

Physical defence traits enhance seedling survival of neotropical tree species

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Summary

1. Physical defence traits of stems and leaves should enhance biomechanical strength and survival of seedlings. For eight neotropical tree species that differ widely in life-history strategies, we compared stem and leaf biomechanical traits of 1 and 7-month-old seedlings grown in the shaded forest understorey and in the laboratory.

2. Material traits (biomechanical traits per unit volume, mass or cross sectional area) were positively associated with seedling survival across species. Shade tolerant species that survive well in the forest understorey had stems and leaves with greater modulus of elasticity (stiffness), fracture toughness (resistance to tear), tissue density and fibre contents, compared to less shade tolerant species.

3. Seedling survival was most strongly correlated with stem tissue density at both 1 and 7 months (Spearman's correlation coefficient $r_s = 0.93$ and 0.90), but was also strongly correlated with leaf density and stem toughness at 7 months ($r_s = 0.93$ and 0.89 , respectively).

4. Multiple material traits were strongly and positively correlated with each other in both stems and leaves. However, these traits varied independently of seed and seedling size among species, indicating the unique importance of physical defence as functional traits.

5. Structural traits of stems that integrate size with material traits, including % critical buckling height, flexural stiffness, work-to-bend and stem flexibility, showed no significant interspecific correlation with seedling survival.

6. Modulus of elasticity and fracture toughness of stems generally increased as seedlings aged from 1 to 7 months, especially in species with high tissue density. In contrast, fracture toughness of leaf mid-vein and lamina showed inconsistent ontogenetic changes across species.

7. These results demonstrate that biomechanical traits including tissue density and fracture toughness should be considered as important functional correlates of seedling survival and overall life-history strategies of tree species.

Key-words: fibre contents, fracture toughness, modulus of elasticity, shade tolerance, tissue density

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Introduction

Natural selection should favour stems and leaves that maximize carbon gain, competitive ability and safety, but that minimize costs of construction and maintenance (Givnish 1995). Because it is often impossible to optimize all these fitness components simultaneously, diverse combinations of functional traits have evolved in relation to ecological niches and life history of the species. Certain functional traits are in a direct trade-off relationship. For example, seedlings and saplings of species that survive well in the forest understorey

exhibit low growth rates (Kitajima 1994; Gilbert *et al.* 2006; Poorter *et al.* 2006). Such growth-survival trade-off probably exists because allocation patterns that place high priority to defence and storage are in conflict with traits that maximize growth rates (Kitajima 1994). Indeed, physical defence traits, such as tough leaves and dense stems are increasingly recognized to be important for survival of tree saplings (Coley 1983; Wright *et al.* 2004; Poorter & Bongers 2006). Yet, importance of these traits for survival of young seedlings remains unexplored.

Young seedlings face a particularly strong trade-off between the need to grow fast in order to compensate for their small size and the need to defend against hazards such as disease (Augsburger 1984a), herbivory

and physical disturbance due to vertebrate activity and litterfall (Clark & Clark 1989; Kitajima & Augspurger 1989; Gartner 1991; Gómez, García & Zamora 2003). For example, Alvarez-Clare (2005) found that 77% of 755 seedlings from eight species of tropical trees transplanted to the forest understorey suffered some type of mechanical damage after 1 year. Investment in physical defence may reduce mortality due to mechanical damage and may be important in explaining large species differences in susceptibility to the multiple mortality agents.

Plant stem and leaves are non-homogeneous composites of tissues, cell types and fibre components. Material strength refers to the resistance expressed per unit area of cross section to be bent or torn (Niklas 1992; Barnett & Jeronimidis 2003). Two important measures of material strength are modulus of elasticity (i.e. stiffness expressed per unit cross sectional area of bending, N m^{-2}) and fracture toughness (i.e. tearing force required per unit cross sectional area of tear, J m^{-2}). Material strength of plant tissue is a function of cell wall volume fraction, of the relative representation of fibre components in cells, and of how fibre-rich cell walls are organized in the tissue (Lucas *et al.* 2000). Overall, material strength should be correlated with fibre density (fibre content per unit volume), which can be broken down into tissue density (dry mass per volume) and relative fibre contents (fibre per unit dry mass). The three main components of plant cell wall fibre: hemicellulose, cellulose and lignin, contribute differently to overall material strength (Lucas *et al.* 2000). All else being equal, high cellulose content should be positively correlated with fracture toughness of leaves and stems because the high tensile strength of cellulose contributes to fracture toughness (Choong 1996). By contrast, high lignin content in stems should be more correlated with stiffness than with toughness, because lignin is a complex polymer with strong covalent bonds (Niklas 1992). Lignin is also known for its anti-fungal properties; pathogen attack often induces localized and systemic increase in lignin production (Humphreys & Chapple 2002). Yet, it is unknown how lignin and other fibre components may be related to species differences in disease susceptibility.

The structural strength of the whole organ (i.e. stem and leaves) to resist tearing and bending is an integrative function of material strength, size and shape. For leaves, the whole structure's resistance against tearing force (such as given by herbivores) is best expressed by force of fracture (N), calculated as the product of fracture toughness (a measurement of the material strength) and leaf thickness. This structural measurement indicates the total force necessary to break leaf tissue considering leaf thickness (Wright & Cannon 2001). For stems, two structural traits that integrate modulus of elasticity (hereafter referred to as E) and stem size (the second moment of area, $I = 0.25\pi r^4$ for a circular cross section with radius r , and height) are often considered. One is flexural stiffness (EI), the

product of E and I , which measures the resistance of the whole stem against bending. The other is critical buckling height, which measures the maximum possible stem height before it buckles under its own weight (see Materials and methods for calculation). Higher the ratio of the actual stem height relative to the critical buckling height ($\% H_{cr}$), lower the safety margin to remain standing against its own weight (Niklas 1998). *A priori*, these whole-stem strength and safety margin measurements may not seem important for small understorey seedlings that do not experience heavy loads or wind exposure. However, they may provide insight on how seedlings cope with non-wind related physical disturbances. For example for saplings from 30 tree species in Bolivia, shade tolerance was positively correlated with material strength (high modulus of rupture and modulus of elasticity, which were correlated with stem density) and structural strength (safety factor for buckling; van Gelder, Poorter & Sterck 2006).

