

This is the [**Accepted**] **Version** of a paper published in the journal *Plant, Cell and Environment*:

Nolf, Markus, Creek, Donna, Duursma, Remko, Holtum, Joseph, Mayr, Stefan, and Choat, Brendan (2015) *Stem and leaf hydraulic properties are finely coordinated in three tropical rainforest tree species*. *Plant, Cell and Environment*, 38 (12). pp. 2652-2661.

<http://dx.doi.org/10.1111/pce.12581>

**Title**

Stem and leaf hydraulic properties are finely coordinated in three tropical rainforest tree species

**Authors**

Markus Nolf<sup>1,2</sup>, Danielle Creek<sup>2</sup>, Remko Duursma<sup>2</sup>, Joseph Holtum<sup>3</sup>, Stefan Mayr<sup>1</sup>, Brendan Choat<sup>2</sup>

**Addresses**

<sup>1</sup> Institute of Botany, University of Innsbruck, 6020 Innsbruck, Austria

<sup>2</sup> Hawkesbury Institute for the Environment, University of Western Sydney, Richmond, New South Wales 2753, Australia

<sup>3</sup> School of Marine and Tropical Biology, James Cook University, Townsville, Queensland 4811, Australia

**Corresponding Author**

Markus Nolf

Institute of Botany, University of Innsbruck, Sternwartestr. 15, 6020 Innsbruck, Austria

Phone: +43 512 507 51012

Fax: +43 512 507 2715

Email: [m.nolf@uws.edu.au](mailto:m.nolf@uws.edu.au)

---

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/pce.12581

**Abstract**

Coordination of stem and leaf hydraulic traits allows terrestrial plants to maintain safe water status under limited water supply. Tropical rainforests, one of the world's most productive biomes, are vulnerable to drought and potentially threatened by increased aridity due to global climate change. However, the relationship of stem and leaf traits within the plant hydraulic continuum remains understudied, particularly in tropical species. We studied within-plant hydraulic coordination between stems and leaves in three tropical lowland rainforest tree species by analyses of hydraulic vulnerability (hydraulic methods and ultrasonic emission (UE) analysis), pressure-volume relations and *in situ* predawn and midday water potentials ( $\Psi$ ).

We found finely coordinated stem and leaf hydraulic features, with a strategy of sacrificing leaves in favour of stems. Fifty percent of hydraulic conductivity ( $P_{50}$ ) was lost at -2.1 to -3.1 MPa in stems and -1.7 to -2.2 MPa in leaves. UE analysis corresponded to hydraulic measurements. Safety margins (leaf  $P_{50}$  – stem  $P_{50}$ ) were very narrow at -0.4 to -1.4 MPa. Pressure-volume analysis and *in situ*  $\Psi$  indicated safe water status in stems but risk of hydraulic failure in leaves. Our study shows that stem and leaf hydraulics were finely tuned to avoid embolism formation in the xylem.

Keywords: plant-water relations, hydraulic vulnerability, tropical rainforest, hydraulic coordination, safety margin, ultrasonic emissions

## **Introduction**

Terrestrial plants require adequate water transport capacity to maintain photosynthesis and growth. Transpiration in leaves induces negative water potential ( $\Psi$ ), i.e. tension in the conducting elements of the xylem, which propagates through twigs, branches, stems, and roots, and enables passively driven water uptake (Tyree & Zimmermann 2002). Limited water supply can cause xylem tension to exceed critical thresholds, leading to the formation of embolism (cavitation), which blocks xylem water transport. Impairment in hydraulic conductance may result in reduced primary production, and even whole-plant mortality (Raven 2002, Hartmann et al. 2013). As a result, strategies to avoid and/or repair embolism in the xylem are critical to plant growth and survival (Johnson et al. 2012a).

Stomatal regulation allows plants to limit the decrease of  $\Psi$  and avoid failure of the water transport system (Sperry & Pockman 1993, Sperry 2000), and stomatal closure was shown to correlate to leaf turgor loss and cavitation-inducing  $\Psi$  for stems and leaves (e.g. Salleo et al. 2001, Cochard et al. 2002, Brodribb et al. 2003, Martorell et al. 2014). Such within-plant hydraulic coordination in respect to leaf traits and hydraulic vulnerability thresholds enables plants to maintain  $\Psi$  within a safe range as conditions become less favourable (Sperry 2000, Brodribb et al. 2003, Sack & Holbrook 2006). Furthermore, the hydraulic vulnerability segmentation hypothesis (Zimmermann & Brown 1971, Tyree & Ewers 1991) predicts that more distal components such as leaves and small branches will protect stems by earlier loss of hydraulic conductivity under drought (e.g. due to xylem embolism, or changes in extraxylary pathways such as aquaporin deactivation or cell shrinkage; Kim & Steudle 2007, Scoffoni et al. 2014). However, the majority of studies focus independently on either stem- (e.g. Maherali et al. 2004, Choat et al. 2012) or leaf hydraulic traits (e.g. Blackman et al. 2010, Scoffoni et al. 2012, Nardini & Luglio 2014). Thus, although hydraulic coordination and vulnerability segmentation of stems and leaves was hypothesised 30 years ago (Zimmermann 1983, Tyree & Ewers 1991), relatively little is known about the relationship between leaf and stem parameters within the plant hydraulic continuum, particularly with reference to the relative vulnerability of stems and leaves to drought-induced dysfunction (e.g. Choat et al. 2005, Meinzer et al. 2008, Chen et al. 2009, Johnson et al. 2011).

Hydraulic coordination may be especially important to tropical rainforest trees. The increasing probability of more prolonged and severe drought stress associated with global climate change (Allison et al. 2011) poses a significant risk to tropical forests (Lewis 2006, Cook & Vizy 2007, Salazar et al. 2007, Phillips et al. 2009), and adaptation towards a more drought-resistant water transport system may be limited by long generation cycles (Aitken et al. 2008, Choat et al. 2012). Rainforest trees are known to exhibit small safety margins between minimum  $\Psi$  observed under natural conditions and  $\Psi$  at 50% loss of conductivity ( $\Psi_{50}$ ) in stems (Meinzer et al. 2009, Meinzer et al. 2008\_ENREF\_5, Choat et al. 2012), yet our knowledge of the effects of drought on rainforest tree species is still limited compared to data available for seasonally dry and arid environments (Choat et al. 2012). Closing this knowledge gap is especially important considering that tropical forests contribute an estimated 30 to 50% of global terrestrial net primary production (Grace et al. 2001, Prentice et al. 2001).

From a methodological perspective, most assays of vulnerability to embolism have been based on measurements of loss in hydraulic conductivity with increasing xylem tension (Sperry et al. 1988, Cochard et al. 2013). These techniques provide direct measures of impairment by embolism but are destructive and often labour-intensive. Measurement of embolism by non-invasive imaging techniques is becoming more widespread but is not field portable (Choat et al. 2010, Brodersen et al. 2013). Embolism formation (cavitation) can be detected using ultrasonic emission (UE) sensors, as tension is released suddenly when water is replaced by air, resulting in acoustic vibrations (Milburn & Johnson 1966, Tyree & Dixon 1983, Ritman & Milburn 1988; Mayr & Rosner 2011; Cochard et al. 2013; Ponomarenko et al. 2014). Monitoring of UE from plant xylem is thus a more automated and less destructive option for vulnerability analysis, offering qualitative information on embolism formation (e.g. Mayr & Rosner 2011, Johnson et al. 2012b, Wolkerstorfer et al. 2012). However, difficulties in signal interpretation (e.g. distinguishing conduit sizes or conductive and non-conductive signal sources) still limit the application of UE analysis in plant hydraulics.

