

The role of ants in the removal of non-myrmecochorous diaspores and seed germination in a neotropical savanna

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Abstract: Ants frequently interact with non-myrmecochorous diaspores on the ground in neotropical savannas. Our objective was to quantify the removal rate of these diaspores by ants and vertebrates in order to test the predator avoidance hypothesis, and to test how diaspore traits influence removal by ants and dispersal distance. We also investigated whether seed cleaning (removal of fruit matter simulating ant activity) can influence seed germination. We performed removal experiments with nine diaspore species in a reserve of cerrado savanna in south-east Brazil. Considerable differences in removal rates were found among the nine species. We found a positive linear relationship between lipid content and removal rates for five diaspore species. Vertebrate predation pressure was low for most species, limiting the benefits that ants can provide to plants to escape predators. Ants displace diaspores up to 25 m, which may increase the chance of a seed hitting a safe site. Smaller diaspores attain longer distances of dispersal than large ones. Seed cleaning increased the germination rate for five out of six species tested in greenhouse experiments. Ant activity can have relevant and possibly lasting effects on seed fate of plants adapted for vertebrate dispersal in the cerrado savanna.

Key Words: ant-plant interaction, cerrado, myrmecochory, secondary seed dispersal, seed predation

INTRODUCTION

Although myrmecochory (i.e. primary seed dispersal by ants) is much more frequent in plants of arid zones of Australia and South Africa (Beattie 1985), recent work in other tropical areas has shown that ants can positively affect seed fate of diaspores (i.e. fruits or seeds that act as the unit of plant dispersal) adapted for vertebrate dispersal (Farji Brener & Silva 1996, Levey & Byrne 1993, Passos & Oliveira 2003). The exploitation of such non-myrmecochorous diaspores by ants usually involves the removal of the diaspore to the ant nest, where the fleshy portion (seed aril or fruit pulp) is fed to larvae and the seed discarded unharmed (Pizo & Oliveira 2001). Because ants quickly remove seeds from predation-prone zones such as the ground surface beneath the plant crown, they reduce the chance of seed encounter by predators (Beattie 1985). This predator-avoidance hypothesis, i.e. a presumed selective advantage of the increased escape

from seed predators as seeds are carried back to ant nests, has been frequently supported in studies with true myrmecochorous diaspores (Giladi 2006). However, the role of ants in providing an escape from post-dispersal seed predators of non-myrmecochorous diaspores has received considerably less attention (Levey & Byrne 1993, Pizo & Oliveira 1998).

Ant behaviour may have been an important selective force in the evolution and maintenance of diaspore traits, since ants show differential response to variation in the degree of seed clumping and morphology of elaiosome-bearing seeds (Hughes & Westoby 1992a). Approaches to explain differences in seed preferences by ants include the foraging strategy hypothesis, under which ants would use diaspore characteristics to increase foraging efficiency and net energy intake (Hughes & Westoby 1992a, Pyke *et al.* 1977). The size of the reward, either absolute or relative to seed size, has been shown to be especially important. Relationships between diaspore traits and removal rates can indicate specific foraging strategies of ants (Hughes & Westoby 1992a). However, adaptations to dispersal by ants may bring significant costs to the plants. Fallen

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diaspores are also attractive to seed predators, usually rodents (Fedriani *et al.* 2004), and intact fruits are especially susceptible to pathogen attack that may kill the seed (Ohkawara & Akino 2005). If removal of the seed's fleshy coat (aril or fleshy pulp) enhances the probability of seed survival and germination, ants may influence seed fate as they clean the seed from fleshy matter. If this is true, seeds deprived of fleshy matter should present a higher germination success than intact diaspores.

The role of ants in diaspore removal and seed fate has largely been neglected in the largest South American savanna known as cerrado (Oliveira-Filho & Ratter 2002). Although the vast majority of plants from this savanna have no visible adaptation for seed dispersal by ants (Gottsberger & Silberbauer-Gottsberger 1983), these insects may influence the fate of large numbers of seeds that fall to the ground spontaneously or are dropped by vertebrate frugivores (Leal & Oliveira 1998). Specific benefits derived from the interaction between ants and diaspores primarily adapted for vertebrate dispersal are still poorly understood in savannas compared with other formations (Vander Wall & Longland 2004). In this study we collected evidence to examine three hypotheses that might account for the role of ants while interacting with non-myrmecochorous diaspores in the cerrado savanna. To test the predator-avoidance hypothesis we compared the relative contribution of ants and vertebrates for the removal of nine non-myrmecochorous diaspore species, and also recorded the dispersal distance provided by ants. In order to test the foraging strategy hypothesis, we measured several morphological parameters of plant diaspores and evaluated their relationship with removal rates by ants. Finally, to evaluate the effect of seed cleaning (i.e. removal of fleshy matter) we compared the percentage of germination of seeds coated in fleshy pulp with that of seeds from which the fleshy portion was removed simulating ant behaviour.

