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HUMBERTO DE PAULA DUTRA

INTERAÇÃO ENTRE FORMIGAS E *URERA BACCIFERA* (URTICACEAE) MEDIADA POR ATRATIVOS DISTINTOS: CORPOS PEROLADOS E FRUTOS

Este exemplar corresponde à redação final da tese defendida pelo(a) candidato (a)
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Resumo

1. O presente trabalho investiga o papel protetor de formigas contra os insetos fitófagos de *Urera baccifera* em uma floresta semidecídua do sudeste do Brasil. *U. baccifera* é utilizada como planta hospedeira por 3 espécies de lepidópteros: *Urbanus esmeraldus* (Hesperiidae), *Pleuroptya silicalis* (Crambidae) e *Smyrna blomfildia* (Nymphalidae)
2. Muitas espécies de formigas forrageiam intensivamente nos arbustos de *U. baccifera,* estando constantemente presentes na planta ao longo do ano, exceto durante o período de deciduidade da planta. Todas as estruturas aéreas, vegetativas e reprodutivas da planta, são visitadas por formigas, especialmente as folhas onde há uma grande produção de corpos perolados (CPs). Além de corpos perolados, formigas também utilizam os frutos de *U. baccifera* como fonte de alimento.
3. CPs são atrativos efetivos de formigas à planta. Arbustos de *U. baccifera* são proporcionalmente mais visitados por formigas do que plantas vizinhas sem CPs. A visitação de formigas a CPs reduz a sobrevivência de lagartas de *S. blomfildia*. Frutos também possuem uma função na atração de formigas aumentando o recrutamento de formigas à planta.
4. Os resultados do experimento mostram que plantas que tiveram suas formigas excluídas foram mais infestadas por herbívoros do que plantas visitadas por formigas no dois anos em que se realizou o experimento, 2003 e 2004.
5. Verificamos uma variação temporal nos resultados da interação formigas-*U. baccifera*. Em 2003 a abscisão foliar ao longo do tempo foi significativamente

maior nas plantas sem formigas do que nas plantas visitadas por r formigas. No entanto, em 2004 os grupos experimentais de plantas não diferiram quanto à abscisão foliar. Os resultados mostram que os benefícios proporcionados pelas formigas aparentemente dependem dos níveis de infestação por herbívoros. Possivelmente os resultados da interação entre formigas e *U. baccifera* estão condicionados pela abundância de herbívoros, de modo que proteção oferecida por formigas só se manifeste quando o nível infestação por herbívoros não é muito elevado.

Summary

1. The present study investigates the protective role of ants against phytophagous insects of *Urera baccifera* in a semi-deciduous forest in south-east Brazil. Three lepidopterans larvae consume the plant: *Urbanus esmeraldus* (Hesperiidae), *Pleuroptya silicalis* (Crambidae) and *Smyrna blomfildia* (Nymphalidae).
2. Many ant species intensively visit shrubs of *U. baccifera* throughout the year, except during leaf fall. Ants forage on all vegetative and reproductive plant structures, but most especially on leaves where they harvest pearl bodies (PBs). Besides PBs ants also use the fleshy fruits of *U. baccifera* as food resource.
3. PBs are effective ant attractants, and *U. baccifera* individuals were significantly more visited by ants than neighboring plants without rewards. Ant visitation to non-reproductive individuals of *U. baccifera* increased larval mortality of *S. blomfildia*, suggesting that ants attracted to PBs reduce herbivore survival. Fruits play a role in ant attraction increasing ant recruitment to *U. baccifera*.
4. Results of an ant-exclusion experiment show that ants were effective in reducing the incidence of lepidopteran larvae. In both 2003 and 2004, herbivores presented higher infestation levels on ant-excluded shrubs than on control shrubs (ants present) of *U. baccifera*.
5. There is a temporal variation in the outcomes of the ant-*U. baccifera* interaction. In 2003 ant-excluded plants have significantly faster leaf abscission rates compared with ant-visited plants. However, ant exclusion had no effect on leaf abscission in 2004. Results show that the outcomes of the interaction may be conditioned by herbivore abundance. At high levels of herbivore infestation,

such as in 2004, ant visitation may not be sufficient to decrease herbivore-induced leaf abscission.

