The Ant Assemblage Visiting Extrafloral Nectaries of *Hibiscus pernambucensis* (Malvaceae) in a Mangrove Forest in Southeast Brazil (Hymenoptera: Formicidae)

by

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ABSTRACT

Ant species visiting extrafloral nectaries (EFNs) of *Hibiscus pernambucensis* were studied in a daily flooded mangrove forest in Picinguaba, Southeast Brazil. Nineteen ant species in five subfamilies were observed visiting the EFNs. The most common species (in order of abundance) were *Camponotus* sp.2, *Brachymyrmex* sp. and *Pseudomyrmex gracilis* during the warm season and *Brachymyrmex* sp., *Camponotus crassus* and *Camponotus* sp.2 during the cold season. A twenty-four hour census showed that ant activity significantly decreased at night, and was positively correlated with air temperature in both seasons. On almost half of the stems no ant was observed and the vast majority of visited stems had only one species present. Less than 1% of sampling sessions showed more than one ant species recorded simultaneously on the same stem. Living termite baits stuck to the plant were attacked by eight ant species. Although ants were more commonly found on new leaves, the percentage of termites attacked was not different between new and old leaves.

Key words: daily activity, foraging, *Hibiscus pernambucensis*, Malvaceae, mangrove, ant-plant interaction, plant defense

INTRODUCTION

Extrafloral nectaries (EFNs) are sugar-producing plant structures not directly related to pollination (Elias 1983). They are extremely variable anatomically and know from virtually every vegetative and reproductive plant part (Elias 1983; Koptur 1992). EFNs occur in at least ninety-three families of angiosperms and have evolved independently many times (Koptur 1992). Ants are the most frequent EFN visitors (Oliveira & Brandão 1991; Koptur 1992).

Ant visitation to EFNs may increase plant fitness by decreasing leaf herbivory, limiting the destruction of flowers and buds and increasing fruit and seed production (Bentley 1977; Koptur 1984; Del-Claro *et. al.*

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1996; Oliveira *et al.* 1999; Labeyrie *et al.* 2001). The effective advantages to the plant may vary in time and space, and may depend on the aggressiveness of the ant species and the presence of specialized herbivores that can overcome ant predation (Koptur 1992). Because just one or a few ant species may effectively protect the plant (Koptur 1992), few studies have focused on the whole ant assemblage (Schemske 1982; Cogni *et al.* 2000), and even fewer have covered the nocturnal ant fauna (see Oliveira & Brandão 1991; Labeyrie *et al.* 2001).

In many ecosystems, EFNs are visited by arboreal ant species as well as by ground-nesting ants that extend their foraging areas by searching for food on the plant substrate (Bentley 1977). Mangroves, on the other hand, form an ecosystem extremely unsuitable to ground-nesting ants, because the sediment is too soft and moist (Clay & Andersen 1996). All the studies regarding ant communities in this ecosystem demonstrate a predominance of arboreal-nesting species (Clay & Andersen 1996; Nielsen 2000; Cole 1983 a and b; Lopes & Santos 1996). Even though sugar solution availability, in the form of EFNs and homopteran honeydew, is an important factor shaping arboreal ant communities (Davidson 1997; Blüthgen *et al.* 2000), the interaction between ants and EFN bearing plants has never been investigated in mangrove ecosystems.

This study investigates the ants that visit the EFNs of the neotropical shrub *Hibiscus pernambucensis* (Malvaceae) in a mangrove forest. *H. pernambucensis* is a shrub frequently found in mangrove and sandy forests of Southeast Brazil. This species bears slender EFNs on the under-leaf surface, near the petiole insertion (Alonso 1977). In the present paper, the following questions were addressed: (1) What is the species composition of the ant assemblage? (2) How does the ant activity vary during the day? (3) Do foraging ants attack the termite baits on plant leaves? (4) Is there variation in ant abundance in different plant parts, and (5) Do ant attacks on termite baits vary among different plant parts?

MATERIALS AND METHODS

Fieldwork was carried out in the "Núcleo Picinguaba" of the "Parque Estadual da Serra do Mar", in Ubatuba (44°55' W; 23°20' S), São Paulo State, SE Brazil. The climate is generally warm and wet. The mean temperature is 24.1°C and the mean monthly precipitation is 331.7 mm in the warm season (October to March), and 18.1°C and 97.6 mm, respectively, in the cold season (April to September). The vegetation of the region is classified as lowland rain forest. There are two rivers on the coastal plain, and some areas are flooded daily during high tide. The study site was located near the Picinguaba River, where *H. pernambucensis* occurs at a density of *ca.* 40-50 individuals per 100 m-transect. Fieldwork was carried out September 4-6, 1999 (cold season) and December 16-21, 1999 (warm season).

