Hydraulic architecture of two species differing in wood density: opposing strategies in co-occurring tropical pioneer trees

KATHERINE A. MCCULLOH[1], DANIEL M. JOHNSON[2], FREDERICK C. MEINZER[3], STEVEN L. VOELKER[4], BARBARA LACHENBRUCH[1] & JEAN-CHRISTOPHE DOMEC[2,5]

[1]*Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR 97331, USA,* [2]*Nicholas School of the Environment, Duke University, Durham, NC 27708, USA,* [3]*USDA Forest Service-Pacific Northwest Research Station, 3200 SW Jefferson Way, Corvallis, OR 97331, USA,* [4]*Department of Biology, Southern Oregon University, Ashland, OR 97520, USA and* [5]*Bordeaux SciencesAgro, UMR 1220 TCEM INRA, 1 cours du général de Gaulle, 33175 Gradignan Cedex, France*

ABSTRACT

Co-occurring species often have different strategies for tolerating daily cycles of water stress. One underlying parameter that can link together the suite of traits that enables a given strategy is wood density. Here we compare hydraulic traits of two pioneer species from a tropical forest in Panama that differ in wood density: *Miconia argentea* and *Anacardium excelsum*. As hypothesized, the higher wood density of *Miconia* was associated with smaller diameter vessels and fibres, more water stress-resistant leaves and stems, and roughly half the capacitance of the lower wood density *Anacardium*. However, the scaling of hydraulic parameters such as the increases in leaf area and measures of hydraulic conductivity with stem diameter was remarkably similar between the two species. The collection of traits exhibited by *Miconia* allowed it to tolerate more water stress than *Anacardium*, which relied more heavily on its capacitance to buffer daily water potential fluctuations. This work demonstrates the importance of examining a range of hydraulic traits throughout the plant and highlights the spectrum of possible strategies for coping with daily and seasonal water stress cycles.

Key-words: *Anacardium excelsum*; *Miconia argentea*; hydraulic conductivity; vulnerability curves; xylem anatomy.

INTRODUCTION

The xylem of plants carries and replaces the enormous quantity of water lost from leaves on a daily basis and is a tissue that varies considerably across species (Wheeler, Baas & Rodgers 2007). Even within a wood type, variability in xylem anatomy can lead to differences in hydraulic function (McCulloh *et al*. 2010), and considerable focus has been on the impact of this variation across species. However, there is also important variation within individuals, and this

Correspondence: K. A. McCulloh. e-mail: kate.mcculloh@ oregonstate.edu

has received somewhat less attention, especially recently (Meinzer *et al*. 2010). For example, conduit diameters narrow and their number per area increase from the bottom to the tops of trees. These axial gradients in anatomy lead to shifts in hydraulic functional traits along water flow paths within plants (Zimmermann 1978; Ewers & Zimmermann 1984; Domec & Gartner 2002; Dunham, Gartner & Ganio 2007)

One parameter of particular interest in the study of hydraulic architecture is wood density. This characteristic has been shown to influence water storage capacity (Meinzer *et al*. 2003, 2008a; Pratt *et al*. 2007; Scholz *et al*. 2007), hydraulic conductivity (Bucci *et al*. 2004; Santiago *et al*. 2004), resistance to drought-induced embolism (Hacke *et al*. 2001; Pratt *et al*. 2007) and, consequently, growth rate (Muller-Landau 2004; King *et al*. 2005; Poorter *et al*. 2008, 2010; Wright *et al*. 2010). Given that the density of the wall material in wood is constant (~1.5 g cm^{-3}; Panshin & de Zeeuw 1980), variation in bulk wood density is largely the result of differences in the proportion of wood volume devoted to lumen space (Gartner, Moore & Gardiner 2004). Recent work has shown that wood density may depend on variation in the dimensions and frequencies of vessels and fibres (Preston, Cornwell & DeNoyer 2006; Poorter *et al*. 2010) or almost exclusively on fibre traits (Martinez-Cabrera *et al*. 2009). The diversity of anatomical patterns leading to observed variation in wood density across ecological gradients suggests that there may be distinct limitations to inferences for plant hydraulic function drawn from such broad surveys without being paired with detailed physiological measurements.

The main goal of this study was to compare co-occurring species differing in wood density to provide insight into the mechanisms by which these strategies affect whole-plant hydraulic function. Research on late successional tropical forest species showed that higher wood density was associated with narrower vessels and lower branch sap flux, and was ultimately linked to lower whole-tree leaf-specific conductance compared with pioneer species that had lower wood density (McCulloh *et al*. 2011). The aforementioned

study was conducted on mature individuals and examined a suite of physiological and anatomical traits from trunks to twigs. However, environmental stresses are more likely to limit growth and survival in smaller individuals due to their less extensive root systems and carbon stores (Greenwood *et al.* 2008). Furthermore, there are at least two difficulties in studying a wide variety of hydraulic parameters using this approach. Firstly, big trees are more complicated architecturally than small trees (Ishii & McDowell 2002). Although mature individuals should show the most variation in characteristics that change along an axial gradient, the sampling required to capture that variability can be prohibitively time consuming or require many assumptions. Secondly, working with small individuals of species differing in wood density often means selecting trees from different successional status or growing environment. For example, slow-growing, late successional species often have higher wood density than fast-growing pioneer species (Enquist *et al.* 1999; Muller-Landau 2004; King *et al.* 2005; Poorter *et al.* 2008, 2010). However, when the late successional species are at a manageable size for extensive measurements, they are often growing in the understory while pioneer species of the same size are often found in gaps. These two environments differ in ways that can strongly influence the hydraulic needs of the individuals. The understory experiences different environmental conditions because it is relatively sheltered with lower radiation, temperature and vapour pressure deficit than the overstory (Ishida, Yamamura & Hori 1992). Furthermore, it has been shown in mature trees that successional status can have a large impact on hydraulic architecture (McCulloh *et al.* 2011), so teasing apart the influence of successional status from growing environment can be problematic. The link between wood density and growth rate may also mean that species with the same successional status often have roughly the same wood density.

We therefore chose to compare two pioneer species from the lowland tropical rainforest of the Republic of Panama that differed in their wood density. *Miconia argentea* and *Anacardium excelsum* are both common species in gaps, secondary forests, and disturbed areas of Central America and northern South America. However, *Miconia* is a relatively slow-growing pioneer (Brokaw 1987) and has denser wood than *Anacardium*. We hypothesized: (1) that the greater wood density in *Miconia* would be associated with it having narrower diameter conduits throughout the plant; (2) that this anatomical difference would lead to lower hydraulic conductivity; (3) that the differences in wood density would also result in lower stem capacitance values in *Miconia* than in *Anacardium*, which would result in *Miconia* being less buffered against daily fluctuations in water potential than *Anacardium*; and (4) that this would result in *Miconia* being more resistant to drought-induced embolism.