Here, we report the first comparative study of biomechanical traits of first-year seedlings, using eight tropical tree species that differ widely in life history strategy, from shade-tolerant species whose seedlings grow slowly but survive well to light-demanding species whose seedlings grow fast but exhibit high mortality. Specifically, we address the following three questions:

1. How do multiple biomechanical traits differ in concert among species? Certain traits should have positive interspecific correlations; in particular both tissue density and fibre contents (per volume and per dry mass) are expected to contribute to material strength. Both material and size should contribute to structural strength, but their relative importance in explaining species differences is unclear. We also predict concordance of tissue density and material strength between leaves and stems because it would not be advantageous to have tough leaves if the plant is likely to be killed because of a weak stem.
2. How do biomechanical properties of stems and leaves change ontogenetically within the first year? If very young seedlings going through rapid initial development by using seed reserves are constrained from having tough stems and roots, then material strength should increase with seedling age. However, because increased size may compensate for weak material strength, material strength may not necessarily increase with size. Furthermore, with ontogenetic development the relationship between material strength and tissue or fibre density may change.
3. Do species with greater material or structural strength survive better in the shaded forest understorey during the first year? In the shaded understorey where replacement of lost tissue is costly relative to photosynthetic income, physical defence should enhance resistance against mechanical damage and possibly disease.

Materials and methods

STUDY SITE, SPECIES AND SEEDLING
SURVIVAL RANKS

The study was conducted in a moist tropical forest within the Barro Colorado Natural Monument (BCNM), Panama (9°10' N, 79°51' W). Climate, flora, and ecological characteristics of the seasonally moist tropical forest in BCNM are well described in Croat (1978) and in Leigh, Windsor & Rand (1982). On average, 78% of the mean annual precipitation of 2600 mm occurs in May–December, and the community-level peak of germination is observed in May–July (Garwood 1983). We selected eight common tree species, whose seedling survival was ranked in two separate studies at ages similar to those measured in this study. The first study quantified % survival of seedlings 2–6 months after transplanting to the forest understorey in low density along 9 km of trails, excluding the initial high mortality due to transplant shock and predation by vertebrates attracted to large seed reserves (S. Alvarez-Clare & K. Kitajima, unpublished data). In the second study, seedlings were transplanted inside three vertebrate exclosures in a forest understorey that received 0.5%–0.8% of total daily photosynthetic photon flux density (% PPF) (K. Kitajima unpublished data), from which we calculated % survival for the first 0–4 months after transplanting and before sample size became reduced by harvests. Species survivals were roughly concordant between these two studies ($r^2 = 0.47$, $N = 8$, $P = 0.06$), but there were differences due to experimental designs and stochastic factors. In order to use a general index of survival in shade, we averaged % survival from these two studies (Table 1).

SEEDLING PREPARATION

In the understorey of a 70-year-old secondary forest in BCNM (light availability = 1.35 % PPF) according to canopy photos), we established three 6 × 6 m common gardens with 1 m tall wire fence with mesh size of 1 cm to exclude ground-dwelling vertebrates. Seeds of study species collected inside BCNM in May–June 2003 were germinated in trays in a nursery with light availability adjusted by shade cloth to 2% PPF. Species mean seed dry mass was determined from 10 to 12 randomly selected seeds after removing seed coat. We transplanted 45 seedlings of each species to random locations (at least 50 cm apart) within each common garden at full expansion of the first photosynthetic organs (leaves for all species except for *Tabebuia rosea*, the only species with photosynthetic cotyledons). Time from germination until leaf expansion varied among species from 1 week for *Anacardium excelsum* to 4 weeks for *Eugenia nesiotica* and *Tetragastris panamensis*. Seedlings that died within the first week after

Table 1. Ecological characteristics of eight tropical tree species used in this study, listed in order of increasing shade tolerance

Sp. Code	Species	Family	Cot. Type	Dispersal	Seed mass (g)	Seedling biomass (g)	Mean % survival
TABR	<i>Tabebuia rosea</i> (Bertol.) DC.	Bignoniaceae	PEF	Wind	0.035 ± 0.007 (12)	0.046 ± 0.024 (23)	45.5
ANAE	<i>Anacardium excelsum</i> (Bertero & Balb.) Skeels	Anacardiaceae	PER	Animal	1.811 ± 0.316 (9)	0.807 ± 0.269 (45)	53.0
CASE	<i>Castilla elastica</i> Sessé in Cerv.	Moraceae	CHR	Animal	0.315 ± 0.005 (8)	0.302 ± 0.208 (44)	65.0
GUSS	<i>Gustavia superba</i> (H.B.K.) Berg	Levythidaceae	CHR	Animal	5.566 ± 1.746 (7)	3.694 ± 1.329 (46)	76.0
TETP	<i>Tetragastris panamensis</i> (Engler) O. Kuntze	Bursaceae	PER	Animal	0.179 ± 0.026 (10)	0.247 ± 0.054 (44)	82.0
BEIP	<i>Beilschmiedia pendula</i> (Sw.) Hemsl.	Lauraceae	CHR	Animal	2.360 ± 0.090 (10)	1.868 ± 0.523 (43)	82.5
ASPC	<i>Aspidosperma cruenta</i> Woods	Apocynaceae	PHR	Wind	0.492 ± 0.002 (6)	0.545 ± 0.127 (45)	87.0
EUGN	<i>Eugenia nesiotica</i> Standl.	Myrtaceae	CHR	Animal	0.474 ± 0.067 (10)	0.399 ± 0.174 (30)	87.5

Cotyledon types classified according to Garwood (1983). PEF, phanerocotylar epigeal reserve; PER, phanerocotylar hypogeal reserve; CHR, cryptocotylar hypogeal reserve. Mean ± SD (N) seed mass without seed coat. Mean ± SD (N) plant biomass 1 month after leaf expansion (T1). Mean % survival was calculated from 0 to 4 months survival of seedlings transplanted to exclosures located in the forest understorey and 2–6 months survival of seedlings transplanted directly into the forest (S. Alvarez-Clare & K. Kitajima unpublished data).

transplanting were replaced. We randomly chose 15 plants per species per garden ($N = 45$) for *in-situ* biomechanical tests and harvest after 1 month (T1). The remaining plants were harvested 6 months later (T2). All harvested plants were kept in a cooler box at 5–10 °C until the laboratory measurements. After measurements of fresh materials, leaves, stems and roots were dried at 60 °C for at least 48 h. The dried material was weighed and saved for fibre analysis.

