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História Natural de *Edessa rufomarginata* De Geer (Heteroptera: Pentatomidae) em vegetação de Cerrado: Aspectos biológicos, ecológicos e comportamentais de um percevejo-do-mato

Este exemplar corresponde à redação final
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Orientador: Prof. Dr. Paulo Sérgio Moreira Carvalho de Oliveira

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Dedicada à minha toda a minha família

(em especial, meus pais, minha irmã e

minhas avós) que sempre esteve lá por mim!

“Se você encontrar um caminho sem obstáculos,
ele provavelmente não leva a lugar nenhum.”
Frank Clark

"Quando você não está feliz, é preciso ser forte para mudar,
resistir à tentação do retorno. O fraco não vai a lugar algum."
Ayrton Senna

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ÍNDICE

RESUMO

Pentatomídeos são importantes pestes agrícolas que podem causar danos diretos e indiretos às plantas e à economia. Entretanto, muitos aspectos de sua biologia e ecologia em ambientes naturais ainda são desconhecidos. Este estudo apresenta informações relacionadas à biologia e ecologia do pentatomídeo *Edessa rufomarginata* (De Geer, 1773) sobre o pequizeiro, *Caryocar brasiliense* Camb. (Caryocaraceae), uma planta típica do cerrado. Fêmeas de *E. rufomarginata* não apresentaram cuidado maternal e ovipuseram em maior freqüência sobre plantas grandes. Entretanto, as freqüências de oviposição em plantas com botões florais/frutos não diferiram estatisticamente daquelas observadas em plantas sem tais estruturas. Ninfas de primeiro instar permanecem agrupadas e dispersam-se na planta hospedeira somente a partir do segundo ínstar. Indivíduos adultos foram mais abundantes sobre ramos maduros e novos de *C. brasiliense*, enquanto as ninfas foram mais abundantes sobre ramos maduros e folhas. Formigas constantemente antenavam o abdômen de ninfas de terceiro a quinto ínstar de *E. rufomarginata*, e por duas vezes, foram observadas consumindo seu exsudato. A interação do percevejo com inimigos naturais**,** como o reduvídeo *Heniartes* sp. também foi observada. Este é um dos primeiros estudos sobre a biologia e ecologia de *E. rufomarginata* em ambientes naturais, bem como um dos primeiros estudos a observar a ocorrência de interações trofobióticas destes insetos com formigas. Assim, mais estudos envolvendo este percevejo são necessários para uma melhor compreensão de sua biologia e ecologia, bem como sua interação com formigas e eventuais inimigos naturais capazes de atuar como agentes de controle biológico em ambientes agrícolas.

Palavras-chaves: *Edessa rufomarginata*, percevejos, Pentatomidae, formigas do cerrado, trofobiose, mirmecofilia, *Caryocar brasiliense*

ABSTRACT

Pentatomids are important crop pests that cause direct and indirect plant and economical damages. However, several biological and ecological features in natural environments remain unknown. The present study presents information concerning the biology and ecology of *Edessa rufomarginata* (De Geer, 1773) on *C. brasiliense* Camb. (Caryocaraceae), a typical plant species from the Brazilian Cerrado savanna. *E. rufomarginata* females did not present maternal care and preferred to laid their eggs more frequently on bigger plants. However, the oviposition frequency was not different between plants with floral buds/fruits and plants without these structures. First-instar nymphs remained aggregated and only dispersed in the second instar onwards. Adults were more abundant on mature and young stems of *C. brasiliense*, whereas nymphs were more abundant on mature stems and leaves. Ants frequently tapped the abdomen of *E. rufomarginata* nymphs with their antennae, and at least on two occasions the ants were seen consuming their honeydew. The interaction of the bug with other natural enemies, such as the predaceous reduviid *Heniartes* sp., was also observed. This is one of the first studies concerning the biology and ecology of *E. rufomarginata* in natural environments, as well as one of the first ones to observe the occurrence of trophobiotic interactions between stink bugs and ants. Therefore, further studies related to this stink bug are necessary to a better understanding of its biology and ecology, as well as its interaction with ants and natural enemies which can potentially act as agents of biological control in agricultural environments.

Keywords: *Edessa rufomarginata*, stink bugs, Pentatomidae, Cerrado ants, trophobiosis, myrmecophily, *Caryocar brasiliense*.

INTRODUÇÃO GERAL

Com mais de 38.000 espécies descritas, divididas em aproximadamente 80 famílias, a subordem Heteroptera (Insecta: Hemiptera) compõe o maior e mais diversificado grupo de insetos hemimetábolos do planeta, os heterópteros ocorrem em todos os continentes (exceto na Antártida) e várias ilhas oceânicas (Triplehorn & Johnson 2004). A diversidade de habitats por eles ocupados e sua variedade de hábitos alimentares são evidências da grande adaptabilidade deste grupo durante sua história evolutiva (Schuh & Slater 1995). Os heterópteros podem ser hematófagos e predadores, mas em sua grande maioria são fitófagos (Schuh & Slater 1995). Como podem causar sérios danos (diretos e/ou indiretos) à plantas economicamente importantes, pelo consumo direto de tecidos e/ou pela facilitação de entrada de patógenos (veja Delabie 2001 e Schaefer & Panizzi 2000 para revisões), muitos destes insetos são importantes pragas agrícolas (Schaefer & Panizzi 2000).

Contendo aproximadamente 4.100 espécies descritas (Grimaldi & Engel 2005; Costa 2006), a família Pentatomidae possui várias espécies de grande importância econômica (Panizzi *et al.* 2000). Os organismos deste grupo de insetos possuem glândulas odoríferas – torácicas (nos indivíduos adultos) ou dorso-abdominais (nas ninfas) – que liberam substâncias de odor desagradável quando os insetos são ameaçados por eventuais inimigos naturais (Howard & Wiemer 1983; Howard 1987). Entretanto, tais substâncias também podem servir como feromônios (Millar 2005). Por esse motivo, eles são popularmente conhecidos como “Maria-fedida”, “Maria-peidona”, “fede-fede”, “fedorento”, “percevejo-fedido”, mas também são conhecidos como “percevejo-do-mato” ou somente “percevejos”, nomes não associados ao odores que podem liberar (Santos 1982; Grazia & Schwertner 2008). Nesta família existem tanto espécies predadoras (subfamília Asopinae), quanto espécies fitófagas (outras subfamílias) (Grazia & Schwertner 2008). Estas últimas podem se alimentar em todas as partes das plantas, entretanto as partes reprodutivas (botões florais, frutos, sementes) são geralmente preferidas como alimento, já que oferecem nutrientes de alta qualidade (e mais compostos nitrogenadas) aos insetos (White 1984; Salisbury & Ross 1992). Uma vez que podem utilizar sementes como sua principal fonte nutricional, vários percevejos são considerados importantes pragas de culturas agrícolas, como por exemplo, a soja ou algodão (Panizzi & Slansky 1985; Todd 1989; Panizzi *et al.* 2000).