MATERIALS AND METHODS

Species collection

Individuals of *M. argentea* (SW.) DC. (Melastomataceae) and *A. excelsum* (Bentero & Balb. Ex Kunth) Skeels

(Anacardiaceae) were collected from a seasonally dry tropical forest in the Republic of Panama. Collection and measurements were made during February 2010, which is during the 3–4 month annual dry season. Small trees ranging in size from 2.7 to 6.3 m in height were collected from the laboratory clearing on Barro Colorado Island and along Pipeline Road in Parque Nacional Soberanía (both are approximately 9°N 79°W). The average height of the eight *Miconia* individuals sampled was 4.4 (±0.8) m, and for the five *Anacardium* individuals was 4.0 (±1.1) m. Both sites are frequently disturbed and all individuals were growing in full or near full (>half the day) sun.

All leaves from the first two individuals collected of both species were counted and measured using a leaf area meter (Li-3100C; Li-Cor Biosciences, Lincoln, NE, USA). From this information, we estimated an average leaf size for each species. For subsequent trees, we counted the number of leaves and calculated the leaf area by multiplying the number by the average area per leaf. We also recorded the number of leaves distal to each stem segment used for hydraulic measurements in order to calculate the leaf-area specific conductivity (see below).

Stem hydraulic measurements

Stems of various diameters were selected from harvested trees for measurements of hydraulic conductivity and vulnerability to drought-induced embolism. The stem segments were taken from a range of locations on saplings ~4 m tall and were selected to represent a range of diameters. Thus, trunks, intermediate and distal branches were all measured. If a stem segment was greater than ~1.5 cm wide, a piece of it was split out along the grain with chisel and hammer (Domec & Gartner 2001). Prior to each measurement, these chiselled segments were wrapped tightly with parafilm to prevent leakage from vessels that had been severed during the preparation. The parafilm was removed after each hydraulic measurement for the air-injection step. The transverse ends of all segments were cut under water with fresh razor blades. Segments were kept as long as possible, but were cut to fit into a container to refill embolized vessels by submerging them in water overnight, which limited lengths to approximately 20 cm. A partial vacuum was applied overnight to the container to draw air out of the stems. Samples were inspected to ensure no bubbles remained on the axial ends before the vacuum was removed and measurements were begun.

After segments were flushed overnight, measurements of hydraulic conductivity (k_h) were begun to construct vulnerability curves. These curves are created from measurements of k_h alternated with radial injection of air at known pressures, which is intended to reduce the conductivity through the formation of embolisms. The 'air-injection' method (Sperry & Saliendra 1994) assumes that vessels embolize at a pressure that has the same magnitude, but opposite sign (i.e. positive versus negative), as *in situ*. Hydraulic conductivity was measured by timing the intervals for water to reach successive gradations on a pipette attached with

tubing to the distal end of the segment, and dividing this volume flow rate by the pressure gradient used to induce flow across the sample. The pressure gradient was created by a hydrostatic pressure head, which was never more than 15 cm high to avoid refilling embolized vessels. Air pressures imposed on stems between each measurement of k_h were increased in 0.3 MPa increments. Vulnerability curves were graphed by comparing the percent loss in k_h relative to the maximum versus the applied pressure.

Other measures of hydraulic function were also computed to compare among the stem segments. The leaf area (k_L)- and xylem area (k_S)-specific hydraulic conductivity were calculated as maximum k_h divided by leaf area distal to the segment and xylem cross-sectional area, respectively.

Recent evidence suggests that segments that contain large numbers of vessels that transit the length of the segment without an endwall (i.e. 'open vessels') have vulnerability curves that differ from those of segments that are long enough to not contain open vessels (Choat *et al.* 2010; Cochard *et al.* 2010; Ennajeh *et al.* 2011). We performed two tests during the dry season of 2011 to assess this issue and its potential impact on our previously collected data. Firstly, we pushed air into one end of stem segments approximately 20 cm long (the length of the segments used the previous year) and held the other end under water to see if bubbles could easily pass through the vessels. If the submerged end emitted bubbles, we assumed that the segment contained open vessels. Secondly, we constructed new vulnerability curves on much longer segments (50–80 cm long) to compare with the measurements made during the 2010 field season.

Small pieces (~1 × 1 cm) of stem (wood and pith) representing the range of diameters of the samples used for hydraulic measurements from both species were used to construct moisture release curves. Eleven samples were measured for *Anacardium* and 18 were used for *Miconia*. For these measurements, the samples were vacuum infiltrated overnight in water. The saturated samples were then blotted on a paper towel to remove excess water, weighed and placed in screen cage thermocouple psychrometer chambers (83 series; JRD Merrill Specialty Equipment, Logan, UT, USA). These chambers were then double bagged and submerged in a cooler of water for 2–3 h to allow the sample to equilibrate with the chamber air. After the equilibration period, the millivolt readings were recorded using a 12-channel digital psychrometer reader (85 series; JRD Merrill Specialty Equipment). Following the measurement, the samples were removed from the chambers, weighed and allowed to dry on the laboratory bench for approximately half an hour before repeating the process (except for the saturation step). The mV output by the psychrometer was converted to MPa based on calibration curves from salt solutions of known water potentials. Samples were measured repeatedly until water potential values reached approximately –4 MPa. Samples were then placed in the drying oven overnight before weighing the dry mass. For each of the repeated measurements, the relative water content (RWC) was calculated as:

$$RWC = \frac{M_f - M_d}{M_s - M_d} \quad (1)$$

where M_f is the sample mass for the measurement, M_d is the dry mass and M_s is the saturated mass of the sample. From RWC, relative water deficit (RWD) was calculated as 1 – RWC. The product of RWD and the mass of water per unit tissue volume at saturation (M_w) yielded the cumulative mass of the water lost at each measurement. M_w was calculated as

$$= \left(\frac{M_s}{M_d} * \rho \right) - \rho \quad (2)$$

where ρ is wood density. Then, by graphing moisture release curves, which compare the cumulative mass of water lost versus the sapwood water potential, the capacitance of the sample could be estimated by plotting a regression to the initial, nearly linear, phase of the plot, which encompassed the likely *in situ* physiological operating range of stem water potential (Meinzer *et al.* 2003, 2008b).

Stem morphological and anatomical measurements

The wood density was measured for all stem segments used for hydraulics. To do this, a short segment (~3 cm long) was cut from one end of the sample, the pith and bark were removed, and the fresh volume was determined by submerging the wood into a cup of water on a scale. The mass difference caused by the sample, which equals the volume of water displaced by the sample, was recorded and converted to volume based on the density of water as 1.0 g cm^{-3} at standard temperature and pressure. The sample was then placed in a drying oven at 100 °C overnight and the dry mass was subsequently measured. Density was calculated as oven-dry mass per green volume.

Vessel and fibre lumen dimensions were measured for a subset of the stems used for hydraulic measurements that represented a range of the diameters. Cross-sections of the stems were made near the midpoint of the segment by hand using fresh razor blades. The sections were mounted in glycerin and images of them were taken using a Photometrics Coolsnap camera (Tucson, AZ, USA) mounted on a Nikon Eclipse E400 microscope connected to a PC using Metavue software (Universal Imaging Corp., Downington, PA, USA). Images were analysed using the freeware software ImageJ (NIH, Bethesda, MD, USA; http://rsbweb.nih.gov/ij/). All vessels bounded by two sets of rays were measured from the cambium to the pith (or the entire cross-section for the larger, chiselled pieces) in three sections of each stem. This resulted in at least 100 vessels measured, or more sections were measured until at least 100 vessels were assessed. The area defined by the rays was also measured. For fibre measurements, 100 fibres were assessed without regard for radial location relative to the cambium, but with more emphasis placed on the ability to clearly see the

dimensions of the much smaller lumen areas. The area of the lumen was measured for both fibres and vessels, and the lumen diameter was calculated by assuming the area was the shape of a circle.

