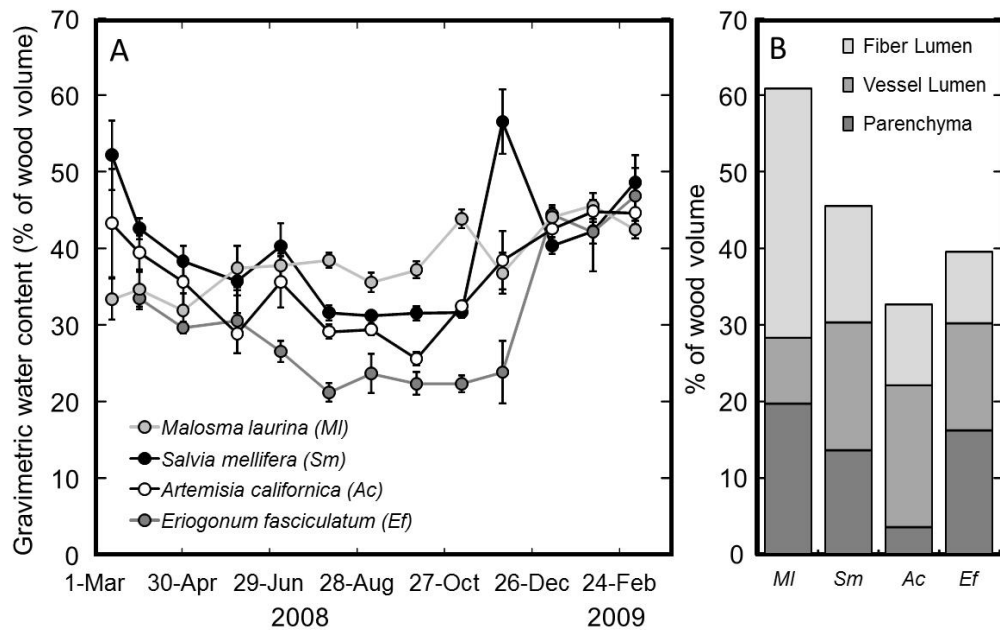
Fig. 3. (A) Gravimetric water contents in the wood of four shrubs species in southern California coastal scrub vegetation measured from March 2008 through February 2010 in Black Star Canyon, Orange County, California, USA. (B) Proportions of fiber lumen, vessels lumens, and living parenchyma in anatomical transverse sections of stem wood for the four species. All proportions are expressed as percent of the transverse sapwood area examined. The remaining percentages (to 100%) are cell walls. Data from Martínez-Cabrera et al. (2009).