

Tolerance to low leaf water status of tropical tree seedlings is related to drought performance and distribution

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Summary

1. Habitat specialization models predict that adaptations to environmental conditions explain species distributions. In tropical rainforests, the ability of the seedlings to survive during drought has been shown to be a key determinant of species distributions. We hypothesize that differences among species in their tolerance to low tissue water status is the mechanism underlying differences in performance during drought.

2. To test this hypothesis we quantified tolerance to low leaf water status for over 20 species from central Panama in greenhouse experiments using two different experimental approaches. Results from both approaches were highly correlated with each other.

3. We found that tolerance to low leaf water status correlated with species drought performance in the field and with their distribution across a gradient of dry season length, with the more desiccation-tolerant species having higher survival in dry relative to irrigated conditions, and occurring in drier areas. These results support the hypothesis that, in tropical forests, tolerance to low tissue water status governs seedling performance during drought, as well as being a determinant of species distribution patterns.

4. Lower tolerance to low leaf water status was correlated with greater stem hydraulic conductance. In addition, all species tested, including both desiccation-sensitive and desiccation-resistant species, showed similar losses of xylem conductance, about 80%, when near death. These results suggest that a principal mechanism by which desiccation leads to plant mortality is the loss of xylem conductivity.

Key-words: drought resistance, dry season, hydraulic conductance, rainfall, water potential

Introduction

Understanding the mechanisms by which abiotic interactions determine the abundance and distribution of organisms is a central goal of ecology. Research in the last two decades in tropical rainforests has demonstrated that rainfall is a key factor. Rainfall varies up to 10-fold between dry, moist and wet tropical forests (Holdridge 1947; Murphy & Lugo 1986;

Clinebell *et al.* 1995; Walsh 1996) and the distributions of many plants correlate with rainfall (Veenendaal & Swaine 1998; Bongers *et al.* 1999; Baltzer *et al.* 2008). As a result, a marked turnover of plant species is associated with this moisture gradient (beta diversity) and such turnover makes a large contribution to regional diversity (Condit *et al.* 2002; Davidar *et al.* 2007). Additionally, wetter forests harbour higher diversity (alpha diversity) than drier forests (Gentry 1988; Clinebell *et al.* 1995; ter Steege *et al.* 2003). Accordingly, understanding the role that rainfall and seasonality play in plant distributions is central to tropical ecology.

Recently we found that drought performance is an important determinant of distribution with respect to the length of the dry season, such that species with poor drought performance are excluded from dry forest (Engelbrecht *et al.*

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2007a). We refer to the ability of a species to survive under field conditions during low water availability as drought performance, a term that encompasses the multiple stresses that may be present under natural drought including changes in pests or nutrients (Tyree *et al.* 2003). In the present study, we have excluded factors such as natural enemies and soil fertility from consideration and focus on the role of drought resistance in drought performance. We define drought resistance as the suite of traits that support survival under conditions of a single stress, low water availability.

Strategies of drought resistance include desiccation delay and tolerance to low tissue water status (Tyree *et al.* 2003). Desiccation delay involves traits that increase access to water and reduce water loss. Deep roots, early stomatal responses, low cuticular conductance, water storage in stem of other organs, osmotic adjustment, and leaf shedding all can contribute to desiccation delay, and can be directly measured. Analysis of plant water status over a drying cycle in a common garden can serve as an integrated, quantitative measure of species differences in desiccation delay. Tolerance to low tissue water status, or desiccation tolerance, is based upon continued plant function despite water loss. The ability of plants to tolerate water deficits is promoted by physiological traits such as greater resistance of the xylem to embolism, permitting continued water transport and gas exchange, or the ability of cells (especially meristems) to remain alive at low relative water content and low water potentials (Ψ). For most species, little is known of the relative importance of desiccation delay and desiccation tolerance in promoting survival during a period of reduced water availability (Tobin, Lopez & Kursar 1999; Tyree *et al.* 2003; Nepstad *et al.* 2007).

Our analysis of five species from Panama suggested that their drought performance depended predominantly on their desiccation tolerance (Tyree *et al.* 2003). Similar methods applied in an independent analysis of floristic changes across a climate gradient in Southeast Asia showed that desiccation tolerance correlated with distribution (Baltzer *et al.* 2008). In the present study we developed a second, independent method to assess desiccation tolerance in which we quantified the water status of plants at 50% mortality. Both the methods are related to the analysis of lethal water status or the degree of stress that causes incipient damage (Moore & Chapman 1986; Ludlow & Muchow 1990; Engelbrecht, Tyree & Kursar 2007b). We extended our data set to over 20 species to further investigate the role of desiccation tolerance in drought performance and distribution.

Additionally, we assessed the mechanism leading to mortality during desiccation. Xylem cavitation is generally believed to be the proximate cause of plant death (Davis *et al.* 2002). Nevertheless few studies correlate vulnerability to loss of hydraulic conductance with the water potential at which plants die. If cavitation causes mortality, the magnitude of the loss of xylem conductance in severely wilted plants is expected to be similar, regardless of whether a species became severely wilted at high or low water potential, as Tyree *et al.* (2003) found for four tropical species. In the present study

we expanded the previous data set to 12 species, including both desiccation-sensitive and desiccation-resistant species.

Methods

SITE AND PLANT MATERIAL

Screenhouse experiments were carried out at the Smithsonian Tropical Research Institute facilities on Barro Colorado Island in the Republic of Panama. We studied 33 species; most are shade-tolerant trees that are common in central Panama (Table 1). Seeds or seedlings were collected from three or more trees in central Panama, mainly in the forests in the Barro Colorado Nature Monument. In this forest the average annual rainfall is 2600 mm, with a pronounced 4-month dry season (Leigh 1999).

TOLERANCE TO LOW LEAF WATER STATUS

Desiccation tolerance was assessed in two ways: by measuring the leaf water status corresponding to 50% of plants having either shoot or plant death (experiment 1), and at the severely wilted stage (experiment 2). Leaf water status was evaluated as leaf water potential (Ψ_{leaf}) and as relative leaf water content (RWC). Leaf water content per leaf dry weight, w_d , was determined as (fresh weight – dry weight)/dry weight. RWC was determined as the ratio of the w_d of droughted plants divided by the w_d of fully hydrated plants and converted to a percent.