Leaf hydraulic measurements

Leaf hydraulic conductance (K_{leaf}) was measured using the timed rehydration method (Brodribb & Holbrook 2003). This method assumes that rehydrating a leaf is equivalent to recharging a capacitor, and calculates K_{leaf} as:

$$K_{leaf} = C \ln(\Psi_o / \Psi_f)/t \qquad (3)$$

where C = leaf capacitance, Ψ_o = leaf water potential before partial rehydration, Ψ_f = leaf water potential after partial rehydration and t = time of rehydration. To make these measurements, branches ~0.5 m-long were taken from the individuals we had already collected and from other, similarly sized trees, and were transported to the lab. Collections were made early in the morning to minimize water stress. In the lab, the branches were re-cut under water and allowed to rehydrate for 4–6 h in distilled water. To construct leaf hydraulic vulnerability curves, stems were then bench dried for periods ranging from 15 min to 2 h before being bagged in thick plastic bags and equilibrated in the dark for 1–2 h. Leaves were measured within 24 h of rehydration and were refrigerated overnight. Adjacent pairs of leaves were taken for measurements of rehydration kinetics in which the first leaf was measured as Ψ_o while the second was rehydrating for a period of t seconds in distilled water before it was used to measure Ψ_f. Water temperature was maintained between 22 and 24 °C. Water potential measurements were made with a 70-bar pressure chamber (PMS Instrument Company, Albany, OR, USA). Between 39 and 45 pairs of leaves were measured for each vulnerability curve.

Values of leaf C were calculated from pressure–volume curves (Scholander *et al.* 1965; Tyree & Hammel 1972) made on leaves from the same branches collected for K_{leaf} measurements. Pressure–volume curves were made on four leaves from each species and created by alternately measuring leaf masses and water potential measurements as a leaf was allowed to air dry and plotting the inverse of the water potential with the corresponding relative water content. The leaf water potential at the turgor-loss point was estimated as the inflection point on this graph. Leaf C-values for each species were determined as the slope of the curve before and after the turgor-loss point on a graph of leaf water potential versus relative water content. All values of percent loss in K_{leaf} shown here are relative to the average value of the bin with the maximum absolute K_{leaf} (i.e. the first bin on the right for *Anacardium* and the second for *Miconia*).

Field water potentials were measured at midday (between 1200 and 1400) on four leaves from each species. Measurements were made on 1, 9 and 18 February 2011, which were all sunny days. Approximately 2 h before the

measurements were made, we sealed plastic bags that had been covered with aluminium foil over a shoot on each of three individuals. This bag allowed the water potential of the leaves to equilibrate with the stems. Midday water potential measurements were made on one to two bagged and unbagged leaves from each individual. The water potentials of bagged samples were assumed to represent stem water potentials.

Statistical analyses

Linear comparisons of measured traits were made using the standardized major axis (SMA) technique. This process was done using the freeware program SMATR (Warton *et al.* 2006; http://www.bio.mq.edu.au/ecology/SMATR/). Means of traits were compared between the two species using Student's *t*-tests. Lines were fitted to vulnerability curves of stems and leaves using all data points, but only mean values are shown in figures for simplicity. Probability levels of $P < 0.05$ were considered significant and those below 0.1 were considered marginal.

RESULTS

Wood density was higher in individuals of *Miconia* (0.59 g cm^{-3}) than *Anacardium* (0.38 g cm^{-3}; *t*-test P value < 0.0001). Despite greater variation in wood density of small diameter stems, only *Miconia* showed a relationship (P value = 0.001) between wood density and stem diameter (Fig. 1a; *Anacardium* P value = 0.8). In contrast, mean vessel diameter increased with stem diameter in both species (Fig. 1b), but showed relationships unique to each species (SMA relationships differed at P value < 0.01), with *Anacardium* exhibiting wider diameter conduits for a given stem diameter. The relationships between mean fibre lumen diameter and stem diameter were also unique to each species, with *Miconia* exhibiting a negative one and *Anacardium* exhibiting a positive relationship (Fig. 1c). The high amount of scatter in the *Miconia* data resulted in only a marginally significant relationship (P value = 0.068). Although both species showed a nearly flat relationship between wood density and stem diameter (Fig. 1a), fibre lumen diameter did decline with greater wood density in both species along a common slope (Fig. 2). When the species were considered separately, the relationship was not significant in *Anacardium* (P value = 0.48), but was in *Miconia* (P value = 0.03), and the *Miconia* relationship was not different from the common slope (P value = 0.66).

Despite the wider conduits in *Anacardium*, the same leaf area was supported by a given stem diameter in both species (Fig. 3, P value < 0.001). This scaling occurred although the average area per leaf of the *Miconia* individuals was 1.5 times that of *Anacardium* (211 cm^2 versus 140 cm^2; *t*-test P value = 0.05). The scaling of hydraulic functional traits with stem diameter between the two species was also nearly identical (Fig. 3, P values < 0.01). The scatter increased when hydraulic conductivity was considered on a leaf area- (k_L Fig. 3b) and wood-area specific basis (k_S; Fig. 3c).
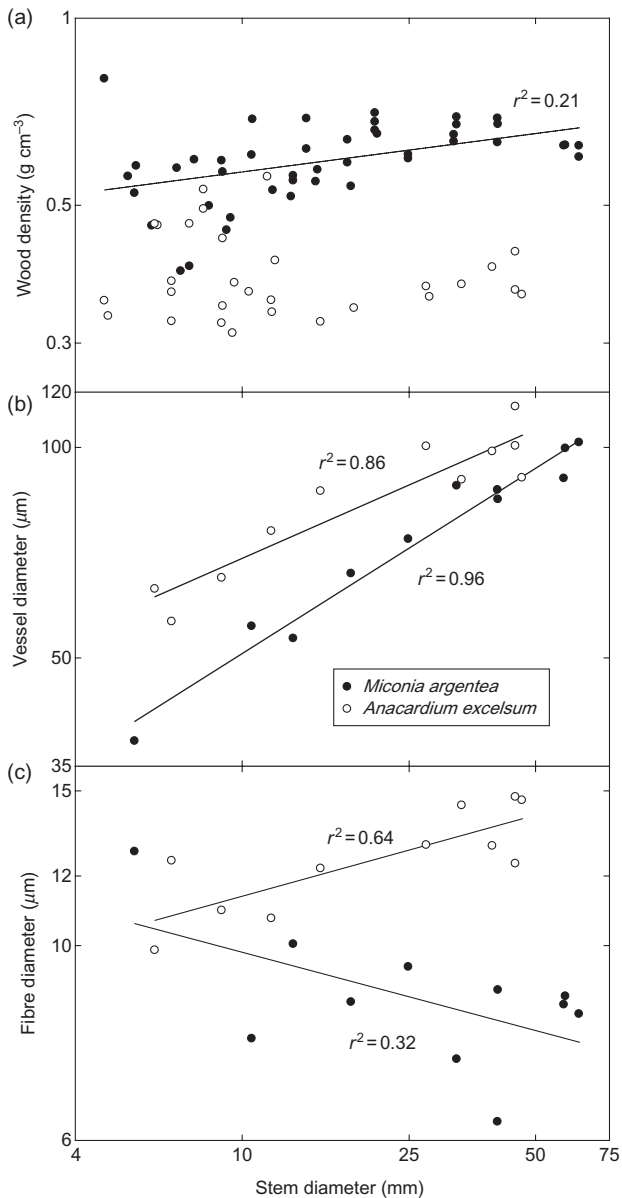
Figure 1. Wood density (a) and vessel diameter (b) and fibre diameter (c) versus stem diameter for two tropical pioneer species on log–log scales. Lines show standardized major axis (SMA) relationships, which were significant at P value < 0.001. The SMA equations are $y = 0.1\,x + 0.5$ for (a), $y = 0.4\,x + 1.3$ for *Miconia* and $y = 0.3\,x + 1.6$ for *Anacardium* in (b), and $y = -0.1\,x + 1.1$ for *Miconia* and $y = 0.1\,x + 0.9$ for *Anacardium* in (c).

