Does leaf shedding protect stems from cavitation during seasonal droughts? A test of the hydraulic fuse hypothesis

Brett T. Wolfe$^{1,2}$, John S. Sperry$^1$ and Thomas A. Kursar$^{1,2}$

$^1$Department of Biology, University of Utah, Salt Lake City, UT 84112, USA; $^2$Smithsonian Tropical Research Institute, PO Box 0843-03092, Balboa, Ancon, Panama

Summary

- During droughts, leaves are predicted to act as ‘hydraulic fuses’ by shedding when plants reach critically low water potential ($\Psi_{\text{plant}}$), thereby slowing water loss, stabilizing $\Psi_{\text{plant}}$ and protecting against cavitation-induced loss of stem hydraulic conductivity ($K_s$).
- We tested these predictions among trees in seasonally dry tropical forests, where leaf shedding is common, yet variable, among species. We tracked leaf phenology, $\Psi_{\text{plant}}$ and $K_s$ in saplings of six tree species distributed across two forests.
- Species differed in their timing and extent of leaf shedding, yet converged in shedding leaves as they approached the $\Psi_{\text{plant}}$ value associated with a 50% loss of $K_s$ and at which their model-estimated maximum sustainable transpiration rate approached zero. However, after shedding all leaves, the $\Psi_{\text{plant}}$ value of one species, Genipa americana, continued to decline, indicating that water loss continued after leaf shedding. $K_s$ was highly variable among saplings within species and seasons, suggesting a minimal influence of seasonal drought on $K_s$.
- Hydraulic limits appear to drive diverse patterns of leaf shedding among tropical trees, supporting the hydraulic fuse hypothesis. However, leaf shedding is not universally effective at stabilizing $\Psi_{\text{plant}}$, suggesting that the main function of drought deciduousness may vary among species.

Introduction

Trees must allow water to evaporate from their leaves (i.e. transpire) in order to maintain the CO$_2$ uptake that supplies photosynthesis. This water is held under tension in a ‘tug of war’ within the soil–plant–atmosphere continuum (Sperry et al., 2002). As water moves down gradients of water potential, plant water potential ($\Psi_{\text{plant}}$) must remain below soil water potential ($\Psi_{\text{soil}}$) for water uptake to feed the transpiration stream. However, as $\Psi_{\text{soil}}$ and $\Psi_{\text{plant}}$ decrease, hydraulic conductance within the soil–canopy continuum decreases as a result of cavitation within soil pore spaces and xylem conduits. These two processes result in a constraint, such that trees must regulate their transpiration rate ($E$) below the critical level ($E_{\text{crit}}$) above which soil–canopy conductance reaches zero and complete ‘hydraulic failure’ occurs. On short timescales, stomata act as valves to control $E$ and $\Psi_{\text{plant}}$ (Sperry et al., 2002). However, as $\Psi_{\text{soil}}$ decreases, $E_{\text{crit}}$ decreases and, at some point, $E_{\text{crit}}$ reaches zero, when any amount of transpiration would cause hydraulic failure. As closed stomata may leak and as water evaporates from leaf cuticles, additional water-conserving responses enhance survival during droughts (Levitt, 1972).

The hydraulic segmentation hypothesis states that, during droughts, the most peripheral plant organs (e.g. leaves) experience the lowest water potential; therefore, if their vulnerability to cavitation is the same as or greater than that of more central organs (e.g. the main stem or bole), the peripheral organs will cavitate before the central organs (Zimmermann, 1983; Tyree et al., 1993; Tyree & Zimmermann, 2002). As cavitation lowers hydraulic conductivity, this response would retain water within the central organs, protecting them from cavitation. Moreover, as cavitation renders the peripheral organs non-functional, the hydraulic segmentation hypothesis also predicts that these organs may be shed or abscised (Tyree et al., 1993). In theory, this hydraulic architecture is adaptive because central organs are more costly than peripheral organs for plants to replace. Thus, leaves are predicted to act as ‘hydraulic fuses’ that break the soil–plant–atmosphere continuum by shedding before stems incur damage, either through local hydraulic failure or at some point before failure by hydraulic stress-induced senescence (Tyree & Sperry, 1988; Tyree et al., 1993). Although the role of stomatal control has received much attention (Klein, 2014), the role of leaf shedding in the context of the hydraulic fuse hypothesis has rarely been tested (Tyree et al., 1993).

Seasonally dry tropical forests contain many tree species that shed some or all of their leaves during annual dry seasons (Axelrod, 1966; Frankie et al., 1974; Condit et al., 2000). In these ecosystems, leaf shedding during drought (i.e. drought deciduousness) may represent a critical response that protects stems as predicted by the hydraulic fuse hypothesis. However, despite its potential importance, the hydraulic function of leaf shedding in tropical plants is poorly understood (Brodribb et al., 2002). It is
also intriguing that the timing, rate and extent of leaf shedding vary considerably among species (Bullock & Solis-Magallanes, 1990; Williams et al., 2008). Such diversity suggests multiple physiological mechanisms for the avoidance of drought-induced mortality. If the hydraulic fuse hypothesis is correct and if leaf shedding is a drought response that protects stems from hydraulic failure, diverse leaf phenologies correspond to divergence among species in hydraulic limits within the soil–canopy continuum. For example, the stems of deciduous species may be more vulnerable to cavitation than those of co-occurring evergreen species. However, this simple prediction has received equivocal support. Some studies have found no difference in vulnerability to cavitation between deciduous and evergreen species (Machado & Tyree, 1994; Sobrado, 1997; Brodribb et al., 2002; Markesteijn et al., 2011), whereas others have found higher vulnerability in deciduous species than in evergreen species (Brodribb et al., 2003; Choaet al., 2005; Lopez et al., 2005; Fu et al., 2012). This suggests that the role of leaf shedding in the maintenance of hydraulic systems of tropical trees deserves a closer mechanistic analysis.

