

The impact of vessel size on vulnerability curves: data and models for within-species variability in saplings of aspen, *Populus tremuloides* Michx

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ABSTRACT

The objective of this study was to quantify the relationship between vulnerability to cavitation and vessel diameter within a species. We measured vulnerability curves (VCs: percentage loss hydraulic conductivity versus tension) in aspen stems and measured vessel-size distributions. Measurements were done on seed-grown, 4-month-old aspen (*Populus tremuloides* Michx) grown in a greenhouse. VCs of stem segments were measured using a centrifuge technique and by a staining technique that allowed a VC to be constructed based on vessel diameter size-classes (D). Vessel-based VCs were also fitted to Weibull cumulative distribution functions (CDF), which provided best-fit values of Weibull CDF constants (c and b) and P_{50} = the tension causing 50% loss of hydraulic conductivity. We show that $P_{50} = 6.166D^{-0.3134}$ ($R^2 = 0.995$) and that b and $1/c$ are both linear functions of D with $R^2 > 0.95$. The results are discussed in terms of models of VCs based on vessel D size-classes and in terms of concepts such as the 'pit area hypothesis' and vessel pathway redundancy.

Key-words: *Populus tremuloides*; air-seeding hypothesis; pit area hypothesis; vessel diameter; Weibull functions.

INTRODUCTION

Water transport is essential for carbon fixation in land-based plants. Water transport commonly happens in a meta-stable state, that is, while xylem water is under negative pressure according to the Cohesion Tension Theory. Xylem conduits containing metastable water are prone to cavitation events, which happen when the continuity of a water column is broken and air displaces water in a xylem conduit. It is amazing to think that the evolution of virtually all land-based organisms (both plant and animal) should depend on a food and energy source that depends on this precarious method of water transport. Hence, studies directed towards how plants accomplish this feat are

important to understanding how plants deal with the environmental factors that enhance the breakdown of meta-stable water.

Vulnerability curves (VCs) are plots of percentage loss of stem hydraulic conductivity, K_h , versus the tension, T , which induces cavitation events, where T = minus the xylem pressure potential. A VC is usually a sigmoid curve and is often characterized by a single value, P_{50} , which equals the T in MPa causing 50% loss of hydraulic conductivity. Since the publication of the first measured VCs in woody plants (Sperry 1985; Tyree & Dixon 1986), many studies have shown considerable variation in vulnerability curves between species (Tyree, Davis & Cochard 1994), where plants from more arid environments tend to have VCs shifted to higher tensions than plants from moist environments. However, there is also considerable within-species variation in VCs. Figure 1 shows two examples from the genus *Populus* demonstrating greater variation in evenly-aged plants grown from wild seeds compared with evenly-aged clonal material.

Sperry & Tyree (1990), LoGullo & Salleo (1991), Hargrave *et al.* (1994), LoGullo *et al.* (1995) and others have noted that large conduits (vessels or tracheids) tend to cavitate before small conduits within a given stem. But no one has ever constructed vulnerability curves for vessel-diameter size classes. There is growing evidence that between-species variation in P_{50} can be explained by differences in mean vessel-diameter ($\bar{D}$) or pit membrane surface area (A_{pit}), where P_{50} is an exponential function of $\bar{D}$ or A_{pit} with an r^2 of 0.72 or 0.75, respectively (Wheeler *et al.* 2005). The functional relationships found by Wheeler *et al.* (2005) have been used to argue for a tradeoff between safety against embolism and efficiency of water transport.

The Hagen–Poiseuille Equation predicts that lumen conductivity to water should increase with the 4th power of diameter (D^4), hence a doubling of diameter will result in a hydraulic conductivity that increases by 16-fold on a per-vessel basis and by fourfold on a cross sectional area basis (Tyree & Zimmermann 2002). Hence, stems with large vessels are more conductive yet more prone to cavitation and therefore there is a tradeoff between stem hydraulic efficiency and stem safety against cavitation.

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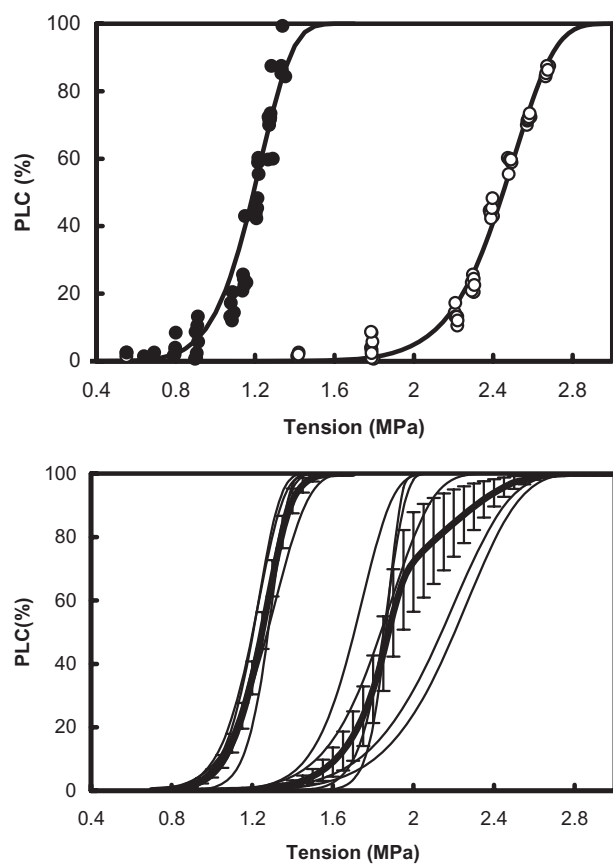


Figure 1. Upper: typical vulnerability curves with points obtained using a Cochard cavitron. Closed symbols: a stem of clone of P38P38, which is a hybrid of *P. balsamifera* × *P. simonii*. Open symbols: a stem of seed-grown aspen. Lower: same clones or species as above but repeated six times for each clone/species. Thick line with error bars is the mean $\pm$ SD of the six different. Weibull fits are indicated by thin lines. Note the much larger variability in the six seed-grown aspens. PLC, percentage loss conductivity.

The purpose of this paper is to investigate the functional dependence of P_{50} on D within stems of one species. Hence, one aim of this paper is to demonstrate how much the stem-to-stem variation in P_{50} can be explained by variation in vessel diameters between stems. The approach taken is to construct VCs for vessels of different diameter by use of vessel-diameter size-classes. In contrast, traditional method uses the analysis of the stem-mean values of P_{50} and $\bar{D}$ between species.