Devido ao seu grande apelo econômico (Panizzi *et al.* 2000), vários aspectos biológicos e ecológicos de pentatomídeos relacionados a ambientes agrícolas como preferência alimentar e *performance* em diferentes plantas hospedeiras (Panizzi 1997, 2000), comportamentos relacionados à oviposição (Panizzi 2006), cuidado maternal (Santos & Albuquerque 2001a, b) e comportamentos ninfais (Lockwood & Story 1986; Hirose *et al.* 2006) já foram estudados. Entretanto, o mesmo não pode ser dito quanto aos aspectos relacionados à sua biologia básica e ecologia em ambientes naturais, que, assim como para muitas espécies da ordem Heteroptera (Schuh & Slater 1995), ainda são bastante fragmentados ou mesmo inexistentes.

Estudos de biologia e ecologia básicas dos pentatomídeos em ambientes naturais são importantes, pois podem revelar informações relevantes para programas de manejo integrado de pestes. Entretanto, a grande diversidade dos grupos que incluem espécies-praga associada à falta de pesquisadores de campo interessados em sua biologia e ecologia básicas, além da pequena quantidade de entomólogos capazes de aferir a real identidade das espécies acabam por desestimular o desenvolvimento da pesquisa de base para muitos dos insetos de interesse econômico. Tomando-se como exemplo o gênero *Edessa* (Fabricius, 1803), existem atualmente 259 espécies descritas, mas ainda restam mais de 200 espécies aguardando descrição e nomenclatura apropriadas (Fernandes & van Doesburg 2000). Assim, na tentativa de se sanar uma ínfima parte deste grande problema e servir como um estímulo para a continuidade de estudos de pentatomídeos em seus ambientes naturais, este trabalho tem como objetivo principal levantar informações básicas sobre a biologia e ecologia de *Edessa rufomarginata* (De Geer, 1773) em vegetação de Cerrado.

Apesar de esta espécie possuir uma distribuição ampla na região neotropical (Silva *et al*. 2004) e causar danos em algumas importantes culturas agrícolas (Panizzi *et al*. 2000), ainda se conhece pouco sobre seus aspectos biológicos e ecológicos. No Cerrado brasileiro, *E. rufomarginata* é constantemente observada se alimentando sobre o pequizeiro *Caryocar brasiliense* Camb. (Caryocaraceae)(Oliveira 1997), uma espécie vegetal muito comum deste bioma (Prance & Freitas da Silva 1973). Durante seus períodos de maior atividade fenológica, o pequizeiro produz uma quantidade considerável de folhas novas, as quais possuem nectários extra-florais capazes de atrair várias espécies de formigas arborícolas do Cerrado (Oliveira & Brandão 1991).

Desta forma, este estudo encontra-se dividido da seguinte maneira: no primeiro capítulo são estudadas informações gerais sobre a biologia e a ecologia de *E. rufomarginata*, com ênfase em: 1) sua fenologia nos pequizeiros, 2) aspectos envolvendo seus hábitos alimentares e uso desta planta como hospedeira, 3) comportamento de oviposição e escolha de plantas hospedeiras e 4) interações com inimigos naturais. Em uma segunda parte, apresentada na forma de uma nota científica, é apresentada a análise química do exsudato produzido pelas ninfas de *E. rufomarginata* e uma breve discussão de sua importância para esta espécie de Pentatomidae no bioma Cerrado.

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Natural history of *Edessa rufomarginata* (De Geer – Hemiptera: Pentatomidae) in the Cerrado savanna: phenology, behavior, and patterns of host plant use

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Keywords: *Edessa rufomarginata*, stink bugs, Pentatomidae, Cerrado ants, trophobiosis, myrmecophily, *Caryocar brasiliense*.

Abstract

Pentatomids are important crop pests that cause direct and indirect plant and economical damages. However, several biological and ecological features in natural environments remain unknown. The present study presents information concerning the biology and ecology of *Edessa rufomarginata* (De Geer, 1773) on *C. brasiliense* Camb. (Caryocaraceae), a typical plant species from the Brazilian Cerrado savanna. *E. rufomarginata* females did not present maternal care and preferred to laid their eggs more frequently on bigger plants. However, the oviposition frequency was not different between plants with floral buds/fruits and plants without these structures. First-instar nymphs remained aggregated and only dispersed in the second instar onwards. Adults were more abundant on mature and young stems of *C. brasiliense*, whereas nymphs were more abundant on mature stems and leaves. Ants frequently tapped the abdomen of *E. rufomarginata* nymphs with their antennae, and at least on two occasions the ants were seen consuming their honeydew. The interaction of the bug with other natural enemies, such as the predaceous reduviid *Heniartes* sp., was also observed. This is one of the first studies concerning the biology and ecology of *E. rufomarginata* in natural environments, as well as one of the first ones to observe the occurrence of trophobiotic interactions between stink bugs and ants. Therefore, further studies related to this stink bug are necessary to a better understanding of its biology and ecology, as well as its interaction with ants and natural enemies which can potentially act as agents of biological control in agricultural environments.

Introduction

Pentatomids, also known as stink bugs, are well adapted to consume liquid food, and the majority of the species compounding this group of insects are phytophagous (Grimaldi & Engel 2005). Some species are considered important crop pests because they cause direct plant damage through the consumption of plant sap, and indirect damage by increasing the infestation of plant pathogens (Buckley 1987; Delabie 2001; Schuh & Slater 1995). Others, such as the predatory species in the subfamily Asopinae, may act as important agents of biological control against defoliating lepidopteran and coleopteran larvae (Grazia *et al.* 1999). Due to their economic interest, many pentatomid species have been extensively studied and some aspects of their biological and ecological features are well documented (Panizzi *et al.* 2000 and references therein).

In the beginning of the reproductive season of their preferred host plants, phytophagous pentatomids emerge, start to feed and reproduce (Grazia & Schwertner 2008). Plant reproductive structures are the main food resource (Schuh & Slater 1995; Grazia & Schwertner 2008): developing fruits, flower buds and seeds are more attractive since they provide a high quality diet (White 1984; Salisbury & Ross 1992) and ensure a higher reproductive success (Panizzi 2000), but plant vascular systems can also be used as feeding resources by some species (Schuh & Slater 1995; Grazia *et al.* 1999).

As soon as the reproductive season of their preferred host plant species finishes, adult pentatomids may start a reproductive diapause under the leaf litter, bark or other shelters (Jones & Sullivan 1981), or may disperse in search of secondary host plants (Grazia & Schwertner 2008). According to Grazia & Schwertner (2008), polyphagy is the rule within the Pentatomidae and their feeding behavior, performance and survival may vary according with the host species (Panizzi 1997; Panizzi 2000). Nonetheless some species are oligophagous (Panizzi 2000).

The adult individuals of some species may remain aggregated over their host plants. The pheromones produced by males partially mediate the formation of such aggregations (Aldrich 1995), and may also mediate male-female mating interactions (Krupke *et al*. 2001). Egg masses may be laid over all plant parts above ground level or on surrounding plants near the main host (Grazia & Schwertner 2008). After emergence, first-instar nymphs remain aggregated, acquiring mutualistic endosymbionts found over the eggshells provided by their mothers during oviposition (Abe *et al*. 1995), and also conserving moisture, an important factor affecting the nymphs' molting process (Lockwood & Story 1986; Hirose *et al*. 2006). During the first instar, nymphs do not feed from plant tissues (Schuh & Slater 1995). Gregariousness continues during second and third instars and nymphs only start to disperse over the host plant in the fourth instar onwards (Grazia & Schwertner 2008).

