The effect of light, seed size and biomass removal on cotyledon reserve use and root mass allocation in *Gustavia superba* seedlings

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Abstract: Some large-seeded tree species have cotyledonary reserves that persist for months after seedling establishment. We carried out two screened growing-house experiments with seedlings of *Gustavia superba* (Lecythidaceae) to test hypotheses proposed to explain why cotyledons are retained. We grew seedlings from large and small seeds in sun and shade to determine if cotyledon reserves supplement photosynthetic carbon gain, and in a second experiment applied defoliation and shoot removal treatments to determine if reserves are allocated to resprout tissue. In each experiment we tracked cotyledonary resource use over time and measured the fraction of seedling biomass allocated to roots and shoots. We found no evidence that light environment, seed size or damage treatment affected the rate of cotyledon resource usage; 20% of the cotyledonary mass remained 9 wk after leaves were fully developed in both sun and shade and 25–30% of the cotyledonary mass remained 6 wk after leaf or shoot removal. Instead, cotyledon reserves appear to be slowly translocated to roots regardless of light environment or seedling damage. Once seedlings are established, lost tissue is replaced using reserves stored in roots; in high light, damaged seedlings had a lower root mass fraction (0.42) than undamaged ones (0.56) when considering the mass of tissue removed and resprout tissue combined. We conclude that cotyledon reserves are important for resprouting during early seedling emergence and establishment, but do not directly contribute to seedling growth or biomass recovery from herbivores at the post-establishment stage. Persistence of cotyledons may ultimately depend on the development of sufficient root mass for reserve reallocation.

Key Words: resprouting, seedling growth, seed reserves, shade tolerance

INTRODUCTION

Large seed reserves are a feature of many tropical woody species (Baraloto & Forget 2007, Foster & Janson 1985, Hammond & Brown 1995, Kelly 1995, Metcalfe & Grubb 1995). The utility of these reserves has been linked to many factors: large seed mass may increase the probability that seeds buried deep in the soil are able to establish (Dalling & Harms 1999, Molofsky & Augspurger 1992), and may confer tolerance to seed predators when partially consumed (Harrington *et al.* 2005, Mack 1998). At the post-establishment phase seed reserves can be used to replace seedling tissue consumed by herbivores (Green & Juniper 2004a, Harms & Dalling 1997) or damaged by falling litter and trampling (Clark & Clark

1989). Large seed mass may also provide a source of energy reserves that can be used to maintain a positive carbon balance or to supplement photosynthetic carbon gain for seedlings that persist for long periods under very low light conditions (Boot 1996; but see Baraloto *et al.* 2005, Myers & Kitajima 2007).

The ability of seed reserves to confer these benefits depends in part on where they are located on the seedling, how long they are conserved by the growing seedling and when they are re-allocated to other structures. In some species, cotyledonary seed reserves are retained for weeks or months after the production of a functional leafy shoot (Dalling & Harms 1999, Edwards *et al.* 2001, Green & Juniper 2004b). It has been suggested that this delayed use of cotyledon reserves may in part be determined by the seedling's light environment (Dalling & Harms 1999, Rose & Poorter 2003). Here we explore how cotyledonary reserves are used in sun and shade in a large-seeded tree species, *Gustavia superba* (H.B.K.)

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Berg (Lecythidaceae). The principal characteristics of the seed ecology of this species are the ability to germinate after substantial pre-dispersal seed damage, the preservation of hypogeal cotyledonary reserves long after the establishment of a functional seedling, and the ability to resprout multiple times following browsing damage (Dalling & Harms 1999, Harms *et al.* 1997). These characteristics may be common among large-seeded tropical tree species (Baraloto & Forget 2007, Harms & Dalling 1997); they have been reported for taxa in a range of families including Lauraceae (*Beilschmiedia*, *Chloro-cardium*, *Endiandra*), Fabaceae (*Carapa*, *Castanospermum*, *Prioria*), Sapindaceae (*Castanospora*) and Calycanthaceae (*Idiospermum*) (Dalling *et al.* 1997, Edwards & Gadek 2002, Edwards *et al.* 2001, Green & Juniper 2004a, b; Harms & Dalling 1997, ter Steege *et al.* 1994).

Our previous experimental studies of *G. superba* seed ecology have examined cotyledonary resource use in the screened growing house under relatively low light conditions (0.4–$1.0\,\mathrm{mol\,m^{-2}\,d^{-1}}$ PAR; Dalling & Harms 1999, Harms *et al.* 1997). These studies showed that significant cotyledonary resources remained attached to seedlings for up to 9 mo after germination. Removal of cotyledonary reserves significantly affected seedling growth for at least the first 3 wk after germination, and were essential for successful resprouting following a shoot removal treatment that simulated browsing damage (Dalling & Harms 1999). Furthermore, cotyledonary resource allocation to resprouting seedlings appears to be tightly regulated; repeated shoot removal from resprouting plants resulted in the production of successively smaller resprouts where each new shoot was constructed from a fixed proportion of remaining cotyledonary reserves (Dalling & Harms 1999).

Gustavia superba cotyledons are especially attractive and vulnerable to insect and mammalian predators (Forget 1992, Sork 1985, 1987), thus retaining cotyledon reserves after emergence may incur costs (Dalling & Harms 1999). As large seedlings that lack cotyledons are also able to resprout, Dalling & Harms (1999) suggested that at some point in ontogeny a shift occurs from dependency on seed reserves to reserves stored in stems and roots for resprouting after defoliation events. A similar suggestion was made by Myers & Kitajima (2007) for other tropical tree species. Thus, cotyledonary reserves may be important for resprouting during the first few weeks after seedling emergence, but once roots and stems become developed cotyledonary tissues may be translocated to other parts of the seedling.

