The beneficial effect of ants on the reproductive success of *Dyckia floribunda* (Bromeliaceae), an extrafloral nectary plant

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Abstract: *Dyckia floribunda* is a perennial herb from the Argentinian Chaco with extrafloral nectaries. Ants visited these nectaries while patrolling inflorescences and infructescences. We anticipated that ants attracted to extrafloral nectaries might protect the reproductive organs, increasing plant reproductive output. To evaluate the possibility of mutualism between *D. floribunda* and ant visitors, we determined whether ant-accessible plants showed a higher seed production than ant-excluded plants. Experimental fieldwork suggested a decrease in fruit set of ant-excluded plants compared with ant-accessible plants but the seed number per fruit was not affected by ant exclusion. Thus, total seed number per plant was highly reduced in treated spikes. Analyses of covariance confirmed these trends, indicating that total seed production per plant was strongly affected by ant exclusion. This study marks the first experimental report of this mutualistic association in Bromeliaceae.

Key words: ant–plant interaction, Bromeliaceae, Chaco, *Dyckia floribunda*, fruit set, seed set, mutualism.

Résumé : Le *Dyckia floribunda*, une herbacée pérenne du Chaco en Argentine, possède des nectaires extra-floraux. Les fourmis visitent ces nectaires en faisant le tour des inflorescences et des infructescences. Les auteurs ont formulé l'hypothèse que les nectaires extra-floraux pourraient protéger les organes reproducteurs et ainsi augmenter le succès reproductif. Afin d'évaluer l'existence de mutualisme entre le *D. floribunda* et les fourmis visiteuses, les auteurs ont vérifié si des plants accessibles aux fourmis produisent plus de graines que des plants non-accessibles aux fourmis. Le travail au champ indique qu'il y aurait une diminution de la mise à fruit chez les plants privés de fourmis, comparativement aux plants visités par les fourmis, mais le nombre de graines par plant n'est pas affecté. Tout de même le nombre total de graines par plant se voit diminué dans les épis traités. L'analyse de covariance confirme ces tendances et indique que la production totale de graines par plant est fortement affectée par l'exclusion des fourmis. Il s'agit de la première démonstration expérimentale de cette association mutualiste chez les Bromeliaceae.

Mots clés : interaction plante–fourmi, Bromeliaceae, Chaco, *Dyckia floribunda*, mise à fruit, mise à graine, mutualisme.

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Introduction

Extrafloral nectaries (EFNs) are morphologically diverse sugar-producing glands that have been recorded for at least 2200 species in more than 90 angiosperm families (Keeler 1989; Koptur 1992). They can be distinguished from floral nectaries by either function (i.e., they are not involved in pollination) or position (i.e., they are located on vegetative parts of the plant or on the outer floral whorls) (Elias 1983; Whitman 1994). A variety of insects, but chiefly ants, are attracted by EFN exudate, which, rich in carbohydrates, is used as a food source (Whitman 1994).

The occurrence of EFNs is an adaptive trait to the plant bearing them when it allows a higher reproductive success, e.g., the increase of the number of propagules. EFNs medi-ate ant–plant interactions by providing food to ants, while these insects prevent the occurrence of herbivory, provide macronutrients, etc. (Bentley 1977; Beattie 1985; Huxley and Cutler 1991; Koptur 1992). Several studies have suggested that ants can protect plants with EFNs against herbivory. On the other hand, other studies have found no evidence of benefit to plant reproductive output.

Although some Bromeliaceae utilize ant guards with rewards of EFNs (Galetto and Bernardello 1992; Koptur 1992) or solid food on stems, leaves, and developing flowers and fruits to deter herbivores, this relationship has largely gone unexplored (Benzing 2000). As Bromeliaceae is a large and important family with a broad adaptation to different environmental conditions, we analysed the importance of the ant–plant relationship in a representative species. We chose a Chaquean species, *Dyckia floribunda* Griseb. (subfamily Pitcairnioideae), in which EFNs, nectar chemical composition, and ant visitors were previously reported (Galetto and Bernardello 1992). This species has yellow flowers that produce floral and extrafloral nectar with different sugar compositions: floral nectar, being actively collected by pollinators, shows a balance between sucrose and hexoses (Bernardello et al. 1991), whereas extrafloral nectar is composed of almost pure sucrose and is harvested mainly by

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ants (Galetto and Bernardello 1992). As the extrafloral nectar secretion period lasts from early differentiated buds to developing fruits (Galetto and Bernardello 1992), it is expected that ants attracted to EFNs might protect the reproductive organs, increasing plant reproductive output. Thus, the aim of this paper is to evaluate whether mutualism exists between *D. floribunda* and ant visitors, specifically measuring whether ant-accessible plants show a higher reproductive success than ant-excluded plants.

