PLANT ANIMAL INTERACTIONS

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Interaction between ants and fruits of *Guapira opposita* (Nyctaginaceae) in a Brazilian sandy plain rainforest: ant effects on seeds and seedlings

Abstract This study examines the dispersal system of *Guapira opposita* in a tropical sandy rainforest in southeast Brazil. *Guapira* trees produce small fruits with a high protein content (28.4%) and low lipid content (0.3%), and the plant is primarily dispersed by birds. Mature fruits of *G. opposita* can fall spontaneously with the pulp intact, or be dropped by birds with bits of pulp attached. In either case, ground-dwelling ants rapidly remove the fruits to their nest (93% after 12 h). The ponerine ants *Odontomachus chelifer* and *Pachycondyla striata* are the main seed vectors among the ants, and together account for 56% (20 of 36) of the ant-fruit interactions recorded on the forest floor. Individual workers of *O. chelifer* and *P. striata* transport single fruits to their nests. Bits of pulp are fed to larvae and worker nestmates, and intact seeds are discarded outside the nest. Germination success in *Guapira* is higher for cleaned seeds (pulp removed) than for seeds coated by pulp. *Guapira* seedlings and juveniles are more frequent close to *Odontomachus* nests than at sites without such nests. Soil samples from *Odontomachus* nests had greater penetrability, and higher concentrations of P, K, and Ca than random soil samples. Field experiments suggest that the association between *G. opposita* seedlings and *O. chelifer* nests can potentially render the plant some protection against herbivores. Results indicate that fruit displacement by ponerine ants play an important role in the biology of *G. opposita* seeds and seedlings in the sandy forest, and illustrate the complex nature of the dispersal ecology of tropical tree species.

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Introduction

In tropical forests, nearly 90% of the trees and shrubs bear fleshy fruits and rely on vertebrate frugivores such as birds, bats, or monkeys for seed dispersal (Frankie et al. 1974; Jordano 1993). However, recent studies have shown that large amounts of the fruit crop produced by tropical trees can reach the forest floor intact, either spontaneously or dropped by vertebrate frugivores (e.g., Pizo and Oliveira 1998; Passos and Oliveira 2002). Because tropical ground-dwelling ants are remarkably abundant and diverse (Byrne 1994), and seeds and fruits constitute a large portion of the litter on the floor of neotropical forests (Foster 1982; Denslow and Gomez-Dias 1990), ant-seed interactions are widespread in these areas. For instance, Pizo and Oliveira (2000) have shown that interactions between ants and seeds/fruits (hereafter diaspores, sensu van der Pijl 1982) involve about 40 ant species and 50 plant species in the Atlantic rainforest of Brazil. These ant-diaspore interactions can modify the fate of seeds (e.g., Roberts and Heithaus 1986; Levey and Byrne 1993), and affect patterns of recruitment (distribution and survival of seedlings) in primarily vertebrate-dispersed species that lack special adaptations for ant-dispersal (Böhning-Gaese et al. 1999; Passos and Oliveira 2002).

Fleshy fruits of tropical forests present a plethora of sizes, shapes, colors, and chemical composition of the edible portion (e.g., Corlett 1996). Consequently, ants in tropical forests interact with a broad range of fruits differing in morphology and nutrient content (Pizo and Oliveira 2000). It has recently been suggested that the outcome of the interaction between ants and diaspores in tropical forests can be largely determined by the size and lipid content of the diaspores (Pizo and Oliveira 2001). Lipids are an important food resource for ants, serving a variety of purposes that include nutrition, physiological constituents, and behavioral releasers (Beattie 1985 and

references therein). According to Pizo and Oliveira (2001), lipid-rich diaspores are more attractive to ants than diaspores with either a low lipid content or those rich in carbohydrates (see Jordano 1993 for main fruit composition patterns). Furthermore, ant effects on seedling recruitment have recently been demonstrated for two lipid-rich tropical species (Böhning-Gaese et al. 1999; Passos and Oliveira 2002).