Plants were 1–3 years old and about 10 to 40 cm tall and kept in a screenhouse to exclude herbivores. Shade cloth reduced the light conditions to 6–7% of that measured in a nearby clearing. Plants were grown in pots of 1.7–1.9 L volume in well-mixed soils collected from the forest and irrigated at least two times per week. For the drought treatment, plants were placed in roofed (rainproof) enclosures and dried to a range of visually assessed wilting stages by withholding watering. Drying times in the greenhouse agreed well with those that we had previously determined in the field for seedlings of shade-tolerant species, 2 weeks to 2 months (Engelbrecht & Kursar 2003; Engelbrecht, Kursar & M.T. Tyree 2005), as well as for seedlings of pioneer species, less than a week (Engelbrecht *et al.* 2006). Sample sizes were 20–47 or 6–31 individuals per species and for experiments 1 and 2, respectively. The only exceptions were *Apeiba aspera* and *Ochroma pyramidale* with 84 and 85 seedlings, respectively. Both species, which typically occur in tree-fall light gaps and have tiny seedlings (< 15 mm high), were grown under high light conditions (30–50% of full sun) in 'row seed flats', with 15–20 plants per flat (25 × 3.2 cm and 3 cm deep; Hummert International, Earth City, MO). Trials for the different species were staggered over a 10-month period.

WATER STATUS AT 50% MORTALITY (EXPERIMENT 1)

For 24 species we assessed plants at a range of desiccation stages for RWC, Ψ_{leaf} , and survival after rehydration. We used these data to calculate the RWC and Ψ_{leaf} at 50% mortality. We term this 'lethal desiccation 50' or LD50 by analogy with the terminology used in dose-response analysis, with $LD50_{\text{RWC}}$ and $LD50_{\Psi}$, representing the RWC and the Ψ_{leaf} at 50% mortality, respectively. Plants were left unwatered for 10–140 days (4 days in *Ochroma* seedlings), giving plants that ranged from only very mildly desiccated to dead. Leaves were sampled for RWC and Ψ_{leaf} ; plants were rewatered and maintained in a well-watered state. In order to maximize the statistical

Table 1. Study species. All species except *Apeiba aspera* and *Ochroma pyramidale* are shade-tolerant and can persist in the forest understorey

Species	Symbol	Family	D_p	Distribution	Classification
<i>Alibertia edulis</i>	Ae	Rubiaceae	81.9	Wet-mid-dry	Dry
<i>Alseis blackiana</i>	Ab	Rubiaceae	nd	Mid-dry	Dry
<i>Andira inermis</i>	Ai	Fabaceae	69.0	Wet-mid-dry	Dry
<i>Apeiba aspera</i>	Aa	Tiliaceae	nd	Wet-mid	Wet
<i>Aspidosperma cruenta</i>	Ac	Apocynaceae	nd	Wet-mid	Wet
<i>Beilschmiedia pendula</i>	Bp	Lauraceae	0	Wet-mid	Wet
<i>Brosimum alicastrum</i>	Ba	Moraceae	96.4	Wet-mid-dry	Dry
<i>Calophyllum longifolium</i>	Bl	Clusiaceae	30.0	Wet-mid	Wet
<i>Chrysophyllum argenteum</i>	Car	Sapotaceae	nd	Wet-mid	Wet
<i>Cojoba rufescens</i> †	Cr	Fabaceae	nd	Wet-mid-dry	Dry
<i>Cordia alliodora</i>	Cal	Boraginaceae	74.2	Mid-dry	Dry
<i>Coussarea curvigemma</i>	Cc	Rubiaceae	nd	Mid-dry	Dry
<i>Crossopetalum parviflorum</i>	Cp	Celastraceae	89.9	Wet-mid	Wet
<i>Dipteryx panamensis</i>	Dp	Fabaceae	100	Mid	
<i>Guatteria amplifolia</i>	Ga	Annonaceae	nd	Wet-mid	Wet
<i>Herrania purpurea</i>	Hpu	Sterculiaceae	58.7	Wet-mid	Wet
<i>Hybanthus prunifolius</i>	Hpr	Violaceae	78.7	Mid	
<i>Lacmellea panamensis</i>	Lpa	Apocynaceae	86.7	Wet-mid	Wet
<i>Licania platypus</i>	Lpl	Chrysobalanaceae	66.7	Mid	
<i>Manilkara bidentata</i>	Mb	Sapotaceae	nd	Wet-mid	Wet
<i>Ochroma pyramidale</i>	Op	Bombacaceae	nd	Wet-mid	Wet
<i>Ouratea lucens</i>	Ol	Ochnaceae	96.7	Mid-dry	Dry
<i>Piper cordulatum</i>	Pc	Piperaceae	nd	Wet-mid-dry	Dry
<i>Piper trigonum</i>	Pt	Piperaceae	17.9	Wet-mid	Wet
<i>Posoqueria latifolia</i>	Pl	Rubiaceae	99.7	Wet-mid-dry	Dry
<i>Pouteria reticulata</i> ‡	Pr	Sapotaceae	65.4	Mid-dry	Dry
<i>Quararibea asterolepis</i>	Qa	Bombacaceae	nd	Mid	
<i>Sorocea affinis</i>	Sa	Moraceae	53.3	Wet-mid-dry	Dry
<i>Swartzia simplex</i> §	Ss	Fabaceae	95.2	Mid-dry	Dry
<i>Tetragastris panamensis</i>	Tp	Bursaceae	78.4	Mid	
<i>Virola multiflora</i>	Vm	Myristicaceae	nd	Wet-mid	Wet
<i>Virola surinamensis</i>	Vs	Myristicaceae	14.2	Wet-mid	Wet
<i>Vochysia ferruginea</i>	Vf	Vochysiaceae	39.6	Wet-mid-dry	Dry

'Symbol' is used in the Figures to identify individual species. D_p is the drought performance in the field experiment. Species with 'nd' were not included in the field experiment. 'Distribution' indicates the occurrence across the Isthmus of Panama and 'Classification' indicates how species distributions were entered into the analyses (see Methods). †*Pithecellobium rufescens* in Croat (1978). ‡*Pouteria unilocularis* in Croat (1978). §*Swartzia simplex* var. *grandiflora*. For authorities consult the Tropicos data base (<http://www.tropicos.org>).

power for identifying the LD50, an excess of plants were desiccated to slightly less than, at the point of, and slightly beyond severely wilted. Three months after rewatering the plant's shoots were scored as alive or dead. For most species, this coincided with the death of the entire plant. Five species, *Cojoba rufescens*, *Crossopetalum parviflorum*, *Hybanthus prunifolius*, *Piper cordulatum* and *Piper trigonum*, had major shoot dieback but did eventually resprout from the roots or lower stem. The reason for using the shoot death criterion is that, because the LD50 analysis is based upon leaf water status, the measurements are only meaningful to the point of hydraulic failure. Once the upper stem has died (or leaf abscission is initiated), the remaining living tissues at the base of the shoot or in the root are not in hydraulic contact with the leaves. Consequently, because this experiment quantifies the leaf water status, it measures the water status of the shoot but not of the surviving tissues. Analyses with the above five species excluded, however, gave very similar results.