The study site was located in the Chaco Serrano, within a continuous forest on the eastern slope of the Sierras Chicas (Argentina) at 650 m above sea level (mean annual temperature 20–22°C, mean annual rainfall 800 mm). The Chaco is a vast region covering the plains of Argentina, Paraguay, Bolivia, and Brazil, mainly covered with semiarid forests and woodlands, with a continental climate (moderate to scarce rains, mild winters, and hot summers). There are members of several plant families (Euphorbiaceae, Fabaceae, Passifloraceae, and Bignoniaceae, among others) that have ant-visited extrafloral nectaries within this phytogeographical region but there is only one previous experimental study (Freitas et al. 2000).

Material and methods

Dyckia floribunda is a terrestrial perennial herb occurring in Argentina, from Jujuy to Córdoba provinces, and Paraguay (Morrone and Zuloaga 1996). It is self-compatible and mainly visited by bees and hummingbirds (Bernardello and Galetto 1995). Plants bloom early in the spring producing ca. 1 m tall inflorescences (mean ± S.D. = 228 ± 63 flowers) that open from the base to the apex. The fruit set per plant is 41 ± 15%. Fruits are septicidal capsules with 123 ± 17 medium-sized seeds mechanically dispersed from December to January (data not shown).

Experiments were conducted in Córdoba (Dept. Colón: El Diquecito) from October to December 1990. Plants growing in a 5 km² area were identified and tagged when their inflorescences were beginning to develop. Individuals growing 1 m apart were used to avoid choosing plants from the same genet. Twenty plants, each one with one inflorescence of similar length and a comparable number of buds, were selected and randomly assigned to both treatments. Ten spikes to which ants had free access were used as controls, whereas in the other ten spikes ants were excluded. The experimental design assumed only winged-herbivore effects. Before flowering, the base of the spike was covered with an oil-based insect repellent (Tanglefoot®) to exclude ants. The repellent was kept at least 20 cm away from the nearest flower, a distance that prevented any influence on the pollinators or flying herbivores. To avoid the formation of natural bridges over the ant-excluded spikes, the adjacent stems of neighbouring plants were removed. Treated plants were checked weekly for repellent integrity until fruit maturation. Spikes were collected prior to seed dispersal. During the experiment, cattle grazing broke three ant-accessible spikes and the actual number of ant-accessible replications was reduced to seven. Spike length and total number of buds were registered for each inflorescence. The total number of buds per spike is a variable that usually correlates with the total number of fruits produced (e.g., Stephenson 1981; Diggle 1997

and references therein). On the other hand, the size of the inflorescence may affect pollinator attraction and may also indicate the nutritional status of the plant (e.g., Galen et al. 1985 and references therein). Thus, these variables were used as covariates to control the differences due to the particularities of the individual plants. In the laboratory, the total number of fruits produced and the number of seeds per fruit were calculated. Seeds were counted in a sample of 7–10 fruits of each infructescence. Finally, the total number of seeds per infructescence was estimated as the total number of fruits by the mean number of seeds per fruit.

Analysis of covariance (ANCOVA) was used to compare the reproductive output (number of fruits, mean number of seeds per fruit, and total number of seed per plant) between ant-accessible and ant-excluded plants, using spike length and initial number of buds per spike as the covariates to control the effects on the dependent variables. Original data were used because they met ANCOVA assumptions. Computer analysis was done with the SPSS statistical package (SPSS Inc. 1999).

Results

In the studied area, five ant species assiduously visited individuals of *D. floribunda*, patrolling their inflorescences and infructescences looking for extrafloral nectar. Occasionally they were seen inside the flowers. Usually, many ant foragers were seen at the same time on the spikes (range 4–41 ants), particularly when ants had their nests close to the plant. Ants belonged to three subfamilies: Formicinae (*Camponotus rufipes*, *Camponotus punctulatus*), Myrmicinae (*Crematogaster quadriformis*, *Zacryptocerus* sp.), and Pseudomyrmecinae (*Pseudomyrmex gracilis*). In general, ants, especially those of the genus *Camponotus*, behaved aggressively, deterring insect visitors. Slower visitors, including flower herbivores, were rapidly deterred. Insect egg removal by ants was not registered.

Flowers and fruits were predated by two main groups: (*i*) larvae of Coleoptera (Curculionidae) were regularly found inside flower buds, predating fertile whorls before they had left the plant to pupate on the ground; and (*ii*) larvae of Lepidoptera (Noctuidae) frequently fed on the seeds, destroying the locule contents. In both cases, ants did not remove the larvae, since they developed inside the reproductive structures. On the other hand, leaves were not predated by any herbivores.