In order to address how leaf shedding relates to the regulation of $\Psi_{\text{plant}}$ and to the avoidance of stem hydraulic failure, we tracked saplings in two seasonally dry tropical forests for leaf phenology, stomatal conductance, $\Psi_{\text{plant}}$ and stem hydraulic conductivity. We focused on saplings because they are amenable to canopy measurements. In addition, because saplings have relatively high mortality during droughts (Enquist & Enquist, 2011), their physiology strongly influences the identity of the species that dominate the forest canopy. We combined our census data with additional measurements of stem vulnerability to cavitation to parameterize a hydraulic model that predicts $E_{\text{crit}}$ in given environmental conditions. We then tested the following predictions of the hydraulic fuse hypothesis: the timing of leaf shedding coincides with key points of hydraulic stress (e.g. $E_{\text{crit}}$ near zero); leaf shedding stabilizes $\Psi_{\text{plant}}$ during seasonal droughts; and saplings maintain stable stem hydraulic conductivity during seasonal droughts.

Materials and Methods

Study sites and species

This study was conducted in two seasonally dry forests in Panama. One forest, the Parque Natural Metropolitano (8°59′N, 79°32′W), is transitional between moist and dry forest (‘transitional forest’), with annual rainfall of 1800 mm. The other forest, the Eugene Eisenmann Reserve (8°31′N, 79°53′W), is a dry forest, with annual rainfall of 1590 mm. Both are mature secondary forests and experience dry seasons from mid-December to May. We measured rainfall in the dry forest with an automated rain gauge (Onset Corp., Bourne, MA, USA) and obtained data from the transitional forest from the Smithsonian Tropical Research Institute. In each forest, we placed a temperature and relative humidity sensor (models HMP50 and CS500; Campbell Scientific, Logan, UT, USA) in the understory (50 cm height). Air temperature and relative humidity were measured every 10 min and hourly mean temperature and minimum and maximum relative humidity were recorded on CR200 data loggers (Campbell Scientific). In general, the dry season in the dry forest is slightly longer, hotter and higher in vapor pressure deficit (VPD) than in the transitional forest. The two dry seasons encompassed by the study (2012 and 2013) fit this pattern, except that the 2013 dry season in the transitional forest extended for c. 30 d longer than normal (Fig. 1i,j; see Supporting Information Table S1). Late in the dry seasons, we measured $\Psi_{\text{soil}}$ in profiles at each site (Fig. S1). $\Psi_{\text{soil}}$ was lower in the dry forest than in the transitional forest (e.g. in 2013, $\Psi_{\text{soil}}$ values at 50 cm depth were $-4.6 \pm 0.7$ and $-2.1 \pm 0.9$ MPa (mean ± SD) in the dry and transitional forest, respectively). In each forest, we chose four tree species that were common as saplings and that were reported to vary in deciduousness and wood density as adults (Table 1). Two species were shared between the forests, and so six species were studied in total. Throughout the text, the study species are referred to by genus name.

Censuses of leaf area, stomatal conductance and plant water potential

Ten healthy-looking saplings of each species were selected for censuses of leaf area and stomatal conductance ($g_s$). Mean ± SD basal diameter ranged among species from 7.2 ± 2.7 mm to 27.3 ± 16.8 mm (Table S2). Censuses were conducted every 3–6 wk from November 2011 to July 2013, more often during the dry seasons. In the transitional forest, three Cojoba, one Cavanillesia and one Annona died, all after November 2012, reducing their sample sizes towards the end of the study. On each census plant, the positions of the leaves along the stems were noted and the length of each leaf was measured to the nearest 1 mm with a ruler. For compound-leaved species (Table 1), the length of the most distal leaflet was measured and the number of leaflets on each leaf was counted. In each census, the presence or absence of previously measured leaves and the number of leaflets were recorded, and any newly produced leaves were measured for length and number of leaflets. Leaves that were incompletely expanded during a census were re-measured for length in the subsequent census.

For each species, 23–94 leaves were collected from nearby saplings and measured for leaf area with an LI-3100 area meter (Li-Cor Biosciences, Lincoln, NE, USA). The relationship between leaf length and area was modeled with simple linear regression on log-transformed values, including number of leaflets as a cofactor for compound-leaved species (Fig. S2). These models were used to estimate the area of each census leaf, the values of which were summed to obtain the total leaf area of each sapling in each census.

During each census, three leaves on each sapling were randomly selected and measured for $g_s$ with a porometer (LI-1600; Li-Cor Biosciences) between 10:00 and 14:00 h. As leaves were not amphistomatic, $g_s$ was measured on the abaxial (lower) side. If saplings had fewer than three leaves during a census, all leaves were measured. The porometer’s cuvette was maintained at <70% relative humidity to reduce measurement error (McDermitt, 1990). Measurements were made within 60 s of placing the cuvette over the leaf.
the leaf to avoid affecting \( g_s \). The porometer was regularly calibrated with a dew point generator (LI-610; Li-Cor Biosciences).

At each census, four saplings of each species located within 50 m of the saplings measured for leaf area and \( g_s \) were measured for leaf water potential (\( \Psi_{\text{leaf}} \)). Most saplings were measured for \( \Psi_{\text{leaf}} \) in only one census; otherwise saplings were left at least 5 months between measurements. At predawn (04:00–06:30 h) and midday (11:00–14:00 h), two leaves were collected from each sapling, sealed in humidified plastic bags, placed in a cooler with ice and measured for \( \Psi_{\text{leaf}} \) with a pressure chamber < 2 h after collection. \( \Psi_{\text{leaf}} \) for each sapling was taken as the average of the two leaves. When plants were leafless, terminal twigs were collected to measure twig water potential (\( \Psi_{\text{twig}} \)) following the same protocol as for \( \Psi_{\text{leaf}} \) except that \( \Psi_{\text{twig}} \) was measured only at predawn. Comparisons with psychrometric measurements of \( \Psi_{\text{twig}} \) confirmed that the pressure chamber measurements accurately assessed \( \Psi_{\text{twig}} \) (Wolfe & Kursar, 2015). As water potential is under a gradient within transpiring plants, we took the mean of \( \Psi_{\text{leaf}} \) at predawn and midday to represent \( \Psi_{\text{plant}} \) for each sapling in each census.