Leaf hydraulic conductance (K_{leaf}) declined more rapidly with water potential in *Anacardium* than in *Miconia* (Fig. 4). *Anacardium* exhibited a steep rise in the percent loss of K_{leaf} and had lost nearly all conductance by approximately −1.3 MPa. In contrast, K_{leaf} declined more slowly in *Miconia* and did not approach complete loss until nearly −2.3 MPa. The pressure at which 50% of the leaf hydraulic conductance was lost (P_{50}) also reflected this difference between the species, with it equalling −1.6 MPa for *Miconia* and −0.9 MPa for *Anacardium*

(95% confidence intervals are not overlapping at P_{50}). Despite *Miconia* having much lower midday leaf water potentials than *Anacardium* (Fig. 4, compare vertical lines), the percent loss of K_{leaf} at midday was roughly equal, which was 29% loss at midday for *Anacardium* compared with 24% loss at midday for *Miconia*. The maximum absolute value of the K_{leaf} was slightly higher in *Anacardium* than *Miconia* (12.1 versus 9.8 mmol m^{-2} s^{-1} MPa^{-1}, respectively).

Measurements of open vessels in stems of approximately 20 cm long made during 2011 revealed that vessels exceeded this length in stems greater than 12 mm in diameter for both species (data not shown). Thus, stems wider than 12 mm diameter were predicted to show artificially high xylem vulnerability (Choat *et al.* 2010; Cochard *et al.* 2010; Ennajeh *et al.* 2011) and these data were discarded. However, the vulnerability curves for narrow stems showed that *Miconia* tended to be more resistant to drought-induced embolism formation than *Anacardium* (Fig. 5): the pressure at which 50% of the hydraulic conductivity (P_{50}) was lost from *Anacardium* stems was lower than for *Miconia* (0.9 versus 1.4 MPa; 95% confidence intervals are not overlapping at P_{50}). The vulnerability curves of these small diameter size classes were also reconfirmed in 2011 with longer stems, which showed the same patterns (data not shown). Stem water potentials at midday differed between the two species as well (t-test P value = 0.03), with *Anacardium* being less negative. Stem water potentials implied that 22 and 27% of the hydraulic conductivity would be lost from the stems at midday for *Miconia* and *Anacardium*, respectively.

Within each species, there was a slight trend for smaller diameter stems to have greater estimated values of capacitance than larger diameter stems, but this difference was not significant (Fig. 6). This distinction is clear in *Anacardium*
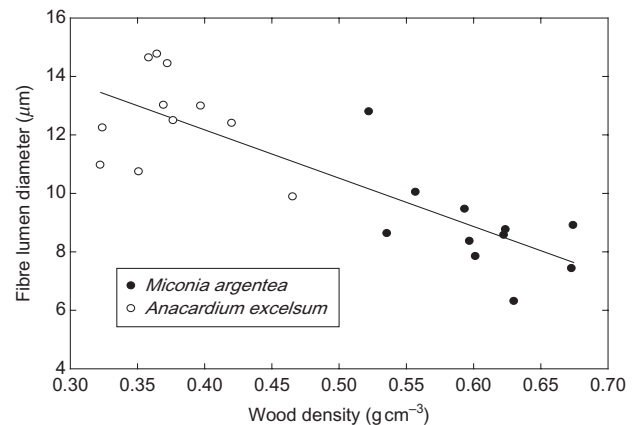

Figure 2. Fibre lumen diameter versus wood density for two tropical pioneer species. The intraspecific slope for the standardized major axis (SMA) relationship in *Anacardium* was not significant, but the significant relationship in *Miconia* did not differ from the interspecific slope, which is shown (P value < 0.01). The SMA equation is $y = -16.5\,x + 18.8$ and the r^2 value is 0.68.
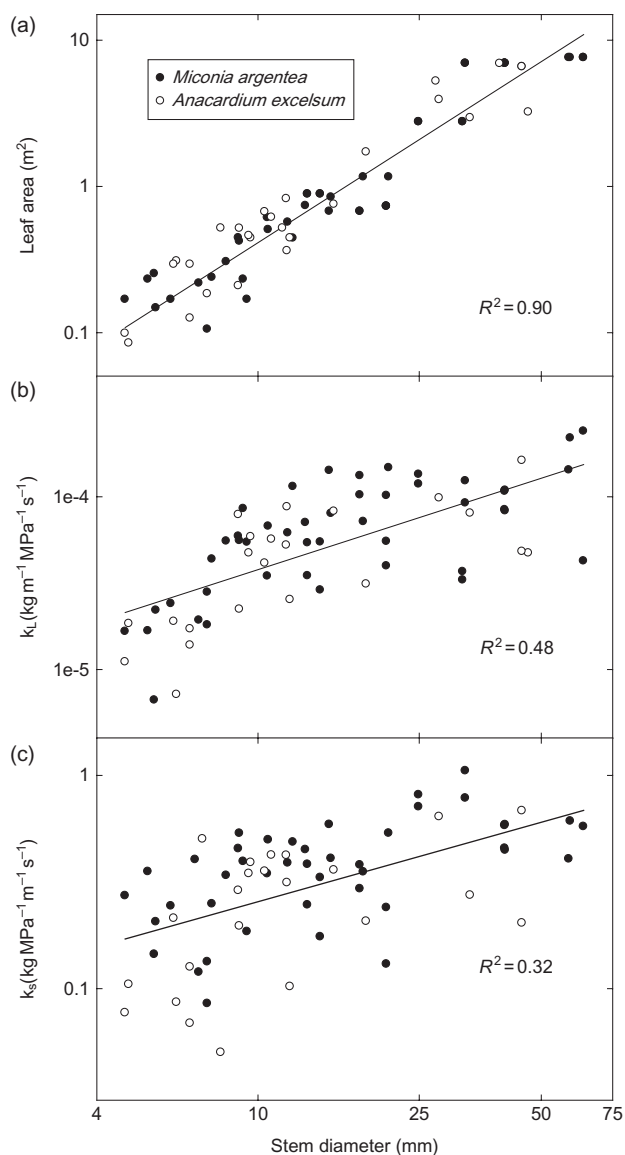
Figure 3. Scaling of leaf area and hydraulic function with stem diameter for two tropical pioneer species on log–log plots. Shown are (a) leaf area, (b) leaf-specific conductivity (k_L) and (c) xylem area-specific conductivity (k_S). The slopes were not different between the two species, so only the common slope is shown for each relationship (P values < 0.05). The standardized major axis (SMA) equations are $y = 1.8\,x - 2.2$ for (a), $y = 0.8\,x - 5.2$ for (b), and $y = 0.5\,x - 1.1$ for (c).

