Seedling Traits Determine Drought Tolerance of Tropical Tree Species

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ABSTRACT

Water availability is the most important factor determining tree species distribution in the tropics, but the underlying mechanisms are still not clear. In this study, we compared functional traits of 38 tropical tree species from dry and moist forest, and quantified their ability to survive drought in a dry-down experiment in which wilting and survival were monitored. We evaluated how seedling traits affect drought survival, and how drought survival determines species distribution along the rainfall gradient. Dry forest species tended to have compound leaves, high stem dry matter content (stem dry mass/fresh mass), and low leaf area ratio, suggesting that reduction of transpiration and avoidance of xylem cavitation are important for their success. Three functional groups were identified based on the seedling traits: (1) drought avoiders with a deciduous leaf habitat and taproots; (2) drought resisters with tough tissues (*i.e.*, a high dry matter content); and (3) light-demanding moist forest species with a large belowground foraging capacity. Dry forest species had a longer drought survival time (62 d) than moist forest species (25 d). Deciduousness explained 69 percent of interspecific variation in drought survival. Among evergreen species, stem density explained 20 percent of the drought survival. Drought survival was not related to species distribution along the rainfall gradient, because it was mainly determined by deciduousness, and species with deciduous seedlings are found in both dry and moist forests. Among evergreen species, drought survival explained 28 percent of the variation in species position along the rainfall gradient. This suggests that, apart from drought tolerance, other factors such as history, dispersal limitation, shade tolerance, and fire shape species distribution patterns along the rainfall gradient.

Key words: Biomass allocation; Bolivia; deciduousness; morphology; survival; tropical forest; water availability; wood density; wilting.

WITHIN THE TROPICS, WATER AVAILABILITY IS THE MOST IMPORTANT ENVIRONMENTAL FACTOR determining tree species richness (Gentry 1988, Poorter *et al.* 2004, ter Steege *et al.* 2006), composition (Hall & Swaine 1976, Bongers *et al.* 2004), and distribution (Bongers *et al.* 1999, Swaine 1996, Holmgren & Poorter 2007). Perhaps the most important component of water availability is the seasonality of its distribution. The length of the dry period may vary from a dry spell of a few days in perhumid wet forests, to a dry season of up to 8 mo in dry monsoon forests (Walter 1985, Walsh 1996). Soil water potential at 20 cm depth can drop during this dry period to values below −2 MPa (Veenendaal *et al.* 1996), suggesting that plant water availability is very low. Seedlings and saplings are affected by this low water availability, and have a reduced leaf water potential and gas exchange (Wright *et al.* 1992, Tobin *et al.* 1999, Cao 2000), leading to a reduction in growth and survival (Engelbrecht & Kursar 2003, Bunker & Carson 2005, Poorter 2005).

During the past decades, tropical forests have experienced a dramatic decrease in annual rainfall, and an increase in dry season length and rainfall variability (Malhi & Wright 2004). Insight into the mechanisms of drought tolerance is needed, if we are to understand and predict species responses to climatic change. The seedling stage is generally considered to be the most important bottleneck for successful regeneration in dry areas, as seedlings, with their limited root system are most vulnerable to drought. Many experiments have been carried out in which seedlings were exposed to fixed treatments of low or high levels of water availability (*e.g.*, Burslem *et al.* 1996, Sack 2004). Although this may show how species partition microsites that differ consistently in water availability, such as wet valley bottoms and dry crests (Gunatilleke *et al.* 2006), it does not demonstrate how species partition sites that differ in seasonality, such as wet and dry forests. An evaluation of species' ability to survive extended periods with little or no water is therefore required (Veenendaal & Swaine 1998).

Species employ three general mechanisms to deal with drought: (1) avoidance—by spending the dry season in dormancy; (2) delay—through increased water uptake and reduced water loss; and (3) physiological tolerance—by being physiologically able to maintain plant functioning at low cell water content. These mechanisms are closely linked to the functional traits of species. Deciduousness, for example, is a trait that confers drought avoidance (Reich & Borchert 1984, Borchert 1994) but it has been more commonly found in the adult than the seedling stage (Hall & Swaine 1981), probably because seedlings do not possess sufficient carbohydrate reserves to replace their leaves annually. High biomass investment in roots and high specific root length enhance water uptake, and low transpiring leaf area and strong stomatal control reduce water loss (Slot & Poorter 2007), both of which contribute to drought delay. Traits that allow drought tolerance are osmotic regulation and the ability to withstand low leaf water potential (Bonal & Guehl 2001, Tyree *et al.* 2003). Understanding how these species traits are correlated allows us to distinguish functional groups of species that respond in a similar way to climate and climatic change (Díaz & Cabido 1997, Lavorel & Garnier 2002). Trait correlations also

provide insight as to whether there is a potential trade-off between drought tolerance and shade tolerance (Smith & Huston 1989, Sack 2004).

Here, we compare seedling functional traits of 38 tropical tree species from dry and moist forest, and quantify the ability of 36 species to survive drought under standardized experimental conditions. Species are often classified as belonging to either wet or dry forests, but in reality they vary gradually and continuously in their distribution along the rainfall gradient (Bongers *et al.* 1999). Here, we use a quantitative 'drought index' (DI) to describe the species' position along this rainfall gradient. We address the following questions: (1) how are seedling traits related to species' position along the rainfall gradient?; (2) how are seedling traits associated and which functional groups can be distinguished?; (3) what is the drought survival of species under standardized conditions?; and (4) which seedling traits are good predictors for drought survival and species distribution?