**Hydraulic conductivity and vulnerability to cavitation**

We searched the area near the census plants (~2 ha) for healthy-looking saplings (110–400 cm tall) to measure for stem hydraulic conductivity. During the 2012 wet season (July–December) and during the subsequent dry season (March–April 2013), eight saplings of each species were collected (four at predawn and four at midday, \( n = 128 \)). For the three species that flushed leaves near
the onset of the wet season (*Cavanillesia*, *Bursera* and *Genipa*; Fig. 1), we also collected eight saplings (four at predawn and four at midday, \( n = 24 \)) that were flushing leaves at the onset of the 2013 wet season (May). For collection, the saplings were cut near the base with pruning shears, sealed in opaque plastic bags humidified with wet paper towels and brought to the laboratory. Additional saplings were collected during the 2012 and 2014 wet seasons and bench dried to assess vulnerability to cavitation. They were air dried in the laboratory for 2 weeks and bench dried to assess vulnerability to cavitation. Additional saplings were collected during the 2012 and 2014 wet seasons and bench dried before subsequent measurements (\( n = 101, 9–16 \) per species in each forest).

In the laboratory, \( \Psi_{\text{leaf}} \) was measured with a pressure chamber on three leaves and averaged. Stem water potential (\( \Psi_{\text{stem}} \)) was assumed to equal \( \Psi_{\text{leaf}} \) because sealing of the stems in opaque bags stopped transpiration (however, see Tobin et al. (2013) for a case in which this method may have failed at low \( \Psi_{\text{stem}} \)). For saplings that were leafless, we measured \( \Psi_{\text{stem}} \) as described earlier for \( \Psi_{\text{twig}} \) of leafless census plants.

The stem was submerged in tap water and a segment of 30 cm in length was removed with pruning shears. We ensured that the stem length between the end cut in the field and the segment measured for hydraulic conductivity was longer than the maximum vessel length (Table S3). The ends of the segment were recut with a fresh razor and the bark within 2 cm of the ends was removed. The segment was attached to a hydraulic conductivity apparatus (Sperry et al., 1988) that measured flow rates with graduated pipettes and perfused with filtered (0.1 \( \mu \)m) and degassed 10 mM KCl. To correct for passive water uptake, flow rates were measured under four pressure heads, in the range 0.98–8.6 kPa, and hydraulic conductivity was calculated as the slope of the regression of the flow rate on the pressure gradient across the stem segment (Torres-Ruiz et al., 2012). Stem area-specific hydraulic conductivity (\( K_s \)) was calculated by dividing conductivity by the segment’s cross-sectional area. The cross-sectional area was calculated by averaging two diameter measurements made with calipers (to 0.1 mm) on each end of the segment.

Vulnerability to cavitation was assessed as the decrease in \( K_s \) with decreasing \( \Psi_{\text{stem}} \). A three-parameter Weibull function was fitted as:

\[
K_s = a \times \exp\left(-\left(-\frac{\Psi_{\text{stem}}}{b}\right)^c\right),
\]

through all \( K_s \) measurements for each species. As multiple factors limit \( K_s \) independently of \( \Psi_{\text{stem}} \) at which \( K_s \) is measured (e.g., previous drought stress, pathogen attack, stem bending), we fitted regressions through the 90th quantile of the measurements, which represents the upper limit of \( K_s \) as a function of \( \Psi_{\text{stem}} \) (Cade & Noon, 2003). We fitted the regressions with the R package quantreg (Koenker, 2013). The Weibull functions were used to calculate \( \Psi_{\text{stem}} \) at 50% and 80% loss of \( K_s \) (\( \Psi_{50} \) and \( \Psi_{80} \), respectively).

It has been reported recently that the excision of stem segments for \( K_s \) measurements while the xylem is under tension produces artifactualy low \( K_s \) values for some species (Wheeler et al., 2013; but see Venturas et al., 2015). As our initial \( K_s \) measurements were liable to this excision artifact, for the measurements made in 2014, we relaxed the xylem tension by sequentially cutting back the stems towards the \( K_s \) segment while the stem was under water. The values of \( K_s \) from 2014 did not differ from the earlier measurements (visual inspection, Fig. 2), and so we included the full dataset in our analyses.
To test whether $K_e$ varied seasonally and diurnally, we set up a two-way ANOVA for each species with season, time of day (predawn vs midday) and their interaction as fixed effects. Linear contrasts were used to compare $K_e$ between seasons and between times of day within a season. The false discovery rate method of Benjamini & Hochberg (1995) was used to correct for multiple comparisons to $\alpha = 0.05$.

**Relationships among leaf shedding, stomatal conductance and vulnerability to cavitation**

In order to test for relationships between leaf shedding and $\Psi_{plant}$, we first calculated the percentage of leaf area relative to the November censuses preceding the dry seasons (PNLA) for each sapling. The November censuses were used as reference points (PNLA $= 100\%$) because that is when most saplings had maximum leaf area (Fig. 1). We then calculated the mean PNLA for each species in each census and used standardized major axis regression to assess its relationship with $\Psi_{plant}$ within each census year (first year, November 2011–August 2012; second year, November 2012–July 2013). To compare among species for sensitivity of leaf shedding in response to $\Psi_{plant}$, we calculated the $\Psi_{plant}$ value at which PNLA was 50% (LA$_{50}$; analogous to the $\Psi_{50}$ value for sensitivity of $K_e$ to $\Psi_{stem}$).