Introdução Geral

Mutualismos

Organismos de diferentes espécies interagem em uma grande variedade de modos. Freqüentemente uma única espécie pode interagir com toda uma comunidade. Por conveniência interações entre espécies são agrupadas de acordo com o tipo de influência que uma exerce sobre a outra. Esta influência pode ser benéfica, prejudicial ou neutra para quaisquer membros da parceria em termos de aptidão (Bronstein 1994). A literatura especializada relata a existência de uma ampla gama de interações entre organismos, parte destas associações confere benefícios mútuos para as espécies envolvidas. Este tipo de interação em que os organismos envolvidos trocam diretamente algum tipo de benefício ou serviço (e. g. comida, defesa e transporte) é conhecido como mutualismo (Ricklefs 2001).

Mutualismos são interações importantes e ubíquas que ocorrem em comunidades naturais, especialmente em ambientes tropicais; apesar do grande e crescente número de estudos que investigam interações mutualísticas, pouco se sabe sobre este tipo de interação, pois grande parte destes se limitam a descrever a história natural das espécies envolvidas (Bronstein 1994).

Interações mutualísticas variam dentro de um contínuo que vai desde interações indiretas, facultativas e difusas até interações obrigatórias, co-evoluídas e altamente especializadas (Bronstein 1994). Interações facultativas envolvem indivíduos de diversas espécies e geralmente consistem de interações intermitentes (Billick & Tonkel 2003). No lado oposto do contínuo estão as associações altamente especializadas ou obrigatórias que envolvem indivíduos de espécies específicas que geralmente vivem em associações íntimas comumente classificadas de simbioses (Herre *et al.* 1999).

Mutualismos entre plantas e formigas

A grande maioria dos estudos de interações mutualísticas investiga relações entre animais e plantas (Bronstein 1994). Uma parte representativa do nosso conhecimento atual sobre interações mutualísticas entre plantas e animais foi adquirida a partir do estudo de interações entre plantas e formigas (Bronstein 1998, Beattie 1985). Em alguns casos a relação entre formigas e plantas consiste na defesa oferecida pelas formigas contra o ataque de herbívoros em troca de alimento e/ou abrigo para a colônia (Beattie 1985, Hölldobler & Wilson 1990). Estas interações podem ser agrupadas em obrigatórias ou facultativas. Em interações obrigatórias as formigas vivem em simbiose com as plantas e a associação é claramente benéfica para ambos (Beattie 1985). Muitos trabalhos demonstram que formigas proporcionam algum tipo de benefício para plantas ao predarem os herbívoros da planta (revisado por Beattie 1985, Koptur 1992). Em contra partida, há um número expressivo de trabalhos que indicam que formigas não proporcionam nenhum benefício para algumas plantas (O'Dowd & Catchpole 1983, Heads & Lawton 1984, Rashbrook *et al.* 1992) ou beneficiam a planta somente em determinadas condições (Barton 1986, Smiley 1986, Horvitz & Schemske 1990, de la Fuente & Marquis 1999, Mody & Linsenmair 2004). O caráter mutualístico das interações entre formigas e plantas pode variar tanto no espaço quanto no tempo. Estas variações podem modular a força das associações mutualísticas alterando os resultados da interação (Barton 1986, Horvitz & Schemske 1990, de la Fuente & Marquis 1999, Del-Claro & Oliveira 2000).

Mutualismos condicionais entre plantas e formigas

Diversos estudos têm mostrado para interações entre plantas e formigas que os benefícios da associação dependem: das espécies de formigas que visitam a planta (Koptur 1984, Rico-Gray & Thien 1989); das taxas de visitação de formigas à planta (Inouye & Taylor 1979, Oliveira *et al.* 1999); da abundância local de herbívoros e formigas (Barton 1986, Smiley 1986, Horvitz & Schemske 1990, de la Fuente & Marquis 1999, Mondor & Linsenmair 2004); da imunidade dos herbívoros à predação por formigas (Lawton & Heads 1984, Heads & Lawton 1985). Deste modo é possível que a ocorrência de benefícios mútuos para formigas e plantas dependa do cenário ecológico em que as interações estão inseridas (Bronstein 1994, 1998), um fenômeno denominado mutualismo condicional (Billick & Tonkel 2003).

