

Secondary Seed Dispersal by Ants of *Ricinus communis* (Euphorbiaceae) in the Atlantic Forest in Southeastern Brazil: Influence on Seed Germination

by

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ABSTRACT

Some seeds rely on fleshy lipid-rich appendages as attractants to ants. By carrying those seeds to their nest, ants play a major role as seed dispersers and can provide suitable conditions for seed germination. *Ricinus communis* (the commonly known castor) seeds are primarily dispersed by **autocory** but probably present secondary dispersal by ants, once they bear a lipid-rich elaiosome. The following questions were addressed: (1) are ants legitimate dispersers of castor seeds?; (2) which ant species interact with the seeds?; and (3) is germination success higher in a predicted scenario met by seeds discarded from ant nest? We compared the removal of seeds with and without elaiosome and we determined which ant species interact with castor seeds and whether there was seed predation by ants. We performed experiments in controlled conditions to evaluate seed germination response to light, temperature and elaiosome. Seeds with elaiosome were removed preferentially and no predation was found, indicating that ants are legitimate seed dispersers. A large coterie of ants (20 species) interacts with castor seeds, especially Myrmicinae species. Elaiosome removal enhanced germination success, as well as alternated temperatures and absence of light. Therefore, by discarding seeds without elaiosome on the pile mound, ants may submit castor seeds to conditions that enhance germination.

Keywords: ant nest, castor, elaiosome, forest edge, myrmecochory, light, temperature.

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INTRODUCTION

Seed dispersal by ants, or myrmecochory, is widespread and occurs in over 60 plant families (van der Pijl 1972, Beattie 1985). Seeds of myrmecochorous species bear an elaiosome, a fleshy lipid-rich appendage that attracts ants (van der Pijl 1972, Beattie 1985, Holldöbler & Wilson 1990). The ants usually carry these seeds to their nest, eat the elaiosome, and discard the seed often in viable condition (Berg 1975, Davidson & Morton 1981, Beattie 1985, Passos & Oliveira 2002). Ants may collect seeds from parental plants (Lu & Mesler 1981, Stiles 1992), soil (Oliveira *et al.* 1995, Guimarães & Cogni 2002), or frugivore feces (Stiles 1992, Pizo *et al.* 2005). The interaction between ants and seeds may result in: (1) modification of seed deposition spectrum and of seed bank dynamics (Levey & Byrne 1993, Pizo & Oliveira 1998, 1999); (2) germination facilities to seeds originally attached to pulp (Culver & Beattie 1978, Oliveira *et al.* 1995, Leal & Oliveira 1998); (3) sapling establishment (Culver & Beattie 1978, Levey & Byrne 1993), and (4) influences on spatial patterns of species primarily dispersed by vertebrates (Böhning-Gaese *et al.* 1999), autocory or abiotic vectors (van der Pijl 1972) and, thereafter, affects plant distribution patterns (Willson 1992, Levey & Byrne 1993, Wang & Smith 2002).

Seeds discarded from ant nest may benefit from nutrients (*e.g.* Passos & Oliveira 2002) and high humidity (Farji-Brener & Ghermandi 2004) available in that microsite, but other abiotic factors may also influence germination success (Fenner 1985). Light and temperature are often regarded in physiological studies, without any further attempt to relate such conditions in which germination is enhanced with seed dispersal. Besides, ants may also affect germination success by changing diaspore features: although studies have shown that pulp removal by ants enhances germination, especially due to reduced fungal attack (*e.g.* Pizo & Oliveira 2001, Guimarães & Cogni 2002), little is known about the influence of elaiosome removal by ants on the germination success (*e.g.* Passos & Ferreira 1996).