According to the pit area hypothesis, the probability of embolism in any vessel increases with total pit area of vessels (Wheeler *et al.* 2005). The pit area hypothesis is a corollary of the air-seeding hypothesis. According to the air-seeding hypothesis, P_{50} will be determined by the largest pit pore in the total pit area of a vessel. The more pit area there is in a vessel, the greater the chances of a large pit pore being found, which is given by the product of the number of pores times the Gaussian or other probability density function (PDF) distribution of pit pore sizes. A full quantitative analysis of this has recently appeared in

Christman, Sperry & Adler (2009). Hence, it might be argued that P_{50} ought to correlate more strongly with mean vessel surface area or mean vessel pit membrane surface area than with $\bar{D}$; however, experimental data do not provide strong support (in terms of R^2) for this notion. We will discuss our results in the context of the pit area hypothesis and argue that using a centrifuge to create VCs places limits on our ability to test the pit area hypothesis when vessels are longer than 3–4 cm, hence vessel diameter is a proxy for pit membrane area when using a centrifuge to measure P_{50} .

MATERIALS AND METHODS

Plant material

Most measurements were done on current-year shoots harvested from 4-month-old seedlings grown from seeds of *Populus tremuloides* Michx collected in Alberta, Canada. *P. tremuloides* is a boreal forest species ubiquitous in Canada from the western edge of the Rocky Mountains to the Atlantic coast and from the southern boarder to the arctic tree-line. Seeds were sown in pots inside a greenhouse on the campus of the University of Alberta. Pots were moved to the lab and branches were cut under water, leaves removed and a central stem-segment 27.4 cm long was cut underwater with a fresh razor blade. Segments were exposed to a desired tension to induce cavitation in a Cochard cavitron and were typically 5–7 mm in diameter at the base.

Cochard cavitron

Two Cochard cavitrons were fabricated at the University of Vermont's Instrumentation and Modeling Facility based on a rotor design described by Cochard *et al.* (2005) and theory as explained in Cochard (2002). The Cochard cavitron is a modification of the classical centrifuge method (Pockman, Sperry & O'Leary 1995; Alder *et al.* 1997) that permits measurement of stem hydraulic conductivity, K_h , while the stem is spinning. VCs are constructed based on the calculated tension ($T = -\text{pressure}$) at the axis of rotation given by:

$$T = 0.25\rho\omega^2R^2, \quad (1)$$

where R = the radius of the stem measured from the axis of rotation to the water at the basal and distal ends, ρ = density of water and ω = the angular velocity of the centrifuge (radians per second). Construction details will be provided to those who write to the corresponding author.

VC measurements in the Cochard cavitron

The VCs were measured by recording K_h at low tension (0.5 MPa) which provides a measurement of maximum $K_h = K_{\max}$. Then as tension (T) is increased, K_h declines and a VC is constructed by plotting percentage loss conductivity (PLC) from $\text{PLC} = 100 \times (K_{\max} - K_h)/K_{\max}$ versus T . Usually,

these curves are fitted to a type of Weibull function called a cumulative distribution function (CDF), that is,

$$f(T; b, c) = \exp\left[-\left(\frac{T}{b}\right)^c\right], \quad (2a)$$

where $f(T; b, c)$ is the Weibull CDF, and b and c are fitting constants. The Weibull CDF is the fractional hydraulic conductivity remaining in the stem $k_h/k_{\max}$, where k_h is the stem conductivity part way through a VC and $k_{\max}$ is the maximum k_h when there is no embolism. Hence, the Weibull curve for VCs in terms of PLC is

$$\frac{PLC}{100} = 1 - f(T; b, c) = 1 - \exp\left[-\left(\frac{T}{b}\right)^c\right], \quad (2b)$$

Common mathematical treatments of the Weibull CDF use λ for b and k for c , but we retain the old symbols used by Sperry and others for consistency with older literature. Excel spreadsheets were used to obtain coefficients b and c by a least-squares method.

In order to get VCs for vessel-diameter size-classes, replicate stems were spun to different endpoints of PLC ranging from 20 to 90%. Once the target PLC was achieved, the stem segment was removed from the cavitron and cut into consecutive 2 cm segments starting at the axis of rotation (centre of the stem segment spun in the cavitron). The two 2 cm segments adjacent to the axis of rotation were treated differently. One was used to visually distinguish conducting from embolized vessels by passing dye (see below). The other adjacent segment was used to measure PLC in a low pressure flow meter (see below).

Measurement of NPLC and PLC on stem segments ≤ 2 cm long

The native level of PLC (NPLC) was measured using stem segments cut above and below the 27.4 cm segment used in the cavitron. All PLC measurements were done in a 'low pressure flow meter' (LPFM) (Sperry, Donnelly & Tyree 1988). Measurements of K_h were done on 2 cm long stem segments 4–8 mm diameter using a pressure head of 3–4 kPa (30–40 cm water). The pressure applied during K_h measurement was small enough to avoid displacement air bubbles from embolized vessels cut open as determined by preliminary experiments. In LPFM measurements, the initial $K_h = K_i$ is the smallest because of embolisms and the maximum $K_h = K_{\max}$ was usually achieved after one or two flushes at 130 kPa for 3 min. Flushes and K_h measurements were done with degassed, 0.1 M KCl solution filtered thru a 0.2 μm filter to remove small particles that might influence measurements.

Most *Populus* sp. are prone to some kind of wound-response that causes K_h to drop rapidly during LPFM measurements and 0.1 M KCl seems to minimize this problem which would otherwise influence the accuracy of PLC measurements. The reason we think it is a wound response is that other solutions such as 0.01 M KCl or NaCl or CaCl_2 at

0.1 M or less do not prevent the rapid decline in K_h . There have been reports of ion-effects on K_h that are reversible, that is, changing solution ion concentrations from 0–5 mM and back to 0 mM concentration causes rapid and reversible changes in K_h in woody stems (Zwieniecki, Melcher & Holbrook 2001). The ion effects we talk about here occur at much higher concentrations, are specific to K^+ ions and prevent irreversible decline in K_h .

Visualization of embolized vessels using stain

Conducting vessels were stained by passing 0.1% stain (basic fuchsin) through 2 cm stem segments embolized in the cavitron. Vessels with stained walls were conducting and vessels with unstained walls were embolized at the time the stain is perfused at a pressure of 3 kPa for 4 min. Basic fuchsin binds strongly to the walls and travels much slower than the water, hence preliminary experiments were done to optimize the timing for perfusion. Stem segments were flushed to eliminate embolisms then stain was passed for various periods of time until all vessels at the midpoint of the segment were stained. The perfusion time used was double the time needed to stain all vessels. After staining vessel walls, the segments were flushed with 0.1 M KCl at 130 kPa for 5 min to remove excess stain.