Apesar da natureza condicional das interações ser conhecida há cerca de três décadas (Paine 1966), apenas mais recentemente começou-se a investigar como as condições ecológicas modificam o resultado dessas interações no tempo e no espaço. Este tipo de abordagem permite um melhor entendimento da dinâmica ecológica e evolutiva destas interações (Cushman & Addicott 1991). Interações mutualísticas entre planta e formigas mediadas por recompensas alimentares constituem um sistema interessante para analisar a natureza condicional deste tipo de interação.

O sistema de estudo

O presente estudo envolve a urtiga *Urera baccifera* (L.) Gaudich (Urticaceae) sua comunidade de formigas e os principais fitófagos que se alimentam da planta: *Urbanus esmeraldus* (Butler) (Hesperiidae: Pyrginae), *Pleuroptya silicalis* (Guené) (Crambidae: Pyraustinae) e *Smyrna blomfildia* (Fruhstorfer) (Nymphalidae: Nymphalinae).

Alguns indivíduos de *Urera baccifera* podem conter mais de 50 formigas forrageando em sua folhagem (Machado & Freitas 2001, Dutra observação pessoal). Machado & Freitas (2001) mostraram que formigas que forrageiam em urtiga podem remover herbívoros potenciais (iscas de cupim) sugerindo que formigas conferem algum benefício à planta na proteção contra herbívoros. A planta oferece duas recompensas alimentares para formigas, frutos e corpos perolados. Corpos perolados, também chamados de glândulas de pérola são estruturas derivadas de tecidos epidérmicos ou sub-epidérmicos, geralmente ricas em lipídeos (O'Dowd 1982). Estas estruturas constituem uma recompensa alimentar para formigas relativamente comum na natureza. No entanto, poucos trabalhos investigaram como a atração de formigas à corpos perolados pode mediar algum benefício à planta (O'Dowd 1982, Beattie 1985, Schupp & Feener 1991).

O objetivo deste trabalho é avaliar o papel dos corpos perolados e frutos na atração de formigas à *U. baccifera*. Para isto serão investigadas as seguintes questões específicas: qual é o padrão de forrageamento de formigas em *U. baccifera e* quais benefícios que formigas oferecem à planta. Além disso, pretende-se investigar se existe alguma variação temporal na interação e identificar quais fatores podem estar condicionando nos resultados da interação entre formigas e urtiga.

CAPÍTULO 1:

Dual ant attraction in the Neotropical shrub *Urera baccifera* (Urticaceae): the role of ant visitation to pearl bodies and fruit pulp in herbivore deterrence

Introduction

Ant-plant associations contributed substantially to our current understanding of animal-plant mutualistic interactions (Bronstein 1998). Mutualistic systems involving ants and plants fall within a continuum from very loose associations to obligate symbiosis (Hölldobler & Wilson 1990). Loose ant-plant relationships are facultative, and may potentially involve individuals from many species. The ecological forces exerted by each species involved, ants and herbivores for example, determine the outcomes of these interactions (Barton 1986, Billick & Tonkel 2003). Recent studies show that the results of ant-herbivore-plant interactions may vary in space and time. Temporal and spatial variations can modulate the strength of the association between ants and their partners (Barton 1986, Horvitz & Schemske 1984, 1995, de la Fuente & Marquis 1999, Del-Claro & Oliveira 2000), a phenomenon termed conditional mutualism (Herre *et al.* 1999). The outcomes of facultative mutualisms depend upon the ecological environment in which they occur (Bronstein 1994, 1998) and also vary with the quality of reciprocal benefits that each partner provides to the other (Koptur 1984, Horvitz & Schemske 1984, Cushman & Whitham 1989, Rico-Gray & Thien 1989, Del-Claro & Oliveira 2000). Therefore the mutualistic nature of facultative ant-plant interactions may vary with changes in the community structure and fluctuations in population dynamics of the organisms involved (Horvitz & Schemske 1990, de la Fuente & Marquis 1999, Del-Claro & Oliveira 2000, Mondor & Linsenmair 2004).