METHODS

SPECIES AND STUDY SITES.—Thirty-eight tropical tree species were selected for the study, of which 36 were included in the drought experiment (Table S1). Seeds of 24 species were collected from a moist semi-evergreen forest (La Chonta) and 16 species from a dry deciduous forest (Inpa) in lowland Bolivia. Two species, *Gallesia integrifolia* and *Spondias mombin*, were collected from both sites. Species differed in their light requirements for regeneration, as indicated by the juvenile crown exposure (CE). The CE indicates the average, population-level light levels experienced by species at 2 m of height, and includes the categories: 1: species in the forest understory; 2: species that receive, on average, lateral light; 3: species that receive, on average, overhead light on part of their crown; and 4: species that receive, on average, full overhead light on their whole crown (Poorter & Kitajima 2007).

Both forests are long-term research sites of the Instituto Boliviano de Investigación Forestal (IBIF), and differ strikingly in climate, forest structure, species richness, and floristic composition (Peña-Claros, M., L. Poorter, A. Alarcón, G. Blate, U. Choque, T. S. Fredericksen, J. Justiniano, C. Leaño, J. C. Licona, W. Pariona, and F. E. Putz, pers. comm.). Inpa (16°6′ S, 61°42′ W), a dry deciduous forest, has an annual rainfall of 1160 mm with a distinct dry period (potential evapotranspiration > precipitation) of 3 mo and a lowest dry season gravimetric soil water content at 10 cm depth of 4.6 ± 0.4 SE percent (L. Poorter, pers. obs.). The forest has an average canopy height of 20 m, stem density (SD) of 437/ha, basal area of 19.7 m²/ha, and species richness of 34 per hectare (all data for trees > 10 cm dbh; M. Peña-Claros *et al.*, pers. comm.). Nearly all canopy trees are deciduous in the dry season. La Chonta (15°47′ S, 62°55′ W), a moist semi-evergreen forest, has an annual rainfall of 1580 mm with a distinct dry period of 1 mo and a lowest dry season gravimetric soil water content at 10 cm depth of 11.9 ± 1.4 percent. Average canopy height is 25 m, SD is 368/ha, basal area is 19.7 m²/ha, and species richness is 59 per hectare. About a third of canopy trees are deciduous in the dry season.

Seeds were germinated at *ca* 15 percent of full sunlight in a nursery in Santa Cruz, Bolivia (16°30′ S, 68°10′ W) in trays with a 50:50 mixture of river sand and organic soil. Young seedlings were transplanted to 200 ml plastic tubes (3 cm wide × 12 cm long) containing 50 percent river sand, 25 percent dry forest topsoil, and 25 percent moist forest topsoil. Seedlings were transferred to two shade houses at IBIF. The shade houses were covered with a light-transparent roof, and roof and walls were covered with neutral density shade cloth. Light level in the shade houses was *ca* 10 percent of full sunlight, typical for small forest gaps. This light level is sufficiently high for pioneers and shade tolerants to survive without problems, and to dry down the soil if no watering occurs. After an acclimatization period of several weeks to months, an initial harvest was carried out.

SEEDLING TRAITS.—Five randomly selected seedlings per species were harvested at the start of the experiment. Height and diameter at the top and base of the stem were measured and leaves were counted. Seedlings were divided into roots, stem, and leaves; and their fresh weight was determined. Leaves were digitalized with, a desktop-scanner (Canon Lide 30 Canon Inc., Tokyo, Japan), and their surface area (cm²) was determined using pixel-counting software (Van Berloo 1998). Total root length was estimated using the line intersect method of Newman (1966). Roots were placed in a transparent water bath over a paper with a 2 × 2 cm grid system, and the number of intersection between the roots and the gridlines were counted. Subsequently, total root length was estimated as $R = \frac{\pi.N.A}{2.H}$ where R is the total length of the root (cm), N is the number of intersections between root and gridlines, A is the area of the rectangle (cm²), and H is the total length of the straight lines of the grid (cm) (Newman 1966). Afterwards, all plant parts were oven-dried for 48 h at 65°C and weighed.

Based on the measurements, we calculated leaf, stem and root dry matter content (LDMC, SDMC, and RDMC; 100 × dry mass per unit fresh mass; %), and leaf, stem and root mass fractions (LMF, SMF, RMF; dry mass/unit dry plant mass; g/g). We further calculated the specific leaf area (SLA; leaf area/unit dry leaf mass; m²/kg), leaf area ratio (LAR; leaf area/unit dry plant mass; m²/kg), specific root length (SRL; root length/unit dry root mass; cm/g), root length per unit plant mass (RLPM; cm/g), and root length per unit leaf area (RLLA; cm/cm²). SD was determined as dry stem mass per unit stem volume (g/cm³). The stem volume (V; cm³) was calculated assuming the shape of a cone; $V = \frac{\pi.L}{12}(D_{top}^2 + D_{top}.D_{base} + D_{base}^2)$, where L is stem length (cm), D_{top} is the diameter at the top of the stem (cm), just under the growth meristem, and D_{base} the diameter at the base of the stem, just above the root. The SD is probably slightly lower than the wood density, as it includes both the bark and the pith. Finally, we scored whether the species showed a deciduous leaf habit in the drought experiment, whether species had simple or compound leaves, and whether species showed a thickened tap root.

DROUGHT EXPERIMENT.—Forty seedlings per species were used for the drought experiment, 20 seedlings per shadehouse. Seedling height and leaf number were measured at the start of the

experiment, and seedlings were assigned in such way to the two shade houses that they did not differ significantly in seedling size. Average seedling height of the species at the start of the experiment was 16.2 cm (range: 4.5–32.1). Tubes containing seedlings were watered up to field capacity after which plants received no additional water. We thus present a worst-case scenario in which seedlings restricted to a limited volume of soil are exposed to sudden drought. Such sudden, short dry spells have also been shown to affect seedling survival in the field within a few days (Engelbrecht *et al.* 2006). The progressive impact of drought on seedlings was assessed from the start of the experiment by monitoring leaf wilting and seedling survival every other day for 36 d. Thereafter, observations were made every fortnight, because the few remaining species showed little change in wilting behavior. We adapted Engelbrecht and Kursar's (2003) five visual wilting stages based on leaf angle, leaf rolling, and necrosis (Table S2). We included 'deciduous' as an additional wilting stage (Table S2). These wilting stages are closely related to the gas exchange and water potential of seedlings of seedlings during progressive drought (Tyree *et al.* 2003, Slot & Poorter 2007). The drought survival experiment was done for eight species in August 2004 and for 28 species in November 2005. There were no significant differences in average drought survival time of species between the two years (*t*-test, *t* = 0.3, *P* = 0.77, df = 34), and the results were therefore pooled.