Similarly, we tested for relationships between $g_s$ and $\Psi_{plant}$. We took the mean $g_s$ for each species at each census and plotted it against $\Psi_{plant}$. Then, for each census year, we fitted a Weibull function through the points with nonlinear regression. Using the intercept of the regression as the maximum $g_s$, we calculated the $\Psi_{plant}$ value at which $g_s$ was 50% of the maximum (SC$_{50}$). We then tested whether leaf responses (SC$_{50}$ and LA$_{50}$) were correlated with points of hydraulic stress in the stem ($\Psi_{50}$ and $\Psi_{80}$) among species using Pearson’s correlation analysis.

**Hydraulic failure parameters**

For each census date, we used a soil–plant–atmosphere model to estimate the percentage loss of hydraulic conductance in the stem network (PLC$_{stem}$) vs in the total soil–canopy path (PLC$_{total}$), and the critical transpiration rate ($E_{crit}$) above which PLC$_{total}$ would go to 100%. The model was developed by Sperry et al. (1998) and upgraded to include stomatal control of $E$ and $\Psi_{leaf}$ (Sperry & Love, 2015; Sperry et al., 2016). The model calculates the steady-state relationship of $E$ vs $\Psi_{leaf}$ (and hence $E_{crit}$) from $\Psi_{soil}$ and vulnerability curves that describe the decline in soil and plant hydraulic conductance with $\Psi$. $E$ predictions assume that $E$ is increasingly limited, as negative $\Psi_{soil}$ and high VPD would otherwise drive the plant closer to $E_{crit}$. The plant therefore exploits, but does not exceed, the available water transport capacity as limited by $E_{crit}$. Only when low $\Psi_{soil}$ drives $E_{crit}$ to zero is PLC$_{total}$ predicted to reach 100%. As details of the model have been described elsewhere (Sperry & Love, 2015; Sperry et al., 2016), here we describe its application.

The model was run for each species–site combination, inputting the time series of paired $\Psi_{soil}$ and midday VPD across all censuses. $\Psi_{soil}$ was set to predawn $\Psi_{leaf}$. Midday VPD was set to the ambient value measured concurrently with $g_s$. The model predicted the corresponding time series of midday $\Psi_{leaf}$, whole-canopy diffusive conductance ($G$ = EVPD, kg h$^{-1}$ m$^{-2}$ basal area), $E_{crit}$, PLC$_{stem}$ and PLC$_{total}$. The model was tuned to obtain the best fit to measured $G$ and $\Psi_{leaf}$, thus maximizing its accuracy for the prediction of $E_{crit}$ and PLC. The measured $G$ was calculated for each census plant and date by multiplying $g_s$ by the leaf area of the canopy and dividing by the stem basal area.

Our stem vulnerability curves (Table S4) were used to represent the plant and Van Genuchten’s (1980) equation for the soil-to-root (rhizosphere) component. The required soil texture parameters were obtained from Leij et al. (1996) for top-horizon soils observed within 200 m of the census plants (clay in transitional forest, sandy clay loam in dry forest; B. L. Turner, pers. comm.). To predict PLC$_{stem}$, we had to input the maximum (no embolism) hydraulic conductance of the stem network. The default stem conductance was set to 25% of the soil–canopy resistance (roots at 50%, leaves at 25%). The 50–50 root–shoot division appears roughly consistent among woody plants (Sperry et al., 1998), as is the 25% value for leaves (Sack & Tyree, 2005). However, the leaf component can shift with plant size (Von Allmen et al., 2015), and so we assessed the consequences of varying the 25–25 stem–leaf breakdown from 20–30 to 30–20.

The model was fitted by iteratively adjusting three unknown inputs: maximum soil–canopy hydraulic conductance ($k_{max}$), maximum $G$ ($G_{max}$) and the average percentage rhizosphere hydraulic resistance. The latter input is the extent to which soil–root hydraulic conductance limits plant water uptake. The lower the surface area of absorbing roots vs leaves, the more ‘vulnerable’ is the Van Genuchten soil hydraulic function relative to the plant’s vulnerability curve, and the greater the percentage rhizosphere resistance. The rhizosphere adjustment can also compensate for missing vulnerability curve information from the plant, in our case the lack of root and leaf vulnerability curves. Missing root or leaf curves prevent us from predicting PLC in these organs, but we can still predict $E_{crit}$ PLC$_{total}$ and PLC$_{stem}$ which was sufficient for our purposes. The $k_{max}$, $G_{max}$ and percentage rhizosphere resistance were iterated manually to maximize the $R^2$ of a single regression through normalized values (i.e. divided by their respective observed average for each species–site combination across the censuses) for measured vs modeled $G$ and $\Psi_{leaf}$.

The best fits were obtained by running the model in irreversible mode where PLC in xylem is permanent (vs reversible mode where PLC in xylem is fully and instantly reversible; Sperry & Love, 2015).

If leaf shedding is a response to diminishing transport capacity and acts to maintain stem $K_e$ as predicted by the hydraulic fuse hypothesis, leaf shedding should be closely associated with drought levels that drive $E_{crit}$ to zero. To test this, we plotted PNLA as a function of $E_{crit}$ and used nonlinear regression to fit Weibull functions through the points for each census year. To test the prediction that leaf shedding slows water loss rates through reduced hydraulic conductance, we plotted the...
model’s output of $PLC_{\text{total}}$ as a function of measured PNLA, and used nonlinear regression to fit a function of the form:

$$PLC_{\text{total}} = b - \exp(\text{PNLA}/a)$$

Eqn 2

Finally, to test whether the saplings regulated $\Psi_{\text{plant}}$, such that the loss of hydraulic conductance in the stem was low compared with that of more peripheral plant components, we plotted $PLC_{\text{stem}}$ against $PLC_{\text{total}}$.

