Do shade-tolerant tropical tree seedlings depend longer on seed reserves? Functional growth analysis of three Bignoniaceae species

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Summary

1. A functional growth analysis was used to determine the duration of strict dependency on seed reserves for energy and nitrogen in three woody Bignoniaceae species (*Tabebuia rosea* DC., *Challichlamys latifolia* K. Schum. and *Pithecoctenium crucigerum* A. Gentry) which differed in cotyledon function (photosynthetic, semi-photosynthetic and storage) and shade tolerance (probability of seedling establishment and survival in the understorey).

2. Seedlings were raised from seeds in sand culture under combinations of three nitrogen levels (daily supply of nutrient solution containing 100, 10 and 0% of 2·6 mm N) and two irradiances (27 and 1% full sun). Time course of biomass, non-cotyledonous biomass and leaf area for 40 days post-germination were compared to identify when the external availability of nitrogen or light began to affect seedling growth.

3. Seedlings of all species became dependent on external energy supply earlier than they did on nitrogen supply. In all species seed nitrogen was sufficient to support positive seedling growth for 40 days in shade, but not in sun.

4. *Tabebuia rosea* with photosynthetic cotyledons responded to light availability earlier than more shade-tolerant species with storage cotyledons. *Challichlamys latifolia*, the most shade-tolerant species, had the highest nitrogen concentration in seeds and was the last to respond to external nitrogen availability. Thus seedlings of the most shade-tolerant species depended on seed reserves for the longest period for both energy and nitrogen.

5. Relative growth rate after seedlings initiated autotrophic growth was in a trade-off relationship with seedling survivorship in the understorey across the three species. *Tabebuia rosea*, the least shade-tolerant species, had the highest positive net carbon balance in sun and shade.

6. Functional morphology of cotyledons and concentration of seed nitrogen deserve as much attention as seed size as correlates of contrasting seedling regeneration strategies.

Key-words: Energy and nitrogen reserves, growth analysis, light availability, seedling establishment, soil nitrogen

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Introduction

The duration of seed-reserve dependency is an important aspect of the ecology of seedling establishment (Fenner 1987; Kitajima & Fenner 2000). Initially, seedlings are completely dependent on seed reserves for all resources except water, but they gradually become dependent on the external supply of resources acquired by shoots (light energy fixed in carbon-based compounds) and roots (mineral nutrients) (Krigel 1967; Fenner 1986). Whether dependency on external sources develops earlier for above- or below-ground resources affects the relative importance of light *vs* soil nutrients during seedling establishment. Among mineral elements, nitrogen is generally the first to become insufficient in supply from seed reserves alone (Fenner 1986; Fenner & Lee 1989; Hanley & Fenner 1997).

The duration of strict seed dependency for a given resource may vary among species in relation to four characteristics: seed size (total seed mass); seed quality (concentration of the focal resource); major function of cotyledons (whether cotyledons serve as photosynthetic or storage organs of seed reserves after germination); and inherent rate of seedling growth and development. These traits are correlated with each

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other according to the species' life-history strategies. Shade-tolerant species tend to have large seeds, storage cotyledons, and inherently slow relative growth rate (RGR) via low specific leaf area (SLA) and leaf area ratio (LAR) (Kitajima 1996a; Leishman *et al.* 2000; Veneklaas & Poorter 1998; Walters & Reich 1999, 2000). In contrast, light-demanding species tend to have small seeds, photosynthetic cotyledons, and inherently fast RGR via high SLA and LAR. Although large-seeded species with storage cotyledons are often assumed to have longer periods of seed-reserve dependency, the differences in duration of seed-reserve dependency between these contrasting regeneration syndromes have not been quantified explicitly. No previous studies have determined whether energy or mineral reserve in seeds is the first to become insufficient to meet seedlings' demands, and whether the results differ between species with different regeneration syndromes.

For quantitative comparisons of periods of seed-reserve dependency among species, a new method was developed to determine when the transition from heterotrophy to autotrophy occurs for a given resource. When seedlings are grown from seeds with or without the supply of a particular resource, the growth rate should be identical as long as seedlings depend completely on seed reserves. However, as seed reserves alone become insufficient in meeting the seedlings' demand, growth of seedlings without an external supply of the focal resource becomes significantly slower than growth of seedlings with a sufficient supply of all necessary resources (Fig. 19·1 of Kitajima 1996b). The point at which this occurs indicates the end of strict seed-reserve dependency. This can be quantified by determining when functional growth curves constructed from frequent harvests become significantly different due to a difference in external resource supply.

Here, this method was applied to three tropical species in the Bignoniaceae differing in morphological and ecological characteristics, in order to ask the following questions: (i) does this transition occur earlier for nitrogen or energy? (ii) do periods of dependency differ among species differing in degrees of shade tolerance and cotyledon functions (photosynthetic *vs* storage)? The main prediction for the second objective was that species with photosynthetic cotyledons begin to utilize light energy earlier than species with storage cotyledons. We also explored whether seed nitrogen concentration was correlated with the dependency periods for these resources.

Methods

STUDY SITE AND SPECIES

The study was conducted on Barro Colorado Island (BCI), Panama, using three woody Bignoniaceae species native to the seasonal moist forest on BCI. All three species have winged seeds that are wind-dispersed during the late dry season (March–April) and germinate during the early rainy season (May–June). *Tabebuia rosea* DC. is a common canopy tree species, while *Challichlamys latifolia* K. Schum. and *Pithecoctenium crucigerum* A. Gentry are common lianas on BCI (see Croat 1978 for nomenclature and site description). They were similar in seed morphology (flat, heart-shaped cotyledons without endosperm) and concentrations of energy (mean ± SD = 27·4 ± 0·8 kJ g^{-1}) and ash (mean ± SD = 3·0 ± 0·5%) in seeds. However, they differed significantly in seed mass, nitrogen concentration in seeds, and cotyledon type after germination (Table 1), as well as in the degree of shade tolerance (Table 2). Although seed mass differed by up to fivefold

Table 1. Morphological and physiological characteristics of seeds and seedling cotyledons for three Bignoniaceae species. Seed mass and nutrient concentrations were determined after removing the seed coat. Light- and CO$_2$-saturated net photosynthetic rates (net P_{max}) of green epigeal cotyledons were measured at 22–23 days after radicle emergence for *T. rosea* and *C. latifolia* in sun, 100% N treatment with a leaf-disk oxygen electrode (Model LD2, Hansatech, Norfolk, UK), by the method of Kitajima (1992) (See also Kitajima, 1996a)

