Plant, Cell & Environment

Plant, Cell and Environment (2010) **33**, 1502–1512 doi: 10.1111/j.1365-3040.2010.02160.x

Measurement of vulnerability to water stress-induced cavitation in grapevine: a comparison of four techniques applied to a long-vesseled species

BRENDAN CHOAT[1,*] WILLIAM M. DRAYTON[2,*] CRAIG BRODERSEN[2], MARK A. MATTHEWS[2], KEN A. SHACKEL[2], HIROSHI WADA[2] & ANDREW J. MCELRONE[3]

[1]*Plant Science Division, Research School of Biology, The Australian National University, Canberra, ACT 0200, Australia and* [2]*University of California, Davis, One Shields Avenue and* [3]*USDA-ARS, Crops Pathology and Genetics Research Unit, Davis, CA 95616, USA*

ABSTRACT

Among woody plants, grapevines are often described as highly vulnerable to water-stress induced cavitation with emboli forming at slight tensions. However, we found native embolism never exceeded 30% despite low xylem water potentials (Ψ_x) for stems of field grown vines. The discrepancy between native embolism measurements and those of previous reports led us to assess vulnerability curve generation using four separate methods and alterations (i.e. segment length and with/without flushing to remove embolism prior to measurement) of each. Centrifuge, dehydration and air-injection methods, which rely on measurement of percentage loss of hydraulic conductivity (PLC) in detached stems, were compared against non-invasive monitoring of xylem cavitation with nuclear magnetic resonance (NMR) imaging. Short segment air-injection and flushed centrifuge stems reached >90 PLC at Ψ_x of-0.5 and –1.5 MPa, respectively, whereas dehydration and long-segment air-injection measurements indicated no significant embolism at $\Psi_x > -2.0$ MPa. Observations from NMR agreed with the dehydration and long segment air-injection methods, showing the majority of vessels were still water-filled at $\Psi_x > -1.5$ MPa. Our findings show *V. vinifera* stems are far less vulnerable to water stress-induced cavitation than previously reported, and dehydration and long segment air-injection techniques are more appropriate for long-vesseled species and organs.

Key-words: *Vitis*; air injection; centrifuge; dehydration; grapevine; nuclear magnetic resonance imaging; vulnerability curves.

INTRODUCTION

Water stress-induced cavitation is seen as one of the principal limitations to plant survival in water-limited environments (McDowell *et al*. 2008; Kursar *et al*. 2009). Xylem cavitation and resultant embolism lead to decreased hydraulic conductivity of the affected organ. This reduced hydraulic capacity influences leaf water status, placing limitations on leaf gas exchange and ultimately the growth and survival of the plant (Rood *et al*. 2000; Davis *et al*. 2002; Brodribb & Cochard 2009). Previous work indicates that the ability of plants to resist drought-induced xylem cavitation is linked to the habitats in which they grow and the level of water-stress experienced (Pockman & Sperry 2000; Maherali *et al*. 2004; Lopez *et al*. 2005; Willson & Jackson 2006; Choat, Sack & Holbrook 2007; Pratt *et al*. 2007). Those adapted to drier environments or that experience more water stress tend to be less vulnerable to cavitation and therefore maintain the ability to transport water to their extremities at more negative water potentials.

Several techniques have been devised to quantify cavitation occurrence and resistance in woody plants. Acoustic transducers and amplifiers have been used for several decades to detect acoustic or ultrasonic emissions associated with cavitation (reviewed in Tyree & Sperry 1989; Johnson *et al*. 2009). Sperry & Tyree (1988) introduced another method to assess vulnerability to cavitation as a percentage loss of hydraulic conductivity (PLC) during dehydration of the plant tissue. From this, a 'vulnerability curve' (VC) can be generated which describes the relationship between PLC and xylem water potential. Because this technique is time consuming and requires multiple stems for the curve, two related techniques have come into frequent use. The first involves the injection of air into stems at progressively higher pressures with the assumption that positive air pressure will simulate negative pressure in the xylem fluid (i.e. the pressure difference across intervessel pit membranes is equal but opposite in sign) (Cochard, Cruiziat & Tyree 1992; Salleo *et al*. 1992; Sperry & Saliendra 1994). The air injection method has been shown to correlate well with the dehydration technique in several species (Cochard *et al*. 1992, 2005; Sperry & Saliendra 1994; Pockman, Sperry & Oleary 1995; Alder *et al*. 1997; Li *et al*. 2008). The second alternative for the generation of VCs uses a centrifuge to create tension in excised sections of xylem (Holbrook, Burns & Field 1995; Pockman *et al*. 1995; Alder *et al*. 1997). When a segment of

Correspondence: A. J. McElrone. Fax: 530 752 0382; e-mail: ajmcelrone@ucdavis.edu

*Authors contributed equally to this manuscript.

xylem tissue is spun in a centrifuge, the water in the vessels experiences a centrifugal force, which is countered by its tensile strength. Both the centrifuge and air injection technique have the advantage of allowing rapid assessment of vulnerability to cavitation via repeated measurements on the same stem. The centrifuge technique has also been modified to allow for measurement while the stem segment is spinning (Cavitron), further increasing the efficiency of measurement (Cochard *et al*. 2005). This technique was further modified by Li *et al*. (2008), which uses the same principal but differs somewhat in design.

In the majority of cases, there is good agreement amongst these techniques (Cochard *et al*. 1992; Pockman *et al*. 1995; Alder *et al*. 1997). There is also a strong correlation between PLC from dehydration VCs and the proportion of xylem vessel stained by dye infiltration of progressively dehydrated stems (Lo Gullo & Salleo 1991; Hietz *et al*. 2008). However, there have also been reports of discrepancies or unrealistically high vulnerability to cavitation when measuring samples from plants with long vessels such as ring porous trees and lianas (Martínez-Vilalta *et al*. 2002; McElrone *et al*. 2004; Cochard *et al*. 2005). Conversely, others have defended the centrifuge technique, arguing that VCs generated with this technique accurately represent the high vulnerability of large vesseled plants and that these plants are able to withstand losing the majority of their conductivity at relatively high water potentials because of high initial conductivities (Hacke *et al*. 2006; Li *et al*. 2008; Taneda & Sperry 2008). Hacke *et al*. (2006) suggest that because ring porous species have very high initial stem hydraulic conductivities, even with 90% loss of conductivity these species retain enough hydraulic capacity to fulfill the requirements of transpiration.

However, the findings of Cochard *et al*. (2005) suggest that the centrifuge technique is inappropriate for measurement of vulnerability to cavitation in the ring porous species, as the 'spin while measuring' (Cavitron) technique produced inconsistent and non-repeatable results for these species. In a more recent study, Cochard *et al*. (in press) showed that VCs generated with the centrifuge matched closely to VCs generated by dehydration for a short vesseled species (*Betula pendula*), but the centrifuge VCs significantly overestimated vulnerability to cavitation in the ring porous species *Quercus robur*, compared with dehydration VCs. There have also been some doubts raised as to the validity of the air injection technique when it is applied to short segments of species and organs with long vessels (Martínez-Vilalta *et al*. 2002; McElrone *et al*. 2004). Because vulnerability to cavitation is an important and widely studied physiological trait, it is essential that any discrepancies between measurement techniques are well understood.

Cavitation has been assessed in both wild and cultivated grapevines in numerous studies for over 50 years. Grapevines are particularly interesting for these studies because they have both long and wide vessels measuring in excess of 1 m and 300 μm, respectively (Zimmermann & Jeje 1981; Chatelet, Matthews & Rost 2006), and are often reported among the most vulnerable woody plant species to water stress-induced cavitation. Scholander *et al*. (1955) were the first to suggest vessels in grapevine stems cavitate at very low tensions. Additional studies report similar cases of high cavitation vulnerability in grapevines (Schultz & Matthews 1988; Wheeler *et al*. 2005; Lovisolo & Schubert 2006), with some studies finding 70% loss of conductivity at tensions less than 0.75 MPa (Tibbetts & Ewers 2000). This is surprising given that cultivated grapevines are commonly grown in dry regions worldwide and are often concurrently subjected to deficit irrigation practices to control vigor and enhance fruit quality (e.g. Chapman *et al*. 2005).