Results

Seasonal changes in leaf area, stomatal conductance and water potential

In both the transitional and dry forests, species exhibited a wide range of dry season deciduousness (Fig. 1). *Cavanillesia* and *Bursera* saplings shed their leaves early during the dry seasons (Fig. 1a,b), whereas, at the other extreme, *Cojoba* saplings did not reduce their leaf area during the dry seasons in either forest (Fig. 1g,h). To varying degrees, the other species were intermediate in deciduousness: *Genipa* shed its leaves later than *Bursera* and remained leafless for 2 months (Fig. 1d); *Annona* shed its leaves gradually through the dry season, but flushed new leaves within days of shedding the old ones (Fig. 1c); *Astronium* maintained its leaf area during the dry season in the transitional forest, but shed nearly 50% of its leaf area in the dry forest (Fig. 1e,f). These patterns were similar between the 2012 and 2013 dry seasons.

All species in both forests had lower $g_s$ during the dry season than during the wet season (Fig. S3). Deciduous species reduced $g_s$ before shedding leaves. *Astronium* and *Cojoba* had lower dry season $g_s$ in the dry forest than in the transitional forest (Fig. S3).

Among species, there was a wide range in seasonal patterns of $\Psi_{\text{plant}}$ (Fig. S4). *Cavanillesia* and *Bursera* maintained $\Psi_{\text{plant}}$ above $-1$ MPa throughout the dry seasons. At the other extreme, *Genipa* reached $-6.5$ MPa during the 2012 dry season and $-4.3$ MPa during the 2013 dry season. Meanwhile, *Annona*, *Astronium* and *Cojoba* had lower $\Psi_{\text{plant}}$ during the dry season than during the wet season. *Astronium* and *Cojoba* reached lower $\Psi_{\text{plant}}$ in the dry forest than in the transitional forest (Fig. S4).

Stem hydraulic conductivity and vulnerability to cavitation

We found a wide range of $\Psi_{50}$ and $\Psi_{80}$ values among species (Fig. 2). For *Astronium* in the dry forest, $K_s$ was generally low, irrespective of $\Psi_{\text{stem}}$ (Fig. 2f); as a Weibull function, or any function, did not fit these data, this population was excluded from the analyses that included vulnerability to cavitation. $K_s$ did not differ significantly between predawn and midday for any species in any season (Fig. 3). $K_s$ differed significantly between seasons in only two cases: *Cojoba* in the transitional forest had lower $K_s$ in the dry season than in the wet season, and *Bursera* had lower $K_s$ in the dry season than during leaf flush at the onset of the wet season (linear contrasts, $P<0.05$; Fig. 3).

Coordination between drought responses and vulnerability to cavitation among species

Within species, $g_s$ generally decreased as $\Psi_{\text{plant}}$ decreased during both dry seasons (Fig. S5). However, during 2012, the nonlinear regressions for *Cavanillesia* and *Bursera* did not converge on a Weibull function, apparently because the saplings had a wide range in $g_s$ throughout the relatively small range in $\Psi_{\text{plant}}$ (Fig. S5). As these species probably had a threshold response that...
could not be fitted via regression, we used the minimum \( \Psi_{\text{plant}} \) as an estimate of \( SC_{50} \) for *Cavanillesia* and *Bursera* during 2012. As for \( g_s \), PNLA generally decreased as \( \Psi_{\text{plant}} \) decreased (Fig. 4; Table S5). However, in the dry forest during 2013, *Cojoba* had higher PNLA at lower \( \Psi_{\text{plant}} \) (Fig. 4h), because it added leaf area in the late wet season and early dry season, then lost leaf area near the onset of the wet season (Fig. 1h). As *Cojoba* in the dry forest never reached LA\(_{50}\) in 2013, we excluded this datum from the LA\(_{50}\) correlation analyses. In general, species had similar SC\(_{50}\), LA\(_{50}\) and minimum \( \Psi_{\text{plant}} \) values between the 2012 and 2013 dry seasons (Fig. 5). Among species, lower SC\(_{50}\) and LA\(_{50}\) were correlated with lower \( \Psi_{50} \) and \( \Psi_{80} \) in both census years (Fig. 5a–d). Minimum \( \Psi_{\text{plant}} \) was not correlated with \( \Psi_{50} \) or \( \Psi_{80} \) during either census year (Fig. 5e,f).

### Hydraulic limits to gas exchange

The hydraulic model successfully fitted the wide swings in measured \( G \) and \( \Psi_{\text{leaf}} \) during the 2 yr of measurement in six of the

![Hydraulic limits to gas exchange](image)

**Fig. 5** Correlations between the stem water potential associated with 50% and 80% loss of hydraulic conductivity (\( \Psi_{50} \) and \( \Psi_{80} \), respectively) and (a, b) the plant water potential (\( \Psi_{\text{plant}} \)) at which stomatal conductance is 50% of maximum (\( SC_{50} \)), (c, d) the \( \Psi_{\text{plant}} \) value at which the leaf area is 50% of the November census before the dry season (LA\(_{50}\)), and (e, f) the minimum \( \Psi_{\text{plant}} \) value reached during the dry season. Species are represented as: *Annona*, open circle; transitional forest *Astronium*, up-pointed triangle; *Bursera*, down-pointed triangle; *Cavanillesia*, open square; transitional forest *Cojoba*, closed diamond; dry forest *Cojoba*, open diamond; *Genipa*, closed circle. Blue and red symbols represent values obtained in the first and second years of the census (2012 and 2013), respectively. When Pearson’s correlation coefficient was \( P < 0.10 \) (statistics shown in each panel), the standardized major axis regression line is drawn as a solid blue or dashed red line for 2012 and 2013, respectively. Dotted 1 : 1 lines are shown for reference.
seven cases (Figs S6, S7). Excluding the poor fit to *Bursera*, the average $r^2$ was 0.79 (range, 0.62–0.94) for $G$ (modeled vs measured) and 0.74 (0.38–0.94) for $\Psi_{\text{leaf}}$. The reasonable model fit to $G$ and $\Psi_{\text{leaf}}$ resulted in reasonable estimates of $E_{\text{crit}}$, $\text{PLC}_{\text{stem}}$, and $\text{PLC}_{\text{total}}$ (Table S6 lists the mean absolute errors of model outputs). Outputs were insensitive to the assumed contribution of stems to continuum resistance: a 20–30% range resulted in <1% change in $E_{\text{crit}}$ and $\text{PLC}_{\text{stem}}/\text{PLC}_{\text{total}}$ (Table S7). The average percentage rhizosphere resistance required to achieve the model fit was consistently high (64–86% excepting *Bursera*, Table S8), indicating either a strong soil limitation or compensation for unknown root and leaf vulnerability.