Figure 4. Percent loss in leaf hydraulic conductance (K_{leaf}) relative to the maximum value for each species versus leaf water potential. Bars indicate standard errors. Relationships within species are defined by three-parameter sigmoid plots. The vertical lines indicate the average (from three individuals on each of 3 d) leaf water potentials at midday for *Miconia* (dashed) and *Anacardium* (solid).

DISCUSSION

The greater wood density of *Miconia* was associated with it having smaller diameter vessels (Fig. 1), stems that were more resistant to drought-induced embolism (Fig. 5), and lower values of capacitance (Fig. 6) than *Anacardium*. In coordination with the stems, the leaves of *Miconia* were also more resistant to loss of hydraulic function with increasing water stress (Fig. 4). Furthermore, fibre diameter increased with stem diameter in *Anacardium*, but decreased in *Miconia*. Despite these differences, the scaling of leaf area and hydraulic properties with increases in stem diameter were indistinguishable between the two species (Fig. 3) and

when trunk versus branch values are compared, and it is also apparent that the branches had greater total amounts of volumetric water released. However, between the two species, *Anacardium* had significantly greater capacitance than *Miconia*. The volumetric water released at the midday water potential, which was calculated as the area defined by the capacitance regression and the vertical line indicating midday stem water potential, was more than two times higher in *Anacardium* than *Miconia*.
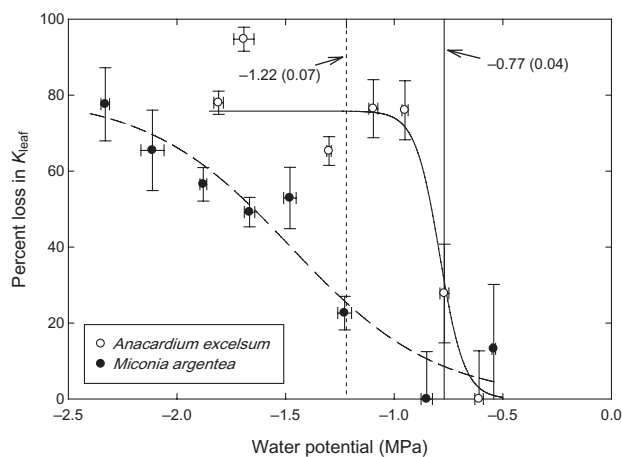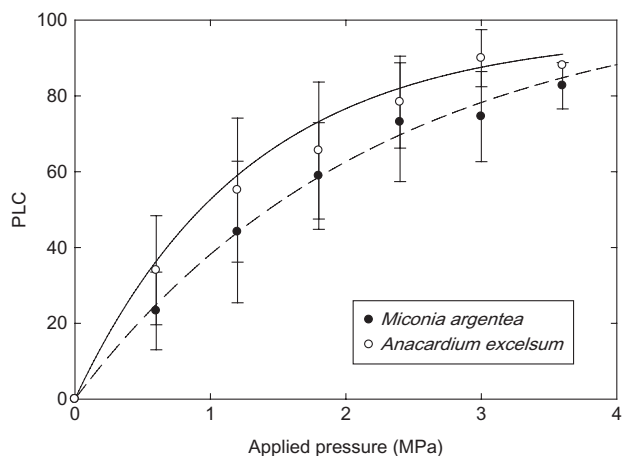

Figure 5. Stem vulnerability curves showing percent loss in hydraulic conductivity (PLC) vs. applied pressure for small diameter branches of two tropical pioneer species. Error bars indicate standard deviations. Relationships within species are defined by two-parameter exponential rise-to-maximum plots.
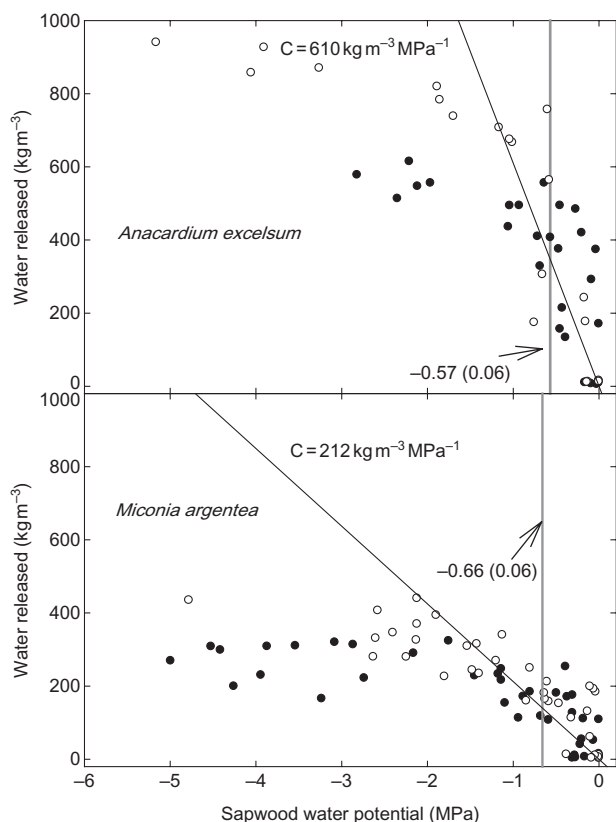
Figure 6. Volumetric water release curves for two species of tropical pioneer species. The initial, linear portion of each curve was used to calculate the capacitance for each species. Within each panel, the open symbols represent branches and the closed symbols are trunks. The grey, vertical lines are the average (from three individuals on each of 3 d) stem water potentials at midday for each species.

fibre diameter declined along a common slope with wood density (Fig. 2). Although the relationship between leaf area and stem diameter was linear on a log–log plot, the relationship remained linear on a plot with linear axis scales, which means that as the trees grow, there would be a non-linear relationship between leaf area : sapwood area. At some point, this would result in less and less leaf area added per given diameter increment. The nearly identical scaling of hydraulic conductivity with stem diameter (data not shown) means that whole-shoot hydraulic conductance would also scale similarly with stem diameter for these two species (McCulloh *et al.* 2010). The interspecific relationship between hydraulic conductivity and stem diameter (not shown) was a strong one ($r^2 = 0.84$), which is somewhat surprising given the greater vessel diameter in *Anacardium* (Fig. 1b). This result could be due to *Anacardium* having shorter vessels, fewer inter-vessel pits per vessel and/or fewer vessels per area. No differences were found in the last parameter (data not shown) and the first two were, unfortunately, not measured. Overall, these results support our hypotheses that greater wood density would be associated with smaller diameter vessels, increased hydraulic safety (more drought-resistant wood) and lower values of capacitance. However,

our prediction that the lower wood density in *Anacardium* would result in greater hydraulic efficiency (i.e. greater hydraulic conductivity) was not supported.