In order to determine the ant assemblage visiting the EFNs, ants were collected during both day and night (total of 30 h) in each of the two seasons. Voucher specimens are deposited in the Museu de Zoologia da Universidade de São Paulo (MZUSP).

The activity of the ant assemblage visiting the EFNs was evaluated through 24-h censuses carried out on 65 tagged stems (*ca.* 3 m apart). The stems (1-2 m tall) were divided into two parts, the "apical portion" (new leaves, buds and flowers) and the "basal portion" (old leaves). Both portions had approximately the same number of leaves. The ants were censused at 2-h intervals; sampling of each stem consisted of recording the number of individual ants of each species on each part of the stem during a period of 15 seconds. In each sampling session, the air temperature near each stem was recorded. One 24-h census was carried out September 5-6, 1999, and one December 17-18, 1999.

The behavior of foraging ants toward potential herbivores was evaluated by using live termite (*Nasutitermes*) workers as baits to evaluate patterns of ant predation (as in Freitas & Oliveira 1996; Dejean *et al.* 2001). These experiments were carried out December 18-21, 1999 between 0700 h and 1600 h. Live termites were glued by the dorsum (legs upwards) in the center of the leaf blade with polar glue (Tenaz®, Loctite Brasil Ltda). Four treatments were carried out, one on each of four different parts of the plant: the apical portion of the stem, the basal portion of the stem, under and upper leaf surfaces. Fifty stems were marked, and each treatment for each stem was randomly assigned by the flip of a coin and carried out in a different day. In all, 200 termites were glued (50 per treatment), and, immediately after, the behavior of foraging ants was observed during 60 min, with a 30-s check at each 10 min interval. The number of workers of each ant species attacking the termite was registered within this period.

RESULTS

In all, nineteen ant species in five subfamilies were recorded visiting the EFNs of *H. pernambucensis*. Sixteen species in four subfamilies were recorded during the 24-h censuses, with ten of these occurring in both seasons, three observed exclusively in the warm season censuses and three only in the cold season (Table 1). The most common species (in order of abundance) were *Camponotus* sp.2, *Brachymyrmex* sp., and *Pseudomyrmex gracilis* (Fabricius) in the warm season; and

Table 1. Number of ants, number of stems occupied by each species and period of activity of ant species visiting the extrafloral nectaries of *Hibiscus pernambucensis* during two 24-h censuses, one in the warm season and another in the cold season, in Picinguaba, Southeast Brazil.

Ant species	No. of individual ants		No. of stems occupied by each species		Period of activity*
	Warm Season	Cold Season	Warm Season	Cold Season	
DOLICHODERINAE					
Azteca sp.	7		6		D, N
FORMICINAE					
Brachymyrmex sp.	42	171	11	31	D
Camponotus crassus	6	36	3	26	D
Camponotus sericeiventris	6	-	5	-	D
Camponotus sp.1	5	16	5	15	N
Camponotus sp.2	58	23	47	21	D
Camponotus (*Myrmothrix*) sp.3	3		2	1	N
Paratrechina sp.		4	-	2	D
MYRMICINAE					
Crematogaster sp.1	21	2	12	2	D
Crematogaster sp.2		1		1	D
Leptothorax sp.	-	1		1	D
Solenopsis sp.	22	12	3	6	D, N
Procryptocerus regularis	1	3	1	3	D
PSEUDOMYRMECINAE					
Pseudomyrmex gracilis	26	12	25	12	D
Pseudomyrmex kuenckeli	3	2	2	1	D
Pseudomyrmex gr. *pallidus*	4	-	4	-	D

* - D = ant species observed during daytime (0600 h – 1800 h), N = ant species observed during nighttime (1800 h – 0600 h).

Brachymyrmex sp., *Camponotus crassus* Mayr, and *Camponotus* sp.2 in the cold season. An additional three ant species were observed visiting the EFNs of *H. pernambucensis* during non-census days: *Dolichoderus attelaboides* (Fabricius) (Dolichoderinae), *Cephalotes pusillus* (Klug) (Myrmicinae), and *Pachycondyla* sp. (Ponerinae).

