

EFFECT OF BATS ON SEED DISTRIBUTION AND GERMINATION OF *CALOPHYLLUM BRASILIENSE* (CLUSIACEAE)

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Abstract. Seed dispersal by vertebrates influences plant reproductive success and consequently population dynamics. By dispersing seeds away from the parent plant, dispersers prevent high seed density below the crown, increasing seed survival. We studied seed dispersal by bats of the tree *Calophyllum brasiliense* (Clusiaceae) in southeastern Brazil in order to examine its consequences for seed distribution, predation, and germination. We assessed tree phenology, recorded visitor bat species, and analyzed seed spatial distribution in a forest fragment. Fruiting occurred from November to July, when the bats *Artibeus lituratus* and *Platyrrhinus lineatus* forage and disperse fruits of *C. brasiliense*. Bat-dispersed fruits were found under 113 feeding roosts, and there were no differences in bat dispersal rates below or away from parent trees. Seed predation on bat-dispersed seeds was greater than on seeds under parent trees. On the other hand, germination rates were greater for seeds with pulp removed by bats than for seeds in non-dispersed fruits. Thus while seed dispersal by bats may not provide an escape from density-dependent mortality, fruit manipulation by bats may provide a secondary benefit for plants in the form of increased seed germination. *Accepted 3 March 2009.*

Key words: Artibeus, Brazil, dispersal effectiveness, escape hypothesis, frugivory, guanandi, Janzen-Connell model, Platyrrhinus, Neotropics.

INTRODUCTION

Seed dispersal is an important step in the reproductive cycle of most plants since it determines the initial spatial distribution of seeds as well as the environment where they will germinate and become seedlings (Howe & Smallwood 1982). There are several possible ways in which seed dispersal may benefit the plant, including (1) escape from density-dependent mortality near the parent plant; (2) higher probability of colonization of new sites; (3) directed dispersal to safe sites. In all cases, seed dispersal improves seed and seedling survival and ultimately increases parental fitness.

The escape hypothesis states that seed and seedling mortality are density- or distance-dependent, and thus are higher near the parent tree (Janzen 1970, Connell 1971). Seed dispersers may actively alter seed distribution, and consequently reduce seed and seedling mortality in the adult neighborhood. The fate of seeds after dispersal by vertebrates has been investigated more intensively in the last three decades for a variety of plant and animal species and the Janzen-Connell model was supported in some studies, but not in others (Chapman & Chapman 1996, Cintra 1997, Fragoso 1997, Jansen *et al.* 2008). Thus beyond distance and density it is possible that the behavior of seed dispersers and predators critically influences seed survival (Nathan & Casagrandi 2004).

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Dispersers may contribute in different ways to plant reproductive success because dispersal effectiveness depends on qualitative and quantitative components that are distinctive to each disperser type (Schupp 1993). In Neotropical forests bats are important seed dispersers and about 250 bat species are partially or totally dependent on plants as a food source (Fleming 1982). Seed dispersal by bats has two general forms. In the first, seeds are ingested and later defecated in small quantities in flight and therefore are scattered randomly. This is one of the more important ways in which plants colonize forest gaps (Fleming & Heithaus 1981). In the second, bats take fruits containing larger seeds to night feeding roosts, consume the edible parts and drop the seeds beneath the roost, close to or distant from the parent plant (Huber 1910, Fleming 1981, Howe 1989). In this last case seed survival could be lowered since their accumulation could make them as vulnerable to density-dependent mortality (attack by seed predators and pathogens) as under the parent plant.

Calophyllum brasiliense Camb. (Clusiaceae), also known as Brazil beauty-leaf or guanandi, is a typical bat-dispersed species (van der Pijl 1957), and here we report on the effect of dispersal by bats on the distribution, predation, and germination of its seeds. Specifically, we tested if bats alter seed distribution and predation rates compared with non-dispersed seeds. Additionally, we tested for germination benefits to seeds by comparing germination rates among bat-dispersed and non-dispersed seeds.

METHODS

Study site and species. The study was carried out in a flooded forest fragment, of approximately 13 ha, in Brotas municipality, São Paulo state (22°16'S, 48°06'W, at 470 m a.s.l.). The regional climate is warm tropical humid, according to Koeppen's classification. *Calophyllum brasiliense* is adapted to the flooded soil (Scarano *et al.* 1997, Marques & July 2000b) and is the most abundant (380 individuals per ha) tree species in the canopy (Marques *et al.* 2003). *Calophyllum brasiliense* occurs in swampy areas from Central America to the coast of southern Brazil and is found in Amazonian forest, Atlantic forest, and in inland forests (Reitz *et al.* 1978). Trees are generally tall (~18 m) and fruits ripen in the canopy (crowns approximately 5 m in diameter). The fruits are large drupes (mean weight \pm SD = 3.74 \pm 0.09 g; N = 267 fruits) with a thick, inodorous, green, lactescent pericarp, and a single spherical and

thick-coated seed (mean diameter \pm SD = 15.8 \pm 1.7 mm, n = 869). Bats are almost the only seed dispersers of *C. brasiliense* in the study site; populations of other mammals are small or absent. Bats scrape the pericarp with their teeth and eat the pulp's juicy material and parts of the epicarp and mesocarp. The endocarp, the seed coat, and endosperm remain intact after feeding. Apart from bat dispersal, seeds are also frequently carried by water if they fall into some of the small rivers within the forest.

