

Carbohydrate storage enhances seedling shade and stress tolerance in a neotropical forest

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Summary

1 To survive in forest understoreys, seedlings must depend on carbohydrate reserves when they experience negative carbon balance imposed by occasional light reduction and tissue loss to herbivores and diseases. We present the first experimental evidence in support of this hypothesis, using seven woody neotropical species.

2 We transplanted seedlings that had recently expanded their first photosynthetic cotyledon or leaf to the forest understorey (1% of full sun) and quantified initial biomass and total non-structural carbohydrate (TNC) in stems, roots and storage cotyledons. We then randomly assigned seedlings to control and two stress treatments: light reduction (0.08% of full sun for 8 weeks) and complete defoliation.

3 First-year survival of control seedlings, a comparative measure of shade tolerance, differed widely among species. The two stress treatments reduced survival and relative growth rates (RGR) of all species. Shade-tolerant species were little impacted by the stress treatments, whereas the two least shade-tolerant species experienced 100% mortality.

4 In all treatments, 8-week and first-year survival was positively correlated with initial TNC pool size in stems and roots. By contrast, survival was generally not correlated with initial TNC concentration in any organ, TNC pools in cotyledons, seed mass or seedling biomass.

5 TNC in stems and roots, but not in cotyledons, decreased in response to light reduction and defoliation over 8 weeks. Leaf area recovery of defoliated seedlings was positively correlated with initial TNC pools in stems and roots.

6 First-year survival in each treatment was negatively correlated with 0–8 week RGR of control seedlings, suggesting higher stress tolerance of species with inherently slow growth rates in shade. RGR of control seedlings from 0 to 8 weeks was negatively correlated with initial TNC pools, but not concentrations, in stems and roots. After 8 weeks, RGR was positive for all species, without clear relationships with survival or TNC.

7 We conclude that carbohydrate storage in stems and roots enhances long-term survival in shade by enabling seedlings to cope with periods of biotic and abiotic stress. Carbohydrate storage is a key functional trait that can explain species differences in growth and survival that lead to species coexistence through niche assembly processes and life-history trade-offs.

Key-words: carbohydrate reserve, carbon balance, growth–survival trade-off, herbivory, life-history strategy, relative growth rate, seed size, seedling survival, shade tolerance, tropical forest

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Introduction

Shade tolerance has long been considered a key functional trait influencing forest composition, structure and dynamics (Warming 1909; Shirley 1943; Baker 1949; Pacala *et al.* 1996; Wright 2002; Wright *et al.* 2003). Juvenile shade tolerance, defined as the ability of

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seedlings and saplings to persist in shaded forest understoreys, varies widely and continuously among coexisting plant species (Augsburger 1984a,b; Kobe *et al.* 1995). In particular, first-year seedlings of many species suffer high density-dependent mortality from disease in the shaded understorey (Augsburger 1984a; Packer & Clay 2000), which may decouple the spatial distribution of seedlings from patterns established during seed dispersal (Augsburger & Kitajima 1992). Shade tolerance determines not only whether a species can establish in a given light environment (Poorter & Arets 2003), but also its life-history strategy and position along the growth–survival trade-off continuum (Hubbell & Foster 1992; Kobe *et al.* 1995; Wright *et al.* 2003; Gilbert *et al.* 2006). Thus, shade tolerance is central among functional traits that contribute to niche assembly and species coexistence.

To persist in the shaded understorey, seedlings must maintain positive net carbon balance, but not necessarily maximize rates of net carbon gain. The realized rate of net carbon gain is the potential rate of net carbon gain minus the rate of tissue loss to herbivores, disease and physical disturbance (Kitajima 1996). In addition, light availability in the understorey is so close to the whole plant light compensation point that even small changes may have large consequences on seedling carbon balance (Montgomery & Chazdon 2002). Although seedlings of many species can acclimate to changing light regimes through adjustment of photosynthesis and respiration (e.g. Walters & Reich 2000), they undoubtedly experience negative carbon balance caused by periods of continuously cloudy weather, canopy dynamics of larger plants and overtopping by litterfall. Hence, no matter how efficiently light energy is converted to biomass, and no matter how well seedlings are defended against herbivory and disease, seedlings in the shaded understorey are likely to experience negative carbon balance imposed by a wide range of biotic and abiotic stresses (e.g. Augspurger 1984a,b; Clark & Clark 1989; Kitajima & Augspurger 1989; Augspurger & Kitajima 1992; Engelbrecht & Kursar 2003; Alvarez-Clare 2005). Thus, we hypothesize that seedlings must rely on stored carbohydrate reserves to meet their energy demands during periods of negative carbon balance.

Carbohydrate storage in seeds, stems or roots can facilitate seedling survival during negative carbon balance, but the importance of each of these carbohydrate sources may shift during ontogeny. During germination and initial development, seedlings rely almost completely on energy stored in seeds, and exhibit negative relative growth rates (RGR, a measure of net carbon gain; Kitajima 2002). Gradually, following development of the first photosynthetic cotyledons or leaves, seedlings achieve positive RGR (Kitajima 2002). Large-seeded species may set aside greater amounts of initial seed reserves in storage (Saverimuttu & Westoby 1996; Leishman *et al.* 2000; Green & Juniper 2004). However, it is not clear whether large storage cotyledons that remain attached to the stem in some species

continue to support seedling energy demands. Transfer of lipids from seeds is negligible, and transfer of carbohydrates from storage cotyledons may be limited to the first few weeks after germination (Ichie *et al.* 2001; Kennedy *et al.* 2004). Energy storage in stems and roots exists mostly as non-structural carbohydrates (Chapin *et al.* 1990), typically measured as the sum of starch and simple sugars, or ‘total non-structural carbohydrate’ (TNC). Unfortunately, little is known about TNC storage in stems, roots and cotyledons of first-year seedlings, nor their relative roles in determining shade and stress tolerance differences among coexisting species.

In this study, we tested the hypothesis that carbohydrate storage enhances survival of first-year seedlings in shade and their tolerance to stresses imposed by tissue loss and periods of very low light. We examined carbohydrate storage, survival and stress tolerance among seven woody species that differ in shade tolerance in terms of first-year seedling survival. We experimentally forced seedlings into negative carbon balance by either reducing light levels below the light compensation point or removing all leaves and photosynthetic cotyledons. We predicted that species with greater TNC storage would exhibit higher survival in shade and greater tolerance of stress imposed by light reduction and defoliation. Thus, the overarching hypothesis of this study was that carbohydrate storage is one of the functional traits underlying growth–survival trade-offs that promote plant species coexistence.