The poor fit for *Bursera* was caused by the unusual result of midday $\Psi_{\text{leaf}}$ being less negative than $\Psi_{\text{soil}}$. This happened during the dry seasons when *Bursera* was essentially leafless. The model cannot predict reverse $\Psi$ gradients, and so, for *Bursera*, it was unable to explain the variation in $\Psi_{\text{leaf}}$ with statistical significance (Fig. S7). Because PLC predictions are $\Psi$ dependent, we did not use them for *Bursera*. However, the model predicted a significant 53% of the variation in $G$ for this species (Fig. S6), and so we accepted $E$ and $E_{\text{crit}}$ outputs for *Bursera*.

Hydraulic limitations within all species were predicted to reduce $E$ during the dry seasons, from maxima of 1.6–14.3 kg h$^{-1}$ m$^{-2}$ to minima of 0.34–2.7 kg h$^{-1}$ m$^{-2}$ (Fig. 6a). PNLA approached zero (full deciduousness) only near the lowest values of $E_{\text{crit}}$, 0.5–2 kg h$^{-1}$ m$^{-2}$ (Fig. 6b), supporting the prediction that leaf shedding is associated with $E_{\text{crit}}$ approaching zero. The relationship between PNLA and $E_{\text{crit}}$ was similar between the two dry seasons ($F$ test, $P = 0.15$; Table S9). $\text{PLC}_{\text{total}}$ predicted by the model was related to the measured PNLA such that, at PNLA $> 80\%$ (only slight leaf loss), $\text{PLC}_{\text{total}}$ was not yet severe, <60%. With greater leaf loss, at PNLA < 60%, $\text{PLC}_{\text{total}}$ increased only slightly (Fig. 6c; Table S10). As a result, measured leaf area reached zero before complete loss of modeled water transport capacity. All species reached relatively high values of $\text{PLC}_{\text{total}}$ during the dry seasons, with *Genipa* reaching the highest (range of 73–97% excluding *Genipa*; *Genipa* = 99.4%), yet $\text{PLC}_{\text{stem}}$ remained relatively low in all species except *Genipa* (range of 5–62% excluding *Genipa*; *Genipa* = 84%; Fig. 6d).

**Discussion**

We found distinct patterns of leaf phenology within each species and population (Fig. 1). Although species fit within ‘evergreen’ and ‘deciduous’ functional groups (evergreen, *Astronium* and *Cojoba*; deciduous, *Cavanillesia*, *Bursera*, *Annona* and *Genipa*), this grouping misses important differences among species. For example, in the transitional forest, *Cavanillesia* reached zero leaf area c. 5 wk earlier than *Annona*. These phenological differences are probably linked to physiological and morphological differences among species and probably influence species performance. The quantification of deciduousness among populations, such as
the time lag between the onset of the dry season and leaf loss, may be more informative than grouping (Méndez-Alonzo et al., 2012). However, we found that the assessment of leaf shedding as a function of $\Psi_{\text{plant}}$ and $E_{\text{crit}}$ (Figs 4, 6) helped to elucidate the mechanisms underlying the diverse patterns of deciduousness among tropical trees.

**Leaf shedding occurs at key points of hydraulic stress**

We found that PNLA decreased precipitously when $E_{\text{crit}}$ was reduced to < 5 kg h$^{-1}$ m$^{-2}$ (Fig. 6b). Likewise, LA$_{50}$ was correlated with $\Psi_{\text{50}}$ and $\Psi_{\text{80}}$ among species (Fig. 5c,d). LA$_{50}$ was above the 1:1 line with $\Psi_{\text{50}}$, indicating that saplings shed half of their leaf area before they experienced levels of cavitation that can lead to hydraulic failure in their stems. Similar effects have been reported for potted seedlings of Mexican dry forest tree species, among which $\Psi_{\text{plant}}$ at 80% leaf loss was correlated with $\Psi_{\text{80}}$ (Pineda-García et al., 2013). Taken together, these results suggest that hydraulic limits drive a universal pattern of leaf shedding among juvenile trees in seasonally dry tropical forests.

Most of the decrease in measured leaf area (from 100% to 0% PNLA) was associated with the increase in model-estimated PLC$_{\text{total}}$ from 60% to 85% (Fig. 6c). As we did not directly parameterize vulnerability to cavitation in the rhizosphere, roots or leaves, we cannot predict their contribution to the soil–canopy bottleneck that drives PLC$_{\text{total}}$ higher as the dry season progresses. Although a high soil limitation was required to fit the model (via high percentage rhizosphere resistance, Table S8), this could be compensating for the unknown vulnerability of roots and leaves (which are typically more vulnerable than stems, see references within Sperry & Love, 2015). Regardless, the prediction of PLC$_{\text{total}}$ $\gg$ PLC$_{\text{stem}}$ as the dry season progresses (Fig. 6d) is consistent with the hydraulic fuse hypothesis – the loss of hydraulic conductance associated with leaf shedding occurred only after major declines in hydraulic conductance other than in the stems. Indeed, most leaf shedding occurred when PLC$_{\text{total}}$ approached levels that were associated with dieback and tree mortality (i.e. 60–90%; Sperry & Love, 2015).