Phenological study. To investigate the seasonal availability of fruits for bats, 28 trees of *C. brasiliense* were observed with binoculars every two weeks from June 1991 for 13 months. Fruiting was the presence of one or more fruits. In this case we considered immature and mature fruit as separate phenological phases.

Bat activity and seed dispersal. Observations of visits by bats were carried out between 17:00 h and 02:00 h in one tree at the forest edge, during the peak of fruiting (March). Bats were captured in mist nets, and some of them were collected for identification. Voucher specimens were deposited in "Museu de História Natural - Universidade Estadual de Campinas".

On the forest floor we observed a large number of seeds with teeth marks clumped together under the bats' night feeding roosts. To show the spatial distribution of seed clumps and trees a 3600 m² plot was delimited in the forest. Bat-generated patches and adult plants (taller than 10 m, Marques & July 2000a) found in the forest were mapped and quantified. In each seed clump, information about the patch size, number of fruits, and predation evidence (endosperm totally or partially destroyed) was collected.

To evaluate the effect of bat activities on seed distribution, ten trees bearing fruits were selected. For each tree one transect was established from its base in a direction that avoided other trees and irregularities in soil level. The population of *C. brasiliense* is dense in this area, but only a small proportion of adults (< 8% of the total; Marques & July 2000a, Fisher & Santos 2001) is effectively reproductive each year. Despite having no information about the origin of each individual seed, we assumed that all seeds in the transect were from the sampled adult and that the bias caused by the presence of conspecifics had no important effects on our results. The length of each transect was twice the radius of the tree canopy

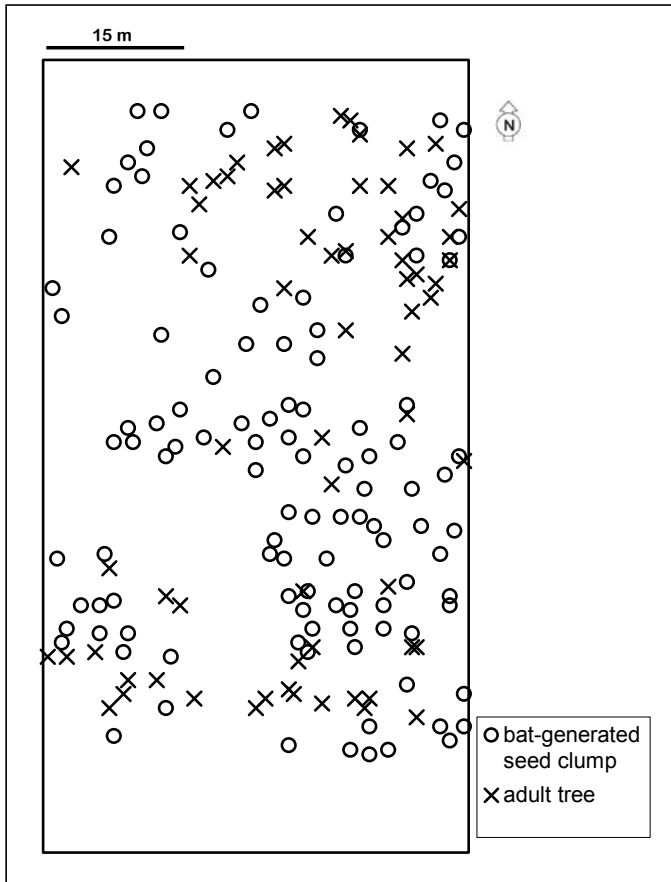


FIG. 1. Spatial distribution of bat-generated seed shadows and *Calophyllum brasiliense* adult trees (taller than 10m) in a 3600-m² area in southeastern Brazil.