In this study, we initially evaluated seasonal native embolism in current year shoots from field grown vines, and found that embolism was much lower (e.g. never exceeded 30% despite Ψ_x reaching –1.33 MPa) than expected based on previous studies. The discrepancy between our field data and previous literature led us to compare VCs generated with the centrifuge, dehydration and air injection techniques to test whether high vulnerability is an artifact of using short segments in some techniques. We also tested whether high pressure flushing, commonly used to remove embolism prior to centrifuge curve generation, alters the vulnerability of stem segments. Because the dehydration, centrifuge and air-injection techniques all require destructive sampling, we also evaluated the amount of embolism with progressive dehydration using nuclear magnetic resonance (NMR) imaging to avoid possible artifacts associated with cutting stems while the xylem fluid is under tension (Holbrook *et al*. 2001).

MATERIALS AND METHODS

Plant material and collection

V. vinifera L. cv. Chardonnay plants were sampled from two locations in northern California; Winters (field-grown) and Davis (both field-grown and greenhouse-grown). Plants from Winters were approximately 10 years old when sampled in July 2007. All samples were collected prior to dawn. Current year shoots 20 nodes or greater were randomly collected at pre-dawn to minimize air entry into the xylem to be measured. Shoots were selected from two adjacent vine rows, avoiding the end five vines. The cut end of the shoot was covered in Parafilm® to reduce water loss and they were transported to the laboratory in white plastic bags that had been previously misted inside to ensure high humidity and to prevent further cavitation. Plants from Davis were collected from the UC Davis vineyards, in May through October 2008 with the sampling protocol the same as that used at the Winters site.

Greenhouse plants were grown in 7.6 L pots filled with one-third peat, one-third sand, one-third redwood compost, with 2.4 kg m^{-2} dolomite lime in a greenhouse [30/20 ± 3 °C; 40/70 ± 10% RH; and natural light with a daily maximum of 1200 μmol photons m^{-2} s^{-1} photosynthetic photon flux density (PPFD)]. All lateral shoots were pinched off as they appeared. Pots were drip irrigated four times a day (at 0600, 0900, 1400, and 1800 h) for 4 min at

7.57 L h^{-1} (2 L d^{-1}) with dilute nutrient solution (90 ppm calcium, 24 ppm magnesium, 124 ppm potassium, 6 ppm nitrogen as NH4, 96 ppm nitrogen as NO3, 26 ppm phosphate, 16 ppm sulphate, 1.6 ppm iron, 0.27 ppm manganese, 0.16 ppm copper, 0.12 ppm zinc, 0.26 ppm boron and 0.016 ppm molybdenum) at pH 5.5–6.0. Each plant was trained to two shoots of approximately 2.2 m length that were the same age and size (plants were excluded where budbreak did not occur at the same time or shoots grew to different sizes). Shoots were harvested by cutting two nodes up from their base before transportation to an adjacent lab (with the exception of the centrifuge study, where they were transported to The University of California, Berkeley) in the same manner as used at the Winters site. Greenhouse grown plants were used for dehydration, air injection, centrifuge, and NMR observation. Field grown plants from the Winters site were also used in dehydration experiments, and measurements of native embolism were made on plants in the UC Davis and Winters vineyards.

Shoot dimensions

Samples used for dehydration and air-injection experiments were cut to a length longer than the maximum vessel length. Maximum vessel lengths were determined for each group of samples. One end of a shoot was attached to a compressed air source at approximately 100 kPa whereas the other end was submerged in a water-filled beaker. The shoot was cut back progressively until air bubbles could be seen exiting the submerged end, indicating at least one xylem vessel was open the entire length of the sample (approximately eight nodes). Therefore, all other samples were kept at eight nodes in length (0.5–0.9 m) except where stated otherwise. Because of the limitations imposed by the centrifuge rotor diameter, all samples measured by centrifuge were approximately 0.145 m in length. Stem segments were taken from the mid-point of the longer segments and contained one node. The number of vessels open at both ends of a 0.145 m segment was measured by injection of silicone elastomer (Rhodorsil RTV 141, Rhodia, Cranbury, NJ, USA) colored red with a pigment (LSDR-11, Dow Corning, Kendallville, IN, USA) (see Sperry, Hacke & Wheeler 2005). Measurements were made on six stems, two samples from each of three current year shoots. All 0.145 m stem segments contained at least one node as internodes this length were rare. Stems were sealed in a pressure chamber with one cut end submerged in elastomer which was infiltrated at 0.15 MPa for 30 min. Samples were then oven dried at 70 °C overnight. Groups of vessels between rays were counted at both ends of the stem with the difference between the proximal and distal end representing the proportion of open vessels.

Cross-sectional area of each end of the sample was measured by micrometer, subtracting the area calculated from the inner diameter (that of the pith) from the area calculated from the outer diameter (excluding the bark). The end with smallest area was used in calculating specific conductivity (K_s).

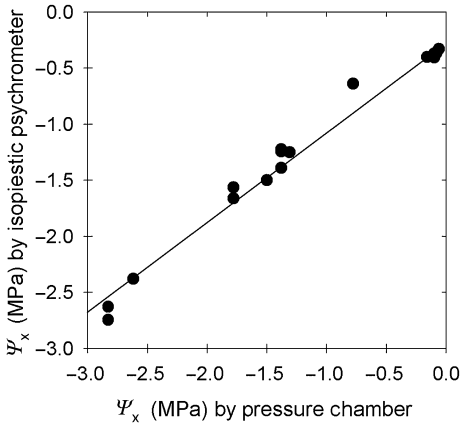

Figure 1. Comparison of isopiestic psychrometer and pressure chamber measurements of xylem water potential (Ψ_x) in Chardonnay grapevines by principle axis analysis (PAA). PAA minimizes the perpendicular distance of the data from the fit line unlike linear regression which minimizes the distance in the *y*-axis direction and assumes that the *x*-axis is correct. The relationship is therefore not influenced by the choice of axes. Best fit line $x = 0.82y - 0.23$.

Water potential measurements

Stem xylem water potential (Ψ_x) was estimated with a pressure chamber (Soil Moisture Equipment Corp., Santa Barbra, CA, USA), by sealing a leaf in a plastic bag covered in aluminum foil for a minimum of 30 min before excision and measurement. To confirm that Ψ_x was accurately represented by the pressure chamber measurement, a preliminary experiment was undertaken comparing the results with those from isopiestic thermocouple psychrometry (Boyer 1995). Stem internode sections from both basal and apical portions of the shoot (Davis, greenhouse-grown Chardonnay) were excised, immediately placed into plastic bags to prevent water loss, stored in a Styrofoam box at ambient temperature, and transported to the laboratory. All further sample preparation and dissection was conducted in a humidity box. Immediately after excision or isolation the shoot segment was carefully placed at the bottom of a psychrometer cup that was coated with melted and resolidified petrolatum (Boyer 1995). Additional petrolatum was used to minimize the exposed cut surface area. A data logger system (CR7, Campbell Scientific, Inc., Logan, UT, USA) was used to detect each voltage change, which allowed us to use the software (PC208, Campbell Scientific, Inc.) to simultaneously monitor the electromotive forces generated by each sample, with a less than ±50 nV noise level, and similar to the existing system of Boyer. The Ψ_x of leaves flanking the node was also measured and it was determined that pressure chamber accurately represents Ψ_x as determined by isopiestic psychrometry (Fig. 1). There was no effect of apical/basal position on Ψ_x by either technique.