Materials and methods

STUDY SITE AND SPECIES SELECTION

The study was conducted in a secondary tropical moist forest on the Buena Vista Peninsula, Barro Colorado Nature Monument, Republic of Panama (9° 10′ N, 79° 51′ W). The site has a mean annual rainfall of *c.* 2700 mm and a pronounced 4-month dry season that usually begins in December (Croat 1978). We chose seven species that spanned a wide range of seedling shade tolerance in terms of first-year seedling survival in the shaded understorey (Table 1). The seven species range from highly shade-tolerant (*Aspidosperma cruenta*) to small-gap-dependent (*Tabebuia rosea*); none of the species is considered to be a pioneer. All species are canopy trees except for *Callichlamys latifolia*, a canopy liana that remains free standing for at least 3 years, even when growing rapidly in treefall gaps (K. Kitajima, personal observation). The negative regression lines between growth rates and survival of first-year seedlings are similar for lianas and canopy trees in this forest, with both groups spanning a comparable range of shade tolerance (Gilbert *et al.* 2006).

The study species also vary in seed mass and cotyledon functional morphology (Table 1). *Aspidosperma cruenta*, *Lacmellea panamensis*, *Castilla elastica* and *Platyopodium elegans* are relatively large-seeded species (244–453 mg dry mass) with hypogeal cotyledons that remain

Table 1 Study species listed in order of decreasing shade tolerance in terms of their first-year survival of control seedlings in the shaded understorey (mean \pm 1 SE; $n = 46\text{--}111$ per species). Also listed are four-letter codes to indicate species in the figures, seed mass (= dry seed mass after removal of seed coats, $n = 6\text{--}64$ per species) and cotyledon types (SH = storage, hypogeal; SE = storage, epigeal; PE = photosynthetic, epigeal). Taxonomic nomenclature follows Croat (1978)

Species	Family	Code	First-year survival (%)	Seed mass (mg)	Cotyledon type
<i>Aspidosperma cruenta</i> Woods.	Apocynaceae	ASPC	96.6 \pm 1.9	453.9 \pm 32.2	SH
<i>Lacmellea panamensis</i> (Woods.) Markg.	Apocynaceae	LACP	91.8 \pm 3.5	237.4 \pm 10.6	SH
<i>Coussareia curvigemmia</i> Dwyer	Rubiaceae	COUC	89.1 \pm 3.4	094.4 \pm 3.6	PE
<i>Callichlamys latifolia</i> (L.C. Rich.) K. Schum	Bignoniaceae	CALL	79.9 \pm 6.1	187.2 \pm 7.2	SE
<i>Castilla elastica</i> Sessé	Moraceae	CASE	57.6 \pm 5.6	203.4 \pm 27.6	SH
<i>Platyopodium elegans</i> J. Vogel	Fabaceae	PLAE	37.9 \pm 8.7	332.3 \pm 10.3	SH
<i>Tabebuia rosea</i> (Bertol.) DC.	Bignoniaceae	TABR	19.3 \pm 5.9	024.2 \pm 1.6	PE

at or below the soil surface and that function as storage organs. The three other species have smaller seeds and green epigeal cotyledons that are elevated above the ground; *Coussareia curvigemmia* and *Tabebuia rosea* have foliaceous photosynthetic cotyledons and *Callichlamys latifolia* has thick cotyledons (2 mm) that function primarily for storage (Kitajima 2002). We intentionally included species with photosynthetic cotyledons as well as those with storage cotyledons at both the tolerant and the intolerant ends of the shade-tolerance spectrum, so that we could evaluate the mechanistic importance of storage cotyledons for survival in shade. For brevity, we henceforth refer to species by their generic names.

We collected recently matured seeds of all species during the late dry season (April) and early wet season (July) of 2003. We planted seeds in plastic trays (5 cm deep) filled with a 1 : 1 mixture of forest soil and washed beach sand. The trays were kept moist in a shade house under light levels similar to those found in the forest understorey (~1% of full sun) until seedlings were transplanted into common gardens located in the field.

COMMON-GARDEN FIELD EXPERIMENT

We established four 7 \times 7 m common gardens in the shaded understorey of the secondary forest on Buena Vista Peninsula. All gardens were separated by at least 50 m and enclosed by 1-m-high fencing to exclude terrestrial mammals. Shortly after individual seedlings of each species had fully expanded their first photosynthetic organ (cotyledons or leaves; Table 1), we transplanted equal numbers of seedlings to random positions in each garden ($n = 36\text{--}95$ total seedlings per species per garden). At this stage, all seedlings should have initiated significant photosynthetic carbon gain (Kitajima 2002). As a result of differences in rates of development, the age of seedlings at leaf or cotyledon expansion ranged from 1 to 16 weeks among the seven species. We trans-

planted all seedlings during the early wet season (May–July). Total initial sample sizes ranged from 144 to 380 seedlings per species, depending on initial seed availability.

Two weeks after transplantation, we randomly assigned seedlings to either control or one of two stress treatments: light reduction (0.08% of full sun) and complete defoliation. The goal of the stress treatments was to force seedlings into negative net carbon balance. For the defoliation treatment, we removed all leaves and foliaceous photosynthetic cotyledons (for *Coussareia* and *Tabebuia*) by clipping at the base of the petiole; storage cotyledons of hypogeal species were not removed. Each seedling assigned to the light-reduction treatment was covered with a cylindrical wire cage wrapped in 90% shade cloth, leaving the bottom 5 cm open for aeration. Shade cages were large enough to provide sufficient room for growth over the duration of the 8-week treatment (15–30 cm in diameter, 20–40 cm in height, depending on seedling size).

We used Li-Cor quantum sensors to measure light levels inside and outside 8–15 shade cages per garden. Light levels outside the shade cages ranged from 0.53% to 1.06% of the light above the forest canopy (overall mean = 0.77% for instantaneous measurements); similar values were obtained from continuous measurements of integrated percentage daily photon flux density (PFD) recorded over 2 days in the centre of each garden. Light reduction by the shade cages was $89 \pm 2\%$ (mean \pm 1 SD). Seedlings inside the shade cages thus experienced only 0.08% of the light above the forest canopy during the 8-week light-reduction treatment.

We recorded seedling survival every week for the first 8 weeks and then every 2 weeks until the end of 1 year. The few seedlings killed by branch falls and herbivory (by subterranean insects for one species in a single garden) were excluded from the study.

We harvested seedlings from all common gardens at three stages: (i) just prior to the application of the

treatments (initial harvest), (ii) 8 weeks after the treatments were initiated (8-week harvest) and (iii) 1 year after the treatments were initiated (1-year harvest). Because *Lacmellea* was erroneously transplanted and harvested before full leaf expansion, we used initial biomass and TNC data from another study located in the same general area (K. Kitajima, unpublished data; $n = 9$ seedlings), but chose not to calculate 0–8 week RGR for this species. In all other species, initial sample sizes ranged from 24 to 48 seedlings. Sample sizes at the 8-week and 1-year harvests ranged from 2 to 41 seedlings per species–treatment combination; lower sample sizes were due to poor survival of some species. We carefully excavated each seedling to retrieve the entire root system, transported all seedlings to the laboratory in polyethylene bags inside an ice-filled cooler and separated them into roots, stems, leaves and cotyledons. We measured leaf and photosynthetic cotyledon area with a leaf area meter. All samples were dried at 100 °C for 1 h (to stop respiration) and then at 60 °C for at least 48 h, after which the dry mass of each sample was recorded.