Proteins are an essential food source for social insects, and ant colonies must get the adequate protein intake to meet the dietary requirements of larvae and functional queens (Hölldobler and Wilson 1990). Comparative studies of the nutrient content of fleshy fruits have revealed that, in general, fruits are extremely poor in protein in comparison with leaves and insects, and that protein-rich fruits are not common (Herrera 1987; Jordano 1993). Ants in the predominantly carnivorous subfamily Ponerinae search the leaf litter for arthropod prey and use the aril or pulp of fruits as a secondary food source (Horvitz and Beattie 1980; Fourcassié and Oliveira 2002; Passos and Oliveira 2002). As main seed vectors on the forest floor, *Odontomachus chelifer* and *Pachycondyla striata* are key species determining the outcome of ant-seed interactions in the Atlantic rainforest in Brazil (Pizo and Oliveira 1998, 2001). The activity of these large ponerines can markedly affect recruitment patterns in lipid-rich species (Passos and Oliveira 2002), but there is no information on ant-induced effects on species with protein-rich fruits.

Ants affect seed distribution through two general mechanisms: by harvesting edible seeds that may subsequently escape predation, or by collecting diaspores to eat an ant-attractive nutritive part (elaiosome, aril, or fleshy pulp) and discarding unharmed viable seeds (van der Pijl 1982; Handel and Beattie 1990). The present study examines the second mechanism by reporting ant activity at fallen fruits of *Guapira opposita* (Vell. & Reitz) (Nyctaginaceae), a primarily bird-dispersed tree producing protein-rich fruits, and by investigating the ants' possible influence on seed fate and seedling recruitment in a coastal forest in southeast Brazil. Three questions were addressed:

1. Which ant species use *G. opposita* fruits on the forest floor?
2. How do ants behave towards the fruits?
3. Can ant-fruit interactions affect seed germination and seedling recruitment in *G. opposita*?

Materials and methods

Study site

Field work was carried out in the sandy plain forest (locally called 'restinga' forest) of the Parque Estadual da Ilha do Cardoso (PEIC), a 22,500 ha island (altitude 0–800 m a.s.l.) located on the coast of São Paulo State (25°03′S, 47°53′W), SE Brazil. Sandy plain forests are characterized by an open canopy, dominant low stature trees, and abundant epiphytes (Oliveira-Filho and Fontes 2000). At PEIC, the sandy plain forest is well-preserved and lies at 2 to 3 m a.s.l., and trees 5–15 m tall form an open canopy with abundant bromeliads on the ground. Mean annual temperature and rainfall are 20.9°C and 3,000 mm, respectively (Barros et al. 1991). The climate is generally warm and wet throughout the year but may be divided into two seasons. There is a colder, drier season from April to August with temperatures dropping to nearly 13°C and rainfall of ca. 500 mm, and a warmer, rainier season from September to March with temperatures reaching 32°C and rainfall of up to 2,600 mm (Oliveira-Filho and Fontes 2000).

The plant species

G. opposita is a tree or treelet distributed in forested areas of tropical South America, extending from Amazonia to southeast Brazil, especially common in the Atlantic forest (Morellato et al. 2000). Fruits occur from January to May and birds are the primary dispersers of *G. opposita* (Galetti 1996; Galetti et al. 2000). Mature black drupes (mean±SD 8.02±0.84 mm length, 7.09±0.49 mm wide, $N=30$) weigh 0.25±0.04 g ($N=30$) and contain one seed each (data from six trees). If not removed by birds in the canopy, the mature fruits fall from parent plants, as recorded in lowland and sandy Atlantic forests for *G. opposita* (Pizo and Oliveira 2000; Passos 2001), in Panama for *G. standleyanum* (Foster 1982), and in the Brazilian 'cerrado' savanna for *G. noxia* (P.S. Oliveira, personal observation). In our study site at PEIC, mature fruits of *G. opposita* can fall spontaneously with the pulp intact, or be dropped by birds with bits of pulp attached. In either case, ground-dwelling ants can rapidly remove the fruit to their nest, or consume the pulp on the spot (see below). The pulp of *G. opposita* has one of the highest protein contents (28.4%) so far described (Jordano 1993), and a negligible amount of lipids (0.5%, Passos 2001; methods follow Bligh and Dyer 1959). Total carbohydrate and ashes account for 66.2% and 4.9% of the dry mass, respectively (Passos 2001).