Both species had midday stem and leaf water potentials that were predicted to impair the hydraulic function of those organs by roughly equal amounts, yet the suite of traits examined here reflects two distinct approaches to the regulation of daily cycles of hydraulic stress. In *Anacardium*, the lower wood density allows for greater capacitance, which buffers the daily fluctuations in water potential (Fig. 6) and is associated with less embolism-resistant wood and leaf tissue, whereas for *Miconia*, the higher density wood is associated with greater resistance to daily water stress in both the stems and leaves. This reliance on capacitance to cushion the daily water deficit observed in *Anacardium* continues to play a vital role in mature individuals, and many other species of low-wood density pioneers (Meinzer *et al.* 2003, 2008a,b), which also show similar values of loss of hydraulic function at midday water potentials (Meinzer *et al.* 2008b). The capacitance measured in the *Anacardium* saplings studied here was approximately two times greater than in the sapwood of mature *Anacardium* (Meinzer *et al.* 2003). This difference is most likely due to the inclusion of pith in samples that were narrow enough for the entire stem diameter to fit in the psychrometer chamber (Fig. 6, open symbols). Our reasoning was that samples this narrow may very well draw on water stored within their pith. An additional source of some capacitive water for these trees could be from embolized vessels, given that the midday water potentials predict that the hydraulic conductivity would be reduced by 27%, assuming they are able to refill each night. The greater resistance to embolism in *Miconia* may confer an advantage by reducing the dry-season mortality compared with other pioneer species (Pearson *et al.* 2003a). *Miconia* also benefits from its investment in drought resistance by having relative growth rates of leaves that are roughly equal to other pioneers during the wet season, but that exceed the others during the dry season (Pearson *et al.* 2003b). Although *Anacardium* was not among the pioneer species studied in the work of Pearson and colleagues, the species that were studied (*Trema micrantha, Cecropia insignis, Luehea seemannii, Ochroma pyramidale* and *Croton bilbergianus*) have wood density more similar to *Anacardium* than *Miconia* (K.A. McCulloh & D.M. Johnson, unpublished results).

The strong dependence on structural tolerance of daily cycles of water stress observed in *Miconia* may be partially responsible for its maximum height being approximately half that of *Anacardium* (~20 m versus ~40 m, respectively; Croat 1978). As a north-temperate conifer (*Pseudotsuga menziesii*) approaches its maximum height (Domec *et al.* 2008), it apparently spirals into a cycle in which the low water potentials prevent tracheids from expanding as much as they could at higher water potentials, resulting in diminished pit dimensions in new growth, which increase the resistance to water stress-induced embolism, but reduce the hydraulic conductivity, forcing a further drop in the water potential necessary to sustain a given water flux,

which further constrains cell expansion. Although there are obvious differences between *P. menziesii* and the species examined here, size-related limitations on cell growth have also been observed in *Fraxinus excelsior, Acer pseudoplatanus, Pinus sylvestris* and a poplar clone (Mencuccini *et al*. 2005), as well as in *Sequoia sempervirens* (Koch *et al*. 2004). Assuming a similar cycle exists for *Miconia* and *Anacardium*, *Miconia* may have a lower maximum height because of greater reliance on a structural versus capacitive strategy.

Miconia may have equivalent leaf relative growth rates to other pioneers (Pearson *et al*. 2003b) because greater flow rates of water through its xylem may support greater stomatal conductance and photosynthesis. Given that the nearly identical scaling of hydraulic conductivity with stem diameter (data not shown) of the two species would predict the same scaling of increases in whole-shoot conductance with stem diameter (McCulloh *et al*. 2010) and that the leaf area scales similarly with stem diameter (Fig. 3), then if we assume that the soil water potentials are the same for these co-occurring species, the lower midday water potentials of *Miconia* would result in greater flow on a leaf-area basis. Consistent with this, stomatal conductance is greater in *Miconia* than in *Anacardium* during the dry season (Meinzer *et al*. 1993, 1995), although this difference is confounded because the *Anacardium* studied were taller than the *Miconia*. This greater hydraulic capacity of *Miconia* presumably yields higher photosynthetic rates (Santiago *et al*. 2004) and the extra carbon required to grow high-density wood at equivalent rates of other, lower-density pioneer species.

Greater wood density has been shown to be associated with greater resistance to drought-induced embolism (Hacke *et al*. 2001; Pratt *et al*. 2007). In fact, it has been proposed that small diameter fibres may be needed to provide the mechanical strength necessary to resist the significant negative pressures experienced under drought conditions (Jacobsen *et al*. 2005). However, given the tradeoff between high wood density and capacitance, it is worth contemplating what benefit high-density wood is providing in terms of stem hydraulics: without the small diameter fibres, the wood density would be lower, the capacitance higher, which would buffer the water potentials, and the plant would not need to tolerate such negative pressures (Höltta *et al*. 2009). Furthermore, recent evaluations of within-plant variation in wood properties suggest that in some circumstances, low-density wood is more biomechanically adaptive while in others high-density wood is (Larjavaara & Muller-Landau 2010; Lachenbruch, Moore & Evans 2011). A possible alternative benefit that the high wood density could be providing is one of defence against decay (Mackensen, Bauhus & Webber 2003; Weedon *et al*. 2009). In our search for suitable species for this study, we noted an apparent tradeoff in which pioneer species either tended to have higher density wood or defences such as mucilage, latex or resins, but not both. Thus, it may be that higher-density wood defends the tree from decay, but then the lack of capacitive ability requires a distinct suite of traits to resist the resulting lower water potentials.

Within each species, stem and leaf vulnerabilities to water stress-induced embolism were tightly coordinated. For neither species were the leaves considerably more or less vulnerable than the stems when comparing their P_{50} values, a metric representing the pressure at which 50% of the hydraulic function has been lost. Previous work has found species where the leaves are more (Tyree *et al*. 1993; Hao *et al*. 2008; Chen *et al*. 2009) and equally (Chen *et al*. 2010) vulnerable than stems, and differences in these strategies may reflect differences in the xylem pressure gradients in the distal portions of the plant. Martin Zimmermann (1983) proposed in his hydraulic segmentation hypothesis that plants would benefit from leaves acting as a discardable buffer that can hydraulically isolate stems. He assumed that stems and leaves were equally vulnerable to embolism within a plant and that the drop in k_L distally (e.g. Fig. 3b) would lead to a drop in xylem pressure that would cause embolism in the leaves. A decade later, Zimmermann's hypothesis was extended by Tyree and colleagues who postulated that a more effective method of isolating the woody portion of the plant would be to have leaves that were more vulnerable to embolism than stems (vulnerability segmentation hypothesis; Tyree *et al*. 1993). Data from different species suggest that both theories may be supported, but the species examined here seem to segment through the original hydraulic hypothesis and not vulnerability when the leaves and small diameter branches are compared.