DATA ANALYSIS.—A principal component analysis (PCA) was carried out to evaluate how seedling traits were associated among each other. The PCA used 15 traits of 40 species. Deciduousness, compound leaves, and the presence of taproots were included as dummy variables (present = 1, absent = 0). The DI, drought survival time, and juvenile CE were not included in the analysis, but later correlated with the species scores along the first and second PCA axis.

A survival analysis was carried out to evaluate differences in survival times among species in the drought experiment. For this analysis, seedlings from both shadehouses were pooled. In 2004, the monitoring period was 2 mo. For two species (*Ceiba samauma* and *Pseudobomax marginatum*), not all seedlings had died by the end of the evaluation period, and these data were right-censored in the survival analysis. However, > 50 percent of the seedlings were still alive after 4 mo of drought (L. Poorter, pers. obs.), and the average survival time of these species was therefore arbitrarily set at 120 d.

The position of species along the rainfall gradient was quantified using a DI. The DI is based on the relative abundance of a species in the dry and moist forest site, and calculated as $DI = 100(\frac{D_{dry}}{D_{dry} + D_{moist}})$, where D_{dry} and D_{moist} are the mean SD (tree/ha) of a given species in the dry forest and moist forest, respectively. Stem densities were calculated from the number of trees >10 cm dbh/ha, for 32 1-ha plots in the dry forest and 48 1-ha plots in the moist forest (M. Peña-Claros *et al.*, pers. comm.). Of the 38 species, ten species occurred only in the moist forest (*i.e.*, DI = 0), seven species occurred only in the dry forest (DI = 100), while the remainder occurred at both sites (DI = 0–100). It must be noted that the DI provides a simplified description of the actual distribution of the species, as it was derived from two forests only. It therefore provides a conservative estimate of the actual species position along the rainfall gradient, as species with a DI of 0 are likely to occur in even wetter forests, whereas species with a DI of 100 are likely to occur in even drier forests. Relationships between seedling traits, survival time, and DI were evaluated with a Pearson correlation. A forward multiple regression was done to evaluate which of the 15 seedling traits are good predictors of the drought survival and the DI of the species. All statistical analyses were done using SPSS 12.0.1.

RESULTS

SPECIES TRAITS VERSUS DROUGHT INDEX.—An initial harvest was conducted at the start of the experiment to evaluate how species differed in their functional traits, and whether these traits are good predictors for species position along the rainfall gradient. Species position along the rainfall gradient was expressed by the DI. RMF, RDMC, SDMC, compoundness, and presence of a thickened taproot were positively correlated, and LAR, LMF, and SMF were negatively correlated with the DI (Table 1; Fig. 1). We evaluated which of the 15 seedling traits were the best predictors of DI with a multiple regression. Compoundness was first included in the analysis, and explained 48 percent of the variation in DI (standardized regression coefficient beta = 0.52, *P* < 0.001). SDMC (beta = 0.28, *P* = 0.013) and LAR (beta = −0.26, *P* = 0.019) were included as second and third variable in the analysis, and explained an additional 10 and 6 percent of the variation.

TRAIT ASSOCIATIONS.—The first and second axis of the PCA explained, respectively, 33 and 25 percent of the trait variation (Fig. 2A). Three clusters of traits can be distinguished, related to deciduousness, dry matter content, and resource capture. On the right of the first axis are species with compound deciduous leaves with a high SLA, a high biomass fraction in roots (RMF), and a taproot. On the top of the second axis are species with high dry matter content in leaves (LDMC), stem (SDMC), and roots (RDMC) and a high SD. At the bottom of the second axis are species with a high resource capture. They have a high SRL, RLLA, and root length per plant mass (RLPM), and a large biomass fraction in leaves (LMF) and leaf area per unit plant mass (LAR). Dry and moist forest species occupy different positions in the multivariate trait space (Fig. 2B). The dry forest species are found in the right and upper part of the PCA, and fall into two different strategies; a group of deciduous species with a high RMF and a taproot (*e.g.*, *S. mombin*, *P. marginatum*, *C. samauma*, and *Amburana cearensis*), and a group of species with dense tissues (*e.g.*, *Guibourtia chodatiana*, *Caesalpinia pluviosa*, *Anadenanthera colubrina*, and *Acacia* sp.). The moist forest species are found in the lower part of the PCA. Interestingly, it is especially the (short-lived) pioneer species that are characterized by a large resource capturing surface of leaves and roots, and these represent a third strategy. Examples of these species are *Trema micrantha*, *Heliocarpus americana*, *Urera caracasana*, *Sapium glandulosum*, and *Jacaratia spinosa* (Fig. 2B).