A microtome (Reichert-Jung 2030 Biocut, Leica, Wetzlar, Germany) was used to cut 30 μm thick cross sections from the middle of stained stem segments. Sections were mounted in glycerin on a glass slide and placed on a microscope (Axioskop 40, Zeiss, Jena, Germany) and photographed with a digital camera (Infinity1-5C, Regent Instruments Inc., Quebec, Canada). Then image analysis software (Win CELL 2007, Regent Instruments Inc.) was used to measure the diameter of every stained (conducting) and unstained (embolized) vessel in pie-shaped wedges.

Percentage vessels in different diameter size-classes were fitted with Weibull PDF:

$$f(x; \lambda, k) = \frac{k}{\lambda} \left(\frac{x}{\lambda}\right)^{(k-1)} \exp\left[-\left(\frac{x}{\lambda}\right)^k\right], \quad (3)$$

where k and λ are fitting constants and x is the bin diameter increment defined as $x = \frac{d_c}{d_w}$, where d_w = bin-width (typically 5 μm) and d_c = the diameter at the centre of the vessel-diameter size-class. The derivative of the CDF (Eqn 2a) equals the Weibull PDF distribution function, Eqn 3, with $b = k$, $T = x$ and $c = \lambda$, but we use different symbols in the CDF and the PDF Weibull equations, because constants used to fit a VC will not be the same value as those used to fit the vessel size PDF.

VCs in each vessel-diameter size-class

Five stem segments were cut under water and mounted in the Cochard cavitron. Each stem was embolized to a different PLC: target values of 20, 35, 50, 70 and 90%. Cross

sections of the stained stem segments within 1 cm of the axis of rotation were taken and all vessels in pie-shaped wedges were photographed at 100× with overlapping photos. Four wedges equally spaced around the circumference were selected and the wedges collectively had 1000–1600 vessels per stem cross section.

Vessel-lumen cross sectional area (A) was measured in μm^2 and converted to equivalent diameter ($D = \sqrt{A/4\pi}$). All vessel diameters were entered into a Microsoft Excel spreadsheet with different columns for embolized-vessels (unstained) and conducting-vessels (stained), and the data-analysis histogram tool was used to divide vessel diameters into vessel-counts in diameter-size classes of $5 \mu\text{m}$ width. The output was the number of vessels versus diameter size-class which was used to create the histogram. The histograms could be used to examine either total counts of all vessels or vessel-counts that were conducting, N_c , or embolized, N_e . Vessel PLC could then be computed in two ways: either % by count in each size-class, PLC_N , or by hydraulic-weighted diameter in the diameter size-class, PLC_H , defined as:

$$PLC_N = \frac{N_e}{N_e + N_c} 100, \quad (4a)$$

$$PLC_H = \frac{\sum_{1bin} D_e^4}{\sum_{1bin} D_e^4 + \sum_{1bin} D_c^4} 100, \quad (4b)$$

where the summations are done within each diameter size-class, hence we end up with a PLC_H or PLC_N for each diameter size-class for each stem. In Eqn 4b D_e and D_c are the measured diameters of embolized (subscript e) and conducting (subscript c) within each size-class (not the mean or median values).

The use of Eqns 4a,b will not necessarily give the same PLC measured by hydraulic methods ($PLC = 100[k_{\max} - k_h]/k_{\max}$) because vessel pathway redundancy might influence the value (see Discussion). In order to check the stain method worked, we also computed the theoretical PLC based on D_e and D_c summed over all the vessels in a stem and compared that with PLC measured by the LPFM method.

$$PLC_{\text{theor}} = \frac{\sum_{\text{stem}} D_e^4}{\sum_{\text{stem}} D_e^4 + \sum_{\text{stem}} D_c^4} 100, \quad (4c)$$

The T in Eqn 1 corresponded to the T that induced PLC_N or PLC_H in each diameter size-class. A vulnerability curve was then computed for each diameter size-class by plotting PLC_N and PLC_H versus T for each size-class. Because we had five points of T versus PLC_N and PLC_H for each of eight diameter size-classes, the five stems provided eight VC for eight diameter size-classes with five points for each VC. Finally, a Weibull CDF function was fitted to each curve using Eqn 2 and the Microsoft Excel solver routine.

Results

The NPLC of our greenhouse-grown aspens had a mean $\pm$ SEM of $2.56 \pm 0.66\%$ ($n = 21$). Native embolism will impact visual estimates of PLC using stains (Eqn 4) so it is important to use the stain-technique on plants that either have low NPLC or reversible native embolism without the complication of cavitation fatigue.

The VCs obtained with the cavitron could be fitted to Eqn 2b with root mean square errors of 2–4% (Fig. 1 upper) and this was sufficient to detect stem to stem variability in VCs (Fig. 1 lower).

Visual estimates of PLC obtained from the stain-technique agreed with PLC measured in the more traditional way with a LPFM (conductivity apparatus). A plot of PLC_{theor} versus PLC measured in an LPFM yielded $y = 0.976x - 9.452$ $R^2 = 0.865$, suggesting that stained-vessel technique yields PLC values close to the traditional method (Fig. 2). The regression line was not significantly different from 1:1 because the slope was not significantly different from 1 and the y-intercept was not significantly different from 0.

Fitting Weibull PDFs to normalized frequency histograms of vessel size

Figure 3 shows the Weibull PDF fit for vessel diameter measurements made on five different aspen seedlings pooled

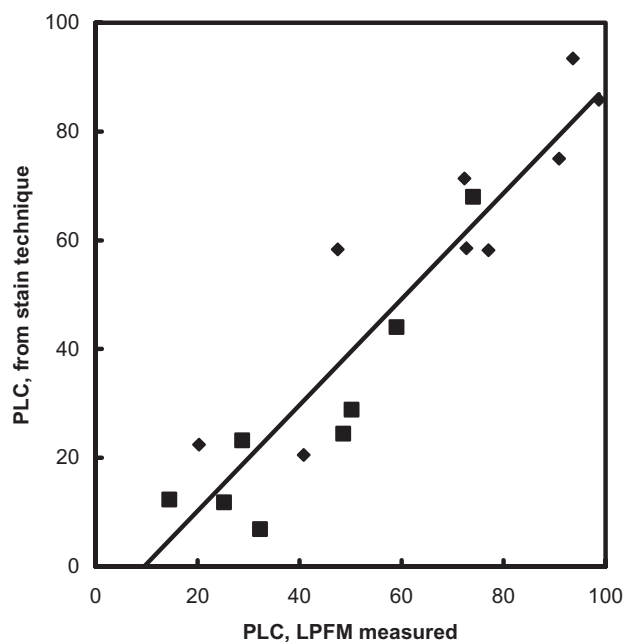


Figure 2. Shown is a plot of percentage loss conductivity (PLC) in 2 cm segments measured by two techniques. Y-axis is the PLC computed from stained and unstained vessels using Eqn 4c. X-axis is the PLC measured on a low pressure flow meter (LPFM; hydraulic measurement). Small diamonds = 4-month-old aspen seedlings. Squares = various other *Populus* clones. The regression line is $y = 0.976x - 9.452$ $R^2 = 0.865$.