Among some pentatomid species, females provide their egg-masses and first-instar nymphs with intense parental care (Eberhard 1975; Santos & Albuquerque 2001a, b; Tallamy & Schaefer 2006), a behavior believed to occur in some species within the Pentatomidae (Schuh & Slater 1995). Although this behavior is sometimes costly to females (Santos & Albuquerque 2001a), it increases brood survival by expelling parasitoids and predators (Santos & Albuquerque 2001b).

Despite the general knowledge concerning the biology and ecology of economically important pentatomids, there is a great lack of specific information concerning Neotropical pentatomids in natural environments (Schuh & Slater 1995).

Especially within the subfamily Edessinae, which includes the highly diverse genus *Edessa* (Fernandes & van Doesburg 2000a, b; Silva *et al*. 2004), basic biological information are badly available. These studies are very important because they might reveal potential agents of biological control, and provide relevant information concerning the behavior and population ecology of these pest species that could be used on Integrated Pest Management Programs (IPMP here on). Additionally, such studies may provide information that may allow future comparative studies on the biology and ecology of the whole Pentatomidae family.

The species *Edessa rufomarginata* (De Geer, 1773) is widely distributed from Mexico to Argentina (Silva *et al*. 2004) and, according to Panizzi *et al*. (2000), is a secondary pest of several crop plants, mostly within the family Solanaceae. The most economically important host plants of this stink bug are tobacco, potato, eggplant, soybean, sunflower, rice and corn (Silva *et al*. 1968; Rizzo & Saini 1987). Although all these crops can be attacked by *E. rufomarginata*, there are very few studies about its biology and ecology (*e.g*. Rizzo & Saini 1987; Fortes & Grazia 1990). In the Brazilian Cerrado savanna, Oliveira (1997) also observed this species feeding and reproducing on *Caryocar brasiliense* Camb. (Caryocaraceae), one of the main representative plant species from the Brazilian Cerrado savanna (Prance & Freitas da Silva 1973), which is commonly visited by ants gathering the nectar produced by Extrafloral Nectaries on its newly-produced leaves (Oliveira & Brandão 1991).

The present study addresses the following questions related to the biology and ecology of *E. rufomarginata* in the Brazilian Cerrado savanna: 1) How is the phenological relationship between *E. rufomarginata* and its main host plant in this biome, *C. brasiliense*?

2) Is host plant size an important feature during oviposition site selection? 3) What is the pattern of host plant use by nymphs and adults? 4) Which parts of *C. brasiliense* are preferentially selected by females for oviposition? 5) When do nymphs disperse? 6) Who are the natural enemies of *E. rufomarginata*, and how does this species behave when they are present? Additionally, a secondary objective of this study is to confirm if *E. rufomarginata* adults and nymphs perform general behaviors observed for other pentatomid species elsewhere in the literature.

Materials and Methods

The study was carried out in a Cerrado area in Itirapina, São Paulo state, south-eastern Brazil (22º 15'S, 47º 49'W, 770 m of altitude). The dry season occurs from April to September (Fall-Winter), while the wet season goes from October to March (Spring-Summer). The Cerrado vegetation at the study site consists of a dense scrub of shrubs, corresponding to the cerrado *sensu stricto* (Oliveira-Filho & Ratter 2002). *C. brasiliense* is very abundant in the area, reaching up to 300 individuals per ha (Oliveira 1997). Data on the biology and ecology of *E. rufomarginata* were obtained from September 2008 to February 2009, and are complementary to previous biological and ecological observations gathered from June 1986 until July 1987.

The phenologies of C. brasiliense *and* E. rufomarginata

From August 1986 and until July 1987, the phenologies of the stink bug and its host plant were obtained biweekly from 80 shrubs of *C. brasiliense* (0.5–1.0 m tall) along a trail in the cerrado. The plant variables measured were: 1) number of leaves, 2) number of inflorescences, 3) number of flower buds, flowers and fertilized flowers, and 4) number of fruits. For the stink bug, the variables measured were: 1) the total number of adults, 2) the total number of nymphs, and 3) the total number of egg masses. During September 2008 and February 2009 such variables were also gathered for both the *C. brasiliense* shrubs and the pentatomid bug. The height of *C. brasiliense* shrubs was not measured. Phenological records are important to record the plant's patterns of leaves, flower and fruit production (Morellato *et al.*2000; Torezan-Silingardi & Oliveira 2004), as well as the insect's populational variations during the study periods.

Biological and ecological features of E. rufomarginata

From September 2008 until February 2009, 75 shrubs of *C. brasiliense* (0,5–2m tall) were used to test whether host plant size and plant reproductive status were important variables affecting host plant selection by *E. rufomarginata* females. The number of leaves, flower buds and fruits of each shrub were measured monthly.

In order to investigate patterns of host plant use by *E. rufomarginata*, as well as the oviposition behavior of females, the crown of *C. brasiliense* shrubs was categorized in three parts: 1) mature stems: brown-grayish stems produced in previous years, and possessing cracks and holes on the bark; 2) young stems: light green, not lignified stems, with no cracks and holes, produced by the plant during the period of the study and 3) leaves: new leaves continuously produced by the shrubs during the study period. Since the *C. brasiliense* shrubs used on the observations were no higher than 2 m, the proportions of mature stems, young stems and leaves available for the use of *E. rufomarginata* adults and nymphs were similar. Once a week during daytime, the number of *E. rufomarginata* individuals (both adults and nymphs) and of egg masses found on each of these plant parts were counted through snapshot scan samplings (*sensu* Altmann 1974). Interactions between *E. rufomarginata* adults and nymphs and other organisms were also observed. Only nymphs in the third, fourth and fifth instars were counted, since first and second instar nymphs remained aggregated on the branch where they hatched.

Forty *E. rufomarginata* adults were collected in the field and reared in the laboratory under controlled conditions (25 ºC ± 2 ºC and 12L:12D); 17 couples were formed. They were fed *ad libitum* with tangerines, peanuts and bean pods in 300 ml plastic cups. Food was replaced three times a week to avoid mold proliferation. A wet cotton ball inside each cup was watered daily to keep adequate humidity levels. Three times a day (mornings at 8:00 h; afternoons at 12:00 and nights at 18:00) the cups were observed in order to record: 1) the frequency of oviposition behavior of females, 2) the number of egg laid, 3) the number of newly-hatched nymphs. These couples were observed during two months. Sporadically, behavior information was also gathered both from field and laboratory observations.

A logistic regression based on the Quasi-Newton iterative method was applied to test whether the number of leaves influenced host plant selection by egg-laying females. Chi-square tests were used to test whether females had preference to oviposit on plants with or without floral buds, and to investigate preferred plant locations. Host plant use by *E. rufomarginata* on each different plant part was compared with a General Linear Model (GLM) module via a one-way ANOVA, followed by Tukey *post hoc* comparisons. The assumptions for all tests were followed and the data was transformed whenever it did not follow the tests' assumptions.

Results

The phenologies of E. rufomarginata *and* C. brasiliense

Except for fruit development and maturation, which occurred during the wet season, most of the phenological events of *C. brasiliense* occurred in the dry season of 1986-1987 and were not observed in any other months throughout that year. Leaves started to senesce and fall at the beginning of February, and in the dry season all shrubs had just few or no leaves at all (Fig. 1A), and during this period both mature and young stems shriveled out. By the end of August, mature stems became active again and structures such as young stems, new leaves and floral buds develop until the beginning of the wet season (Figs. 1.1A and 1.1B). During October-November, all flowers were completely opened. Fruit maturation occurred in the end of the wet season, three to four months after flowers had been opened, but no new fruit was produced in February-March 1987 (Fig. 1B). From September 2008 to February 2009 the same patterns were observed (Fig. 1D and E), however the percentage of *C. brasiliense* shrubs producing fruits was lower than that observed during 1986-1987 wet season (Fig. 1E).