In this study we investigated the interaction between seeds of the castor plant *Ricinus communis* L. (Euphorbiaceae) and ants in a forest edge of the Atlantic Forest in southeastern Brazil. Castor is a shrub or treelet that occurs worldwide as a colonizing species. The seeds are primarily dispersed by auto-

cory (*e.g.* Stebbins 1974, Fenner 1985, Willson 1992) but probably present secondary dispersal by ants, once they bear a lipid-rich elaiosome (van der Pijl 1972). Although castor seed is often regarded in literature as typically myrmecochorous (*e.g.* van der Pijl 1972), no study to the extent of our knowledge has documented the interaction between ants and those seeds. We addressed the following questions: (1) are ants legitimate dispersers of castor seeds?; (2) which ant species interact with the seeds?; and (3) is germination success higher in a predicted scenario met by seeds discarded from ant nest?

METHODS

Study area

Fieldwork was undertaken between October 2002 and February 2003 and consisted of castor seed removal experiments in three areas located on the edge of a fragmented forest, the Reserva Municipal Mata de Santa Genebra (22°48'34"-50'13"S and 47°06'15"-07'33"W), Campinas, Southeast Brazil. This fragment has approximately 250 ha of residual Atlantic Forest vegetation, in which mesophilous semideciduous forest is predominant (Leitão-Filho 1995). The criteria to define the study areas was presence of great abundance of castor plants in clusters which were not connected between the areas.

Experiment

Seed removal by ants on the forest edge

In order to determine whether the elaiosome of castor seeds is attractive to ants, we compared the removal of seeds with and without elaiosome. One intact seed and one without the elaiosome (manually removed) were placed side by side (Byrne & Levey 1993) on white filter paper (4 cm x 4 cm). The white filter paper allows ants interacting with seeds to be more easily visualized (Pizo & Oliveira 2001). There is no interference of filter paper on ants' behavior, once they do not seem to distinguish between filter paper and the litter around it (Byrne & Levey 1993). We placed 30 samples of filter paper with seeds around each of the three study areas. There was a 5 m distance between samples, ensuring that ants of different colonies explore seeds in different samples (Pizo & Oliveira 2000, 2001). We observed the experiment on an hourly basis between 0830 h and 1830 h and we counted removed seeds, which were not replaced. We repeated the experiment once for each

area. We used chi-square test to test for differences among seed removal in both treatments (Zar 1996).

In order to determine whether ants remove castor seeds exclusively to consume the elaiosome and to identify ant species interacting with the seeds, we created two experimental groups. The first one was composed by 15 samples consisting of two intact seeds on white filter paper (4 cm x 4 cm). The second experimental group was identical but the fact that the elaiosome was removed. Samples of both groups were placed in alternate fashion around each of the three study areas. We observed the experiment on an hourly basis between 0830 h and 1830 h and we collected ants interacting with seeds. We used soft brush to collect ants and we preserved the specimens in alcohol 70%. We replaced seeds removed during the experiment and we repeated the experiment once for each area. The specimens were deposited in the Museu de Zoologia da Universidade de São Paulo (MZUSP).

Each time both experiments were checked and there were ants interacting with seeds, we registered what was happening at the moment. So, no further effort was made to determine ants' behavior or dispersal distances.

Seed germination in controlled conditions

In order to determine germinative responses of castor seeds to temperature, light and presence or absence of elaiosome, we performed eight germination treatments. We used two germination cameras with white light, one with a constant temperature of 30° C and other with alternate temperatures of 20° C and 30° C. We regulated both cameras for uninterrupted 12 hour light and 12 hour darkness. In the alternated temperature camera, dark period was coincident with 20° C period. In order to reproduce natural conditions with more fidelity, we did not perform any kind of pre treatment with seeds, such as application of fungicide.

The eight germination treatments consisted in combinations of light (present or absence), temperature (alternated or constant) and elaiosome (present or absence). Seeds kept in constant darkness were placed in Petri dishes inside black plastic bags, and seeds exposed to light were placed in Petri dishes inside transparent plastic bags, only for humidity retention. There were five Petri dishes for each treatment, each dish with 25 castor seeds newly harvested, totalizing 125 seeds per treatment. We humidified the Petri dishes with

distilled water whenever necessary. We considered a seed germinated when there was protrusion of the hypocotile-radicular axis. Seeds kept in constant darkness were observed inside dark room with green light. We carried out the experiment for 30 days, recording the number of germinated seeds.