Several unrelated plant species attract ants by providing nest sites (Vasconcelos 1991, Rocha & Bergallo 1992, Davidson & Mckey 1993, Fonseca 1994) and/or food resources, such as extrafloral nectar and food bodies (Bentley

1977, Beattie 1985, Koptur 1992, Heil & Mckey 2003). The term food-body comprises a wide variety of epidermal structures that have often been interpreted as ant-attractants (O'Dowd 1982, Beattie 1985, Schupp & Feener 1991). Food bodies do not form a distinct group of epidermal structures, because of the great diversity of these structures in higher plants (Beattie 1985). Notwithstanding, O'Dowd (1982) attempted to define the unifying features for a food bodies' subset named "pearl bodies" (hereafter PBs). O'Dowd (1982) describes PBs as single to multicelled lipid rich structures with a spherical or elongated shape, and lustrous pearl-like appearance, that are produced on leaves and stems of plants. Plants bearing PBs occur over a wide range of taxa, comprising more than 50 genera (O'Dowd 1982).

Although, the harvesting of PBs by ants has long been reported in the botanical literature (O'Dowd 1979, 1980, Risch *et al.* 1977, Risch & Rickson 1981, see also O'Dowd 1982 and references therein), the role of PBs in mediating plant protection remains largely overlooked (Schupp & Feener 1991, Marquis & Braker 1994). In fact, ant-derived benefits to PB-bearing plants were investigated mostly in symbiotic systems especially in Paleotropical myrmecophyte species (Fiala & Maschwitz 1990, Fiala *et. al.* 1994, Heil *et al.* 1997, Heil *et al.* 2001, but see Letourneau 1983, Vasconcelos 1991, Fonseca 1994). It seems that, for these highly integrated ant-plant associations, ants are effective to provide protection against herbivores (Letourneau 1983, Fiala *et al.* 1989, 1994 , Vasconcelos 1991, Fonseca 1994, Heil *et al.* 1997, Heil *et al.* 2001).

Though PBs bearing plants are widespread and well known, few studies have directly investigated the outcomes for facultative, non-symbiotic, ant-PBs-plant interactions (Fiala *et al.* 1994, Heil *et al.* 2001). Studies examining Paleotropical *Macaranga* (Euphorbiaceae) species provided the first experimental results demonstrating that facultative associations with ants, mediated by PBs, could be profitable for plants (Whalen & Mackay 1988, Fiala *et al.* 1994, Heil *et al.* 2001). Possibly, these are the only studies investigating the role of PBs on non-symbiotic interactions. In turn, no study conducted experimental approaches exploring the role of PBs in a non-symbiotic Neotropical ant-plant interaction.

Ants foraging on plant foliage do not feed exclusively dependent on PBs and extrafloral nectar and incorporate a broad variety of vegetal material in their diets, including fruits (Rico-Gray 1993, Leal & Oliveira 1998). In tropical environments, fruits may represent a substantial portion in ants' diet (Hölldobler & Wilson 1990, Rico-Gray 1993, Leal & Oliveira 1998, Pizo & Oliveira 2000). The heavy fruit fall together with the great diversity and density of ants in Tropical forests, engender a wide range of interactions between ants and fleshy fruits (Leal & Oliveira 1998, Pizo & Oliveira 2000, Passos & Oliveira 2003). Ant-fruit interactions occur mostly on the ground, though, some studies reported ants climbing the plant to collect the fruits (Pizo 1998, Lu & Mesler 1981, Wheelwright 1985, Machado & Freitas 2001).

Urera baccifera (L.) Gaudich. (Urticaceae), is a nettle usually found in secondary environments (Francis 2000) that offer fruits and PBs as food rewards to ants (Schupp & Feener 1991, Machado & Freitas 2001 - Fig. 1). Machado & Freitas (2001) reported up to 50 ant individuals foraging on a single *U. baccifera* plant, and their results point to a protective role of visiting ants against potential herbivores. Three species of Lepidopterans use *U. baccifera* as host plant, namely: *Urbanus esmeraldus* (Butler) (Hesperiidae: Pyrginae), *Pleuroptya silicalis* (Guené)

(Crambidae: Pyraustinae) and *Smyrna blomfildia* (Fruhstorfer) (Nymphalidae: Nymphalinae). The present study investigates the protective role of ants against phytophagous insects of *U. baccifera*'s in a semi-deciduous forest in south-east Brazil. Five questions were addressed: 1) What is the ant foraging patterns on shrubs of *U. baccifera;* 2) does the presence of PBs or fruits increase ant visitation to *U. baccifera*; 3) does ant visitation to PBs reduce herbivores infestation levels on the plant, and does it affect leaf survival; 5) do ant-derived benefits to *U. baccifera* present temporal variation?