E. rufomarginata adults were observed on *C. brasiliense* shrubs from August to December 1986 (Fig. 1C) and they were not observed again during the next months when such observations took place. Egg masses and nymphs were observed from October 1986 to January 1987. Increased abundance of *E. rufomarginata* egg masses and nymphs was clearly associated with the host plant's reproductive phase. The same phenological patterns were observed for *E. rufomarginata* during 2008-2009 (Fig. 1F), however none egg was found during December 2008 and January 2009.

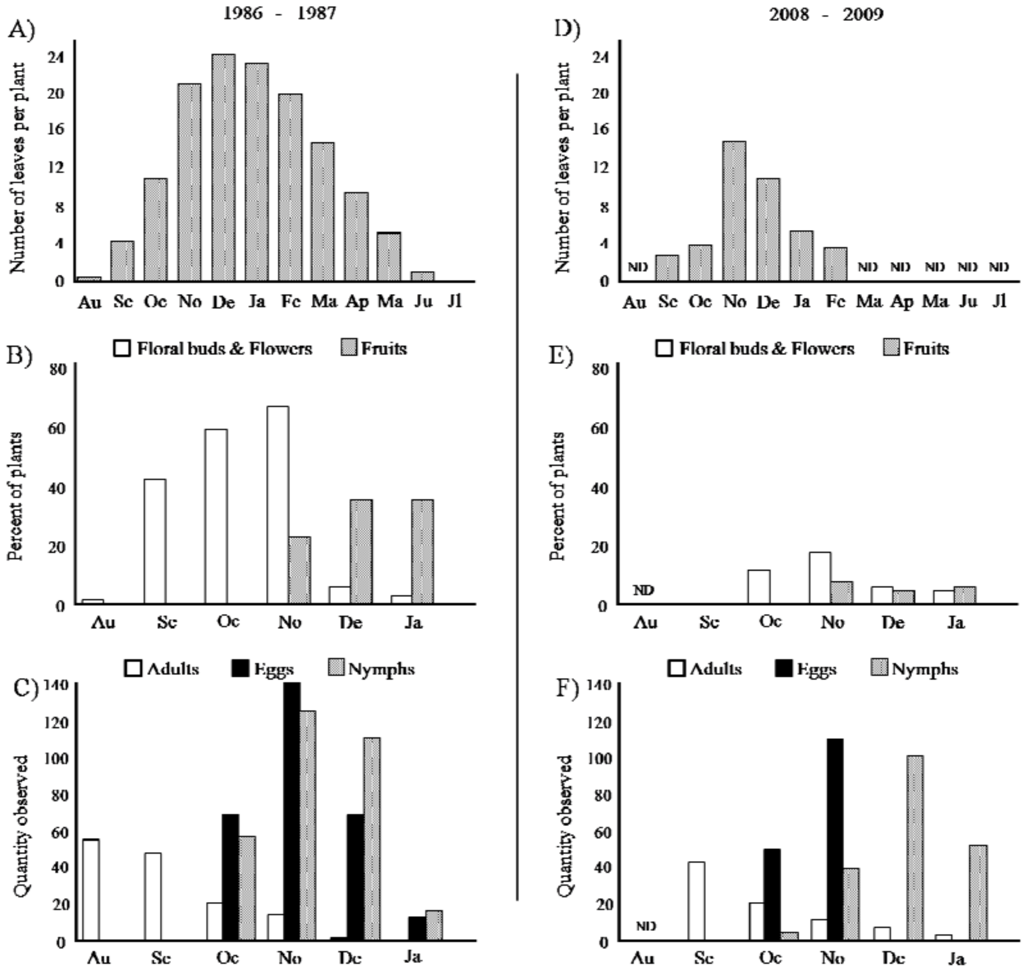

Figure 1. Phenologies of *C. brasiliense* and *E. rufomarginata* during August 1986 and July 1987 in Itirapina, Brazil. A) Leaves of *C. brasiliense* shrubs per month in 1986-1987; B) Floral buds, flowers and fruits produced by *C. brasiliense* in 1986-1987; C) Phenology of *E. rufomarginata* on *C. brasiliense* in 1986-1987; D) Leaves of *C. brasiliense* shrubs per month between September 2008 and February 2009; E) Floral buds, flowers and fruits produced by *C. brasiliense* in 2008-2009 ; F) Phenology of *E. rufomarginata* on *C. brasiliense* in 2008-2009. ND means No Data. Ja, Fe, Ma, Ap, Ma, Ju, Jl, Au, Se, Oc, No, and De are the months within a year.

Host plant use by E. rufomarginata

Eighteen egg-masses were observed on mature stems of *C. brasiliense*, five were observed on young stems and none occurred on leaves. Mature stems were used more frequently as oviposition sites by females compared to young stems ($\chi^2 = 14.695$; g.l. = 1; $p < 0.01$). In fact, two egg-masses deposited on young stems fell from the plant. Sixteen egg-masses were recorded on plants that did not develop floral buds/fruits during the reproductive season, while seven were found on plants with reproductive structures ($\chi^2 = 1.935$; g.l. = 1; $p = 0.164$). Egg-masses were more frequently observed on plants with increased quantity of leaves ($\chi^2 = 13.791$; g.l. = 1; $p < 0.01$; Fig. 2).