STUDY SITE

Diaspore removal experiments were carried out in the reserve of the Estação Experimental de Itirapina (22°12'S, 47°51'W), south-east Brazil. Average annual rainfall is 1360 mm, concentrated mostly in the warm/wet season (December to March). A dry/cold season occurs from April to November. Mean annual temperature is 21.8 °C (data from 1994 to 2004 from the reserve's climatological station). The vegetation at the study site is the cerrado *sensu stricto*, the typical savanna-like vegetation that grows on sandy, nutrient-poor soils (Oliveira-Filho & Ratter 2002). The vegetation is characterized by 50–80% of ground cover by small palms (*Syagrus petraea* (Mart.) Becc., *Attalea geraensis* Barb. Rodr.), shrubs (*Miconia albicans* (Sw.) Triana, *Campomanesia pubescens* (DC.)

O. Berg), and 4–6-m-tall trees (*Miconia rubiginosa* (Bonpl.) DC., *Xylopia aromatica* (Lam.) Mart., *Pouteria torta* (Mart.) Radlk). A fair amount of herbaceous vegetation covers the soil (*Echinolaena inflexa* (Poir.) Chase, *Paspalum* spp., *Bromelia balansae* Mez). Further floristic details of the study site are given by Giannotti (1988).

METHODS

Diaspore removal experiments: ants versus vertebrates

To examine the contribution of ants to diaspore removal on the savanna floor we performed field experiments with fruits collected from nine plant species at the study site: *Erythroxylum pelleterianum* A. St.-Hil (Erythroxylaceae), *Miconia albicans* (Sw.) Triana, *M. fallax* DC., *M. rubiginosa* (Bonpl.) DC. (Melastomataceae), *Myrcia lingua* (O. Berg.) Mattos & D. Legrand (Myrtaceae), *Ocotea pulchella* Mart. (Lauraceae), *Ouratea spectabilis* (Mart. ex Engl.) Engl. (Ochnaceae), *Psychotria tricholoba* Müll Arg. (Rubiaceae), and *Stryphnodendron adstringens* (Mart.) Coville (Mimosaceae). Large numbers of ripe fruits or seeds frequently fall from these trees, either naturally or dropped by vertebrate frugivores. During the fruiting period of each species, diaspore removal by ants only (exclosure treatment) was compared to combined removal by ants and vertebrates (open control) through a paired experiment on the savanna floor. We assume that removal of diaspores by vertebrates is equal to predation. This is based on data showing that small seeds are indeed eaten rather than dispersed by small rodents (Vieira *et al.* 2003). Tinamous and doves, the birds most likely to remove such diaspores on the floor, are also recognized as seed predators by cracking the seeds in their gizzards (Schubart *et al.* 1965). Vertebrates were excluded from treated diaspores with the aid of a wire cage (17 × 17 × 8 cm), fenced on top and sides with mesh (1.5 cm) and staked to the ground (see Roberts & Heithaus 1986 for a similar method). Control diaspores were placed outside the cage, 15 cm away. At each station, treatment and control categories each received ten diaspores of a single plant species, and were exposed for 24 h. After this period we recorded the ant species interacting with diaspores, and the number of diaspores missing. The diaspores were distributed under fruiting plants of the same species to simulate natural conditions under which they are found by ants and vertebrates. Experimental diaspores were marked with a small dot of a permanent ink marker (Testors, USA) to distinguish them from naturally fallen ones. The ink mark has no detectable effect on ant behaviour (Passos & Oliveira 2002). We kept a minimum distance of 10 m between replicates to provide independent discoveries by different ant colonies (Levey & Byrne 1993, Pizo & Oliveira

2000). The number of replicates varied between species according to diaspore availability. To compare diaspore removal of treated (accessible to ants only) and control (accessible to vertebrates and ants) diaspores of each plant species we used Wilcoxon paired sample tests, except for *O. spectabilis* and *P. tricholoba* for which we used Mann–Whitney U-tests due to small sample sizes.