The lethal desiccation state was determined in a dose-response analysis of survival as a function of leaf RWC or Ψ_{leaf} . The data were fitted using probit regression (PROC PROBIT; SAS Institute 1999). The probability of survival was modelled as:

$$\Phi^{-1}(p) = \beta_0 + (\beta_1 \times \text{RWC}) \text{ and } \Phi^{-1}(p) = \beta_0 + (\beta_1 \times \Psi_{\text{leaf}}) \quad \text{eqn 1}$$

where $\Phi^{-1}(p)$ is the inverse of the cumulative normal probability function (Young & Young 1998). Untransformed values for RWC and Ψ_{leaf} were entered into the model. Implementation of the 'INVERSECL' option of SAS computed the water status at a range of survival probabilities and also implemented Fieller's theorem to give the 95% confidence limits. A worked example of the calculation of confidence intervals is provided in Collett (2003). To obtain RWC and Ψ_{leaf} at 50% mortality, we set $P = 0.5$ in eqn 1 and obtain $\Phi^{-1}(p) = 0$. Values at 50% mortality then equal:

$$LD50_{\text{RWC}} \text{ or } LD50_{\Psi} = -\beta_0/\beta_1 \quad \text{eqn 2}$$

Appendices S1 and S2 contain values for the intercept (β_0), beta (β_1), LD50 and SE (from Fieller's theorem) (see Supplementary Material). As shown in Fig. 1a,b, we also calculated the probability of survival as a function of RWC or Ψ_{leaf} as follows:

$$p = \Phi(\beta_0 + (\beta_1 \times \text{RWC})) \text{ and } p = \Phi(\beta_0 + (\beta_1 \times \Psi_{\text{leaf}})) \quad \text{eqn 3}$$

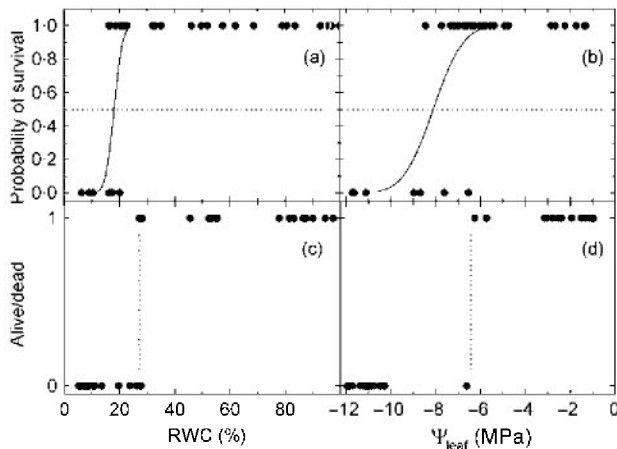


Fig. 1. Survival as a function of percent leaf relative water content, RWC, and leaf water potential, Ψ_{leaf} . (a) *Aspidosperma cruenta* (28 individuals alive, 11 dead), with $LD50_{\text{RWC}} = 18.1\%$. (b) *Cojoba rufescens* (28 alive, 8 dead), with $LD50_{\Psi} = -8.11$ MPa. In a and b the $LD50$'s were determined by probit regression (solid lines). The horizontal dashed lines indicate 50% survival and cross the regression lines at the $LD50$'s. (c) *Pouteria reticulata* (26 alive, 22 dead), with $LD50_{\text{RWC}} = 27.2\%$. (d) *Lacmellea panamensis* (16 alive, 16 dead), with $LD50_{\Psi} = -6.4$ MPa. In c and d the $LD50$'s were determined by interpolation (see Methods) and are denoted by the vertical dashed lines.

where Φ is the cumulative normal probability function (see Young & Young 1998 for a worked example). For a given Φ (RWC) or Φ (Ψ_{leaf}), the value for P was obtained from a table of the cumulative normal probability function (as found in statistics texts, for example, Table A in Moore & McCabe 2003). In some species, no variation was observed: all plants above a specific water status lived and all of those below a specific water status died (Fig. 1c,d). Such data were inappropriate for statistical analysis and the $LD50$'s were estimated by interpolation. We report the midpoint between the water status of the plant that died at the highest and that of the surviving plant with the lowest water status. All three values are reported in Appendices S1 and S2.

WATER STATUS AT THE SEVERELY WILTED STAGE (EXPERIMENT 2)

For 28 species, we assessed the RWC and Ψ_{leaf} of severely wilted plants represented by, SW_{RWC} and SW_{Ψ} , respectively. We also include data for five species from Tyree *et al.* 2003. The drying time to reach the severely wilted state varied from 15 to 52 days. We defined severely wilted plants as those with 25–50% leaf necrosis (modified from Tyree *et al.* 2003). Additionally we restricted the severely wilted state such that more than 40% of plants had to survive when irrigation was resumed. To that end, a subset of 6–20 severely wilted plants per species were rewatered and scored for survival 3 months later. Survivorship averaged 77% across species (range 43–100%, with eight species at 100%). When the visual definition proved inadequate (*Lacmellea panamensis*, *Swartzia simplex* and *Posoqueria latifolia*, all plants dead), we redefined severely wilted as any visual symptom that occurred just before plants die and repeated the trial if enough plants were available (*P. latifolia*).