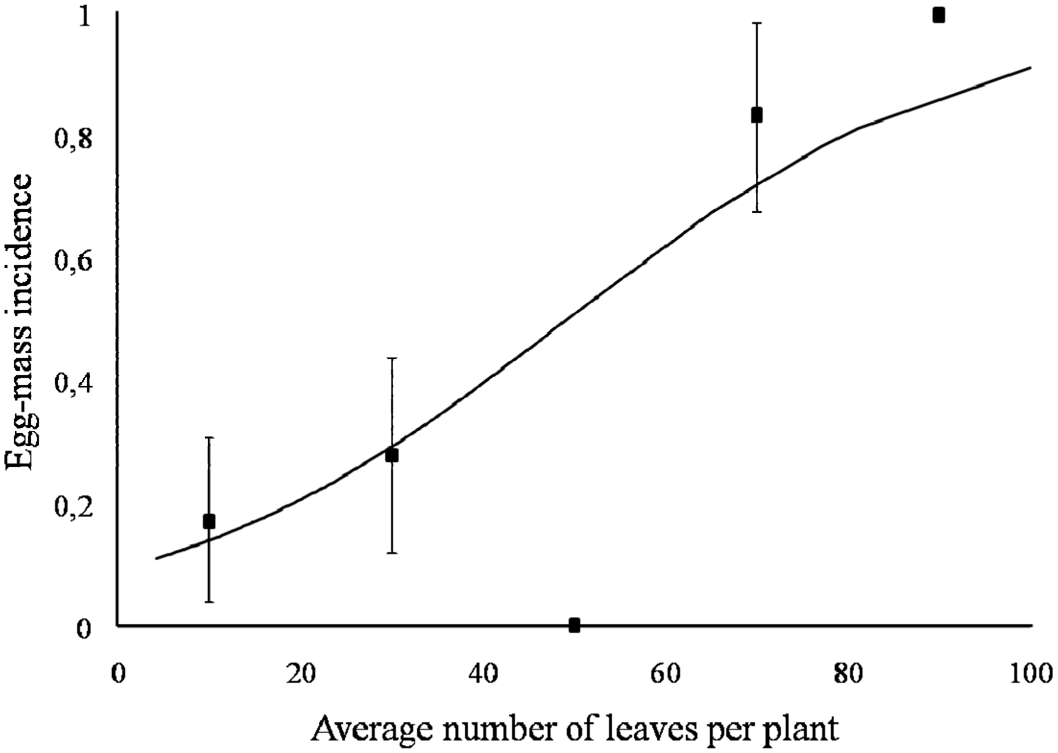

Figure 2. Preference of oviposition site by E. rufomarginata on C. brasiliense, according to host plant size. Plotted are the predicted probability of egg mass incidence (curve) and the mean ± 95% Confidence Interval of egg masse incidences according to each leaf quantity class (filled squares with bars), n=75 plants.

Adults and nymphs of *E. rufomarginata* spent considerable time feeding on particular plant parts. Both stages were frequently observed feeding on mature and young stems, whereas floral buds and fruits were rarely used as feeding resources by *E. rufomarginata*. The pentatomids did not feed on leaves, but they used these structures as resting and protection sites against natural enemies. Snapshot observations revealed that adults were more abundant on mature stems than on leaves or young stems ($F_{2,30} = 3.518$; g.l. = 2; p = 0.042; Fig. 3A). Nymphs, however, were more abundant on mature stems compared to young stems or leaves ($F_{2,30} = 4.703$; g.l. = 2; p = 0.017; Fig. 3B).

Interactions between E. rufomarginata *and other organisms*

When disturbed, adults and nymphs of *E. rufomarginata* vibrated their antennae intensely and usually hid themselves at protected sites among the foliage. They could also jump off the plant and hide among the leaf litter. Adults could fly off to neighboring plants. Upon disturbance, both adults and nymphs frequently secreted volatile substances from their metathoracic (adults) or dorso-abdominal (nymphs) scent glands.

Pheidole sp. ants (Myrmicinae) were once observed preying upon *E. rufomarginata* eggs (Fig. 4A). A large worker of *Pachycondyla villosa* (Ponerinae) was observed preying upon a third-instar nymph. Nymphs and adults of the predacious reduviid *Heniartes* sp. (Hemiptera: Reduviidae) were observed sucking up the body contents of both adults and nymphs of *E. rufomarginata* (Fig. 4B; n = 6). No vertebrate species was observed preying upon *E. rufomarginata*. However, insectivorous vertebrates (armadillos, lizards and birds) were commonly found at the study area and could potentially prey upon this stink bug.

Other interactions involving ants were observed at the study site. Frequently, *Camponotus rufipes* (Formicinae) tapped the abdomen of third to fifth-instar nymphs feeding on *C. brasiliense*. On two such occasions, the *E. rufomarginata* nymphs secreted droplets of honeydew, which was promptly collected by the ants. Adult individuals also were able to secrete a similar substance but no ants were seen consuming it. However, *E. rufomarginata* (both nymphs and adults) did not flick away their honeydew droplets, unlike other hemipteran species form the Cerrado savanna (see Del-Claro & Oliveira 1996).

Oviposition behavior, egg-masses, and nymph gregariousness

Two oviposition events were observed in the field. The female’s genital plaques opened and closed repeatedly before the eggs were sequentially expelled from the body. After having laid an egg on the host plant, the female touches it with the last tarsomere of a hind leg. The whole oviposition process lasts 20-25 minutes, after which the females fly away and abandon the eggs.

Twenty three egg masses were observed over *C. brasiliense* shrubs, and none of them were guarded by females. Generally, the egg masses contained 14 bluish-green eggs, divided in two rows of seven eggs each. This pattern varied only twice: one egg-mass had only 10 eggs while other one only had one egg. The average number of eggs laid by the females was 13.26 (SD = 2.799 eggs; n = 23). The heights of the egg-masses above ground level varied from 21 cm to 112 cm ($\overline{X}$ = 63.4 cm; n = 23; SD = 27.72 cm). Three darkened

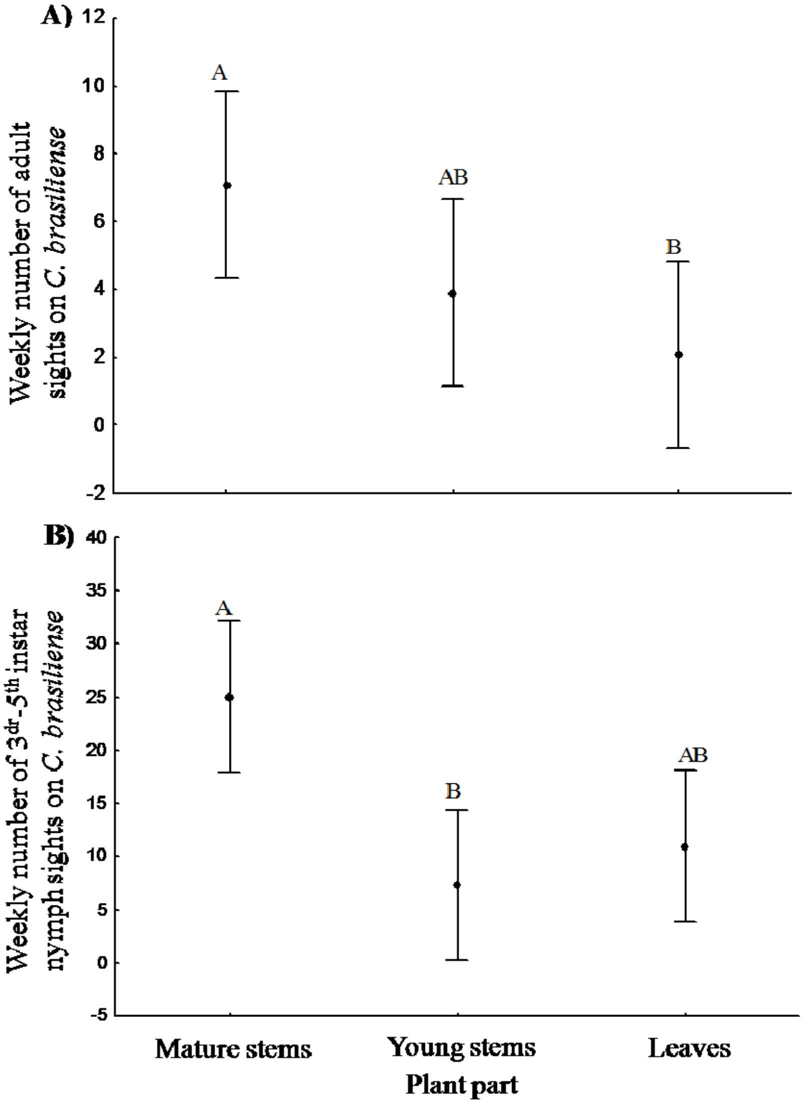

Figure 3. Occurrence of *E. rufomarginata* individuals on different C. brasiliense plant parts. A) Adults. B) Nymphs. Vertical bars represent 95% confidence intervals. Bars with the same letter represent means which are not statistically different according to post-hoc Tukey test, α = 0.05. (Data used in nymph abundance analyses were log+1 transformed, but the B graph presents untransformed data).