Parameter	*T. rosea* n	Mean ± SD	*C. latifolia* n	Mean ± SD	*P. crucigerum* n	Mean ± SD
Seed characteristics						
Seed mass (mg)	12	34·6 ± 6·8	6	187 ± 18	6	46.1 ± 5·3
N concentration (mg g^{-1})	3	37·4 ± 1·8	3	82·3 ± 3·8	3	43·1 ± 1·1
Cotyledon characteristics						
Main function	Photosynthetic		Storage		Storage	
Position	Epigeal		Epigeal		Hypogeal	
Exposure	Phanerocotylar		Phanerocotylar		Cryptocotylar	
Area at radicle emergence (cm^2)*	6	1·5 ± 0·2	7	3·7 ± 0·5	7	1·7 ± 0·2
Area at full expansion (cm^2)*	51	10·4 ± 1·7	47	4·5 ± 0·8	28	2·1 ± 0·4
Net P_{max} (µmol O$_2$ m^{-2} s^{-1})	4	15·6 ± 1·5	4	5·4 ± 3·2		ND
Net P_{max} (µmol O$_2$ g^{-1} s^{-1})	4	0·51 ± 0·06	4	0·06 ± 0·01		ND
Mean number per plant at 40 days	24	2·0	158	1·7	10	1·0

*Total area of the two cotyledons of plants in sun, 100% N.

Table 2. Survival and growth characteristics of seedlings for the three species. Field survival data for naturally dispersed seeds and established seedlings were collected during 1995–2001 on BCI (S. J. Wright, unpublished results, see Methods for explanation). Recruitment probability = ratio of seedling recruits to trapped seeds adjusted for the sampling area difference; post-establishment survival = probability of seedling survival from first to second year; sample size (n) = initial number for each type of survival calculation pooled for the six census years. RGR of total seedling mass at 29 days after radicle emergence (when all species have mature true-leaves) was calculated as the instantaneous slope of the third-order polynomial fit to $\log_e$(total biomass) (cf. left panels of Figs 2–4). RGR of non-cotyledonous mass during the autotrophic phase (= period indicated for each species) was calculated as the linear regression slope for $\log_e$(non-cotyledonous biomass) vs time (cf. centre panels of Figs 2–4)

Parameter	*T. rosea*		*C. latifolia*		*P. crucigerum*	
Seedling shade tolerance	n	ratio	n	ratio	n	ratio
Recruitment probability	1321	0·011	58	0·310	347	0·047
Post-establishment survival	35	0·343	50	0·700	62	0·435
RGR of total seedling mass at 29 days (mg g^{-1} day^{-1})						
Reference (100% N, sun)	99		56		75	
Sun, 0% N	42		48		27	
Shade, 100% N	22		8		17	
RGR of non-cotyledonous mass (mg g^{-1} day^{-1}, mean ± SE)						
Period used for regression (days)	9–42		13–45		16–40	
Reference (100% N, sun)	125 ± 4		75 ± 5		86 ± 9	
Sun, 0% N	71 ± 3		55 ± 5		40 ± 11	
Shade, 100% N	42 ± 3		31 ± 4		32 ± 5	

among species, this variation was much smaller than the interspecific variation in this community (10^5-fold maximal difference among 74 woody species, Kitajima 1996a).

The degrees of shade tolerance of the three species have been quantified in a long-term study of seed rain and seedling recruitment (S. J. Wright, unpublished data). A summary of the community-wide trends found in this data set has been published elsewhere (Harms *et al.* 2000).

Seed rain (number of seeds per m^2) was quantified by weekly censuses of 200 1 m^2 traps placed within a 50 ha permanent forest plot between 1994 and 2000. Newly recruited seedlings were tagged and counted in 600 1 m^2 plots and censused during January and March each year from 1995 to 2001. Three plots per seed trap were placed at 2 m from the trap at three sides, leaving one side unoccupied for access to the trap.

The ratio of the total number of newly recruited seedlings to the total number of seeds trapped, adjusted for the difference in sampling areas, reflected post-dispersal seed survival and seedling establishment probabilities in the shaded understorey during the first 8–10 months after germination (May–January). This 'recruitment probability' is calculated as the first index of shade tolerance. The second index of shade tolerance was the survival probability of the recruited first-year seedlings to the next year's census ('post-establishment survival'). Both indices of shade tolerance indicate that *C. latifolia* was the most shade-tolerant, *P. crucigerum* was intermediate, and *T. rosea* was the least shade-tolerant species (Table 2, significant species difference at $P = 0·0001$ and $0·002$ for recruitment and post-establishment probability, respectively, by a log-likelihood ratio test). Relative

to other species sampled in the same study, these three species were intermediately (*T. rosea*) to highly (*C. latifolia*) shade tolerant. Relatively low shade tolerance of *T. rosea* was also shown in a comparative study of post-germination survivorship of nine tree species in the shade (35% at 2 months, 1% at 1 year) *vs* gaps (87% at 2 months, 31% at 1 year) (Augspurger 1984a). Field observations of the relative abundance of these seedlings in the shaded understorey *vs* illuminated sites, a commonly used index of shade tolerance (Rosevear, Yong & Johnson 2001), are also consistent with these ranks of shade tolerance (K. Kitajima, unpublished results).

The similar embryonic cotyledons of the three species develop into completely different cotyledon types after germination (Table 1). Cotyledons of *T. rosea* and *C. latifolia* are lifted above-ground (epigeal) and free of seed coat (phanerocotylar). Cotyledons of *T. rosea* expand in area by 575% after emergence to become thin (0·4 mm in the centre) and photosynthetic (Table 1). In contrast, semi-photosynthetic cotyledons of *C. latifolia* expand little (22% increase in area) and remain thick (1·8 mm in the centre). The primary function of *C. latifolia* cotyledons is storage of seed reserves, because their photosynthetic capacity per unit mass is unlikely to be sufficient to export assimilates (Kitajima 1992). Completely non-photosynthetic storage cotyledons of *P. crucigerum* remain inside the seed coat (cryptocotylar) at ground level (hypogeal) throughout their life span. Naturally dispersed seeds were collected from the forest floor at several locations (minimum of three parents) in March–April 1990. Seeds of each species were pooled and stored in plastic bags in an air-conditioned laboratory until the beginning of the experiment several weeks later.

GROWTH CONDITIONS

Seedlings were grown in a screened enclosure in a large clearing adjacent to the forest. Six benches, covered with clear plastic to avoid rainwater, were randomly assigned to sun and shade light treatments (27 and 1·2% of total daily photon flux density, PFD, above canopy, respectively). The means for total daily PFD were 6·77 and 0·29 mol photons m^{-2} day^{-1}, respectively (means for 12–24 days with quantum sensors and LI-1000 data logger, Li-Cor, Nebraska, USA). Shading in the sun treatment was from the nearby forest edge and the structure of the enclosure and the plastic over the benches. The shade treatment was covered with multiple layers of shade cloth that is neutral in wavelength transmission. The shade treatment was comparable to the typical shade in tropical rainforest understoreys (Canham *et al*. 1990), while the sun treatment corresponded to the typical light availability in large tree-fall gaps, which is optimal for growth of most tropical tree seedlings (Poorter 1999; Valladares *et al*. 2000).