Ants visited the EFNs of *H. pernambucensis* throughout 24 hours, but few individuals were observed at night (Fig. 1). The mean number of ants per stem during the day (0600h – 1800h) (warm season: 0.48 ± 0.06; cold season: 0.66 ± 0.13) was much higher than during the night (1800 h - 0600 h) (warm season: 0.05 ± 0.01; cold season: 0.08 ± 0.03) ($p < 0.05$; paired t-test). Ant activity was similar during the censuses carried out in the two different seasons (Fig. 1). The number of ant

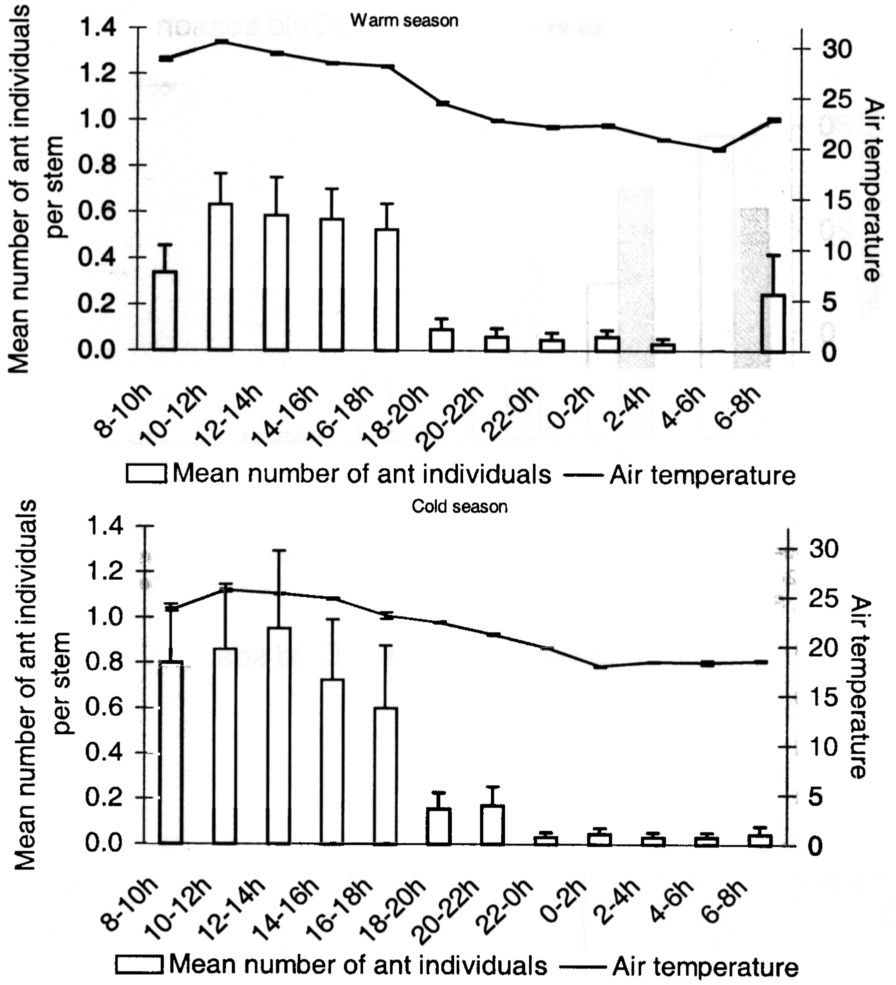

Fig. 1. Variation of the mean number of ant individuals per stem of *Hibiscus pernambucensis* and the air temperature (ºC) during a 24 h census in the warm (a) and cold (b) seasons in Picinguaba, southeast Brazil. Values are means of 65 tagged stems. Vertical bars show the standard error.

individuals in each sampling session was positively correlated with air temperature in both seasons (warm season: Spearman $r_s = 0.901$; cold season: $r_s = 0.970$).

During the 24-h census, no ant was recorded in 22 (34%) of the 65 tagged stems in the warm season and in 29 (45%) in the cold season (Fig. 2). Many stems were visited by just one ant species (Fig. 2). In the case of stems visited by more than one ant species, the different species usually visited the EFNs in different hours of the day. Most sampling