The ant assemblage removing diaspores

To increase the number of records of ants interacting with diaspores and to measure the distance of displacement we also conducted systematic samplings. We placed diaspores of four selected plant species on the floor of the cerrado, and recorded the ants removing them in diurnal and nocturnal samplings. The following plants were selected for these surveys: *Erythroxylum pelleterianum* (Erythroxylaceae), *Ocotea pulchella* (Lauraceae), *Miconia albicans* and *M. rubiginosa* (Melastomataceae). The number of diaspores used to attract ants varied according to their availability (from 40 to 100 diaspores), and samplings were carried out during the fruiting period of each species. Fresh diaspores of each species were placed at sampling stations 10 m apart, 1–2 m off a transect that crossed the study site. At each sampling station, two diaspores of a single species were placed on a small piece of white filter paper (4 × 4 cm) to facilitate visualization. The filter paper apparently did not influence ant behaviour (Pizo & Oliveira 2000). Diurnal samplings began at 08h00, and nocturnal ones at 19h00. At each period we walked slowly along the transect over 2 h and recorded the ants removing diaspores. We followed ants carrying diaspores until they reached the nest or disappeared in the leaf litter. The distance of displacement was then measured. At any time during the study, opportunistic observations of ants removing diaspores were also recorded. Voucher specimens of ants and plants are deposited in the entomological collection of the Universidade Federal Rural do Rio de Janeiro (CECL), and at the herbarium of the Universidade Estadual de Campinas (UEC), respectively.

Diaspore traits affecting removal by ants

To investigate the influence of diaspore traits on removal by ants we measured several morphological and chemical traits of plant diaspores and evaluated their relationship with removal rates by ants. Diaspore morphology was characterized by fresh mass, fresh mass of seeds, and pulp/seed ratio. Measurements were taken on fruits of 1–5 plants per species, and from 1–10 fruits per individual according to diaspore availability. Ripe fruits were frozen and brought to the laboratory for measurements. For the

chemical analyses, diaspores were selected based solely on their availability. We limited the chemical analyses to lipid concentration because lipids are among the main elicitors of diaspore removal by ants (Hughes *et al.* 1994, Pizo & Oliveira 2001). Fruit pulp was obtained from ripe fruits collected at the plant crown, or from recently fallen ones. Lipids were analysed according to the methods described in AOAC (2000). Mean diaspore removal inside enclosure cages (access only to ants) was related to per cent lipid of the fruit pulp, fruit fresh mass, and pulp/seed ratio (all log-transformed) with linear regression. For seven species for which we had records of the displacement distance, we performed the same analysis using the mean or the maximum dispersal distance (log-transformed) as the dependent variable to evaluate the effect of diaspore traits on the distance of dispersal by ants.

Seed germination tests

We evaluated the effect of ant–diaspore interactions on seed germination through greenhouse experiments. Six plant species observed interacting with ants in the field were used in the experiments: *Copaifera langsdorffii* Desf. (Fabaceae), *Erythroxylum pelleterianum* (Erythroxylaceae), *Guapira noxia* (Netto) Lundell (Nyctaginaceae), *Miconia albicans*, *M. rubiginosa* (Melastomataceae) and *Ouratea spectabilis* (Ochnaceae). Diaspores from at least three individuals of each species were collected in the field and mixed to form a bulk sample. Diaspores were then sorted into two groups and subjected to one of the following treatments: removal of aril/pulp (to simulate removal by ants), or control (manipulated by us but without removal of fleshy part). Both groups were sown in plastic trays (37 × 28 cm) containing regularly moistened vermiculite. Fruits of both treatments were sown interspersed with each other in a systematic design type in the greenhouse of the Universidade Estadual de Campinas. Fruits were buried 1 cm in the substrate, 2 cm apart from each other, and checked at 7–10-d intervals. Fruits of *Miconia* spp. were sown on the surface of the vermiculite, because these species require light to germinate (Hoffmann 1996). Since the number of seeds varies with fruit size in *Miconia* (from 5 to 25 seeds), we first separated fruits by size to control for the number of seeds used in each treatment. We then assigned an equal number of diaspores from each size category to both treatment groups. Seeds were removed from single fruits and sown in a cluster about the same diameter as the fruit. A fruit of the same size was sown as control. When a seed germinated, the whole cluster (or fruit) was removed from the experiments. Germination experiments ran until seeds presented signs of decay or until no new germination was recorded for 2 mo. We used G-tests to compare the final percentage of germination between treatments.