Figure 4. A) *Pheidole* sp. workers attacking an egg mass of *E. rufomarginata*. B) *Heniartes* sp. adult feeding on *E. rufomarginata*. Photos by: A) Daniel P. Silva; B) Sebástian Sendoya.

egg-masses were found in the field, in which cases no hatching was observed after three weeks.

No mating was observed under laboratory conditions, indicating that females were already fertilized in the field. Females oviposited only once, and died soon after doing this. Seventeen egg masses were obtained. Seven of them were laid during the mornings and nine during the afternoons. No egg was laid at night. Egg masses had in general 14 eggs, but this number also varied twice: one of them had only 11 eggs, while the other had 27 eggs. The average number of eggs laid per females was 14.53 (SD = 3.279 eggs; n = 17). Nearly 74% of the eggs laid were fertilized, whereas 26% had no embryo. Nymphs hatched from 82% of the fertilized eggs, 7 to 10 days after oviposition ($\overline{X}$ = 8.75 days; n = 17; SD = 1.00 day).

After hatching, first-instar nymphs remained grouped at the oviposition site consuming the liquid from inside the eggshells. Occasionally they also consumed the contents from the unviable eggs. On one occasion, first-instar nymphs also consumed the contents of all eggs from an adjacent egg mass, from which no nymph ever hatched. No first-instar nymphs were recorded consuming plant sap. After molting, second-instar nymphs presented increased movement rates on *C. brasiliense* shrubs and no longer aggregate near eggshells, but still present some of gregariousness. Second instar nymphs feed on plant sap. From the third-instar onwards, nymphs become solitary. Nymphs of different instars (probably from different oviposition events), however, were once observed together on a *C. brasiliense* shrub.

Discussion

Phenological relationships

The phenological patterns of *C. brasiliense* observed in Itirapina corresponded those recorded for *C. brasiliense* in other Cerrado areas (Leite *et al.* 2006; Vilela *et al.* 2008) as well as those recorded for other Cerrado tree species, which concentrate their production of vegetative and reproductive tissues (new stems, leaves, floral buds, flowers and fruits) on the end of the dry season and beginning of the wet one (Batalha & Martins 2004). Since water is a key resource to plant species within the Brazilian Cerrado savanna (Oliveira *et al.* 2005), this phenological pattern is an important physiological mechanism to save water and nutrients during the dry season (Batalha & Martins 2004).

The phenology *E. rufomarginata* was expected to match that of *C. brasiliense* since this tree species is the pentatomid's main host plant in the Cerrado of Itirapina. However, when *C. brasiliense* shrubs dry out, *E. rufomarginata* adults may respond in two different ways: 1) they may start a reproductive diapause, as do other pentatomid species (Jones & Sullivan 1981), or 2) they may search for alternative host plants (Panizzi 1997; Panizzi 2000). Since *E. rufomarginata* dormant individuals were not found, and no bug was observed feeding/reproducing over other plant species, further studies are needed to assess the fate of *E. rufomarginata* during the dry season, as well as, if *E. rufomarginata* occurring in other Cerrado areas only use *C. brasiliense* as their main host plant or if other species may serve as secondary host for these stink bugs.

Host plant use by E. rufomarginata

Egg attachment on mature and young stems may differ due to their different physical characteristics (lignified or soft bark, presence/absence of cracks and holes, different rates of tissue growth), and *E. rufomarginata* females may distinguish such traits and prefer mature stems as oviposition sites. Among species from several insect groups, such plant characteristics (tissue toughness, plant part age, presence/absence of plant structural defenses, plant nutritious quality) are very important during host plant selection, serving as cues to egg-laying females during oviposition. This is true especially to species within Lepidoptera (Thompson & Pellmyr 1991 and references therein), beetles (Réu & Del-Claro 2005), gall-inducing insects (Stein & Price 1995) and within Hemiptera (Backus 1988; Romani *et al*. 2005).

Generally, the pentatomid diet is based on the consumption of the sap from plants with developing reproductive structures (Panizzi 2000), once they to the stink bugs (and other herbivorous insects) a high quality food (White 1984; Salisbury & Ross 1992). However, since *E. rufomarginata* was observed feeding itself mainly from *C. brasiliense* vascular system and rarely from the plant's reproductive structures, plant architecture and structure may be a more important feature than sap quality for this pentatomid. Plants with more leaves may provide better microhabitats for insects that use them (Raghu *et al*. 2004), protecting adults and, especially, nymphs and egg batches from desiccation and may also serve as surrogates of plant quality to egg-laying females (Thompson & Pellmyr 1991). In fact, the abundance of *E. rufomarginata* nymphs was high among *C. brasiliense* leaves. Bigger plants (with many leaves in this case) may also provide more protection against natural enemies (Price *et al*. 1980; Jeffries & Lawton 1984), and better feeding sites (Heisswolf *et al*. 2005) when compared with smaller plants. Considering the 'Plant Vigor Hypothesis' (Price 1991), bigger *C. brasiliense* shrubs (with more leaves) may be more nutritious than smaller ones (with fewer leaves) and, consequently, they may provide higher performance to *E. rufomarginata* nymphs, being preferred by egg-laying females as the host plants for their offspring (Jaenike 1978). Such relationship was proven to be truth for many species of phytophagous insects (Thompson 1988), but not for others (Forrister *et al.* 2009).

Finally, *E. rufomarginata* nymphs and adults were more abundant on *C. brasiliense* mature stems compared to young ones. Since *Edessa* species have a shorter rostrum than other pentatomid species (Panizzi & Machado-Neto 1992 , A.R. Panizzi pers. obs.), it could be expected that *E. rufomarginta* would be more abundant over *C. brasiliense* young stems, which are softer and not lignified and could provide the insects with an easier access to the plant's sap vessels. However, since these plant parts are the main route taken by ants collecting nectar from EFNs on *C. brasiliense* new leaves (Oliveira 1997), ants may exert a strong interference on *E. rufomarginata* feeding activities on young stems. Oliveira (1997) indeed observed *E. rufomarginata* adults and nymphs avoiding ants in such plant parts for several times and this ant avoidance may explain why their abundances were higher *C. brasiliense* mature stems.

Interactions between E. rufomarginata *and other organisms*

Field observations revealed *Heniartes* sp. individuals consuming *E. rufomarginata* over some *C. brasiliense* shrubs. Many predaceous reduviids were already successfully used in IPMPs (Nishi *et al.* 2004; Grundy 2007) and *Heniartes* sp. can potentially act as biological control agents against *Edessa* species on crop plants. However, further biological and ecological investigation is needed to confirm the real status of this reduviid species as a biological control agent.