MEASUREMENT OF BIOMECHANICAL TRAITS

We used a portable universal tester to determine modulus of elasticity (E) and fracture toughness for stems and leaves as described by Lucas *et al.* (2001). Tissue density (g cm^{-3}) was calculated for leaves and stems as dry mass per unit volume. Specific leaf area (SLA) was calculated as the ratio of leaf area and leaf total dry mass. We calculated leaf volume as total leaf area multiplied by the lamina thickness, and stem volume from stem length and diameter in the middle, assuming a perfect circular cross section and no taper. For leaves, force of fracture (N) was calculated as the product of fracture toughness (N m^{-1}) and lamina thickness (m). For stems, two measurements of structural strength were performed: (i) flexural stiffness (EI) was measured as the product of the second moment of area and modulus of elasticity (as described in the Introduction); and (ii) percent (%) critical height was calculated for each seedling stem according to the formula given by Greenhill (1881):

$$H_{cr} = 1.26(E/w)^{1/3} (d_b)^{2/3}$$

where E , modulus of elasticity (N m^{-3}), w , fresh weight per unit volume (N m^{-3}) and d_b , diameter at base (m). The ratio of the actual stem height to H_{cr} multiplied by 100 is % H_{cr} .

Fibre fractions

Fibre contents of dried and ground leaves and stems were determined with a fibre analyzer system (ANKOM Technology, NY, USA). Each ground sample was enclosed in a chemical resistant bag, and treated with a series of increasingly aggressive extractants to determine % neutral detergent fibre (% NDF, total fibre), % acid detergent fibre (% ADF, including cellulose, lignin, insoluble ash) and % (lignin and insoluble ash), followed by % ash determination with combustion at 500 °C (Ryan, Melillo & Ricca 1989). From these, % hemicellulose, % cellulose and % lignin was calculated by subtraction. Mass of non-fibre contents (including ash) + hemicellulose + cellulose + lignin add up to 100% of the original dry mass. Lignin (or cellulose) per unit volume was calculated by multiplying % lignin by tissue density and dividing by 100.

In-situ field measurements of stems

We measured resistance of stems to bending by compressive and tension using rooted intact plants in the field. To estimate work-to-bend a 2 L plastic container was mounted on a 30 cm² Styrofoam platform and hung from a tripod with a spring balance just above the seedling. Then, water was poured slowly into the container, until the weighted platform deflected the stem to 70%–60% of its original height. Assuming that acceleration was nil, water weight (force) times vertical displacement, was calculated as work to bend the seedling. Stem flexibility was measured following Holbrook & Putz (1989) by pulling the stem horizontally in four directions with a spring balance for 20° from vertical and calculating angular deflection per average applied force (radians per N). In the case of *E. nesiotica*, the stem was bent to 40°, because the force required to bend the stem 20° was too small to be detected in its small seedlings.

STATISTICAL ANALYSES

Species means for biomechanical traits were log-transformed to meet normality assumptions for ANOVA to test effects of species ($N = 8$), harvest time ($N = 2$) and their interactions. To test if means differed between T1 and T2 within each species, *t*-tests with Bonferroni corrections were used. To explore general functional relationships between traits, Spearman correlations were calculated for each harvest ($N = 8$). We explored how the relationship of material strength with tissue or fibre density changed over time, by performing an ANCOVA using harvest time as the main factor and density as the covariate. All analyses were performed using JMP IN 4.0 (SAS Institute Inc., Cary, NC, USA) with a significance level of $\alpha = 0.05$.

Results

STEM BIOMECHANICS

Material strength of seedling stems differed significantly among species, as well as between the two seedling ages. Modulus of elasticity (E) of the seedling stems varied 20-fold among species (Fig. 1a). Most species increased their resistance to bending (E) during the 6-months period between T1 and T2 resulting in significant time effect without a species \times time interaction. Stem fracture toughness also varied among species and between harvests (Fig. 1b), but the degree of increase in fracture toughness varied among species. While *E. nesiotica* increased its mean fracture toughness threefold from 1 to 6 months after leaf expansion, *A. excelsum* and *T. panamensis* showed no increase (Fig. 1b). Stem tissue density also varied among species and between harvests, remaining constant or increasing from T1 to T2 to various degrees in all species (with points close or above to the 1 : 1 line) except in *A.*

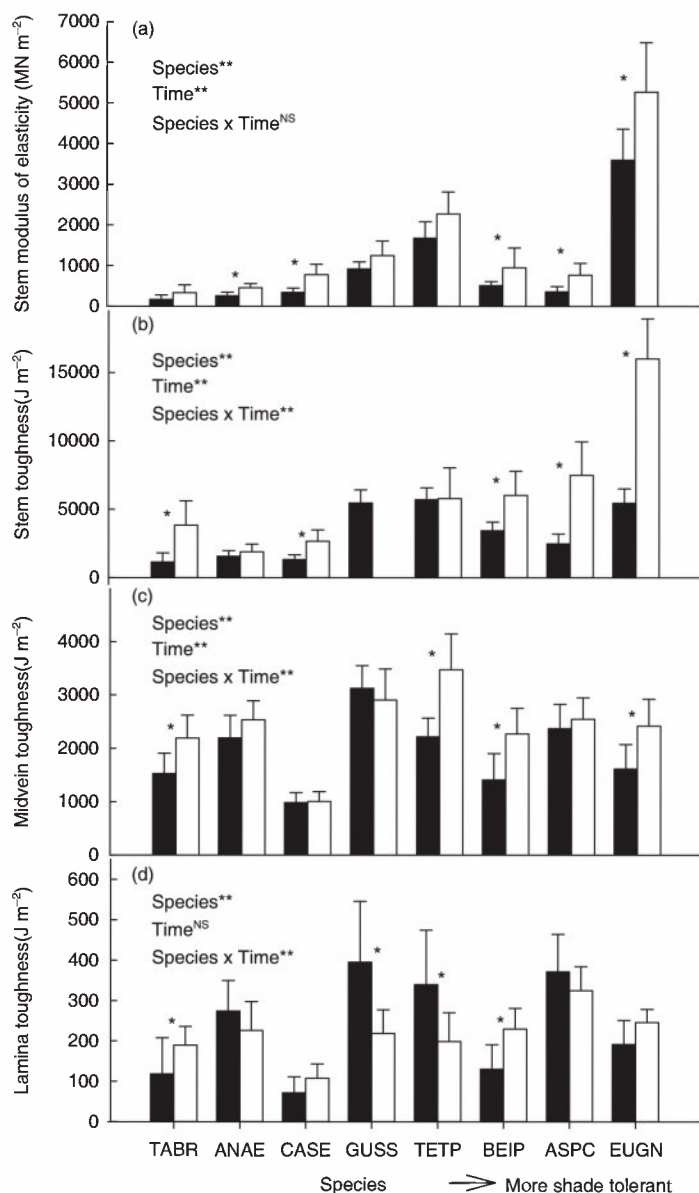


Fig. 1. Species means (± 1 SD) for material biomechanical traits of stems and leaves for seedlings of eight tree species in order of increasing shade tolerance from left to right (see Table 1 for species code). Closed and open bars indicate 1 month (T1) and 7 months (T2) after first leaf expansion: (a) modulus of elasticity of stems (E); (b) stem fracture toughness; (c) mid-vein fracture toughness; and (d) lamina fracture toughness. Also shown are results from two-way ANOVAs (** $P < 0.001$). Asterisks above bars indicate significant difference between T1 and T2 ($P < 0.006$, due to Bonferroni correction). No data were available for stem toughness of GUSS at T2 because the stem diameter (> 3 mm) exceeded the size limit for the tester.

cruenta (Fig. 2a). Species differed in % NDF ($F = 57.7$, $P < 0.001$; Fig. 3a), as well as in individual fibre fractions ($F_{\text{hemicellulose}} = 25.3$, $P < 0.001$, $F_{\text{cellulose}} = 30.7$, $P < 0.001$ and $F_{\text{lignin}} = 38.4$, $P < 0.001$).