Table 1. Growth form, primary dispersal agent, mean masses of fresh diaspores, seeds, and pulp/seed ratio of the plant species studied (N = 1–10 diaspores from 1–5 plants). Seeds of *S. adstringens* have no fleshy portion, thus pulp/seed ratio was set to be zero.

Plant species	Growth form	Primary dispersal	Diaspore mass (g)	Seed mass (g)	Pulp/seed ratio
<i>Erythroxylum pelleterianum</i>	Shrub	Bird	0.15	0.041	2.63
<i>Miconia albicans</i>	Shrub	Bird	0.07	0.009	6.78
<i>M. fallax</i>	Shrub	Bird	0.25	0.015	16.5
<i>M. rubiginosa</i>	Tree	Bird	0.12	0.012	11.7
<i>Myrcia lingua</i>	Tree	Bird	0.23	0.082	2.04
<i>Ocotea pulchella</i>	Tree	Bird	0.16	0.100	0.693
<i>Ouratea spectabilis</i>	Tree	Bird	0.52	0.338	0.525
<i>Psychotria tricholoba</i>	Shrub	Bird	0.11	0.033	2.44
<i>Stryphnodendron adstringens</i>	Tree	Abiotic	0.09	0.090	0

RESULTS

The ant assemblage removing diaspores

We recorded 27 ant species interacting with diaspores in the field (Appendix 1). The number of ant species recorded per diaspore species varied from zero (for *Psychotria tricholoba*) to 19 (for *Erythroxylum pelleterianum*). Most species (18 out of 27) did not remove diaspores, and just cleaned the seed (i.e. removed the fleshy part) or collected liquids from the diaspore on the spot. This was especially true for *Camponotus* spp. and myrmicine ants in the genera *Pheidole*, *Solenopsis* and *Wasmannia*. Diaspore removal was recorded among ants in the genera *Atta*, *Pheidole*, *Dinoponera*, *Ectatomma*, *Odontomachus* and *Pachycondyla*, the last four usually regarded in the literature as primarily carnivorous.

Diaspore traits and their removal by ants and vertebrates

Diaspore traits differed greatly among species used in the removal experiments (Table 1). For instance, diaspore size varied from 0.07 g in *Miconia albicans* to 0.52 g in *Ouratea spectabilis*. Due to limited diaspore availability, per cent lipid content data were obtained for five species only. The amount of lipids in the fleshy portion of diaspores varied from 0.78% fresh mass in *Miconia rubiginosa* to 31.6% fresh mass in *Ocotea pulchella*, a 40-fold difference.

Diaspore removal differed between caged and open treatments only for two species (Table 2), and ants were likely to be the main source of removal for most diaspores in both treatments because no other invertebrates were observed removing diaspores. Removal at open controls was significantly correlated with removal at caged treatments across the plant species studied ($r = 0.75$; $N = 9$; $P = 0.02$).

To test the foraging strategy hypothesis we related the removal rates of diaspores inside enclosure cages with the following seed parameters: per cent lipid content of

Table 2. Results from diaspore removal experiments on the savanna floor. Ten diaspores of each species were set out under an enclosure cage (access to ants; vertebrates excluded) paired with an open control (access to all animals). The number of diaspores removed was recorded after 24 h. N refers to the number of replicates; ns indicates no significant difference ($P > 0.05$).