Background data on treatment of seeds by ants

Previous field and laboratory observations on the treatment of *G. opposita* fruits and seeds by ants have enabled us to confirm that the fleshy pulp, rather than the seed, acts as an ant-attractant, and that the interactions between carnivorous ponerine ants and fruits represent dispersal rather than granivory. After frequently observing large ponerine ants (*O. chelifer* and *P. striata*) in the field transporting fallen *G. opposita* fruits to nests, we used captive colonies of one focal ant species (*O. chelifer*) to document food consumption by the ants, and the fate of seeds inside the nest (a similar method was used by Levey and Byrne 1993). We excavated and collected three colonies of *O. chelifer* containing 290 to 650 workers each, and abundant brood (eggs, larvae, and pupae). Colonies were cultured at 25°C in plastic boxes (40×30 cm) under natural light. Each artificial nest consisted of 10 glass test tubes covered by translucent red shelters and containing water trapped behind a cotton plug. Colonies were fed every 2 days with cockroaches and synthetic ant diet (Hölldobler and Wilson 1990), even during trials. Trials ($N=10$) consisted of placing a *G. opposita* fruit at a spot 20 cm from the nest entrance. Ant-fruit interactions were monitored for 30 min initially and then at 8–12 h intervals. Workers carried the fruit into the nest, and fed bits of pulp to larvae and worker nestmates. Cleaned intact seeds were deposited on refuse piles outside the nest with no evidence of seed predation by the ants. These observations support previous reports on treatment of seeds by primarily carnivorous ponerines (e.g., Horvitz and Beattie 1980; Hughes and Westoby 1992; Pizo and Oliveira 2001). This contrasts with other ant-seed systems in which ant-induced seed movements are caused by both seed predation and 'true' myrmecochory (see Garrido et al. 2002). Indeed, even small myrmicine ants (see below), which recruit nestmates and collectively collect pulp material from *G. opposita* fruits on the spot, also leave the intact cleaned seed on the leaf litter (Pizo and Oliveira 2000; Passos and Oliveira 2003). Therefore, previous knowledge that the edible fleshy pulp mediates ant-attraction to *G. opposita*

fruits allowed us to perform the field experiments on removal rates by ants using intact fruits (i.e., with pulp) (similar methods were used by Horvitz and Schemske 1986a; Passos and Oliveira 2002).

Ant-fruit interactions

Interactions between ants and fruits were surveyed by placing single fruits on the forest floor at 10 m intervals along an 800 m transect at the study site. Fruits were set on small pieces (4×4 cm) of white filter paper to ensure that ground-dwelling ants were visible. Filter paper apparently had no effect at excluding some ant species, or other potential seed dispersers (see Pizo and Oliveira 2000). Fruits were distributed at 0700 hours and 1500 hours and checked at 15 min intervals for 2 h (n=90 fruits). We recorded the ants which were attracted to the fruits, as well as whether they removed the fruits and/or recruited nestmates to exploit them on the spot. We followed ants carrying fruits until they entered their nests or disappeared in the leaf litter. The distance of fruit displacement was then measured.

Fruit removal by ants was assessed experimentally by distributing marked fruits (small dot of Enamel paint, Testors, Rockford, USA) along one transect, at intervals of 10 m to ensure independent discoveries by different ant colonies (Kaspari 1993). The marking procedure apparently had no effect on ant response (Passos and Oliveira 2003). Each fruit was placed directly on the forest floor under a wire cage (17×17×8 cm, 1.5 cm mesh) closed at the top and staked to the ground to permit free access by ants and exclude vertebrates (Roberts and Heithaus 1986). Fruits were set out at ca. 1800 hours, and their removal was checked after 12 h. A given fruit was considered removed if not found within a 30 cm radius of the cage (N=30 fruits). We interrupted every trial subjected to rainfall.

Seed germination

To test if fruit cleaning (i.e., pulp removal) had any effect on G. opposita seed germination, we sowed intact fruits (control), as well as fruits whose pulp had been removed by us (treatment), in plastic trays (40×40 cm) containing regularly moistened vermiculite. A total of 40 fruits were used in each treatment. Fruits in both treatments were placed in separate trays that were kept in the greenhouse of the Universidade Estadual de Campinas. Fruits were buried 1 cm into the substrate, 3 cm apart from each other, and checked for germination at 7-day intervals until seeds germinated or presented signs of decay. We used chi-square tests to evaluate seed germination. Given that some ants may secrete fungicidal substances from their metapleural glands (Beattie et al. 1986), thus potentially

rendering an additional benefit for seed germination, cleaning of fruits is probably a conservative simulation of the services provided by the ants (see Pizo and Oliveira 2001).