Most stem material traits were correlated with each other at each ontogenetic stage (Table 2). Mechanical traits, such as fracture toughness and modulus of elasticity (E) were positively correlated with tissue density (dry mass per unit volume) and % fibre contents (Table 2). Fracture toughness increased with ontogeny much more strongly in species with high fibre and dry mass per volume (significant time-covariate interaction

Table 2. Pair-wise correlations for material and structural traits of seedling stems among eight tree species

	E (MN m ²)	Toughness (J m ⁻²)	Density (g cm ⁻³)	% NDF	% Cellulose	% Lignin	I (mm ⁴)	EI (N m ²)	% H_r	Seedling mass (g)
Material traits										
E		0.90 (0.002)	0.71 (0.047)	0.95 (< 0.001)	0.88 (0.004)	0.65 (0.090)	-0.29 (0.493)	0.05 (0.911)	-0.62 (0.102)	0.00 (0.100)
Toughness	0.61 (0.148)		0.64 (0.0856)	0.81 (0.015)	0.90 (0.002)	0.55 (0.160)	-0.02 (0.955)	0.29 (0.492)	-0.55 (0.160)	0.24 (0.570)
Density	0.81 (0.015)	0.86 (0.014)		0.60 (0.120)	0.45 (0.260)	0.17 (0.693)	-0.26 (0.531)	-0.14 (0.736)	-0.05 (0.911)	0.04 (0.911)
% NDF	0.95 (< 0.001)	0.68 (0.094)	0.86 (0.007)		0.88 (0.004)	0.76 (0.028)	-0.29 (0.493)	0.05 (0.911)	-0.55 (0.160)	-0.07 (0.867)
% Cellulose	0.74 (0.037)	0.68 (0.093)	0.71 (0.047)	0.79 (0.021)		0.67 (0.071)	0.05 (0.911)	0.29 (0.493)	-0.55 (0.160)	0.12 (0.779)
% Lignin	0.83 (0.01)	0.43 (0.337)	0.55 (0.160)	0.79 (0.021)	0.83 (0.010)		-0.26 (0.531)	0.00 (0.100)	-0.55 (0.160)	-0.17 (0.693)
Size-related traits										
I	-0.48 (0.233)	-0.82 (0.023)	-0.83 (0.010)	-0.62 (0.102)	-0.69 (0.058)	-0.17 (0.693)		0.93 (< 0.001)	0.40 (0.320)	0.93 (< 0.001)
EI	0.00 (0.100)	-0.43 (0.337)	-0.35 (0.385)	-0.19 (0.651)	-0.45 (0.260)	0.26 (0.531)	0.81 (0.015)	0.93 (< 0.001)	0.095 (0.823)	0.93 (< 0.001)
% H_r	-0.74 (0.036)	-0.64 (0.119)	-0.60 (0.120)	-0.74 (0.037)	-0.88 (0.004)	-0.57 (0.139)	0.38 (0.352)	0.05 (0.911)	0.02 (0.955)	0.24 (0.570)
Seedling mass	-0.02 (0.955)	-0.29 (0.492)	-0.29 (0.492)	-0.21 (0.610)	-0.43 (0.289)	0.24 (0.570)	0.74 (0.037)	0.98 (< 0.001)		

Shown are Spearman correlation coefficients for species means at two harvests ($N = 8$ each) along with P values in parentheses. Data for harvest 1 (1 month) are shown above the diagonal and data for harvest 2 (7 months) are shown below the diagonal. Bold type indicates $P < 0.05$. Modulus of elasticity (E) and fracture toughness (Toughness) were measured by a universal tester. Density refers to dry mass per unit volume, while fibre fractions per unit mass are expressed in % (NDF, neutral detergent fibre). The second moment of area (I) is a function of stem radius (see the text). Structural measurements included % critical buckling height (% H_r) and flexural stiffness (EI).

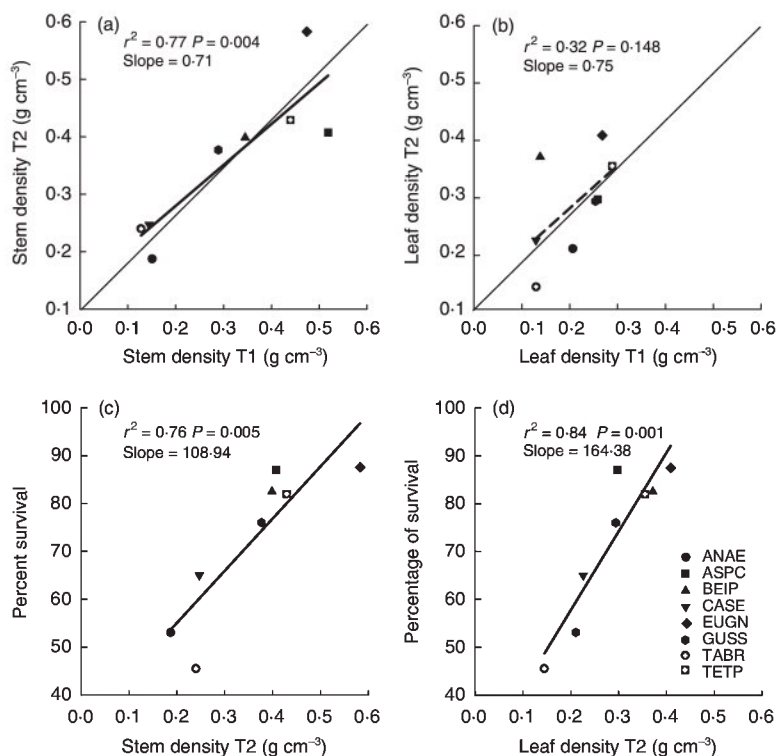


Fig. 2. Relationships between stem and leaf density at different developmental stages and between density and survival for seedlings from eight tree species: (a) stem density at 1 and 7 months after leaf expansion (T1 and T2, respectively); (b) leaf density at T1 and T2; (c) seedling survival as a function of stem density; and (d) seedling survival as a function of leaf density. See Table 1 for species codes.

in ANCOVA, Table 3). In other words, ontogenetic development of tougher tissue occurred only in species with high fibre and mass content per volume. Likewise, the positive effect of lignin per volume upon E increased with ontogeny.