Plant species	N	Number of diaspores removed (mean \pm SD)		P
		Enclosure treatment	Open control	
<i>Erythroxylum pelleterianum</i>	5	5.6 \pm 3.9	6.8 \pm 4.0	ns
<i>Miconia albicans</i>	16	3.1 \pm 3.4	4.3 \pm 4.0	ns
<i>M. fallax</i>	5	5.6 \pm 4.1	5.6 \pm 4.2	ns
<i>M. rubiginosa</i>	33	3.2 \pm 4.4	4.0 \pm 4.5	0.02
<i>Myrcia lingua</i>	5	3.8 \pm 4.8	4.2 \pm 5.3	ns
<i>Ocotea pulchella</i>	5	6.2 \pm 3.0	4.8 \pm 4.8	ns
<i>Ouratea spectabilis</i>	3	1.0 \pm 1.7	2.3 \pm 0.6	ns
<i>Psychotria tricholoba</i>	3	0.0 \pm 0.0	0.3 \pm 0.6	ns
<i>Stryphnodendron adstringens</i>	7	1.6 \pm 1.9	5.7 \pm 4.2	0.05

the fleshy portion, fruit mass and pulp/seed ratio. We applied stepwise regression analysis using the backward selection procedure from STATISTICA software, version 5 (c1996, STATSOFT, INC.). The final model included only lipid content of the fleshy portion which explained most of the variation in the removal rates ($y = 2.6x + 2.5$; $N = 5$; $t = 13.3$; $P < 0.001$; $r^2 = 0.98$) (Figure 1). Additional variables (fruit mass and pulp/seed ratio) when included in the model were not significant. Stepwise or univariate regression analysis without lipid data gave similar results. A negative, but non-significant, correlation was found between lipid content of the fleshy portion and pulp/seed ratio ($r = -0.85$; $N = 5$; $P = 0.07$).

Ants disperse diaspores to considerable distances (up to 25.4 m), but there is also great variation among the distances achieved per diaspore species (Table 3). No relationship was detected between the mean dispersal distance and diaspore traits. However, the maximum distance of dispersal recorded was negatively influenced by diaspore mass ($y = -11.7x + 1.7$; $N = 7$; $t = -5.04$; $P = 0.004$; $r^2 = 0.80$).

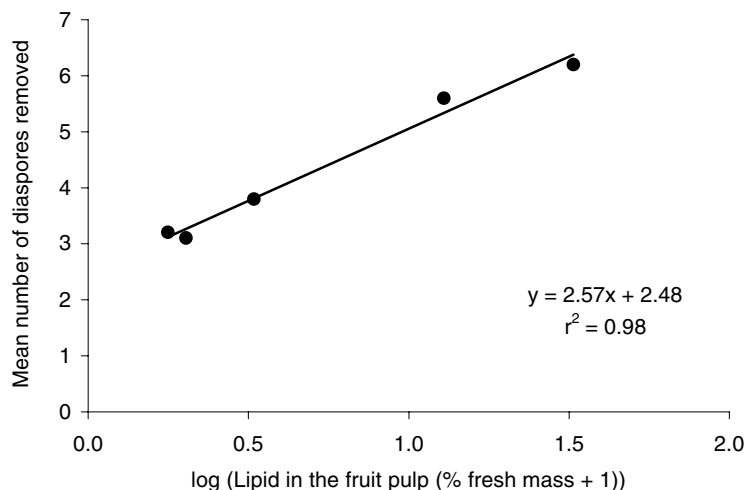


Figure 1. Effect of lipids on diaspore removal by ants in the cerrado savanna. Mean number of diaspores (out of 10) removed by ants in 24 h as a function of per cent lipid in the fleshy portion (amount of lipid relative to total fresh mass) for five plant species.

Table 3. The mean distance (m) that ants disperse diaspores in the cerrado savanna, range and sample size.

Plant species	Distance of displacement (m)		
	Mean	Range	N
<i>Erythroxylum pelleterianum</i>	1.46	0.1–7.1	22
<i>Miconia albicans</i>	5.91	2.0–13.9	12
<i>Miconia fallax</i>	1.90	–	1
<i>Miconia rubiginosa</i>	6.54	0.2–14.6	25
<i>Myrcia lingua</i>	3.20	2.3–4.6	3
<i>Ocotea pulchella</i>	4.52	0.1–9.0	2
<i>Stryphnodendron adstringens</i>	17.7	10.0–25.4	3

Seed germination tests

The removal of fruit pulp simulating ant activity increased seed germination of five out of six plant species tested (Table 4). Greenhouse conditions apparently were not adequate for the germination of *Ouratea spectabilis*. Pulp removal increased germination success by 35–77% compared with controls. For species with germination success > 40% in the control group, we also compared the

germination rate. In such cases seed cleaning increased germination speed, as recorded for *Miconia albicans* (Kolmogorov–Smirnov test: $D_{max} = 0.90$; $P < 0.001$) and *Copaifera langsdorffii* ($D_{max} = 0.55$; $P < 0.01$).