Materials and Methods

Study Area

Fieldwork was carried out from 2000 to 2004 in Santa Genebra Reserve in the County of Campinas, São Paulo, South-eastern Brazil (22°49'45"S, 47°06'33"W). The climate is the Cwa type by the Köeppen classification, defined as warm and wet, with a dry winter and a wet summer. The average annual rainfall is 1381.2 mm and the mean annual temperature is 21.6 °C. Most of the reserve area is covered by semi deciduous mesophytic forest (Morellato 1995). The experiments were performed with *U. baccifera* shrubs located along a 1200 m trail within the forest.

Study organisms

Urera baccifera is a thin-stemmed shrub, 1.5 to 2 m height that occasionally becomes a tree let. The most identifiable trait of the species is the presence of many sharp, stinging-spines on the leaves, branches and stem (Francis 2000). *Urera baccifera* is a typical pioneer species, moderately shade intolerant, distributed from Mexico to Brazil (Francis 2000, Martins & Rodrigues 2002). The plant starts to abscise its leaves during the fruiting season (from early April to late June), sprouting again in August. Individuals of *U. baccifera* produce a few hundreds of small white single-seeded fruits (width 0.54 ± 0.06 cm; length 0.56 ± 0.05 cm; weight 0.07 ± 0.02 g; N = 50 fruits). These fruits are rich in carbohydrates (79.6% of fruits dry mass) and proteins (16.3 of fruits dry mass – H. P. Dutra unpublished data)

Urera baccifera is primarily dispersed by the capuchin-monkey (Cebidae: *Cebus apella* – H. P. Dutra, personal observation); and also by birds: *Saltator similis* (Cardinalidae), and 3 Thraupidae species *Thlypopsis sordida*, *Tachyphonus coronatus* and *Tricothraupis melanops* (W. R. Silva, personal communication). At least three large ant species also act as primary seed dispersers: two myrmicine species (*Acromyrmex* sp., *Atta sexdens*) and a large ponerine (*Pachycondyla villosa*), which climb onto the plant and remove the entire fruit to their nests (Machado & Freitas 2001, H. P. Dutra, personal observation, Fig. 1 A B).

Ants also forage intensely on vegetative individuals of *U. baccifera* in search of PBs, which are found in all aerial structures of the plant: stems, leaves, and pedicels of inflorescences and infrutescences. Plants reared in the lab accumulate thousands of these structures, although, in the field we seldom see *U. baccifera*'s PBs in the field because they are promptly removed by foraging ants or easily detached from the plant. Preliminary analysis showed that *U. baccifera*'s PBs have glucose on their constitution.

Three lepidopteran larvae are the principal insect herbivores of *U. baccifera.* The skipper *Urbanus esmeraldus* rests inside a cone-shaped shelter constructed by cutting a small piece of leaf and by fastening it with silk. A new shelter is built after moulting; *Pleuroptya silicalis* a leaf-tying moth that feeds inside its shelter; the butterfly *Smyrna blomfildia* construct frass chain, a stick-like structure formed by feces and silk where 1st – 3rd larvae rest. *S. blomfildia* probably is the main herbivore of *U. baccifera,* since a single full grow larva may defoliate the entire plant (H. P. Dutra, personal observation).

Patterns of ant visitation at *U. baccifera*

The seasonality of ant visitation to *U. baccifera* was evaluated by regular ant censuses (every 15-20 days) on 17 plants (1-2 m height) from May 2000 to April 2001. At each census, individual branches were searched for ants during 40 s, and the number of ants was recorded. Ants were classified according to their location on the plant (stem, leaves, flowers or fruits). All censuses were carried out from 12:00 to 14:00 h. Air temperature (1.5 m above ground) was recorded at the beginning of each sampling session. The phenological state of each plant was also recorded. We performed a linear regression analysis for correlations between temperature records and number of ants visiting the plant on each census.

Pearl bodies: ant attraction and herbivore survival

To see whether PBs increased ant density over that expected on non-rewarding plants, 76 experimental plant pairs were tagged. Each pair consisted of a vegetative shrub of *U. baccifera* (1-2 m height) and the nearest similar-sized plant without ant-attractants. We avoided plants bearing extrafloral nectaries and plants hosting honeydew-producing hemipterans or lepidopterans. The number of ants present on each plant was recorded upon our arrival. Ant recruitment to experimental plants was compared using a Wilcoxon test. The number of plants occupied by ants in experimental pairs was compared using a chi-square test, assuming the expected ratio of 1:1 to *U. baccifera* and plants without ant-attractants.