Structural traits integrating size and material traits also varied among species and stages, but without any obvious relationship with seedling survival. All species had relatively low percentage (%) critical height (% H_{cr} , ranging 14%–28%) indicating that these seedlings had a large margin of safety before buckling under their own weight (Fig. 4a). However, light-demanding species tended to have higher % H_{cr} , reflecting a tendency towards stem elongation, rather than diameter increment during early developmental stages. Flexural stiffness (EI), as well as the two field-based measurements performed on whole stems, work-to-bend and stem flexibility also showed significant species differences (Fig. 4b–d). As expected by biomechanical theories, EI was correlated with work-to-bend ($r_s = 0.96$, determined only at T1) and stem flexibility ($r_s = -0.93$ and -0.95 for T1 and T2, respectively). These three traits were strongly correlated with second moment of area (I) ($P < 0.014$, Table 2). Flexural stiffness increased and stem flexibility decreased, as stems developed ontogenetically with seedling age and size (Fig. 4b,d).

Table 3. Results of analyses of covariance (ANCOVA) to test the effect of ontogeny (1 and 7 months after leaf expansion) on the functional relationship of stem modulus of elasticity (MOE) or fracture toughness (toughness) with stem density (dry mass per volume), cellulose per volume or lignin per volume as covariate

	Stem MOE		Stem toughness	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Stage	0.1	0.730	4.6	0.056
Density	15.8	0.002	34.9	< 0.001
Interaction	2.9	0.110	11.8	0.005
Model	5.9	0.010	16.7	< 0.001
Stage	0.3	0.58	7	0.023
Cellulose	33.1	< 0.001	43.3	< 0.001
Interaction	2.8	0.12	8.8	0.013
Model	12.2	< 0.001	10.5	< 0.001
Stage	24.8	< 0.001	27.2	< 0.001
% Lignin	55.1	< 0.001	31.7	< 0.001
Interaction	19.3	< 0.001	23.9	< 0.001
Model	20.1	< 0.001	13.8	< 0.001

At each stage, there was a positive correlation between stem MOE and toughness with each covariate (Table 2). A significant interaction reflected that stems strength increased with seedling age only in species with high density. *F* and *P* values are shown in bold when significant ($P < 0.05$).

Material traits of stem tissues were correlated with each other at both stages. However, they were uncorrelated with indicators of size (I and seed or seedling mass) and structural traits incorporating size at T1 (Table 2). In contrast, at T2, I was negatively correlated with fracture toughness and with tissue density (Table 2), which is expected if size compensates for weak material. The % H_{cr} was negatively correlated with modulus of elasticity (E), % NDF, and % cellulose at T2, indicating that species with stronger material had a lower % H_{cr} and hence a greater safety margin.

LEAF BIOMECHANICS

Material biomechanical traits of leaves differed among species, but directions and magnitudes of change between T1 and T2 were not consistent across species. Typically, the first fully expanded leaf of each plant was measured at T1, whereas a leaf below the newest leaf (which could be the oldest or the one in the middle position) was measured at T2. In general, for each species at a given stage, mid-vein fracture toughness (Fig. 1c) was less than stem toughness (ca. $\times 1/2$; Fig. 1b), but much higher (ca. $\times 10$) than lamina toughness (Fig. 1d). All individual fibre fractions (Fig. 3b) varied among species ($F_{\text{NDF}} = 5.5$, $P < 0.001$, $F_{\text{hemicellulose}} = 36.2$, $P < 0.001$, $F_{\text{cellulose}} = 6.9$, $P < 0.001$ and $F_{\text{lignin}} = 24.4$, $P < 0.001$) but with disparity between the two harvests. Only % cellulose and % lignin were significantly correlated between the two harvests ($P = 0.05$). The two measured leaf structural traits, force of fracture and SLA, differed among

