

SHORT COMMUNICATION

**Multiple resprouting from diaspores and single cotyledons in the Australian tropical tree species *Idiospermum australiense***

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In 1999, Dalling & Harms simulated 100% above-ground herbivory on seedlings of *Gustavia superba*, a large-seeded species from Barro Colorado Island, Panama, and showed the remarkable ability for cotyledons to regenerate up to eight new shoots. They used this evidence to propose that cotyledon size (at least in this species) was adaptive in surviving pre- and early post-germination hazards (Dalling & Harms 1999). In this note we describe the first record of multiple resprouting in an Australian tropical plant species. *Idiospermum australiense* (Diels) S. T. Blake (Calycanthaceae) exhibits characteristics similar (but not identical) to *G. superba* that support the contention of Dalling & Harms (1999).

*Idiospermum australiense* is a rare canopy tree species (Briggs & Leigh 1996) that occurs in restricted populations in lowland tropical rain forests in north Queensland, Australia. The species produces the largest dicotyledonary diaspore in the Australian flora. Fresh diaspores can weigh up to 225 g and comprise between two and six thick, starchy cotyledons (modal cotyledon number is four) released within an ephemeral, corky seed coat (Worboys 1999). Diaspores are gravity dispersed and the seed coat rapidly decays, exposing cotyledons to the immediate environment prior to germination. Germination behaviour of *I. australiense* in the field is unusual, and may take either of two possible fates. Diaspores remain intact, in which case a single individual shoot is produced; or diaspores separate into component cotyledons, each of which produces an

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independent shoot (Edwards *et al.* 2001). Both behaviours are observed (R. Jago *pers. comm.*) though separation of cotyledons is less common (*pers. obs.*).

Total reserve mass in intact diaspores and in single cotyledons appears greater than required to produce a single shoot. Twelve weeks after germination, seedlings may reach heights up to 2 m, but cotyledons remain turgid and attached to the developing shoot (Edwards *et al.* 2001). In this context reserve mass in *I. australiense* is 'over-sized' for germination alone, much like oaks (*Quercus*) and *Gustavia* (Anderson & Frost 1996, Dalling & Harms 1999, Soneson 1994). Here we examine the effects of diaspore fate on seedling growth and test the hypothesis that 'extra' reserves allow for multiple resprouting.

Thirteen diaspores were collected. *Idiospermum australiense* is extremely rare, and numbers were kept small to avoid potential adverse effects on population structure within natural populations. Diaspores were randomly divided into two groups (control and treatment). Each of the six diaspores within the control group was kept intact (mean diaspore mass = 110.1 g, SE = 10.5 g). In the treatment group, diaspores were separated into their cotyledons by mechanically breaking the connection between them (mean cotyledon mass = 27.3 g, SE = 4.1 g). The seven treatment diaspores yielded 27 cotyledons (six diaspores with four cotyledons, one diaspore with three cotyledons). All intact diaspores and separated cotyledons were weighed to the nearest 0.01 g and planted into 4-litre pots in 50:50 rain-forest soil and potting mix. Pots were placed on laboratory benches in 40% full sun and received 10 min of fine-spray mist watering three times each day. Germination was scored as the time of shoot emergence because *I. australiense* exhibits hypogeal germination (cotyledons remain on soil surface and the shoot emerges from the cotyledon; Wilson 1979).

Emerging shoots were allowed to grow for 12 wk. At this time the height of the apical bud from the soil surface was measured and shoots harvested (simulating 100% above-ground herbivory) by cutting the stem as close as possible to the soil surface. All shoots were oven-dried at 38 °C for 72 h, after which the shoot was divided into stem and leaf fractions. For each replicate in both treatments the mass of total above-ground shoot plus the mass of leaves was determined to the nearest 0.01 g.

All diaspores and cotyledons were left *in situ* and monitored for the production of new shoots. Emerging shoots were allowed to grow for a further 12 wk after which time we again simulated 100% above-ground herbivory as described above. This procedure was repeated until each diaspore or cotyledon had produced three 12-wk-old shoots.