We carried out an ant exclusion experiment to evaluate if ant visitation to PBs reduce herbivore survival. We avoided using fruiting plants in this experiment to ensure that PBs were the only ant attractants. We used 80 similar sized plants (1-2 m height) and randomly assigned them as control or treatment. Treatment plants had their ants excluded by applying a sticky barrier of tree Tanglefoot (Tanglefoot CO. Grand Rapids, MI, USA) to the basis of their stem every 15 days. Grass bridges providing aerial access of ants to treated plants were regularly pruned. Ants had free access to control individuals of *U. baccifera*. Control plants received Tanglefoot on only side the stem, so that ants could still access the plant foliage. We chose *S. blomfildia* for this experiment because it was the most abundant herbivore when we conducted the experiment in February 2003. Ant effects on *S. blomfildia* larvae survival were evaluated by placing one larva (1st or 2nd instar) on the first full grown leaf (4th to 6th in relation to the plant apex) on each plant in either experimental group. Test larvae were obtained from plants that were not used in the experiment, and kept individually in plastic boxes. To ensure that larvae were securely established on experimental plants we waited until they build their frass-chains. Ant predation on larvae was measured as the number of larvae that disappeared after 4 days. Larval survival in experimental plants was compared using chi-square tests.

Fruits as ant attractants

We experimentally tested the role of fruits as ant attractants by tagging 74 non-reproductive individuals of *Piper amalago* (Piperaceae), each designated as treatment or control plants by the flip of a coin. Treatment plants received 10-15 fresh fruits of *U. baccifera* that were trespassed with a polyester sewing line (Corrente®) and tied on a branch. Control plants received a leaf of *U. baccifera* trespassed with sewing line and tied to a branch. Experimental plants were established in the morning and ants in both experimental groups were censused at 1 h intervals from 13:00 h to 17:00 h. Samplings at each plant consisted of recording the number of ants during a standard period of 20s. Ant activity on experimental plants along successive censuses was analyzed by repeated-measures ANOVA. We performed log x+1 transformation on the data.

Effects of ants on herbivores infestation, and on leaf survival

The protective role of visiting ants against herbivores of *U. baccifera* was tested with ant-exclusion experiment in two years. In 2003 and 2004, before the beginning of the fruiting period, we tagged similar sized (0.6 - 2.0 m height) *U. baccifera* individuals in the same phenological state (no buds, flowers or fruits). We randomly assigned each plant as a treatment or control (24 and 29 in 2003, and 26 and 30 in 2004, respectively). Each plant had one branch selected for records of herbivores. Ants were prevented from climbing on experimental branches of treatment plants by applying a sticky barrier of Tanglefoot to the trunk base. Ants had free access to control individuals of *U. baccifera,* which were applied Tanglefoot on only side of the trunk.

Infestation levels by *S. blomfildia, U. esmeraldus*, and *P. silicalis* were determined by counting the number of larvae of each species present on tagged branches. Since herbivory may affect leaf survival (Núnez-Farfán & Dirzo 1989, Blundell & Peart 2000), we also counted the initial number of leaves, and the number of abscised leaves of tagged plants. We conducted weekly herbivore and leaf censuses on both years during 7 weeks. Herbivore infestation levels and leaf survival on experimental plant groups along successive censuses were analyzed by repeated-measures ANOVA. We performed log x+1 transformation on the data.

RESULTS

Patterns of ant visitation at *U. baccifera*

The ant assemblage recorded foraging on shrubs of *U. baccifera* comprises a total of 17 ant species from 10 genera distributed in four subfamilies, as follows: Formicinae, *Camponotus abdominalis, Camponotus. lespesi, Camponotus crassus, Camponotus sericeiventris, Camponotus* sp. 1, *Camponotus* sp. 2 and *Paratrechina* sp.*;* Myrmicinae: *Acromyrmex* sp., *Atta sexdens, Crematogaster* sp. 1, *Crematogaster* sp. 2, *Cephalotes pusillus, Pheidole* sp. 1, *Pheidole* sp. 2., and *Solenopsis* sp.; Ponerinae: *Pachycondyla villosa;* Pseudomyrmecinae: *Pseudomyrmex* sp. Except for *Atta sexdens* and *Acromyrmex* sp. that were only seen collecting fruits, all other species exploit both PBs and fruits of *U. baccifera.*