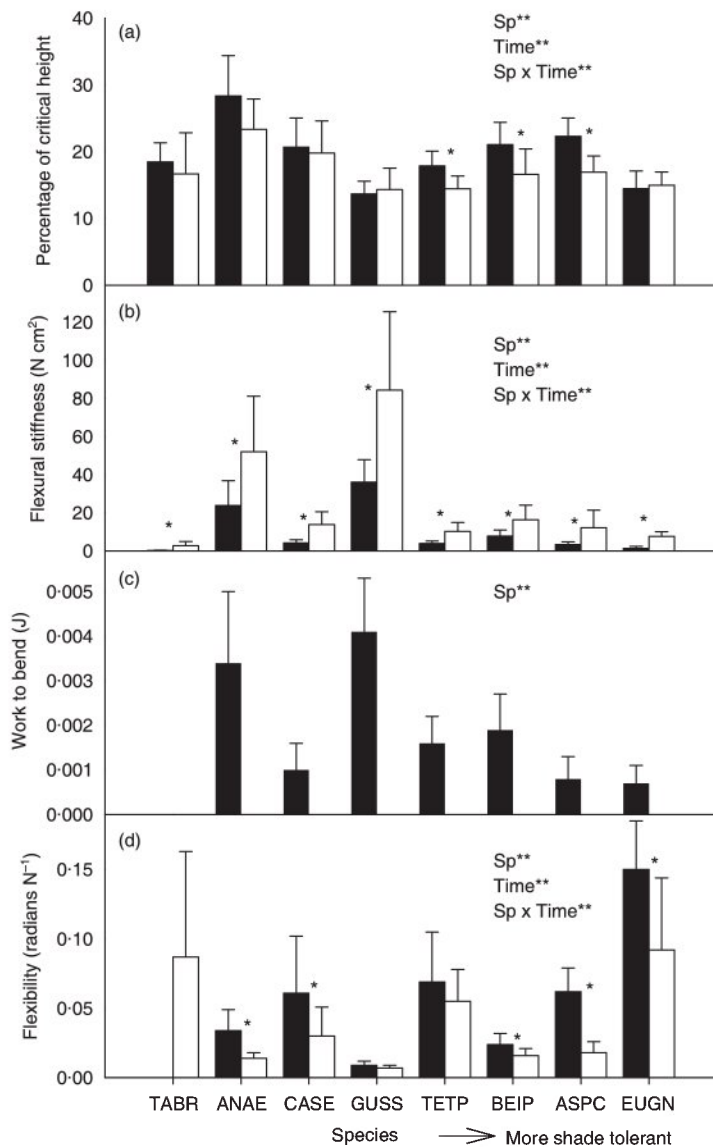


Fig. 4. Species means (± 1 SD) for structural biomechanical traits of stems for seedlings of eight tree species, shown in order of increasing shade tolerance from left to right (see Table 1 for species codes). Closed and open bars indicate 1 (T1) and 7 months (T2) after first leaf expansion: (a) % critical height; (b) flexural stiffness; (c) work-to-bend; and (d) flexibility. Also shown are results from two-way ANOVAs (** $P < 0.001$). Asterisks above bars indicate significant difference between T1 and T2 ($P < 0.006$, due to Bonferroni correction). Work-to-bend was only measured at T1. Work-to-bend and flexibility could not be measured for TABR at T1 because of small size of seedlings.

RELATIONSHIP BETWEEN SEEDLING BIOMECHANICS AND SURVIVAL

Several material traits of stems and leaves were positively correlated with species survival ranks (Table 6). Stem tissue density at both T1 and T2, as well as leaf tissue density at T2, exhibited the highest correlations with survival (Table 6, Fig. 2c,d). Stem fracture toughness at T2 also showed a high rank correlation with survival (Table 6). Although individual fibre fractions exhibited no significant correlation with survival, % NDF in both stems and leaves was positively correlated

Table 5. Pair-wise Spearman correlation coefficients (with P values) between stem and leaf tissues for a given type of trait (fracture toughness, density and % NDF) at a given harvest (T1 and T2). $N = 8$ species

Biomechanical trait	T1	T2
Fracture toughness ($J m^{-2}$)		
Lamina	0.67 (0.071)	0.75 (0.052)
Mid-vein	0.57 (0.139)	0.29 (0.535)
Density ($g cm^{-3}$)	0.81 (0.015)	0.90 (0.002)
% NDF	0.88 (0.004)	0.21 (0.610)

Table 6. Spearman correlation coefficients (r_s) between % survival (Table 1) and seedling biomechanical traits of stems and leaves ($N = 8$ species). T1 and T2, two harvest times (1 and 7 months after first leaf expansion, respectively)

	T1		T2	
	r_s	P	r_s	P
Stem				
Material traits				
E	0.79	0.021	0.69	0.058
Toughness	0.60	0.120	0.89	0.007
Density	0.93	< 0.001	0.90	0.002
% NDF	0.74	0.037	0.71	0.047
% Hemicellulose	0.50	0.120	0.52	0.183
% Cellulose	0.48	0.233	0.45	0.260
% Lignin	0.38	0.352	0.60	0.120
Structural traits				
% H_{cr}	-0.17	0.693	-0.38	0.352
EI	-0.17	0.693	-0.12	0.779
Work-to-bend	-0.68	0.094	-	-
Flexibility	0.54	0.215	0.19	0.651
Leaf				
Material traits				
Lamina toughness	0.33	0.420	0.76	0.028
Mid-vein toughness	0.21	0.610	0.31	0.456
Density	0.64	0.086	0.93	< 0.001
% NDF	0.81	0.015	0.45	0.260
% Hemicellulose	0.33	0.420	0.14	0.736
% Cellulose	0.36	0.385	-0.05	0.912
% Lignin	0.43	0.289	0.24	0.570
Structural traits				
SLA	-0.52	0.183	-0.60	0.120
Force of fracture	0.29	0.493	0.048	0.911

with survival. Contrary to material properties, none of the stem and leaf structural properties incorporating size, or total seedling mass, were correlated with seedling survival ($P > 0.05$, Table 6).

Discussion

ASSOCIATION OF SEEDLING BIOMECHANICS WITH SURVIVAL

Our study is the first to demonstrate the importance of biomechanical material strength of stems and leaves for survival of young tree seedlings. In general, species that establish and survive well in the shaded forest

understorey had stiff and tough stems, and these two aspects of material strength were associated with each other, especially at early developmental stages. Furthermore, both traits were associated with high tissue density and lignin, and cellulose contents per unit mass and volume, which presumably reflect species differences in vascular cambium maturation, cell wall volume fraction, and secondary cell wall development (Lucas *et al.* 2000; Niklas *et al.* 2000). Notably, shade-tolerant species had greater fibre and mass per unit stem volume, and the positive effects of these traits upon fracture toughness and modulus of elasticity increased with age (Table 3). It is possible that the vascular cambium started to produce secondary xylem ('wood') and secondary phloem between the two ontogenetic stages, but to a greater degree in shade-tolerant species. These findings are congruent with van Gelder *et al.* (2006), who found that saplings of shade-tolerant species, in terms of their distribution relative to light environment, have greater stem wood density and stiffness than saplings of light-demanding species. Our study is a more direct demonstration of the survival advantage of tissue density and biomechanical strength in the shaded forest understorey.

In general, biomechanical traits of stems and leaves varied in concert. Seedlings of shade-tolerant species had tougher and denser stems and leaf laminae than less shade-tolerant species (Tables 2 and 3, Fig. 2). However, stem biomechanical traits were better correlated with seedling survival, especially at very young developmental stages. Perhaps, physical defence of stems has immediate consequence on whole plant survival of young seedlings, whereas leaf physical defence becomes more important in older seedlings (Table 6). In a study with 2548 species, Wright *et al.* (2004) found that leaf mass per area (LMA) explained 42% of the variation in leaf life span, indicating that thicker, denser leaves usually live longer. Leaf survival is important for maintenance of positive net carbon balance in light-limited forest understoreys, where cost of leaf replacement is high (King 1994), suggesting that perhaps shade tolerance requires both tough stems and leaves.

Material traits were more important for survival than size-dependent structural traits (i.e. flexural stiffness, % critical height). This was a surprise given that previous studies comparing many taxa found a positive association of large seeds and seedlings with survival in the shaded understorey (Moles & Westoby 2004; Poorter & Rose 2005); however, it is not uncommon to find no correlation when relatively small number of species are compared (e.g. among 19 species in Augspurger 1984b; 15 species in Kitajima 1994). Large seedling size does confer an advantage for emergence out of litter (Molofsky & Augspurger 1992), light acquisition (Turner 1990) and size of carbohydrate storage pool (Myers & Kitajima 2007). However, large but physically undefended seedlings probably do not survive well. While size may compensate for weak material, such compensation was only suggested by a

non-significant negative correlation of stem fracture toughness and tissue density with I (Table 2).