Seeds were randomly assigned to light treatments and planted in plastic flats filled with moist vermiculite. As soon as the radicle emerged, 6–14 days after planting (radicle length <10 mm for *C. latifolia*, <5 mm for the other species), seedlings were transplanted individually to plastic pots filled with 230 cm^3 1 : 1 mix of sand and vermiculite and randomly assigned to three nitrogen treatments (100% N; 10% N; 0% N) within each light regime. Each plant was marked with a plastic stake to identify the exact date of radicle emergence (0 days after germination). Within each light treatment, locations of pots were rotated every third day within and among benches.

Each pot was initially flushed with 75 ml of the designated solution, then saturated daily with 20 ml of the same solution. One-fifth strength modified Johnson's solution (Epstein 1972) was used as the reference nutrient solution (100% N treatment) with the following macronutrient concentrations: NO_3^-, $2·2 \times 10^{-3}$ M; NH_4^+, $4·2 \times 10^{-4}$ M; K, $1·2 \times 10^{-3}$ M; Ca, $8·0 \times 10^{-4}$ M; Mg, $2·0 \times 10^{-4}$ M; S, $2·0 \times 10^{-4}$ M; P, $4·0 \times 10^{-4}$ M. The solution for the 10% N treatment had the same composition as the reference treatment, except that 90% of the NO_3^- and NH_4^+ was replaced with Cl^- and K^+, respectively. For the 0% N treatment, all nitrogenous ions were replaced with Cl^- and K^+. The nitrogen concentration in the reference solution was similar to what seedlings experience in the field. Soil on BCI supplies moderate amount of N (total N = 4·7–5·3 mg g^{-1} dry soil; $CaCl_2$-extractable NO_3^-–N = 12·9–15·8 µg g^{-1}; NH_4^+–N = 0·6–1·7 µg g^{-1}, Yavitt & Wieder 1988). The nitrogen concentration of the 100% N solution was comparable to the highest concentration recorded in stream water on BCI (M. Keller and R. Stallard, personal communications).

There were six total treatment combinations (two light levels times three nitrogen levels) per species

for *T. rosea* and *C. latifolia*. Because of limited seed availability, only two nutrient treatments (100% N and 0% N) were possible in each light regime for *P. crucigerum*. The frequency of harvests differed among species because of differences in seed availability; three plants were harvested from each treatment daily for *T. rosea*, every second day for *C. latifolia*, and every third day for *P. crucigerum* for 30–40 days after radicle emergence. Because differences in seedling size among nitrogen treatments were not evident in shade at that time, seedling harvests were continued at longer intervals (5–7 days) up to 60 days in shade, in order to check the possible occurrence of later size differences among nitrogen treatments in shade. Seedling survival was 100% in all treatments. Soilborne pathogens, which may cause high mortality of seedlings in the field and nursery (Augspurger 1984b), did not cause mortality in this experiment using pathogen-free soil medium. In a study of longer-term deprivation of mineral nutrients (12 weeks), Hanley & Fenner (1997) did not observe any elevated mortality.

At harvest, after rinsing roots carefully with water to remove soil medium, each plant was separated into stem, roots and leaves to determine leaf area. Dry mass of seeds and seedling parts was determined after drying at 65 °C to constant values. For seeds, tissue concentration of Kjeldahl nitrogen (determined with Nessler colorimetry, Allen 1974; Table 1). For seedlings, concentration and total content of nitrogen were determined for *T. rosea* and *C. latifolia* at approximate ages when seedling growth started to respond to light or nitrogen deficiency. Tissue N was not analysed for *P. crucigerum* for logistical reasons.

GROWTH ANALYSIS

Several measures of plant size, including total seedling mass (including and excluding cotyledons) and total photosynthetic area, were examined with and without log transformation as a function of time, and compared among treatments for each species. Total photosynthetic area was the total projected area of leaves plus green cotyledons for *T. rosea* and *C. latifolia*, while it included only leaf area for *P. crucigerum* (which had non-photosynthetic cotyledons). Photosynthetic cotyledons of *T. rosea* increased their size more rapidly and achieved greater area (by 6%) and mass (by 35–76%) in high than in low light (data not shown). In contrast, storage cotyledons of *C. latifolia* and *P. crucigerum* lost mass continuously, and these time courses did not differ among treatment types. The purpose of the log transformation was to homogenize the variance between early and late samples. It was also convenient because the instantaneous slope of log_e-transformed biomass against time is RGR (Hunt 1982). Cubic (third-order) polynomials were chosen for curve-fitting because they provide mathematically simple and reliable estimates of confidence intervals (Hunt 1982; Wickens & Cheeseman 1988). The time at
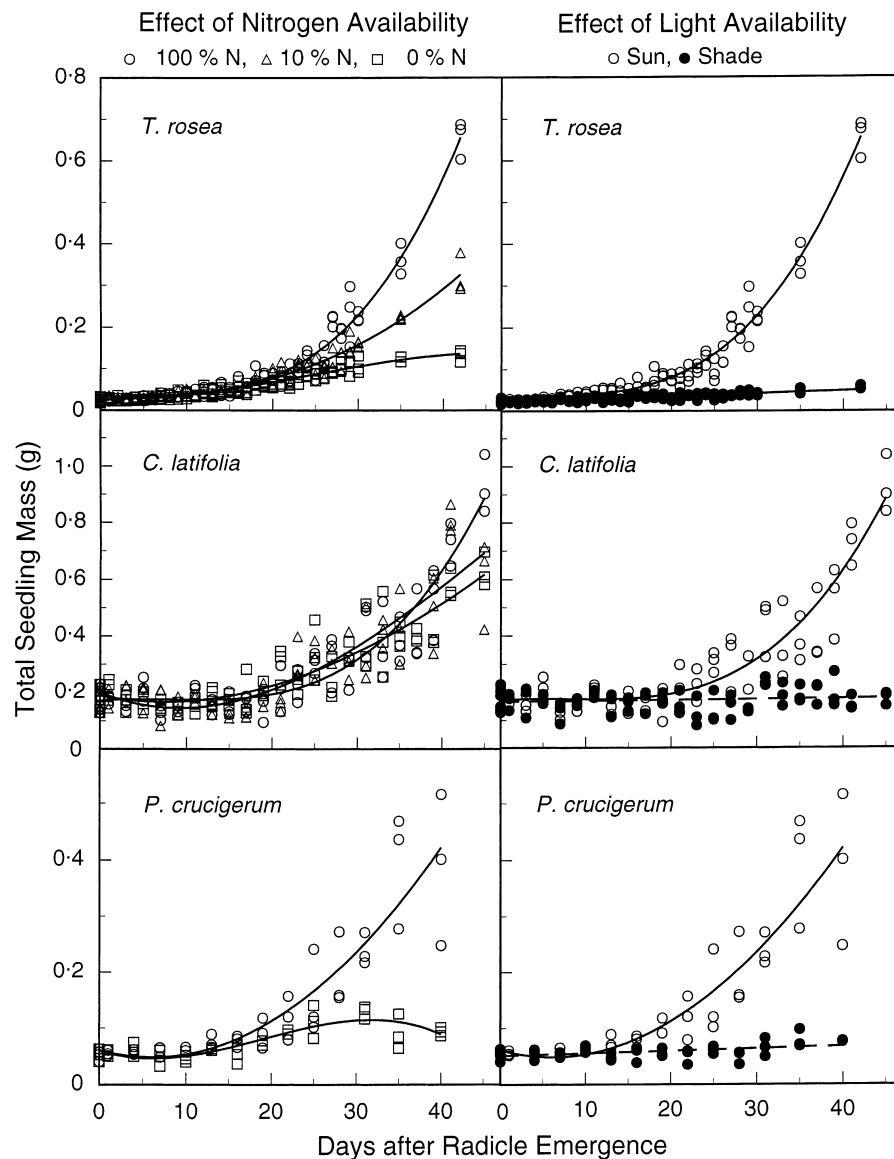
Fig. 1. Total seedling dry mass (g) of the three species for 40–45 days after radicle emergence under three nitrogen treatments in sun (left), and under two light regimes with 100% N (right). See Table 1 for species names. Results for 'shade, 10% N' and 'shade, 0% N' are not shown because they overlap completely with 'shade, 100% N'. Cubic polynomial or simple regressions are shown (solid lines for significant, broken lines for non-significant trends, respectively). No 10% N treatment existed for *P. crucigerum*. Vertical axis scales differ among species.