In the present study, the coordination of stem and leaf hydraulic properties was examined in three tropical lowland rainforest tree species. We analysed the vulnerability of stems and leaves to drought-induced loss of conductivity using both hydraulic measurements and UE analysis, as well as pressure-

volume relations and *in situ* predawn and midday  $\Psi$ . We hypothesised that stem- and leaf hydraulic parameters should be coordinated in these species such that stomatal regulation prevents hydraulic failure in stem and leaf xylem. Leaves were hypothesised to be equally or more vulnerable to drought-induced hydraulic decline than stems, and safety margins (stem- vs. leaf hydraulic vulnerability within species) to be relatively narrow.

## Materials and Methods

### *Study site, measurement period, and selected species*

Our study site was the Daintree Rainforest Observatory Research Facility (Cape Tribulation, Queensland, Australia; S 16.104°, E 145.449°), which operates a canopy crane in a tropical lowland rainforest at 40 m elevation. Mean air temperature was 24.8 °C in 2012, and 24.9 °C in the 30-year mean (1961–1990; Fig. 1). Mean temperatures were calculated from gridded data, by averaging daily minimum and maximum temperatures (Bureau of Meteorology 2013). The local weather station (Liddell 2013) recorded an actual mean air temperature of 25.6 °C in 2012 (based on daily 24-hour averages), but long-term data is not available. Total annual precipitation at the nearest weather station, Cape Tribulation Store, was 3609 mm in 2012, and 4141 mm in the 30-year average (1961–1990; Bureau of Meteorology 2013), where > 70% of the annual rain typically falls in the wet season (December to April; Fig. 1). Total rainfall in November 2012 was only 11 mm (30-year average: 177.4 ± 35.1 mm), but *in situ*  $\Psi_{\text{leaf}}$  showed that trees were still well-hydrated and did not experience drought stress. All measurements were carried out in December 2012.

Three native, co-occurring and abundant evergreen tree species from different families were chosen for our study: *Elaeocarpus grandis* (syn. *E. angustifolius*, Elaeocarpaceae) is a fast-growing, early successional species with relatively sturdy lanceolate leaves, which regenerates in available gaps and also persists in the canopy of mature phase forests (Rosetto et al. 2004, Hyland et al. 2010). The species typically occurs along the banks of creeks in subtropical rainforests, but is less habitat-specific in more humid environments (Boland et al. 2006). *Syzygium sayeri* (Myrtaceae) is an intermediate to late successional species with rigid ovoid leaves, which is frequently found along, but not restricted to, creeks and watercourses (White et al. 2004, Hyland et al. 2010). *Dysoxylum papuanum* (Meliaceae) is

another species typical of intermediate to late succession stages (White et al. 2004), with relatively weak, thin compound leaves known to grow on a variety of sites within well-developed rainforest (Hyland et al. 2010).

#### *Sample collection for in situ water potential determination*

Two fully developed undamaged leaves (*E. grandis*, *S. sayeri*) or leaflets on different leaves (*D. papuanum*) were excised from each of three trees per species in the canopy at approx. 20 to 30 m height, between 04:00 and 05:30 a.m. within a two day period (sunrise: 05:37). Leaves were immediately sealed in individual plastic bags and placed in a cool box until  $\Psi_{\text{leaf}}$  measurements in the lab. Two more leaves or leaflets per tree were enclosed in plastic wrap and aluminium foil in the morning. These leaves were collected between 11:30 and 13:00 on the same day for midday  $\Psi_{\text{stem}}$  determination. Midday  $\Psi_{\text{leaf}}$  was measured on two additional, unwrapped leaves from fully sun-exposed branches.

#### *Sample collection for hydraulic and acoustic analyses*

Branches of 1 to 2 m length were sampled from the rainforest canopy at approx. 20 to 30 m height before sunrise, immediately re-trimmed (approx. 10 cm) under water and covered with dark plastic bags. After transport to the laboratory (10 min., cut ends remained under water), they were re-cut (5 to 10 cm) and rehydrated at ambient temperature (approx. 25 to 30 °C) for at least 4 h. Only one branch per tree was used for each method, with sample sizes of three (pressure volume- and ultrasonic emission analysis) or six to eight trees (stem and leaf hydraulic vulnerability analysis), respectively.

#### *Water potential determination*

Water potential was measured as described in Scholander et al. (1965), using a pressure chamber (Model 1505D, PMS Instrument Co., USA). When necessary, the lamina of sampled leaves was partially incised along the middle leaf vein for easier sealing into the system. One to two  $\Psi$  measurements were averaged per tree and dataset (predawn  $\Psi_{\text{leaf}}$ , midday  $\Psi_{\text{stem}}$ , and midday  $\Psi_{\text{leaf}}$ ) for

*in situ* determination, and two measurements were averaged per sampled branch and desiccation step in the vulnerability and pressure-volume analyses.

### *Pressure-volume analysis*

Leaf turgor dynamics and parameters of bulk tissue water relations were determined via pressure-volume analysis (Tyree & Hammel 1972, Sack et al. 2011), using one leaf each from three trees per species. Sample leaves were excised from rehydrated branches under water and dehydrated slowly on the bench-top.  $\Psi_{\text{leaf}}$  and leaf mass (weighed to 0.001 g) were measured at intervals. Leaf areas were measured using a digital camera and ImageJ (Ver. 1.46; Schneider et al. 2012). Leaf dry masses were determined after desiccation in a drying oven for at least 48 h at 80 °C. The turgor loss point (TLP) was estimated as the point of transition between curvilinear and linear portions of the graph of  $1/\Psi_{\text{leaf}}$  vs. the relative water content (RWC). Osmotic potential at full turgor was calculated from the intersection between the extended linear portion of the same graph and the y-axis (Kirkham 2004). The bulk modulus of elasticity was defined as the slope of RWC vs. pressure potential at and above TLP. Bulk leaf capacitance (adjusted for the mass of water per leaf area) was determined from the slopes of the pressure-volume relationship between full turgor and TLP, and separately, below TLP (Brodribb & Holbrook 2003):

$$C = \Delta\text{RWC}/\Delta\Psi_{\text{leaf}} \cdot (\text{DM}/\text{LA}) \cdot (\text{WM}/\text{DM})/M \quad (1)$$

where RWC is the leaf relative water content (%), DM is the leaf dry mass (g), LA is the leaf area ( $\text{m}^2$ ), WM (g) is the mass of leaf water at 100% RWC (WM = fresh mass - dry mass), and M is the molar mass of water ( $\text{g mol}^{-1}$ ).

### *Stem hydraulic vulnerability*

Hydraulic vulnerability of stem xylem to drought-induced embolism was analysed using the bench-top dehydration method described by Sperry et al. (1988). Briefly, conductivity measurements were made before and after removal of embolism, at increasingly negative levels of  $\Psi_{\text{stem}}$  during bench-top desiccation of branches. Prior to conductivity measurements, branches were equilibrated for at least 30 min in dark plastic bags, and their  $\Psi_{\text{stem}}$  was determined using two neighbouring leaves or branchlets.