TABLE 1. *Pearson correlation between functional traits of seedlings of tropical tree species, their survival time during drought (SurvT), survival time during drought for evergreen species only (STever), juvenile crown exposure (CE), and drought index (DI). N varies from 36 to 40 species (except for correlations with STever, for which N varies from 27 to 31). For trait abbreviations see Table S1. Correlations in bold are significant at P < 0.05, correlations in bold and italics are significant at P < 0.01.*

| Trait	SurvT	STever	DI	CE	LAR	SLA	LMF	SMF	RMF	LDMC	SDMC	RDMC	SD	SRL	RLLA	RLPM	Comp	Dec
DI	0.27	**0.53**																
CE	0.26	0.02	**0.36**															
LAR	−0.24	−0.14	**−0.51**	−0.26														
SLA	**0.52**	−0.09	−0.06	0.28	0.22													
LMF	**−0.50**	−0.06	**−0.39**	**−0.48**	**0.72**	**−0.47**												
SMF	−0.19	−0.23	**−0.36**	−0.04	−0.01	0.02	−0.05											
RMF	**0.48**	0.23	**0.54**	**0.36**	**−0.49**	0.30	**−0.65**	**−0.73**										
LDMC	**−0.52**	0.17	0.31	**−0.39**	−0.14	**−0.57**	0.24	−0.19	−0.02									
SDMC	**−0.41**	0.11	**0.51**	−0.20	−0.28	**−0.59**	0.14	**−0.31**	0.15	**0.79**								
RDMC	**−0.45**	0.01	**0.34**	−0.24	−0.06	**−0.48**	0.29	**−0.32**	0.05	**0.68**	**0.83**							
SD	**−0.43**	**0.44**	0.23	**−0.33**	−0.05	**−0.55**	**0.32**	−0.03	−0.20	**0.69**	**0.70**	**0.62**						
SRL	**−0.33**	−0.04	−0.21	0.07	0.24	−0.14	0.26	**0.36**	**−0.46**	0.04	0.01	0.08	0.20					
RLLA	−0.28	0.02	0.06	0.27	−0.21	−0.19	−0.13	0.31	−0.15	0.24	0.17	0.10	0.21	**0.81**				
RLPM	**−0.33**	−0.03	−0.08	0.16	0.10	−0.15	0.13	0.28	−0.30	0.14	0.09	0.11	0.23	**0.96**	**0.91**			
Comp	**0.43**	0.25	**0.69**	**0.33**	−0.31	0.25	**−0.41**	**−0.35**	**0.55**	0.17	0.29	0.16	0.08	**−0.51**	−0.28	**−0.41**		
Dec	**0.85**	–	0.08	0.23	−0.22	**0.53**	**−0.49**	−0.16	**0.46**	**−0.58**	**−0.45**	**−0.42**	**−0.59**	**−0.41**	**−0.36**	**−0.41**	0.30	
Tapr	**0.56**	0.15	**0.41**	0.13	**−0.33**	0.14	**−0.37**	−0.24	**0.43**	−0.04	0.00	−0.20	−0.12	**−0.40**	−0.17	−0.31	**0.47**	**0.33**
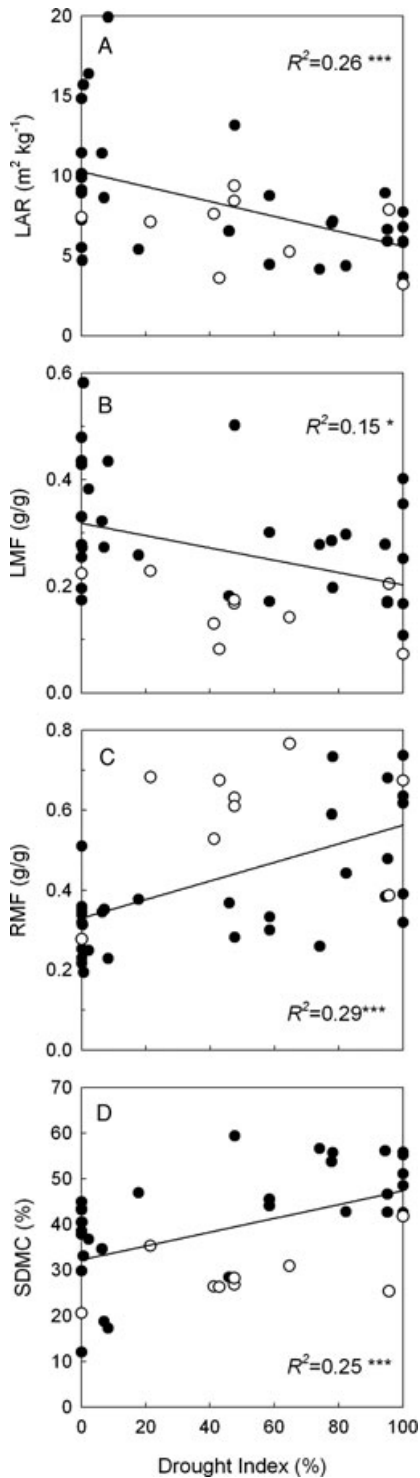
FIGURE 1. Relationship between functional traits and drought index of 38 tropical tree species (two species sampled at both sites). Evergreen species are represented by filled symbols, deciduous species by open symbols. (A) Leaf area ratio (LAR), (B) leaf mass fraction (LMF), (C) root mass fraction (RMF), (D) stem dry matter content (SDMC). Regression lines, coefficients of determination, and significance levels are shown. $*P < 0.05$; $***P < 0.001$.
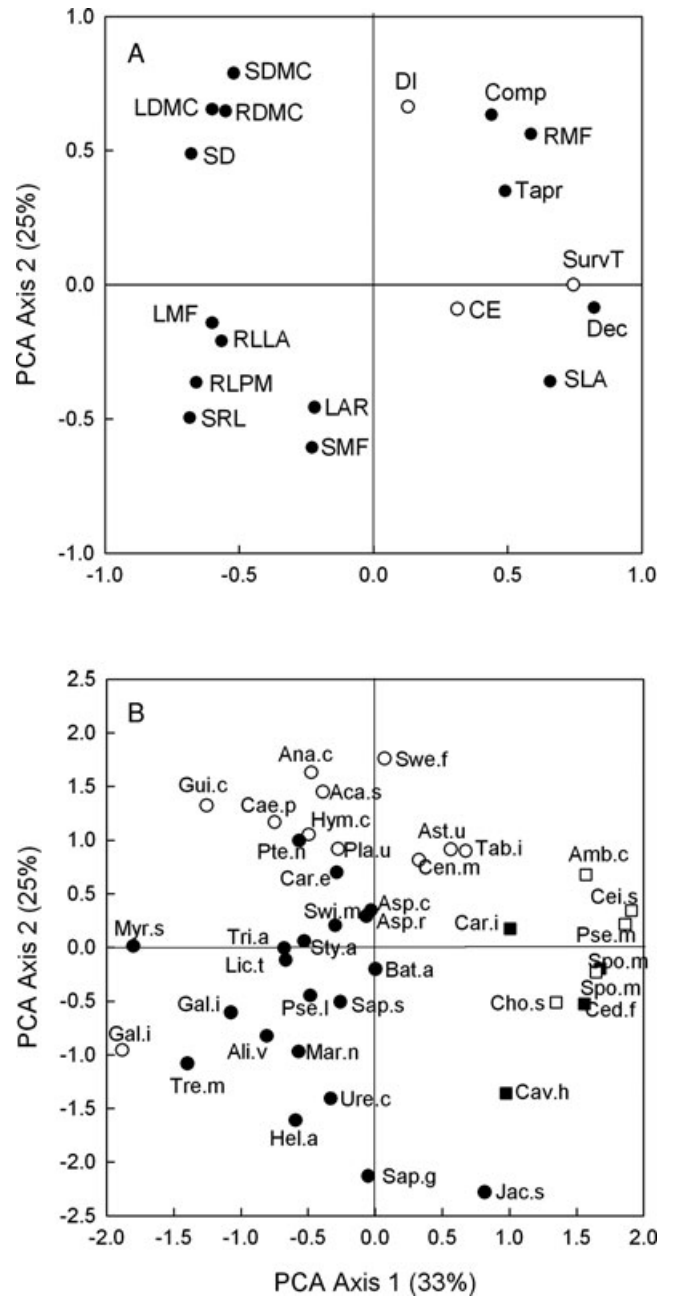