MEASUREMENT OF LEAF WATER POTENTIALS (Ψ_{LEAF}) AND RELATIVE WATER CONTENT (RWC)

Before the start of each species trial the total leaf area of each plant was assessed (Tyree *et al.* 2002; Engelbrecht & Kursar 2003). In the morning, leaf samples were collected for water status measurements and plants were photographed (Nikon Cool Pix 1000). The images were used for retrospective analyses of the wilting states. Leaf water potentials, Ψ_{leaf} , were determined using thermocouple psychrometers. Leaves were dried and cleaned with Kimwipes and, 5–15 min later, 5–6 mm² leaf discs were taken using leaf-cutter psychrometers (Merrill Engineering, Logan, UT). For well-watered controls, on the day prior to the measurements, pots were immersed in water for 5 min, drained for 30 s and the shoot and pot were enclosed in a plastic bag until the measurement. In experiment 1 two leaf discs were collected per psychrometer and four measurements were made per plant, in experiment 2, one leaf disc was collected per psychrometer and ten measurements were made per plant. We collected tissue that appeared to be living, usually from the youngest leaves.

We used a CR7 data logger with A3769 psychrometric modules (Campbell Instruments, Logan, UT) in psychrometric mode after 3–5 h equilibration in a water bath at 23.5–25.5 °C using cooling times of 15 s (for 0.3–4 MPa) and 45 s (for 4–8 MPa). The psychrometers were calibrated with NaCl solutions. Ψ_{leaf} was determined by psychrometry down to –8 to –9 MPa. Water potentials between –9 and –12 MPa were estimated from the water content per leaf disc (Tyree *et al.* 2002). Following the Ψ_{leaf} determination, we measured the leaf disk fresh weight to the nearest 0.001 mg (Sartorius MC5 microbalance; Precision Weighing Machines, Bradford, Massachusetts). The disk was dried for 2.5 days at 60 °C and reweighed (dry weight). Low values of leaf water status that were over two SEs from the mean for the plant were removed on the assumption that such leaf segments were not in hydraulic contact with the stem.

DROUGHT-PERFORMANCE UNDER FIELD CONDITIONS AND SPECIES DISTRIBUTIONS

Seedling drought performance in the forest understorey was assessed for 48 species in the field in the Barro Colorado Nature Monument during 2000–2001 and 2002–2003, with eight species used in both experiments. Drought performance in the field, D_p , was calculated based on the ratio of survival in dry and wet plots:

$$D_p = 100 \times S_d/S_w \quad \text{eqn 4}$$

where S_d and S_w were relative survival in non-irrigated and irrigated plots, respectively. Results of the field experiments have been reported (Engelbrecht & Kursar 2003; Engelbrecht *et al.* 2007a). Eighteen of the species in the present study also had D_p values.

A pronounced rainfall gradient over a distance of only 65 km and at less than 100 m above sea level occurs across the Isthmus of Panama. Rainfall ranges from 1650 mm year⁻¹ at the Pacific side to 3000 mm year⁻¹ at the Caribbean side, and dry season length varies from 150–115 days (Engelbrecht *et al.* 2007a). To relate desiccation tolerance to distribution, we classified species as present or absent in the Pacific, middle, or Caribbean regions of the Isthmus (Table 1). Occurrence was determined from field observations, collection locales of herbarium specimens, the Missouri Botanical Garden's Tropicos data base (<http://www.tropicos.org>), the Centre for Tropical forest Science data sets (<http://ctfs.si.edu/datasets/>) and Engelbrecht *et al.* (2007a). Species found on the Pacific side, including those with

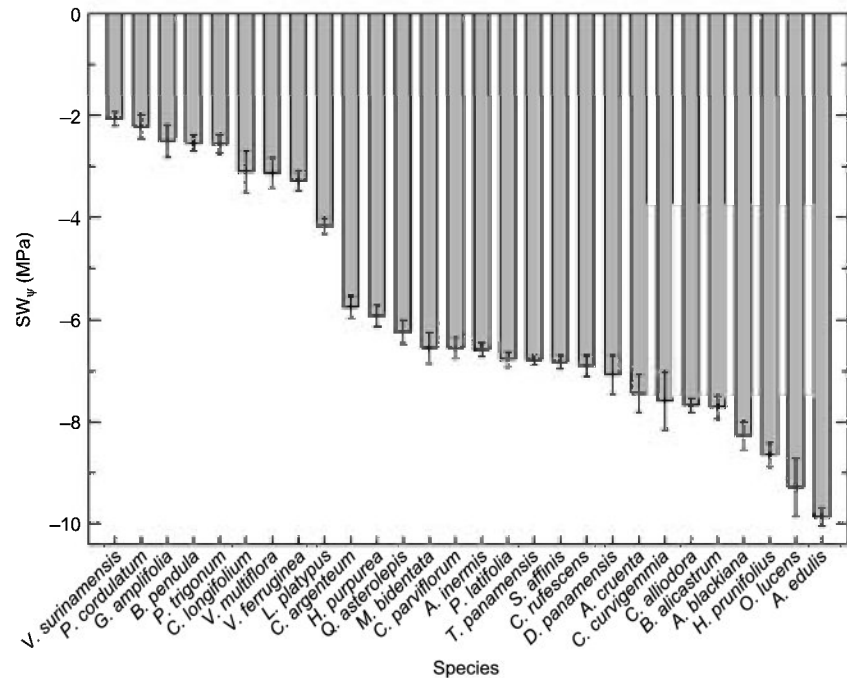


Fig. 2. Leaf water potentials of severely wilted seedlings for 28 species. Data are averages \pm 1 SE. See Table 1 for species names.

distributions in the Pacific plus middle or all across the Isthmus, were classified as dry-side species. Species distributed in the Caribbean only or in the Caribbean plus middle were classified as wet-side species (Table 1). Species only found in the middle were excluded. Thus, 14 of our study species occurred in dry forests, and 14 were restricted to wetter forests.

STEM HYDRAULIC CONDUCTANCE

Hydraulic conductance was determined on the whole stem (with the leaves removed) using a vacuum method and a Sartorius CP2250 balance (with an accuracy of 0.05 mg, Precision Weighing Machines, Bradford MA, USA; Kolb, Sperry & Lamont 1996; Tyree *et al.* 2003). Flow was determined at the following sequence of vacuum pressures: 0, -24, -47, -71, -59, -36, -12 and 0 kPa and k_{ws} was obtained by linear regression of flow as a function of pressure (corrected to 25 °C). To account for differences in plant size, we scaled conductance to the total leaf area, giving leaf-specific hydraulic conductance or k_{ws} ($\text{kg s}^{-1} \text{m}^{-2} \text{MPa}^{-1}$). We used the leaf area before plants had been drought-stressed so that stem hydraulic conductance was scaled to the same leaf area in watered and wilted plants. Thus, our calculation of stem conductance was not adjusted to the leaf area present at the time of the measurement of the severely wilted plants (having 25–50% loss of living leaf area). All k_{ws} data are in Appendix S3.