which the growth curves of plants under contrasting conditions depart from each other was determined as the earliest date when 95% confidence intervals for the two departing curves did not overlap. JMP v.2 (SAS Institute, Cary, USA) was used for curve fitting and other statistical analyses.

Results

TIME COURSE OF SEEDLING GROWTH

Biomass of all species responded to nitrogen and light treatments by the end of the experiment (Fig. 1). In sun, nitrogen availability started to affect seedling growth between 15 and 40 days after radicle emergence, but at different times for different species.

Growth of plants in 0% N and 10% N started to drop below the reference growth (100% N) at about the same time. The final biomass of plants in 10% N was intermediate between those of 100% N and 0% N treatment. Hereafter, the timing of response to nitrogen availability is reported only for the contrasts between 0% N and 100% N. In shade, nitrogen availability caused no difference in seedling growth of any species; the growth curves overlapped completely (data not shown). For this reason the effect of light is examined only by contrasting 'shade, 100% N' to the reference 'sun, 100% N'. The effects of nitrogen availability occurred later than those of light availability in *T. rosea* and *C. latifolia*, as is evident by comparing the timing of growth curve separation between the left and right panels of Fig. 1.
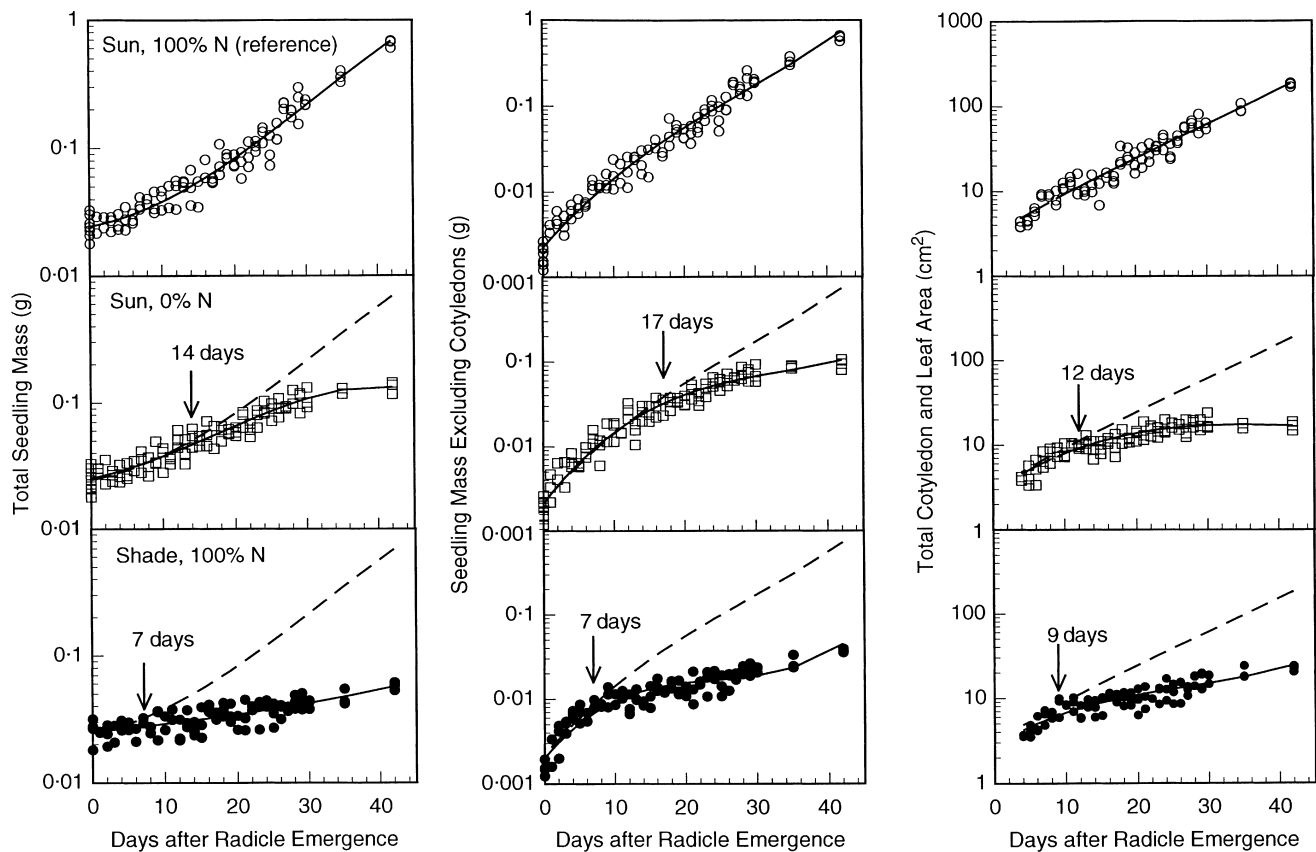
Fig. 2. Total seedling mass (left), total seedling mass excluding cotyledons (centre), and total photosynthetic area (total of leaf and cotyledon areas, right) of seedlings of *T. rosea* with highly photosynthetic cotyledons under reference conditions (sun, 100% N), nitrogen-deficient conditions (sun, 0% N), and light-limited conditions (shade, 100% N). Broken line indicates fitted curve for reference plants. Arrows indicate dates at which growth curves for the given conditions depart significantly from the reference curve (no overlap of 95% confidence intervals). Seedling mass data are from Kitajima & Fenner (2000).