Although we did not measure microhabitats conditions on soil, inside ant nest and on the pile mound, we assumed three different scenarios to castor seed germination based on literature: (1) seeds with elaiosome exposed to light and alternated temperatures, as a result of no interaction between castor seeds and ants; (2) seeds without elaiosome kept in both constant darkness and temperature, for those removed to the ant nest but not discarded outside it on a pile mound; (3) seeds without elaiosome exposed to light and alternated temperatures, as a result of being discarded outside ant nest after elaiosome removal. The effects of light, temperature and presence of elaiosome on germination were investigated using a nominal logistic regression (Dobson 2002).

RESULTS

Ants removed 75.6% of the intact castor seeds and only 40.6% of seeds without elaiosome in the three study areas ($\chi^2 = 18.990$, $df = 1$, $\alpha = 0.05$). Seeds without elaiosome were always removed from the same sample from which intact seeds had been already removed. Ants used the elaiosome as a handle to carry the seeds to their nest. We did not observe ants detaching the elaiosome from seeds in order to take only the appendage to their nest. Smaller ants were recorded recruiting many nest mates to samples, while bigger ants used to remove seeds alone or in small groups of two or three individuals. No removal was observed when only seeds without elaiosome were placed on filter paper in the second experiment. Therefore, ants might regard only the elaiosome as food source, without consuming the seed itself. We performed 44 collections of ants interacting with intact castor seeds. We found 20 ant species of four subfamilies and the more abundant species was the Myrmecinae *Pheidole* sp.1, which occurred in 40.91% of collects (Table 1).

We strongly believe that ants were the only agents removing castor seeds in the study areas. This is because these seeds are highly toxic (Windor 2004) and we are not aware of any specialized predator or any other possible vector removing seeds in Santa Genbra. After all, this fragment is extremely affected

Table 1. Occurrence of subfamilies and species of ants interacting with seeds of *Ricinus communis* in three study areas in Southeast Brazil.

Subfamily	Occurrence (%)	Species	Occurrence (%)
		<i>Pheidole</i> sp.1	40.91
		<i>Pheidole</i> sp.2	11.36
		<i>Pheidole</i> sp.3	9.09
		<i>Pheidole</i> sp.4	6.82
		<i>Pheidole</i> sp.5	4.55
		<i>Pheidole</i> sp.6	4.55
		<i>Pheidole</i> sp.7	2.27
Myrmicinae	93.18	<i>Pheidole</i> sp.8	2.27
		<i>Pheidole</i> sp.9	2.27
		<i>Solenopsis</i> sp.1	6.82
		<i>Solenopsis</i> sp.2	4.55
		<i>Solenopsis</i> sp.3	9.09
		<i>Solenopsis</i> sp.4	2.27
		<i>Oligomyrmex eidmanni</i>	2.27
		<i>Atta sexdens piriventris</i>	4.55
Formicinae	2.27	<i>Camponotus silvestrii</i>	2.27
Ectatomminae	2.27	<i>Ectatomma edentatum</i>	2.27
		<i>Odontomachus chelifer</i>	2.27
Ponerinae	6.82	<i>Pachycondyla striata</i>	2.27
		<i>Pachycondyla marginata</i>	2.27

by anthropogenic disturbance and, especially at the edge, no mammals such as rodents may be seen during the hours at which the experiments were carried out.

The nominal logistic regression was significant ($\chi^2 = 365.44$, $P < 0.0001$) and all factors tested markedly affected the probability of germination. The probability of germination of castor seeds was enhanced by absence of light ($\chi^2 = 64.57$, $P < 0.0001$, odds ratio = 3.88), by alternated temperatures ($\chi^2 = 228.07$, $P < 0.0001$, odds ratio = 15.24) and by removal of the elaiosome ($\chi^2 = 64.57$, $P = 0.003$, odds ratio = 1.62).