DISCUSSION

We found evidence that ants are an important source of diaspore removal on the floor of the cerrado savanna, and that this activity is influenced by the lipid content of the fleshy portion. Diaspores are transported to the nests up to 25 m, and seeds deprived of the fleshy part showed higher germination rates than intact seeds. Taken together these sources of evidence indicate that ants can influence the fate of non-myrmecochorous diaspores in the cerrado.

Fast removal of vulnerable seeds by non-granivorous ants supports the predator-avoidance hypothesis (Manzaneda *et al.* 2005, Pizo & Oliveira 1998, Roberts & Heithaus 1986). However, the interpretation of these results is more difficult when seed predation pressures

Table 4. Results of G-tests on the germination of selected plant species in the cerrado. Treatment refers to seeds from which the fleshy part (aril or pulp) was manually removed to simulate ant activity; control seeds were just hand manipulated, but without removal of fleshy part. Per cent differences between treatment and control groups in final germination are indicated. Positive differences indicate increased seed germination in treatment compared with control group.

Plant species	Number of seeds sown		Number of germinated seeds		% difference	G-value
	Treatment	Control	Treatment	Control		
<i>Copaifera langsdorffii</i>	21	12	18	5	+ 43%	7.0**
<i>Erythroxylum pelleterianum</i>	60	60	31	0	+ 52%	54.0***
<i>Guapira noxia</i>	16	13	11	0	+ 69%	18.6***
<i>Miconia albicans</i>	60	60	60	39	+ 35%	33.6***
<i>Miconia rubiginosa</i>	30	30	24	1	+ 77%	42.7***
<i>Ouratea spectabilis</i>	20	20	4	0	–	–

** $P < 0.01$; *** $P < 0.001$.

are weaker (Giladi 2006). Differences between the mean diaspore removal in enclosure treatments and open controls indicated that predation levels (removal of diaspores by rodents and granivorous birds) are small to moderate for most species, with differences ranging from 0% (as in the case of *M. fallax*) to 38% (for *M. albicans*), most of which are not significant. Exceptions were *O. spectabilis* and *S. adstringens*, with removal rates at open controls 100% and 250% higher than caged treatments, respectively. However, the large variance found in removal rates indicates great spatial variation within the study site and in the potential benefits ants may render to plants, as found by other authors working at larger spatial scales (Fedriani *et al.* 2004, Manzaneda *et al.* 2005). In addition, not all ants recorded interacting with diaspores are recognized as good dispersers. *Atta* and *Pheidole* are considered as potentially important seed consumers, although they can also disperse some seeds (Dalling & Wirth 1998, Farji Brener & Silva 1996, Hughes & Westoby 1992b, Leal & Oliveira 1998, Levey & Byrne 1993). However, we believe that most diaspores have a higher probability of survival when removed by ants for three reasons. First, ants can remove seeds to considerable distances (up to 25 m), far from the parent-offspring conflict zone under the parent crown (Retana *et al.* 2004). Second, seed cleaning behaviour can increase the germination percentage and speed of some plant species, minimizing the time seeds are exposed to predators or pathogens (Oliveira *et al.* 1995). Third, seedlings of several plants investigated in this study are found mainly in refuse piles of ant nests (A. V. Christianini & P. S. Oliveira unpubl. data). Some of this evidence, however, cannot be exclusively linked to the predator-avoidance hypothesis. Indeed, in a review of the evidence for the evolution of myrmecochory, Giladi (2006) found several examples of studies showing simultaneous support to non-competing hypotheses such as the predator-avoidance and the directed-dispersal hypothesis. Our findings could reflect weak selection pressure from vertebrate predators, which can also be affected by spatial or temporal heterogeneity (Manzaneda *et al.* 2005, and references therein). Our experimental design does not allow us to examine the historical selective forces that shaped ant dispersal of non-myrmecochorous diaspores, but give some insights on seed preference by ants in cerrado.