TNC ANALYSIS

We quantified TNC concentrations (mg TNC per mg dry biomass) and total pool sizes (TNC concentration \times biomass) in stems, roots and storage cotyledons at the initial and 8-week harvests. TNC was not determined at 1 year because of low sample sizes. We did not measure TNC in leaves and photosynthetic cotyledons, because TNC levels in leaves fluctuate daily in response to photosynthetic activity and export of starch out of the leaves (Graham *et al.* 2003). To obtain the minimum of 10 mg of ground sample necessary for TNC analysis, we pooled samples from 2–6 individuals from each common garden. Consequently, sample sizes ranged from four to eight pooled samples per species at the initial harvest (except for *Lacmellea*), and from one to eight at the 8-week harvest. The pooled samples were ground with a ball mill and a subsample of 10–16 mg was used for TNC extraction.

Following the method of Marquis *et al.* (1997), we extracted soluble sugars with 80% ethanol in a shaking water bath at 27 °C, followed by two additional 2-h extractions the next day. Starch in the remaining sample was digested to glucose with amyloglucosidase (Sigma A-7255). We measured concentrations of simple sugars and starch using a phenol/sulphuric acid colorimetric assay (Dubois *et al.* 1956; Ashwell 1966). TNC pool size (mg glucose) for a given organ type was calculated as the product of TNC concentration and individual organ biomass.

DATA ANALYSIS

To distinguish the effects of large TNC reserves from the advantages of large seedling size *per se*, we quantified initial and 8-week seedling sizes in terms of both total

biomass and structural biomass. Structural biomass was calculated by subtracting TNC pool sizes in stems, roots and cotyledons from total seedling biomass. Similarly, we calculated short-term RGR of total (RGR_{tot}) and structural (RGR_{str}) biomass. RGR_{str} measures allocation to structural biomass independent of allocation to storage. We calculated RGR using the following formula:

$$RGR \text{ (mg mg}^{-1} \text{ week}^{-1}) = [\ln(\text{biomass at 8 weeks}) - \ln(\text{mean initial biomass})]/8 \text{ weeks.}$$

We also calculated longer-term RGR_{tot} from 8 weeks to 1 year; longer-term RGR_{str} could not be calculated because TNC was not measured at 1 year. Cotyledon biomass (if present) was included in all biomass and RGR calculations.

We tested for differences among species and treatments in TNC concentration and pool size, biomass, RGR and leaf area using ANOVA, including common garden as a random factor. When necessary, we applied logarithmic transformation (for biomass and TNC pool size) and arcsine square root transformation (for proportional data including survival and TNC concentration) to achieve normality and homogeneity of variance. We used the Kaplan–Meier method to estimate first-year survivorship curves for each species by treatment combination, and a proportional hazards model to test for effects of species, treatment, and species by treatment interactions on seedling survival times (see Fox 2001). Seedlings harvested for biomass measurements and those that survived beyond the final census were considered ‘right-censored’. We performed all statistical analyses using JMP version 4.0.4 (SAS Institute, Cary, NC, USA).

Results

SEEDLING SURVIVAL

Species differed widely in first-year survival of control seedlings (19–97%) and in their sensitivity to the stress treatments (Fig. 1). Hereafter, we use first-year survival of control seedlings as a comparative measure of seedling shade tolerance (Table 1). The light-reduction and defoliation treatments reduced survival of all species, but more shade-tolerant species were less impacted by the two stress treatments (Fig. 1). The proportional hazards model indicated significant effects ($P < 0.0001$) of species ($\chi^2 = 806.9$, d.f. = 6), treatment ($\chi^2 = 389.3$, d.f. = 2) and species by treatment interactions ($\chi^2 = 73.2$, d.f. = 12) on seedling survival times. During the first 8 weeks, the four least shade-tolerant species experienced substantial mortality in both stress treatments. The three most shade-tolerant species, by contrast, exhibited only minor reductions in survival from 0 to 8 weeks. For most species, the negative effect of light reduction on survival persisted beyond 8 weeks, the time at which the shade cages were removed. In some

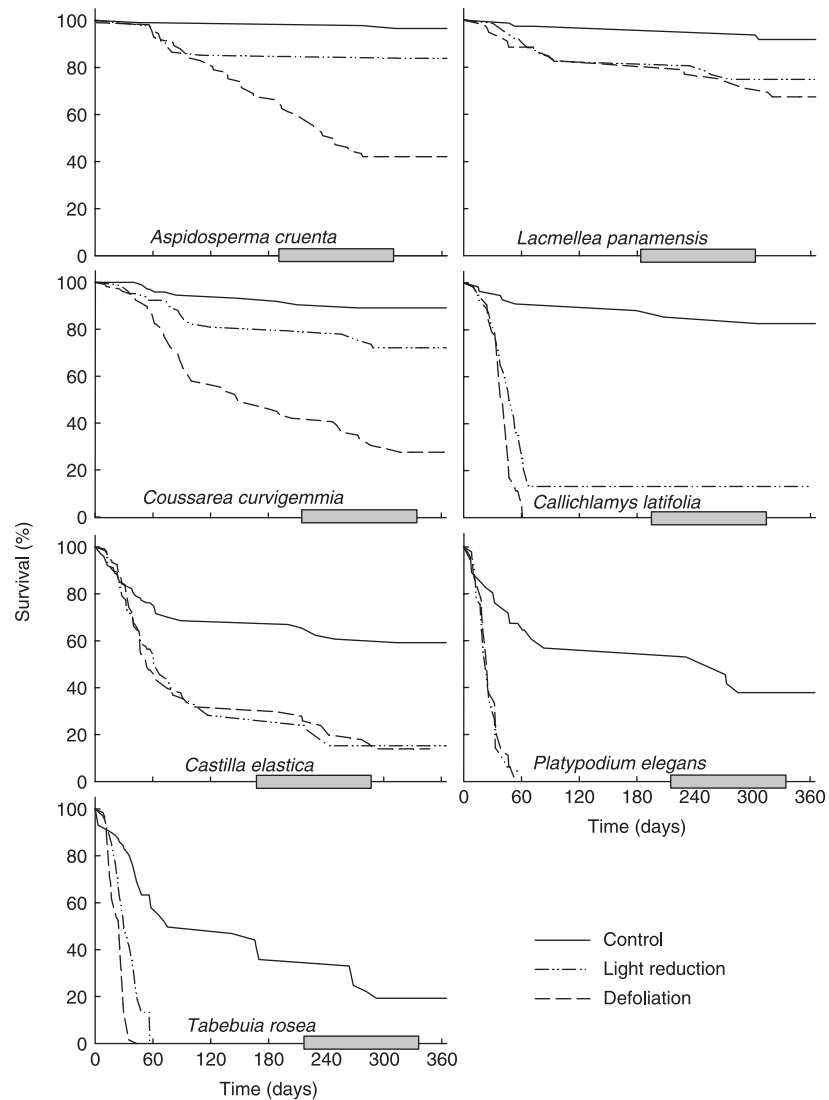


Fig. 1 Seedling survivorship curves in the control, light-reduction and defoliation treatments. Species are shown in the order of first-year survival (%) of control seedlings (Table 1). Time zero represents the start of the treatments; treatments were initiated 2 weeks after the full expansion of the first set of photosynthetic organs (see Methods for details). Shaded bars show the approximate timing of the dry season, which differs among panels because of interspecific differences in the timing of seed dispersal, germination and first leaf expansion.