Hydraulic measurements

Hydraulic conductivity was measured as described in Sperry *et al.* (1988). If present, any remaining leaves and

lateral shoots were removed and the cut surfaces were sealed using superglue and accelerant (Loctite® 409, Henkel North America, Rocky Hill, CT, USA). The shoot was cut underwater, removing the eight-node buffer zone from the basal end along with any artificially introduced embolism that may have been present from the cut made in the field. The cut end was then shaved using a fresh razor blade and flexible tubing was attached while preventing air from contacting the cut surface. The process was repeated with the shoot's apical end, leaving a sample approximately eight nodes (0.5–0.9 m) in length and minimizing the chance of open vessels exceeding the sample length unless otherwise stated. The flexible tubing on the basal end of the sample was then attached to an elevated Mariotte bottle (McCarthy 1934) (which maintains a constant upstream pressure), and the apical tubing was attached to a balance (Sartorious AG, Goettingen, Germany). The Mariotte bottle, balance, and tubing were filled with ultra-pure, degassed 10 mM KCl solution and care was taken not to expose the cut ends to air. A pressure head of 7.0 kPa was used for measurements on long segments with no open vessels. On short segments, a pressure head of 1.6 kPa was used to avoid displacing bubbles from the large open vessels present in these segments.

Hydraulic conductance (K_h) was calculated as the quotient of the mass flow rate of solution through the sample (F) and the pressure gradient (ΔP) along its length. K_h was expressed on a cross-sectional area basis to give specific hydraulic conductivity (K_s). F was plotted in real time to indicate when a steady-state was reached (generally after 60 s) and this value was used to calculate K_s. This initial or pre-flush K_s was termed K_i and was compared with the maximum conductivity of the same shoot with all embolism removed (K_{max}). K_{max} was determined as described below in the 'Vulnerability curves' section of Methods.

Native conductivity and embolism

V. vinifera cv. Chardonnay shoots from the Davis site were harvested at three times (anthesis, veraison, and post-harvest) through the 2008 growing season. The date of collection and equivalent number of days after anthesis (DAA) are as follows: 20 May (14); 13 July (69); and 28 October (175). Stem water potential was measured on three leaves for each shoot using the pressure chamber as described above. Leaves were bagged and foiled before harvesting and allowed to equilibrate for 30 min. Shoots were then transported back to the laboratory where Ψ_x was assessed by pressure chamber and PLC was measured. Five shoots were measured at each time period to evaluate native embolism.

Vulnerability curves

Embolism was induced by three techniques: bench-top drying; centrifuge; and air-injection. Bench-top drying (Tyree & Dixon 1986; Sperry & Tyree 1988) involved placing the recently harvested shoots on a lab bench for varying amounts of time to achieve a range of Ψ_x. Plastic bags were placed over three individual leaves (apical, middle and basal) of the shoot from the beginning of the drying process to maintain the ability to measure them by pressure chamber subsequently. The shoots were allowed to dry for between 0 and 72 h at room temperature before being returned to the large plastic bags for a minimum of 1 h to equilibrate. K_i and mean Ψ_x of the three leaves were then measured. The shoot was then flushed with ultra-pure, degassed 10 mM KCl solution at a minimum of 150 kPa for 15 min in each direction, before K_{max} was assessed. In order to compare degrees of cavitation or embolism between shoots with different K, the percentage loss of conductivity (PLC) was calculated for each shoot relative to K_{max}:

$$PLC = 100 \times \left(1 - \frac{K_i}{K_{max}}\right) \quad (1)$$

Vulnerability curves were then generated by plotting PLCs against their corresponding Ψ_x values.

The centrifuge technique (Pockman *et al*. 1995; Alder *et al*. 1997) involved using shorter shoot samples because of the limitations in centrifuge rotor dimensions (RC5C Plus, Sorvall, Kendro Laboratory Products, Asheville, NC, USA). The rotor design was as in Alder *et al*. (1997) except it held three shoot segments, each 145 mm in length. The shoots were prepared in a similar fashion to those described in the hydraulic measurement section above except the sample was kept to 145 mm with a single node in the center. They were then flushed with ultra-pure, degassed 10 mM KCl solution at a minimum of 150 kPa for 15 min in each direction, before K_{max} was assessed. To test whether this flushing process altered the vulnerability of stem segments, we also used non-flushed stem segments (referred to as native sap centrifuge below) harvested from the middle of long stem segments (~2 m) that were fully hydrated overnight. Stem hydration was conducted by submerging the segment in a water bath for ~24 h. Measurements segments 145 mm in length were then carefully removed from the middle of the submerged segment.

Shoot segments were placed in the rotor with each end sitting in a Plexiglas reservoir that maintained the cut ends immersed in water while the rotor was spinning. They were spun for 4 min at a velocity that was equivalent to a given Ψ_{stem} before being remeasured for hydraulic conductivity. Shoots were then spun at successively higher velocities to achieve tensions of 0.25, 0.5, 0.75, 1.0 and 1.5 MPa, with hydraulic measurements taken after each speed increase, to produce a vulnerability curve.

For the air-injection technique (Salleo *et al*. 1992; Sperry & Saliendra 1994), the same plant material was used as for bench-top drying. Shoots were prepared as described above for hydraulic measurements, and were mounted in a double-ended pressure sleeve with both ends protruding. Air injection measurements were performed on both long segments (~0.9 m) and short segments (0.145 m) similar in length to those used in the centrifuge technique. A single excised petiole was left unsealed (from where the leaf had

been removed) within the double-ended pressure sleeve to act as the air entry location into the xylem. The basal end of the shoot was connected to a valve that could select between a high-pressure source of ultra-pure, degassed 10 mM KCl solution at 150 kPa, and a Mariotte bottle containing the same solution. The shoot was then flushed for 30 min to remove any embolism that may have been present before a hydraulic measurement was taken to establish K_{max}. The air pressure in the chamber was then increased to a target value for 10 min before the pressure was reduced back to atmospheric levels and another hydraulic measurement was taken and PLC calculated. Air pressure was increased to successively higher levels with hydraulic measurements taken after each. This was performed on five to six stems to produce vulnerability curves from the mean PLC values at each pressure level.

Shoot length PLC comparison

Plants were dried in pots to a range of different Ψ_x by withholding water for between 0 and 4 days. Each shoot was harvested and assessed for PLC as described under hydraulic measurements and bench drying earlier. Observations were paired with the first shoot section from a plant being eight nodes long while the second shoot was the length used in the centrifuge (145 mm). The two shoots did not necessarily have the same Ψ_x but as they originated from the same drying pot and root system the values were close enough to allow comparison of the PLC of each shoot section, and to assess the effect of sample length within one technique.

NMR Imaging

NMR imaging was undertaken on greenhouse grown Chardonnay vines. Vines were allowed to dry for between 1 and 4 days in the greenhouse after irrigation tubes were removed. The potted vines were transferred to the NMR facility (University of California, Davis) and Ψ_x was measured immediately with a pressure bomb using bagged and foiled leaves as described above. NMR measurements were performed with a 7 Tesla (300 MHz), horizontal wide-bore magnet (180 mm diameter), with a BioSpec spectrometer (Bruker Biospin Instruments, Fremont, CA, USA). A microgradient set (60 mm i.d.), capable of approximately 950 mT m^{-1}, was mounted in the magnet bore and a volume coil (35 mm i.d.) was used for both radio frequency (RF) excitation and reception. The distal end of each shoot was wrapped with plastic film to allow the shoot to be passed through the RF coil without damage to the leaves. All measurements were made on internodes of current year shoots, which were centered in the RF coil using foam supports. Six transverse image slices of the internode were simultaneously acquired using a gradient-echo pulse sequence, with a tip angle of 30°, a repetition time (TR) of 600 ms, and an echo time (TE) of 3.0 ms. The slices were 2.0 mm thick with a separation of 2.5 mm between them. Slices were averaged four times to increase signal-to-noise ratio. The images were acquired as data arrays of 256 × 256 pixels with a field of view of 12 mm (in-plane resolution of approximately 50 μm). Each image required a scanning time of 10 min and 14 s. The gradient-echo method was chosen over spin echo because it provides better contrast due to the local field heterogeneity at the boundary of anatomical structures. Upon completion of the NMR and PLC measurements transverse stem sections were taken from the same region and imaged using light microscopy.