At least for the five species for which data on lipid content of fruit pulp were available, our data suggest that ants collect diaspores on the basis of high lipid content, i.e. maximizing the amount of lipids ingested per unit of diaspore mass handled. This finding supports the foraging strategy hypothesis. Animals are thought to adjust their foraging strategies to optimize their net rate of energy intake (Pyke *et al.* 1977). Lipids are an important food resource for ants, serving a variety of purposes that include nutrition, physiological constituents and

behavioural releasers (Beattie 1985, and references therein). The fatty acid composition of lipid-rich diaspores and typical myrmecochorous seeds is closely similar (Hughes *et al.* 1994, Pizo & Oliveira 2001), and therefore it is not surprising that ants responded positively to lipid-rich diaspores in the present study. The fleshy portion of lipid-rich diaspores should provide a higher caloric reward to ants, since average gross-energy equivalents of lipids (38.9 kJ g^{-1}) are much larger than equivalents for protein (17.2 kJ g^{-1}) and carbohydrates (17.2 kJ g^{-1}) (see Izhaki 2002, and references therein). Water and carbohydrates are often the predominant reward in fruit pulp (Jordano 1995). Preference of ants for diaspores with a high absolute reward has been shown in other studies (Mark & Olesen 1996, Peters *et al.* 2003), but diaspore size (Gorb & Gorb 1995, Pizo & Oliveira 2001) and pulp/seed ratio (Hughes & Westoby 1992a) may also play a role.

We found that the maximum dispersal distances provided by ants in the cerrado were constrained by diaspore size, which suggests a disproportionate increase in the costs of transport of larger diaspores as travel distances of a forager ant increase. It is recognized that forager ants become more selective as the distance of a resource patch from the nest increases. Selectivity is reduced when resources are scarce (see Traniello 1989, and references therein). Larger diaspores should demand more time and energy to be brought to the nest, exposing the ants to higher risks of predation and/or to superior competitors. In resource-rich habitats large and low-rewarding diaspores would be removed at lower rates when found far from nests, thus achieving shorter distances of dispersal by ants. Future studies should investigate the role of resource availability and net energy gain on the distance of seed dispersal by ants.

The distances of displacement of diaspores found in this study (mean for all events 5.04 m) were larger than the mean 0.96 m global estimate of myrmecochorous dispersal distances (Gómez & Espadaler 1998), and larger than most distances recorded in other studies on seed dispersal by ants in the Neotropics (Ness *et al.* 2004). The larger distances recorded in the cerrado likely result from the many records of *Attini* (mainly *Atta* spp.) carrying diaspores. Dispersal distances by *Atta* were larger than those found for all other species (A. V. Christianini, unpubl. data). Similar displacement distances recorded for *Attini* ants in another cerrado locality reinforce this hypothesis (Leal & Oliveira 1998). *Atta* workers are among the largest ants in the local ant assemblage. Ant body size bears a positive linear relationship with dispersal distances (Ness *et al.* 2004), which is possibly linked with the density and distribution of the ant nests (Gómez & Espadaler 1998). Nevertheless all studies report great variance around the distances of dispersal. Many diaspores are also dropped and not recovered during transport to the nest (Dalling & Wirth 1998, Leal &

Oliveira 1998). From a plant's perspective, it means that seeds can be spread out at variable distances from the parent plant, and at variable densities (Dalling & Wirth 1998, Passos & Oliveira 2002), which can be important for a plant that needs to reach a particular microsite for regeneration. *Miconia* species, for instance, need particular conditions for germination and establishment (Hoffmann 1996), and dispersal to long distances should increase the probability of a seed hitting a safe site (Green 1983).

Even large-seeded diaspores may benefit from the interaction with ants. For instance seed cleaning by ants may reduce fungal attack on fallen fruits (Oliveira *et al.* 1995, Ohkawara & Akino 2005), and pulp removal increased germination success of most species tested in this study. For those species dispersed near the end of the wet season and with no seed dormancy (as in the case of most species tested in this study) seed cleaning may also be advantageous because early emergence maximizes the length of the first growing season and allows time for the growing of a larger and deeper root system. This might ensure survival during the first dry season when seedlings are in general more susceptible to death during periods of water shortage (Moles & Westoby 2004, Passos & Oliveira 2004), which is particularly severe in the study site.