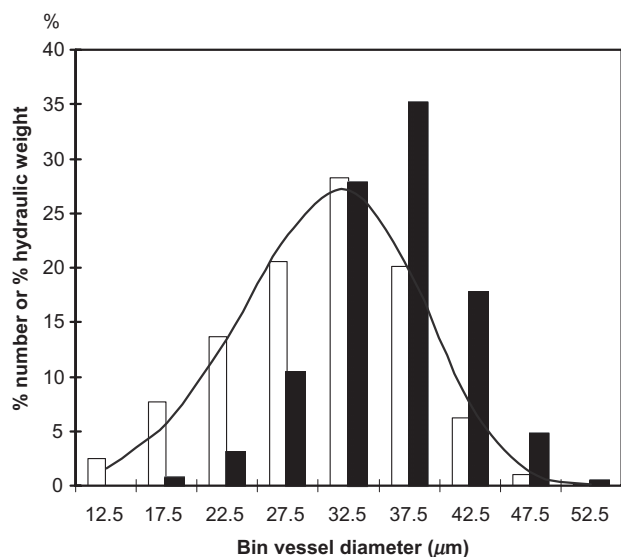


Figure 3. Probability density function (PDF) of vessel count (white bars) and PDF as hydraulic contribution (hatched bars) using the same data as in upper graph. Hydraulic weighted values = $N_i D_i^4 / \sum_{bins} N_i D_i^4$. Note that smaller vessel diameter contribute very little to whole stem hydraulic conductivity.

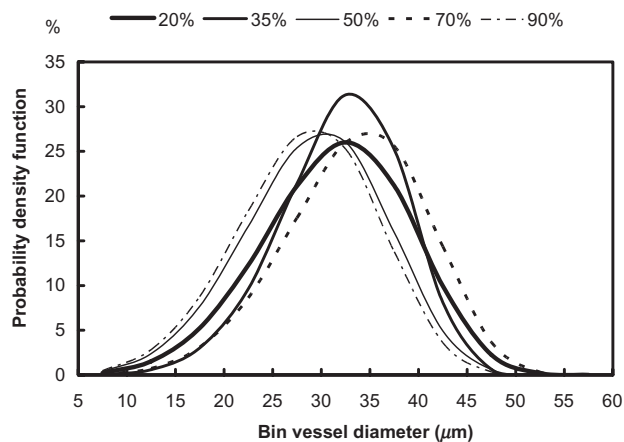


Figure 4. Variation between branches in Weibull probability density function fits for all vessel (stained and unstained) in all five branches; the branches are identified by the target percentage loss conductivity to which they were spun in the Cochard cavitron.

into one data set of >6300 points (open bars). The Weibull PDF mimics the measured normalized frequency histogram (points) on large as well as smaller data set of >500 points. There was some uncertainty about percentages in the two smallest size classes because many small vessels were found near the cambium and it was not obvious which vessels were mature and which were still growing. Ideally, measurements should be confined to mature vessels. However, the impact of small vessels on whole stem hydraulic conductivity was small based on hydraulically weighted contributions

(open bars versus hatched bars) as has been reported by others (Ewers & Fisher 1989). Figure 4 shows the variability in vessel PDF distributions in vessel diameters between individual plants. Variation in vessel diameter accounts for part of the variation in P_{50} between branches as explained in the discussion. One Weibull PDF curve is shown for each plant identified by the target-PLC they were spun to in the Cochard cavitron.

Multi-year-old stems of some species do not have vessel-diameter PDFs that follow the Weibull distribution (e.g. *Salvia mellifera* Hargrave *et al.* 1994). Our observed Weibull distributions may be the consequence of doing all measurements on 4-month-old seedlings.

Weibull PDFs of embolized versus conducting vessels

The PDFs of embolized versus conducting vessels were different; two examples are illustrated in Fig. 5. As hypothesized, the PDF of the embolized vessels was shifted towards the larger diameter size classes compared with conducting vessels. We were able to fit Weibull PDFs to all

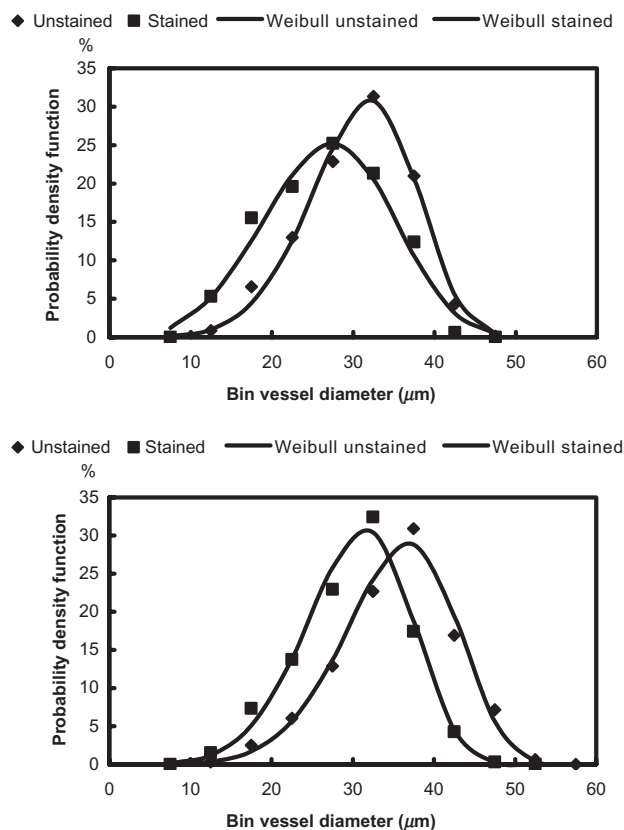


Figure 5. Plotted are probability density functions (PDFs) of conducting (stained) vessels and embolized (unstained). The solid lines are Weibull PDF fits. Upper: Stems embolized in Cochard cavitron until percentage loss conductivity (PLC) = 46% (50% target). Lower: Stems embolized in cavitron until PLC = 72% (70% target).