Measurements were made on 10 vines at progressively greater levels of water stress. The number of cavitated vessels in each stem cross section was established by comparing NMR images to light microscope cross sections of each internode. The resolution of NMR images did not allow us to identify smaller vessels below 50 μm in diameter, therefore only vessels above this diameter were included in counts on light micrographs. This approach was adopted because smaller vessels will contribute a much smaller fraction of the hydraulic conductivity through the internode than the larger vessels. The number of large water-filled vessels, visible as bright areas in the cross section, was then counted and subtracted from the total number of large vessels. Additionally, light microscopy images were superimposed on the NMR images, so vessel locations could be aligned between the two image types. Vessels larger than 50 μm in diameter were identified in the light microscopy image, and then compared with the NMR image to determine the status of the vessel (filled versus not filled). Vessel area was calculated using the 'elliptical marquee' selection tool in Adobe Photoshop CS3 (Adobe Systems Incorporated, San Jose, CA, USA). The area of each vessel was measured and used for calculating the theoretical PLC based on vessel diameter and the Hagen–Poiseulle equation.

Statistics and vulnerability curve analysis

The vulnerability curve data were fitted to an exponential sigmoidal equation after Pammenter & Vander Willigen (1998). Pressure data for air-injection was assumed to have negative sign for the purpose of statistical testing.

$$PLC = \frac{100}{1 + e^{a(\psi - b)}} \quad (2)$$

Coefficients a and b were determined from a linearized form of Eqn 2

$$\ln\left(\frac{100}{PLC} - 1\right) = a\psi - ab \quad (3)$$

using a mixed model analysis of variance in SAS (version 9; SAS Institute Inc., Cary, NC, USA) with population level parameters as fixed effects and individual plant level parameters as random effects. Information about plant level variation was borrowed from the repeated measures methods (centrifuge and air-injection) to estimate that for the singlicate measured method (dehydration), as the

distributions were assumed similar. The transformed data were compared at three levels of Ψ_x (–0.5, –2.0 and –3.5 MPa) using a Tukey–Kramer comparison at 95% confidence. The results of this were then related back to the untransformed curves for comparison.

RESULTS

Vessel length measurements

Maximum vessel length determined by air injection was approximately eight nodes or 0.5–0.9 m. Therefore, all other samples were kept at approximately eight nodes in length except where stated otherwise. Elastomer injections on 145 mm long stem segments indicated that 75.1% of vessels were open at both ends in segments of this length.

Water potential measurements

To ensure the accuracy of Ψ_x measurements on dehydrated stems, pressure chamber measurements on bagged leaves were compared with isopiestic psychrometry of stem internodes adjacent to these leaves (Fig. 1). Principle axis analysis confirmed that pressure chamber measurements accurately reflect Ψ_x as determined by isopiestic psychrometry (Fig. 1). This demonstrated that the low vulnerability to cavitation measured with the dehydration technique did not result from leaves becoming hydraulically isolated from the stem and reaching a water potential lower than that of the true Ψ_x.

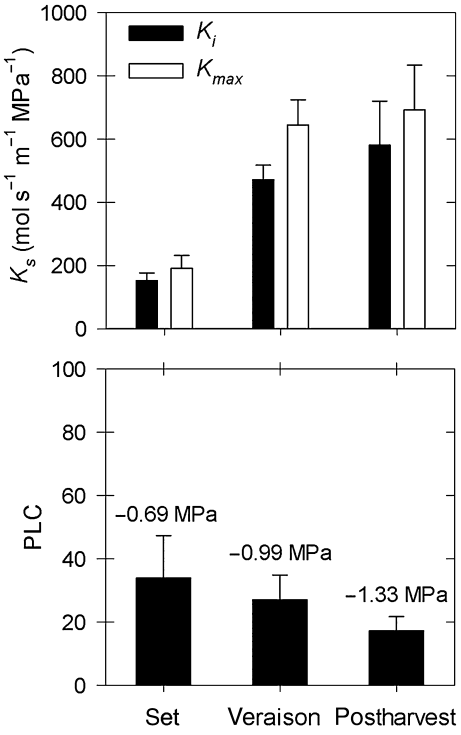

Figure 2. Native and maximum specific hydraulic conductivity of field grown *Vitis vinifera* cv. Chardonnay at three phenological stages; set, veraison and postharvest (top panel). Percent loss of hydraulic conductivity caused by embolism (PLC) calculated from K_i and K_{max} with corresponding mean Ψ_x values for each phenological stage (bottom panel). Data represent the mean ± 1 SE for $n \geq 5$ replicate samples.

Native embolism

Both mean native (K_i) and mean maximum conductivity (K_{max}) of stems increased during the growing season (Fig. 2). There was no difference in PLC throughout the season despite Ψ_x decreasing from –0.69 to –1.33 MPa (Fig. 2). The PLC measured at –1.33 MPa was 18%, which is similar to the VCs generated by dehydration and air-injection techniques, but inconsistent with the centrifuge curve. Similarly, low PLC was measured across the season in field grown *V. vinifera* cv. 'Cabernet sauvignon' vines (data not shown; mean PLC <10% at Ψ_x of –1.43 MPa), demonstrating that this response is not unique to Chardonnay.

Vulnerability curves

The vulnerability of Chardonnay stems measured by the various techniques ranged from a Ψ_{50} (i.e. Ψ_x at 50% loss of conductivity) of –0.21 MPa for the short segment air injection to –2.97 MPa for the dehydration technique on field-grown plants (Fig. 3, Table 1). All methods were compared with each other at three levels of Ψ_x: –0.5, –2.0 and –3.5 MPa (Table 2). There was no significant difference between dehydration vulnerability curves conducted on field-grown or greenhouse-grown Chardonnay at any value of Ψ_x tested ($P > 0.05$; Table 2). The dehydration technique was also assessed on short segments (i.e. segment lengths equal to those used in the centrifuge technique) and did not differ significantly than dehydration on longer segments from the field and greenhouse (Fig. 3; hollow diamonds). The long segment air-injection technique agreed with the dehydration technique ($P > 0.05$; Fig. 3, Tables 1 & 2) with low PLC at $\Psi_x > -2.0$ MPa. Conversely, stems subjected to the short air injection technique exhibited extremely high vulnerability to cavitation reaching ~100 PLC by $\Psi_x = -0.5$ MPa. Flushed stems from the centrifuge technique also exhibited high vulnerability to cavitation across all three levels of Ψ_x compared with those from the dehydration and long-segment air injection techniques ($P < 0.05$; Table 2). Stems from the flushed centrifuge and short-segment air injection techniques had the only $\Psi_{50s} > -2$ MPa (Table 1) and had the steepest slopes, meaning conductivity was lost across a more narrow range of water potentials than by the other techniques as Ψ_x decreased (Fig. 3, Table 1). Interestingly, vulnerability to cavitation measured with the native sap centrifuge technique was similar to the flushed centrifuge technique at low tensions (i.e. $\Psi_x \geq -0.5$ MPa), was different than all other techniques at $\Psi_x = 1.0$ MPa, and was similar to dehydration and long-segment air injection techniques at high tensions (i.e. $\Psi_x \leq -2.0$ MPa) (Fig. 3, Tables 1 & 2).