Two to five distal branchlets were taken from each large branch harvested for hydraulic measurements. Sample branchlets were cut off and sequentially retrimmed to 5 to 10 cm length under water, in order to relieve xylem tension and avoid the cutting artefact (Wheeler et al. 2013). Flow rates were measured with a liquid mass flow meter (LiquiFlow L13-AAD-11-K-10S, Bronkhorst High-Tech B.V., Netherlands) using a filtered (0.22  $\mu\text{m}$ ) solution of distilled water with 2 mmol KCl. The relative difference in flow (PLC, percent loss of conductivity) before and after repeated flushing at high pressure was plotted against  $\Psi_{\text{stem}}$  to create stem hydraulic vulnerability curves.

#### *Leaf hydraulic vulnerability*

Leaf hydraulic conductance was measured on non-light-acclimated leaves using a modified, non-steady-state leaf rehydration technique (Brodribb & Cochard 2009) and bench-top dehydration. Sample branches were dried for up to 96 h under ambient conditions without direct sunlight or, for later stages of dehydration, in a room at 20 to 25  $^{\circ}\text{C}$  under laboratory lighting. Flow rates for 41 to 46 leaves per species were determined via timed output of a digital balance (CPA225D, Sartorius, Germany) with computer interface, recording the initial decline in mass of a small water container connected via hydraulic tubing to the leaf petiole.

Prior to measurement,  $\Psi$  was determined from two neighbouring leaves on the same branchlet of an equilibrated (bagged for  $\geq 30$  min.) branch. Branchlets were used for measurement only if the difference in  $\Psi$  was  $\leq 10\%$ . A sample leaf was excised under water, immediately connected with the petiole to the hydraulic measurement system and placed under moist paper towel to prevent transpiration. The initial flow rate of filtered (0.22  $\mu\text{m}$ ) distilled water was recorded at an interval of 1 s, averaged over the first 5 s, and adjusted for leaf area and water viscosity. PLC was calculated as

$$\text{PLC} = 100 - k_{\text{leaf}} / k_{\text{leaf max}} \cdot 100 \quad (2)$$

where  $k_{\text{leaf}}$  is the adjusted leaf hydraulic conductance, and  $k_{\text{leaf max}}$  is the maximum  $k_{\text{leaf}}$  measured for each species. No cutting effect (Wheeler et al. 2013) was expected for leaves excised under water (Scoffoni & Sack 2014). Leaf hydraulic vulnerability curves were created by plotting PLC vs.  $\Psi$ .

#### *Curve-fitting for hydraulic vulnerability analysis*

The Weibull function as reparameterized by Ogle et al. (2009) was fitted to vulnerability curves:

$$\begin{aligned}
 k &= k_{sat} \left(1 - \frac{X}{100}\right)^p \\
 p &= \left[ \left( \frac{P}{P_x} \right)^{\frac{P_x S_x}{V}} \right] \\
 V &= (X - 100) \ln \left(1 - \frac{X}{100}\right)
 \end{aligned}
 \tag{3}$$

where  $k$  is the expected hydraulic conductivity at X% loss of conductivity,  $k_{sat}$  is the saturated hydraulic conductivity (i.e.  $k$  at 0 MPa),  $P$  is the positive-valued xylem water potential ( $P = -\Psi$ ),  $P_x$  is the  $\Psi$  at X% loss of conductivity, and  $S_x$  is the slope of the vulnerability curve at  $P = P_x$ .  $P_{12}$ ,  $P_{50}$ , and  $P_{88}$  were defined as  $\Psi$  at 12, 50, and 88% loss of conductivity, respectively. We fit Eq. 3 using non-linear least squares (function *nls* in R 3.0.1, R Core Team 2013). We used a bootstrap resampling technique to arrive at confidence intervals for the fitted parameters, by fitting the curve to resampled data (999 replicates). The fitting routines were implemented as an R package (*fitplc*, available from Duursma 2014).

#### *Ultrasonic emission analysis*

Ultrasonic emissions (UE) of stems and leaves were recorded during dehydration of two to three water-saturated branches per species in a temperature controlled room (20 to 25 °C, no direct sunlight). For each branch, one 150 kHz pre-amplified resonance sensor (PK15I, Physical Acoustics Corporation, Germany) was clamped to a small region of exposed xylem of the main branch axis where the cortex had been removed. Another sensor was placed on the upper side of a fully expanded leaf's midrib (proximal third of leaf, loaded with a small weight of approx. 100 g for stability and acoustic coupling) in close proximity to the branch xylem sensor. Silicone lubricant was used to improve acoustic coupling and prevent transpirational losses at the prepared areas. Peak definition time, hit definition time, and hit lockout time were 200, 400, and 2  $\mu$ s, respectively. Signals of 35 db or greater amplitude were recorded using AEWin™ for USB (Ver. E3.35, Physical Acoustics

Corporation, Germany) for at least 70 h, which was long enough to reach 100% loss of hydraulic conductivity.  $\Psi_{\text{stem}}$  and  $\Psi_{\text{leaf}}$  were measured periodically. Plots of relative cumulative UE and corresponding  $\Psi$  vs. time (Fig. 3) enabled to estimate the  $\Psi$  at the onset of hydraulic decline, although UE continued beyond total loss of hydraulic conductivity. Furthermore, time-dependent rates of stem UE per minute (15 minute bins) were calculated and related to the maximum rate per stem. The corresponding  $\Psi$  at maximum UE rate was calculated by linear interpolation (Fig. 4).

### *Statistical analysis*

Data for vulnerability curves were pooled per species and organ (stem/leaf) prior to curve-fitting, and statistically tested for differences using bootstrapped confidence intervals (CI,  $n = 999$  bootstrap estimates). Other results were successfully tested for homoscedasticity (Levene test), and further analyzed using pairwise, Bonferroni-corrected Student's t-tests. All tests were performed at a probability level of  $P < 0.05$  using R (Ver. 3.0.1, R Core Team 2013). Values are given as mean  $\pm$  standard error (SE) or mean (lower CI, upper CI), respectively. SE was not computed when  $n < 3$ .

## **Results**

### *In situ water potentials*

Predawn  $\Psi_{\text{stem}}$  and  $\Psi_{\text{leaf}}$  were between -0.3 and -0.5 MPa in all species (Tab. 1). By midday,  $\Psi_{\text{leaf}}$  declined to minima of -1.2 to -1.6 MPa, whereas  $\Psi_{\text{stem}}$  remained between -0.7 and -1.0 MPa. No significant differences were found between species. *Elaeocarpus grandis* had both the least negative predawn  $\Psi$  (-0.3 MPa), as well as the greatest difference between midday  $\Psi_{\text{leaf}}$  and  $\Psi_{\text{stem}}$  ( $\Delta\Psi = 0.79$  MPa).