The honeydew secreted by *E. rufomarginata* nymphs probably contains important nutrients, such as sugars and proteins (Auclair 1963; Blüthgen *et al.* 2004), which are capable to appease the ants and decrease their willingness to attack (Stadler & Dixon 2008). These substances are commonly found in the honeydew produced by several groups of herbivorous insects, such as riodinid and lycaenid butterflies (DeVries 1997; Pierce *et al.* 2002), and many auchenorrhynchous and sternorrhynchous hemipterans (Delabie 2001). The trade-offs faced by these insects while maintaining myrmecophilous interactions have also been extensively assessed elsewhere (see Stadler & Dixon 2005 for a review). However, since such interactions are poorly known among heteropterans (Waldkircher *et al.* 2004), further studies concerning *E. rufomarginata* and Cerrado ants are certainly needed to unveil the trade-offs of myrmecophily for each species involved.

Oviposition behavior, egg-masses, and nymph gregariousness

Panizzi (2006) hypothesized that egg-laying females may provide better egg positioning and attachment to the substrate when they touch the eggs during oviposition. Considering that *E. rufomarginata* females behaved that way, their eggs may be better positioned and attached to the oviposition substrate. Although Panizzi (2006) did not relate egg attachment to nymph hatchability or survival, eggs weakly attached to the oviposition substrate seem more prone to fall off the plant, which indeed occurred twice with two egg masses oviposited over *C. brasiliense* young stems.

Under controlled laboratory conditions during the two months when they were observed, the average number of eggs laid per female, egg viability, and egg development time were similar to those found by Fortes & Grazia (1990). The gregariousness of *E. rufomarginata* first-instar nymphs may provide them with a more humid environment, preventing them from desiccation, and previous studies have already stressed out the importance of a humid micro-environment during nymphal development (Lockwood & Story 1986; Hirose *et al*. 2006). Since desiccation may cause the loss of cuticular softness and lubrication in insects (Guarneri *et al*. 2002), nymphal emergence and molting, as well as their development and survival may be affected. However, the acquisition of endosymbiotic mutualists from the eggshells may also account for the gregariousness of first-instar nymphs (Buchner 1965; Abe *et al*. 1995; Fukatsu & Hosokawa 2002). Such information corroborate that found for other species and, in the future, may be used in studies involving the comparative biology for the diverse Pentatomidae family.

Conclusions

The phenological relationships of *E. rufomarginata* and its host plant, *C. brasiliense*, at Itirapina match one another. Shortly after the beginning of *C. brasiliense* reproductive season, the stink bugs were already present over the plant’s stems, leaves and reproductive structures, but they disappear from the plant in the end of its reproductive season. The present study did not reveal the fate of the pentatomids during the dry season and new studies may answer such question.

The females's oviposition frequencies over plants with and without reproductive structures were not different, but the plant size (measured by the quantity of leaves) may be a very important factor assessed by the females during the oviposition processes. Plants with more leaves, may provide the insect with more protection against natural enemies and may offer a high quality diet to the stink bugs.

The production of sugary honeydew by *E. rufomarginata* nymphs may decrease the Cerrado ants' willingness to attack them. Consequently, these nymphs may be assuring a enemy-free space that may result in a high fitness of this species during the individual's lifetime, and future studies involving this pentatomid should assess the trade-offs involved in such trophobiotic interactions.

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New record of a trophobiotic interaction: ants and the stink bug *Edessa rufomarginata* (De Geer – Heteroptera: Pentatomidae) in the Brazilian Cerrado Savanna

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Ants comprise a very diverse and abundant group of animals in all ecosystems worldwide (Hölldobler and Wilson 1990). During their foraging activities, ants can feed on liquid resources provided by plants and myrmecophilous insects, as well as on herbivorous insects found on the plants (Carroll and Janzen 1973; Oliveira and Pie 1998; Del-Claro and Torezan-Silingardi 2009). Ants are the main group of insects visiting plants bearing extrafloral nectaries (EFNs), and their antiherbivore deterrence has been proved to be important for several EFN-bearing plant species (Koptur 1992; Bronstein 1998; Bronstein *et al.* 2006). Since all life stages of phytophagous insects (eggs, immature and adults) may be preyed by ants (Heads and Lawton 1985), ants are considered as important selective agents throughout the evolutionary history of the herbivorous insects (Rico-Gray and Oliveira 2007).

Herbivorous species developed many features to overcome ant predation (Heads and Lawton 1985; Freitas and Oliveira 1992; Dyer 1995; Portugal and Trigo 2005; Sendoya *et al*. 2009). One of these tactics is the production of sweet appeasing resources to ants. These substances are rich in sugars, proteins and vitamins (Auclair 1963; Blüthgen *et al.* 2004), and are very attractive to ants. Usually referred to as trophobiosis, these interactions arose among several ant-attended insect groups such as lycaenids (Pierce *et al.* 2002) , riodinids (DeVries 1997) and hemipterans (Delabie 2001). Among the order Hemiptera, the majority of the described interactions occur within the suborders Auchenorrhyncha and Sternorrhyncha, and just a few within the suborder Heteroptera (within Coreidae and Plataspididae; Delabie 2001).The present scientific note describes the first record of a trophobiotic interaction involving a species of Pentatomidae, the stink bug *Edessa rufomarginata* (De Geer, 1773), and foliage-dwelling ants.

Fieldwork was carried out in the Brazilian Cerrado savanna, which covers about 25% of the Brazilian territory (Oliveira-Filho and Ratter 2002) and hosts a diverse community of ants and EFN-bearing plants (Oliveira and Leitão-Filho 1987; Oliveira and Brandão 1991). *Caryocar brasiliense* Camb. (Caryocaraceae) is a typical cerrado plant possessing EFNs on floral buds and shoot tips, which are constantly visited by over 30 ant species (Oliveira and Brandão 1991; Oliveira 1997).

The pentatomid *E. rufomarginata* also feeds on *C. brasiliense* and ants were thought to be one of its natural enemies, because bugs and eggs were more frequently seen on ant-free than on ant-visited plants (Oliveira 1997). However, after recent observations from September 2008-February 2009, the interaction between *E. rufomarginata* nymphs and ants proved to be more complex than previously thought. Some ant species, such as *Pachycondila villosa* (Ponerinae), do attack the nymphs of this pentatomid (Fig. 1A). However, on several occasions (n>10), *Camponotus* ants were observed tapping their antennae on the abdomen of third to fifth instar nymphs, which did not seem to be disturbed by the ants. Indeed, on two occasions *Camponotus rufipes* were observed consuming the honeydew excreted by nymphs of *E. rufomarginata* (Fig. 1B).

The presence of sugars in *E. rufomarginata* honeydew was investigated. Eight nymphs (third- and fourth-instar) were hand-stimulated to secrete the honeydew, which was sampled on a small piece of filter paper. The filter paper was immediately stored in a portable freezer (0º C), brought to the laboratory and stored at -20°C until analysis. Gas chromatography-mass (GC-MS) was used to estimate the relative amount of sugars in honeydew. The filter paper with the honeydew was

Figure 1 – A) *Pachycondyla villosa* worker carrying a recently captured *E. rufomarginata* nymph. B) *Camponotus rufipes* ant consuming honeydew produced by a fourth instar nymph of *E. rufomarginata* (Photos by A: Paulo S. Oliveira and B: Sebastían Sendoya).

extracted in 5 ml EtOH. The solvent was dried on vacuum and the ethanol extract was acetylated with 300 µl pyridine: AcO_2 (1:1) for 48 hours. The reaction was dried overnight on air flux and recovery in 300 µl EtOAc for GC-MS analysis at EI mode on a Hewlett Packard-6890 GC system with a fused capillary column (30 m x 0.25 mm x 0.25 µm, HP-5MS, Crossbond 5% phenyl–95% dimethylpolysiloxane) directly coupled to a selective mass detector Hewlett Packard 5973. Conditions of injection were: injector temperature 290°C; oven temperature program 160-300°C, 4°C/min, 5 min at 300°C; transference line temperature: 300°C; injection mode split 20:1; carrier gas He: 1 ml/min, constant flow; sample volume 1.0 ml. The characterization of the sugars was performed by comparison of MS fragmentation with NIST Mass Spectral Search Program (Version 2.0f, 2008), acoording to Biemann *et al*. (1963).