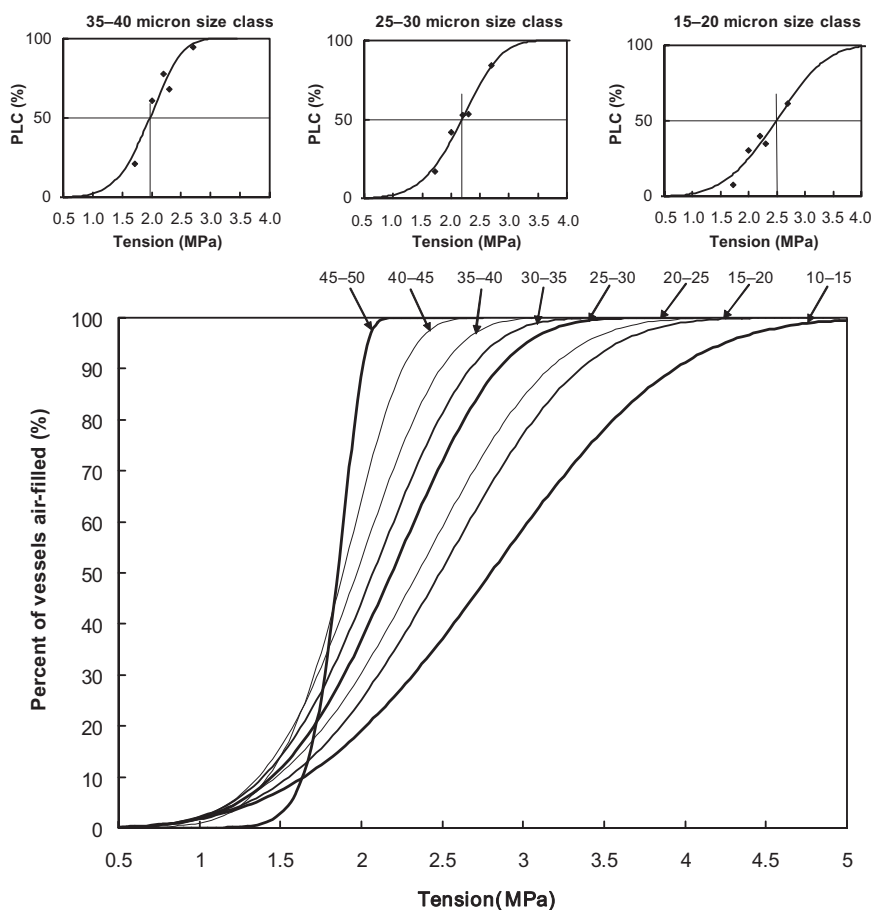


Figure 6. Vulnerability curves (VCs) for vessels computed from the number of stained (conducting) and unstained (embolized) vessels (Eqn 4a). Upper three small panels show the typical Weibull cumulative distribution function curves fitted to data points. Lower graph shows the best fit Weibull range in μm . Curves based on hydraulic-weighted conductivity (Eqn 4b) were similar. The symbols in the upper curves represent five stems spun to different target percentage loss conductivities (PLCs). The VC for the largest size class is an outlier liner because of scatter of points (not shown) where three are all >98%, one is at 90% and one at 20%, and the number of vessel in that size class is quite low (≤ 10 in 4 out of 5 segments), hence the fit may not be reliable.

normalized frequency histograms. The Weibull PDFs were generally fitted with an RMS error of 1.5% (range 0.92 to 2.2%).

VCs for vessels of different diameter size-class & Weibull CDF fits

The VCs for each diameter size-class were computed from the ratio of embolized to total vessels in each size-class. Three examples of data points fitted with Weibull CDF are shown in Fig. 6 (top). As diameter decreased, P_{50} shifted to higher tensions and the slope of the Weibull CDF decreased. The large graph in Fig. 6 shows the family of Weibull CDF fits without data points. The Weibull CDF fits were least certain for the largest and smallest vessel-diameter size-classes because relatively few vessels were in these size classes. The PLC values computed from the stained and unstained vessels were based on Eqns 4a,b and the values correspond to theoretical PLC that tends to underestimate PLC values by a few percent compared with PLC measured in an LPFM on 2 cm segments.

Dependence of P_{50} and Weibull CDF constants on vessel diameter size-class

The Weibull CDF fits yield best-fit values of P_{50} and the two Weibull CDF constants: b and c in Eqn 2. Weibull CDF fits

for data from both Eqns 4a,b were obtained, that is, for theoretical PLCs computed based on numbers of conducting and embolized vessels and based on hydraulically weighted values, respectively. The P_{50} values from Weibull CDF fits did not differ significantly whether Eqns 4a or 4b were used.

The value of b represents the tension at which $\text{PLC} = 63.2\%$ and hence can be thought of as a P_{63} . When $T = b$, the exponent of Eqn 2b = 1, therefore the PLC is the same regardless of the value of c , hence P_{63} is a unique characteristic value of the Weibull CDF. The unique property of b can be seen in Fig. 7, where regressions of P_{50} , b and c are shown versus bin vessel diameter. P_{50} is an exponential function of D with a very high $R^2 = 0.997$. The value of P_{63} ($=b$) was a linear function of D ($R^2 = 0.956$) and the value of $1/c$ was a linear function of D ($R^2 = 0.945$).

DISCUSSION

Wheeler *et al.* (2005) did non-linear fits of between-species values of the stem-mean P_{50} versus stem-mean vessel diameter, $\bar{D}$, and found a fit equivalent to the form $P_{50} = a\bar{D}^\alpha$ with an R -squared value of 0.72. A slightly higher R^2 (0.75) was found when P_{50} was regressed against mean vessel pit membrane area, A_p . The higher R^2 value (0.75 versus 0.72) provides slight but not compelling evidence that A_p may determine values of P_{50} , but the notion deserves serious