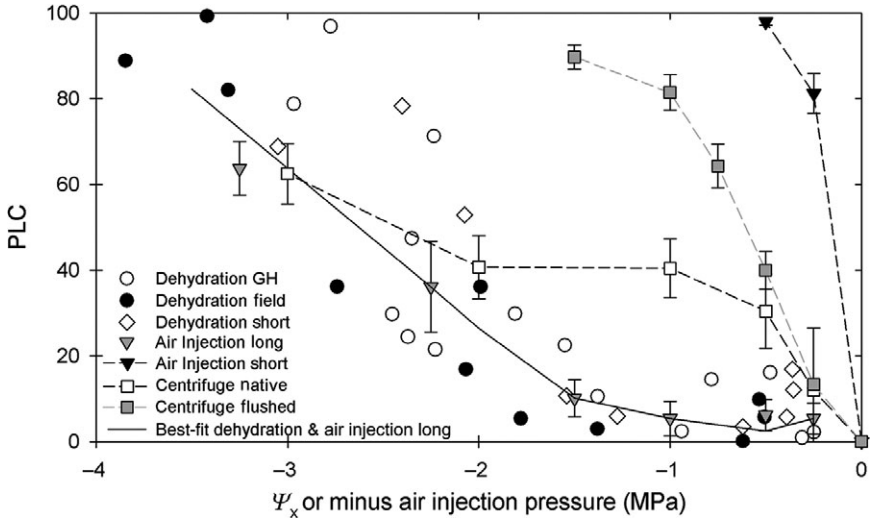

Figure 3. Vulnerability to embolism curves generated by centrifuge, bench top dehydration and air injection methods. Dehydration methods were performed on both long segments from the field and greenhouse and on short segments with a length equal to those used in the centrifuge (see methods for details). Air injection methods were also performed on segments longer than the longest vessel and with a length equal to those used in the centrifuge. Centrifuge methods were performed on segments pre-flushed prior to measurements and those with native sap. As curves generated by the bench top dehydration and air injection methods were not significantly different (see results Tables 1 & 2), a single best fit curve was fit to this data collectively. Data for centrifuge and air injection measurements represent the mean ± 1 SE.

NMR Imaging

NMR imaging revealed that the number of cavitated vessels was relatively low at $\Psi_x > -2.0$ MPa (Fig 4a,b). The number of large cavitated vessels increased sharply at around –2.0 MPa, similar to the VCs generated by dehydration and air-injection but differing from centrifuge curves (Figs 4c,d & 5). NMR image analysis showed that the percentage of empty vessels observed in the cross section increased as a function of decreasing Ψ_x in a similar manner to the increase observed for PLC in bench top dehydration VCs (Fig. 5). After completion of the NMR for each vine, stem PLC was measured and revealed values similar to those measured by the dehydration and air-injection techniques (Fig. 5). PLC was <17% in all samples with a Ψ_x higher than –1.6 MPa (Fig. 5).

Table 1. Exponential sigmoidal function parameters for vulnerability curves. Coefficient estimates were generated for each method on the transformed data. These were then used to produce the best fit curves for each method. *n* represents number of individual plants used to make the measurements for each technique. Coefficient *a* represents the slope of the vulnerability curve (higher values are steeper) whereas *b* represents the Ψ_{50} (i.e. Ψ_x at 50% loss of conductivity). No SE is given for parameter *b* as it was calculated from *a* and *ab*

	PLC		
Method	*a* ± SE	*ab* ± SE	*b*
Dehydration Field ($n = 14$)	2.20 ± 0.29	–5.61 ± 1.05	–2.97
Dehydration Greenhouse ($n = 15$)	1.85 ± 0.20	–4.26 ± 0.63	–2.17
Air injection long ($n = 6$)	1.42 ± 0.34	–4.21 ± 0.52	–2.30
Air injection short ($n = 6$)	37.72 ± 6.29	–7.69 ± 1.12	–0.21
Centrifuge native ($n \geq 6$)	0.64 ± 0.21	–1.43 ± 0.35	–2.19
Centrifuge flushed ($n = 6$)	3.01 ± 0.36	–2.10 ± 0.37	–0.70

DISCUSSION

Our results indicate the flushed centrifuge and short segment air-injection techniques overestimate vulnerability to water stress-induced embolism in *V. vinifera* across all Ψ_x. This can be seen from the comparison of the VCs for these methods with those of the dehydration and long segment air-injection VCs (Fig. 3). The possibility that covered leaf water potential does not accurately reflect stem xylem water potential was ruled out by comparison with isopiestic psychrometer measurements of xylem water potential.

Table 2. Percent loss of conductivity (PLC) at three levels of xylem water potential (Ψ_x) for each vulnerability technique. Pairwise comparison took place on linearized data, however untransformed estimates are given for ease of comparison. Untransformed estimates within a column followed by the same letter indicate the transformed data are not significantly different at the $P = 0.05$ level

	PLC		
Method	Ψ_x –0.5	Ψ_x –2.0	Ψ_x –3.5
Dehydration field	1.1 a	23.0 a	88.9 a
Dehydration greenhouse	3.4 a	36.5 a	90.3 a
Air injection long	2.9 a	20.1 a	67.8 a
Air injection short	99.9 c	100.0 b	100.0 b
Centrifuge native	25.2 b	47.0 a	69.9 a
Centrifuge flushed	35.7 b	98.1 b	100.0 b

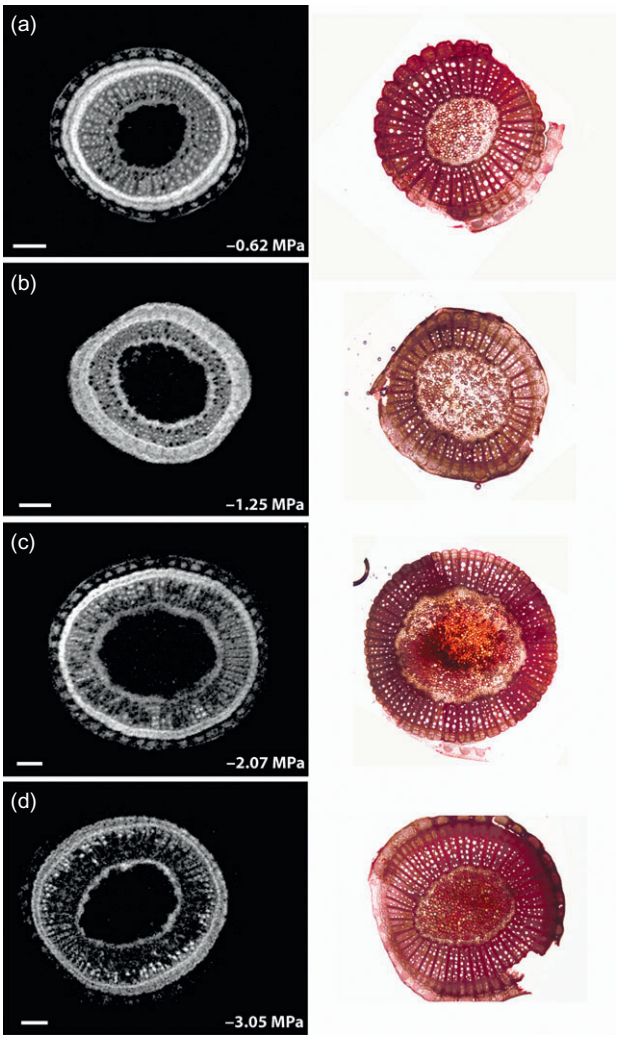

Figure 4. Nuclear magnetic resonance (NMR) images (a–d, left column) and light microscopy cross sections (e–h, right column) of *Vitis vinifera* cv. Chardonnay stems. Water filled xylem vessels can be seen as bright areas in the xylem visualized with the NMR. Cavitated vessels are seen as black areas against background xylem depending on contrast with surrounding xylem tissue. Each image represents a separate stem with water stress increasing from image (a to d). Xylem water potential for each plant is shown at the bottom of each NMR image, and scale bars indicate a distance of 1 mm.