Experimental fieldwork suggested a decrease in the reproductive success of the ant-excluded plants compared with the controls (Figs. 1–3). ANCOVA analyses confirmed the expected trends between ant-accessible and treated plants (Table 1), indicating that reproductive success was strongly affected by ant exclusion in this bromeliad.

Discussion

In *D. floribunda*, EFNs are very simple, nonstructural, nonvascularized nectaries that do not possess a specially differentiated nectar-producing tissue (Galetto and Bernardello 1992). As such, they could potentially be located in any part of the plant. However, they only function in the reproductive organs and are not present on the hardened rosette leaves with spines. Thus, ants do not provide protection to the leaves that otherwise have mechanical defence. This fact is

Fig. 1. Box-plot of number of fruits per plant in ant-accessible and ant-excluded plants (n = 7 and 10 individuals, respectively) of *Dyckia floribunda*. Data are median ± interquartiles. Ant-excluded plants showed a significant reduction of the fruit set.
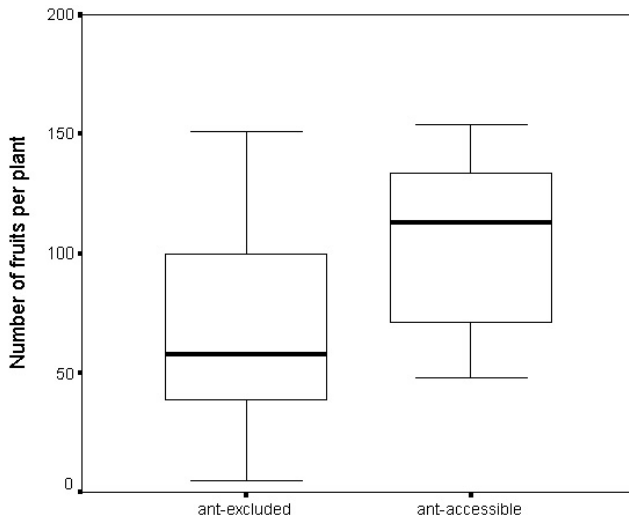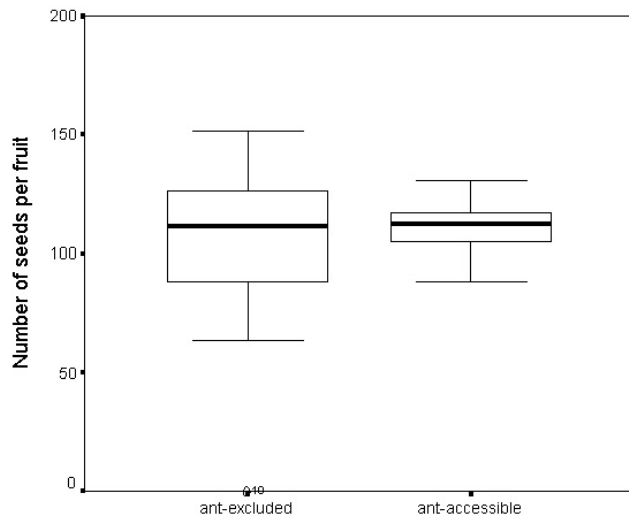

Fig. 2. Box-plot of number of seeds per fruit in ant-accessible and ant-excluded plants (n = 7 and 10 individuals, respectively) of *Dyckia floribunda*. Data are median ± interquartiles. Seed number per fruit was not affected by ant exclusion.

Fig. 3. Box-plot of number of seeds per plant in ant-accessible and ant-excluded plants (n = 7 and 10 individuals, respectively) of *Dyckia floribunda*. Data are median ± interquartiles. The total seed number per plant was highly reduced in the treated spikes.
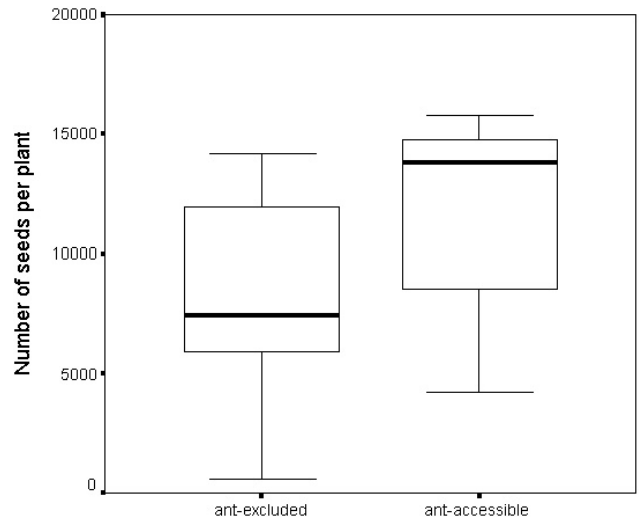

Table 1. Analyses of covariance to compare the reproductive output in *Dyckia floribunda* between ant-accessible and ant-excluded plants (treatment) with spike length and initial number of buds per spike as covariates.