WHEN DO SEEDLINGS START RESPONDING TO EXTERNAL LIGHT AND N AVAILABILITY?

In Figs 2–4 the timings of response to external nitrogen and light treatments are shown more closely for each species. Cubic polynomials were fitted to log-transformed values of total seedling mass; total seedling mass excluding cotyledons (non-cotyledonous mass); and total photosynthetic area (total of leaf and cotyledon area for *T. rosea* and *C. latifolia*, and total leaf area for *P. crucigerum*) plotted against time. In all cases cubic polynomial fitting had $R^2 > 0.9$.

In *T. rosea*, with fully photosynthetic cotyledons, seedling growth was affected by external light availability as early as 7–9 days after germination (comparison of bottom panels, Fig. 2). Biomass growth responded to light availability slightly earlier (7 days) than photosynthetic area (9 days). The response to external nitrogen availability occurred later (12–14 days) than that to light availability (7–9 days), regardless of which variable was used to measure growth. Photosynthetic area responded to external nitrogen availability earlier (12 days) than did the total biomass (14 days) and non-cotyledonous biomass (17 days).

In *C. latifolia*, with semi-photosynthetic storage cotyledons, the response of biomass to external light availability occurred at 16–20 days, but earlier than the response of photosynthetic area at 33 days (bottom panels, Fig. 3). Seedling biomass did not respond to nitrogen availability for 45 days, while photosynthetic area started to respond to nitrogen at 35 days. If the total leaf area (instead of the total of cotyledon and leaf area) was plotted, the result would be identical because the total cotyledon area of *C. latifolia* remained constant after cotyledon emergence. As in *T. rosea*, the response of biomass growth to light occurred earlier than that to external nitrogen availability (20 *vs* >45 days for total biomass, 16 *vs* >45 days for non-cotyledonous biomass). The response of leaf area occurred at similar times for light and nitrogen (33 *vs* 35 days).

In *P. crucigerum*, which had non-photosynthetic storage cotyledons, the biomass response to light occurred between 9 and 13 days, while leaf area responded at 24 days (bottom panels, Fig. 4). In this species, seedling biomass responded to light availability only slightly earlier than to nitrogen availability (13 *vs* 16 days for total biomass, 9 *vs* 12 days for non-cotyledonous biomass), but leaf area response occurred slightly earlier for nitrogen (21 days) than for light (24 days). Because of longer sampling intervals and smaller total sample size, the band of 95% confidence intervals associated with each fitted curve was wider in *P. crucigerum* than in the other two species.
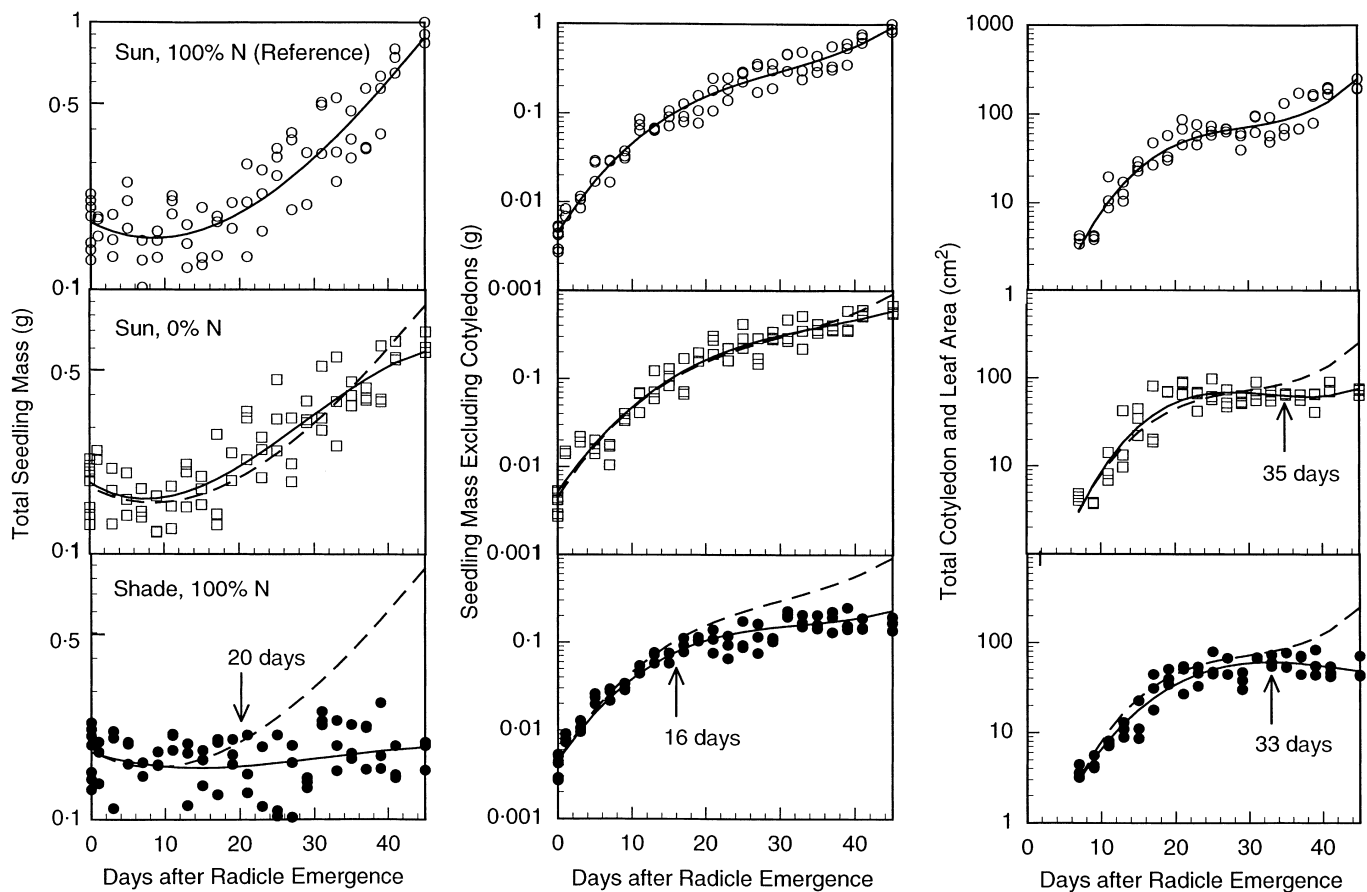
Fig. 3. The same type of data and analysis as Fig. 2 for epigeal seedlings of *C. latifolia* with storage cotyledons with low photosynthetic capacity.

