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Regeneration from Cotyledons in *Gustavia superba* (Lecythidaceae)¹

Key words: Barro Colorado Island; cotyledon; *Gustavia superba*; *Lecythidaceae*; Panamá; seedling; regeneration; vegetative reproduction; *Tontelea richardii*.

SEVERAL WOODY TROPICAL PLANTS ARE able to regenerate completely, *i.e.*, to reproduce vegetatively, from removed ramets. For example, in the event of breakage, new plants can arise from the excised branches or leaves of many species of shrubs (Gartner 1989, Kinsman 1990, Sagers 1993).

In this note, we report the previously undescribed phenomenon of plant regeneration, under natural conditions, from fragments of the cotyledons of a tropical tree. There is a rich literature on regeneration from tissue and organ cultures of cotyledons of woody species, including tropical woody species (*e.g.*, La Rue 1933, Sinnott 1960, Bonga & Durzan 1982, Jain *et al.* 1995). However, we have found no references to regeneration from cotyledons under natural conditions in woody tropical species and no discussion of the potential ecological significance of this phenomenon.

Gustavia superba (Lecythidaceae) is a medium-sized (10–20 m tall), tree of lowland Tropical Moist

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TABLE 1. *The regeneration responses of 35 cotyledons of Gustavia superba, each cut transversely to yield two halves, one distal and one proximal to the previous point of attachment to the seedling. To be included in the tallies for this table, a cotyledon half must have produced at least one shoot with \geq two leaves and roots.*

Both halves	Distal half only	Proximal half only	Neither half
5 (14%)	2 (6%)	14 (40%)	14 (40%)

and Wet Forest (*sensu* Holdridge 1967) of Central America and northern South America (Croat 1978, Prance and Mori 1979). Adults of *G. superba* produce indehiscent fruits containing several large seeds (Sork (1987) reports 5–50 seeds/fruit, and a mass of 3–15 g/seed; Forget (1992) reports an average of seven viable seeds/fruit, with a range of 1–22 seeds/fruit, and an average mass of 12.6 g/seed, $N = 50$). Most of the volume and mass of each seed consists of 2–4 (model = 3) cotyledons (K. Harms and J. Dalling, pers. obs.).

The cotyledons of *G. superba* remain inside the seed coat upon germination and they are not lifted above the soil surface by the emerging seedling. According to the classification scheme of Garwood (1983), seedlings of *G. superba* are therefore of Type C, *i.e.*, hypogeal and cryptocotylar (*sensu* Duke 1969). Although they turn green upon exposure to light, K. Kitajima (pers. comm.) found no stomates on cotyledons of *G. superba*, suggesting minimal photosynthetic activity.

In the process of working with *G. superba* seedlings in a screened growing house on Barro Colorado Island (BCI), Panamá, we observed that whole cotyledons often break off from recently germinated seedlings. Furthermore, we noticed that some of these excised cotyledons sprouted and gave rise to individual plantlets separate from the seedlings from which they had become removed. In this note we describe a simple experiment in which we demonstrate the capacity of cotyledonary fragments of *G. superba* to regenerate.

In August 1994, we collected fallen fruits of *G. superba* from beneath ten adult trees on BCI. We removed the mature, intact seeds of these fruits and placed 35 of them on the surface of 4 cm of forest soil in seed germination trays in a screened growing house on BCI (most of the unused seeds were damaged by the pre-dispersal seed-boring moth, *Carmenta foraseminis*, Harms & Aiello 1995). In September 1994, following germination and expansion of the first pair of leaves, we removed one cotyledon from each seedling. We then cut the 35 cotyledons transversely to their previous point of attachment to the seedling shoot, resulting in two equal-sized cotyledon halves, one distal and one proximal. The distal and proximal halves of each cotyledon were placed in separate seed trays. The 70 cotyledon halves were watered and checked for signs of shoot production every other day until March 1995.

Of the 35 original cotyledons, 21 (60%) had at least one of its halves give rise to a shoot with \geq two leaves and roots by March 1995 (Table 1). An additional distal half produced a shoot but had not yet flushed its first leaves upon termination of the experiment in March 1995. In two cases, a single cotyledon half (in one case proximal and in the other distal) simultaneously produced two leaf-bearing shoots with roots. Of the 21 cotyledons that produced leaf-bearing shoots, five had both the distal and proximal halves produce shoots, each with \geq two leaves and roots. In all, 19 of the proximal halves and seven of the distal halves produced shoots with \geq two leaves and roots (Table 1).