(thus the areas beneath and away from the tree's crown were similar). Along each transect a set of 1 x 1-m quadrats (a total of 62 quadrats in the whole 0.3-ha area) was delimited on the forest floor. Within those quadrats all fruits were counted and their dispersal pattern was estimated. Bat teeth marks indicated bat dispersal, while absence of marks on the fruit indicated dispersal by other means, such as gravity or water. To test if the number of fruits removed by bats is different beneath or away from the parent tree, we compared proportions of bat-dispersed and non bat-dispersed fruits in quadrats using a chi-square test (Zar 1999). In addition, to test if seed predation rate in bat-clumps (fruits counted in the clumps under night feeding roosts) is different from the rate in parent-clumps (fruits counted in quadrats below adult trees) we compared

proportions of damaged (seeds with endosperm and/or embryo destroyed) and undamaged seeds using a chi-square test.

Germination tests. In order to test if pericarp removal by bats affects seed germination, we collected fruits on the ground and estimated the percentage of pulp that remained (% of fruit area) attached to the seed. All fruits had been dispersed by bats (presence of teeth marks) up to one week before the germination experiment. Fruits were divided into four classes based on the percentage of pulp remaining on the seed (0-25%, 26-50%, 51-75%, 76-100%) and submitted to a germination test. Each fruit class had 20 seeds, which were sterilized in 1% sodium hypochlorite and maintained in regular moisture on a gerbox plate with sieved and sterilized sand in a B.O.D. germination chamber (Fanem 147), at 25 ±

TABLE 1. Number of fruits dispersed by bats and by other means counted in transects beneath and away from the crown of 10 *Calophyllum brasiliense* trees in southeastern Brazil. ($\chi^2 = 1.04$, $P > 0.05$, $N = 10$).

	Non bat-dispersed	Bat-dispersed	Total
Beneath crown	1,767	76	1,843
Away from crown	597	32	629
Total	2,364	108	2,472

2° C and a photoperiod of 12 h light/12 h dark, for 12 weeks. The germinating seeds were counted every week, and differences between classes in germination rate were subjected to survival analysis (Allison 1995).

RESULTS

Fruiting and frugivory. Fruiting occurred from November to July. Immature fruits ripened during approximately two months (November and December), and mature fruits remained on the trees for six months.

Platyrrhinus lineatus and *Artibeus lituratus* (Chiroptera: Phyllostomidae) were observed harvesting fruits. *Platyrrhinus lineatus* was the smaller species and started its foraging activity around 18:00 h, when groups of two to four individuals were observed flying above the tree crown. From 18:30 h onward bats began to forage at a higher density. The rates of visits decreased as the night progressed. *Artibeus litu-*

TABLE 2. Number of damaged and undamaged seeds found under 113 bat feeding roosts and beneath the crown of 10 *Calophyllum brasiliense* trees (fruits dispersed by bats or other means) in southeastern Brazil. ($\chi^2 = 71.04$, $P < 0.01$, $N = 10$)

	Undamaged seeds	Damaged seeds	Total
Under bat feeding roosts	3880	162	4042
Under conspecific trees	1841	2	1843
Total	5721	164	5885

ratus (the larger species) foraged alone from 20:00 h onwards with a similar behavior but at a ~50% lower frequency than *P. lineatus*.

Bats consumed the fruits only rarely directly in the fruit tree but transported them mostly away from the tree, probably to night feeding roosts. There the pulp was eaten and the seed dropped on the ground. Feeding roosts were probably occupied by a single individual that returned to the fruiting tree several times.

There were 113 bat-generated patches and 66 adult plants in the study area (Fig. 1). Each patch (approximately 1m² in area) contained an average of 57.8 fruits (SD = 67; $N = 70$). All seeds under the roosts were of *C. brasiliense*.

Effect of bat activity. The number of fruits not dispersed by bats was 23 times greater than the number of bat-dispersed fruits in transects (Table 1). There was no difference ($\chi^2 = 1.04$, $P > 0.05$, $N = 10$) between the proportion of bat-dispersed fruits below or away from trees (4.1% and 5% respectively, Table 1). Seeds of *C. brasiliense* were damaged at a greater rate under bat feeding roosts (4%) than below the fruiting trees (0.11%) ($\chi^2 = 71.04$, $P < 0.01$, $N = 10$, Table 2).

Bats strongly reduced the pericarp cover of the pulp remaining on the fruits (45 ± 32%, $n = 461$). Fruits with a higher proportion of pulp remaining germinated at a lower rate than fruits with a lower proportion of pulp ($\chi^2 = 27.0$, SD = 3, $P < 0.001$). Fruits with over 75% of pulp remaining did not germinate after 12 weeks (Fig. 2).

DISCUSSION

Our results for *Calophyllum brasiliense* showed that proportions of bat-dispersed seeds are the same below or away from the parent tree and that seed predation among bat-dispersed seeds was greater than in non-dispersed seeds beneath parent trees. This suggests that seed dispersal by bats does not improve plant survival by avoiding seed predation beneath parent trees. On the other hand, seeds benefited from bat dispersal by germinating more rapidly.