The hydraulic strategy associated with high- versus low-density wood in co-occurring species is common in many habitats (Swenson & Enquist 2007), yet the tradeoffs of each approach are not entirely understood. *Miconia* has higher relative growth rates than other low-wood density pioneers (Pearson *et al*. 2003b) and is approximately 26 times more abundant than *Anacardium* in a long-term census plot on Barro Colorado Island (Hubbell, Condit & Foster 2005). However, in highly disturbed locations, these species coexist and the strategy of *Anacardium* is clearly not a hindrance to success. The results presented here highlight the importance of examining multiple hydraulic traits (Meinzer *et al*. 2009, 2010). Focusing solely on stem vulnerability curves would have ignored the impact of capacitance in *Anacardium* as an additional measure of safety. By studying a coordinated suite of traits, the different strategies employed by plants to resist diurnal and seasonal water stress, and the tradeoffs associated with them, can be explored more thoroughly.

ACKNOWLEDGMENTS

REFERENCES

Brodribb T.J. & Holbrook N.M. (2003) Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. *Plant Physiology* **132**, 2166–2173.

Brokaw N.V.L. (1987) Gap-phase regeneration of three pioneer tree species in a tropical forest. *Journal of Ecology* **75**, 9–19.

Bucci S.J., Goldstein G., Meinzer F.C., Scholz F.G., Franco A.C. & Bustamante M. (2004) Functional convergence in hydraulic architecture and water relations of tropical savanna trees: from leaf to whole plant. *Tree Physiology* **24**, 891–899.

Chen J.-W., Zhang Q., Li X.-S. & Cao K.-F. (2009) Independence of stem and leaf hydraulic traits in six *Euphorbiaceae* tree species with contrasting leaf phenology. *Planta* **230**, 459–468.

Chen J.-W., Zhang Q., Li X.-S. & Cao K.-F. (2010) Gas exchange and hydraulics in seedlings of *Hevea brasiliensis* during water stress and recovery. *Tree Physiology* **30**, 876–885.

Choat B., Drayton W.M., Brodersen C., Matthews M.A., Shackel K.A., Wada H. & McElrone A.J. (2010) Measurement of vulnerability to water stress-induced cavitation in grapevine: a comparison of four techniques applied to a long-vesseled species. *Plant, Cell & Environment* **33**, 1502–1512.

Cochard H., Herbette S., Barigah T., Badel E., Ennajeh M. & Vilagrosa A. (2010) Does sample length influence the shape of the xylem embolism vulnerability curves? A test with the Cavitron spinning technique. *Plant, Cell & Environment* **33**, 1543–1552.

Croat T.B. (1978) *Flora of Barro Colorado Island*. Stanford University Press, Stanford, CA, USA.

Domec J.-C. & Gartner B.L. (2001) Cavitation and water storage in bole xylem segments of mature and young Douglas-fir trees. *Trees* **15**, 204–214.

Domec J.-C. & Gartner B.L. (2002) Age- and position-related changes in hydraulic versus mechanic dysfunction of xylem: inferring the design criteria for Douglas-fir wood structure. *Tree Physiology* **22**, 91–104.

Domec J.-C., Lachenbruch B., Meinzer F.C., Woodruff D.R., Warren J.M. & McCulloh K.A. (2008) Maximum height in a conifer is associated with conflicting requirements for xylem design. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 12069–12074.

Dunham S.M., Gartner B.L. & Ganio L.M. (2007) Bayesian analysis of Douglas-fir hydraulic architecture at multiple scales. *Trees* **21**, 65–78.

Ennajeh M., Simões F., Khemira H. & Cochard H. (2011) How reliable is the double-ended pressure sleeve technique for assessing xylem vulnerability to cavitation in woody angiosperms? *Physiologia Plantarum* **142**, 205–210.

Enquist B.J., West G.B., Charnov E.L. & Brown J.H. (1999) Allometric scaling of production and life-history variation in vascular plants. *Nature* **401**, 907–911.

Ewers F.W. & Zimmermann M.H. (1984) The hydraulic architecture of eastern hemlock (*Tsuga canadensis*). *Canadian Journal of Botany* **62**, 940–946.

Gartner B.L., Moore J.R. & Gardiner B.A. (2004) Gas in stems: abundance and potential consequences for tree biomechanics. *Tree Physiology* **24**, 1239–1250.

Greenwood M.S., O'Brien C.L., Schatz J.D., Diggins C.A., Day M.E., Jacobson G.L., White A.S. & Wagner R.G. (2008) Is early life-cycle success a determinant of the abundance of red spruce and balsam fir? *Canadian Journal of Forest Research* **38**, 2295–2305.

Hacke U.G., Sperry J.S., Pockman W.T., Davis S.D. & McCulloh K.A. (2001) Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* **126**, 457–461.

Hao G., Hoffmann W.A., Scholz F.G., Bucci S.J., Meinzer F.C., Franco A.C., Cao K. & Goldstein G. (2008) Stem and leaf hydraulics of congeneric tree species from adjacent tropical savanna and forest ecosystems. *Oecologia* **155**, 405–415.

Höltta T., Cochard H., Nikinmaa E. & Mencuccini M. (2009) Capacitive effect of cavitation in xylem conduits: results from a dynamic model. *Plant, Cell & Environment* **32**, 10–21.

Hubbell S.P., Condit R. & Foster R.B. (2005) Barro Colorado Forest census plot data. URL http://ctfs.arnarb.harvard.edu/webatlas/datasets/bci

Ishida A., Yamamura Y. & Hori Y. (1992) Roles of leaf water potential and soil-to-leaf hydraulic conductance in water use by understory woody plants. *Ecological Research* **7**, 213–223.

Ishii H. & McDowell N. (2002) Age-related development of crown structure in coastal Douglas-fir trees. *Forest Ecology and Management* **169**, 257–270.

Jacobsen A.L., Ewers F.W., Pratt R.B., Paddock W.A. III & Davis S.D. (2005) Do xylem fibers affect vessel cavitation resistance? *Plant Physiology* **139**, 546–556.

King D.A., Davies S.J., Nur Supardi M.N. & Tan S. (2005) Tree growth is related to light interception and wood density in two mixed dipterocarp forests of Malaysia. *Functional Ecology* **19**, 445–453.

Koch G.W., Sillett S.C., Jennings G.M. & Davis S.D. (2004) The limits to tree height. *Nature* **428**, 851–854.

Lachenbruch B., Moore J.R. & Evans R. (2011) Radial variation in wood structure and function in woody plants, and hypotheses for its occurrence. In *Age- and Size-Related Changes in Tree Structure and Function* (eds F.C. Meinzer, B. Lachenbruch & T.E. Dawson), pp. 165–192. Springer-Verlag, New York, USA.

Larjavaara M. & Muller-Landau H. (2010) Rethinking the value of high wood density. *Functional Ecology* **24**, 701–705.

McCulloh K.A., Sperry J.S., Lachenbruch B., Meinzer F.C., Reich P.B. & Voelker S. (2010) Moving water well: comparing hydraulic efficiency in twigs and trunks of coniferous, ring-porous and diffuse-porous saplings from temperate and tropical forests. *The New Phytologist* **186**, 439–450.

