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The Outcomes of an Ant-Treehopper Association on *Solanum lycocarpum* St. Hill: Increased Membracid Fecundity and Reduced Damage by Chewing Herbivores

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A Associação Formigas-Cigarrinhas sobre *Solanum lycocarpum* St. Hill: Aumento na Fecundidade do Membracidae e Redução no Dano por Herbívoros Mastigadores

RESUMO - Interações entre formigas e hemípteros (Auchenorryncha e Sternorryncha) são importantes ferramentas para o estudo do impacto de relações multitróficas sobre a diversidade de artrópodes na vegetação. No presente estudo, através de manipulação experimental no campo, investigou-se no cerrado o impacto de uma dessas relações multitróficas para quatro membros da interação: formigas, o hemíptero (*Enchenopa brasiliensis* Strümpel, a planta hospedeira (*Solanum lycocarpum* St. Hill) e herbívoros. A variação nos efeitos da relação ao longo do tempo também foi avaliada. Os resultados permitiram verificar que formigas se beneficiam de uma fonte alimentar rica e previsível no tempo e espaço, enquanto que os membracídeos atendidos por formigas sobreviveram melhor. Os resultados mostram também que o benefício da relação pode se estender sobre a fecundidade dos membracídeos. A presença da associação formiga-hemíptero teve um impacto significativo sobre a redução da herbivoria por mastigadores na planta hospedeira.

PALAVRAS-CHAVE: Mutualismo, interação multi-trófica, associação formiga-Hemiptera, Solanaceae

ABSTRACT - Interactions between ants and hemipterans (Auchenorryncha e Sternorryncha) are important to understand the impact of multitrophic relationships on arthropod diversity on vegetation. The present field study experimentally investigates the outcomes of a multispecies interaction involving four components: ants, hemipteran trophobionts (*Enchenopa brasiliensis* Strümpel, chewing herbivores, and host plant (*Solanum lycocarpum* St. Hill). The occurrence of temporal variation in the outcomes of the relationships was also investigated. Results showed that ants were benefited by a rich food source predictable in time and space, while membracids survived better under ant attendance. Ant tending also had a positive impact on treehopper fecundity. Presence of ant-hemipteran associations was demonstrated to benefit the host plant through a significant reduction in herbivory caused by chewing insects.

KEY WORDS: Mutualism, multitrophic interaction, ant-Hemiptera association, Solanaceae, Membracidae

Honeydew-producing hemipterans (Auchenorryncha and Sternorryncha) can function as insect analogs of extrafloral nectaries if tending ants can deter other herbivores associated with the plant (Carroll & Janzen 1973). Hemiptera (mainly aphids, scales and membracids) are phloem-feeding insects that produce honeydew, an antattractant formed by a mixture of water, sugars, amides, amino acids, and proteins (Auclair 1963, Buckley 1987). Honeydew droplets that fall on the ground and leaves beneath a group of treehoppers are used as cues by ants, which find the treehoppers and begin tending activity (Del-Claro & Oliveira 1996). Ant-hemipteran relationships are usually considered mutualistic (Way 1963) because tending ants harvest the energy-rich fluid around the clock (Del-Claro & Oliveira 1999) and in turn provide a range of benefits to the hemipterans, including protection against natural enemies and increased fecundity (Bristow 1983, Cushman & Whitham 1989, Billick & Tonkel 2003).

The outcomes of mutualistic interactions are strongly dependent upon the ecological conditions in which they occur (Thompson 1982, 1988; Bronstein 1994). This is true also for ant-hemipteran interactions because antderived benefits may vary with factors such as the size and developmental stage of hemipteran aggregations, species and behavior of ant partner, and predator abundance (Cushman & Whitham 1989, Brenton & Addicott 1992, Del-Claro & Oliveira 2000). Ant-Hemiptera interactions can positively affect plant fitness if the benefits of antderived protection from herbivory outweigh the losses incurred by hemipteran feeding (Carroll & Janzen 1973, Janzen 1979). During the past three decades a number of experimental studies on ant-plant-hemipteran interactions have been conducted, most of which in temperate habitats (e.g. Bristow 1983, 1984; Buckley 1987). Only more recently has this type of study been performed in tropical habitats (Dansa & Rocha 1992; Del-Claro & Oliveira 1993, 1996, 1999, 2000; Stefani et al. 2000), and only one evaluated the impact of an ant-hemipteran interaction on the host plant (Oliveira & Del-Claro 2005). In the present paper we focus on the association between the honeydewproducing treehopper Enchenopa brasiliensis Strümpel (Membracidae), its tending ants, and the impact of the interaction on the host plant, Solanum lycocarpum St. Hill (Solanaceae) in a cerrado area in Central Brazil. We conducted a two-year field study to address the following questions: 1 - Do tending ants benefit the membracid? 2 -Do tending ants reduce damage to the host plant by chewing herbivores? 3 - Do ant-derived benefits vary with time, or host plant quality?