Although leaf shedding was associated with hydraulic stress, we found no evidence that it was associated with hydraulic failure; $E$ remained below $E_{\text{crit}}$ for all species (Fig. 6). As hydraulic failure before leaf shedding would impede nutrient resorption (Marchin et al., 2010), trees adapted to seasonally dry conditions may be programmed to senesce and abscise leaves before hydraulic failure occurs. Leaves on our study trees commonly turned yellow or red before shedding (B. T. Wolfe, pers. obs.), which suggests that senescence occurred (Munné-Bosch & Alegre, 2004). How environmental conditions, such as drought and light, interact with endogenous factors, such as leaf age, to induce senescence is still poorly understood (Munné-Bosch & Alegre, 2004; Giraldo & Holbrook, 2011; Schippers et al., 2015). Various models predict that the optimal time for leaf senescence occurs as net CO$_2$ assimilation becomes unfavorable (reviewed by Givnish, 2002). Others predict that leaf senescence occurs in response to water stress, such as a loss of hydraulic conductance in petioles (Tyree et al., 1993; Brodribb et al., 2003). These models are essentially indistinguishable under natural drought conditions because water stress, stomatal closure and the resultant reduced net CO$_2$ assimilation co-occur. However, when droughts are out of season or extreme, leaves may act as hydraulic fuses without a senescence–abscission response. For example, during extreme droughts, leaves attached to stems that experience high losses in $K_e$ are often described as turning brown on the stem or abscising while green, suggesting that a loss of $K_e$ inhibits leaf senescence (Marchin et al., 2010; Nardini et al., 2013).

From our censuses, we cannot discern whether our ‘evergreen’ species would shed leaves under drought conditions in which their $E_{\text{crit}}$ was closer to zero and, conversely, whether our ‘deciduous’ species would maintain leaves under less intense droughts. Studies that manipulate water availability in seasonally dry forests should address these issues. One such study, on Barro Colorado Island (BCI), Panama, found that dry season irrigation did not affect leaf shedding in canopy trees of 16 out of 19 species, despite effectively raising $\Psi_{\text{leaf}}$ (Wright & Cornejo, 1990). However, most canopy trees on BCI access soil water (i.e. high $E_{\text{crit}}$) during the relatively mild dry season, such that leaf shedding phenology is probably driven by leaf production, which coincides with the dry season peak in irradiance (Wright & van Schaik, 1994). By contrast, when 1-yr-old seedlings near BCI were irrigated during the dry season, leaf shedding was significantly reduced in 25 of 28 species (Engelbrecht & Kursar, 2003). Probably, the effect of irrigation was stronger for seedlings because unirrigated seedlings have poor dry season soil water access. Together, these results suggest that, when $E_{\text{crit}}$ approaches zero, water stress overrides irradiance as a driver of leaf phenology.

**Leaf shedding may not stabilize $\Psi_{\text{plant}}$ during seasonal droughts**

During both dry seasons, $\Psi_{\text{plant}}$ of *Genipa* declined even after saplings shed all of their leaves, putting them at risk of hydraulic failure (i.e. $\Psi_{\text{plant}}$ < $\Psi_{\text{80}}$; Figs 5f, S4). In contrast with dynamic failure caused by $E$ > $E_{\text{crit}}$, this would be static failure (*sensu* Tyree & Sperry, 1988), in which $\Psi_{\text{stem}}$ and $K_e$ decline without a significant xylem pressure gradient produced by transpiration. Although a reduction in the evaporative surface area through leaf shedding certainly slows water loss, the pattern exhibited by *Genipa* does not support a central prediction of the hydraulic fuse hypothesis – that leaf shedding protects stems from hydraulic stress. Similarly, after adult trees of several species in Costa Rican dry forests shed all of their leaves, they experienced stem diameter contraction associated with water loss (Daubenmire, 1972; Reich & Borchert, 1984).

Although *Cavanillesia* and *Bursera* had stable $\Psi_{\text{plant}}$ after shedding leaves, they also had relatively stable $\Psi_{\text{plant}}$ during the onset of the dry season, before shedding leaves (Fig. 5). These species have high stem hydraulic capacitance that maintains high $\Psi_{\text{plant}}$ even with significant water loss (Wolfe & Kursar, 2015). For *Cavanillesia*, direct measurements of stem water content revealed no difference between the wet and dry seasons, suggesting that leaf shedding contributed to the complete retention of stem water.
(Wolfe & Kursar, 2015). Meanwhile, *Bursera* had significantly lower stem water content in the dry season than in the wet season, suggesting that either leaf shedding did not stop water loss or that water loss occurred before leaf shedding (Wolfe & Kursar, 2015), and so it is unclear whether leaf shedding retained stem water. By contrast, *Annona* had $\Psi_{\text{plant}}$, that decreased at the onset of the dry season, before the saplings shed leaves, and then stabilized after leaf shedding (compare Figs 1c and S4c). This pattern suggests that leaf shedding was effective at stopping water loss in *Annona*.

The effectiveness of leaf shedding in stopping water loss depends on several factors that were not measured. For example, the permeability of the stem surface to water vapor would affect the rate at which leafless stems lose water. This trait probably varies widely among tropical tree species, considering the great diversity of bark traits (Rosell et al., 2014). Likewise, low root radial hydraulic conductance would help to maintain $\Psi_{\text{plant}} > \Psi_{\text{soil}}$ by slowing the rate at which water diffuses from roots to dry soil. Root radial hydraulic conductance is highly dynamic on timescales of minutes to hours through aquaporin regulation (Javot & Maurel, 2002), and on longer timescales through mechanisms such as root shrinkage (Nobel & Cui, 1992) and suberization (Lo Gullo et al., 1998). In order for leaf shedding to stop water loss from trees rooted in dry soil, it must be coordinated with root responses. The lack of correlation between minimum $\Psi_{\text{plant}}$ and $\Psi_{\text{soil}}$ or $\Psi_{\text{soil}}$ among species (Fig. 5e,f) could reflect differences among species in this coordination.

Do saplings maintain stable $K_s$ during normal dry seasons?