Bats, including *Artibeus lituratus* and *Platyrrhinus lineatus*, are abundant and important seed dispersers in the tropics (Fleming 1981, Zortéa & Chiarello 1994). The large fruit-eating bat *A. lituratus* was found eating *C. brasiliense* fruits at various Atlantic forest sites in Brazil (Fischer & Santos 2001), and has already been identified as an effective seed disperser of this plant (Mello *et al.* 2005). In Brotas these bats

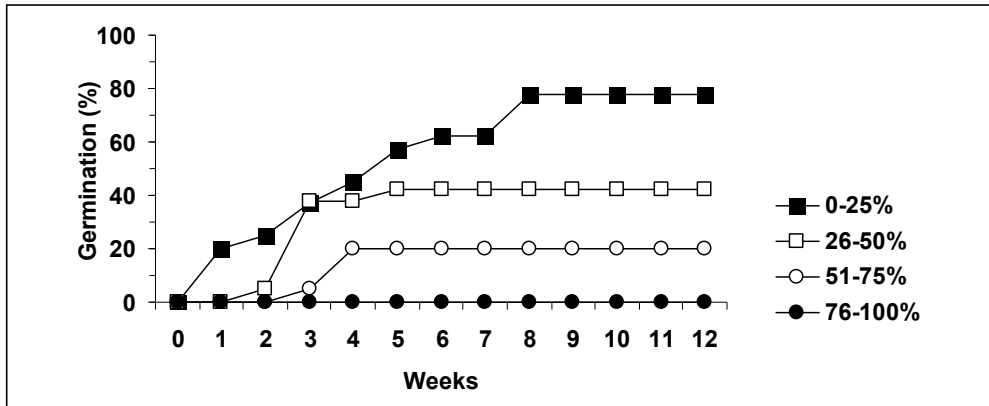


FIG. 2. Germination rate of *Calophyllum brasiliense* seeds in the laboratory, with different percentages of remaining pericarp.

consume *C. brasiliense* fruits during most of its seven-month fruiting season, so the fruits are probably an important element in their diet.

The behavior of the bats and the morphology of *C. brasiliense* fruits generate different models of seed distribution. By accumulating fruits at night roosts bats act as clump-dispersers (Howe 1989), since 113 groups of an average 57 seeds were observed in the area. Nevertheless, when carrying fruits in flight bats can accidentally drop some of them on the forest floor, so acting also as scatter-dispersers of *C. brasiliense* seeds (Fleming & Heithaus 1981, Howe 1989). Thus, depending on where bats drop the fruits, they can generate various kinds of seed shadow.

Bat-dispersed seeds of *C. brasiliense* are found both under night feeding roosts and beneath the parent tree. Carrying fruits to feeding roosts does not change the proportion of fruits present near adult trees or at larger distances. Bat-generated seed shadows probably have a higher density of seeds (number of seeds by area) than seed shadows beneath parent trees, which might explain the increased predation of *C. brasiliense* seeds under bat feeding roosts. In contrast to low predation rates ($\approx 4\%$) in Brotas, our results show that bat-dispersal did not reduce seed predation. Bat dispersal appears to increase both seed clumping and seed predation. Plants that rely on clump-dispersers could have developed chemical or mechanical defenses against seed predators and be able to recruit seedlings even from seeds located near parent trees (Howe 1989). This strategy could result

in a population with an aggregated spatial distribution of adults and saplings such as observed for *C. brasiliense* at our study site (Marques & July 2000a). Nevertheless bats are not the only seed disperser, since fruits may also be water-dispersed (Fischer & Santos 2001). Hence the original bat-generated seed shadow is expected to change after flooding periods (November to March), when the effects of density might be lower than observed here.

Under each bat feeding roost, fruits exhibited different proportions of surviving pulp. It is possible that the differing amounts of pulp remaining are due to bat satiation or their limited ability to consume the fruit. The faster germination rate caused by removal of the pericarp reduces the time during which seeds are susceptible to predators or pathogens. Since seeds of *C. brasiliense* are viable for 4-5 months (Marques & July 2000b), and after emergence seedlings survive for a long time (Marques & July 2000a), a faster germination may provide an important reproductive advantage for this tree species. Additionally, bats may provide an extra benefit to *C. brasiliense* by removing preferentially larger fruits and thus dispersing mainly larger seeds which tend to germinate faster and survive better, which could improve the parent tree's fitness (Mello *et al.* 2005).

Bat-dispersed species such as *C. brasiliense*, which have large seeds and aggregated dispersed fruits, probably depend more on bats as "peelers" of fruits than as long-distance seed dispersers. Future studies

on bat-dispersed plant demography should consider bats' effects on seed dispersal as a more comprehensive process.

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