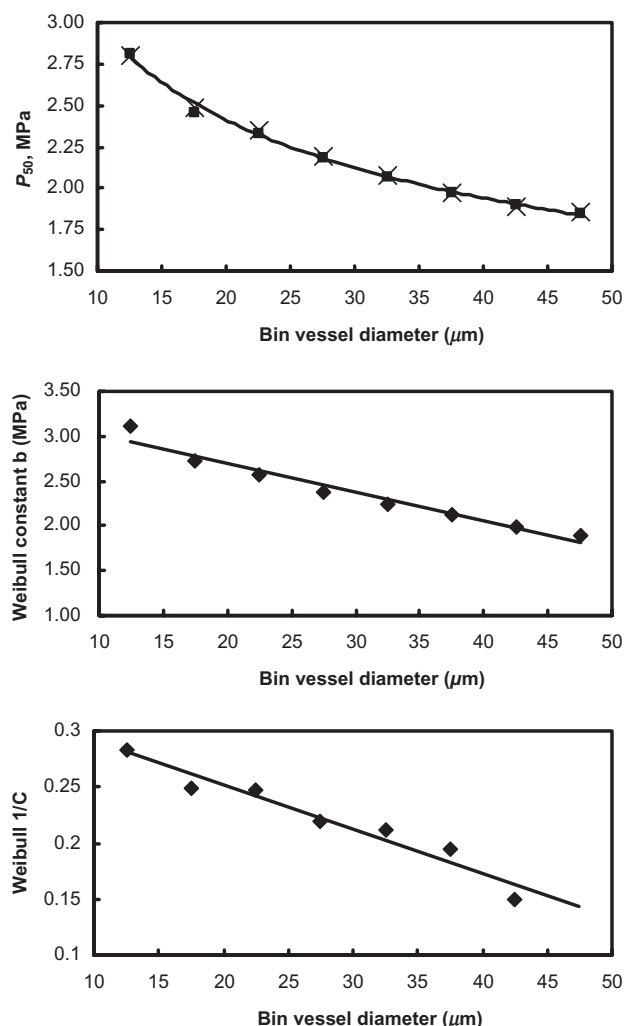


Figure 7. Weibull parameters from the best fit Weibull curves versus vessel diameter size class in μm . Upper: The P_{50} regression is $Y = 6.166 D^{-0.3134}$ $R^2 = 0.997$ and x's and squares refer to actual and hydraulic D -values, respectively. Middle: b parameter $Y = b = -3.243 \cdot 10^{-2} D + 3.351$ $R^2 = 0.956$. Bottom: c parameter $Y = 1/C = -3.92 \cdot 10^{-3} D + 0.3299$ $R^2 = 0.945$.

consideration because it is consistent with the air-seeding hypothesis (Tyree & Zimmermann 2002) and makes sense in terms of the 'pit area hypothesis' (Hacke *et al.* 2006; Sperry, Hacke & Pittermann 2006).

We found a much higher R -squared >0.99 , and we found linear transforms that allow us to predict the Weibull CDF constants (c and b) from vessel diameter, D . We found $1/C$ linearly correlated with D , and b was linearly related to D . The latter correlation was surprising given that b and P_{50} describe similar processes. The value of P_{50} is the tension at which $\text{PLC} = 50\%$, and the value of b gives the tension at which $\text{PLC} = 63.2\%$, hence we can think of b as being a P_{63} (see Eqn 2b).

Our ability to predict c and b from D within a species gets us a step nearer to predicting variations in VC within a species. Figure 4 shows the measured variation in measured vessel-diameter PDFs. By comparing Fig. 4 with VCs by

diameter size-class in Fig. 6, we can conclude qualitatively that the stems with vessel-diameter PDFs shifted to the right in Fig. 4 will be more vulnerable to cavitation (lower P_{50}) than the stems with PDFs shifted to the left. However, vessel diameter cannot explain all the variation in VCs between stems (see below). Two other factors of importance might be: (1) pathway redundancy for water flow in stems much longer than the median vessel length; and (2) the impact of pit area fraction on VCs.

Vessel-diameter PDFs and VCs

That vessel-diameter PDFs do not account for enough range in the variation of VCs is easily illustrated with our limited data set. In the discrete model (Eqn 6), we assume vessel K_h scales with vessel lumen hydraulic conductivity. The vessel K_h will be given by the hydraulic resistance of the lumen plus the hydraulic resistance of the end walls. Studies have shown these resistances to be approximately equal, hence $K_h \approx K_{\text{lumen}}/2$ (Sperry, Hacke & Wheeler 2005; Wheeler *et al.* 2005; Hacke *et al.* 2006). The maximum lumen conductivity of vessels in i^{th} diameter size-class (D_i) will be

$$K_{\text{max},i} = N_i \pi D_i^4 / 128 \eta, \quad (5a)$$

from the Hagen–Poiseuille Law. If the conductivity of a whole vessel is a fraction, f , of the lumen conductivity for every size-class, then we can compute the maximum whole-stem conductivity from:

$$K_{\text{max}} = f \frac{\pi}{128 \eta} \sum_{\text{bins}} D_i^4, \quad (5b).$$

Furthermore, Eqn 2b provides % loss of K_{max} if we use the bin values of c and b , c_i and b_i , computed from the regressions in Fig. 7. Hence, our discrete vessel-diameter model for PLC is given by

$$\begin{aligned} \frac{\text{PLC}_{\text{discrete}}}{100} &= \frac{\sum_{\text{bins}} \left\{ k_{\text{max},i} \left(1 - \exp \left[- \left(\frac{T}{b_i} \right)^{c_i} \right] \right) \right\}}{\sum_{\text{bins}} k_{\text{max},i}} \\ &= \frac{\sum_{\text{bins}} \left\{ N_i D_i^4 \left(1 - \exp \left[- \left(\frac{T}{b_i} \right)^{c_i} \right] \right) \right\}}{\sum_{\text{bins}} N_i D_i^4}, \end{aligned} \quad (6)$$

Equation 6 can be used to compute a VC (PLC versus T) but the reader has to keep in mind that T changes with position in a stem when a centrifuge is used to measure a VC (see Fig. 9). The maximum range of curves for our five stems is illustrated by the two pairs of extreme curves in Fig. 8 (two thick and two thin dashed lines). If all vessels are longer than the stem segment spun in a centrifuge, then the PLC of all vessels in the stem cross section will be determined by the T at the axis of rotation and this extreme is given by the pair with thick dashed lines. We argue that nearly the same curves would result if T did not change with

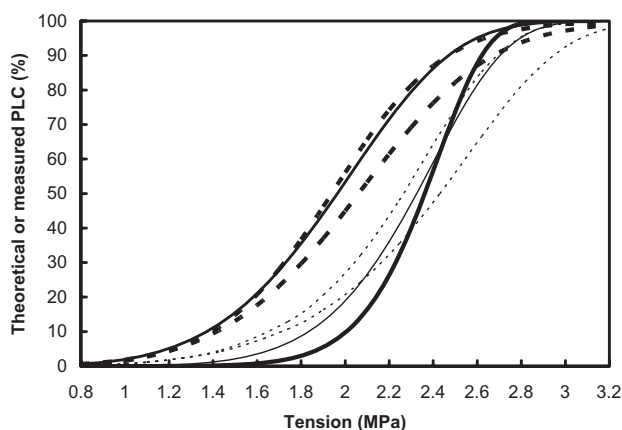


Figure 8. The theoretical model for percentage loss conductivity (PLC) (Eqn 6) was computed for the five aspen stem segments in this study and this resulted in a family of five curves that were closely clustered for each of two cases assuming the centrifuge method (1) does or (2) does not shift vulnerability curves (VCs) to the right. In order to reduce clutter in this figure, only the two extremes are shown in each case; the other three simulations were in between the plotted extremes. (1) The thick dashed lines give the model output, assuming the centrifuge method does not shift the VC to right, which might happen in stems with long vessels. (2) The thin dashed lines give the model output, assuming the centrifuge method shifts the VC the maximum amount to the right as predicted by Cochard *et al.* (2005), which might happen if vessels are short. The solid lines are the best-fit lines for the measured VCs (the Weibull cumulative distribution functions); data points for the best fit curves are omitted to reduce clutter in the figure. Two of five stems were not included because the centrifuge experiment was halted after less than 50% loss of hydraulic conductivity and hence the curves are less certain.