In this study we explored how light availability, seed mass and biomass removal affected cotyledonary resource usage and biomass allocation to roots. If cotyledonary persistence primarily functions to permit long-term seedling survival under very low light conditions in forest understoreys (Foster 1986, Osunkoya *et al.* 1984), then

we predict (1) that the rate of cotyledonary resource use will be higher in the shade than in the sun. If larger seeds maintain a greater proportion of reserves in storage as the seedling develops (i.e. the 'reserve effect' hypothesis, Leishman *et al.* 2000, Westoby *et al.* 1996; but see Baraloto *et al.* 2005, Green & Juniper 2004b), then we predict (2) a relatively lower rate of cotyledonary use for seedlings produced from larger seeds. If cotyledonary reserves are an adaptation to permit seedling resprouting after shoot damage (Baraloto & Forget 2007, Dalling & Harms 1999, Green & Juniper 2004a), then we predict (3) higher cotyledonary use in damaged than in undamaged seedlings. On the other hand, if root reserves, rather than cotyledon reserves, are used to replace shoot tissue after damage (Baraloto & Forget 2007, Myers & Kitajima 2007), then we predict (4) a lower root biomass fraction (RMF) for damaged seedlings compared with undamaged ones when considering both the tissue removed by defoliation and resprout tissue combined.

Thus, in this study we carried out two experiments. We first analysed the effects of light availability on cotyledon persistence and biomass allocation to roots of *G. superba* seedlings through the first 4 mo after germination. Then, we analysed the effects of light availability and seed size on the response of seedlings to different biomass removal treatments. In particular, we evaluated how light availability and seed size affect cotyledon resource usage and root biomass allocation after seedling damage.

METHODS

Species description and study site

Gustavia superba is a common subcanopy tree of humid tropical forests of Central America (Prance & Mori 1979). At the study site, Barro Colorado Island in central Panama (described in detail in Leigh 1999), *G. superba* is abundant in 60–100-y-old secondary forest and on exposed peninsulas along the shore of the island (Croat 1978). *Gustavia superba* produces large (150–600 g) indehiscent fruits that fall to the ground and are broken or opened by scatterhoarding rodents, which subsequently bury some of the seeds (Forget 1992, Sork 1985). Fruits contain 2–20 seeds varying in size from 3–30 g. Seeds lack a protective seed coat and consist mostly of cotyledonary tissue (Dalling & Harms 1999). Germination of *G. superba* seeds occurs within a few weeks of dispersal and may be hypogeal if seeds are buried (Molofsky & Augspurger 1992), or semi-hypogeal when seeds remain on the soil surface (Kitajima 1992). Seeds successfully germinate from small cotyledonary fragments (Harms *et al.* 1997), or following extensive damage by larvae of sesiid moths (Dalling & Harms 1999, Harms & Aiello 1995). Leaf area is produced in flushes of 3–12 leaves that developed very

quickly, expanding from 20% of full size to full size in 4 d (Aide 1991, Aide & Londoño 1989).

Experimental design

In July 2000, we collected seeds from fallen intact fruits beneath *c.* 10 *G. superba* trees along Barbour trail on Barro Colorado Island. Seeds were checked for insect infestation (Harms & Aiello 1995) and then stored in dark moist conditions to allow seeds containing insect larvae to be detected. After 1 mo, all rotten or infested seeds were discarded. The remaining intact seeds were weighed individually and ordered according to their fresh mass. Unusually large or small seeds were discarded to leave a subset of 200 seeds (13.80 ± 0.36 g, mean $\pm$ SE; range: 4.12–32.52 g). Of these seeds, ten were randomly selected and oven-dried at 70 °C for 48 h to estimate initial dry mass. The remaining seeds were then randomly subdivided into two groups for use in the experiments described below. For Experiment 1 a set of 42 seeds was randomly allocated to two light treatments (both treatments had similar mean seed mass: *t*-test $= -0.18$; df $= 40$; P $= 0.86$; high light $= 12.95 \pm 1.08$ g and low light $= 13.21 \pm 1.08$ g). For Experiment 2 the remaining seeds were divided into 'small' (10.06 ± 0.24 g; mean $\pm$ SE; range 6.61–11.98 g) and 'large' seeds (18.22 ± 0.66 g; range 12.88–27.89 g) and then also allocated to two light treatments in a stratified random manner to ensure equality of mean seed masses.

In August 2000, seeds from both experiments were placed in a screened growing house under the two light conditions. A 'sun' treatment simulated light conditions in small treefall gaps ($7\text{–}12 \, \text{mol m}^{-2} \, \text{d}^{-1}$ PAR; approximately 10% of full sun), and the 'shade' treatment simulated forest understorey conditions ($0.4\text{–}0.8 \, \text{mol m}^{-2} \, \text{d}^{-1}$ PAR; approximately 0.6% of full sun). Seeds were sown individually on the surface of large (7.5 L) pots to prevent seedlings from becoming pot-bound, and were filled with a 3:1 mixture of forest soil and rinsed sea sand. Pots were watered once a week as required. Several seedlings developed two or more concurrent above-ground sprouts. These seedlings were removed from the data prior to analyses (Dalling & Harms 1999, Green & Juniper 2004a).

Experiment 1: Light effects on cotyledonary resource use and biomass allocation

To determine how light availability influences cotyledon persistence and biomass allocation we followed seedling growth over the first 4 mo after seed germination. We tracked the germination and leaf expansion date of each seedling, and harvested three seedlings from each light treatment at seven time periods (4, 7, 12, 20, 34, 55 and 90 d after first full leaf expansion). At harvest time we measured seedling height and calculated leaf area from regression equations developed in a separate field study (Barberis 2001). Seedlings were then separated into leaves, stem, root and cotyledon and their dry mass estimated. For each harvest date we calculated the proportion of cotyledonary mass remaining (i.e. cotyledon dry mass at the end/initial seed dry mass calculated by regression; CR/CI). We also calculated the mass fraction of biomass (excluding cotyledons) allocated to root (RMF), stem (SMF) and leaves (LMF), where RMF + SMF + LMF = 1. Finally, we calculated the mass fraction of biomass allocated to cotyledons (CMF).