Distribution of seedlings and juveniles

Ponerine species, in particular O. chelifer, are abundant at the study site, and accounted for a great number of the ant-seed interactions recorded for G. opposita (Passos and Oliveira 2003). The number of seedlings and juveniles of G. opposita growing on nests of O. chelifer as compared to control areas (without ant nests) was determined in May 2000 by establishing paired experimental plots (0.5×0.5 m). Experimental nests were located by following ant workers attracted to tuna baits placed on the forest floor. We tagged 40 O. chelifer nests, and established a control plot 2.5 m (random direction) away from each nest. Seedlings and juveniles were counted at the end of the G. opposita fruiting period (May), when they were abundant at the study site ('juvenile' refers to a plant that no longer has cotyledons). Seedling mortality was evaluated by re-checking the same experimental plots for the number of seedlings and juveniles at the end of the dry season (September). Differences in the number of seedlings and juveniles growing in treatment and control plots in May and September 2000 were analyzed using Wilcoxon paired-sample sign rank tests (Zar 1999). We compared the soil composition in experimental plots by collecting soil samples from O. chelifer nests (n=40) and control plots (n=40). Samples were air-dried and soils were analyzed for total nitrogen, macro-nutrients, pH, and organic matter. Since physical soil properties such as drainage and aeration may be modified locally by ant nests (Farji-Brener and Medina 2000), and may affect seedling establishment and growth, we evaluated soil penetrability in refuse piles of O. chelifer nests and adjacent plots (n=40). At each location, we released a sharpened wire stake (30 cm long) from the inside top of a 1.5 m high plastic PVC tube. The depth reached by the stake into the ground was the estimate of soil penetrability for that location. Differences in soil variables between nest and control plots were analyzed using Wilcoxon paired-sample sign rank tests.

In order to evaluate if seedlings growing near O. chelifer nests can gain protection from ants against herbivores, we performed an experiment using dipteran larvae. We placed one live larva on each of two seedlings growing on an ant nest, and on an adjacent plot without an ant nest. To permit free access by ants and exclude vertebrates, the seedlings were protected with wire cages (17×17×8 cm, 1.5 cm mesh) closed at the top and staked to the ground. The number of larvae attacked by O. chelifer ants, or by other ant species, in either experimental plot was recorded at 30-min intervals from 0700 to 0930 hours. Differences in the numbers of

Table 1 Ant behavior toward fallen fruits of *Guapira opposita* in the Atlantic rainforest of Cardoso Island, SE Brazil. Frequency is expressed as the percentage of records for each ant species relative to the total number of interactions observed at fruits (36). The behavior of the ant species attracted to the fruits is indicated in parentheses. Species of ants are arranged in alphabetical order under each subfamily. Key to ant behavior: *C* clean seeds by removing portions of the pulp on the spot, no displacement; *E* inspect or manipulate diaspore, no removal; *R* remove diaspores more than 5 cm; *T* try to remove diaspores, displacement <5 cm

Ant subfamily and species	Percentage [behavior at fruits (n=90)]
Ponerinae	
Odontomachus chelifer	27.8 (R, T)
Odontomachus sp.	2.8 (E)
Pachycondyla striata	27.8 (R)
Myrmicinae	
Acromyrmex aspersus	2.8 (C)
A. subterraneus	8.3 (E, R)
Crematogaster sp. 1	5.5 (C)
Crematogaster sp. 2	2.8 (C)
Pheidole sp. 1	8.3 (C)
Pheidole sp. 3	8.3 (E)
Solenopsis sp. 1	2.8 (E)
Strumigenys sp.	2.8 (E)

larvae attacked by ants were analyzed using Wilcoxon paired-sample sign rank tests (n=30 plot pairs).

Results

Ant-fruit interactions

Eleven ant species were attracted to fruits of *G. opposita* at PEIC (Table 1). The most frequent species recorded on fruits were the large ponerines *O. chelifer* and *P. striata* that together accounted for 56% of the ant-fruit interactions, and were the main seed vectors among the ants (Table 1). *O. chelifer* and *P. striata* displaced fruits to considerable distances (*O. chelifer*: mean±SD=2.18 ±1.18 m, range 0.90–4.00 m, n=8; *P. striata*: 1.28 ±0.58 m, range 0.50–2.30 m, n=10). *O. chelifer* and *P. striata* carried fruits into their nests in 87.5% and 40.0% of the records, respectively. Large attines such as *Acromyrmex subterraneus* may occasionally remove fruits. Other ants (mainly *Pheidole* and *Crematogaster*) typically recruited nestmates to remove the pulp on the spot, without displacing the seeds (Table 1). Ants removed 93% of the fruits used in removal experiments during the 12-h period.