We used two-way ANOVA to test for differences between diaspore treatment and harvest period in: shoot height, leaf mass and total shoot mass. Because we anticipated a possible effect of diaspore or cotyledon size on each of these growth parameters (see Dalling & Harms 1999) we performed these analyses using both absolute values and values standardized against initial reserve mass. We used initial reserve mass to standardize across all harvest periods because

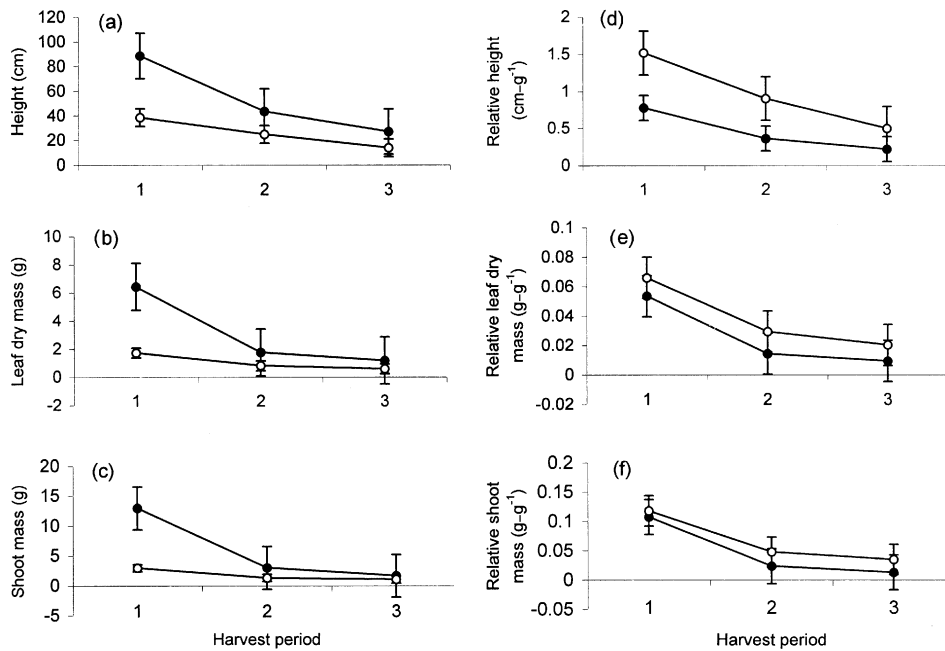


Figure 1. Mean (and SE) height (a), leaf mass (b) and total shoot mass (c) in 12-wk-old stems of successive resprouts in intact diaspores (closed circles) and single cotyledons (open circles) of *Idiospermum australiense*. Figure 1 d-f shows relative height (d), relative leaf mass (e) and relative total shoot mass (f) for same shoots.

our small sample size would not permit destructive sampling at each harvest.

All six intact diaspores and 23/27 separate cotyledons germinated to produce an initial shoot. The remaining cotyledons failed to germinate and deteriorated, apparently due to fungal infection. When we harvested above-ground biomass after 12 wk, all six intact diaspores and 22 separate cotyledons produced resprouts. One intact diaspore and five separated cotyledons failed to produce more than two shoots. Thus, by the end of the experiment 5/6 intact diaspores and 17/27 single cotyledons had resprouted after two rounds of complete excision of all above-ground (photosynthetic) material. All individuals (in both treatments) that failed to resprout deteriorated in a manner identical to the four cotyledons that failed to germinate at the beginning of the experiment. Because reserve mass in individuals that produced stems was apparently healthy and intact at time of harvest, we conclude that the deterioration we observed between harvest and resprouting was also due to fungal infection rather than to depletion of available reserves in construction of stems.

Absolute height, leaf mass and total shoot mass were significantly associated with diaspore fate. Across all harvest periods, shoots from intact diaspores were consistently taller than shoots arising from single cotyledons (treatment  $F = 74.3$ ,  $df = 1$ ,  $P < 0.0001$ ) (Figure 1a). There was also a significant reduction in height in both treatments across harvest periods (harvest  $F = 64.3$ ,  $df = 2$ ,