Ants were present on plants all over the year, except during the period of leaf fall (July-August). On average we recorded 0.39 ±0.05 SE ants per plant. We recorded a seasonal variation on the number of ants visiting *U. baccifera* (Fig. 2a), and ant activity was positively related with temperature (Fig. 2b). During the summer, we registered peaks of 1.29 ±0.36 SE ants per plant. We also observed an increase of ant visitation during the fruiting period, May-June 2000 and April 2001 (Fig. 2a). Ants forage on all aerial vegetative and reproductive structures, but most especially on leaves. Ants were also seen harvesting PBs produced on the stems and pedicels of inflorescences. During the fruiting season, May to June 2003 and April 2004, ants were mostly found feeding on fruit pulp (Fig. 2c).

Pearl bodies: ant attraction and herbivore survival

The number of ants visiting shrubs of *U. baccifera* was significantly higher than on neighboring plants without PBs (Wilcoxon; $z = -3.51$, $p < 0.001$ - Table 1). The number of *U. baccifera* individuals occupied by ants was also significantly different from that recorded for neighboring plants without PBs ($\chi = 11.95$, D. F. = 1, $p < 0.001$ – Table 1). These results show that ants preferably forage on *U. baccifera* than on neighboring plants, suggesting that PBs are effective ant attractants.

The ant exclusion experiment showed that larval mortality is influenced by ant presence ($\chi^2 = 6.24$, D. F. = 1, $p < 0.05$). After 4 days, 55% of *S. blomfildia* larvae had disappeared from the ant-visited branches whereas only 26.5% had disappeared from the ants excluded branches, indicating that ants have a negative impact on *S. blomfildia* larval survival.

Fruits as ant attractants

Ant activity on shrubs of *Piper amalago* having *U. baccifera*'s fruits attached, was significantly higher than on *P. amalago* plants having an attached leaf of *U. baccifera* (Repeated measures ANOVA; $F_{1,72} = 14.70$; $p < 0.0001$ - Fig. 3). During the censuses we recorded up to 33 ants on *P. amalago* plants with *U. baccifera* fruits attached.

Effects of ants on herbivores infestation, and on leaf survival

Both in 2003 and 2004, herbivores presented higher infestation levels on ant excluded than on control shrubs of *U. baccifera*, indicating that ants were effective in reducing incidence of Lepidoptera larvae on this plant (Fig. 4, see also Table 2). *Smyrna blomfildia* accounted for most of the herbivores records in both years, but in 2004 we observed an increase in the number of *U. esmeraldus* larvae. In both years we recorded low infestation levels of the moth *P. silicalis* (Table 3). Predation on *U. esmeraldus* larvae by the large ponerine ant *Pachycondyla villosa* was recorded twice. We also observed once the formicine *Camponotus crassus* preying upon *S. blomfildia* larva. In all ant predation records larvae were feeding on leaves outside their refuges. On many occasions larvae of *S. blomfildia* and *U. esmeraldus* that were seen feeding on a leaf, outside their refuges, quickly returned to their interior in consequence of ant presence on that leaf, similar to registers obtained for *Eunica bechina* (Nymphalidae: Nymphalinae, Freitas & Oliveira 1996).

A significant interaction between ant treatment and time was identified for leaf survival in 2003, indicating that ant-excluded plants have faster leaf abscission rates (Fig. 5, see also Table 2). This pattern was not observed in 2004, hence, ant treatment had no effect in leaf abscission in this year.

Discussion

Most studies dealing with ant-PBs systems investigated highly specialized ant-plant associations and showed that PBs-harvesting ants benefit the plant through protection against herbivores (Letourneau 1983, Fiala *et al.*1989, 1994, Fiala & Maschwitz 1990, Vasconcelos 1991, Heil *et al.* 1997, Fonseca 1994, Heil *et al.* 2001). While the role of PBs in symbiotic ant-plant interactions is well documented, a sweeping statement about the role of PBs in ant-plant facultative systems is not yet achievable due to the lack of experimental studies investigating these systems (but see Whalen & Mackay 1988, Fiala *et al.* 1994, Heil *et al.* 2001). Here, we take a step towards filling this gap.