Experiment 2: Light and seed-size effects on response to simulated herbivory

To determine how light and seed size affect the ability of seedlings to resprout we tracked the germination and leaf expansion of individual seedlings as before. Three weeks after full leaf expansion, eight seedlings from each light and seed-size treatment combination were allocated to one of the following damage treatments: (1) control (no damage) (2) 100% defoliation, (3) 100% defoliation and stem excision just above the cotyledonary attachment. These damage levels were designed to simulate common damage suffered by *G. superba* seedlings when attacked by insect herbivores (treatment 2) or when browsed by mammals or damaged by falling debris (treatment 3). At the time of treatment application we dried and weighed the leaves and stem harvested from each plant, and measured the leaf area harvested.

Six weeks after treatment application, seedlings were harvested and separated into leaves, stem, root and cotyledons. Plant height, leaf number, leaf area, root length and dry mass were measured, and the specific leaf area (SLA; leaf area per unit leaf dry mass) was calculated. For each combination of light, seed size and biomass removal treatment we calculated the leaf area ratio (LAR; leaf area per unit total plant dry mass) and biomass allocation (excluding cotyledons) to root (RMF), stem (SMF) and leaf (LMF). As in the first experiment, we also calculated the mass fraction of biomass allocated to cotyledons (CMF). These variables were calculated for the final harvest mass only (H), and also for the sum of the harvest mass and the mass of excised tissue removed when treatments were applied (H+T). Finally, we estimated the proportion of the initial cotyledon mass remaining by the end of the experiment (CR/CI). During the experiment several seedlings were damaged by agoutis, which entered the screened growing house, reducing the final sample size to 74 seedlings.

Table 1. Summary statistics for general linear models performed on plant trait data of *Gustavia superba*. Bold values denote significant differences (P < 0.05). Where the Time × Light interaction was not significant general linear models were re-run omitting the interaction term.

Variables	df	Time (logdays)		Light		Time × Light	
		F	P	F	P	F	P
RMF	1, 37	33.8	**<0.0001**	3.6	0.0648	14.8	**0.0005**
SMF	1, 37	0.1	0.882	1.2	0.282	9.9	**0.0032**
LMF	1, 38	38.8	**<0.0001**	3.1	0.0883		
Stem length	1, 38	12.7	**0.0010**	32.2	**<0.0001**		
Root length	1, 37	25.2	**<0.0001**	3.0	0.0945	6.3	**0.0169**
Leaf number	1, 37	21.1	**<0.0001**	1.9	0.175	5.0	**0.0309**
Biomass without cotyledons	1, 37	29.7	**<0.0001**	2.3	0.135	6.8	**0.0130**
Cotyledonary mass	1, 38	20.7	**<0.0001**	0.1	0.917		
CMF	1, 38	109.2	**<0.0001**	7.4	**0.0099**		
CR/CI	1, 38	42.7	**<0.0001**	0.3	0.619		
Biomass with cotyledons	1, 38	0.1	0.897	2.4	0.126		

Statistical analyses

Experiment 1

The effects of light treatment on (1) time to seedling emergence, (2) time from seedling emergence to the stage of fully expanded leaves, and (3) the initial number of expanded leaves were analysed using Mann–Whitney U-tests. The effect of light treatment on seedling height and leaf area at full leaf expansion was analysed with t-tests. Both variables were log_{10}-transformed to improve the homogeneity of variances.

Differences in cotyledon mass persistence (CR/CI), plant architecture (height, leaf area and biomass) and biomass allocation (RMF, SMF, LMF and CMF) through time between light environments were analysed separately with general linear models. Light environment was used as a categorical factor and time (in log_{10} d after leaves were fully expanded; logdays) was treated as a covariate. Data were analysed for residual normality and homoscedasticity. All variables were either log_{10}- or arcsine-transformed to improve the homogeneity of variance. The slopes of the relationships between the transformed response variables and logdays were compared using a model that included the logdays × transformed response term. Where the interaction term was not significant, the model was refitted, assuming a common slope, and intercepts were compared. F-tests were run considering Type III Sums of Squares.

Experiment 2

General linear models were used to analyse the effects of seed size, light environment, different damage levels and their interactions on (1) time to seedling emergence, (2) time from seedling emergence to the stage of fully expanded leaves, and (3) seedling height, leaf number and leaf area at the time of full leaf expansion and at treatment application time. Variables were log_{10}-transformed to improve homogeneity of variances.

The effects of seed size, light environment and different biomass removal intensities on leaf number, stem length

(height), root length, leaf area, total biomass, root mass, stem mass, leaf mass, cotyledon mass, SLA, LAR, RMF, SMF, LMF and CMF were analysed separately with general linear models. Variables were log_{10}- or arcsine-transformed to improve the homogeneity of variance where appropriate. F-tests were run considering Type III Sums of Squares. As there were no effects of seed size or its interaction with habitat and damage treatments for all allocation variables, we pooled the small- and large-seed data and ran the tests again, without including the seed size factor. All tests were done using SAS 8.0 (SAS Institute Inc., Cary, North Carolina, USA).

RESULTS

Experiment 1

Light treatment did not affect the timing of seedling emergence (Sun median = 24 d and shade median = 22 d; Mann–Whitney U-test: U = 463; N = 21 for both samples; P = 0.77), but affected the time elapsed between emergence and full leaf expansion (U = 284; N = 21 for both samples; P < 0.001). Seedlings grown in the sun grew faster (median = 13 d) than seedlings grown in the shade (median = 22 d). At the time of full leaf expansion there were no differences between seedlings grown in high or low light either in leaf number (median for both samples = 5 leaves; U = 468; N = 21 for both samples; P = 0.67) or in leaf area (Sun = 130.0 cm^2 and shade = 133.7 cm^2; t-test: t = −0.12; df = 40; P = 0.91). However, seedlings grown in the sun were shorter compared to those in the shade (Sun = 12.1 cm and shade = 18.6 cm; t = −5.53; df = 40; P < 0.001).