$P < 0.0001$ ) (Figure 1a). The absolute reduction in shoot height was, however, much greater in shoots from intact diaspores (treatment  $\times$  harvest  $F = 13.3$ ,  $df = 2$ ,  $P < 0.0001$ ) resulting in a reduction in the absolute difference in stem heights between treatments across time. Identical results were shown in both other parameters of leaf and total shoot dry mass (leaf mass: treatment  $F = 40.8$ ,  $df = 1$ ,  $P < 0.0001$ ; harvest  $F = 38.4$ ,  $df = 2$ ,  $P < 0.0001$ ; treatment  $\times$  harvest  $F = 28.6$ ,  $df = 2$ ,  $P < 0.0001$ . Total shoot mass: treatment  $F = 58.4$ ,  $df = 1$ ,  $P < 0.0001$ ; harvest  $F = 55.7$ ,  $df = 2$ ,  $P < 0.0001$ ; treatment  $\times$  harvest  $F = 29.9$ ,  $df = 2$ ,  $P < 0.0001$ ) (Figure 1b, c). These results suggest that the size of above-ground stems became progressively more similar between treatments with successive harvests. This was not the case in *G. superba* where shoot mass decreased at constant proportion across sequential harvests (Dalling & Harms 1999).

When standardized for initial reserve mass, stems arising from single cotyledons were taller and allocated greater amounts of dry mass to leaves across all harvest periods (Figure 1d, e) (relative shoot height: treatment  $F = 48.0$ ,  $df = 1$ ,  $P < 0.0001$ ; harvest  $F = 37.8$ ,  $df = 2$ ,  $P < 0.0001$ ; treatment  $\times$  harvest,  $F = 3.06$ ,  $df = 2$ ,  $P = 0.06$ . Relative leaf mass: treatment  $F = 14.7$ ,  $df = 1$ ,  $P < 0.001$ ; harvest  $F = 81.9$ ,  $df = 2$ ,  $P < 0.0001$ ; treatment  $\times$  harvest  $F = 0.22$ ,  $df = 2$ ,  $P < 0.80$ ). Relative total shoot mass, however, did not differ between treatments (Figure 1f) (relative total shoot mass: treatment  $F = 2.7$ ,  $df = 1$ ,  $P = 0.11$ ; harvest  $F = 13.1$ ,  $df = 2$ ,  $P < 0.0001$ ; treatment  $\times$  harvest  $F = 0.44$ ,  $df = 2$ ,  $P = 0.65$ ). Relative height, leaf dry mass and total shoot mass declined in successive shoots in both treatments (a significant effect of harvest in all analyses). However, the magnitude of the effect did not differ between treatments across successive harvests (no significant treatment  $\times$  harvest interaction). This suggests that the proportion of available reserve mass utilized in the production of successive shoots is contingent on initial reserve mass.

Regeneration in *I. australiense* displays the potential for two alternate fates; diaspores may remain intact, or separate into component cotyledons. The ability to germinate and produce multiple resprouts was demonstrated in both groups. Unlike *G. superba*, the morphological basis for this ability in *I. australiense* is unknown at this time (Harms *et al.* 1997). Resprouting per se may not be surprising, since average cotyledon mass exceeds 25 g and thus even single cotyledons present large stored reserves. These alternative regeneration possibilities, however, represent alternate solutions to the size–number trade-off in offspring provisioning within a single species and the patterns we observed reflect differences more commonly found between species. For example, seedlings arising from intact diaspores (larger reserve masses) produced taller seedlings with greater leaf mass, while diaspores that separate into individual cotyledons produced, on average, four independent seedlings. More strikingly, relative investment in height and leaf mass were consistently greater in the individual cotyledon (smaller reserve mass) across harvest periods. Total relative shoot mass was not different between treatments, implying comparatively

weaker stems due to lower mass per unit stem height (Edwards *et al.* 2001). Furthermore, these results suggest that allocation may be explained by reserve mass alone, and that the pattern of seedling growth is not an intrinsic feature of individual species per se.

We have shown the first reported occurrence of multiple resprouting in an Australian plant species. Evidence of resprouting from this study and from the neotropics (Dalling *et al.* 1997, Dalling & Harms 1999) implies that the phenomenon may not be as isolated as previously considered. The ability to resprout is contingent on stored reserves remaining on or under the soil surface (hypogeal-type germination). Hypogeal germination is evolutionarily labile, and has evolved independently many times (Wright *et al.* 2000) most often in association with large reserve mass (Dalling & Harms 1999). Many species in tropical floras produce seeds with stored reserves (i.e. in excess of 5 g; Dalling & Harms 1999; Grubb & Metcalfe 1996) and display hypogeal germination. It is not unreasonable, therefore, to predict more examples of multiple resprouting will be found. Indeed, to fully understand large seed size in tropical floras, it must now be determined whether the ability to resprout is rare in nature or rare only in the current literature.

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