Seed germination

While 100% of the cleaned seeds of *G. opposita* germinated in 10 days, only 20% of the pulp-coated seeds germinated in the same period (χ^2=50.05, P<0.0001) and 55% rotted in 20 days. The presence of pulp reduced the germination success in this species (100% for cleaned seeds against 45% for intact fruits; χ^2=30.34, P<0.001).

Distribution of seedlings and juveniles

By the end of the fruiting period (May) *G. opposita* seedlings and juveniles were more abundant in the vicinity of *O. chelifer* nests than in random plots without nests (seedlings: Z=−4.947, P<0.000, n=40; juveniles: Z=−3.818, P<0.000, n=40; Fig. 1a). Although the number of seedlings in ant nests decreased through the dry season (Fig. 1b), in September both *G. opposita* seedlings and juveniles were still more frequent in nests than in random plots (seedlings: Z=−4.560, P<0.000, n=40; juveniles: Z= −4.219, P<0.000, n=40).

The soil analyses indicated that the concentrations of phosphorus, potassium, and calcium are significantly higher at *O. chelifer* nests than at random locations. Soil samples from *O. chelifer* nests also had higher pH, while random locations had more organic matter, and higher cation exchange capacity and acid potential (H+Al) (Table 2). Soil penetrability was greater in *O. chelifer* nests than in random plots without nests (Table 2).
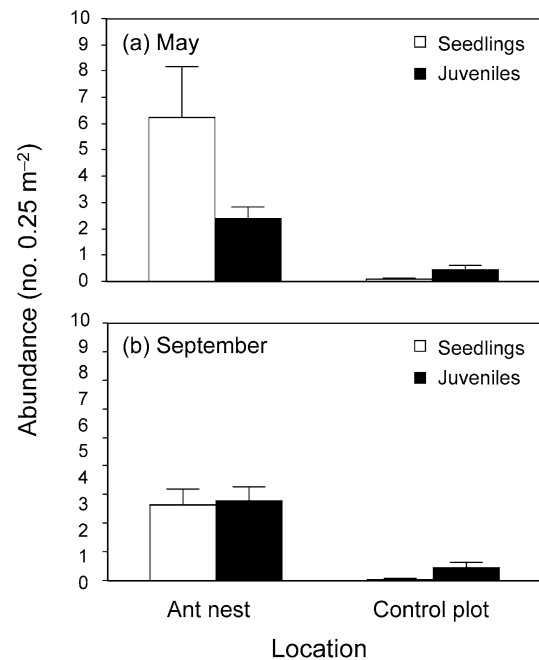

Fig. 1a, b Quantity of seedlings and juveniles of *Guapira opposita* in nests of *Odontomachus chelifer* (n=40) and in respective adjacent control plots (n=40). **a** Beginning of dry season (May); **b** end of dry season (September). Data are means+1 SE

Table 2 Comparison of soil variables from nests of *Odontomachus chelifer* (n=40), and random spots on the forest floor (n=40). Total N concentration in g kg^{-1}; K, Ca, Mg, H+Al concentrations, and CEC (cation exchange capacity) are in mmol dm^{-3}; P concentration in mg dm^{-3}; organic matter in g dm^{-3} (values are means). Soil penetrability in cm

Soil variable	*O. chelifer*	Random	Wilcoxon's P
Total nitrogen	1.21	1.40	0.594
Phosphorus	13.88	11.20	0.024
Potassium	1.45	1.24	0.041
Calcium	5.13	2.26	<0.001
Magnesium	2.95	2.58	0.244
Organic matter	43.98	58.40	0.003
CEC	72.16	137.05	<0.001
H+Al	62.55	131.08	<0.001
pH	3.88	3.24	<0.001
Penetrability	10.21	6.21	<0.001

Live dipteran larvae placed on seedlings growing near *O. chelifer* nests were attacked by ants in greater numbers than those in control plots without nests (Fig. 2; Z=15.42, P=0.003). *O. chelifer* accounted for 97% of the attacks to larvae near the ant nests: attacks by other ants (small myrmicines) were more common (27%) in the control plots (Fig. 2).