### *Leaf turgor dynamics and leaf hydraulic conductance*

Turgor was lost at  $\Psi_{\text{leaf}}$  (TLP) of  $-2.1 \pm 0.1$  MPa in *Dysoxylum papuanum*,  $-2.2 \pm 0.3$  MPa in *E. grandis*, and  $-1.9 \pm 0.1$  MPa in *Syzygium sayeri* (Tab. 1), respectively. The osmotic potential at full water saturation ranged between  $-1.5 \pm 0.2$  MPa (*S. sayeri*) and  $-1.9 \pm 0.1$  MPa (*D. papuanum*). Absolute capacitance was between  $223 \pm 9$  and  $343 \pm 48$  mmol m<sup>-2</sup> MPa<sup>-1</sup> at full turgor ( $C_{\text{FT}}$ ), and

between  $597 \pm 88$  and  $1420 \pm 151$   $\text{mmol m}^{-2} \text{MPa}^{-1}$  after turgor loss ( $C_{\text{TLP}}$ ). Only  $C_{\text{TLP}}$  showed significant differences across species (Tab. 1).

The maximum whole-leaf hydraulic conductance observed for each species was  $3.5 \text{ mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$  (*D. papuanum*),  $20.2 \text{ mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$  (*E. grandis*) and  $3.1 \text{ mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$  (*S. sayeri*), respectively (Tab. 1).

#### *Hydraulic vulnerability*

All three species had high (less negative) hydraulic vulnerability thresholds as could be expected for plants growing in this tropical high rainfall environment. *S. sayeri* was the most vulnerable species, with 50% loss of conductivity ( $P_{50}$ ) occurring at  $-2.1$  MPa in stems and  $-1.7$  MPa in leaves. *D. papuanum* had stem and leaf  $P_{50}$  of  $-2.6$  MPa and  $-2.2$  MPa, respectively. In *E. grandis*, the  $P_{50}$  of stems ( $-3.1$  MPa) was  $1.4$  MPa more negative than the  $P_{50}$  of leaves ( $-1.7$  MPa). Safety margins (leaf  $P_{50}$  - stem  $P_{50}$ ) were between  $0.4$  and  $1.0$  MPa (Tab. 2, Fig. 2). Induction of embolism in stems occurred at  $\Psi$   $0.4$  to  $1.2$  MPa more negative compared to leaves, with stem and leaf  $P_{12}$  (12% loss of conductivity) of  $-1.2$  and  $-0.7$  MPa (*D. papuanum*), respectively, as well as  $-1.9$  and  $-0.66$  MPa (*E. grandis*), and  $-1.2$  and  $-0.5$  MPa (*S. sayeri*) (Fig. 2). Statistically significant differences ( $P < 0.05$ ) were found between stem and leaf hydraulic parameters in *E. grandis* ( $P_{12}$ ,  $P_{50}$ ) and *S. sayeri* ( $P_{88}$ ).

#### *Ultrasonic emission analysis*

Due to varying dehydration dynamics, Figs 3 and 4 showcase individual curves, whereas further data are presented in Figs S1 to S3. Curves of cumulative ultrasonic emissions (UE) in stems started to rise near the hydraulically measured  $P_{12}$  in *E. grandis* and *S. sayeri* and reached their steepest slope near the hydraulic  $P_{50}$  (Figs 3, S2, S3). In *D. papuanum*, the hydraulic  $P_{12}$  coincided with the first recorded UE, but a rise was not visible in the cumulative curves since 95 to 99% of signals were observed after branches had reached their  $P_{50}$  (Figs 3, S1).

In all species, UE continued beyond complete loss of hydraulic conductivity as measured with hydraulic techniques, sometimes reaching another steep increase at  $\Psi$  below  $P_{88}$ . No end point for quantitative analysis could be determined from the cumulative curves or from acoustic parameters

such as signal amplitude or absolute energy. Leaf UE started close to the species' leaf  $P_{12}$  and midday  $\Psi_{\text{leaf}}$  (Figs 3, S1 to S3). In all leaves, the vast majority of UE was observed at  $\Psi$  below leaf  $P_{50}$ , and UE only reached a final plateau in *D. papuanum*.

Time-dependent maximum emission rates were  $63.2 \pm 29.8$  hits per minute for all stems and  $2.4 \pm 0.6$  hits per minute for all leaves. UE rate maxima in stems were reached at approx. -2.8 MPa (*D. papuanum*), -2.3 MPa (*E. grandis*), and -2.3 MPa (*S. sayeri*; Tab. 2, Fig. 4). Leaf UE rates peaked at  $\Psi_{\text{leaf}}$  between -1.1 and -5.4 MPa (Fig. 4), but were generally very low (< seven hits per minute; Figs S1 to S3) and thus time-dependent leaf UE rates could not be used for analysis. During the dehydration process,  $\Psi_{\text{stem}}$  and  $\Psi_{\text{leaf}}$  within branches were first equilibrated at approx. -2.8 (*D. papuanum*), -2.0 (*E. grandis*), and -2.1 MPa (*S. sayeri*).

### **Discussion**

Stem and leaf hydraulic traits were finely coordinated in the studied species. Under the well hydrated conditions observed in this study, all three tree species maintained daily midday water potentials ( $\Psi$ ) at or above their thresholds for the onset of hydraulic decline ( $P_{12}$ ) in stems, and at or above the point of 50% loss of hydraulic conductivity ( $P_{50}$ ) in leaves. The turgor loss point of leaves indicated that stomata would close at or below  $\Psi$  inducing significant conductivity losses ( $P_{50}$ ) in leaves, but before reaching  $P_{50}$  in stems, thus protecting stems from embolism formation. Vulnerability analysis revealed narrow safety margins between the vulnerability of stems and leaves (leaf  $P_{50}$  - stem  $P_{50}$ ) and a strategy of sacrificing leaves in favour of stems if water potentials declined as a result of drought stress.

The hydraulic vulnerability of branches and stems in woody species has been widely studied with  $P_{50}$  values ranging between -14.1 and -0.2 MPa across all biomes (Maherali et al. 2004, Choat et al. 2012). Tropical rainforest species represent the most hydraulically vulnerable group, with a mean  $P_{50}$  of  $-1.8 \pm 0.2$  MPa ( $n = 75$ ; Choat et al. 2012). Our study species are within the expected range of hydraulic vulnerability for tropical rainforest trees (Tab. 2), and add to the limited pool of data for this biome. A literature survey on leaf hydraulic vulnerability revealed a mean leaf  $P_{50}$  of  $-1.8 \pm 0.1$  MPa across 93 angiosperm tree species, with individual species values ranging from -4.3 to -0.1 MPa (suppl. Tab. 1). In contrast, gymnosperms were found to be more resistant to drought (e.g. Brodribb et al. 2014) with a

mean leaf  $P_{50}$  of  $-3.2 \pm 0.2$  MPa across 65 species (range:  $-7.5$  to  $-0.8$  MPa; suppl. Tab. 1). In tropical biomes, leaf  $P_{50}$  was between  $-0.8$  and  $-2.9$  MPa. Leaves analysed in this study were less vulnerable than the compiled average for tropical angiosperms (mean  $P_{50}$  of  $-2.9 \pm 0.1$  MPa), but  $P_{50}$  were also within the recorded range. Since leaf-hydraulic measurements were based on non-light-acclimated leaves, extraxylary factors such as aquaporins may contribute to a higher leaf hydraulic vulnerability *in situ* (Guyot et al. 2012). While all three species were hydraulically very vulnerable, *S. sayeri* was the most vulnerable species as demonstrated by its hydraulic vulnerability, least negative midday  $\Psi$ , and least negative  $\Psi_{TLP}$ .