Dehydration and long segment air-injection VCs were also confirmed by observations of native embolism in field grown vines and by non-destructive NMR measurements of cavitation. Although previous studies have stated the air-injection technique produced unrealistic VCs for long-vesseled species, it is possible that segments used contained open vessels (Martínez-Vilalta *et al*. 2002). We found that air-injection measurements on short grapevine stem segments with open vessels produced VCs even more vulnerable than those generated with the flushed centrifuge method. This is similar to the findings of Cochard *et al*. (2005), who reported that air-injection measurements on stem segments with many open vessels produced unreliable data.

Cochard *et al*. (2005) observed that the centrifuge method produced inconsistent and unrepeatable results for ring porous tree species. However, there was relatively little variation in flushed centrifuge VCs for individual stems of *V. vinifera* measured here, or VCs generated for ring porous *Quercus robur* in a more recent paper (Cochard *et al*. in press), indicating that results were repeatable and that the overestimation was caused by systematic rather than random error. Although the data of Cochard *et al*. (in press) relate to the spin while measuring (Cavitron version of the centrifuge technique), the work of Li *et al*. (2008) indicates that both the spin and the original gravity measurement versions of the centrifuge technique produce similar curves for long-vesseled species. Given that our results were obtained with the gravity measurements, it is clear that the overestimation of vulnerability for long-vesseled species occurs in both spin and gravity versions of the centrifuge technique.

The cause of systematic error in the centrifuge VCs is unclear. One possibility is that the effect of segment length is produced by a bias of large open vessels on stem K_h. In short segments, the dynamics of K_h loss will be dominated almost completely by the large open vessels because of the high hydraulic resistance of intervessel pit connections (Choat, Cobb & Jansen 2008). Past studies have shown that larger vessels are more vulnerable to cavitation than smaller vessels within a stem (Lo Gullo & Salleo 1991; Hargrave *et al*. 1994). Therefore, if VCs on short segments are controlled primarily by the vulnerability characteristics of large open vessels and these vessels tend to cavitate at

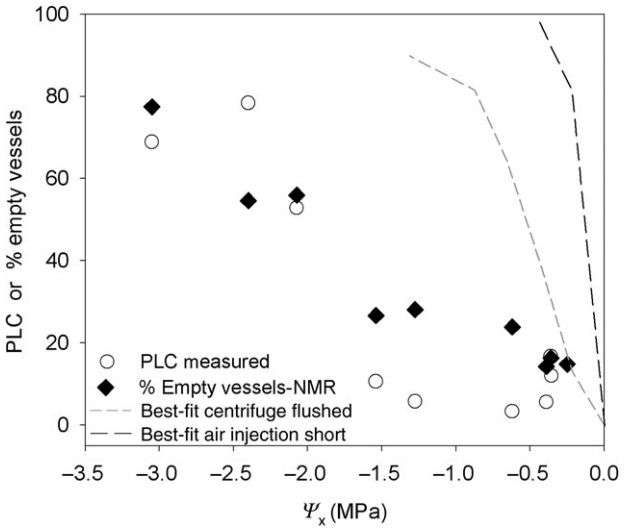

Figure 5. The percentage empty xylem vessels observed in nuclear magnetic resonance (NMR) image slices and the percent loss of conductivity (PLC) for the same stems as a function of xylem water potential (Ψ_x). PLC was measured on stems immediately after the vines were used in the NMR measurements. The best fit line for the flushed centrifuge technique and air injection on short segments (Fig. 3) are presented for comparison with data from stems used in the NMR analysis.

higher Ψ_x than small vessels, the overestimation of vulnerability by the centrifuge may be caused primarily by a sampling bias towards larger vessel size classes. However, if this were the case then we would expect to see a similar error in dehydration curves made on short stems. This was not the case, as measurements made on long stem segments with no open vessels were not significantly different from measurements made on short segments. This suggests that the error is not caused by segment length but rather caused by protocol of the centrifuge measurements.

Cochard *et al*. (2005) suggest that the high vulnerability measured with the centrifuge technique may be caused by particles or micro-bubbles that enter the stem when it is flushed or during the measurement. In species with shorter vessels such particles would be prevented from entering the stem by intervessel pit membranes, which have sufficiently small pore sizes to trap gas or detritus that may have entered the perfusate (Choat *et al*. 2008; Jansen, Choat & Pletsers 2009). Introduction of microbubbles via perfusate could explain differences between the flushed centrifuge and dehydration curves (samples contained native xylem sap during stem dehydration), and could also apply to the differences we documented between the flushed and native centrifuge techniques at high tensions (i.e. $\Psi_x \leq -1.0$ MPa). However, centrifuged stems containing native sap were still more vulnerable at low tensions (i.e. $\Psi_x = -1.0$ MPa) suggesting that micro-bubble introduction is not solely responsible for the high vulnerability we documented with the centrifuge technique. For air-injection on long segments, negative pressure is avoided and therefore the cavitation could not be nucleated by particles or micro-bubbles in perfusate. Another candidate for increasing the vulnerability of open-ended vessels is the ease with which water can drain from them. Water-filled reservoirs placed at either end of a segment, which submerge the sample ends by centripetal force as the sample spins (at 54 rad s^{-1} for a sample length of 275 mm), are thought to prevent the displacement of water from the stem (Alder *et al*. 1997). However, there is still a significant period when air may enter the sample, between when the centrifuge starts spinning and when the water in the reservoirs covers the sample ends. Open-ended vessels (as were present in our grapevine samples) could therefore be emptied or have air bubbles introduced before the reservoir water reaches them. This problem is exacerbated if the stem is not exactly centered with respect to the axis of rotation as a pressure difference is set up between the two vessel menisci. Vessel drainage that occurs just as the centrifuge starts spinning may contribute some to the apparently high vulnerability of segments with many large, open-ended vessels, however, drainage cannot be solely responsible because it should have caused massive PLC even at the lowest tension generated by the centrifuge (mean PLC was <20% at 0.25 MPa for the centrifuged segments – Fig. 3). Given that we found no significant difference between the flushed and native centrifuge curves at low tensions, it is likely that vessel drainage or air introduction that occur when the centrifuge starts to spin contribute to the higher cavitation with this technique at $\Psi_x > -1.0$ MPa.

NMR measurements suggest that many of the primary xylem vessels close to the pith were not filled with sap, even when Ψ_x was >–0.3 MPa. As Ψ_x became more negative, more empty vessels were observed, although the percentage of cavitated vessels did not increase over 35% until Ψ_x dropped below –2.0 MPa. Interestingly, some of the larger vessels towards the outer edge of the growth ring remained conductive even at very low water potentials (–3.0 MPa). These non invasive measurements provide the most convincing evidence that dehydration and long-segment air injection curves are producing the most physiologically relevant measure of vulnerability to cavitation in *V. vinifera*. The results are consistent with a previous NMR study of grapevine in which only a small proportion of vessels were embolized at leaf water potentials down to –2.2 MPa (Holbrook *et al*. 2001). Measurements of native embolism in field grown vines also indicate that PLC at Ψ_x of –1.33 MPa was well below what could be expected from centrifuge VCs. In fact, native PLC was greatest early in the season when Ψ_x was higher at –0.69 MPa. This result may be explained by developmental variation in vulnerability to embolism. Early in the season, the primary xylem will be responsible for a greater proportion of stem K_h. Images from the NMR show that primary xylem conduits adjacent to the pith become embolized before the larger secondary xylem vessels as the plant is dehydrated (Fig. 4a and b). This greater vulnerability of primary xylem vessels is also supported by measurements on other woody species, and most likely related to large areas of primary wall exposed by partial secondary thickening of the conduits (Mencuccini & Comstock 1997; Choat *et al*. 2005).