species, there were slight increases in mortality during the dry season (designated by the grey bars in Fig. 1). First-year survival percentages were strongly and positively correlated between any two treatments ($n = 7$ species; $P = 0.002$, $r = 0.93$ for control vs. light reduction; $P = 0.04$, $r = 0.76$ for control vs. defoliation; $P = 0.005$, $r = 0.90$ for light reduction vs. defoliation). Thus, species that survived well as control seedlings in the shaded understorey also survived well when they experienced the additional stress of heavy shading and defoliation.

TNC CONCENTRATION AND POOL SIZE

Both initial TNC concentrations and initial TNC pool sizes in stems, roots and cotyledons differed significantly among species (Fig. 2). Stem TNC concentration (range = 6–25%) was positively correlated with root TNC concentration (4–21%) among species ($P < 0.02$,

$r = 0.84$, $n = 7$), but root concentrations were lower than stem concentrations in all species (paired t -tests, $P \leq 0.01$). Cotyledon TNC concentration, by contrast, was not significantly correlated with either root or stem TNC concentration. Initial TNC concentrations showed no apparent relationship with shade tolerance differences among species (Fig. 2a–c), whereas initial TNC pool sizes in stems and roots were conspicuously high for the two most shade-tolerant species (Fig. 2d,e). Stem TNC pool size was positively correlated with root TNC pool size ($P = 0.0002$, $r = 0.98$). Thus, the results of statistical correlations involving TNC pool size were approximately identical for stems and roots. Hereafter, we only report the results using TNC pools in stems and roots combined (= root TNC pool plus stem TNC pool per individual).

Initial TNC pool size in stems and roots was positively correlated with initial total seedling biomass ($P < 0.02$,

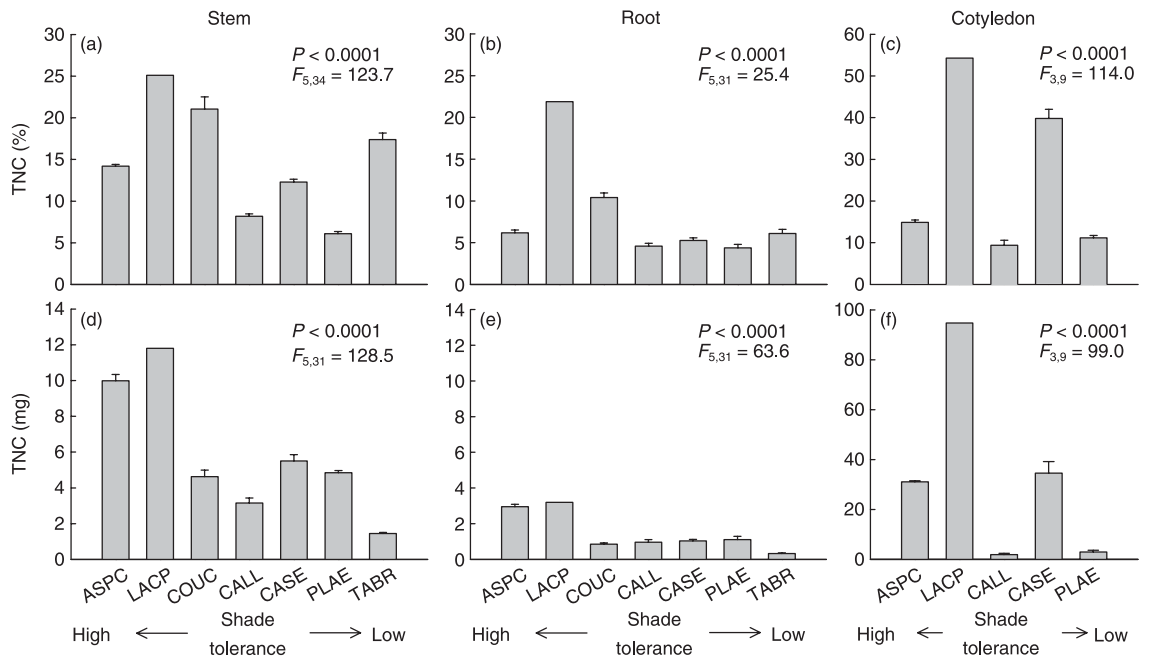


Fig. 2 Initial total non-structural carbohydrate (TNC) reserves in stems, roots and cotyledons for seven woody neotropical forest species. Species are arranged in order of their shade tolerance (see Table 1 for species codes). (a–c) TNC concentration (percentage of organ biomass) and (d–e) TNC pool size (TNC concentration \times organ biomass). Cotyledon TNC was quantified only for species with storage cotyledons. The result of a one-way ANOVA to test for differences among six species (excluding LACP, see Methods) is shown in each panel. Note the differences in the scales of the vertical axes. Error bars represent 1 SEM.

Table 2 Effects of species, treatment and common garden on total non-structural carbohydrate (TNC) concentration and pool size in stems, roots and cotyledons 8 weeks after the light-reduction and defoliation treatments (ANOVA F and P , NS for $P > 0.10$). Species by treatment interactions did not have a significant effect on any variable except stem TNC pool size ($F = 2.7$, $P = 0.01$)

Variable	Stem			Root			Cotyledon		
	d.f.	F	P	d.f.	F	P	d.f.	F	P
TNC concentration (%)									
Species	6	68.3	< 0.0001	6	75.3	< 0.0001	2	0.9	NS
Treatment	2	16.4	< 0.0001	2	15.0	< 0.0001	2	3.7	0.04
Garden	3	3.3	0.02	3	5.7	0.001	3	0.2	NS
Error	97			96			20		
TNC pool size (mg)									
Species	6	54.1	< 0.0001	6	93.7	< 0.0001	2	5.1	0.01
Treatment	2	19.9	< 0.0001	2	38.5	< 0.0001	2	1.6	NS
Garden	4	2.4	0.06	4	9.7	< 0.0001	3	0.6	NS
Error	97			96			20		

$r = 0.85$), but not with seed mass ($P > 0.10$), even though seed and seedling mass were positively correlated ($P < 0.004$, $r = 0.92$). TNC pool size in storage cotyledons, by contrast, was not correlated with seed mass or initial seedling biomass ($P > 0.45$, $n = 5$). There was also no relationship between TNC concentrations in stems, roots or cotyledons and total seedling biomass ($P > 0.14$ for all correlations). Thus, TNC concentrations and pool sizes could not be predicted from seed size, even though TNC pool size in stems and roots was correlated with seedling biomass.