Material and Methods

E. brasiliensis is a small phloem-sucking insect that forms aggregations of nymphs and a few adults. The nymphs are very similar in color to adults (pale green), and complete development (1st nymphal instar to adult) in around 23 days. Previous observations (Stefani *et al.* 2000) revealed that this membracid species is very common on shrubs of *S. lycocarpum*, feeding on leaves and apical meristems, generally being tended by at least one ant species. The host plant is a common shrub (1-3m tall) in cerrado vegetation, and fields of cattle farms in Minas Gerais State, southeastern Brazil. *S. lycocarpum* does not produce nectar, floral or extrafloral, as reward to insects. Pollen is the floral resource collected by bumblebees through buzz pollination behavior (Oliveira-Filho & Oliveira 1988).

The field study was conducted in the cerrado area ("dirty field cerrado" sensu Oliveira-Filho & Ratter 2002) at Gloria's Farm / Federal University of Uberlândia, Uberlândia/MG, southeast of Brazil (18° 57'S; 48° 12'W). In this farm *S. lycocarpum* occurs at a density of nearly 100 individuals per hectare. Two sets of experiments were performed in one hectare of this farm. We used shrubs of *S.*

lycocarpum infested by the membracid *E. brasiliensis*, and selected plants had similar phenological and developmental state, and size (1-2m tall). The membracid clutches were all tended by ants, had almost the same age $(1^{st} - 2^{nd} nymphal instars)$, and had similar number of individuals.

In the first experiment, named Plant-Experiment (PE), performed between March and June 1996 (middle to end of rainy season), we tagged 35 shrubs. By the flip of a coin 24 plants were designated as control group, and the other 11 plants as treatment plants. The control plants were maintained in its natural state, no manipulations were performed. In the treatment group, ants were manually removed from the plant, and the ant exclusion was maintained by the application of a resin in the trunk (Tree Tanglefoot Pest Barrier, Tanglefoot®), 25 cm above the ground. Stem and grass bridges that could allow ants to climb onto treatment plants were removed.

In the second experiment, named Stem-Experiment (SE), conducted between May and August 1997 (dry season), 24 plants were selected in the same manner as in 1996, and in the same area. However, in this case each plant had at least two stems infested by membracids. In general, four or more stems were infested per plant. In each shrub we tagged two similar stems containing clutches of *E. brasiliensis* of similar age and number of individuals. By the flip of a coin, each branch was designated as control or treatment. Control branches were maintained in its natural state while treatment branches had ants excluded with tanglefoot. Non experimental stems had ants and membracids removed in either plant class.

In both, 1996 and 1997, plants in each class were inspected weekly after ant exclusion on the first day of the experiments. During each visit we recorded the number of hemipterans (nymphs, adults and new egg masses), the number and species of ants present, and the number of natural enemies of membracids. Also on the first and last days of the experiments, we quantified the level of damage caused by chewing herbivores on leaves of entire plants (PE) or stems (SE). In PE, the entire surface area of ten leaves (three of the tip of main trunk, four of a central branch, and three of a lower branch) was drawn on a graded paper (mm) to evaluate the proportion of tissue lost by each leaf (lost leaf area in number of points X 100 / total leaf area in number of points). The same general procedure was used in SE, but in this case herbivory was estimated sampling the nearest ten leaves of the apical meristem of each stem with (control) or without (treatment) ant access.