Based on dehydration, long segment air-injection, and NMR data, *V. vinifera* is predicted to suffer significant embolism only below Ψ_x –2.0 MPa. Given the range of stem water potentials generally experienced in field grown grapevines is between –0.3 and –1.8 MPa, it appears grapevine does not suffer significant stem embolism in the field (Barbe *et al*. 2005). This is supported by measurements of native embolism which show that PLC is below 30% at all points of the season. However, it is possible that grapevine would experience greater levels of stem embolism under other growing conditions and there is evidence that vulnerability to cavitation varies between cultivars (Alsina *et al*. 2007). It is also possible that cavitation occurs to a greater degree in other organs such as petioles or roots (Schultz 2003; Lovisolo *et al*. 2008).

The results of the present study contrast with some recent studies that have shown good agreement between centrifuge VCs and native embolism measured in ring porous tree species and vines (Li *et al*. 2008; Taneda & Sperry 2008). These authors suggest that ring porous species have a large proportion of extremely vulnerable vessels, but because the K_{max} is very high relative to diffuse porous species, ring porous species are able to operate at only a fraction of their K_{max}. However, it is possible that the K_{max} measured in these experiments is exaggerated by xylem vessels in previous years of growth that are not functional under natural conditions and are perhaps more vulnerable to cavitation than

the current years' xylem because of age-related and stress effects (Sperry, Perry & Sullivan 1991; Melcher, Zwieniecki & Holbrook 2003; Hacke *et al*. 2001). This issue may also have played a role in the study of Tibbetts & Ewers (2000), who reported similarly high vulnerability in *V. riparia* using the dehydration technique. Differences between VCs from Tibbetts & Ewers (2000) and the present work could also relate to interspecific differences between *V. vinifera* and *V. riparia*. However, measurements on *V. riparia* in that study were made on 2–4 year old stems and initial PLC of between 40 and 70% was present when samples were collected from the field (Tibbetts & Ewers 2000). In the present study, only current year shoots of *V. vinifera* were used and the problem of including older growth rings was avoided.

We conclude that the centrifuge and short segment techniques are inappropriate for *V. vinifera* as it produces erroneously high vulnerability. Given doubts raised by Cochard *et al*. (2005, in press), we suggest that the centrifuge technique is inappropriate for all species and organs (i.e. roots) with long vessels, that will result in a high proportion of vessels cut open in the experimental stem segment. This is especially important given that resistance to cavitation has been suggested as a useful screening trait for breeding programs, and the centrifuge offers the quickest means of assessment that would likely be adopted by a large audience of scientists (Cochard *et al*. 2005). When working with plant material in which a high proportion of vessels are continuous through the length of the stem or root segment, we suggest that VCs generated by the centrifuge technique are checked against either the dehydration technique or the air injection technique using stem segments that are longer than the maximum vessel length. If available, NMR is an extremely valuable technique that is capable of non-invasive repeated measures on the same stem. However, NMR facilities available for plant based measurements are rare and it is recognized that this technique cannot be used in the majority of cases. Finally, we emphasize that our results do not conflict with the strong agreement between the centrifuge, air injection and dehydration VCs observed for many species with short vessels (Pockman *et al*. 1995; Alder *et al*. 1997; Cochard *et al*. 2005).

ACKNOWLEDGMENTS

This work was funded in part by a National Science Foundation award (IOS-0818479), a California Department of Food and Agriculture contract (01-0712), and a USDA-ARS CRIS project (5306-21220-004-00). W.M.D. received financial support from Horticulture and Agronomy block grants through U.C. Davis and from the Ralph and Saralee Kunde, Serenity Vineyards, Wine Spectator and the Albert and Pearl Winkler scholarships. Thanks to P.K. Bostwick, E.M. Kriedler, L. Mercenaro, F.P. Palladino for help with data collection; to M.M. Anderson and Martinez Orchards for providing experimental material; and to G. Gambetta for useful discussions about the data sets.

REFERENCES

Alder N.N., Pockman W.T., Sperry J.S. & Nuismer S. (1997) Use of centrifugal force in the study of xylem cavitation. *Journal of Experimental Botany* **48,** 665–674.

Alsina M.M., De Herralde F., Aranda X., Save R. & Biel C. (2007) Water relations and vulnerability to embolism are not related: experiments with eight grapevine cultivars. *Vitis* **46,** 1–6.

Barbe C., Van Leeuwen C., Shackel K.A. & Choné X. (2005) Worldwide use of stem water potential to manage vine water deficit with irrigation in estate vineyards. *American Journal of Enology and Viticulture* **56,** 301A–302A.

Boyer J.S. (1995) *Measuring the Water Status of Plants and Soils*. Academic Press, San Diego, CA, USA.

Brodribb T.J. & Cochard H. (2009) Hydraulic failure defines the recovery and point of death in water-stressed conifers. *Plant Physiology* **149,** 575–584.

Chapman D.M., Roby G., Ebeler S.E., Guinard J.X. & Matthews M.A. (2005) Sensory attributes of Cabernet Sauvignon wines made from vines with different water status. *Australian Journal of Grape and Wine Research* **11,** 339–347.

Chatelet D.S., Matthews M.A. & Rost T.L. (2006) Xylem structure and connectivity in grapevine (Vitis vinifera) shoots provides a passive mechanism for the spread of bacteria in grape plants. *Annals of Botany* **98,** 483–494.

Choat B., Lahr E.C., Melcher P.J., Zwieniecki M.A. & Holbrook N.M. (2005) The spatial pattern of air seeding thresholds in mature sugar maple trees. *Plant, Cell & Environment* **28,** 1082–1089.

Choat B., Sack L. & Holbrook N.M. (2007) Diversity of hydraulic traits in nine *Cordia* species growing in tropical forests with contrasting precipitation. *New Phytologist* **175,** 686–698.

Choat B., Cobb A.R. & Jansen S. (2008) Structure and function of bordered pits: new discoveries and impacts on whole-plant hydraulic function. *New Phytologist* **177,** 608–625.

Cochard H., Cruiziat P. & Tyree M.T. (1992) Use of positive pressures to establish vulnerability curves – further support for the air-seeding hypothesis and implications for pressure-volume analysis. *Plant Physiology* **100,** 205–209.

Cochard H., Herbette S., Barigah T., Ennajeh M. & Vilagrosa A. (2010) A pitfall in the centrifugation techniques for measuring xylem vulnerability to cavitation. *Plant, Cell & Environment* doi: 10.1111/j.1365-3040.2010.02163.x

Cochard H., Damour G., Bodet C., Tharwat I., Poirier M. & Ameglio T. (2005) Evaluation of a new centrifuge technique for rapid generation of xylem vulnerability curves. *Physiologia Plantarum* **124,** 410–418.

Davis S.D., Ewers F.W., Sperry J.S., Portwood K.A., Crocker M.C. & Adams G.C. (2002) Shoot dieback during prolonged drought in *Ceanothus* (Rhamnaceae) chaparral of California: a possible case of hydraulic failure. *American Journal of Botany* **89,** 820–828.

Hacke U.G., Stiller V., Sperry J.S., Pittermann J. & McCulloh K.A. (2001) Cavitation fatigue. Embolism and refilling cycles can weaken the cavitation resistance of xylem. *Plant Physiology* **125,** 779–786.

Hacke U.G., Sperry J.S., Wheeler J.K. & Castro L. (2006) Scaling of angiosperm xylem structure with safety and efficiency. *Tree Physiology* **26,** 619–701.

Hargrave K.R., Kolb K.J., Ewers F.W. & Davis S.D. (1994) Conduit Diameter and Drought-Induced Embolism in *Salvia mellifera* Greene (Labiatae). *New Phytologist* **126,** 695–705.

Hietz P., Rosner S., Sorz J. & Mayr S. (2008) Comparison of methods to quantify loss of hydraulic conductivity in Norway spruce. *Annals of Forest Science* **65,** 502.

Holbrook N.M., Burns M.J. & Field C.B. (1995) Negative xylem pressures in plants – a test of the balancing pressure technique. *Science* **270,** 1193–1194.