The ability of large-seeded species to regenerate from removed cotyledons or cotyledonary fragments may be favored in the forest understory, where seedlings have a high probability of being damaged by herbivores (*e.g.*, Denslow 1980, Clark & Clark 1985) and falling debris (*e.g.*, Uhl 1982, Aide 1987, Clark & Clark 1991). Sork (1985, 1987) and Forget (1992) found that seeds of *G. superba* are both dispersed (scatter-hoarded) and consumed by rodents on BCI. Forget (1992) found that agoutis (*Dasyprocta punctata*) in the process of scatter-hoarding the seeds of *G. superba*, usually dig up and consume the cotyledons after germination. Cotyledons are sometimes carried away from seedlings and partial cotyledon damage occurs (Forget 1992), potentially leaving cotyledons and cotyledon fragments to regenerate on their own. Whole seeds are also commonly fragmented before or during the processes of

removal from a fallen fruit, transport, and burial (K. Harms and J. Dalling, pers. obs.). When fragmentation of large seeds is common, the ability to regenerate from cotyledonary fragments should increase the chances of any seed producing at least one established seedling.

Further studies are required to determine whether or not fragments of ungerminated seeds are able to regenerate and to determine the anatomical basis for regeneration in *G. superba* (e.g., were the regenerated plantlets we observed the products of somatic embryogenesis?). Although the phenomenon of natural cotyledonary regeneration in woody tropical species has not been reported previously, we suspect that this ability may be widespread. In a related study, Dalling (unpubl. data) found that the large-seeded liana, *Tonatelea richardii* (Hippocrateaceae), is able to produce functional seedlings (with roots, shoots, and leaves) from both the proximal and distal halves of cotyledons removed from ungerminated seeds. In order to determine the extent to which regeneration from cotyledons occurs in other species, we encourage investigators to conduct similar experiments on a wide variety of large-seeded tropical species.

We have shown that seeds of *G. superba*, when broken into separate pieces after germination, are capable of producing >1 individual plant under natural conditions in a growing house. Knowledge of the extent to which this capability translates into fitness consequences for individuals and into demographic consequences for natural populations awaits observations and experiments in the field. It is necessary to determine the frequency with which cotyledons are damaged and to determine the probability that removed or damaged cotyledons are able to regenerate, grow, and reproduce in nature.

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Influence of Aerial *Azteca* Nests on the Epiphyte Community of Some Belizean Orange Orchards¹

Key words: ant gardens; ant nests; *Azteca*, Belize; diversity; epiphytes.

ANTS ARE FREQUENTLY A MAJOR component of the invertebrate biomass and diversity of tropical forest canopies. Ant species building aerial nests made of papery or cardboard-like material called carton, are often numerically dominant among the ants (Madison 1979a, Wilson 1987, Davidson 1988, Tobin 1991). Certain epiphytes are largely restricted to aerial ant nests, *i.e.*, obligate ant nest users (subsequently referred to as “obligate” or “ant garden” epiphytes), and many of them produce specialized ant-dispersed seeds to ensure dispersal to suitable substrates, *i.e.*, nests (Ule 1901a, b; Madison 1979a; Benzing 1990, 1991a, b). Consequently aerial ant nests with associated epiphytes have become known as “ant gardens”. Although it has been suggested that the relationship between the garden and the ants is casual (Wheeler 1921, Weber 1943), the most recent observations suggest that it is a complex mutualism in some epiphyte species at least (*e.g.*, Kleinfeldt 1978, Madison 1979a, Beattie 1985, Davidson 1988, Davidson and Epstein 1989, Hölldobler & Wilson 1990, Yu 1994).

In one of the few references to the possible effects of ants on non-obligate ant nest epiphytes (*i.e.*, species not largely restricted to carton), Longino (1986) suggested that the accumulation of ant-provided substrates including carton nests, carton covered trails on branches, and carton-covered homopteran colonies on twigs, promotes epiphyte establishment in tropical lowland rain forests. Ants may also benefit non-obligate epiphytes by consuming or driving off potentially harmful herbivores (*e.g.*, Janzen 1972, Olmsted & Dejean 1987). On the other hand, Weir and Kiew (1986) found that tree crowns with ant nests were largely free of epiphytes and they attributed this to removal by the ants. Davidson *et al.* (1988) have reviewed pruning by ants and have suggested an explanation for its origin in reducing the threat of invasions of alien ants. Ants may also have a negative impact on epiphytes by protecting colonies of homopteran insects (Way 1963, Hölldobler & Wilson 1990) that provide the ants with sugary secretions, but suck phloem sap leading to the decline of epiphytes and/or epiphyte hosts (*e.g.*, Buckley 1987, Rico-Gray & Thien 1989, Steyn 1954). Thus aerial-nesting and carton-producing ants can have positive, negative, or both effects on the non-garden epiphyte community.

The present study utilized orange orchards in central Belize, where (1) some of the factors affecting epiphyte distribution (*e.g.*, climate, host species, tree height and density) are essentially constant; and (2) aerial nests are frequent enough to permit the collection and analysis of quantitative data thus enabling an objective approach to the influence of aerial-nesting ants. The orchards are inhabited by aerial-nesting *Azteca* ants that produce carton nests, carton-covered trails and also produce carton over colonies of homopteran insects which they protect.

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