Total biomass without cotyledons increased with time (Figure 1a). However, overall, the net transfer of resources out of the cotyledons resulted in a non-significant change in total seedling biomass with time (Table 1, Figure 1b). Throughout the study period, seedlings increased their
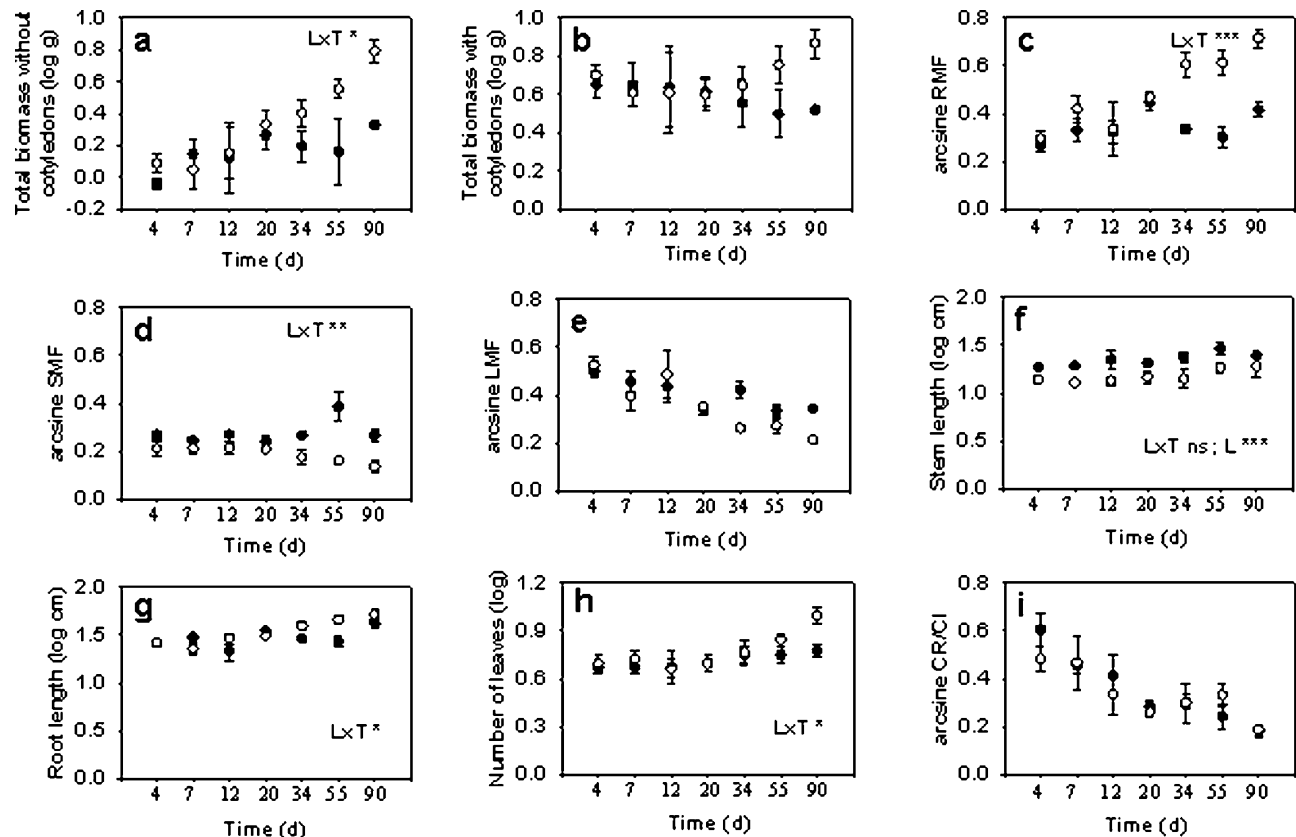
Figure 1. Variation in plant size (a, b, f, g, h), biomass allocation (c, d, e), and cotyledon resource use (i) in relation to time after leaves were fully developed for *Gustavia superba* seedlings grown in sun (empty circles) and shade (filled circles). N = 3 for each Time × Light treatment combination. Mean and SE are shown. L×T denotes Light × Treatment interactions. Note that Time (d) is plotted on a log scale.

non-cotyledon root mass fraction as well as their leaf number, stem and root length, and decreased their leaf mass fraction; stem mass allocation was unchanged (Table 1; Figure 1c–h). Cotyledonary mass, and the fraction of cotyledonary reserves remaining (CR/CI) decreased (Table 1, Figure 1i). There were significant interactions between time since leaf expansion (logdays) and light environment for several variables (Table 1). Seedlings grown in the sun had longer and heavier roots (Figure 1c, g), whereas seedlings grown in the shade allocated more to stem biomass (Figure 1d). In contrast there was no difference in the proportion of cotyledon mass remaining (CR/CI) at harvest between light environments (Figure 1i).

Experiment 2

The effects of light environment on the timing of emergence, time to full expansion, leaf number, area and stem length were similar to those reported for Experiment 1. Seed mass did not affect the timing of seedling emergence (large seeds = 19.9 ± 1.2 d; small seeds = 20.6 ± 1.4 d; $F_{1,62} = 0.15$, P = 0.69), or the time elapsed between

emergence and full leaf expansion (large seeds = 13.6 ± 0.4 d; small seeds = 14.6 ± 0.5 d; $F_{1,62} = 2.21$, P = 0.14). At the stage of full leaf expansion seedlings from large seeds had more leaves (large seeds = 5.0 ± 0.1 leaves and small seeds = 4.4 ± 0.1 leaves; $F_{1,62} = 7.41$, P = 0.008), and a higher leaf area (large seeds = 160.7 ± 1.07 cm^2 and small seeds = 115.6 ± 1.07 cm^2; $F_{1,62} = 11.62$, P = 0.001) compared with those from small seeds.

There were no significant differences in seed size, seedling emergence or growth among biomass removal treatments before treatment application (all P > 0.05). At treatment time, seedlings that originated from larger seeds had higher leaf mass ($F_{1,62} = 17.9$, P < 0.01), and leaf area ($F_{1,62} = 13.3$, P < 0.01) than those from smaller seeds. In contrast, there were no differences in these variables between light treatments (all P > 0.05). There were no differences among biomass removal treatments for leaf mass and leaf area (both P > 0.05).