ECOLOGICAL ROLE OF TISSUE DENSITY

Tissue density (dry mass investment per unit volume) was strongly correlated with both modulus of elasticity and fracture toughness of stems and leaves, and more importantly, it was the strongest correlate of seedling survival among various material traits quantified in this study (Table 6, Fig. 2c,d). This finding is in accordance with results obtained for older woody plants, which show that stem wood density is strongly associated with key life-history traits, including juvenile shade tolerance, maximum adult stature, age of maturity and seed production (Wright *et al.* 2003; Muller-Landau 2004; van Gelder *et al.* 2006). In leaves, both regional and global data analyses suggest that leaf density is strongly associated with leaf life span and adaptation to low resource environments of shade and infertile soils (Wright & Cannon 2001; Wright *et al.* 2004).

High tissue density provides not only mechanical strength, but also additional ecological advantage for juvenile persistence in the shaded forest understorey. This is evident because leaf and stem density exhibited a stronger correlation with seedling survival than mechanical strength. One additional advantage of high tissue density may be increased resistance to pathogenic disease. Species differences in first-year seedling mortality in the shaded understorey largely reflect their differences in disease susceptibility (Augspurger 1984a). Furthermore, the higher the wood density of adults and saplings, the lower the overall seedling mortality in shade (Augspurger 1984b; Kitajima 1994). The current study demonstrates directly that high tissue density of seedling stems contributes to seedling survival. In stems, high tissue density also meant that a greater proportion of the biomass was invested in fibre (% NDF), specifically in cellulose and lignin in older seedlings (Table 2). While lignin and cellulose contents per unit mass were not significantly correlated with survival at this early stage, higher tissue density of shade-tolerant species means greater cellulose and lignin contents per unit volume of stems ($r_s = 0.90$ for survival vs. lignin per unit volume and for survival vs. cellulose per unit volume). Greater fibre contents per unit tissue volume may serve a dual purpose, enhancing resistance to pathogenic microbes as well as mechanical strength.

CHANGES OF MECHANICAL TRAITS DURING STEM DEVELOPMENT

For stems, both material and structural strength increased during the 7 months of observed stem development. This reflects not a simple increase in tissue density, but rather a greater positive effect of biomass, cellulose and lignin per stem volume on

fracture toughness and modulus of elasticity (Table 3). It is reasonable to expect that seedlings going through rapid initial development using seed reserves to be constrained from having tough and stiff tissues. Indeed, at 1 month after the first leaf expansion stems of shade-intolerant species (e.g. *T. rosea*, *C. elastica*) had similar moduli of elasticity to those of understorey herbs (Niklas 1995; Cooley, Reich & Rundel 2004). In these species, seedlings relied on primary tissues for mechanical support. In contrast, shade-tolerant species (e.g. *T. panamensis* and *E. nesiotica*) had moduli of elasticity similar to the values found in adult wood (Niklas 1992). These shade-tolerant species probably had more developed vascular cambium and advanced secondary growth than shade-intolerant species, especially at T2 (7 months after leaf expansion). In other words, the denser stems of shade-tolerant species show greater ontogenetic change in stem anatomy and mechanical strength than the less dense stems of shade-intolerant species. Preliminary anatomical examination of stem cross sections of a few species confirms this view (S. Alvarez-Clare, unpublished observation).

In some species, fracture toughness of leaves did not show an increase between 1 and 7 months after the expansion of the first leaf, even decreasing in some cases. This trend is difficult to interpret partly because we sampled the most representative mature leaf in the middle, rather than the same first leaf for 7-month-old seedlings. Thicker or older leaves may decrease fracture toughness as size compensates for material strength. Lucas & Pereira (1990) also found that leaf fracture toughness decreased over time, which may reflect an increase in parenchymatous tissue and air species in thicker and older leaves.

In summary, tissue density and biomechanical strength of seedling stems and leaves are previously unexplored but significant functional correlates of life-history strategies of tree species. Seedlings that survive well in the shaded understorey were physically well defended with high tissue density in both leaves and stems, even very early in ontogeny. Additional functional traits also contribute to the suite of functional traits associated with the overall life-history strategy of shade-tolerant tree species. No matter how physically well protected, seedlings may experience occasional periods of negative carbon gain due to bad weather, litterfall cover, herbivory and physical disturbances. During these periods, for both seedlings and saplings, maintenance of carbohydrate reserves is important for survival in the shaded understorey where replacement of lost tissue is difficult due to limited net carbon gain (Myers & Kitajima 2007; Poorter & Kitajima 2007). Across a broad range of taxa, large seed and seedling size, storage cotyledons, tissue density and carbohydrate reserves are associated with juvenile shade tolerance. Such convergent trait association, however, may be somewhat modified by taxon-specific traits, such as chemical poisons. For example, *A. cruenta*, the second most shade tolerant among the eight species studied

here, had relatively low material strength and low carbohydrate concentration in stems and roots (Myers & Kitajima 2007). Instead, its high survival may be attributable to presence of poisonous alkaloids (obscurinervine and obscurinervidine, Harper *et al.* 1993) as well as its relatively large size. For more rigorous analysis of evolutionary and ecological associations of multiple functional traits, future studies should compare a larger number of species with statistical methods that take into account phylogenetic relationships.

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References

- Alvarez-Clare, S. (2005) *Biomechanical Properties of Tropical Tree Seedlings as a Functional Correlate of Shade Tolerance*. MSc Thesis, University of Florida, Gainesville.
- Augsburger, C.K. (1984a) Seedling survival of tropical tree species: interactions of dispersal distance, light gaps, and pathogens. *Ecology*, **65**, 1705–1712.
- Augsburger, C.K. (1984b) Light requirements of neotropical tree seedlings: a comparative study of growth and survival. *Journal of Ecology*, **72**, 777–795.
- Barnett, J.R. & Jeronimidis, G., eds. (2003) *Wood Quality and its Biological Basis*. Blackwell Publishing, Oxford, UK.
- Choong, M.F. (1996) What makes a leaf tough and how this affects the pattern of *Castanopsis fissa* leaf consumption by caterpillars. *Functional Ecology*, **10**, 668–674.
- Clark, D.B. & Clark, D.A. (1989) The role of physical damage in the seedling mortality regime of a neotropical rain forest. *Oikos*, **55**, 225–230.
- Coley, P.D. (1983) Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecological Monographs*, **53**, 209–233.
- Cooley, A.M., Reich, A. & Rundel P. (2004) Leaf support biomechanics of neotropical understorey herbs. *American Journal of Botany*, **91**, 573–581.
- Croat, T.B. (1978) *Flora of Barro Colorado Island*. Stanford University Press, Stanford, UK.
- Gartner, B.L. (1991) Structural stability and architecture of vines vs. shrubs of poison oak, *Toxicodendron diversilobum*. *Ecology*, **72**, 2005–2015.
- Garwood, N.C. (1983) Seed germination in a seasonal tropical forest in Panama: a community study. *Ecological Monographs*, **53**, 159–181.
- Gilbert, B., Wright, S.J., Kitajima, K. & Muller-Landau, H.C. (2006) Life-history trade-offs in tropical trees and lianas. *Ecology*, **87**, 1281–1288.
- Givnish, T.J. (1995) Plant stems: biomechanical adaptation for energy capture and influence on species distributions. *Plant Stems Physiology and Functional Morphology* (ed. B.L. Gartner), pp. 3–49. Academic Press, San Diego, California.