To investigate possible benefits provided by tending ants to *E. brasiliensis* during a single reproductive cycle, we recorded the number of adults, nymphs and egg masses on the first and 14th day of both experiments (SE and PE). Data were transformed ($\sqrt{x+1}$) and compared using repeated measures ANOVA (r.m. ANOVA). We observed that when a group of *E. brasiliensis* had success in the infestation of a host, commonly there was generation overlapping in that plant. Thus, variation through time (four months) in hemipteran survivorship and herbivory levels after the experimental manipulations were compared with r.m. ANOVA after arc sin $\sqrt{x+1}$ transformation of the data.

Results and Discussion

Ten ant species were found tending E. brasiliensis during the study. In the PE experiment the most common ants were Pheidole sp. and Camponotus crassus Mayr and in the SE, C. crassus and Ectatomma quadridens Fabr. were the principal ant attendants (Table 1). These ants are commonly associated with honeydew producing-hemipterans and extrafloral-nectary plants in cerrado (Oliveira & Brandão 1991). Recently, Del-Claro & Oliveira (1999) showed that 21 different ant species establish associations with the membracid Guayaquila xiphias Fabr., on Didymopanax vinosum March. (Araliaceae). Ants visited G. xiphias day and night, and the simultaneous occurrence of more than one ant species tending the same aggregation on a given plant was rarely recorded (Del-Claro & Oliveira 1999). In the case of *E. brasiliensis*, three or more ant species are able to simultaneously tend a treehopper clutch on the same plant. While the system studied by Del-Claro & Oliveira (1999) was located in a well-preserved cerrado area, the present study was developed in a very disturbed field, inside an experimental farm. The contrasting characteristics of the two habitats can possibly explain the observed differences in the formation of anthemipteran associations. For example, the lack of other or additional sugar sources in the farm site could be forcing different ant species to exploit the same resource simultaneously. However, factors such as the biological and behavioral differences between treehoppers and ants

in the two areas also should be considered.

Nymphs and adults of E. brasiliensis were benefited by ant attendance. In the presence of ants the impact of natural enemies was reduced in both types of experiments, comparing plants (PE) or comparing stems (SE) in the same plant (Fig. 1). The benefit of ant attendance also occurred through different times and seasons (Fig. 2). Billick & Tonkel (2003) had similar results to the treehopper Publilia modesta Uhler. However, while in some sites ants had a greater positive effect on nymph survivorship, aggregations at those sites produced proportionally fewer adults due to negative density dependent effects (Billick & Tonkel 2003). Predatory bugs (Hemipteran, Reduviidae) and spiders (Salticidae) were the main natural enemies of *E. brasiliensis* in the study site. Other authors also showed that protection against Salticidae, Reduviidae, and parasitoid wasps are among the main benefits provided by ants to treehoppers and aphids (Buckley & Gullan 1991, Cushman & Addicott 1991, Paro et al. 2001).

Ant-derived benefits to treehoppers can vary with time and/or species of ant partner (Billick & Tonkel 2003, Cushman & Whithan 1989, Del-Claro & Oliveira 2000). Although ants had positive effects on the survivorship of *E. brasiliensis* in both years of the study and in two distinct seasons, and the benefit was not dependent of plant quality (Figs. 1 and 2), in the first year the number of individuals reduced during the experiment (Fig. 2A), while in the second year it increased (Fig. 2B). In the experiments performed in the end of the rainy season, first year (EP), the strong rains

Subfamily	Plant experiment (PE)	%
Formicinae	Camponotus aff. blandus	1.9
	C. crassus Mayr	25.5
	C. ruffipes Fabr.	19.9
	<i>C</i> . sp.1	3.7
Myrmicinae	Crematogaster sp. Lund	4.7
	Pheidole sp. Westwood	34.7
Ponerinae	Ectatomma quadridens Fabr.	5.6
Dolichoderinae	Unidentified species	14.1
Subfamily	Stem Experiment (SE)	⁰∕₀
Formicinae	C. crassus Mayr	40.5
	C. ruffipes Fabr.	12.2
Myrmicinae	Crematogaster sp Lund	3.3
	Pheidole sp. Westwood	4.4
Ponerinae	Ectatomma quadridens Fabr.	35.4
Pseudomyrmecinae	Pseudomyrmex aff. gracillis	2.2
	P. aff. pallidus	2.2

Table 1. Ant species tending *E. brasiliensis* (Hemiptera: Membracidae) on shrubs of *S. lycocarpum* (Solanaceae) in dirty field cerrado. % - indicates the percentage of individuals in relation to total observed.