Seed size had no effect on biomass allocation patterns (SLA, LAR, RMF, SMF and CMF were not significantly different between large and small seeds; all P > 0.05; data not shown). However, at harvest time, seedlings produced from large seeds had greater total biomass, greater root, stem, leaf and cotyledon mass, and larger leaf area and leaf

Table 2. Summary statistics (F and P values) for general linear models performed on plant size of *Gustavia superba* for two data sets (a) excluding the biomass of removed tissues and (b) including the biomass of removed tissues. Bold values denote significant differences (P < 0.05). Light: high vs. low light. Seed size: Small seeds vs. large seeds. Biomass removal: control (no damage), 100% defoliation, and 100% defoliation and shoot excision. For all other interactions there were no significant differences (P > 0.05).

Variables	Light $F_{1,62}$	P	Seed size $F_{1,62}$	P	Biomass removal $F_{2,62}$	P	L × T $F_{2,62}$	P
Without removed tissues								
Total biomass with cotyledons	8.55	**0.0048**	44.9	**<0.0001**	4.60	**0.0137**	2.97	0.0588
Total biomass without cotyledons	13.38	**0.0005**	30.8	**<0.0001**	27.2	**<0.0001**	2.63	0.0802
Cotyledonary mass	0.93	0.338	22.6	**<0.0001**	2.36	0.103	0.66	0.518
Root mass	13.10	**0.0006**	23.6	**<0.0001**	5.30	**0.0075**	5.18	**0.0083**
Stem mass	0.35	0.554	26.9	**<0.0001**	84.1	**<0.0001**	1.42	0.250
Leaf mass	17.22	**0.0001**	10.5	**0.0020**	47.4	**<0.0001**	0.32	0.724
Leaf area	6.37	**0.0143**	10.4	**0.0021**	25.8	**<0.0001**	2.38	0.101
Leaf number	16.10	**0.0002**	5.69	**0.0202**	22.8	**<0.0001**	0.63	0.536
Stem length	21.62	**<0.0001**	1.53	0.220	26.4	**<0.0001**	2.91	0.0620
Root length	1.21	0.275	3.89	0.0529	1.63	0.203	3.83	**0.0270**
With removed tissues								
Total biomass with cotyledons	8.95	**0.0040**	46.10	**<0.0001**	0.20	0.8182	3.20	**0.0474**
Total biomass without cotyledons	12.54	**0.0008**	33.84	**<0.0001**	4.01	**0.0230**	3.59	**0.0335**
Stem mass	0.02	0.8753	24.76	**<0.0001**	2.96	0.0590	0.97	0.3853
Leaf mass	13.93	**0.0004**	24.40	**<0.0001**	0.45	0.6409	1.62	0.2054
Leaf area	1.42	0.2384	25.72	**<0.0001**	1.24	0.2969	2.49	0.0910

number than seedlings from small seeds (Table 2; Figure 2 a–g). Cotyledonary persistence (CR/CI) was unaffected by seed mass ($F_{1,62} = 1.98$; P = 0.164). Moreover, there were no significant interactions of seed mass with light environment or biomass removal (for all size and biomass allocation variables P > 0.05; data not shown).

As observed in Experiment 1, seedlings grown in high light were shorter, but had more leaves, leaf area, and biomass (root and shoot mass) compared with seedlings grown in the shade (Table 2, Figure 2). Biomass removal (i.e. leaf or shoot removal 3 wk after leaves were fully expanded) reduced leaf number, leaf area and total biomass by the end of the experiment (Table 2, Figure 2). However, most of these treatment effects on biomass variables disappeared when the tissue biomass harvested at treatment time was added to the final harvest biomass (Table 2).

As expected, removal of shoot or foliar tissue modified most biomass allocation patterns at harvest time (Table 3, Figure 3a–f). Thus cotyledon mass fraction (CMF) ranged from 0.3 in control plants to almost 0.6 in damaged seedlings (Table 3; Figure 3d). However, biomass removal did not affect the proportion of cotyledonary mass remaining by the end of the experiment (CR/CI; Figure 3g). Non-defoliated seedlings had higher LMF, higher LAR and lower SLA than seedlings from both shoot and leaf removal treatments (Table 3; Figure 3c, e, f). They also had lower RMF and higher SMF than those from the shoot removal treatment, but not than those from the leaf removal treatment (Table 3; Figure 3a, b).

There were no interactions between seed size and damage treatments for any size or allocation variable (all P > 0.05; data not shown). In contrast, there were significant interactions of light environment with

the damage treatments for several size and allocation variables (Tables 2 and 3; Figures 2 and 3). Excluding cotyledonary mass, only seedlings that were in the no-damage treatment grew larger and had higher root mass, in high versus low light (Table 2; Figure 2a, c). In low light, there were no differences among biomass removal treatments in RMF when tissue biomass harvested at treatment time was added to the final harvest data (Table 3; Figure 3h). In contrast, in high-light conditions, seedlings of both damage treatments had lower RMF when tissue biomass harvested at treatment time was added to the final harvest data than undamaged seedlings (Figure 3h). However, it should be noted that RMF following resprouting was not related to the resprout biomass (Figure 4a, b), nor was it related to the sum of total biomass produced at harvest and resprout shoot biomass (Figure 4c, d).