Results

TOLERANCE OF LOW LEAF WATER STATUS

The capacity to tolerate low leaf water status varied widely among species. The lethal leaf water status assessed as $LD50_{RWC}$ varied from 58.5% for *Calophyllum longifolium* down to 7.0% for *Hybanthus prunifolius*, that is, some species

showed 50% mortality after they had lost 40% of their leaf water content relative to the fully hydrated leaf, whereas others tolerated a loss of 93% of their leaf water. $LD50_{\psi}$ ranged from -1.9 MPa for *Virola surinamensis* down to -12 MPa (or less) for *Alseis blackiana* and *Chrysophyllum argenteum*. The 95% confidence limits for $LD50_{RWC}$ and $LD50_{\psi}$ were large, often 50–100% of $LD50_{RWC}$ and $LD50_{\psi}$ (not shown). From a regression of confidence intervals as a function of sample sizes (not shown), we estimated that about 50 plants would be required to decrease the 95% confidence interval to < 25% of the modelled LD50.

Desiccation tolerance assessed as leaf water status of severely wilted plants, SW_{RWC} , showed a similarly wide variation: from 61.1% in *Virola surinamensis* to 16.2% in *Hybanthus prunifolius*. Hence species were severely wilted after losing 40–84% of their leaf water. SW_{ψ} varied from -2.1 MPa for *V. surinamensis* to -9.9 MPa for *Alibertia edulis* (Fig. 2).

Regression analysis indicated that the relationship between RWC and Ψ_{leaf} was linear in both experiments and not significantly different from each other (Fig. 3a,b). Our two independent assessments of desiccation tolerance were highly significantly correlated with each other. The slopes, 0.98 for RWC and 1.11 for Ψ_{leaf} , were not significantly different from 1.0 (Fig. 3c,d), indicating that the two experiments assessed similar physiological states. The LD50 experiment may give lower water status values than the severely wilted experiment if leaves were in the process of abscission (e.g., species with low leaf dry weight per leaf area). For three of four such species we noted that the critical water status at the LD50 was indeed lower than at the severely wilted stage (Fig. 3c,d filled circles, Ab Car, Hpr but not Cc).

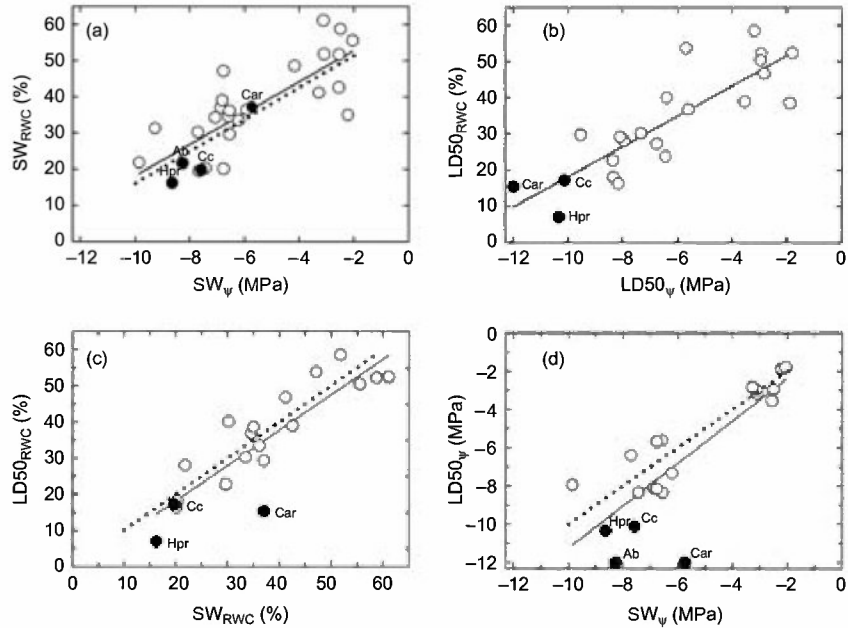


Fig. 3. The relationship between critical leaf water potentials and leaf relative water contents (a, b) and the relationship between desiccation tolerance assessed with the two different experimental approaches (c, d). (a) The relationship between the relative water content and leaf water potential of severely wilted plants, SW_{RWC} and SW_{ψ} , respectively ($SW_{RWC} = 61.9 + 4.40 \times SW_{\psi}$, $R^2 = 0.64$; $P < 0.0001$, $n = 28$); (b) The relationship between the relative water content and leaf water potential at 50% mortality, $LD50_{RWC}$ and $LD50_{\psi}$, respectively ($LD50_{RWC} = 60.0 + 4.18 \times LD50_{\psi}$, $R^2 = 0.72$; $P < 0.0001$, $n = 22$). The regression from b is shown in a as a dashed line; these are not significantly different from each other. (c) The relationship of $LD50_{RWC}$ with SW_{RWC} (slope = 0.98, $R^2 = 0.76$, $P < 0.0001$, $n = 20$). (d) Relationship of $LD50_{\psi}$ with SW_{ψ} (slope = 1.11, $R^2 = 0.68$, $P < 0.0001$, $n = 20$). The dotted lines in c and d are for a slope of 1.0. Four species with low dry leaf weight per area which may have low values for $LD50_{RWC}$ and $LD50_{\psi}$ (see Results) are represented by filled circles. These are identified by symbols (see Table 1).