The samples contained three main kinds of sugar. The disaccharide trehalose was the main sugar found in *E. rufomarginata* honeydew and accounted for 94.1% of the carbohydrates in the sample. The honeydew also contained small proportions of two monosaccharides, glucose (4.7%) and sorbose (1.2%). A control filter paper did not contain sugars. Honeydew composition and attraction to ants varies accordingly to the species (Auclair 1963; Blüthgen *et al.* 2004), but trehalose usually make up for more than 35% of the sugar contents within the honeydew, once it is the major sugar found in the insect's haemolymph (Hölldobler and Wilson 1990). Thus, the high concentration of this sugar in the honeydew samples from *E. rufomarginata* nymphs is not uncommon. After trehalose, monosaccharides are the main constituents of honeydew of myrmecophilous hemipterans (Hölldobler and Wilson 1990). The absence of more specific saccharides such as melezitose, commonly found in more specialized trophobiotic interactions between hemipterans and ants (Kiss 1981), suggests that the relationship between *E. rufomarginata* and ants is occasional in nature.

A previous study with the butterfly *Eunica bechina* (Nymphalidae) showed that egg-laying females avoid ovipositing on *C. brasiliense* ant-visited plants (Sendoya et al. 2009). Even though *Eu. bechina* eggs are not consumed by ants, caterpillars can be fiercely attacked by ants foraging over *C. brasiliense*. Therefore, eggs laid on ant-free plants are more prone to develop into adult butterflies than those laid on plants visited by aggressive ants (Sendoya *et al*. 2009). Given that *E. rufomarginata* nymphs cannot fly and present a low mobility compared to adults, honeydew production may appease the ants and, consequently, decrease their willingness to attack (Stadler and Dixon 2008). Because *E. rufomarginata* feeds on an EFN-bearing plant that attracts many ant species (Oliveira and Brandão 1991), honeydew secretion may be especially important as a defense mechanism against natural enemies (including ants), as documented for other trophobiont species in Cerrado (Del-Claro and Oliveira 1993, 1996, 1999).

Generally, ant-trophobiont interactions incur in several costs (physiological and developmental restrictions) and benefits (enemy-free space, higher reproductive success) for both the ants and trophobionts (Stadler *et al.* 2003). Such trade-offs have been extensively assessed elsewhere for many riodinid and lycaenid butterflies and auchenorrhynchous and sternorrhynchous hemipterans (see Hölldobler and Wilson 1990; Stadler and Dixon 2005 for reviews). However, these interactions are poorly known among heteropterans (Delabie 2001), and according to Waldkircher *et al.* (2004), the wide range of feeding habits among the Heteroptera accounts for the rareness of myrmecophilous records found within this group (Table 1). Therefore, further studies involving *E.*

Table 1 – All known records and possible records of trophobiotic interactions between ants and heteroperans. An asterisk (*) indicates that true trophobiosis has not been documented yet.

Taxa	Location	Citation
PLATASPIDAE		
Coptosoma sp. (Laporte)	Sri Lanka	Green (1900) *apud* Waldkircher *et al.* (2004)
Coptosomoides myrmecophilus (China)	Sumatra	China 1931 *apud* Waldkircher *et al.* (2004)
Tropidotylus servus (Dolling)	Malaysia	Maschwitz *et al.* (1987)
Tropidotylus minister (Dolling)	Malaysia	Maschwitz *et al.* (1987)
Caternaultiella rugosa (Schouteden)	Cameroon	Dejean *et al.* 2000, 2002; Gibernau & Dejean 2001
Tetrisia vacca (Webb)	Malaysia	Waldkircher *et al.* (2004)
COREIDAE		
Hygia (Trichocolpura) cliens (Dolling)	Malaysia	Maschwitz *et al.* (1987)
Cloresmus spp.	Malaysia	Maschwitz *et al.* (1987)
Notobius affinis (Dallas)	Malaysia	Maschwitz *et al.* (1987)
Amorbus obscuricornis (Westwood)	Tasmania	Steinbauer (1996)
PENTATOMIDAE		
Eurystethus microlobatus (Ruckes)	Brazil	Guerra *et al.* (pers. comm.)
Lincus spathuliger (Breddin)*	Surinam	Stahel (1954)
Edessa rufomarginata (De Geer 1773)	Brazil	Present study.

rufomarginata and their attending ants are necessary to assess the trade-offs inherent to myrmecophily in this stink bug and others Pentatomidae species.

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CONSIDERAÇÕES FINAIS

1. As fenologias do pentatomídeo *E. rufomarginata* e de sua planta hospedeira no cerrado, o pequizeiro *C. brasiliense*, são relacionadas. A partir do início da estação reprodutiva do pequizeiro, os pentatomídeos começam a ser encontrados sobre seus ramos, folhas e estruturas reprodutivas, desaparecendo no fim da estação reprodutiva da planta. Neste estudo não foi determinado se os percevejos mudam de planta hospedeira ou se entram em diapausa reprodutiva quando a alimentação sobre o pequi se torna ineficaz. Novos estudos devem tentar responder a essa pergunta.

2. As freqüências de oviposição das fêmeas de *E. rufomarginata* em plantas com e sem botões florais/ frutos não foram significativamente diferentes, mas a quantidade de folhas da planta hospedeira pode ser um fator importante durante a seleção das fêmeas por locais de oviposição. Plantas grandes, com mais folhas, podem oferecer mais proteção contra inimigos naturais e constituírem melhores fontes de alimentação para os percevejos.

3. A conservação da umidade e a obtenção de organismos endossimbiontes são as principais hipóteses para explicar as agregações de ninfas de *E. rufomarginata* durante seu primeiro ínstar de desenvolvimento. Futuros estudos envolvendo esta espécie devem analisar quais microorganismos ocorrem sobre os ovos, e de que forma o risco de dessecação relaciona-se com o comportamento de gregário das ninfas.

4. As ninfas de *E. rufomarginata* produzem um exsudato rico em substâncias açucaradas atrativas às formigas do Cerrado. As ninfas de *E. rufomarginata* aparentemente diminuem a motivação das formigas em atacá-las ao oferecerem às formigas tal substância. Adicionalmente, as ninfas podem estar assegurando um espaço livre de inimigos, uma vez que eventuais inimigos naturais poderão ser afastados pelas formigas que as estão atendendo. Futuros estudos com *E. rufomarginata* deverão avaliar os custos e benefícios para cada um dos envolvidos nesta interação trofobiótica.

5. A biologia e ecologia das espécies de reduvíideos que se alimentam de conteúdos corpóreos de *E. rufomarginata* devem ser estudadas, uma vez que estas espécies podem vir a ser utilizadas em programas de manejo integrado de pestes contra espécies praga do gênero *Edessa*.