DISCUSSION

Light effects on cotyledonary resource use and biomass allocation

In common with several large-seeded tropical tree species (Dalling & Harms 1999, Dalling *et al.* 1997, Edwards & Gadek 2002, Edwards *et al.* 2001, Green & Juniper 2004b), less than half of the cotyledonary mass of *G. superba* was used for seedling construction (about 40% when first leaf expansion occurred). About 20% of the cotyledonary mass of *G. superba* still remained 90 d after first full leaf expansion in both high- and low-light environments. Thus, our results did not support our first hypothesis that the retention of cotyledonary
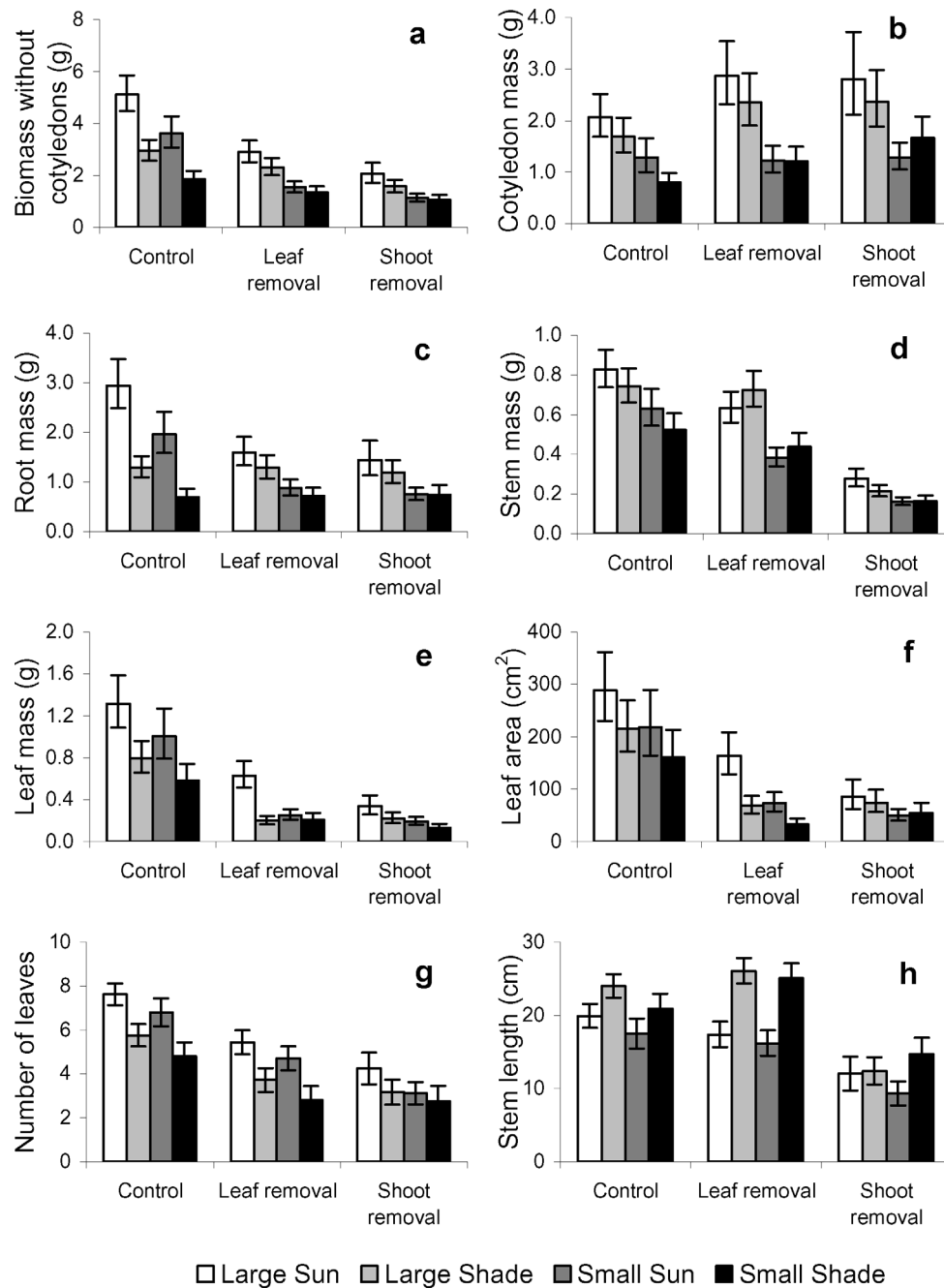
Figure 2. Effects of light, seed size and biomass removal on total seedling biomass excluding cotyledons (a), biomass components (b–e), leaf area and number (f–g) and stem length (h) for *Gustavia superba* seedlings at the time of harvest. Mean and SE are shown.

tissue after seedling emergence provides resources to supplement photosynthetic carbon gain under low-light conditions. This is consistent with observations made on *Chlorocardium rodiei* seedlings by ter Steege *et al.* (1994). Based on the assumption that all *Chlorocardium* seedlings were 1 y old, they found no difference in cotyledonary mass along a gradient of light availability. However, in an accompanying experiment *Chlorocardium* seedling survival over 8 wk was strongly negatively affected by cotyledon removal (ter Steege *et al.* 1994).

In agreement with previous field studies, *G. superba* seedlings grown in high light were larger than those from low light (Molofsky & Fisher 1993, Sork 1987), and had higher root:shoot ratios than seedlings grown in low light (Barberis 2001). These differences in biomass allocation between light environments may simply be related to ontogeny (i.e. 'apparent plasticity'; Weiner 2004, Wright & McConnaughay 2002). As *G. superba* produces leaves in flushes (Aide 1991), and we recorded differences in root mass but not in leaf or stem mass between

Table 3. Summary statistics (F and P values) for general linear models performed on allocation variables for *Gustavia superba* plants growing in different light environments (high vs. low), and under different biomass removal treatments (control (no damage), 100% defoliation, and 100% defoliation and shoot excision) for two data sets (a) excluding the biomass of removed tissues and (b) including the biomass of removed tissues. Bold values denote significant differences ($P < 0.05$).