In conclusion secondary seed dispersal by ants can have relevant and possibly lasting effects on the fate of non-myrmecochorous diaspores in the cerrado savanna (see also Passos & Oliveira 2002, 2004). Ant activity can enhance seed germination, reduce seed predation by increasing dispersal distance from the parent plant, and produce a fine tuning of directed dispersal following longer primary dispersal by vertebrates (Giladi 2006, Horvitz & Le Corff 1993). At the moment we cannot discuss if ants are a selective force towards a particular suite of traits of non-myrmecochorous diaspores in cerrado. A large assemblage of vertebrate frugivores primarily disperses the seeds in this savanna (Gottsberger & Silberbauer-Gottsberger 1983), and fruit and seed traits are strongly constrained by phylogenetic inertia (Jordano 1995). Ignoring secondary dispersal, however, may produce a misleading picture of the seed dispersal loop of cerrado plants (Vander Wall & Longland 2004).

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Appendix 1. Ants recorded exploiting diaspores on the floor of cerrado. Plant species abbreviations: *Erythroxylum pelleterianum* (Eryp); *Miconia albicans* (Mica); *M. fallax* (Micf); *M. rubiginosa* (Micr); *Myrcia lingua* (Myrl); *Ocotea pulchella* (Ocop); *Ouratea spectabilis* (Ours); *Psychotria tricholoba* (Psyt); *Stryphnodendron adstringens* (Stra). Ant species are in alphabetical order, following a local species list (A. V. Christianini unpubl. data). Values inside cells indicate the number of records each ant species interacted with each diaspore species. Key to ant behaviour: C = clean seeds through pulp removal on the spot, no displacement; I = inspect or collect liquids, no displacement; R = remove diaspores \geq 5 cm; T = try to remove diaspores, displacement < 5 cm.

Ant species	Behaviour	Eryp	Mica	Micf	Micr	Myrl	Ocop	Ours	Psyt	Stra
<i>Atta laevigata</i> (Fr. Smith)	R	–	–	–	11	–	–	–	–	–
<i>Atta sexdens rubropilosa</i> Forel	I, R	3	21	3	29	8	2	–	–	3
<i>Azteca</i> sp. 2	C	2	–	–	–	–	–	–	–	–
<i>Azteca</i> sp. 3	I	1	–	–	–	–	–	–	–	–
<i>Camponotus renggeri</i> (Emery)	I	2	–	–	–	–	–	–	–	–
<i>Camponotus rufipes</i> (Fabricius)	I	1	–	–	–	–	–	–	–	–
<i>Dinoponera australis</i> Emery	R	6	–	–	1	–	1	–	–	–
<i>Ectatomma opaciventre</i> Roger	R	–	–	–	7	–	–	–	–	–
<i>Mycocepurus</i> sp.	C, I	2	–	–	–	–	–	–	–	–
<i>Odontomachus chelifer</i> (Latreille)	I, R	5	1	–	3	–	–	–	–	–
<i>Oligomyrmex</i> sp.	C	–	–	–	–	–	–	1	–	–
<i>Pachycondyla striata</i> Fr. Smith	R	19	1	–	2	–	–	–	–	–
<i>Pachycondyla villosa</i> (Fabricius)	R	1	–	–	1	–	–	–	–	–
<i>Pheidole gr tristis prox allarmata</i>	C	–	–	–	1	–	1	–	–	–
<i>Pheidole</i> sp. 2	C, R	6	–	1	–	1	–	–	–	1
<i>Pheidole</i> sp. 4	C	5	–	–	–	–	–	–	–	–
<i>Pheidole</i> sp. 5	C, T, R	13	1	–	–	1	1	–	–	–
<i>Pheidole</i> sp. 6	C	–	–	–	1	–	–	1	–	–
<i>Pheidole</i> sp. 7	C	8	2	–	3	–	–	2	–	–
<i>Pheidole</i> sp. 8	I, R	–	–	–	–	–	3	–	–	–
<i>Pheidole</i> sp. 15	C	2	–	–	–	–	–	–	–	–
<i>Pheidole</i> sp. 16	C	–	–	–	–	–	2	–	–	–
<i>Pheidole</i> sp. 17	C, R	2	–	–	–	–	–	–	–	–
<i>Solenopsis</i> sp. 1	C	1	–	–	–	–	–	–	–	–
<i>Solenopsis</i> sp. 2	I	1	–	–	–	–	–	–	–	–
<i>Solenopsis</i> sp. 3	C	–	–	–	–	–	1	–	–	–
<i>Wasmannia auropunctata</i> (Roger)	C	7	1	–	–	–	–	–	–	–
Total number of records at diaspores		87	27	4	59	10	11	4	0	4