#### *Daily minimum water potential and embolism avoidance*

Midday  $\Psi_{stem}$  under well-watered conditions remained above the stem  $P_{12}$  in all study species (Tab. 1). This is in accordance with previous findings for tropical and temperate trees under non-drought conditions (e.g. Choat et al. 2006, Meinzer et al. 2009, Johnson et al. 2011). In contrast, midday  $\Psi_{leaf}$  were  $0.5$  to  $0.9$  MPa more negative than the threshold for reductions in whole-leaf conductance ( $P_{12}$ ; Tabs 1 and 2), but remained at or above leaf  $P_{50}$  in all species. Leaf turgor was lost at  $\Psi$  (TLP) within  $0.5$  MPa of leaf  $P_{50}$  in all species (Tabs 1 and 2), indicating that leaves faced losses of  $k_{leaf}$  of up to  $50\%$  before stomata close (Brodrigg et al. 2003, Martorell et al. 2014\_ENREF\_2). \_ENREF\_5Thus, all species maintained a safe water status in stems while risking hydraulic failure in leaves under the well-hydrated conditions observed during this study.

#### *Coordination of stem and leaf hydraulic vulnerability*

Our study showed a high degree of coordination between stem and leaf hydraulic features. In all three species, leaves were more vulnerable to loss of hydraulic conductance than stems, with stem hydraulic parameters ( $P_{12}$ ,  $P_{50}$ ) being  $0.4$  to  $1.4$  MPa more negative than respective leaf parameters.

Midday  $\Psi_{leaf}$  was predicted to cause a  $33$  to  $49\%$  midday decline in whole-leaf conductance, which is in agreement with previous studies (Bucci et al. 2003, Brodrigg & Holbrook 2004, Bucci et al. 2012\_ENREF\_2) and may serve to protect stems from embolism formation by reducing further water loss (Brodrigg & Holbrook 2003, Zhang et al. 2009).

Comparing safety margins between leaves and stems (leaf  $P_{50}$  - stem  $P_{50}$ ), values between -0.1 MPa (leaves minimally less vulnerable than stems) and +2.4 MPa (leaves much more vulnerable than stems) are reported in the literature for 26 angiosperm species from temperate (Hacke & Sauter 1996, Cochard et al. 2002, Johnson et al. 2011) and tropical biomes (Brodribb & Holbrook 2003, Brodribb et al. 2003, Bucci et al. 2008, Hao et al. 2008, Chen et al. 2009, Chen et al. 2010). The overall mean safety margin across these 26 species was  $0.9 \pm 0.2$  MPa, with very narrow safety margins of 0.5 MPa or less in 3 temperate and 8 tropical species. In contrast, Bucci et al. (2013) reported broader safety margins of 0.8 to 2.6 MPa for cold desert species. Our observed hydraulic safety margins were very narrow at 0.4 MPa in *D. papuanum* and *S. sayeri*, with a broader safety margin of 1.4 MPa in *E. grandis* (Tab. 2, Fig. 2). The higher hydraulic vulnerability of leaves is in accordance with the hydraulic vulnerability segmentation hypothesis (Zimmermann & Brown 1971, Tyree & Ewers 1991), which states that terminal components of the hydraulic system are more expendable, i.e. leaves that are more prone to hydraulic failure may serve to protect more proximal and more “expensive” stems from embolism. It is also possible that declines in  $K_{\text{leaf}}$  associated with extra-xylary components of the pathway (e.g. aquaporins) may have occurred at higher water potentials but were not detected by our measurements (Guyot et al. 2012). If this were the case then these transient declines in  $K_{\text{leaf}}$  would provide greater safety margins between leaf and stem.

#### *Ultrasonic emissions*

Ultrasonic emission (UE) data supported hydraulic vulnerability analysis, as hydraulically measured stem  $P_{12}$  corresponded to an initial rise in the cumulative curves in two species, and to the first occurrence of UE in the third (Fig. 3). In two species, high acoustic activity in stems, i.e. a steep rise in cumulative signals, was observed near their hydraulic  $P_{50}$ . Leaf UE started near hydraulic  $P_{12}$  and midday  $\Psi_{\text{leaf}}$ , which is in accordance with previous studies (Hacke & Sauter 1996, Kikuta et al. 1997, Nardini & Salleo 2003). The alignment of UE and hydraulic  $P_{12}$  adds weight to the observation that plants operate close to their hydraulic limits on the level of leaves (e.g. Hacke & Sauter 1996; current study) and stems (Choat et al. 2012).

In contrast to UE counts, the  $\Psi_{\text{stem}}$  at maximum rate of stem UE signals corresponded well with hydraulic  $P_{50}$  in *D. papuanum* and *S. sayeri*, and coincided with the point at which the  $\Psi$  of stems and leaves was equilibrated in all species (Tab. 2, Fig. 4). Furthermore, we observed a first small UE rate peak at or just above midday  $\Psi_{\text{stem}}$ , where a short rise to 5 to 25% of maximum rate was followed by a subsequent decline in signals (Fig. 4), indicating again that hydraulic coordination was fine-tuned towards avoiding significant embolism formation in stems. Use of stem UE rates thus may be considered as an alternative to cumulative UE counts for analysis of ultrasonic data.

Accepted Article



### *Conclusion*

Our results illustrate that even in tropical rainforests, trees operate at water potentials close to those causing the induction of embolism in stems and loss of hydraulic conductance in leaves. The three studied species maintained water potentials at levels that avoided embolism formation in the stem xylem. Leaf hydraulic conductance was predicted to decline at midday during peak periods of transpiration. Hydraulic characteristics of stems and leaves were finely tuned: hydraulic coordination was apparent within leaves (leaf  $P_{12}$ , midday  $\Psi_{\text{leaf}}$ , TLP), within stems (stem  $P_{12}$ , midday  $\Psi_{\text{stem}}$ ), and between leaves and stems (stem and leaf  $P_{12}$ ,  $P_{50}$ , and  $P_{88}$ ). Balanced plant-water relations would enable the studied species to optimise photosynthesis and growth within a hydraulically safe range under sufficient water supply. When water supply becomes limited, within-plant coordination is predicted to reduce further water loss (midday  $\Psi_{\text{stem}}$ , midday  $\Psi_{\text{leaf}}$ ) and limit the extent of hydraulic damage by sacrificing leaves in favour of stems.

The observed, very narrow safety margins provide further evidence that species from wet tropical forests would be at risk of mortality if the predicted aridification associated with climate change occurs in this biome. A more complete dataset of tropical rainforest hydraulics will be essential for a better understanding of the impact of global climate change on this important, productive ecosystem.

### ***Acknowledgements***

We thank Chris Blackman (Macquarie University) for sharing his methodical expertise, Sebastian Pfautsch (University of Western Sydney) for the loan of scientific equipment, Peter Byrnes and Andrew Thompson (Daintree Rainforest Observatory) for excellent technical assistance on site, Ivan Hanigan (Australian National University) for help with climate data grid processing. Markus Nolf is a recipient of a DOC-fellowship of the Austrian Academy of Sciences and a Student Research Grant of the Daintree Rainforest Observatory. Brendan Choat was supported by an Australian Research Council Future Fellowship (FT130101115).

## References

Aitken S.N., Yeaman S., Holliday J.A., Wang T. & Curtis-McLane S. (2008) Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evolutionary Applications* 1, 95-111.