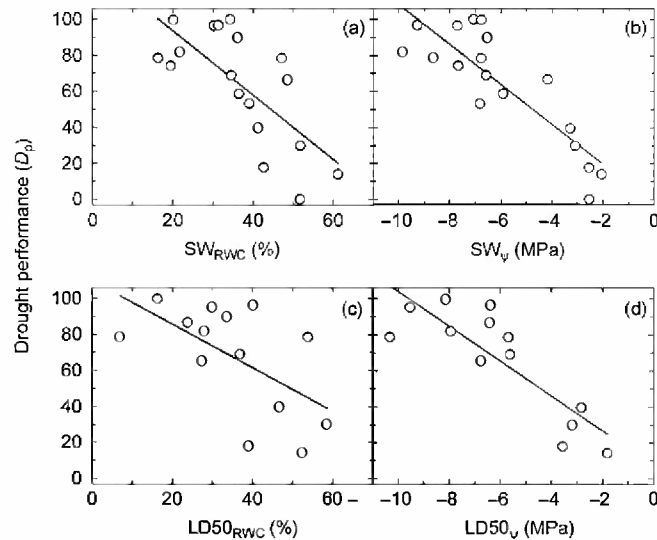


Fig. 4. The relationship between species tolerance of low leaf water status and their drought performance (D_p) in the forest understorey. Regression of D_p with the relative water content and leaf water potential of severely wilted plants SW_{RWC} and SW_{ψ} , respectively, and at 50% mortality, $LD50_{RWC}$ and $LD50_{\psi}$, respectively: (a) SW_{RWC} ($R^2 = 0.51$, $P = 0.0009$, $n = 18$); (b) SW_{ψ} ($R^2 = 0.73$, $P < 0.0001$, $n = 18$); (c) $LD50_{RWC}$ ($R^2 = 0.35$, $P = 0.024$, $n = 14$); (d) $LD50_{\psi}$ ($R^2 = 0.71$, $P = 0.0003$, $n = 13$).

THE RELATIONSHIP OF TOLERANCE TO LOW LEAF WATER STATUS WITH PLANT DROUGHT PERFORMANCE AND DISTRIBUTIONS

Drought performance (D_p), assessed as the percent survivorship in unwatered relative to irrigated plots in the forest understorey

(Engelbrecht & Kursar 2003; Engelbrecht *et al.* 2007a) varied considerably in our study species from very low, 0% for *Beilschmiedia pendula* to very high, 100% for *Dipteryx panamensis*. The D_p values from the field experiments were significantly correlated with desiccation tolerance assessed as SW_{RWC} and SW_{ψ} (Fig. 4a,b) as well as $LD50_{RWC}$ and $LD50_{\psi}$ (Fig. 4c,d).

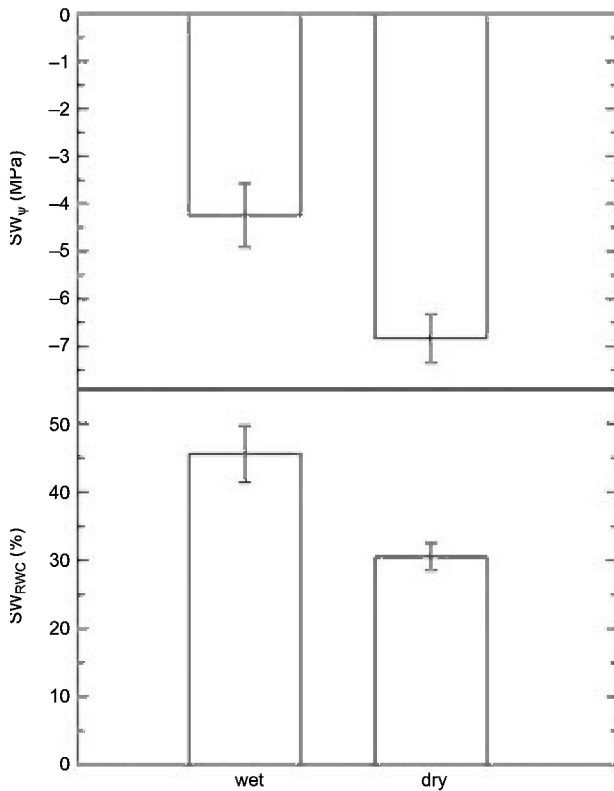


Fig. 5. Desiccation tolerance of species occurring at the dry side of the Isthmus of Panama (dry), and of species restricted to the wetter forests (wet). SW_{RWC} and SW_{ψ} are the RWC and Ψ_{leaf} , respectively, of severely wilted plants. (a) Leaf water potential of severely wilted plants, SW_{ψ} (t -test, $P < 0.005$, $n = 25$ species). (b) Leaf relative water content of severely wilted plants, SW_{RWC} (t -test, $P = 0.002$, $n = 25$ species). Data are averages \pm 1 SE.

If desiccation tolerance is a determinant of plant distributions, we predicted that species typical of drier forests should have higher desiccation tolerance, that is, lower SW_{RWC} , SW_{ψ} , $LD50_{RWC}$ and $LD50_{\psi}$, than species restricted to wetter forests. As expected we found lower SW_{RWC} and SW_{ψ} for the species from the drier side of the Isthmus (Fig. 5). The $LD50$'s of species from the wet and dry sides did not differ, nor was there an obvious trend.

STEM HYDRAULIC CONDUCTANCE

The leaf-area-specific stem hydraulic conductance, k_{ws} , of well-watered control plants varied among species by a factor of 4.3, from 3.74 to $16.1 \times 10^{-5} \text{ kg s}^{-1} \text{ m}^{-2} \text{ MPa}^{-1}$. For severely wilted plants, k_{ws} , which we scaled to the total leaf area present before plants were desiccated (see Methods), strongly decreased and varied among species by a factor of 9.0, from 0.54 to $4.85 \times 10^{-5} \text{ kg s}^{-1} \text{ m}^{-2} \text{ MPa}^{-1}$ (Fig. 6, upper panel). In contrast to the large variation among species in k_{ws} , the variation in loss of conductance was relatively small: from the well-watered to the severely wilted stage species lost 79.8% ($\pm 3.1\%$, 1 SE) of their leaf-specific stem conductance, with a range of 61.1–95.3% (Fig. 6, lower panel).