FIGURE 2. Principal component analysis of 15 seedling traits of 38 tropical tree species (two species sampled at both sites). (A) The loading plots for the first axis (explained variation is 33%) and second axis (explained variation is 25%) are shown. Survival time during drought (SurvT), drought index (DI), and juvenile crown exposure (CE) were not included in the PCA analysis, but later correlated with the PCA axes (indicated by open symbols). (B) Species loadings. Moist forest species are indicated by filled symbols, dry forest species by open symbols, evergreen species by circles, and deciduous species by squares. Trait and species abbreviations are given in Table S1.

Deciduousness is an important component of the drought tolerance strategy of species. Deciduousness (as a dummy variable) was positively associated with SLA and RMF, and negatively with SD, tissue dry matter content (LDMC, SDMC, and RDMC), and root length per unit root mass (SRL), plant mass (RLPM), and leaf area (RLLA) (Table 1).

DROUGHT SURVIVAL.—Species varied largely in their wilting response to drought (Fig. 3). Some species progressed rapidly through all wilting stages (*e.g.*, *Licaria triandra*; Fig. 3A), whereas other species passed through the stages more gradually and died (*e.g.*, *G. chodatiana*; Fig. 3F). Other species postponed desiccation by spending considerable time in the intermediately dehydrated stage, after which they shed their leaves and entered a deciduous stage, which could last for months (*e.g.*, *S. mombin*, *A. cearensis*, *Chorisia speciosa*; Fig. 3D,G,H). Species differed greatly in their survival response to drought (Fig. 4). Average survival time until 50 percent of seedlings died ranged from 9 d (*Triplaris americana*) to 180 d (*A. cearensis*). Dry forest species, on average, survived longer (61.8 d) than moist forest species (24.7 d) ($t = 2.3$, df $= 17.9$, $P = 0.033$), also when deciduous species were excluded from the analysis (average: dry $=$ 23.3 d, moist $=$ 18.6 d; $t = 2.85$, df $= 26$, $P = 0.008$).

SPECIES TRAITS VERSUS DROUGHT SURVIVAL.—Survival time of species was related to their functional traits using a multiple forward regression. Survival time was only significantly related to deciduousness and the presence of a taproot. Both factors enhanced survival, explaining 74 percent of the variation. Deciduousness was first included in the analysis and its standardized regression coefficient was larger (beta $= 0.75$, $P < 0.001$) than that of taproot (beta $= 0.23$, $P < 0.01$), indicating that it is a stronger determinant of drought survival. Deciduous species, on average, survived markedly longer (110 d) than evergreen species (20 d; $t = 4.5$, df $= 7.0$, $P = 0.003$). When the nine deciduous species were excluded from the analysis, survival time was only positively correlated to SD (beta $= 0.44$, $P = 0.018$, $R^2 = 0.20$, $N = 28$; Fig. 5A). Presence of taproot was not significant here, probably because it is closely associated with deciduousness ($\chi^2 = 4.2$, df $= 1$, $P < 0.05$); 67 percent of the deciduous species had a taproot, compared to 29 percent of evergreen species. Survival time was not related to DI ($r = 0.28$, $P = 0.10$, $N = 36$) when all species were included, but was significantly and positively correlated with the DI when the deciduous species were excluded ($r = 0.53$, $P = 0.004$, $N = 28$; Fig. 5B).

DISCUSSION

SPECIES TRAITS VERSUS DROUGHT INDEX.—In moist forests, light levels in the understory are persistently lower than in dry forests (Coomes & Grubb 2000), due to a high leaf area index and less seasonality in leaf cover (Parker *et al.* 2005). Light is therefore a more limiting resource in moist forests, and species can enhance their light interception by investing more biomass into stems and leaves to overtop neighboring plants, and by producing thin leaves with a

high SLA, thus increasing the leaf area per unit plant mass. Species from moister forests (with a lower DI) were indeed characterized by a higher biomass fraction in stem and leaves and by a higher LAR (Fig. 1) (*cf* Hoffmann & Franco 2003), but not by a higher SLA (Table 1). In dry forests, water availability is substantially lower during the dry season. Species from drier forests (with a higher DI) reduced water loss with lower transpiring leaf area per unit plant mass (Fig. 1) and compound leaves (Table 1). Small leaflets have a lower boundary layer resistance, which allows for better convective cooling of leaves (Parkhurst & Loucks 1972). Similarly, among 216 European woody species, compound-leaved species were found in more arid sites (Niinemets 1998). Compound-leaved species are also able to drop individual leaflets, rather than whole leaves, thus allowing plants to fine-tune leaf area during drought stress.