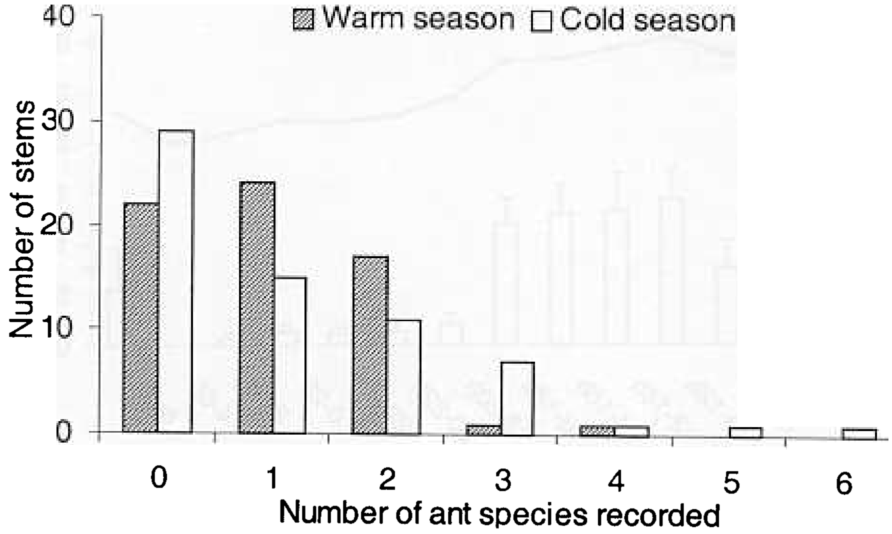

Fig. 2. Frequency of *Hibiscus pernambucensis* stems visited by different numbers of ant species during one 24-h census (12 sampling periods) in the warm season and one in the cold season in Picinguaba, Southeast Brazil. N=65 stems in each season.

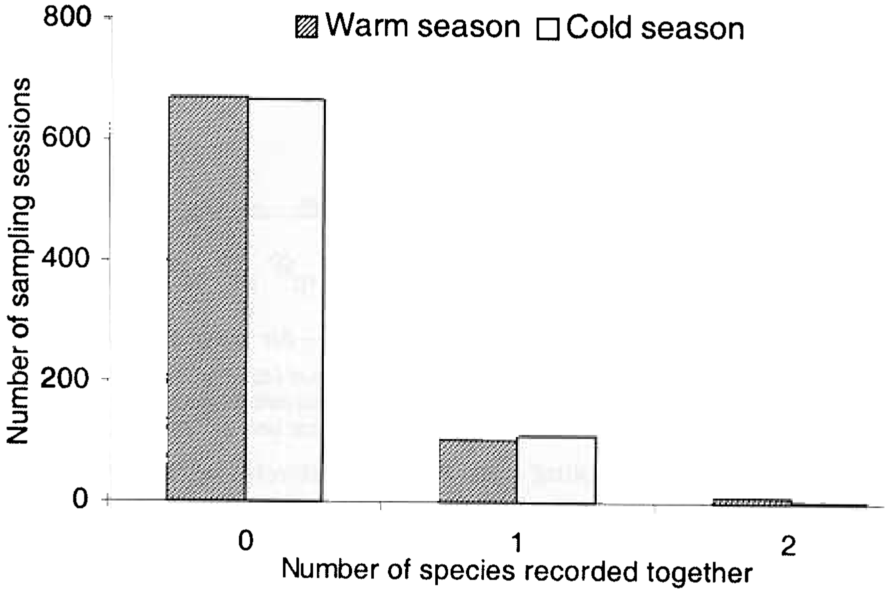

Fig. 3. Frequency of sampling sessions in which 0, 1 or 2 ant species were observed simultaneously on the same stem of *Hibiscus pernambucensis* during one 24-h census (12 sampling periods) in the warm season and one in the cold season in Picinguaba, Southeast Brazil. N=780 sampling periods in each season.

periods showed only one ant species on the same stem, with two or more species on the same stem observed in only 9 (1%) sampling periods in the warm season and 4 (less than 1%) in the cold season (Fig. 3).

The number of ants on the apical portion of the stem (warm season: 0.21 ± 0.06; cold season: 0.31 ± 0.09) greatly surpassed that of the basal portion (warm season: 0.05 ± 0.01; cold season: 0.05 ± 0.02) ($p < 0.05$; paired t-test). On the other hand, the number of baits removed was not different in the four different parts of the plant (Table 2) ($p > 0.05$; X^2 test). Eight ant species were observed attacking the termite baits: *Camponotus* sp.2 (n=45), *Pseudomyrmex gracilis* (n=24), *Camponotus sericeiventris* (n=15), *Crematogaster* sp.1 (n=6), *Pseudomyrmex kuenckeli* (n=3), *Procryptocerus regularis* (n=1), *Pseudomyrmex* gr. *pallidus* (n=1), and *Solenopsis* sp. (n=1). These ant species showed different behavior while attacking the baits. Large ants (0.8 to 1.2 cm), like *Pseudomyrmex gracilis* and *Camponotus sericeiventris*, attacked and retrieved the bait alone. Small ants (0.2 to 0.5 cm), like *Camponotus* sp.2 and *Crematogaster* sp.1, recruited many workers to retrieve the bait to the nest.