Throughout the dry season, for all species except *Genipa*, the modeled $\text{PLC}_{\text{stem}}$ remained at $< 65\%$, whereas $\text{PLC}_{\text{total}}$ reached 73–97% (Fig. 6d). This result is consistent with the hydraulic segmentation hypothesis, that in that stem hydraulic conductance was protected from cavitation relative to the more peripheral components. For most species, the model predictions of low $\text{PLC}_{\text{stem}}$ concurred with direct measurements of $K_c$, which did not vary between the wet and dry seasons (Fig. 5). The model prediction of 84% $\text{PLC}_{\text{stem}}$ in *Genipa* appears contradictory to direct measurements, where $K_c$ did not vary between seasons (Figs 3b, 6d). However, unlike the other species, some field-collected (not bench-dried) *Genipa* saplings indeed reached $\Psi_{\text{stem}} < \Psi_{\text{soil}}$, whereas the *Genipa* saplings that maintained near-wet season $K_c$ also maintained near-wet season $\Psi_{\text{stem}}$ (Fig. 2). On average, the $K_c$ saplings maintained higher dry season water potential than the census saplings used to parameterize $\Psi_{\text{soil}}$ in the model (2013 census, mean ± SE: $-3.2 ± 0.4$ vs $-4.3 ± 1.1$ MPa), possibly because they were larger (Tables S2, S3). Taller saplings were necessary to prevent cavitation in the $K_c$ segments during collection (Table S3). Thus, both field measurements and model predictions suggest that the census saplings that reached lower $\Psi_{\text{plant}}$ experienced high $\text{PLC}_{\text{stem}}$. Considering that none of the *Genipa* census saplings died during the study, this species probably possesses mechanisms to recover $K_c$, either through embolism refilling or xylem production.

We are aware of only one other study from the tropics with comparable seasonal $K_c$ measurements (Sobrado, 1993). Two other studies that measured $K_c$ across seasons are not comparable because petioles were included in the $K_c$ segments (Brodrribb et al., 2002) or the segments were rehydrated before $K_c$ measurements (Ishida et al., 2010), precluding inferences of seasonal change in native $K_c$ within stems. Sobrado (1993) found that, within distal branch segments of adult trees in a Venezuelan dry forest, four deciduous species had 65–92% lower $K_c$ in the dry season than in the wet season, whereas two evergreen species did not have significantly lower $K_c$. Thus, the deciduous species in the study of Sobrado (1993) lost more $K_c$ than the saplings in our study (Figs 3, 4). The results of Sobrado (1993) do not support the prediction that leaf shedding protects stems from hydraulic stress, yet it is unclear whether leaf shedding preceded or followed the loss of $K_c$. As native $K_c$ is considered to be a key factor affecting drought performance and survival (McDowell et al., 2013), more studies should address the conditions, traits and behaviors that are associated with changes in $K_c$ during (seasonal) droughts.

Conclusions

Among species in seasonally dry tropical forests, diverse patterns of leaf phenology coincide in that leaf shedding occurs as saplings reach hydraulic limits that reduce $E_{\text{crit}}$ (and, by extension, CO$_2$ assimilation) to near zero. This is consistent with the prediction that leaf shedding is cues to occur before leaves are cut off from their water source through hydraulic failure, although the cues remain unclear. The extension of this relationship to adult trees could improve leaf phenology parameters in terrestrial biosphere models (Powell et al., 2013). However, our results and those of others suggest that leaf shedding does not always stop water loss or the loss of $K_c$ in stems, contrary to the predictions of the hydraulic fuse hypothesis. Rather than drought deciduousness acting universally as a water conservation strategy, its main function (or ‘ultimate cause’) may vary among species and situations, acting as a means for respiration reduction, nutrient re-utilization and leaf-to-root area balancing.

Acknowledgements

B.T.W. was supported by a short-term fellowship from the Smithsonian Tropical Research Institute, a University of Utah (UU) Graduate Research Fellowship, a UU Global Change and Sustainability Center grant and a National Science Foundation (NSF) GK-12 Program fellowship from the UU’s Think Globally Teach Locally Program. Funding was provided by NSF grant DEB-0444590 to T.A.K. We thank Joe Wright for advice and support, Sarah Bruemmer for laboratory assistance, and Parque Natural Metropolitano and Eugene Eisenmann Reserve for study site access.

Author contributions

B.T.W., J.S.S. and T.A.K. designed the experiment. B.T.W. conducted the field and laboratory work. B.T.W. and J.S.S. analyzed the data. B.T.W., J.S.S. and T.A.K. wrote the manuscript.
References


Pérez R. 2008. Árboles de los bosques del Canal de Panamá. Panama City, Panama: Smithsonian Tropical Research Institute.


**Supporting Information**

Additional Supporting Information may be found online in the Supporting Information tab for this article:

**Fig. S1** Dry season soil water potential during the study period.

**Fig. S2** Relationship between measured and predicted leaf area for each study species.

**Fig. S3** Twenty-one-month time course of stomatal conductance for each study species.

**Fig. S4** Twenty-one-month time course of plant water potential for each study species.

**Fig. S5** Stomatal conductance as a function of plant water potential for each study species.

**Fig. S6** Modeled vs measured canopy diffusive conductance for each study species.

**Fig. S7** Modeled vs measured midday leaf water potential for each study species.

**Table S1** Dry season characteristics during the study period

**Table S2** Characteristics of saplings used for censuses of leaf area and stomatal conductance

**Table S3** Characteristics of stems used for hydraulic conductivity measurements

**Table S4** Parameters of Weibull functions used to quantify vulnerability to cavitation

**Table S5** Parameters of regression of percentage of November leaf area vs plant water potential

**Table S6** Mean absolute errors of hydraulic model outputs

**Table S7** Sensitivity of hydraulic model outputs to partitioning of plant organ resistance

**Table S8** Hydraulic model parameters that were fitted with iteration

**Table S9** Parameters of nonlinear regressions of percentage of November leaf area vs $E_{\text{crit}}$

**Table S10** Parameters of nonlinear regressions of PLC$_{\text{total}}$ vs percentage of November leaf area

Please note: Wiley Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the New Phytologist Central Office.