Species from drier forests may enhance water capture by investing more biomass in roots, and by producing thin roots with a high SRL, thus increasing the RLPM. Species from drier forests were indeed characterized by a higher RMF (*cf* Hoffmann & Franco 2003), but did not have a higher SRL or RLPM (Table 1). Foraging a large soil volume for water probably makes little sense in dry forests, as during the dry season, water content in the topsoil falls to uniformly low-levels. Instead, trees may store water in a large root system (high RMF), consisting of a thick taproot with a low SRL. Similarly, in Australia, woody species from dry areas have a lower SRL than species from wet areas, which probably enhances the ability of roots to penetrate dry soil (Wright & Westoby 1999, Nicotra *et al.* 2002). In natural environments, soil water availability increases strongly with soil depth, especially in the dry season (Engelbrecht *et al.* 2005). Species may therefore explore the moister deeper soil layers by producing deep roots (Poorter & Hayashida-Oliver 2000). We did not evaluate rooting depth in our experimental setup, but other studies found that seedlings from drier forests make deeper roots than those from wetter forests (Nicotra *et al.* 2002, Paz 2003; L. Markesteijn & L. Poorter, pers. obs.), by investing more biomass in the primary root axis, and diverting less to lateral roots (Nicotra *et al.* 2002).

Perhaps the most surprising result was the strong correlation between stem dry matter content and the DI. Similarly, Wright and Westoby (1999) found that species from drier areas had higher plant dry matter content than those from wetter areas. The SDMC represents the ratio of woody stem biomass over stem fresh mass. The fresh mass also includes water present in the symplast, vessels, and extracellular spaces. Tree species with a high SDMC are therefore likely to have narrow vessels with thick cell walls (Castro-Diez *et al.* 1998), and it is especially these stem traits that make trees less vulnerable to xylem cavitation (Hacke *et al.* 2001). Xylem cavitation is generally considered to be the most important cause of tree mortality in dry habitats (Cavender-Bares *et al.* 2004). A high SDMC may also enhance plant resistance to fire, wind, and grazing, which are other important causes of plant mortality in drier and more open plant communities.

The multiple regression indicated that dry forest trees are best characterized by compound leaves, stems with a high dry matter content, and low LAR, suggesting that reduction of transpiration
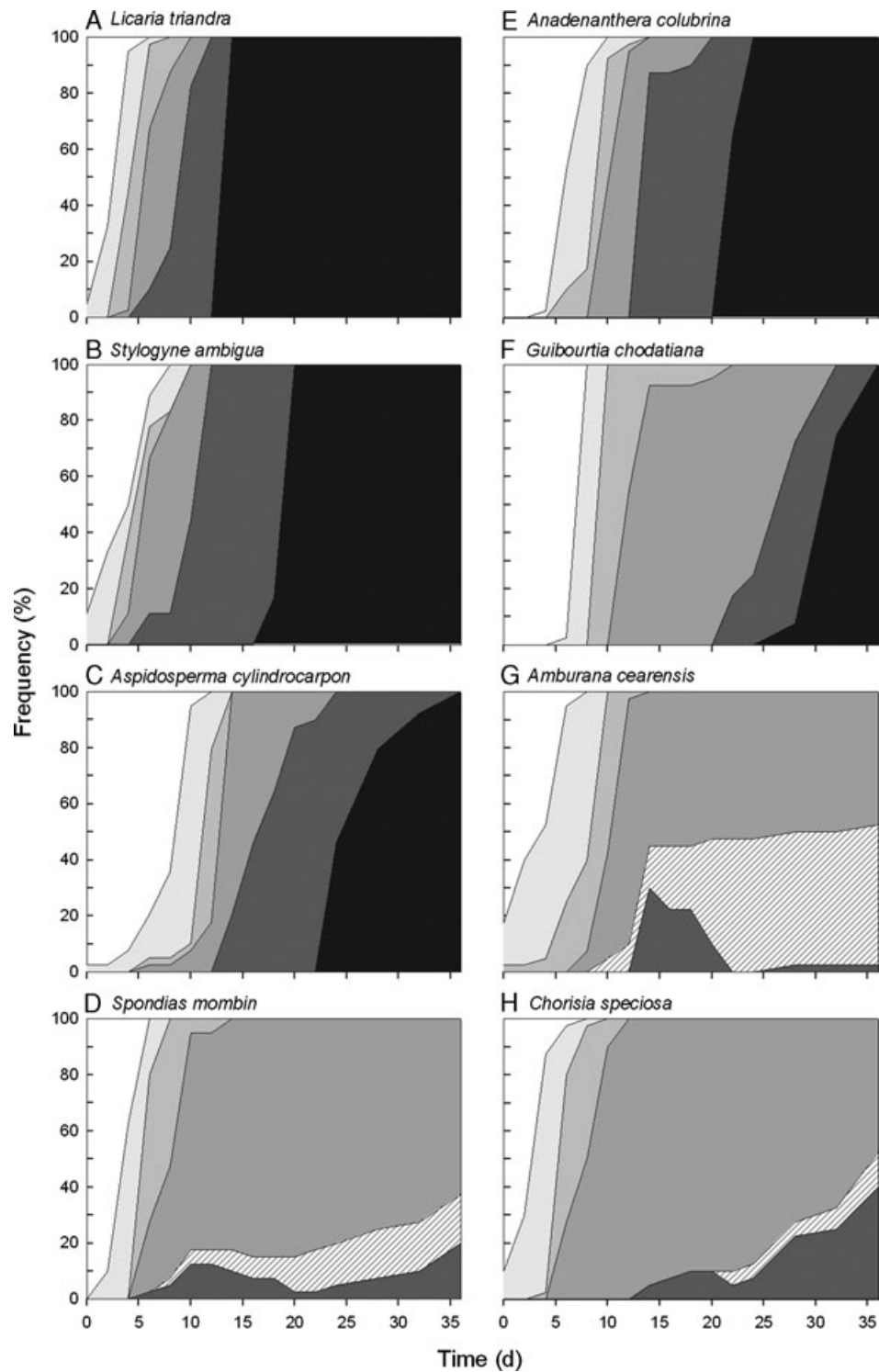
FIGURE 3. Time course of wilting of moist forest (left panels) and dry forest (right panels) tree species exposed to experimental drought. (A) *Licaria triandra*, (B) *Stylogyne ambigua*, (C) *Aspidosperma cylindrocarpon*, (D) *Spondias mombin*, (E) *Adenanthera colubrina*, (F) *Guibourtia chodatiana*, (G) *Amburana cearensis*, (H) *Chorisia speciosa.* Forty seedlings per species were exposed to drought. Different shading refers to different wilting stages: white: normal, dotted: slightly wilted, gray: intermediately wilted, dark gray: severely wilted, very dark gray: nearly dead, hatched: deciduous, black: dead.
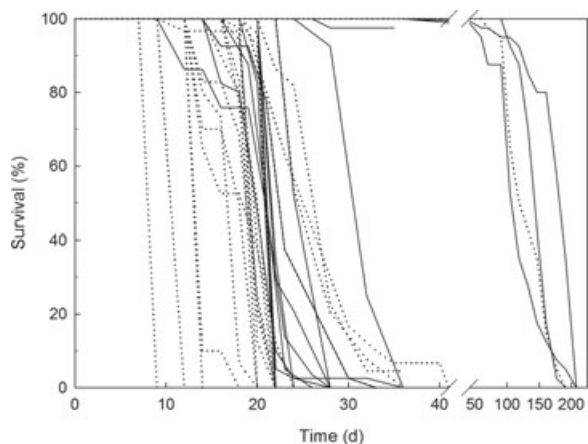
FIGURE 4. Survival curve of seedlings ($N = 40$ per species) of 21 moist forest species (dotted lines) and 15 dry forest species (continuous lines) exposed to experimental drought.