Consequently, the response date is estimated less accurately for this species than for the others.

RGR DURING SEED-DEPENDENT AND AUTOTROPHIC PERIODS

In all species, change of log(total biomass) was characterized by a short lag phase of 5–10 days, followed by high RGR (= the instantaneous slope of the curve; left panels of Figs 2–4). During the initial lag phases RGR was slightly, but not significantly, negative (most apparent in *C. latifolia*, Fig. 4, left). During the post-lag phase, RGR gradually increased to relatively constant high and low rates in sun and shade, respectively. However, under 0% N in sun, biomass growth rate decelerated to zero as the individuals exhausted all the initial nitrogen capital in the seed. The instantaneous slope at 29 days for the third-order polynomial fit shown in left panels of Figs 2–4 were calculated as representative values of RGR after all species became autotrophic (Table 2). In contrast to the time course of log(total biomass), the time course of log(non-cotyledonous mass) lacked a lag phase and exhibited an initial period of high RGR, reflecting the rapid transfer of seed reserves, followed by a period of slower and relatively constant growth primarily supported by autotrophic resource acquisition (centre panels of Figs 2–4). The RGR of non-cotyledonous mass (= initial

slope) did not differ significantly among treatments and species during the initial seed-dependent phase (mean slope = 216 mg g^{-1} day^{-1}). However, during the later autotrophic phase the estimated RGR for non-cotyledonous biomass differed among species and treatments (Table 2). The calculated RGR during the later autotrophic phase for reference plants (sun, 100% N), which indicates the inherent RGR of the species, varied inversely with shade tolerance of the species; it was fastest for the least shade-tolerant *T. rosea* and slowest for the most shade-tolerant *C. latifolia*.

TIME COURSE OF NITROGEN CONTENT

Total N content of *T. rosea* plants in all treatments decreased by approximately 30% from seed to 7 days after radicle emergence, then increased when nitrogen was available in the soil (Fig. 5, top). The tissue N concentration (Fig. 5, bottom) did not differ among treatments at 7 days when growth of plants started to respond to light availability. By the time nitrogen availability started to affect plant biomass in sun (12 days), both the total content and concentration of nitrogen were significantly different among nitrogen treatments in sun, but not in shade. Total N content of 0% N plants in sun continued to decrease until 12 days, then no further change occurred through 35 days. Although total N content increased in 10% and 100% N treatments in
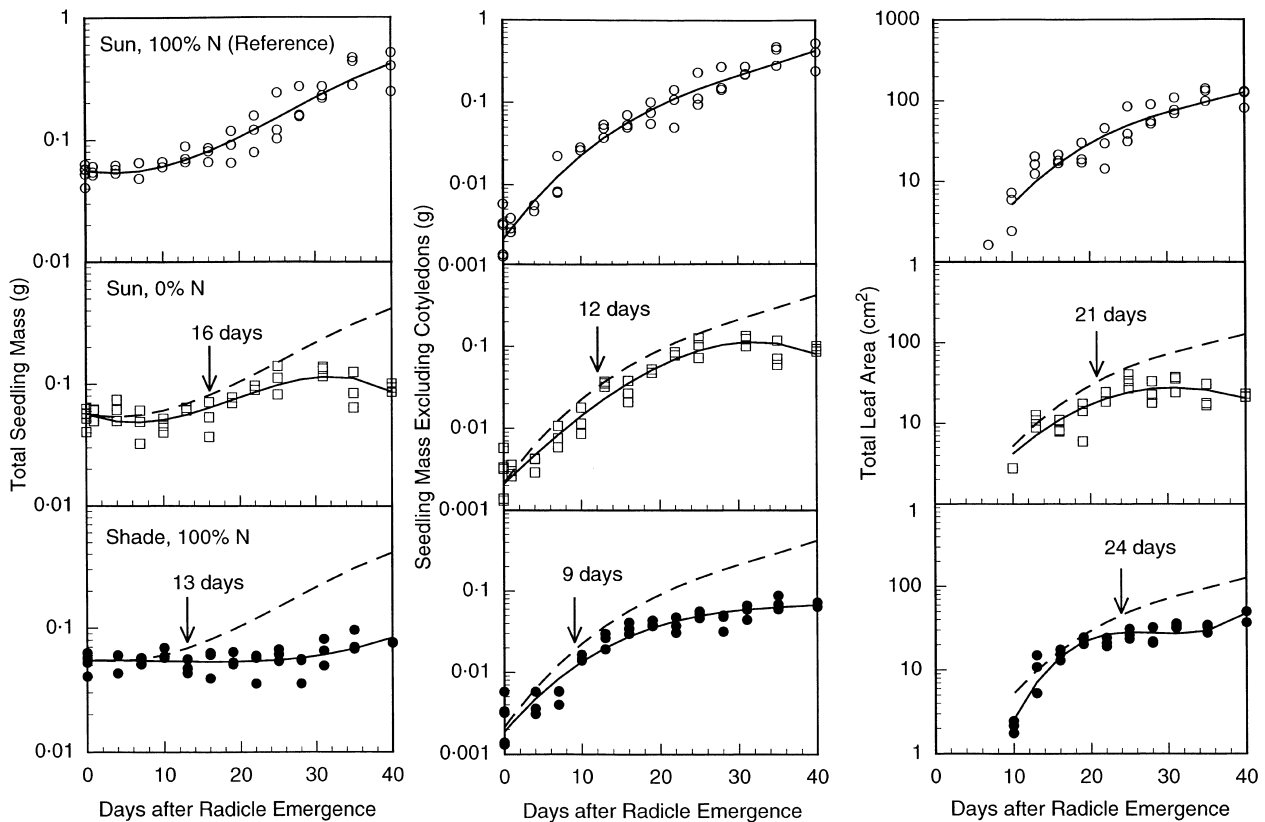
Fig. 4. The same type of data and analysis as Fig. 2 for seedlings of *P. crucigerum* with non-photosynthetic storage cotyledons.
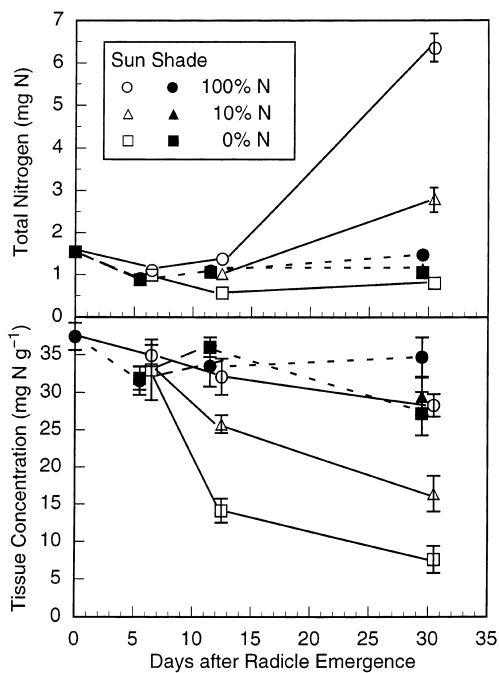