Eight-week TNC concentrations and pool sizes in stems and roots were significantly lower in the light-reduction and defoliation treatments relative to the

control (Table 2; see Table S1 in Supplementary Material for species means and standard errors). Yet, 8-week TNC concentrations and pool sizes in stems and roots were strongly and positively correlated between any two treatments, as well as with the initial values ($P = 0.0002$ – 0.02 ; $r = 0.86$ – 0.98 for all correlations). Among the five species with storage cotyledons, three species retained their cotyledons over 8 weeks (means = 0.53, 0.67, 0.15 cotyledons per individual for *Aspidosperma*, *Lacmellea* and *Castilla*, respectively), while all individuals in the other two species (*Callichlamys* and *Platypodium*) had abscised cotyledons by 8 weeks. Cotyledon TNC concentrations were significantly higher in the stress treatments than in the control (Tables 2 & S1), a pattern

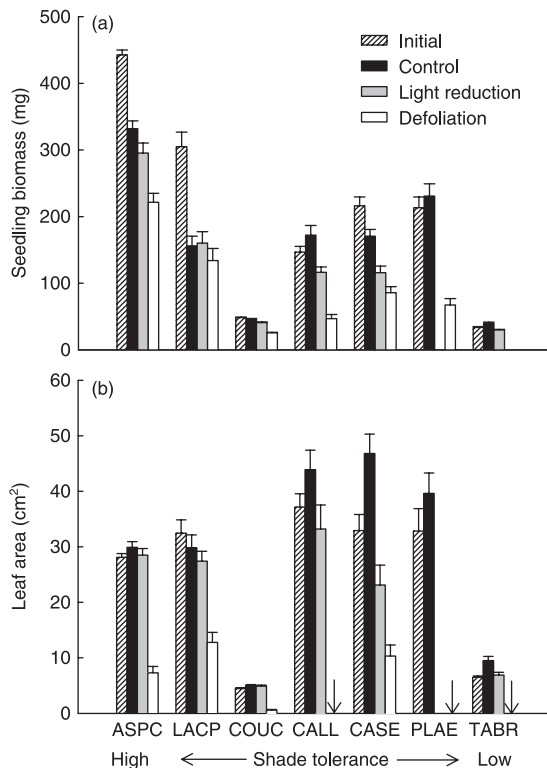


Fig. 3 Total seedling (a) biomass and (b) leaf area at the initial and 8-week seedling harvests. Arrows in (b) indicate complete lack of new leaf growth after defoliation; otherwise, a missing bar indicates that no seedlings survived the treatment. At 8 weeks, species, treatment and species–treatment interactions had significant effects on both variables: (a) $P < 0.0001$, $F_{6,400} = 269.0$ for species, $F_{2,400} = 72.9$ for treatment; $P = 0.0003$, $F_{8,355}$ for interaction; (b) $P < 0.0001$, $F_{6,365} = 126.8$ for species, $F_{2,365} = 165.1$ for treatment, $F_{8,355} = 9.2$ for interaction. Error bars represent 1 SEM. See Table 1 for species codes.

that runs counter to the hypothesis that seedlings utilize cotyledon TNC under stress. Furthermore, cotyledon TNC pool sizes showed no response to the stress treatments. Thus, seedlings apparently relied on TNC stored in stems and roots, but not in cotyledons, to survive the stress treatments.

SEEDLING BIOMASS, LEAF AREA AND GROWTH

Light reduction and defoliation decreased total seedling biomass and leaf area relative to the initial values, as well as relative to the control at 8 weeks, in most species (Fig. 3). At 8 weeks, shade-tolerant species showed little difference in biomass and leaf area between the control and light-reduction treatments. Large biomass differences between 0 and 8 weeks for the two most shade-tolerant species reflected decreases in the total mass of storage cotyledons (Fig. 3a). The three most shade-tolerant species also showed minimal leaf area growth over 8 weeks in the control treatment, but they exhibited significant growth of new leaves in response to the defoliation treatment (Fig. 3b). By contrast, the four less shade-tolerant species showed substantial leaf

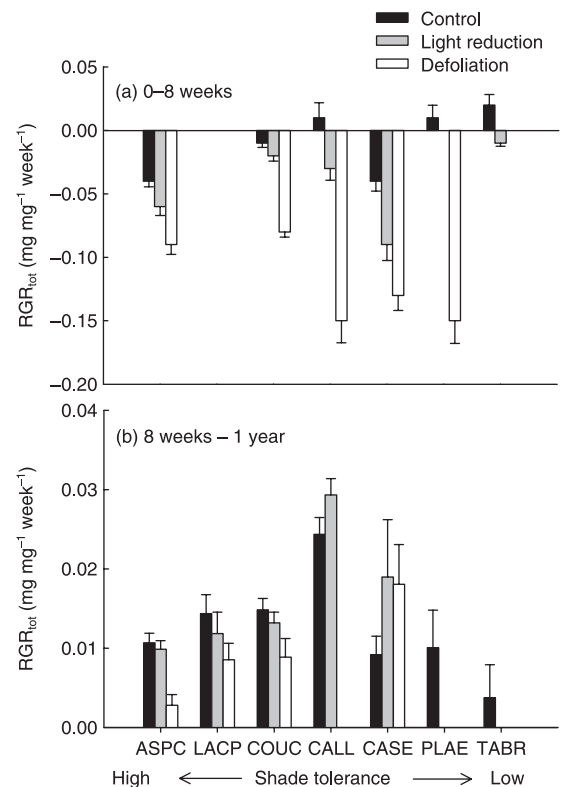


Fig. 4 Seedling growth rates in the control, light-reduction and defoliation treatments. (a) Short-term relative growth rate of total biomass (RGR_{tot}) from 0 to 8 weeks. (b) Longer-term RGR_{tot} from 8 weeks to 1 year. RGR_{tot} from 0 to 8 weeks was not calculated for LACP (see Methods); otherwise, a missing bar indicates that no seedlings survived the treatment. Species, treatment and species–treatment interactions had significant effects on RGR_{tot} in both time intervals: (a) $P < 0.0001$, $F_{5,344} = 17.6$ for species; $F_{2,344} = 100.6$, $P < 0.0003$, $F_{6,291} = 4.3$ for interaction; (b) $P < 0.0001$, $F_{4,338} = 13.9$ for species; $F_{2,338} = 11.7$ for treatment; $P = 0.005$, $F_{6,311} = 3.1$ for interaction. Error bars represent 1 SEM. See Table 1 for species codes.

area growth in the control treatment, but none or little after defoliation (Fig. 3b). Of these species, the three that did not produce new leaves experienced 100% mortality in the defoliation treatment (Fig. 1).