and avoidance of xylem cavitation are important elements for the success of dry forest species. Wet season performance might be also important for the success of dry forest species. Many compound-leaved dry forest species belong to the Fabaceae (Table S1), which are nitrogen fixers. They are characterized by higher leaf nitrogen concentrations and a higher photosynthetic potential than non-fixing species. This allows high rates of photosynthesis when water is available and accumulation of carbon reserves for new foliage production after drought (cf Eamus & Prior 2001).

DROUGHT SURVIVAL.—Our survival analysis showed that species vary widely in their dehydration and survival response to drought (Figs. 3 and 4). Responses varied from sudden dehydration and death within 9 d for the most extreme moist forest species, to leaf abscission and survival up to 6 mo for the most extreme dry forest species. Dry forest species survived drought for, on average, twice as long as moist forest species, indicating that they can bridge a longer dry season. Dry season survival under field conditions may be much higher than in our experiment, because wild seedlings grow in unlimited soil volume and benefit from incidental rains. The difference in survival time between dry and moist forest species is likely to be more marked under field conditions, since dry forest species in particular are able to survive with the little water remaining in the soil. In a moist forest in Panama (2600 mm annual rainfall), Engelbrecht and Kursar (2003) evaluated the drought survival of seedlings of 28 species in the forest understory. Fifty-seven percent of species showed increased mortality in response to drought, but as many as 43 percent of the species could easily withstand five and a half months of drought, suggesting that they are well adapted to the seasonal drought in the area.

SPECIES TRAITS AND DROUGHT SURVIVAL: THE IMPORTANCE OF DE-CIDUOUSNESS AND TOUGH TISSUES.—The multiple regression analysis showed that, under extreme dry conditions, drought avoidance through leaf abscission is the best drought survival strategy, and that
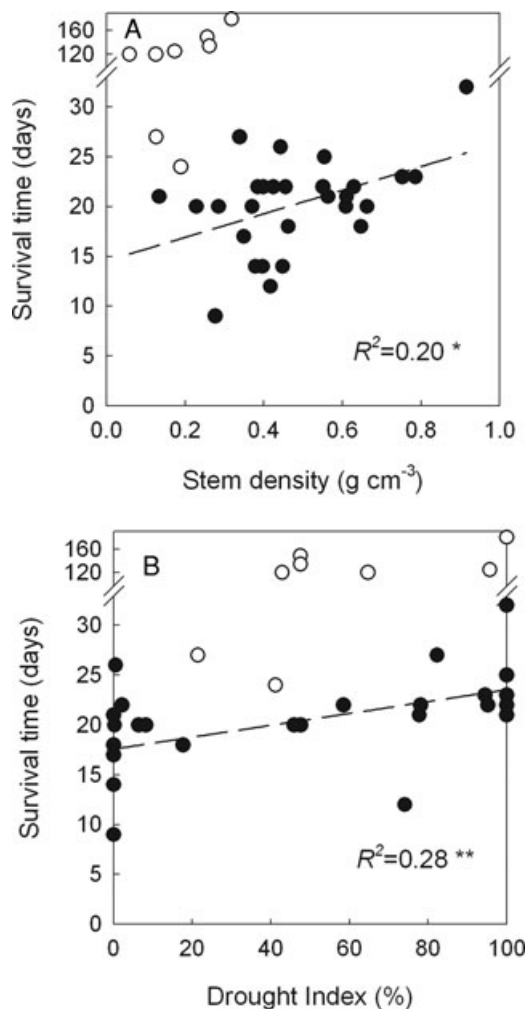