DISCUSSION

Castor seeds with elaiosome are more attractive to ants than seeds without elaiosome, a pattern demonstrated before by other authors for myrmecochorous seeds (O'Dowd & Hay 1980, Davidson & Morton 1981, Lu & Mesler 1981, Stiles 1992, Byrne & Levey 1993). Almost all ants regard elaiosomes as food sources, as demonstrated by the fact that ants from four subfamilies (Formicinae, Myrmecinae, Ectatomminae and Ponerinae) removed seeds with elaiosome. For some species, lipidic compounds found in the elaiosome act as

physiological constituents and behavioral releasers (Beattie 1985, Marshall *et al.* 1979), Although some ants such as harvest ants are primarily granivores (Holldöbler & Wilson 1990), in our study no ant removed seeds when only seeds without elaiosome were placed on filter paper on the second experiment, suggesting that ants are acting as legitimate dispersers of castor seeds. We believe ants may have removed seeds without elaiosome when seeds with and without this appendage were placed on filter paper on the first experiment due to the chemical stimuli emitted by the elaiosome of the intact seed, inducing ants to remove both seeds in the sample. Besides, Berg (1975) described that inexperienced workers may carry seeds without elaiosome to their nest, whereas more experienced workers exclusively remove intact seeds.

Castor seeds without elaiosome are found on the pile mound. Seeds discarded outside ant nest after elaiosome removal is a pattern recorded for ant-dispersed plants (Berg 1975, Handel 1978, Culver & Beattie 1978, Horvitz & Beattie 1980, Horvitz 1981, Davidson & Morton 1981). Outside the ant nest, discarded seeds are exposed to other agents transportation (Stebbins 1974, Horvitz & Beattie 1980, O'Dowd & Hay 1980), but may as well germinate on the pile mound, where soil usually shows higher concentration of organic matter and nutrients than its vicinities (Culver & Beattie 1978, Davidson & Morton 1981, Horvitz 1981, Horvitz & Schemske 1986, Higashi *et al.* 1989).

It is often found in literature that seed cleaning by ants increases germination success by allowing water and nutrients to enter the seed or by reducing pathogen attack that begins in the fleshy portion of the seed (Augspurger 1990, Oliveira *et al.* 1995, Pizo & Oliveira 1998, Guimarães & Cogni 2002, Passos & Oliveira 2002). Studies show that the elaiosome of castor seed plays an important role in absorbing water from the soil and transferring it to the rest of the seed during germination, thus, enhancing germination success (Lisci *et al.* 1995, Bianchini & Pacini 1996). Although, our results do not confirm this and are more accordingly to the results of Lagôa & Pereira (1987). These authors found that elaiosome removal of castor seed speeded germination rate, since inhibitory substances are found in the elaiosome. Thus, the removal of this appendage by ants may be determinant to the germination of viable seeds in natural systems (Lagôa & Pereira 1987).

The higher germination success of seeds in the absence of light and under alternate temperatures suggests that the best scenarios for castor seed germination is a mixture of the predicted ones and is met by seeds discarded from ant nest, but covered by a thin layer of litter, or eventually abandoned inside the nest, but in shallow depths. In these situations castor seeds may benefit from temperature and light conditions. Many species that respond to alternate temperatures form seed banks, an important mechanism that limits seedling emergence to suitable sites for sapling establishment (Fenner 1985, Pons 1992). Therefore, castor seed germinative response and its high longevity (I.F.M. Válio, pers. comm.) indicate seed bank formation.

In conclusion, the elaiosome of castor seed is attractive to different species of ants. By discarding seeds without elaiosome on the pile mound, ants constraint the intrinsic inhibition for seed germination and may submit the seeds to conditions that enhance germination success. Ungerminated seeds on the pile mound may be incorporated into soil to form seed banks, a temporal dispersal mechanism still to be studied for castor.

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