position in the stem. Cochard stated that if the vessels are short, then the impact of the cavitron method ought to be to shift the VCs constructed from Eqn 6 to the right in Fig. 8. Using the calculation methods in Cochard *et al.* (2005), this theoretical shift was computed and plotted in Fig. 8 (thin dashed lines). Tentatively, it appears that the actual range of variation in VCs measured in the cavitron is more than predicted by Eqn 6 as shown by the solid lines in Fig. 8, hence we may have to look to additional causes of variation. This suggests that the model in Eqn 6 might need to be improved to account for the impact on VCs of vessel length distributions and pathway redundancy.

Pathway redundancy, pit area fraction and VCs

Vessel redundancy was first discussed qualitatively by Zimmermann (1983) and first treated quantitatively in a simple model by H. Cochard in Tyree *et al.* (1994). Below is a semi-quantitative introduction to help explain the issue. Water flow pathways in vessels are complex because any one vessel will be connected to two or more adjacent vessels. Zimmermann (1983, fig. 3.13) pointed out qualitatively that increased interconnection between vessel elements can decrease the relative impact of embolisms on loss

of stem hydraulic conduction. H. Cochard was the first to assess quantitative impact of embolisms in stems with multi-connected conduits (see fig. 10a Tyree *et al.* 1994). The impact of pathway redundancy is quantified by changing connectivity $\langle k \rangle$ = the average number of connections per conduit in stem-segments much longer than the average vessel length. In Cochard's unit pipe model, $\langle k \rangle = 2$ and 50% loss of hydraulic conductivity occurred when just 13% of the conduits were 'theoretically' cavitated. At higher $\langle k \rangle$ values, the 50% loss point was delayed until a greater percentage of embolized vessels occurred, that is, 20, 29 and 30% for $\langle k \rangle$ of 4, 6 and 8, respectively. Loepfe *et al.* (2007) did a more complete analysis and simulated the effect of increasing $\langle k \rangle$ on the shape of VCs. They found that increasing $\langle k \rangle$ should make the VC steeper (larger c constant) but should also decrease P_{50} (see fig. 3 in Loepfe *et al.* 2007). A full discussion of this topic is beyond the scope of this paper but readers might want to refer also to Ellmore, Zanne & Orians 2006; Zanne *et al.* 2006; and Ewers *et al.* 2007.

From the said analysis, it is clear that differences in $\langle k \rangle$ between stems will explain why there is some extra variation in VC between individual stems not accounted for by bin D . In addition, measuring PLC on stem segments that are short in comparison with average vessel length will not reflect the impact of pathway redundancy. The fact that the PLC measured with the LPFM on 2 cm segments agrees well with the PLC computed dye method (Eqn 4) suggests that the 2 cm segments had little redundancy.

Another source of variation in VCs might be explained by the variation between stems in the pitfield fraction (the fraction of the vessel surface area occupied by pits),

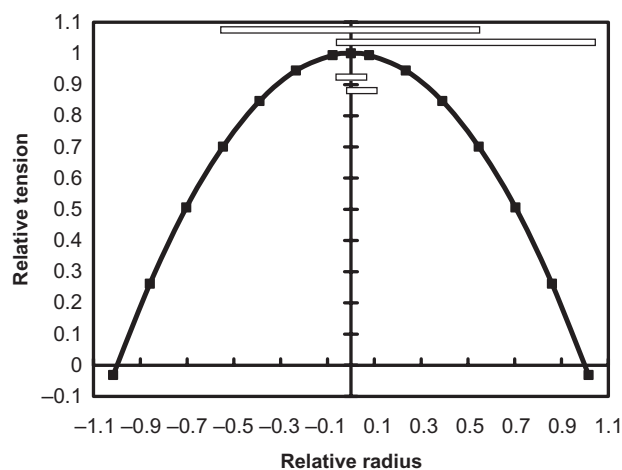


Figure 9. Shown are two vessels (two long and two short) and a line showing the theoretical tension profile in a cavitron. Relative tension is tension at an x -position relative to the maximum tension at the centre. Relative radius is x -position relative to the maximum radius. All tension profiles are identical in relative terms regardless of the stem size or rotor RPM. Note that the tension is relatively uniform over the short vessel but falls off rapidly towards the end of long vessels especially when the vessels begin and end off-centre with respect to the axis of rotation.

assuming the pit area hypothesis is correct. We tentatively reject this notion for the data in this paper because variation in pit fraction would also destroy the high correlations we found between bin D and the Weibull parameters (P_{50} , b and c), because the pit area hypothesis postulates that the Weibull parameters are a function of pitfield area.

The high correlations we found between the Weibull parameters and bin D surprised us because weaker correlations were found between the Weibull parameters species-mean $D(\bar{D})$ when comparisons are made between species. Below is our tentative guess as to the cause.

Pressure profiles in a cavitron, vessel length and the pit area hypothesis

Cochard *et al.* (2005) discussed how pressure profiles in the cavitron might shift VCs when vessels are short compared with the length of the stem in the cavitron. The effect is to shift VCs to the right (Fig. 8), hence Cochard was surprised when VCs measured by other methods were quite close to those measured by the cavitron. Hence, something is shifting the cavitron curves (where T changes with position) back into agreement with standard methods (where T is approximately constant along the stem). A contributing factor to this might be vessel length as illustrated in Fig. 9. A long vessel which passes through the axis of rotation is likely to be cavitated by the high T quite near the axis of rotation but the embolism will extend into regions where the T is low enough to be below the threshold that would induce cavitation. Hence, Cochard's computational method will overestimate the shift to the right as illustrated in Fig. 9 in species with long vessels.

Vessel lengths and the tension profiles in a cavitron might also diminish the rigor of tests of the pit area hypothesis. In Fig. 9 is a plot of the T -profile and superimpose some possible placements of vessels within this T -profile. In our aspen stems, the average vessel length was about 3 cm which is about 0.1 times the stem length in the cavitron. Hence, the vessels near the axis of rotation will experience nearly the same T over their entire vessel-length. On the other hand, species with quite long vessel-lengths will experience large differences in T over their vessel-lengths. This fact poses a problem with regard to rigorous testing of the pit area hypothesis.