Light reduction and defoliation decreased relative growth rate of total biomass (RGR_{tot}) over the short term (Fig. 4a). All seedlings in the stress treatments, as well as control seedlings of three species, experienced negative 0–8 week RGR_{tot} (Fig. 4a). The very negative RGR_{tot} of defoliated seedlings reflected the removal of leaves and cotyledons. Shade-tolerant species experienced small changes in 0–8 week RGR_{tot} between the control and stress treatments (Fig. 4a). Relative growth rate of structural biomass (RGR_{str}) showed similar patterns as 0–8 week RGR_{tot} (data not shown).

From 8 weeks to 1 year, surviving seedlings of all species showed positive RGR_{tot} in all treatments (Fig. 4b). In most species, RGR_{tot} did not differ significantly between the control and stress treatments (Tukey's HSD test, $P > 0.05$), suggesting that most seedlings had recovered from the stress treatments by 1 year. The only

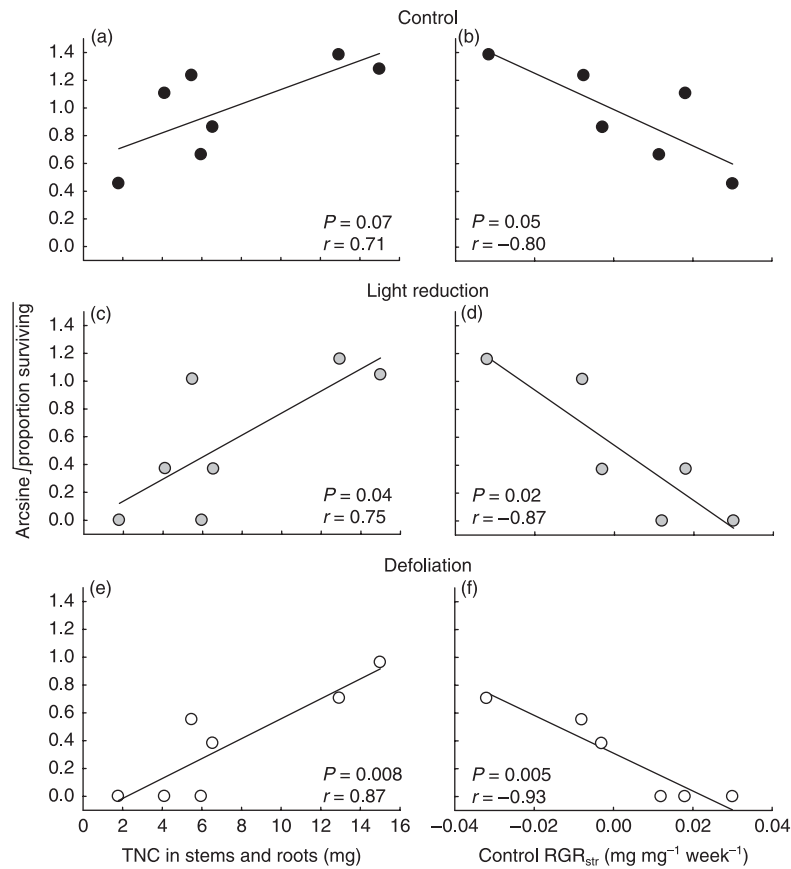


Fig. 5 First-year seedling survival in each treatment plotted against (a, c, e) initial carbohydrate pool size in stems and roots, and (b, d, e) 0–8 week relative growth rate of structural biomass (RGR_{str}) in the control treatment. Each point represents a species mean. Survival proportions were arcsine-square root transformed to improve normality in the residuals.

exception was the most shade-tolerant species (*Aspidosperma*), which had significantly lower RGR_{tot} in the defoliation relative to the control treatment ($P < 0.05$; Fig. 4b).

RELATIONSHIPS BETWEEN SEEDLING SURVIVAL AND FUNCTIONAL TRAITS

In all treatments, first-year seedling survival was positively correlated with initial TNC pool size in stems and roots (Fig. 5a,c,e; Table 3). The same pattern held for survival from 0 to 8 weeks (Table 3). The relative impact of the stress treatments on each species, measured as the ratio of first-year survival of stressed to control seedlings, was also positively correlated with initial TNC pool size in stems and roots in both the defoliation ($P = 0.005$, $r = 0.90$) and the light-reduction ($P = 0.04$, $r = 0.75$) treatments. By contrast, survival was generally not correlated with either TNC concentrations in any organ or TNC pool size in cotyledons (Table 3). The only exception was in the defoliation treatment, in which first-year survival was positively correlated with initial TNC pool size in cotyledons ($P = 0.03$), as well as TNC concentrations in stems ($P = 0.06$) and roots ($P = 0.03$) (Table 3). As in the control and light-reduction treatments, however, the TNC variable most strongly

correlated with survival in the defoliation treatment was TNC pool size in stems and roots ($P = 0.008$) (Fig. 5e; Table 3).

In all treatments, first-year seedling survival was negatively correlated with 0–8 week RGR_{str} of control seedlings (Fig. 5b,d,f). The relationship was less strong, but also negative, with RGR_{tot} of control seedlings ($r = -0.63$ – 0.88 , data not shown). Both 0–8 week RGR_{str} and RGR_{tot} of control seedlings, in turn, were negatively correlated with initial TNC pool size in stems and roots (Fig. 6a for RGR_{str} ; $P = 0.03$, $r = -0.83$ for RGR_{tot}). By contrast, 0–1 year RGR_{tot} was not significantly correlated with initial TNC pool size in stems and roots ($P > 0.3$, $r = -0.42$). In addition, RGR_{tot} and RGR_{str} were uncorrelated with TNC concentration in any organ ($P > 0.5$ for all correlations). Thus, survival was positively correlated with initial TNC pool size, and both survival and initial TNC pool size were negatively correlated with inherent RGR in shade over the short term (as indicated by control seedling RGR).

Survival and RGR were not significantly correlated with seed mass, initial seedling biomass, structural seedling biomass or initial leaf area in any treatment (see Table 3 for survival; $P > 0.10$ for all correlations). Total leaf area at 8 weeks, however, was positively correlated with survival of defoliated seedlings from

Table 3 Pearson correlation coefficients for relationships between seedling traits and survival in the control (C), light-reduction (LR) and defoliation (D) treatments over three time periods. Listed are correlations between survival and TNC concentration, TNC pool size, biomass and leaf area at the beginning of each period (e.g. initial TNC concentration vs. 0–8 week survival; 8 week TNC concentration vs. 8 week – 1 year survival), as well as within-treatment correlations between survival and RGR during each period. Only the five species with storage cotyledons were used for correlations with cotyledon TNC. Empty cells indicate that correlations were not examined because of low sample size ($n < 5$ species). NS = $P \geq 0.10$