Allison I., Bindoff N.L., Bindschadler R.A., Cox P.M., de Noblet N., England M.H., ..., Weaver A.J. (2011) The Copenhagen Diagnosis: Updating the world on the latest climate science. Elsevier, Oxford.

Blackman C.J., Brodribb T.J. & Jordan G.J. (2010) Leaf hydraulic vulnerability is related to conduit dimensions and drought resistance across a diverse range of woody angiosperms. *New Phytologist* 188, 1113-1123.

Blackman C.J. & Brodribb T.J. (2011) Two measures of leaf capacitance: insights into the water transport pathway and hydraulic conductance in leaves. *Functional Plant Biology* 38, 118-126.

Brodersen C.R., McElrone A.J., Choat B., Lee E.F., Shackel K.A. & Matthews M.A. (2013) In vivo visualizations of drought-induced embolism spread in *Vitis vinifera*. *Plant Physiology* 161, 1820-1829.

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

Brodribb T.J., Holbrook N.M., Edwards E.J. & Gutiérrez M.V. (2003) Relations between stomatal closure, leaf turgor and xylem vulnerability in eight tropical dry forest trees. *Plant, Cell and Environment* 26, 443-450.

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

Brodribb T.J. & Holbrook N.M. (2004) Diurnal depression of leaf hydraulic conductance in a tropical tree species. *Plant, Cell and Environment* 27, 820-827.

Brodribb T.J. & Holbrook N.M. (2006) Declining hydraulic efficiency as transpiring leaves desiccate: two types of response. *Plant, Cell and Environment* 29, 2205-2215.

Brodribb T.J., McAdam S.A., Jordan G.J. & Martins S.C. (2014) Conifer species adapt to low-rainfall climates by following one of two divergent pathways. *Proceedings of the National Academy of Sciences of the United States of America* 111, 14489-14493.

- Bucci S.J., Scholz F.G., Campanello P.I., Montti L., Jimenez-Castillo M., Rockwell F.A., ..., Goldstein G. (2012) Hydraulic differences along the water transport system of South American *Nothofagus* species: do leaves protect the stem functionality? *Tree Physiology* 32, 880-893.
- Bucci S.J., Scholz F.G., Goldstein G., Meinzer F.C., Franco A.C., Zhang Y. & Hao G.-Y. (2008) Water relations and hydraulic architecture in Cerrado trees: adjustments to seasonal changes in water availability and evaporative demand. *Brazilian Journal of Plant Physiology* 20, 233-245.
- Bucci S.J., Scholz F.G., Goldstein G., Meinzer F.C. & Sternberg L.D.L. (2003) Dynamic changes in hydraulic conductivity in petioles of two savanna tree species: factors and mechanisms contributing to the refilling of embolized vessels. *Plant, Cell and Environment* 26, 1633-1645.
- Bucci S.J., Scholz F.G., Peschiutta M.L., Arias N.S., Meinzer F.C. & Goldstein G. (2013) The stem xylem of Patagonian shrubs operates far from the point of catastrophic dysfunction and is additionally protected from drought-induced embolism by leaves and roots. *Plant, Cell and Environment* 26, 2163-2174.
- Bureau of Meteorology (2013) Climate Data Online, [WWW document] URL <http://www.bom.gov.au/climate/data/> [accessed 2013-08-08]
- 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.
- Choat B., Ball M.C., Luly J.G., Donnelly C.F. & Holtum J.A.M. (2006) Seasonal patterns of leaf gas exchange and water relations in dry rain forest trees of contrasting leaf phenology. *Tree Physiology* 26, 657-664.
- 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 and Environment* 33, 1502-1512.
- Choat B., Lahr E.C., Melcher P.J., Zwieniecki M.A. & Holbrook N.M. (2005) The spatial pattern of air seeding thresholds in mature sugar maple trees. *Plant, Cell and Environment* 28, 1082-1089.
- Choat B., Jansen S., Brodribb T.J., Cochard H., Delzon S., Bhaskar R., ..., Zanne A.E. (2012) Global convergence in the vulnerability of forests to drought. *Nature* 491, 752-755.

Cochard H., Badel E., Herbette S., Delzon S., Choat B. & Jansen S. (2013) Methods for measuring plant vulnerability to cavitation: a critical review. *Journal of Experimental Botany* 64, 4779-4791.

Cochard H. & Tyree M.T. (1990) Xylem dysfunction in *Quercus*: vessel sizes, tyloses, cavitation and seasonal changes in embolism. *Tree Physiology* 6, 393-407.

Cochard H., Coll L., Le Roux X. & Améglio T. (2002) Unraveling the effects of plant hydraulics on stomatal closure during water stress in walnut. *Plant Physiology* 128, 282-290.

Cook K.H. & Vizy E.K. (2007) Effects of twenty-first-century climate change on the Amazon rain forest. *Journal of Climate* 21, 542-560.

Duursma R. (2014) Fit vulnerability curves in R (R package) [WWW document] URL <https://bitbucket.org/remkoduursma/fitplc/> [accessed 2014-07-04]

Grace J., Malhi Y., Higuchi N. & Meir P. (2001) Productivity of tropical forests. In *Terrestrial Global Productivity* (eds Roy J, Saugier B, Mooney H.A.), pp 401-426. Academic Press, San Diego.

Guyot G., Scoffoni C. & Sack L. (2012) Combined impacts of irradiance and dehydration on leaf hydraulic conductance: insights into vulnerability and stomatal control. *Plant Cell and Environment* 35, 857-871.

Hacke U. & Sauter J. (1996) Drought-induced xylem dysfunction in petioles, branches, and roots of *Populus balsamifera* L. and *Alnus glutinosa* (L.) Gaertn. *Plant Physiology* 111, 413-417.

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

Hartmann H., Ziegler W., Kolle O. & Trumbore S. (2013) Thirst beats hunger – declining hydration during drought prevents carbon starvation in Norway spruce saplings. *New Phytologist* 200, 340-349.

Hyland B.P.M., Whiffin T. & Zich F.A. (2010) Australian tropical rainforest plants. Trees, shrubs and vines. Version 6.1 [WWW document] URL <http://keys.trin.org.au/key-server/data/0e0f0504-0103-430d-8004-060d07080d04/media/Html/index.html> [accessed 2013-09-18]

Johnson D.M., McCulloh K.A., Meinzer F.C., Woodruff D.R. & Eissenstat D.M. (2011) Hydraulic patterns and safety margins, from stem to stomata, in three eastern US tree species. *Tree Physiology* 31, 659-668.

Johnson D.M., McCulloh K.A., Woodruff D.R. & Meinzer F.C. (2012a) Hydraulic safety margins and embolism reversal in stems and leaves: Why are conifers and angiosperms so different? *Plant Science* 195, 48-53.

Johnson D.M., McCulloh K.A., Woodruff D.R. & Meinzer F.C. (2012b) Evidence for xylem embolism as a primary factor in dehydration-induced declines in leaf hydraulic conductance. *Plant, Cell and Environment* 35, 760-769.

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

Kim Y.X. & Steudle E. (2007) Light and turgor affect the water permeability (aquaporins) of parenchyma cells in the midrib of leaves of *Zea mays*. *Journal of Experimental Botany* 58, 4119-4129.