Both drought performance (D_p) and SW_{ψ} , measured under field and lab conditions respectively, were highly correlated

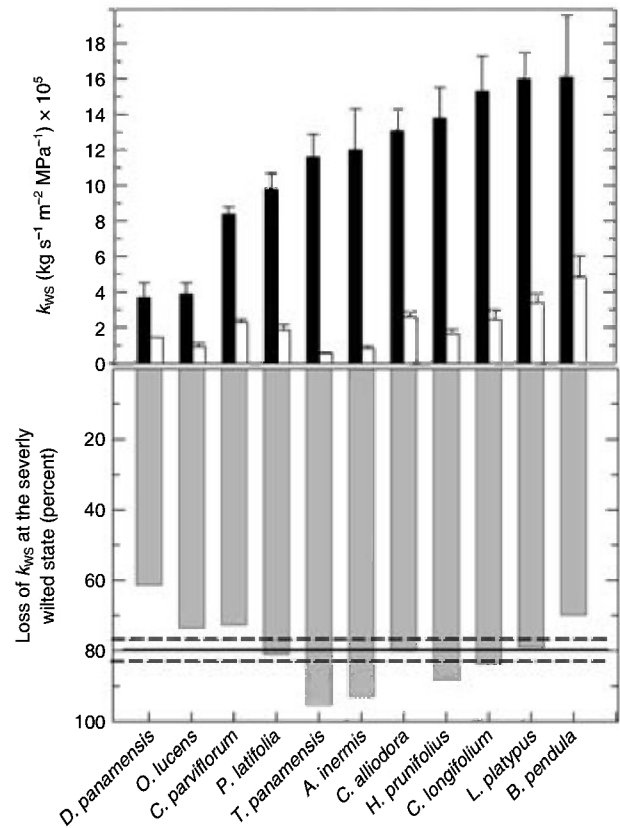


Fig. 6. Whole stem hydraulic conductance. Upper panel. stem conductance of well-watered (filled bars) and severely wilted plants (open bars). Stem conductance was scaled by the initial leaf area present before droughting (see Methods). Data are averages \pm 1 SE. Lower panel. Percent loss of stem hydraulic conductance in severely wilted plants. The average is indicated by the solid line and 1 SE by the dashed lines.

with k_{ws} . Species with higher D_p and lower SW_{ψ} had lower k_{ws} in both the well-watered and the severely wilted plants (Fig. 7). There was no relationship of k_{ws} with SW_{RWC} , $LD50_{RWC}$ or $LD50_{\psi}$.

Discussion

THE CONTRIBUTION OF TOLERANCE OF LOW LEAF WATER STATUS TO DROUGHT PERFORMANCE

Our study species, all common in Central Panama, gave the full range of drought performance, D_p , from 0% to 100% (Engelbrecht & Kursar 2003; Engelbrecht *et al.* 2007a). Such large differences among species in their performance during drought could be due to a number of mechanisms. One strategy by which species may vary in drought performance, desiccation tolerance, is the focus of this study. All of our measures of the capacity to tolerate low leaf water status, $LD50_{\psi}$, $LD50_{RWC}$, SW_{ψ} and SW_{RWC} , showed wide variation among species and were highly correlated with D_p , as determined in field experiments (Fig. 4). The significant correlation of desiccation tolerance with drought performance in the field has two implications. The results suggest that drought performance was indeed mainly driven by the lack of water

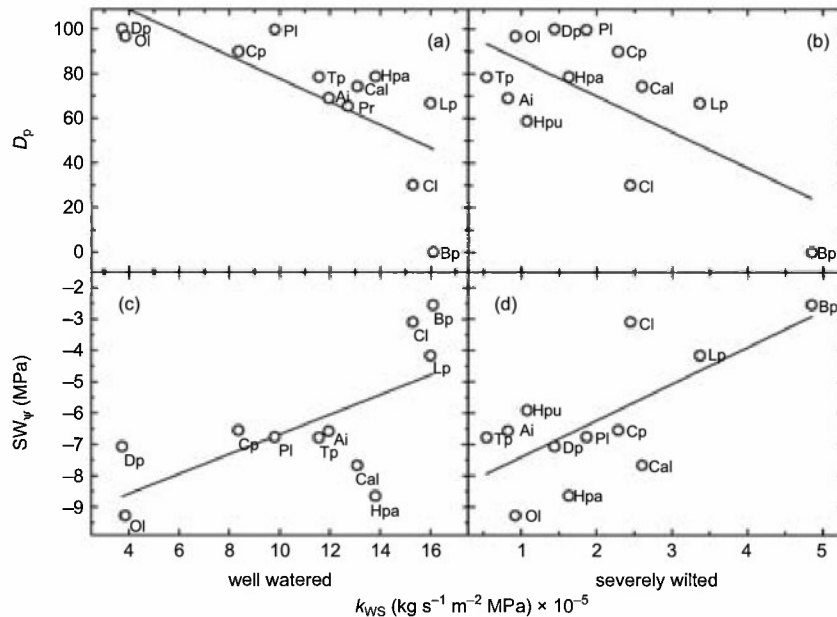


Fig. 7. Relation of drought performance and desiccation tolerance with stem hydraulic conductance. Drought performance, D_p , as a function of k_{ws} of (a) well-watered plants, and of (b) severely wilted plants. The Ψ_{leaf} of severely wilted plants, SW_{ψ} , as a function of k_{ws} of (c) well-watered, and of (d) severely wilted plants. (a) $R^2 = 0.54$, $P = 0.006$, $n = 12$; (b) $R^2 = 0.52$, $P < 0.013$, $n = 12$; (c) $R^2 = 0.42$, $P < 0.031$, $n = 11$; (d) $R^2 = 0.49$, $P < 0.012$, $n = 12$. The species are identified by symbols (see Table 1).

and by differences among species in their ability to cope with low water availability, rather than by the multiple further stresses that may act during drought, such as changes in pests or soil nutrients. Second, the results strongly suggest that, in tropical forest seedlings and saplings, desiccation tolerance is a principle strategy by which species cope with low water availability.

A second strategy leading to differences in drought performance is desiccation delay, or avoidance. In other studies we found that several surrogates for desiccation delay, such as seedling size, rooting depth, root : shoot ratio and cuticular conductance, were unrelated to species drought performance (unpublished data). As well, we found that minimum dry-season water potentials in the field, an integrated measure of desiccation delay, did not correlate with either desiccation tolerance or drought performance (unpublished data). We can therefore rule out that a correlation between desiccation tolerance and delay led to a spurious correlation of desiccation tolerance with drought performance and that, in reality, desiccation delay promotes drought performance. Instead the study species cope with low water availability primarily by desiccation tolerance. Nevertheless, for saplings or established trees and for seedlings in habitats that experience very dry surface soil, desiccation delay through deeper roots could be an important survival strategy (Padilla & Pugnaire 2007).