According to the pit area hypothesis, the P_{50} is driven by the total surface area of all pits in vessels. The pit area hypothesis is the logical consequence of the air-seeding hypothesis wherein the T causing a cavitation is determined by surface tension of water and the diameter of the largest pore in the vessel wall. The pit area hypothesis assumes the largest pore is located in just one of the many pit membranes in a vessel. The probability of a cavitation would depend on the size of the largest pore in all the pit membranes in any given vessel assuming tension is uniform along the entire vessel length. But most tests of the pit area hypothesis are performed in a cavitron where the assumption of uniform tension is not satisfied. The stochastic argument is that the probability of a large pore in a vessel

increases as pit-surface-area increases. Keeping this in mind, it is not surprising to us that Wheeler *et al.* (2005) found similar R^2 values for plots P_{50} versus A_p (pit area) and P_{50} versus $\bar{D}$.

The cavitron has an 'active zone' of characteristic length (L_c) where most of the cavitations probably occur near to the axis of rotation because the tension is most near the centre. The cavitron rotor used by Wheeler *et al.* (2005) used only 14.2 cm long segments (L) but vessel lengths (L_v) ranged from 1.6 to 13 cm. Given that $L_v \cong L$ in some species and given that T falls to zero near the ends of the stem segments, one might predict that most of the cavitations in long vessels will be seeded only in the shorter characteristic length (L_c , maybe 0.25 of the stem length?) around the axis of rotation in the rotor where tension is largest and uniform. Although the authors might believe they are testing for how P_{50} changes with $A_p = F_p \pi \bar{D} L_v$, where F_p = the pit area fraction, they are in fact testing for how P_{50} changes with $F_p = \pi \bar{D} L_c$ where $L_c < L_v$ for several species in their study. Average vessel diameter, $\bar{D}$, would be a proxy for A_p for most of the species provided F_p is constant between species. But in Wheeler *et al.* (2005) F_p ranges over a factor of 10 (0.016–0.161). Keeping this in mind, it surprised us that R^2 for P_{50} versus A_p is not much better than P_{50} versus $\bar{D}$, that is, $R^2 = 0.75$ and 0.72 , respectively. Does the R^2 improve any if we ignore L_v in the calculation of A_p but instead insert the characteristic length, L_c , and, hence, calculate A_p in the zone of maximum tension? What is the value of L_c ?

An approximate answer to these two questions was obtained by doing repeated regressions of P_{50} versus pit area = $A_p F_p \pi \bar{D} L^*$, where $L^* = L_v$ when $L_v < L_c$ and $L^* = L_c$ when $L_v > L_c$. So if vessels are too long, then cavitations were assumed to be seeded only over a part of the vessel length, that is, the characteristic length, L_c . We assumed a reasonable value of L_c would equal the value with the highest R^2 . Using the data from Wheeler *et al.* (2005) provided by U. Hacke, we computed the graphs in Fig. 10, where it can be seen that the maximum R^2 (0.78) occurred with $L_c = 3.8$ cm. These results lend more support to the pit area hypothesis even though the R^2 is still not dramatically better than the R^2 for P_{50} versus $\bar{D}$. We tentatively attribute our high $R^2 \geq 0.994$ results to two possible causes: (1) The average vessel length was within the uniform T -profile; and (2) the value of F_p was probably about the same for all vessels within aspen stems.

The lower R^2 values in Wheeler *et al.* (2005) might be attributed to difficulties resulting from: (1) using mean values of diameter ($\bar{D}$) and ignoring the impact of diameter PDFs; (2) obtaining accurate values of F_p ; and (3) errors caused by doing a regression of P_{50} versus A_p when $L_v > L_c$ because of the known T gradients. The pit area hypothesis or 'rare pit' hypothesis has been more rigorously tested in a paper that appeared while ours was under review (Christman *et al.* 2009). In the authors' opinion, the methods used in Christman *et al.* (2009) avoid the problems discussed above and we hope our analysis will 'drive home' the reason why the centrifuge technique is less than optimal for testing the pit area hypothesis.

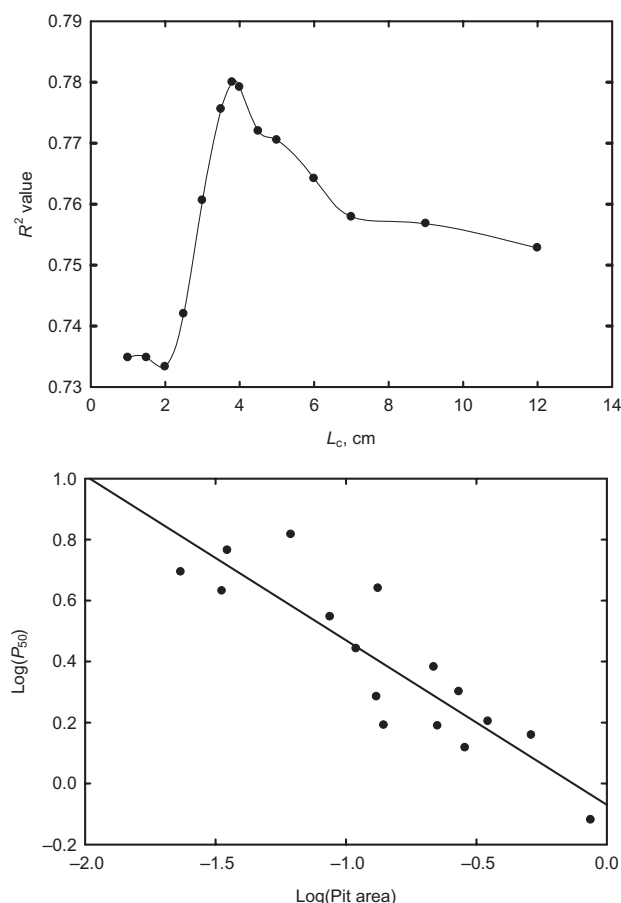


Figure 10. Upper: Impact of the value of L_c on the R^2 on a regression like that in the lower graph. Each point plotted is the R^2 value from a new regression P_{50} versus pit area where a new calculation on pit area is made = $F_p \pi \bar{D} L^*$, where $L^* = L_v$ when $L_v < L_c$ and $L^* = L_c$ when $L_v \geq L_c$. Each value of L^* is a guess on the 'correct' value of L_c . The criterion of 'correctness' is the value of L_c shown on the x-axis that gives the highest R^2 . Had there not been a clear single-peak maximum value of R^2 , then the notion of a characteristic length, L_c , might be discounted. Lower: Shows the regression with the highest R^2 value with $L_c = 3.8$ cm.

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