Source of variation	df	MS	F	P
No. of fruits per plant				
Treatment	1	5 663	4.33	0.05
Spike length	1	4 911	3.81	0.07
No. of buds per spike	1	258	0.20	0.66
Error	13	1 288		
No. of seeds per fruit				
Treatment	1	38	0.07	0.80
Spike length	1	35	0.06	0.81
No. of buds per spike	1	265	0.48	0.50
Error	12	556		
No. of seeds per plant				
Treatment	1	76 677 960	4.60	0.05
Spike length	1	60 433 052	4.33	0.06
No. of buds per spike	1	151 987	0.01	0.92
Error	12	13 934 445		

expected because plants that receive ant protection generally provide food reward at the time periods and on some parts (e.g., new vegetative growth, developing buds, flowers, or fruits) where they are more vulnerable to herbivore attack (cf. Bentley 1977).

Dyckia floribunda produces abundant highly concentrated extrafloral nectar from the outer whorl of tepals (Galetto and Bernardello 1992). The secretion period includes its whole reproductive period: from developing buds to almost mature fruits. During this period, ants visit the plants both nocturnally and diurnally to feed on nectar. This association ends after seed development, since no reward is offered to the ants. The four ant genera recorded in *D. floribunda* have been regularly registered visiting EFNs in different plant

taxa from tropical and subtropical regions of the New World (cf. Oliveira and Brandão 1991). Ant activity on the inflorescences presumably disturbs herbivore oviposition on buds or flowers, as was reported in other plant species bearing EFNs on reproductive organs (cf. Oliveira 1997). Once the herbivores have laid their eggs, larvae begin their development undisturbed, as they grow enclosed within the flower or fruit, where they are inaccessible to the visiting ants. These endophytic herbivores might benefit from ant recruitment, when ants protect them from their antagonists (e.g., parasitoids).

The interaction between ants and *D. floribunda* is considered to be mutualistic because ant-excluded plants decrease their reproductive performance in comparison with ant-accessible plants. At the same time, nectar is a nutritious

food reward for ants, ants are aggressive towards insects, and spikes are reproductively vulnerable to insect feeding. Even though ant attendance may represent a disadvantage for the plant because they can diminish pollinator visits and parasitoid activity on herbivores, this may be outweighed by the benefits of ant activity decreasing herbivore attack.

Although we have observed some ants within the flowers and there are some reported cases of ant pollination in angiosperms (Peakall et al. 1991), in the studied species and location ants seem not to play a pollination role, considering the low ant visit frequency and the scarce possibility of pollen transfer.

Ant-derived benefits related to plant reproductive success, however, may vary in space and time (cf. Oliveira 1997), and selective pressures to enhance reproductive output may lead the plant to develop alternative strategies, e.g., some individuals of the population could bloom earlier or later to diminish herbivore attack. If that would be the case, individuals with a phenological displacement would show a higher reproductive success, independently of ant activity. In addition to seed output, other potentially important components of plant fitness are seed dispersal and seedling establishment. Ants do not play any role in seed dispersal or seedling establishment of *D. floribunda*. Thus, herbivore deterrence by ants influences predispersal seed performance, but does not affect postdispersal stages.

EFN–ant relationships have been reported in a wide range of angiosperms and even in a fern species (cf. Bentley 1977; Beattie 1985; Huxley and Cutler 1991; Koptur 1992). On the other hand, specialized ants in symbiosis with plants are represented disproportionately in particular taxonomic categories of ants (Davidson and McKey 1993). Independently of these opposite trends, the relationship between ants and plants mediated by EFNs ranges from simple opportunism to mutual benefit and onto complex multiple interactions (Koptur 1992). The ant–plant association may be continuous throughout the year if plants bear EFNs on vegetative parts, or be restricted to the reproductive season if EFNs are located on flowers or fruits.

Even though this is the first experimental report in the family, this mutualistic association might be widespread in Bromeliaceae, a plant group with approximately 2400 terrestrial and epiphytic species. For instance, it is already known that terrestrial *Dyckia* and *Deuterocohnia* (Galetto and Bernardello 1992) and epiphytic *Tillandsia* (Koptur 1992) have ant-visited EFNs.

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