Kikuta S.B. (2003) Ultrasound acoustic emissions from bark samples differing in anatomical characteristics. *Phyton-Annales Rei Botanicae* 43, 161-178.

Kikuta S.B., Lo Gullo M.A., Nardini A., Richter H. & Salleo S. (1997) Ultrasound acoustic emissions from dehydrating leaves of deciduous and evergreen trees. *Plant, Cell and Environment* 20, 1381-1390.

Kirkham M.B. (2004) Principles of soil and plant water relations. 1st edition. Elsevier Academic Press, Amsterdam, Netherlands.

Lewis S.L. (2006) Tropical forests and the changing earth system. *Philosophical Transactions of the Royal Society B* 361, 195-210.

Liddell M. (2013) Baseline Climate Data 2012, Far North Queensland Rainforest Supersite, Cape Tribulation Node. Document ID lloyd.238.14 [WWW document] URL <http://www.tern-supersites.net.au/knb/metacat/lloyd.238.14/html> [accessed 2013-09-09]

- Maherali H., Pockman W.T. & Jackson R.B. (2004) Adaptive variation in the vulnerability of woody plants to xylem cavitation. *Ecology* 85, 2184-2199.
- Martorell S., Diaz-Espejo A., Medrano H., Ball M.C. & Choat B. (2014) Rapid hydraulic recovery in *Eucalyptus pauciflora* after drought: linkages between stem hydraulics and leaf gas exchange. *Plant, Cell and Environment* 37, 617-626.
- Mayr S., Beikircher B., Obkircher M. & Schmid P. (2010) Hydraulic plasticity and limitations of alpine *Rhododendron* species. *Oecologia* 164, 321-330.
- Mayr S., Rosner S. (2011) Cavitation in dehydrating xylem of *Picea abies*: energy properties of ultrasonic emissions reflect tracheid dimensions. *Tree Physiology* 31, 59-67.
- 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., Woodruff D.R., Domec J.-C., Goldstein G., Campanello P.I., Gatti M.G. & Villalobos-Vega R. (2008) Coordination of leaf and stem water transport properties in tropical forest trees. *Oecologia* 156, 31-41.
- Milburn J.A. & Johnson R.P.C. (1966) The conduction of sap. II. Detection of vibrations produced by sap cavitation in *Ricinus* xylem. *Planta* 69, 43-52.
- Nardini A. & Luglio J. (2014) Leaf hydraulic capacity and drought vulnerability: possible trade-offs and correlations with climate across three major biomes. *Functional Ecology* 28, 810-818.
- Nardini A. & Salleo S. (2003) Effects of the experimental blockage of the major veins on hydraulics and gas exchange of *Prunus laurocerasus* L. leaves. *Journal of Experimental Botany* 54, 1213-1219.
- Phillips O.L., Aragão L.E.O.C., Lewis S.L., Fisher J.B., Lloyd J., López-González G., ..., Torres-Lezama A. (2009) Drought sensitivity of the Amazon rainforest. *Science* 323, 1344-1347.
- Ponomarenko A., Vincent O., Pietriga A., Cochard H., Badel E. & Marmottant P. (2014) Ultrasonic emissions reveal individual cavitation bubbles in water-stressed wood. *Journal of the Royal Society Interface* 11. doi: 10.1098/rsif.2014.0480.

Prentice I.C., Farquhar G.D., Fasham M.J.R., Goulden M.L., Heimann M., Jaramillo V.J., ... Wallace D.W. (2001) The carbon cycle and atmospheric carbon dioxide. In *Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change* (eds Houghton JT, Ding Y, Griggs DJ), pp 183-237. Cambridge University Press, Cambridge.

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>

Raven J.A. (2002) Selection pressures on stomatal evolution. *New Phytologist* 153, 371-386.

Ritman K.T. & Milburn J.A. (1988) Acoustic emissions from plants: ultrasonic and audible compared. *Journal of Experimental Botany* 39, 1237-1248.

Rosetto M., Jones R. & Hunter J. (2004) Genetic effects of rainforest fragmentation in an early successional tree (*Elaeocarpus grandis*). *Heredity* 93, 610-618.

Sack L. & Holbrook N.M. (2006) Leaf hydraulics. *Annual Review of Plant Biology* 57: 361-381.

Sack L., Pasquet-Kok J. & PrometheusWiki contributors (2011) Leaf pressure-volume curve parameters. PrometheusWiki [WWW document] URL [http://www.publish.csiro.au/prometheuswiki/tiki-pagehistory.php?page=Leaf pressure-volume curve parameters](http://www.publish.csiro.au/prometheuswiki/tiki-pagehistory.php?page=Leaf_pressure-volume_curve_parameters) [accessed 2013-08-13]

Salazar L.F., Nobre C.A. & Oyama M.D. (2007) Climate change consequences on the biome distribution in tropical South America. *Geophysical Research Letters* 34, L09708.

Schneider C.A., Rasband W.S. & Eliceiri K.W. (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9, 671-675.

Scholander P., Bradstreet E., Hemmingsen E. & Hammel H. (1965) Sap pressure in vascular plants: negative hydrostatic pressure can be measured in plants. *Science* 148, 339-346.

Scoffoni C., McKown A.D., Rawls M. & Sack L. (2012) Dynamics of leaf hydraulic conductance with water status: quantification and analysis of species differences under steady state. *Journal of Experimental Botany* 63, 643-658.

Scoffoni C. & Sack L. (2014) Are leaves 'freewheelin'? Testing for a Wheeler-type effect in leaf xylem hydraulic decline. *Plant, Cell and Environment* 38, 534-543.

- Scoffoni C., Vuong C., Diep S., Cochard H. & Sack L. (2014) Leaf shrinkage with dehydration: coordination with hydraulic vulnerability and drought tolerance. *Plant Physiology* 164, 1772-1788.
- Sperry J.S. (2000) Hydraulic constraints on plant gas exchange. *Agricultural and Forest Meteorology* 104, 13-23.
- Sperry J.S., Donnelly J.R. & Tyree M.T. (1988) A method for measuring hydraulic conductivity and embolism in xylem. *Plant, Cell and Environment* 11, 35-40.
- Sperry J.S. & Pockman W.T. (1993) Limitation of transpiration by hydraulic conductance and xylem cavitation in *Betula occidentalis*. *Plant, Cell and Environment* 16, 279-287.
- Tyree M.T. & Dixon M.A. (1983) Cavitation events in *Thuja occidentalis* L.? *Plant Physiology* 72, 1094-1099.
- Tyree M.T. & Ewers F.W. (1991) The hydraulic architecture of trees and other woody plants. *New Phytologist* 119, 345-360.
- Tyree M.T. & Zimmermann M.H. (2002) Xylem structure and the ascent of sap. 2nd edition. Springer, Berlin, Germany.
- 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.
- Wheeler J.K., Huggett B.A., Tofte A.N., Rockwell F.E. & Holbrook N.M. (2013) Cutting xylem under tension or supersaturated with gas can generate PLC and the appearance of rapid recovery from embolism. *Plant, Cell and Environment* 36, 1938-1949.
- White E., Tucker N., Meyers N. & Wilson J. (2004) Seed dispersal to revegetated isolated rainforest patches in North Queensland. *Forest Ecology and Management* 192, 409-426.
- Wolkerstorfer S.V., Rosner S. & Hietz P. (2012) An improved method and data analysis for ultrasound acoustic emissions and xylem vulnerability in conifer wood. *Physiologia Plantarum* 146, 184-191.
- Zhang Y.J., Meinzer F.C., Hao G.Y., Scholz F.G., Bucci S.J., Takahashi F.S.C., ..., Goldstein G. (2009) Size-dependent mortality in a Neotropical savanna tree: the role of height-related



adjustments in hydraulic architecture and carbon allocation. *Plant, Cell and Environment* 32, 1456-1466.