Fig. 5. Total N content (top) and tissue N concentration (bottom) of *T. rosea* seedlings as a function of age and light × nitrogen treatments (*n* = 3 seedlings per treatment per age, mean ± SD). Seedlings were sampled at the ages when their growth started to respond to external light availability but not nitrogen availability (7 days); when they also started to respond to external nitrogen availability (12 days); and at a later date when the difference due to either light or nitrogen treatment was very large (30 days). Positions of points are staggered horizontally to avoid an overlap of symbols.

sun after 12 days, tissue N concentration decreased during this period. Throughout the duration of the experiment, all plants in shade had a similar or higher tissue N concentration than the reference plants (sun, 100% N). Although nitrogen treatment did not affect growth in shade, the difference in tissue N concentration between 100% N and 0% N became significant at 30 days ($P < 0.05$; Fig. 5, bottom).

In *C. latifolia*, tissue N concentration was not different between 0% N and 100% N in sun at 13 days (mean ± SD = 74 ± 4 mg N g^{-1}). At 25 days, before seedling growth started to be affected by external N availability, tissue N concentration was significantly different between 100% N and 0% N in sun (mean ± SD = 53 ± 2 and 43 ± 2 mg N g^{-1}, respectively), but not between 100% N and 10% N (pooled mean ± SD = 52 ± 3 mg N g^{-1}). These tissue N concentrations were much higher than the highest tissue N concentration observed in *T. rosea* (37 mg N g^{-1}). There was no effect of N treatment on tissue N concentration of *C. latifolia* in shade at 25 days (70 ± 4 mg N g^{-1}), which was higher than in sun.

Discussion

IMPLICATIONS FOR SEEDLING REGENERATION ECOLOGY

The diversity of seed size and cotyledon morphology among tropical dicotyledonous species (Garwood 1996; de Vogel 1980) must be a product of natural

selection for contrasting life-history traits, even though seedling survival is strongly subject to stochastic events (Hubbell *et al.* 1999). In broad community-wide comparisons, shade-tolerant species tend to have large seeds and storage cotyledons, while light-demanding species tend to have small seeds and photosynthetic cotyledons (Hladik & Miquel 1990; Kitajima 1996a). The results reported here point to a likely functional reason underlying this trend: *T. rosea*, with photosynthetic cotyledons, switched earlier from seed reserves to light than did the other species with storage cotyledons. *Tabebuia rosea* also exhibited the fastest growth rates of non-cotyledonous biomass in sun and shade, after all species initiated autotrophic growth (Table 2). This is a conservative test for a faster RGR being associated with species with photosynthetic cotyledons than with those with storage cotyledons, because non-cotyledonous RGR reflects only the autotrophic carbon gain in species with photosynthetic cotyledons (*T. rosea*), while it reflects the total of transfer of remaining seed reserves plus autotrophic carbon gain in species with storage cotyledons (*C. latifolia* and *P. crucigerum*). Hence the poor establishment and survival ability of *T. rosea* seedlings in the field, relative to the other two species, cannot be attributed to a poor carbon balance. Instead, the results of this study support the trade-off between growth rates and survival probabilities observed in comparative studies of tropical tree species (Kitajima 1994; Poorter 1999; Veneklaas & Poorter 1998).

DURATION OF SEED-RESERVE DEPENDENCY FOR ENERGY *VS* NITROGEN

There were two findings common among the three species irrespective of shade tolerance and cotyledon morphology. Seedling growth of all species became affected by external energy supply (= light) earlier than external nitrogen supply from soil. All species, both shade-tolerant and -intolerant, exhibited positive biomass growth in deep shade (1·2% full sun) for 45 days after germination (Figs 2–4; Table 1), during which their nitrogen demands were completely met by maternal provisioning in the seed. Biomass growth of *T. guyacan* in an identical experiment also exhibited earlier response to light (7 days) than to nitrogen (18 days) (K. K., unpublished results). These results support the notion that light availability is the primary determinant of the spatial and temporal patterns of tree seedling recruitment in forests (Augspurger 1984a, 1984b; Coomes & Grubb 2000; Pacala & Rees 1998). In a comparative study of 10 temperate trees, seedling growth and survival were affected by nitrogen availability only in the two most light-demanding species, and only under high light availability (Walters & Reich 2000). Soil nutrient availability, however, may later become a co-limiting factor for growth and survival of established shade-tolerant seedlings (Burslem, Grubb & Turner 1995, 1996; Grubb & Coomes 1997;

Latham 1992; Lewis & Tanner 2000; Lusk, Contreras & Figueroa 1997; Walters & Reich 1996).

The timing of growth-curve divergence due to deficiency of a given resource differed by several days, depending how growth was measured (as total mass, non-cotyledonous mass or photosynthetic area), although this choice had little effect on the rank order of the timing of a switch for light *vs* nitrogen. In all species, the shade-acclimation response of increasing SLA and LAR (data not shown) apparently contributed to the slower response of leaf area than biomass to light availability. On the other hand, total photosynthetic area responded to nitrogen availability before seedling mass in *T. rosea* and *C. latifolia*, perhaps because leaf area expansion was regulated directly by nitrogen availability (Hirose & Kitajima 1986; Potter & Jones 1977).