FIGURE 5. Drought survival time of seedlings of 36 tropical tree species versus (A) stem density, and (B) drought index. Evergreen species are represented by filled symbols, deciduous species by open symbols. The regression line has been fitted for the evergreen species only. Coefficients of determination, and significance levels are shown. $*P < 0.05$; $**P < 0.01$.

the presence of a thickened taproot is also important. In fact, most deciduous species have a taproot, and water stored therein may allow deciduous plants to support evaporational water loss through the bark, and maintain sufficiently high cell water content to maintain metabolic processes, and flush again at the onset of the rainy season. If deciduous species were excluded, SD became the best predictor of drought survival, probably due to its close link with resistance to xylem cavitation. PCA showed that dry forest species follow the same two strategies: drought avoidance through deciduousness, and drought resistance through tough and dense tissues (Fig. 2B). These two strategies were also found among drought-adapted Californian chaparral shrubs (Ackerly 2004), suggesting that they represent common avenues of plant adaptation to dry habitats (cf Eamus & Prior 2001).

A third strategy is followed by the light-demanding pioneers from the moist forest (Fig. 3B), characterized by an extensive root

system, with fine roots with a high SRL and high RLPM (*cf* Paz 2003). Such pioneers may forage not only for water, but especially for nutrients to meet the high nutrient requirements that accompany fast growth rates and high turnover rates of leaves and roots (*cf* Ryser 1996, Reich *et al.* 1998, Poorter & Bongers 2006).

We evaluated seedling traits related to biomass allocation, morphology, and phenology. Tyree *et al.* (2003) found that drought tolerance is closely related to physiological traits; species able to tolerate low leaf water contents and leaf water potentials also survive longest in dry conditions. Hence, the physiological ability of cells and meristems to remain alive in dry conditions is also an important component of drought survival.

The drought survival of evergreen species is linked to species position along the rainfall gradient, with species that survive longest having higher abundances in drier forests (Fig. 5B). Similarly, in Panama the drought survival of seedlings was a good predictor of species position along local and regional gradients in water availability; species with a high drought survival were more abundant on the dry plateau, and in drier forests (Engelbrecht *et al.* 2005, 2007).

IS THERE A TRADE-OFF BETWEEN DROUGHT TOLERANCE AND SHADE TOLERANCE?—Smith and Huston (1989) hypothesized that there is a trade-off between species' ability to tolerate shade and drought. Species from dry habitats should allocate more biomass to roots to capture water while those from moist, shaded habitats should invest more into leaves to capture light. For the Bolivian tropical tree species, such a trade-off between shade and drought tolerance has indeed been identified; the correlation between DI and juvenile CE (the inverse of shade tolerance) was 0.36 ($N = 37$, $P = 0.03$; Table 1). Similar trade-offs have been found for a large set of temperate tree species (Niinemets & Valladares 2006). The underlying trade-off in biomass allocation to leaves and roots was also found ($r = -0.65$, $N = 40$, $P < 0.001$), and species from moist habitats had indeed a large LMF while those from dry habitats a high RMF (Fig. 1). Yet, Smith and Huston's paradigm does not fully apply; the high biomass fraction in leaves enables moist forest species to capture more light, but a high root biomass fraction does not enable dry forest species to acquire more water, because their thick taproots are not efficient in water uptake (*cf* Craine *et al.* 2002) and do not result in a higher RLPM (Table 1). Instead, roots of dry forest species may have a storage function for water to overcome the dry season, or a storage function for carbohydrates to resprout after fire (Hoffmann *et al.* 2004).

CONCLUSIONS

In this study, we evaluated how seedling traits affect whole plant performance under dry conditions, and how drought survival, in turn, determines the distribution pattern of tree species. Three functional groups were identified based on seedling traits: (1) drought avoiders with a deciduous leaf habit and taproots; (2) drought resisters with tough tissues and a high SD; and (3) light-demanding

moist forest species with a large foraging capacity for belowground resources. Until recently, it was thought that drought survival of seedlings is primarily dependent on physiological traits (*e.g.*, Tyree *et al.* 2003), but this study indicates that phenological and morphological traits are also important. Deciduousness explained a high proportion (69%) of interspecific variation in drought survival. Apparently, there is a cost associated with such a drought avoidance mechanism, since only 22 percent of the studied species are deciduous in the seedling stage. Among the evergreen species, SD explained most variation in drought survival (20%), but 80 percent of the variation remained unexplained. This suggests that physiological traits and trait characteristics of later ontogenetic stages co-determine the drought tolerance of this large group of evergreen species.

Drought survival in itself could not explain species distribution along the rainfall gradient, because it was primarily determined by deciduousness, and species that are deciduous in the seedling stage are found in both dry and moist forests (Fig. 5B). Among the evergreen species, drought survival was a reasonable but modest predictor (28%) of species position along the rainfall gradient. This predictive power might be enhanced once better and more refined estimates of species position along the rainfall gradient become available (*e.g.*, Bongers *et al.* 1999). Nevertheless, it suggests that, in addition to drought tolerance, other factors such as biogeographic history (Killeen *et al.* 2005), dispersal limitation (Daws *et al.* 2005), and shade and fire tolerance (Hoffmann 1999) also shape the distribution patterns of tree species along the rainfall gradient.

ACKNOWLEDGMENTS

SUPPLEMENTARY MATERIAL

The following supplementary material for this article is available online at: www.blackwell-synergy.com/loi/btp

Table S1. List of 38 species with codes, density of stems > 10 cm dbh in dry and moist forest, drought index, juvenile crown exposure, survival time during drought, and seedling traits.

Table S2. Characterization of different wilting stages, based on visual leaf and stem characteristics (from Engelbrecht & Kursar 2003).

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