Variables	Light $F_{1,68}$	P	Biomass removal $F_{2,68}$	P	L × T $F_{2,68}$	P
Without removed tissues						
CR/CI	3.36	0.0713	2.13	0.127	0.53	0.594
CMF	4.31	**0.0416**	38.8	**<0.0001**	0.22	0.806
SLA	55.4	**<0.0001**	19.3	**<0.0001**	1.92	0.154
LAR	0.50	0.480	7.16	**0.0015**	4.66	**0.0127**
RMF	1.24	0.268	45.7	**<0.0001**	8.26	**0.0006**
SMF	27.5	**<0.0001**	43.2	**<0.0001**	6.99	**0.0017**
LMF	4.41	**0.0395**	34.6	**<0.0001**	5.12	**0.0085**
With removed tissues						
CMF	2.79	0.0994	9.97	**0.0002**	0.15	0.860
LAR	12.8	**0.0006**	22.8	**<0.0001**	2.87	0.0636
RMF	7.01	**0.0101**	8.52	**0.0005**	5.48	**0.0062**
SMF	27.1	**<0.0001**	2.20	0.119	5.68	**0.0052**
LMF	0.29	0.591	9.39	**0.0003**	2.57	0.0838

light environments, high-light plants may have been accumulating carbohydrate reserves in their roots prior to the next leaf flush. This is possible as *G. superba* has a coarse taproot (López & Kursar 1999, Tyree *et al.* 1998), which is likely to store reserves as observed in other tropical species (Ichie *et al.* 2001). Differences in RMF between high- and low–light-grown plants, despite similar rates of reduction of cotyledonary resources, probably therefore reflect a combination of cotyledonary resource and photosynthate translocation to roots in high-light plants, and possibly, cotyledonary resource allocation to shoot mass in low-light plants.

### Light and seed size effects on response to simulated herbivory

Our results did not support our second hypothesis that cotyledonary persistence depends on seed size. We predicted a lower rate of cotyledonary resource use for seedlings produced from larger seeds based on the 'reserve effect' hypothesis (Leishman *et al.* 2000, Westoby *et al.* 1996), which states that larger seeds maintain a greater proportion of reserves in storage as the seedling develops. The absence of a seed size effect on seedling growth or allocation after seedling damage is consistent with the results observed by Green & Juniper (2004a), and with our earlier result where sequential resprout mass produced by individual seeds was a fixed proportion remaining cotyledonary mass (Dalling & Harms 1999). In agreement with Baraloto & Forget (2007) we did not find any interaction between seed size, light and damage treatment. Thus there is no evidence for a 'reserve effect' in *G. superba* at least at the range of seed masses included in this experiment.

Biomass removal (either as leaf or shoot tissue) did not affect cotyledonary use (i.e. CR/CI) despite affecting

biomass allocation to roots, stem and leaves. Thus our data did not support our third hypothesis. If cotyledon reserves are not used for resprouting after biomass removal, it follows that seedlings must use reserves from other organs. It is likely that reserves came from roots, because in high light, damage-treated seedlings had a lower RMF than undamaged ones when all biomass was included (i.e. sum of harvest biomass plus tissue removed when treatments applied). Surprisingly, however this pattern was not observed for seedlings grown in low light, perhaps because the resprout biomass was insufficient to cause a detectable reduction in RMF.

Using observations of biomass allocation through time in our first experiment, we can conclude that, at the time that damage treatments were applied (i.e. 20 d after leaves fully expanded), there would have been no differences between light treatments in RMF (Figure 1a). Forty-two days later, at harvest time, there was an interaction between light and biomass removal treatments for root mass and RMF, such that only seedlings in the no damage treatment grew larger and had higher root mass in high versus low light. Thus, there were no differences in RMF between light treatments for leaf or shoot removal treatments. The absence of a light treatment effect on biomass allocation in the damage treatments may be explained by the time to leaf flushing. While there were no differences in the timing of leaf flush between light treatments for leaf or shoot removal treatments ($P > 0.05$, data not shown), undamaged seedlings grown in high light flushed earlier than those in the shade (17 ± 2 d vs. 28 ± 3 d, respectively; $P < 0.05$). Based on the lack of differences in root biomass allocation and timing of leaf flush between light environments, it seems that the rate of reserve translocation from root to shoot after damage was similar between light environments. To detect differences between light treatments in reserve translocation, future experiments need to be longer and
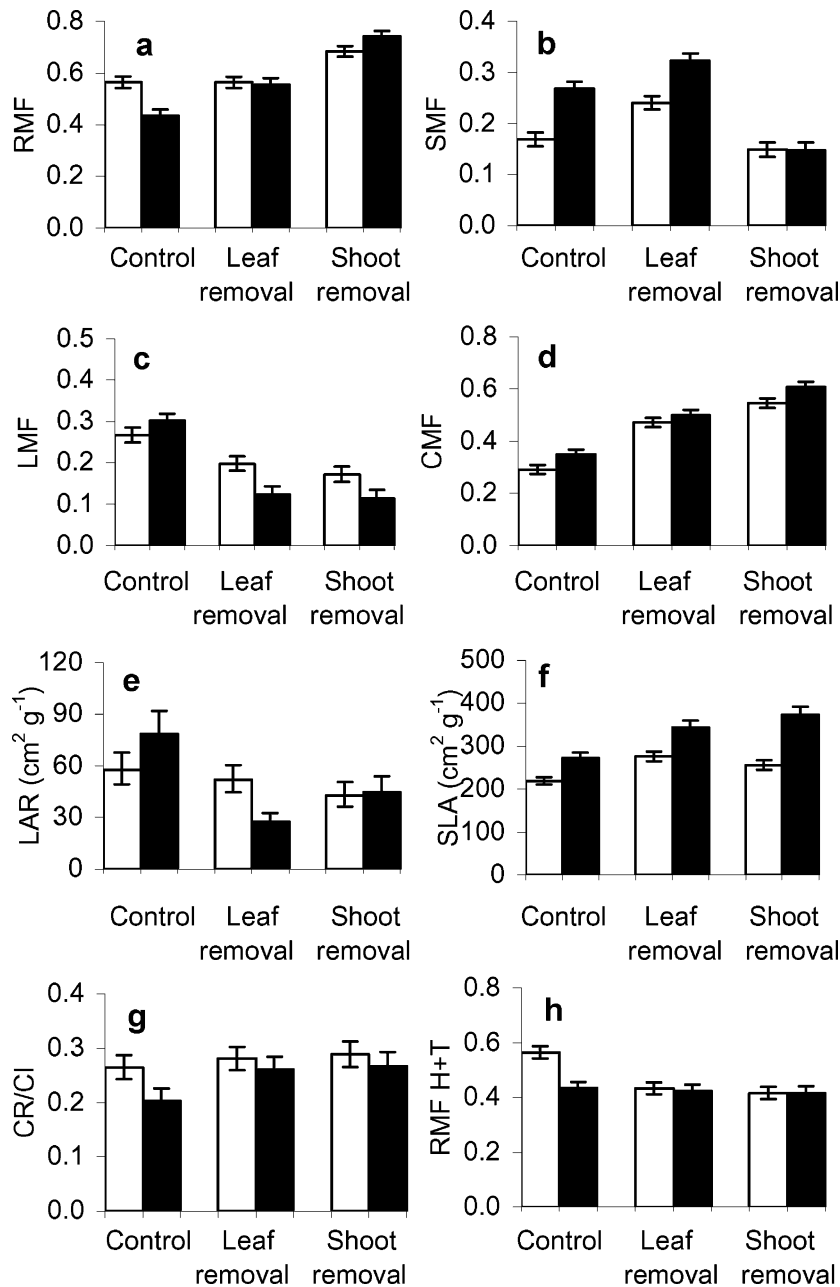
Figure 3. Effects of light and biomass removal on biomass fractions (a–d, h), leaf area allocation (e–f) and cotyledon resource usage (g) at harvest time for *Gustavia superba* seedlings grown in high light (open bars) and low light (filled bars). Mean and SE are shown. H+T denotes the biomass harvested at treatment time added to the resprout biomass.