EFFECTS OF SEED NITROGEN CONCENTRATION

The most shade-tolerant *C. latifolia* had nitrogen enrichment in seeds (Table 1) and, consequently, a proportionally longer dependency on seed nitrogen reserves than *T. rosea* and *P. crucigerum*. This was surprising because the relative importance of nitrogen limitation is expected to be greater in tree-fall gaps, on which the latter two species are more dependent for initial establishment. Nitrogen concentration in *C. latifolia* seeds (82 mg g^{-1}) was unusually high, ranking among the top few per cent of 1253 plant species for which seed nitrogen concentration was examined in a large economic botanical survey (Barclay & Earl 1974). Other species that have similarly high nitrogen concentration in seeds are mostly in the Proteaceae, including species that inhabit relatively open sites on nurient-poor soils in Australia (Milberg, Pérez-Fernández & Lamont 1998). Tissue nitrogen concentration of *C. latifolia* in 'sun, 0% N' was still high (42 mg g^{-1}) at 25 days, even after being diluted by biomass growth. What is the adaptive significance of nitrogen enrichment in the seeds of *C. latifolia*, seedlings of which primarily establish in energy-limited environments? Trenching experiments have shown that nitrogen availability in the understorey may be significantly reduced by root competition in some tropical rainforests (Coomes & Grubb 2000; Lewis & Tanner 2000). Although the soils on BCI are not deficient in nitrogen (Yavitt & Wieder 1988; Yavitt 2000), avoiding the energy costs associated with nitrogen uptake and assimilation by starting with enough nitrogen capital is advantageous for seedling establishment in shade. Across ecosystems differing in soil nitrogen availability, the importance of nitrogen capital in seeds has been shown for both legumes and non-legumes (Fenner & Lee 1989; Hanley & Fenner 1997; Milberg *et al.* 1998). The ecological significance of differences in seed nutrient concentration among species (Fenner 1983; Grubb & Burslem 1998; Grubb & Coomes 1997)

deserves further evaluation in relation to their seed size, developmental morphology, seed-reserve dependency period, and preferred microhabitats for regeneration (e.g. soil type, topography, gaps).

Tabebuia rosea and *C. latifolia* lost approximately 30% of the total nitrogen capital in seeds during germination (1·54 mg N per seed to 1·1 mg per seedling at 7 days in *T. rosea*; Fig. 5; 15·4 mg N per seed to 11·0 mg N per seedling at 13 days in *C. latifolia*), presumably due to leaching, as reported for other species (Simon & Harun 1972). Not surprisingly, soil nitrogen availability had significantly affected tissue N concentration before it affected growth in sun. In shade, even after the initial leaching loss of seed N reserves, seedling tissue N concentration remained at or above the value for the reference plants (sun, 100% N). *Tabebuia rosea* seedlings in shade showed a significant luxurious consumption under high N availability (significantly higher N content and concentration for 100% N plants than for 10 or 0% N plants in shade, $P = 0·05$, Student–Newman–Keuls multiple comparison test).

EFFECTS OF COTYLEDON PHOTOSYNTHETIC FUNCTION

As predicted, the seedling mass of *T. rosea* with photosynthetic cotyledons responded to light availability earlier (7 days) than that of *C. latifolia* (16–20 days) and *P. crucigerum* (9–13 days) with primarily storage cotyledons (Figs 2–4). *Tabebuia rosea* and *P. crucigerum* had similar seed mass, but *T. rosea* with photosynthetic cotyledons responded to light availability earlier than *P. crucigerum*. *Tabebuia guyacan*, a congener of *T. rosea* with slightly smaller seed size (26·3 mg) but similarly photosynthetic cotyledons, responded to light at the same time (7 days) as *T. rosea* in an identical experiment (K. K., unpublished results).

Photosynthetic capacity per unit mass of *C. latifolia* cotyledons was low, and marginally enough to cover the cotyledons' own respiration even under saturating light (Table 1). Unlike thinner dual-function cotyledons that export their autotrophic carbon gain (e.g. *Acer* spp. studied by Ampofo, Moore & Lovell 1976), the green, thick cotyledons of *C. latifolia* act primarily as storage organs and export negligible photosynthate, like those of *Phaseolus* spp. (Lovell & Moore 1970). The later timing of growth curve divergence due to light deficiency for *C. latifolia* (16–20 days) than for *P. crucigerum* (9–12 days) was the opposite of what would be expected if the small photosynthetic capacity of *C. latifolia* matters.

Overall, a larger seed size resulted in a proportionally larger initial seedling size and a slightly longer period of support by seed reserves. The total seedling mass at the time of growth curve divergence due to light availability differed among species (0·03 g for *T. rosea*; 0·16 g for *C. latifolia*; 0·05 g for *P. crucigerum*) at the same magnitude as seed mass differences (Table 1). The duration of seed-reserve dependency should also

be negatively correlated with the developmental rate and inherent RGR of the species. The three species had similar RGR of non-cotyledonous biomass during complete seed-reserve dependency (= initial slopes of plots in centre panels of Figs 2–4), although it became significantly different after autotrophic carbon gain became important (Table 2). Thus the rate of seed reserve utilization *per se* was not the reason for the difference in duration of strict seed-reserve dependency among the three species.

ADVANTAGE OF FUNCTIONAL GROWTH ANALYSIS

Cotyledon retention time is not equal to the dependency periods for any specific resource type. This is obvious for highly photosynthetic cotyledons that continue to function like true leaves (Harris, McKender & Smith 1986; Lovell & Moore 1971; Marshall & Kozlowski 1974). Photosynthetic cotyledons of *T. rosea* were fully functional for more than 80 days. Abscissions of storage-type cotyledons of *C. latifolia* and *P. crucigerum* occurred as early as 30 and 28 days, respectively. However, some cotyledons of these species had not abscised as late as 80 days, even though almost all their mass was lost at about 40 days (data not shown). Storage cotyledons of some other species may remain attached to the stem for a long period after seed reserves cease to be exported, but are kept as an insurance that would allow recovery from accidental shoot loss (Harms & Dalling 1997).

Despite the large numbers of plants required for functional growth analysis, the method used here is promising in estimating seed-reserve dependency periods for specific types of resources. The number of plants necessary for destructive harvests may be reduced by estimating biomass and leaf area nondestructively with allometric equations. More species need to be compared with the method used here before any generalization can be made on the effects of cotyledon functional morphology, seed size and seed quality on the duration of seed-reserve dependency. These traits are correlated with each other across a broad range of taxa in relation to their life-history strategies. The method can also be applied to studies in which the duration of seed-reserve dependency is of interest, such as comparisons between species native to poor *vs* rich soil, and between pioneer *vs* climax species. It would be particularly interesting to quantify the period of seed-reserve dependency for energy, nitrogen and phosphorus in relation to the establishment of mycorrhizal symbiosis (Janos 1980).

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