McCulloh K.A., Meinzer F.C., Sperry J.S., Lachenbruch B., Voelker S.L., Woodruff D.R. & Domec J.-C. (2011) Comparative hydraulic architecture of tropical tree species representing a range of successional stages and wood density. *Oecologia* **167**, 27–37 .

Mackensen J., Bauhus J. & Webber E. (2003) Decomposition rates of coarse woody debris – a review with particular emphasis on Australian tree species. *Australian Journal of Botany* **51**, 27–37.

Martinez-Cabrera H.I., Jones C.S., Espino S. & Schenk H.J. (2009) Wood anatomy and wood density in shrubs: responses to varying aridity along transcontinental transects. *American Journal of Botany* **96**, 1388–1398.

Meinzer F.C., Goldstein G., Holbrook N.M., Jackson P. & Cavelier J. (1993) Stomatal and environmental control of transpiration in a lowland tropical forest tree. *Plant, Cell & Environment* **16**, 429–436.

Meinzer F.C., Goldstein G., Jackson P., Holbrook N.M., Gutierrez M. & Cavelier J. (1995) Environmental and physiological regulation of transpiration in tropical forest gap colonizing species during the dry season: the influence of boundary layer and hydraulic properties. *Oecologia* **101**, 514–522.

Meinzer F.C., James S.A., Goldstein G. & Woodruff D. (2003) Whole-tree water transport scales with sapwood capacitance in tropical forest canopy trees. *Plant, Cell & Environment* **26**, 1147–1155.

Meinzer F.C., Campanello P.I., Domec J.-C., Gatti M.G., Goldstein G., Villalobos-Vega R. & Woodruff D.R. (2008a) Constraints on physiological function associated with branch architecture and

wood density in tropical forest trees. *Tree Physiology* **28,** 1609–1617.

Meinzer F.C., Woodruff D.R., Domec J.-C., Goldstein G., Campanello P.I., Gatti M.G. & Villalobos-Vega R. (2008b) Coordination of leaf and stem water transport properties in tropical forest trees. *Oecologia* **156,** 31–41.

Meinzer F.C., Johnson D.M., Lachenbruch B., McCulloh K.A. & Woodruff D.R. (2009) Xylem hydraulic safety margins in woody plants: coordination of stomatal control of xylem tension with hydraulic capacitance. *Functional Ecology* **23,** 922–930.

Meinzer F.C., McCulloh K.A., Lachenbruch B., Woodruff D.R. & Johnson D.M. (2010) The blind men and the elephant: the impact of context and scale in evaluating conflicts between plant hydraulic safety and efficiency. *Oecologia* **164,** 287–296.

Mencuccini M., Martinez-Vilalta J., Vanderklein D., Hamid H.A., Korakaki E., Lee S. & Michiels B. (2005) Size-mediated ageing reduces vigour in trees. *Ecology Letters* **8,** 1183–1190.

Muller-Landau H.C. (2004) Interspecific and intersite variation in wood specific gravity of tropical trees. *Biotropica* **36,** 20–32.

Panshin A.J. & de Zeeuw C. (1980) *Textbook of Wood Technology: Structure, Identification, Properties, and Uses of the Commercial Woods of the United States and Canada* 4th edn. McGraw-Hill, New York, NY, USA.

Pearson T.R.H., Burslem F.R.P., Goeriz R.E. & Dalling J.W. (2003a) Interactions of gap size and herbivory on establishment, growth and survival of three species of neotropical pioneer trees. *The Journal of Ecology* **91,** 785–796.

Pearson T.R.H., Burslem F.R.P., Goeriz R.E. & Dalling J.W. (2003b) Regeneration niche partitioning in neotropical pioneers: effects of gap size, seasonal drought and herbivory on growth and survival. *Oecologia* **137,** 456–465.

Poorter L., Wright S.J., Paz H., *et al.* (2008) Are functional traits good predictors of demographis rates? Evidence from five neotropical forests. *Ecology* **89,** 1908–1920.

Poorter L., McDonald I., Alarcón A., Fichtler E., Licona J.-C., Peña-Claros M., Sterck F., Villegas Z. & Sass-Klaassen U. (2010) The importance of wood traits and hydraulic conductance for the performance and life history strategies of 42 rainforest tree species. *The New Phytologist* **185,** 481–492.

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. *The New Phytologist* **174,** 787–798.

Preston K.A., Cornwell W.K. & DeNoyer J.L. (2006) Wood density and vessel traits as distinct correlates of ecological strategy in 51 California coast range angiosperms. *The New Phytologist* **170,** 807–818.

Santiago L.S., Goldstein G., Meinzer F.C., Fisher J.B., Machado K., Woodruff D. & Jones T. (2004) Leaf photosynthetic traits scale with hydraulic conductivity and wood density in Panamanian forest canopy trees. *Oecologia* **140,** 543–550.

Scholander P.F., Hammel H.T., Bradstreet E.D. & Hemmingsen E.A. (1965) Negative hydrostatic pressure can be measured in plants. *Science* **148,** 339–345.

Scholz F.G., Bucci S.J., Goldstein G., Meinzer F.C., Franco A.C. & Miralles-Wilhelm F. (2007) Biophysical properties and functional significance of stem water storage tissues in neotropical savanna trees. *Plant, Cell & Environment* **30,** 236–248.

Sperry J.S. & Saliendra N.Z. (1994) Intra- and inter-plant variation in xylem cavitation in *Betula occidentalis*. *Plant, Cell & Environment* **16,** 279–287.

Swenson N.G. & Enquist B.J. (2007) Ecological and evolutionary determinants of a key plant functional trait: wood density and its community-wide variation across latitude and elevation. *American Journal of Botany* **94,** 451–459.

Tyree M.T. & Hammel H.T. (1972) The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. *Journal of Experimental Botany* **23,** 267–282.

Tyree M.T., Cochard H., Cruiziat P., Sinclair B. & Ameglio T. (1993) Drought-induced leaf shedding in walnut: evidence for vulnerability segmentation. *Plant, Cell & Environment* **16,** 879–882.

Warton D.I., Wright I.J., Falster D.S. & Westoby M. (2006) Bivariate line-fitting methods for allometry. *Biological Reviews of the Cambridge Philosophical Society* **81,** 259–291.

Weedon J.T., Cornwell W.K., Cornelissen J.H.C., Zanne A.E., Wirth C. & Coomes D.A. (2009) Global meta-analysis of wood decomposition rates: a role for trait variation among tree species? *Ecology Letters* **12,** 45–56.

Wheeler E.A., Baas P. & Rodgers S. (2007) Variations in dicot wood anatomy: a global analysis based on the InsideWood database. *IAWA Journal* **28,** 229–258.

Wright S.J., Kitajima K., Kraft N.J.B., *et al.* (2010) Functional traits and the growth-mortality trade-off in tropical trees. *Ecology* **91,** 3664–3674.

Zimmermann M.H. (1978) Hydraulic architecture of some diffuse-porous trees. *Canadian Journal of Botany* **56,** 2286–2295.

Zimmermann M.H. (1983) *Xylem Structure and the Ascent of Sap*. Springer-Verlag, Berlin, Germany.