TOLERANCE TO LOW LEAF WATER STATUS AS A DETERMINANT OF DISTRIBUTION IN TROPICAL RAINFOREST

Many observations point to drought as a key determinant of tree abundance and distribution in tropical forests (Gentry 1986; Condit 1998; Veenendaal & Swaine 1998; Bongers *et al.*

1999). We recently showed that seedling drought performance is important in shaping species distributions in tropical forests: species occurring on the drier side of the Isthmus have higher drought performance, D_p , as measured in our field experiments than species restricted to wetter forests (Engelbrecht *et al.* 2007a). In the present study we hypothesized that desiccation tolerance of seedlings determines drought performance, and thus shapes species distributions. As predicted, we found higher desiccation tolerance for the species occurring on the drier side of the Isthmus than on the wetter side, in particular, lower SW_{RWC} and SW_{ψ} (Fig. 5). The fact that we find a strong relationship between desiccation tolerance and species distribution, even though the gradient in drought length across the Isthmus of Panama is only moderate (from 115 to 150 days, Engelbrecht *et al.* 2007a), indicates that species are very sensitive to differences in drought regimes. In addition, a recent study in Southeast Asia obtained similar results, with species found in drier forests able to tolerate lower Ψ_{leaf} and RWC (Baltzer *et al.* 2008).

THE ROLE OF STEM HYDRAULIC CONDUCTANCE AND VULNERABILITY TO CAVITATION FOR MORTALITY DURING DESICCATION

We found that species with lower leaf-area-specific stem hydraulic conductance, k_{ws} , had greater desiccation tolerance and drought performance (Fig. 7). The mechanism behind such a relationship could be a trade-off between greater xylem conductance and lower resistance to cavitation. This mechanism has frequently been suggested although the relationship has not been consistently demonstrated (Brodribb & Hill 1999; Pockman & Sperry 2000; Hacke *et al.* 2006).

To test whether a threshold loss of about 80% of stem conductance leads to mortality in most species, as suggested by Tyree *et al.* (2003), we carried out a comparable experiment with seven more species and obtained similar results. The loss of conductance at the severely wilted stage was $79.8\% \pm 3.1\%$, with a range of 61.1–95.3% (Fig. 6, lower panel). For these species, a much larger variation was found in desiccation tolerance, with SW_{ψ} values between -2.5 and -9.3 MPa, as well as in k_{ws} , with values between 3.74 and $16.1 \times 10^{-5} \text{ kg s}^{-1} \text{ m}^{-2} \text{ MPa}^{-1}$ (Fig. 7). Our results are consistent with the hypothesis that loss of xylem function may cause death during desiccation (Davis *et al.* 2002). Hence, the crucial mechanism that allows cell and plant survival despite water loss may be the prevention of xylem embolism. Our results show that, in tropical rainforest habitats, those species with higher leaf-specific shoot conductance (which may trade off with resistance to cavitation) had worse drought performance, were less desiccation tolerance and may be excluded from drier habitats. Contrasting results were obtained in a study of two Mediterranean species in which the species with higher resistance to cavitation had lower seedling survivorship during drought (Vilagrosa *et al.* 2003).

COMPARISON OF THE TWO METHODS FOR ASSESSING TOLERANCE TO LOW LEAF WATER STATUS

In this study we assessed desiccation tolerance in two ways: as the water status of plants in a severely wilted state (SW_{RWC} and SW_{ψ}) an approach that was based on an earlier study (Tyree *et al.* 2003). The second assesses the leaf water status associated with 50% mortality ($LD50_{RWC}$ and $LD50_{\psi}$). This approach was developed *a priori* and is based on well-established concepts of dose-response analysis from toxicology (Collett 2003).

We found more statistically significant relationships with distribution and k_{ws} using SW_{RWC} and SW_{ψ} than with $LD50_{RWC}$ or $LD50_{\psi}$ (Figs 5 and 7), suggesting that the severely wilted method may better reflect the critical physiological processes. To address, in part, the issue that the severely wilted method depends on a subjective, visual scoring of stress, we rewatered severely wilted plants and scored these for survival (see the discussion of *Lacmellea*, *Swartzia* and *Posoqueria* in Methods). The LD50 approach was independent of subjective assessments of plant appearance, so that it might be expected to be the more reliable method. Nevertheless, a major drawback of the LD50 method is that it requires considerably larger samples sizes, 50 plants, to reach reasonably small confidence intervals (vs. 5–10 for the severely wilted approach). Also, for extremely stressed plants, some leaves or leaf segments that initiate abscission may not be in hydraulic contact with the stem, leading to an overestimation of desiccation tolerance (see Results).

While both methods have their drawbacks, the values that we independently obtained by the two methods were highly significantly linearly related with slopes close to 1.0 (Fig. 3c,d). This suggests that both methods assessed very similar physiological states of the plants, and augments our confidence in both methods. The relationships with ecological parameters

(drought performance and distribution) and with physiological parameters (hydraulic conductance) furthermore suggest that they are useful for resolving ecological and physiological questions.

CONCLUSIONS

Ours and other studies indicate that drought significantly impacts the dynamics, distribution and habitat specialization of tropical rainforest species. Drought also may significantly impact productivity and carbon balance at the ecosystem level (Schoor 2003). At the same time, climate projections for tropical regions include increased evapotranspiration, changes in drought conditions and large changes in local rainfall resulting from displacements in tropical rain belts (Christensen *et al.* 2007). Our ability to predict how such changes will impact tropical forests will be of decisive ecological significance. Hence relating desiccation tolerance with distribution, such as described herein, will become increasingly necessary.

Acknowledgements

Authors thank N. Anaya, M. Rios, G. Rivas, R. Perez, S. Aguilar, and T. Brenes-Arguedas for assistance with determining species distributions, D. Feener for statistical advice, J. Sperry for comments on the text and the Autoridad Nacional del Ambiente of Panama for research and collection permits. The project was funded by the Andrew W. Mellon Foundation and the USDA Forest Service.

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Received 26 February 2008; accepted 18 August 2008
Handling Editor: Ken Thompson

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Relative water content data.

Appendix S2. Water potential data.

Appendix S3. Stem conductance data.

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