Figure 1. Number of adults, nymphs, and egg masses of *E. brasiliensis*, compared between the first (initial) and 14^{th} day of Plant Experiments (PE) and Stem Experiments (SE). White bars indicate control groups (ants present) and black bars indicate treatment ones (ants excluded). Statistical differences (P < 0,05; r.m. ANOVA) are indicated (*).

of the end of March were possibly the main factor accounting for the reduction in the number of membracids (Stefani *et al.* 2000). Del-Claro & Oliveira (1999) also pointed out that strong summer rains could remove a large numbers of treehoppers from their host plants in cerrado. In the second year (SE), by May, *S. lycocarpum* plants had a higher vegetative growth, producing a large amount of young leaves in a period with a small number of natural enemies in the field (Stefani *et al.* 2000). This factor, probably favored the increase of E. brasiliensis during dry season.

Del-Claro & Oliveira (2000) showed that the presence of tending ants can have a positive impact on treehopper fecundity (see also Bristow 1983). In the PE experiment, ant exclusion resulted in a significant increase in the production of new egg masses by *E. brasiliensis*. However, such a benefit did not occur when comparing stems of the same plant. On the contrary, the data suggested that stems without ants presented an increase in the number of new egg masses (Fig. 1). Del-Claro & Oliveira



herbivores in different ways, and this may result in positive, negative, or neutral consequences for plants (Bronstein 1994, Beattie & Hughes 2002). The results of the PE and SE experiments suggested that ant-Enchenopa associations benefit the host plant, S. lycocarpum, through reduction in leaf herbivory (Fig. 3). Although Rico-Gray & Thien (1983) showed that by protecting sap-feeding hemipterans from their natural enemies, honeydew-gathering ants could negatively affect plant fitness by increasing hemipteran damage to the host plant, the outcomes of such plant-ant-herbivore systems are highly variable. For example, *Formica* ants tending Publilia treehoppers attack leaf-chewing beetles and reduce folivory to goldenrod (Solidago sp.) (Messina 1981). Moreover, Bach (1991) demonstrated that sanitation by tending ants reduces fungi-induced leaf damage (see also Queiroz & Oliveira 2001). Oliveira & Del-Claro (2005) showed that three types of herbivores (thrips, chewing beetles, and leaf-mining caterpillars) were negatively affected by ants, and their damage was reduced on plants hosting anttreehopper associations. Thus, the E. brasiliensis-ants-S. lycocarpum system are apparently conditioned by a number of factors (e.g., time, habitat type, identity, abundance, and behaviour of species partners, severity of herbivore damage) that may influence its final outcomes (Bronstein 1994).

This study provides additional evidence that the relationship between ants, honeydew-producing hemipterans and their host plants can result in positive effects for all participants in the interaction (Messina 1981, Oliveira & Del-Claro 2005). Ants receive an energy-rich and predictable liquid resource (e.g. Tobin 1994, Davidson *et al.* 2003), and in turn increase treehopper survival and reduce herbivore damage to the host plant.



Figure 3. Leaf herbivory in *S. lycocarpum*, observed between the first and last day of Plant (A) and Stem (B) Experiments, depending on the presence (white bars) or absence (black bars) of tending ants. Statistical differences (P < 0,01; r.m. ANOVA) are indicated (**).



Figure 2. Variation in the number of E. brasiliensis through

time depending on presence (white bars) or absence (black bars)

of tending ants during Plant (A) and Stem (B) Experiments. In

March, ends the summer with strong tropical rains. In May,

plants had the main vegetative growing. Statistical differences

(1996) showed that ants are important partners of treehoppers

during the establishment of new aggregations. When one female

of G. xiphias finds a new host plant, droplets of honeydew are

used as cues to ground dwelling ants to find the treehoppers.

When the association with ants is established, the female

migrates to the apical portions of the plant to produce an

(P < 0.05; r.m. ANOVA) are indicated (*).

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