Zimmermann M.H. (1983) Xylem structure and the ascent of sap. 1st edition. Springer, Berlin, Germany.

Zimmermann M.H & Brown C.L. (1971) Trees: structure and function. Springer, New York, USA.

Accepted Article

### Tables and table legends

Tab. 1: *In situ* water potentials (predawn, midday leaf, midday stem; MPa), maximum leaf hydraulic conductance ( $k_{\text{leaf max}}$ ;  $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ ), and pressure-volume curve parameters (turgor loss point ( $\Psi_{\text{TLP}}$ ; MPa), osmotic potential at full water saturation ( $\Pi_0$ ; MPa), absolute capacitance at full turgor ( $C_{\text{FT}}$ ;  $\text{mmol m}^{-2} \text{MPa}^{-1}$ ) and absolute capacitance below turgor loss point ( $C_{\text{TLP}}$ ;  $\text{mmol m}^{-2} \text{MPa}^{-1}$ )).  $n = 3$ . In each row, different superscript letters indicate significant differences ( $P < 0.05$ ) across species. Mean  $\pm$  standard error.

	<i>D. papuanum</i>	<i>E. grandis</i>	<i>S. sayeri</i>
Predawn $\Psi_{\text{leaf}}$	$-0.50 \pm 0.07^a$	$-0.29 \pm 0.06^a$	$-0.34 \pm 0.10^a$
Midday $\Psi_{\text{leaf}}$	$-1.56 \pm 0.28^a$	$-1.62 \pm 0.31^a$	$-1.23 \pm 0.50^a$
Midday $\Psi_{\text{stem}}$	$-1.01 \pm 0.07^a$	$-0.83 \pm 0.08^a$	$-0.75 \pm 0.26^a$
$k_{\text{leaf max}}$	3.53	20.19	3.13
$\Psi_{\text{TLP}}$	$-2.12 \pm 0.11^a$	$-2.16 \pm 0.26^a$	$-1.86 \pm 0.14^a$
$\Pi_0$	$-1.85 \pm 0.06^a$	$-1.76 \pm 0.17^a$	$-1.50 \pm 0.19^a$
$C_{\text{FT}}$	$230.7 \pm 11.1^a$	$223.3 \pm 8.8^a$	$342.8 \pm 48.3^a$
$C_{\text{TLP}}$	$1419 \pm 150.7^a$	$597.3 \pm 88.2^b$	$1192.2 \pm 150.4^{ab}$

Tab. 2: Hydraulic vulnerability of stems and leaves. Water potential ( $\Psi$ ; MPa) at 12 ( $P_{12}$ ), 50 ( $P_{50}$ ) and 88% ( $P_{88}$ ) loss of conductivity and hydraulic safety margins (leaf  $P_{50}$  - stem  $P_{50}$ ; MPa), and  $\Psi$  at maximum rate of ultrasonic emissions in stems ( $P_{\max\text{UErate}}$ , MPa;  $n = 3$  (*D. papuanum*), 3 (*E. grandis*), 2 (*S. sayeri*)). In each row, different superscript letters indicate significant differences ( $P < 0.05$ ) across species. Asterisks indicate significant differences between stems and leaves of one species ( $P < 0.05$ ). Mean values and 95% confidence interval (in parentheses).

	<i>D. papuanum</i>	<i>E. grandis</i>	<i>S. sayeri</i>
Stem $P_{12}$	-1.18 <sup>a</sup> (-1.55, -0.77)	-1.87 <sup>a</sup> (-2.68, -1.31)	-1.15 <sup>a</sup> (-1.51, -0.56)
Stem $P_{50}$	-2.63 <sup>a</sup> (-2.97, -2.34)	-3.06 <sup>a</sup> (-3.41, -2.63)	-2.10 <sup>a</sup> (-2.47, -1.87)
Stem $P_{88}$	-4.48 <sup>a</sup> (-5.27, -3.71)	-4.24 <sup>a</sup> (-4.94, -3.21)	-3.13 <sup>a</sup> (-3.30, -2.60)
Leaf $P_{12}$	-0.74 <sup>a</sup> (-1.44, >-0.74)	-0.66 <sup>a*</sup> (-1.22, >-0.66)	-0.48 <sup>a</sup> (-0.86, >-0.43)
Leaf $P_{50}$	-2.24 <sup>a</sup> (-2.77, -1.73)	-1.66 <sup>a*</sup> (-2.06, -1.09)	-1.72 <sup>a</sup> (-1.96, -1.40)
Leaf $P_{88}$	-4.66 <sup>a</sup> (-6.15, -3.77)	-3.03 <sup>b</sup> (-3.48, -2.55)	-4.01 <sup>a*</sup> (-5.25, -4.45)
Safety margin (leaf $P_{50}$ - stem $P_{50}$ )	0.39	1.40	0.38
Stem $P_{\max\text{UErate}}$	-2.84 ± 0.64 <sup>a</sup>	-2.25 ± 0.16 <sup>b</sup>	-2.31 <sup>ab</sup>

### Figure legends

Fig. 1: Monthly temperature and precipitation at Cape Tribulation Store. Mean monthly temperature in 2012 (red dashed line) and 30-year average (grey dashed line). Total monthly precipitation in 2012 (blue solid line) and 30-year average (1961–1990; grey boxplot). Data: Bureau of Meteorology (2013).

Fig. 2: Vulnerability curves (solid lines) of stems (a, c, and e; open symbols) and leaves (b, d, and f; closed symbols) in *Dysoxylum papuanum* (a and b; circles), *Elaeocarpus grandis* (c and d; triangles) and *Syzygium sayeri* (e and f; squares), measured using hydraulic techniques. Vertical lines indicate hydraulic  $P_{12}$ ,  $P_{50}$  and  $P_{88}$  (solid), *in situ* midday water potential (dashed) and turgor loss point (dotted).

Fig. 3: Cumulative ultrasonic emissions (solid lines) and corresponding water potential (open circles, dashed lines) in stems (a, c, and e) and leaves (b, d, and f) of *Dysoxylum papuanum* (a and b), *Elaeocarpus grandis* (c and d) and *Syzygium sayeri* (e and f). Dashed vertical lines and arrows indicate the timing of *in situ* midday water potential ( $\Psi_{\text{midday}}$ ) and hydraulically measured vulnerability thresholds ( $P_{12}$ ,  $P_{50}$ , and  $P_{88}$ ), respectively.

Fig. 4: Progress of ultrasonic emission (UE) rates (% of maximum rate, solid lines) and water potential (open circles, dashed lines) in stems of *Dysoxylum papuanum* (a), *Elaeocarpus grandis* (b), and *Syzygium sayeri* (c) during the first 40 h of branch desiccation. Vertical lines indicate the maximum UE-rate for each branch (solid) and *in-situ* midday stem water potential (dashed). Example curves obtained from individual branches.