Seedling trait	Survival (%)								
	0–8 weeks			0–1 year			8 weeks – 1 year		
	C	LR	D	C	LR	D	C	LR	D
TNC concentration (%)									
Stem	NS	0.67*	NS	NS	NS	0.72**	NS	NS	NS
Root	NS	NS	NS	NS	NS	0.78**	NS	NS	NS
Cotyledon	NS	NS	NS	NS	NS	NS	NS	NS	NS
TNC pool size (mg)									
Stem and root	0.68*	0.66*	0.78**	0.71*	0.75**	0.87***	0.72*	0.76*	NS
Cotyledon	NS	NS	NS	NS	NS	0.90***	NS	NS	NS
Biomass (mg)									
Seed	NS	NS	NS	NS	NS	NS	NS	NS	NS
Total	NS	NS	NS	NS	NS	NS	NS	NS	NS
Structural	NS	NS	NS	NS	NS	NS	NS	NS	NS
RGR ($\text{mg mg}^{-1} \text{week}^{-1}$)									
RGR _{tot} : 0–8 weeks	NS	NS	0.95**	NS	NS	0.95***	NS	NS	0.95***
RGR _{str} : 0–8 weeks	-0.76*	NS	0.87**	-0.80**	NS	0.92***	-0.76**	NS	0.97***
RGR _{tot} : 8 weeks – 1 year	NS	-0.95***		NS	NS		NS	NS	
RGR _{tot} : 0–1 year	NS			NS			NS		
Leaf area (cm^2)	NS	NS	NS	NS	NS	NS	NS	NS	0.81**

* $P < 0.10$; ** $P \leq 0.05$; *** $P < 0.01$.

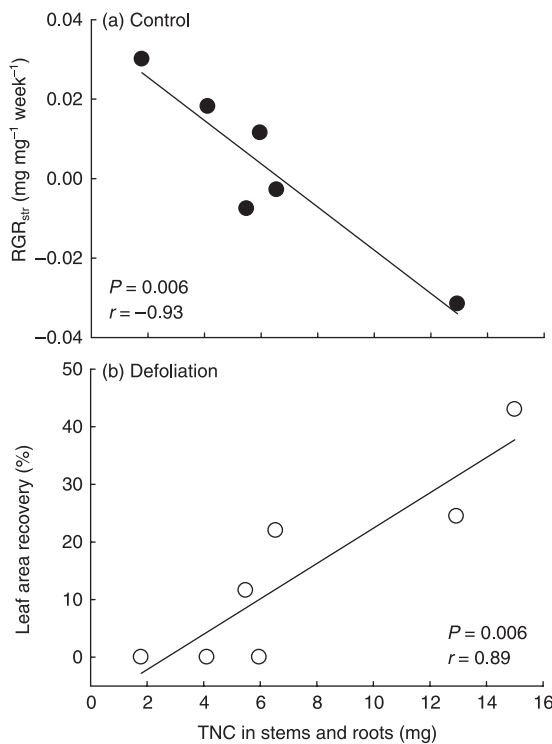


Fig. 6 Relationship between initial carbohydrate pool size in stems and roots and (a) 0–8 week relative growth rate of structural biomass (RGR_{str}) in the control treatment, and (b) leaf area recovery in the defoliation treatment. Leaf area recovery = leaf area of defoliated seedlings at 8 weeks/leaf area of control seedlings at 8 weeks. Each point is a species mean.

8 weeks to 1 year ($P = 0.04$, $n = 6$, Table 3). Likewise, 0–8 week RGR_{tot} and RGR_{str} of defoliated seedlings were positively correlated with their survival from both 0–8 weeks and beyond 8 weeks (Table 3). In other words, survival after defoliation depended on the extent to which defoliated seedlings grew new leaves from 0 to 8 weeks. Leaf area growth of defoliated seedlings, relative to the leaf area of control seedlings at 8 weeks (percentage leaf area recovery), was positively correlated with TNC pool size in stems and roots (Fig. 6b), as well as with TNC pool size in cotyledons ($P = 0.009$, $r = 0.95$, $n = 5$). Thus, the greater the initial TNC pool size in stems and roots, the greater the leaf area recovery, biomass growth rate and survival following the defoliation treatment. At the same time, there was no obvious advantage of large seedling size *per se* for survival in shade, even though greater stem and root biomass may facilitate the storage of larger pools of TNC.

Discussion

CARBON BALANCE AND SURVIVAL IN THE SHADED UNDERSTOREY

This study provides the first experimental support for the hypothesis that carbohydrate storage enhances tolerance of seedlings to both biotic and abiotic stresses in forest understoreys. TNC pools in stems and roots were positively correlated with survival in shade, especially

when seedlings experienced additional stresses of light reduction and defoliation (Fig. 5a,c,e). Our experimental sites were located in the deeply shaded understorey; control seedlings in our study received on average only 0.8% of full sun, and those in the light-reduction treatment received only 0.08% for 8 weeks, a light level that would put them below the light compensation point. Carbohydrate storage enabled survival through periods of negative carbon balance that seedlings are likely to encounter in the understorey, providing them time to acclimate to new light environments and the resources necessary to recover from tissue loss.

The three most shade-tolerant species exhibited a remarkable degree of tolerance to light reduction and defoliation. When understorey light levels fall below the light compensation point, seedlings may utilize TNC to meet their respiratory demands. This mechanism, potentially coupled with down-regulation of respiration rates (Walters & Reich 2000) and other acclimation responses, may explain why the more shade-tolerant species exhibited smaller changes in survival (Fig. 1) and carbon balance (Fig. 4a) in response to our light-reduction treatment. In this situation, seedlings can use TNC to avoid falling deeper into negative carbon balance. Likewise, seedlings that experience intense herbivory or browsing can utilize TNC both to maintain metabolic function over the short term and to replace lost tissue over the long term (Fig. 6b).

Over the long term, seedlings must achieve net positive net carbon balance to persist in shade. For example, surviving seedlings of all species in the light-reduction treatment exhibited negative RGR during the 8-week treatment (Fig. 4a), but achieved positive RGR over the long term once the shade cages were removed (Fig. 4b). Likewise, seedlings that recovered leaf area after defoliation subsequently achieved positive RGR. Thus, we propose that seedlings rely on TNC reserves to survive short-term periods of negative carbon balance, thereby enabling net positive RGR over the long term.

TNC STORAGE AND THE SURVIVAL ADVANTAGES OF LARGE SEED SIZE IN SHADE

We found that seedling survival (over both the short and the long term) was positively correlated with TNC pool size in stems and roots in all treatments (Fig. 5), whereas the correlations between survival and seed or seedling size were not significant. The lack of a correlation between survival and seed/seedling size may have been influenced by low sample size ($n = 7$ species). Nevertheless, our results do suggest that TNC storage may provide an important functional mechanism to explain why larger-seeded species often have a survival advantage over smaller-seeded species in shade (Moles & Westoby 2004). In contrast, previous studies have postulated that large seed size enhances survival because of longer energetic support by seed reserves (the 'larger-seed-slower-deployment effect' or 'reserve effect';

Saverimuttu & Westoby 1996; Leishman *et al.* 2000; Green & Juniper 2004). Although the reserve effect can enhance survival over the short term, energy export from storage cotyledons may become negligible after expansion of the first leaves, even in species with storage cotyledons that remain attached (Ichie *et al.* 2001; Kennedy *et al.* 2004). Thus, longer-term survival advantages may accrue not from continued energy export from storage cotyledons but from other advantages associated with large seed size, such as large seedling size (Leishman *et al.* 2000).