Discussion

The fruits of *G. opposita* are rapidly removed by ants on the forest floor, and the species composition of the ant
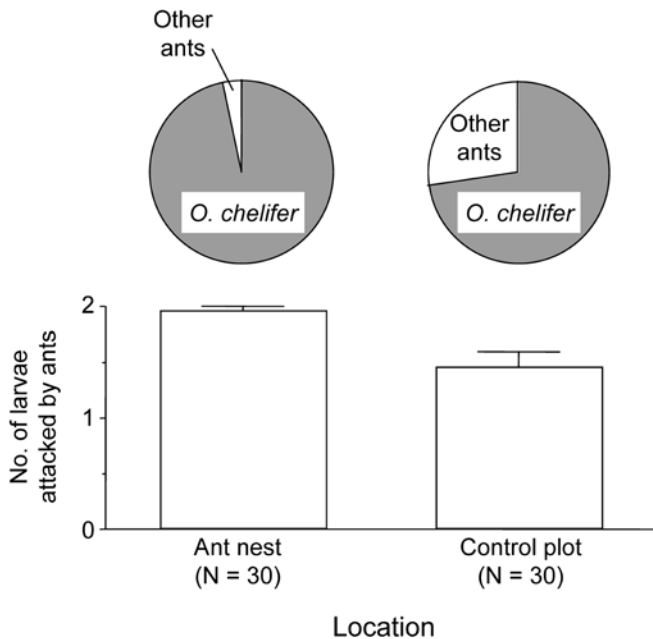
Fig. 2 Distribution of attacks by ants (mean+1 SE) on live dipteran larvae placed on seedlings growing in *O. chelifer* nests, and in respective control plots (two larvae per location). Pie charts show the proportion of larvae attacked by *O. chelifer* and other ants (small myrmicines) in either experimental plot

assemblage exploiting the protein-rich fruits of this species is similar to that found for lipid-rich arillate species (Horvitz and Beattie 1980; Horvitz 1981; Pizo and Oliveira 1998). However, the percentage occurrence of ponerines (*O. chelifer* and *P. striata*) at fruits of *G. opposita* is much higher (27.8% for each species) than that recorded for other plant species (Pizo and Oliveira 1998; Passos and Oliveira 2002; but see Horvitz and Schemske 1986b). Chemicals mediate the behavior of ants toward potential food items, and lipids are the major attractant in the interactions between ants and diaspores of myrmecochorous species (Marshall et al. 1979; Brew et al. 1989). Ants in the subfamily Ponerinae are predominantly carnivorous (Hölldobler and Wilson 1990), and the lipid-rich elaiosome can be regarded as an arthropod prey mimic (Carroll and Janzen 1973). Lipid content is apparently also important in ant attraction to non-myrmecochorous fleshy diaspores in Brazilian Atlantic forests, with lipid-rich arillate seeds being especially attractive to ponerine ants (Pizo and Oliveira 1998, 2001; Passos and Oliveira 2002). Although the role of proteins as ant attractants in diaspores is still to be tested, our results with *G. opposita* suggest that the fruits might complement the protein intake in colonies of primarily carnivorous ponerines, especially in areas with low litter arthropod biomass such as the sandy forest at PEIC (Pizo et al. 2004). The estimates of the displacement of *G. opposita* fruits by *O. chelifer* and *P. striata* are similar to those reported for other plant species bearing lipid-rich diaspores in neotropical forests (e.g., Horvitz and Beattie 1980 for *Calathea* spp.; Passos and Oliveira 2002 for *Clusia cruiva*; Pizo and Oliveira 1998 for *Cabralea*

canjerana). In contrast, small myrmicine ants such as *Pheidole* spp. do not displace fruits, but recruit nestmates and consume the pulp on the spot.