Holbrook N.M., Ahrens E.T., Burns M.J. & Zwieniecki M.A. (2001) In vivo observation of cavitation and embolism repair using magnetic resonance imaging. *Plant Physiology* **126,** 27–31.

Jansen S., Choat B. & Pletsers A. (2009) Morphological variation of intervessel pit membranes and implications to xylem function in angiosperms. *American Journal of Botany* **96,** 409–419.

Johnson D.M., Meinzer F.C., Woodruff D.R. & McCulloh K.A. (2009) Leaf xylem embolism, detected acoustically and by cryo-SEM, corresponds to decreases in leaf hydraulic conductance in four evergreen species. *Plant, Cell & Environment* **32,** 828–836.

Kursar T.A., Engelbrecht B.M.J., Burke A., Tyree M.T., El Omari B. & Giraldo J.P. (2009) Tolerance to low leaf water status of tropical tree seedlings is related to drought performance and distribution. *Functional Ecology* **23,** 93–102.

Li Y.Y., Sperry J.S., Taneda H., Bush S.E. & Hacke U.G. (2008) Evaluation of centrifugal methods for measuring xylem cavitation in conifers, diffuse- and ring-porous angiosperms. *New Phytologist* **177,** 558–568.

Lo Gullo M.A. & Salleo S. (1991) Three different methods for measuring xylem cavitation and embolism – a comparison. *Annals of Botany* **67,** 417–424.

Lopez O.R., Kursar T.A., Cochard H. & Tyree M.T. (2005) Interspecific variation in xylem vulnerability to cavitation among tropical tree and shrub species. *Tree Physiology* **25,** 1553–1562.

Lovisolo C. & Schubert A. (2006) Mercury hinders recovery of shoot hydraulic conductivity during grapevine rehydration: evidence from a whole plant approach. *New Phytologist* **172,** 469–478.

Lovisolo C., Tramontini S., Flexas J. & Schubert A. (2008) Mercurial inhibition of root hydraulic conductance in Vitis spp. rootstocks under water stress. *Environmental & Experimental Botany* **63,** 178–182.

McCarthy E. (1934) Mariotte's bottle. *Science* **80,** 100.

McDowell N., Pockman W.T., Allen C.D., *et al*. (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist* **178,** 719–739.

McElrone A.J., Pockman W.T., Martínez-Vilalta J. & Jackson R.B. (2004) Variation in xylem structure and function in stems and roots of trees to 20 m depth. *New Phytologist* **163,** 507–517.

Maherali H., Pockman W.T. & Jackson R.B. (2004) Adaptive variation in the vulnerability of woody plants to xylem cavitation. *Ecology* **85,** 2184–2199.

Martínez-Vilalta J., Prat E., Oliveras I. & Pinol J. (2002) Xylem hydraulic properties of roots and stems of nine Mediterranean woody species. *Oecologia* **133,** 19–29.

Melcher P.J., Zwieniecki M.A. & Holbrook N.M. (2003) Vulnerability of xylem vessels to cavitation in sugar maple. Scaling from individual vessels to whole branches. *Plant Physiology* **131,** 1775–1780.

Mencuccini M. & Comstock J. (1997) Vulnerability to cavitation in populations of two desert species, *Hymenoclea salsola* and *Ambrosia dumosa*, from different climatic regions. *Journal of Experimental Botany* **48,** 1323–1334.

Pammenter N.W. & Vander Willigen C. (1998) A mathematical and statistical analysis of the curves illustrating vulnerability of xylem to cavitation. *Tree Physiology* **18,** 589–593.

Pockman W.T. & Sperry J.S. (2000) Vulnerability to xylem cavitation and the distribution of Sonoran desert vegetation. *American Journal of Botany* **87,** 1287–1299.

Pockman W.T., Sperry J.S. & Oleary J.W. (1995) Sustained and significant negative water pressure in xylem. *Nature* **378,** 715–716.

Pratt R.B., Jacobsen A.L., Ewers F.W. & Davis S.D. (2007) Relationships among xylem transport, biomechanics and storage in stems and roots of nine Rhamnaceae species of the California chaparral. *New Phytologist* **174,** 787–798.

Rood S.B., Patino S., Coombs K. & Tyree M.T. (2000) Branch sacrifice: cavitation-associated drought adaptation of riparian cottonwoods. *Trees – Structure and Function* **14,** 248–257.

Salleo S., Hinckley T.M., Kikuta S.B., Lo Gullo M.A., Weilgony P., Yoon T.M. & Richter H. (1992) A method for introducing xylem embolism *in situ* – experiments with a field grown tree. *Plant, Cell & Environment* **15,** 491–497.

Scholander P.F., Love W.E. & Kanwisher J.W. (1955) The rise of sap in tall grapevines. *Plant Physiology* **30,** 93–104.

Schultz H.R. (2003) Differences in hydraulic architecture account for near-isohydric and anisohydric behaviour of two field-grown *Vitis vinifera* L. cultivars during drought. *Plant, Cell & Environment* **26,** 1393–1405.

Schultz H.R. & Matthews M.A. (1988) Resistance to water transport in shoots of *Vitis vinifera* L. – relation to growth at low water potential. *Plant Physiology* **88,** 718–724.

Sperry J.S. & Tyree M.T. (1988) Mechanism of water stress-induced xylem embolism. *Plant Physiology* **88,** 581–587.

Sperry J.S. & Saliendra N.Z. (1994) Intra-plant and inter-plant variation in xylem cavitation in *Betula occidentalis*. *Plant, Cell & Environment* **17,** 1233–1241.

Sperry J.S., Donnelly J.R. & Tyree M.T. (1988) A method for measuring hydraulic conductivity and embolism in xylem. *Plant, Cell & Environment* **11,** 35–40.

Sperry J.S., Perry A.H. & Sullivan J.E.M. (1991) Pit membrane degradation and air-embolism formation in aging xylem vessels of *Populus tremuloides* Michx. *Journal of Experimental Botany* **42,** 1399–1406.

Sperry J.S., Hacke U.G. & Wheeler J.K. (2005) Comparative analysis of end wall resistivity in xylem conduits. *Plant, Cell & Environment* **28,** 456–465.

Taneda H. & Sperry J.S. (2008) A case-study of water transport in co-occurring ring- versus diffuse-porous trees: contrasts in water-status, conducting capacity, cavitation and vessel refilling. *Tree Physiology* **28,** 1641–1651.

Tibbetts T.J. & Ewers F.W. (2000) Root pressure and specific conductivity in temperate lianas: exotic *Celastrus orbiculatus* (Celastraceae) vs. native *Vitis riparia* (Vitaceae). *American Journal of Botany* **87,** 1272–1278.

Tyree M.T. & Dixon M.A. (1986) Water-stress induced cavitation and embolism in some woody plants. *American Journal of Botany* **73,** 725–725.

Tyree M.T. & Sperry J.S. (1989) Vulnerability of xylem to cavitation and embolism. *Annual Review of Plant Physiology and Plant Molecular Biology* **40,** 19–38.

Wheeler J.K., Sperry J.S., Hacke U.G. & Hoang N. (2005) Intervessel pitting and cavitation in woody Rosaceae and other vesseled plants: a basis for a safety vs. efficiency trade-off in xylem transport. *Plant, Cell & Environment* **28,** 800–812.

Willson C.J. & Jackson R.B. (2006) Xylem cavitation caused by drought and freezing stress in four co-occurring *Juniperus* species. *Physiologia Plantarum* **127,** 374–382.

Zimmermann M.H. & Jeje A.A. (1981) Vessel length distribution in stems of some American woody plants. *Canadian Journal of Botany-Revue Canadienne De Botanique* **59,** 1882–1892.

Received 14 October 2009; received in revised form 14 March 2010; accepted for publication 29 March 2010