- Gómez, J.M., García, D. & Zamora, R. (2003) Impact of vertebrate acorn- and seedling-predators on a Mediterranean *Quercus pyrenaica* forest. *Forest Ecology and Management*, **180**, 125–134.
- Greenhill, G. (1881) Determination of the greatest height consistent with stability that a vertical pole or mast can be made, and the greatest height to which a tree of given proportions can grow. *Proceedures of the Cambridge Philosophical Society*, **4**, 65–73.
- Harper, J.K., Dalley, N.K., Owen, N.L., Wood, S.G. & Cates, R.G. (1993) X-ray structure and C-13 NMR assignments of indole alkaloids from *Aspidosperma cruenta*. *Journal of Crystallographic and Spectroscopic Research*, **23**, 1005–1011.
- Holbrook, N.M. & Putz, F.E. (1989) Influence of neighbors on tree form: effects of lateral shade and prevention of sway on the allometry of *Liquidambar styraciflua* (sweet gum). *American Journal of Botany*, **76**, 1740–1749.
- Humphreys, J.M. & Chapple, C. (2002) Rewriting the lignin roadmap. *Current Opinion in Plant Biology*, **5**, 224–229.
- King, D.A. (1994) Influence of light level on the growth and morphology of saplings in a Panamanian forest. *American Journal of Botany*, **81**, 948–957.
- Kitajima, K. (1994) Relative importance of photosynthetic traits and allocation patterns as correlates of seedling shade tolerance of 13 tropical trees. *Oecologia*, **98**, 419–428.
- Kitajima, K. & Augspurger, C.K. (1989) Seed and seedling ecology of a monocarpic tropical tree, *Tachigalia-versicolor*. *Ecology*, **70**, 1102–1114.
- Leigh, E.G., Jr., Windsor, D.M. & Rand, S.A., eds. (1982) *The Ecology of a Tropical Forest: Seasonal Rhythms and Long-term Changes*. Smithsonian Institution Press, Washington D.C.
- Lucas, P.W. & Pereira, B. (1990) Estimation of the fracture toughness of leaves. *Functional Ecology*, **4**, 819–822.
- Lucas, P.W., Beta, T., Darvell, B.W., Dominy, N.J., Essackjee, H.C., Lee, P.K.D., Osorio, D., Ramsden, L., Yamashita, N. & Yuen, T.D.B. (2001) Field kit to characterize physical, chemical and spatial aspects of potential primate foods. *Folia Primatologica*, **72**, 11–25.
- Lucas, P.W., Turner, I.M., Dominy, N.J. & Yamashita, N. (2000) Mechanical defences to herbivory. *Annals of Botany*, **86**, 913–920.
- Moles, A.T. & Westoby, M. (2004) What do seedlings die from and what are the implications for evolution of seed size? *Oikos*, **106**, 193–199.
- Molofsky, J. & Augspurger, C.K. (1992) The effect of leaf litter on early seedling establishment in a tropical forest. *Ecology*, **73**, 68–77.
- Muller-Landau, H. (2004) Interspecific and intraspecific variation in wood specific gravity of tropical trees. *Biotropica*, **36**, 26–32.
- Myers, J.A. & Kitajima, K. (2007) Carbohydrate storage enhances seedling shade and stress tolerance in a neotropical forest. *Journal of Ecology*, **95**, 383–395.
- Niklas, K.J. (1992) *Plant Biomechanics. An Engineering Approach to Plant Form and Function*. University of Chicago Press, Chicago.
- Niklas, K.J. (1995) Plant height and the properties of some herbaceous stems. *Annals of Botany*, **75**, 133–142.
- Niklas, K.J. (1998) The influence of gravity and wind on land plant evolution. *Review of Palaeobotany and Palynology*, **102**, 1–14.
- Niklas, K.J., Molina-Freaner, F., Tinoco-Ojanguren, C. & Paolillo, D.J. (2000) Wood biomechanics and anatomy of *Pachycereus pringlei*. *American Journal of Botany*, **87**, 469–481.
- Poorter, L. & Bongers, F. (2006) Leaf traits are good predictors of plant performance across 53 rain forest species. *Ecology*, **87**, 1733–1743.
- Poorter, L. & Rose, S. (2005) Light-dependent changes in the relationship between seed mass and seedling traits: a meta-analysis for rain forest tree species. *Oecologia*, **142**, 378–387.
- Poorter, L. & Kitajima, K. (2007) Carbohydrate storage and light requirements of tropical moist and dry forest species. *Ecology*, **88**, 1000–1011.
- Ryan M.G., Melillo, J.M. & Ricca, A. (1989) A comparison of methods for determining proximate carbon fractions of forest litter. *Canadian Journal of Forest Research*, **20**, 166–171.
- Turner, I.M. (1990) Tree seedling growth and survival in a Malaysian rain forest. *Biotropica*, **22**, 146–154.
- van Gelder, H.A., Poorter, L. & Sterck, F.J. (2006) Wood mechanics, allometry, and life-history variation in a tropical rain forest tree community. *New Phytologist*, **171**, 367–378.
- Wright, I.J. & Cannon, K. (2001) Relationships between leaf lifespan and structural defenses in a low-nutrient, sclerophyll flora. *Functional Ecology*, **15**, 351–359.
- Wright, I.J., Reich, P.B., Westoby, M. *et al.* (2004) The worldwide leaf economics spectrum. *Nature*, **428**, 821–827.
- Wright, S.J., Muller-Landau, H.C., Condit, R. & Hubbell, S.P. (2003) Gap-dependent recruitment, realized vital rates, and size distributions of tropical trees. *Ecology*, **84**, 3174–3185.

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