to include different times of damage application. It would also be useful to include a low light level below the light compensation point, to prevent photosynthate accumulation during the experiment (see Myers & Kitajima 2007).

Overall, these results partially support our fourth hypothesis and also the suggestion of Dalling & Harms (1999) that cotyledonary reserves are important for resprouting mainly in the first few weeks after seedling emergence, when shoot damage is most likely to occur. Once roots become developed however, cotyledonary reserves are

slowly translocated to roots. It should be noted here that we tracked resource allocation using biomass fractions. Increases in RMF and SMF are assumed to reflect greater allocation to carbon storage, however the same pattern would also be observed if plants invested heavily in structural tissue for defence (Canham *et al.* 1999). As pointed out by Baraloto *et al.* (2005), future studies will require not only measurements of cotyledon and seedling biomass through time, but also quantification of structural vs. non-structural carbohydrates in stem and root tissue (Myers & Kitajima 2007).
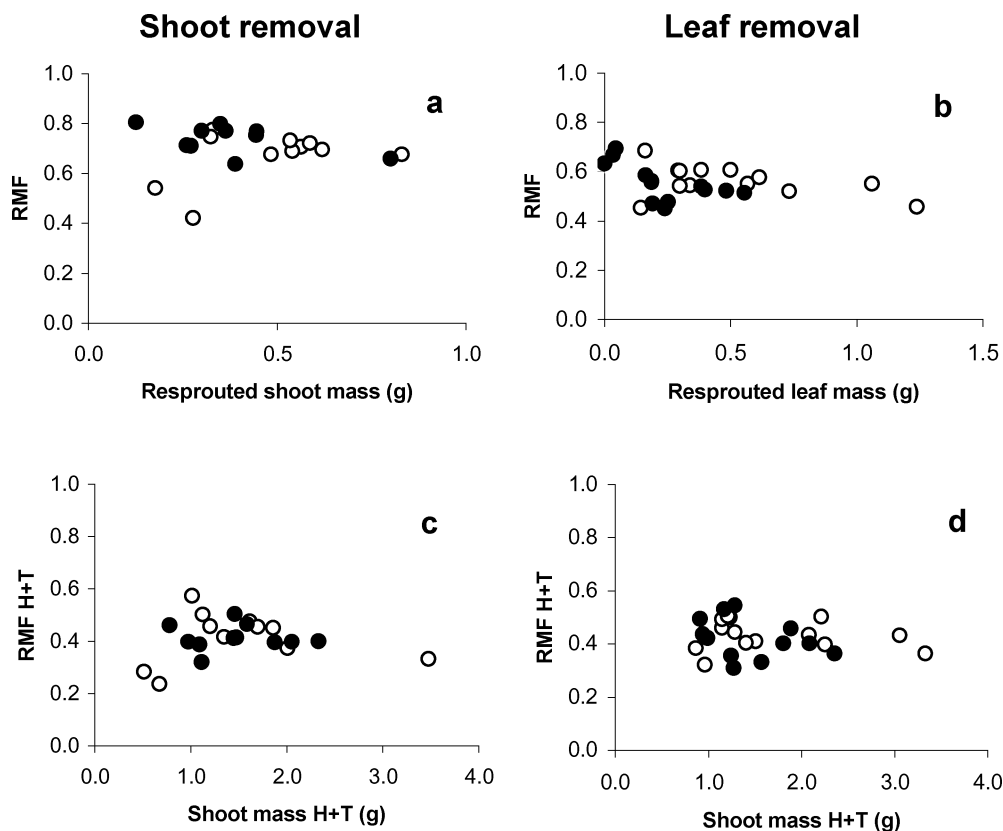
Figure 4. Variation of root mass fraction in relation to resprouted (a, b) and total shoot biomass (c, d) for shoot (a, c) and leaf removal (b, d) treatments in high light (open circles) and low light (filled circles) environments for *Gustavia superba* seedlings. H+T denotes the biomass harvested at treatment time added to the resprouted biomass.

The question that still remains is why *G. superba* retains its cotyledons for weeks after germination, exposing them to damage or removal from scatter-hoarding rodents. A plausible explanation is that cotyledons are retained for as long as necessary for the seedling to develop a taproot to translocate the reserves into. Furthermore, exposed cotyledons that are removed by scatter-hoarders have the potential to develop elsewhere; even small cotyledon fragments of *G. superba* are capable of sprouting into new seedlings through apparent somatic embryogenesis (Harms *et al.* 1997, Harrington *et al.* 2005).

ACKNOWLEDGEMENTS

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