Table 2. Number of baits (living termites) removed by ants in four different parts of *Hibiscus pernambucensis* stem during the warm season in Picinguaba, Southeast Brazil.

PORTION OF THE STEM	Leaf face	removed	not removed
APICAL	upper	26	24
	under	19	31
BASAL	upper	28	22
	under	24	26

DISCUSSION

The number of ant species (19) visiting the EFNs of *H. pernambucensis* was similar to that observed in other EFN-bearing plants in tropical habitats (reviewed by Oliveira & Brandão 1991). Studies regarding mangrove ant communities also reported similar species number: (i) Clay & Andersen (1996) reported 16 ant species in an Australian mangrove, (ii) Lopes & Santos (1996) reported 22 species in a mangrove in Santa Catarina, Brazil (*ca.* 700 Km south of Picinguaba) and (iii) Nielsen (2000) reported 10 species in the canopy of a mangrove tree species in Australia. Taking into account that only part of the ant community visits EFNs (McKey *et al.* 2001) and the fact that just one plant species was studied, the number of species in the entire ant community of the area should be higher. The vast majority of species recorded are arboreal-nesting ants, a fact contrasting with studies in other ecosystems, where many ground-nesting ants climb up on the vegetation and forage on the EFNs (Bentley 1977; Schemske 1982). The absence of ground-nesting ants must be caused by the unsuitable conditions of the sediment (too soft and moist) (see Clay & Andersen 1996) and is currently under investigation (Cogni *et al.* in prep).

As in other arboreal ant communities, an ant mosaic was reported in tropical mangroves (Adams 1994). These mosaics are exclusively foraging territories, produced by intra and interspecific competition (Hölldobler & Wilson 1990; Adams 1994; Dejean *et al.* 2000). In the present study, most of the stems were visited by just one ant species, a fact that suggests the existence of an ant mosaic in the mangrove studied here. The foraging territory of each colony should also incorporate more than one plant, since the ants walk from one plant to the other by connections formed by branches and fallen wood (see Adams and Levings 1987). The stems that were never visited by ants, on the other hand, should have no connections, and so must be inaccessible to the ants. The importance of vegetation connections to ant visitation was also demonstrated in other studies (Schemske 1982; Apple & Feener 2001), and must be especially important in a environment without ground continuity all the time.

The pattern of continuous ant activity reported in the present study is similar to those observed in different tropical ecosystems, such as the Brazilian *cerrado* (savanna-like vegetation) (Oliveira & Brandão 1991), Mexican sand dunes and desert (Oliveira *et al.* 1999; Blom & Clark 1980 respectively), moist forest of Costa Rica (Koptur 1984), tropical French Guiana forest (Labeyrie *et al.* 2001), a suburban area of southeast Brazil (Cogni *et al.* 2000), and also in temperate habitats (Beckmann & Stucky 1981). However, the low ant visitation at night in the present study is different from data reported in the studies above cited, and additional field work in mangrove vegetation should be carried out to confirm if this is a general pattern of this vegetation or a local phenomenon.

Even though just four ant species were observed at night, a day-to night turnover in species composition was clear. In addition, the ant activity was significantly correlated with temperature. This segregation of daily foraging schedules among sympatric ant species is common in assemblages of ants and generally results from different humidity and temperature ranges tolerated by each species (Hölldobler & Wilson 1990). This pattern was reported in other nectar-gathering ant assemblages and should permit temporal resource partitioning (Oliveira *et al.* 1999; Cogni *et al.* 2000; Labeyrie *et al.* 2001; Orivel & Dejean 2001).

Although ants are most commonly found on the apical portion of the stems, they forage over the whole plant and actively attack potential herbivores. The termite bait results show that, even though EFNs are localized on the under side of leaves and active only on the apical leaves, ants attack potential herbivores with the same frequency on different parts of the plant. These results suggest that even in an extreme environment, like the mangrove, ants should protect the plant against herbivores. These ant defenses in mangrove vegetation are promising topics to be further studied in this system.

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