Earlier studies also hypothesized that seed reserves set aside in storage cotyledons may serve as emergency savings that seedlings utilize to replace tissue lost to natural enemies (Kitajima 1996; Harms & Dalling 1997; Green & Juniper 2004). In our study, however, TNC stored in cotyledons was not mobilized in response to experimental defoliation, whereas TNC concentration and pool size in stems and roots both decreased. Moreover, defoliated seedlings of *Coussarea*, a relatively small-statured species with photosynthetic cotyledons, had higher survival (Fig. 1) and recovered more of their original leaf area (Fig. 6b) than seedlings of three of the species with large storage cotyledons. These results suggest a rapid ontogenetic shift in seedling dependency on seed reserves to TNC stored in stems and roots for recovery from defoliation events.

TNC pool sizes in stems and roots are expected to correlate with seed size, because of an allometric relationship between seed size and the initial seedling size at the first leaf stage (e.g. Kitajima 1994). However, we found no significant correlation between TNC pool size and seed size, despite the fact that TNC pool size and seedling size were positively correlated. The decoupling of TNC pool size in stems and roots from seed size may reflect species differences in tissue TNC concentration and allocation patterns. Because we initiated the stress treatments 2 weeks after full expansion of the first photosynthetic organs, and because seedlings had been grown in shade (1% of full sun) from seeds, it is unlikely that a large fraction of the TNC stored in stems and roots was produced *de novo* by photosynthesis. A more plausible explanation is that the majority of TNC found in stems and roots at the beginning of our experiment came from energy reserves transferred from endosperm and cotyledons during germination and initial development. Collectively, these patterns suggest that large seed and seedling size may contribute indirectly to longer-term survival in shade by enhancing the size of TNC pools in stems and roots.

TNC STORAGE AND SEEDLING GROWTH– SURVIVAL TRADE-OFFS

We propose that seedling TNC storage is a functional trait that covaries with the position of species along the growth–survival trade-off continuum (Hubbell & Foster 1992; Kobe *et al.* 1995; Wright *et al.* 2003; Gilbert *et al.* 2006). Across our seven study species, TNC pool

size was positively and negatively correlated with survival and inherent RGR in shade, respectively, and the latter two traits were negatively correlated with each other during the early seedling stage (Fig. 5). Thus, at the 'slow growth–high survival' end of the trade-off continuum, seedlings of shade-tolerant species employ conservative carbon allocation strategies that sacrifice growth rates in order to ensure survival. Additional functional traits associated with this conservative allocation strategy include low specific leaf area (SLA), low leaf area ratio (LAR), slow leaf turnover rates (Kitajima 1994, 1996; Veneklaas & Poorter 1998), high tissue density (Kitajima 1994; Wright *et al.* 2003) and high biomechanical tissue strength (Alvarez-Clare 2005). This suite of traits ensures positive carbon balance over the long term, through steady and slow growth rates. A contrasting suite of traits are found in opportunistic species that grow quickly in treefall gaps, in which fast growth is disproportionately important for light competition. These opportunistic and conservative strategies can promote coexistence by allowing species to partition forest light environments.

During the first 8 weeks, RGR was negatively correlated with TNC pool size and survival. These negative correlations were strongest when RGR was expressed in terms of structural biomass (RGR_{str}). This finding underscores the importance of distinguishing between structural and non-structural biomass when linking biomass allocation patterns to seedling growth and survival (Canham *et al.* 1999). Survival of control seedlings beyond 8 weeks, however, was not significantly correlated with RGR. This result may reflect a decoupling of growth and survival over longer time-scales, or simply could have been driven by limited sample sizes caused by the extremely high mortality of less shade-tolerant species. Previous studies involving larger numbers of species have shown that growth–survival trade-offs can persist well into later ontogenetic stages, in both seedlings (Baraloto *et al.* 2005; Gilbert *et al.* 2006) and saplings (Kobe *et al.* 1995; Wright *et al.* 2003; Gilbert *et al.* 2006). Surprisingly, TNC concentration was not correlated with RGR_{str} or RGR_{tot} , even though such a correlation is expected if greater proportional allocation to storage is the direct physiological cause for slow RGR (Kobe 1997). The negative correlation between TNC pool size and RGR probably reflects covariation among suites of functional traits, rather than a cause–effect relationship mediated by a single trait. For example, a negative RGR–TNC pool size correlation could be driven by correlations between each of these variables and seed or seedling size (small seed size may concomitantly increase RGR but decrease TNC pools).

Three other studies have examined the role of TNC reserves for juvenile tree growth and survival in shade. Using pair-wise comparisons of two temperate deciduous and evergreen tree species at the sapling stage, Kobe (1997) found higher root TNC concentration in more shade-tolerant species. In contrast, we found that

TNC pool size, but not TNC concentration, was linked to shade tolerance. Canham *et al.* (1999) found little evidence for a link between TNC storage in roots and stems and shade tolerance across 2-year-old seedlings of four temperate deciduous tree species. However, they found that TNC increased survival following defoliation, as in our study. All seven of our study species are evergreen as seedlings, some of which survive in much shadier environments than those typically found in temperate forest understoreys (Canham *et al.* 1990). Most recently, Poorter & Kitajima (in press) found positive relationships between survival and both TNC concentration and pool size in saplings of 49 species of tropical trees. They also found that TNC concentration and pool size were related to differences in light requirements among species (at the sapling stage), but not with height growth rates. Taken together with our results, these studies demonstrate that TNC storage plays a significant role in juvenile survival, but there is currently little support for a potential trade-off between allocation to TNC and growth rates.

Our study highlights the critical importance of TNC storage for first-year seedling survival and stress tolerance in forest understoreys. The strength of our study lies in the experimental approach to testing the extent to which seedlings rely on carbohydrate reserves to survive periods of negative carbon balance imposed by biotic and abiotic stress. Further generalization of the interspecific trends suggested in this study will be augmented by surveys of TNC across larger numbers of species, during both the first year that is critical for seedling recruitment, as well as at later juvenile stages. These endeavours should enhance our mechanistic understanding of the ecological processes influencing the organization of forest tree communities.

Conclusions

Carbohydrate storage is a central component of the life history of shade-tolerant tree species. TNC reserves, especially in stems and roots, enable shade-tolerant seedlings to survive through short-term periods of negative carbon balance imposed by both biotic and abiotic stresses, and thus to achieve positive carbon balance over the long term. Carbohydrate storage is a key functional trait that can explain species differences in growth and survival that promote species coexistence through niche assembly processes and life-history trade-offs.

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Supplementary material

The following supplementary material is available for this article:

Table S1 Means and standard errors of total non-structural carbohydrate (TNC) concentrations and pool sizes in stems, roots, and cotyledons at the 8-week seedling harvest

This material is available as part of the online article from: <http://www.blackwell-synergy.com/doi/full/10.1111/j.1365-2745.2006.01207.x>

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