Pulp removal by us increased *G. opposita* germination success. Seed cleaning by ants may reduce fungal attack on fallen fruits, facilitating germination in some species (Horvitz 1981; Oliveira et al. 1995; Leal and Oliveira 1998; Pizo and Oliveira 1998). *G. opposita* seedlings emerge shortly before the dry season, thus seedling mortality due to drought stress can be severe in this species (see Fig. 1b). Seed cleaning in *G. opposita* may be advantageous because early emergence maximizes the length of the first growing season and allows time for the development of a potentially large root system, which might ensure survival through the first dry season (see Garwood 1983; Traveset 1998). The data on the number of seedlings and juvenile plants before and after the dry season indicate that, although seedlings suffered a heavy mortality during this period due to desiccation (L. Passos, personal observation), juvenile plants survived much better. These results are in agreement with the observed pattern for tropical rainforest trees, in which rates of survivorship improve as seedlings become larger and older (Lieberman 1996; Traveset 1998).

G. opposita seed dispersal by *O. chelifer* had a marked effect on the distribution pattern of seedlings and juveniles of this species, supporting the view that large ponerines are key species mediating ant-seed interactions in tropical forests. Although some plant species bear diaspores which are morphologically adapted for sequential dispersal by vertebrates first and then by ants (Davidson 1988; Kaufmann et al. 1991), the effect of ants on seed recruitment found here is devoid of any morphological specialization by the plant for ant dispersal. It has recently been suggested that the importance of primary dispersal by vertebrates for plants bearing fleshy fruits greatly exceeds that of secondary dispersal by ants (Böhning-Gaese et al. 1999). The distribution patterns of *G. opposita* seedlings and juveniles, however, suggest that dispersal by ants strongly affects recruitment in this species [but see Horvitz and Le Corff (1993) for a discussion of the scale of bird and ant dispersal]. Although distances of seed displacement by ants may be small, ant-induced seed movement may lead to non-random spatial recruitment patterns in *G. opposita*.

The association between ants and diaspores could have arisen without special adaptations on the part of the plants or ants. Considering that ant-diaspore interactions are common on the forest floor of tropical forests (Pizo and Oliveira 2000), we predict that ant-induced effects on recruitment will probably hold for other tropical species, particularly those presenting small and nutritious diaspores (Passos and Oliveira 2002).

Seed dispersal, germination, and early seedling growth/survival are the most critical stages in determining where plants recruit within a landscape (Herrera et al. 1994; Schupp 1995). Ant nests are known to have specific temperature, moisture, texture, and nutrient characteristics (Hölldobler and Wilson 1990; Farji-Brener and Medina

2000) that may be important conditions for seed germination and seedling establishment (Horvitz 1981). *O. chelifer* nests are significantly richer in phosphorous, potassium, and calcium, and the ants also increase soil penetrability, which might improve the performance of *G. opposita* seedlings (see Horvitz 1981; Levey and Byrne 1993). Moreover, our data suggest that the association of *G. opposita* seedlings with *O. chelifer* nests can potentially render the plant some protection against herbivores, as expressed by the ants' predatory behavior toward dipteran larvae. Indeed, ant-derived protection of seedlings growing on ant nests has already been demonstrated (Davidson and Epstein 1989). Given that herbivore-induced injury was seen in many *G. opposita* seedlings and juveniles (L. Passos, personal observation), an anti-herbivore role of *O. chelifer* deserves further investigation. Drought stress and herbivory seem to be important sources of seedling mortality in *G. opposita*, as reported for other tropical species (Steven 1994; Lieberman 1996; Basset 1999). However, since *G. opposita* seedlings are strongly clumped at *O. chelifer* nests, additional density-related mortality factors should also be considered, as well as other possible factors affecting establishment and survivorship (see Auspurger and Kelly 1984; Howe and Schupp 1985; Howe 1989).

The location of suitable sites for seedling establishment is unpredictable, but some sites are associated with higher probabilities of survival than others (Schupp 1993). The nests of *O. chelifer* seem to be suitable sites for *G. opposita* seedlings/juveniles, but results with other species in the same area (Passos and Oliveira 2002, 2003) suggest that the quality of such microsites may vary with the requirements of the plant species involved (see also Schupp 1993; Denslow et al. 1987).

Recent studies on seed dispersal ecology in the tropics have emphasized the role of secondary dispersers and their impact on the seed fate of primarily vertebrate-dispersed species (e.g., Levey and Byrne 1993; Böhning-Gaese et al. 1999; Pizo et al. 2004). This study also illustrates the complex nature of the dispersal ecology of tropical tree species. Untangling the complexity of such dispersal systems is crucial for understanding the evolutionary relationship between frugivores and fruiting plants, as well as their role in determining the spatial structure of plant populations (Wang and Smith 2002).

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