Our data confirmed that possession of PBs by *Urera baccifera* increases ant density in relation to non-rewarding plants. Although some authors argue that PBs are only attractive for specialized ants (Fiala & Maschwitz 1990, 1992, Fiala *et al.* 1994), we demonstrated that PBs of *U. baccifera* were attractive to a wide variety of ant taxa. These results indicate a low specificity of the association, supporting the facultative character of this mutualism. Ants respond positively to larger food resource concentrations (O'Dowd 1979, Koptur 1984, McKey 1984) and the efficacy of ant attraction is usually enhanced when PBs are offered together with other food rewards (Fiala & Maschwitz 1991). Our ant censuses reinforce this tendency by showing that dual ant attraction with PBs and fruits increased ant recruitment to *U. baccifera*. Fruits probably play an essential role increasing the diversity of ants associated with *U. baccifera*, especially because at least two ant species (*Atta sexdens* and *Acromyrmex* sp.) climb the plant just to gather fruits. Although previous work (Machado & Freitas 2001) suggested that fruits may attract ants, we experimentally demonstrated that fruits per se are effective ant attractants, characterizing a dual ant attractant system for *U. baccifera* with two distinct food rewards to ants, fruits and PBs.

Fruits and PBs seemed to have a significant nutritional value for their ant consumers, because PBs often have relatively large amounts of lipids, carbohydrates and proteins (O'Dowd 1982, Heil *et al.* 1997, 1998) and fruits of *U. baccifera* are rich in carbohydrates and proteins (Dutra *et al.,* unpublished data). Since ants also consume herbivores present on plant foliage, we can infer that PBs and fruits do not meet all specific nutritional requirements of ants visiting *U. baccifera*.

Our results from ant censuses indicated that ants were present on shrubs of *U. baccifera* throughout the year. Some authors suggest that it is difficult to observe PB collection because ants promptly remove them (O'Dowd 1979, Schupp & Feener 1991, Marquis & Braker 1994). However our continual observations in the field allowed us to see PB collection several times, especially after the leaf fall period when individuals of *U. baccifera* produced new leaves.

The absence of ants during the plant leaf fall period may be explained by two non-exclusive factors. First, it is possible that under natural conditions plants stop or diminish PB production during this period. Second, the leaf abscission per se reduces the potential ant-foraging area of a plant and consequently the number of PBs available for ants. Some studies demonstrated that ant-plants could increase PBs production when ants are present (Risch & Rickson 1981, Heil *et al.* 1997) thus it would be interesting to investigate if PB production by *U. baccifera* fluctuates with ant activity and vice-versa.

Seasonal variation in ant activity on *U. baccifera* is also linked to temperature oscillations. Ant response to temperature has been already registered for other ant-plant systems (Lawton & Heads 1984, Oliveira *et al.* 1995, Oliveira *et al.* 1999, Cogni *et al.* 2000). We believe that temperature could determine ant recruitment to *U. baccifera,* however, it is necessary diligence on the interpretation of this data because other factors, biotic or abiotic, apart from temperature may also affect ant visitation.

Machado & Freitas (2001) showed that fruiting individuals of *U. baccifera* have high ant visitation rates, which decrease residence time of live termites, and reduce *S. blomfildia* larval survival. However, the authors were unable to identify and isolate the effects of PBs and fruits. Our data demonstrate that ant visitation to PBs reduced the survival of *S. blomfildia* caterpillars. Moreover, ant-exclusion experiments demonstrate that ants benefit the plant reducing herbivore infestation levels. These results confirm the deterring capacities of PB-attracted ants on non-symbiotic systems (Fiala *et al.* 1994).

Although, all lepidopteran species using *U. baccifera* as a host plant apparently larval strategy or refuge in order to escape from predators (*e. g.* Frass chains, leaf shelters), it seems that ants were able to overcome the protection offered by those refuges and prey upon caterpillars. The reduced herbivore infestation levels on shrubs without ants could be explained by four non-exclusive factors: 1) ant aggressive behavior towards ovipositing females (Janzen 1967, Inouye & Taylor 1979, Schemske 1980); 2) by adult lepidopterans host plant choice (Freitas & Oliveira 1996, Freitas *et al.* 1999); 3) egg removal by ants (Letourneau 1983, Barton 1986); or 4) larval predation by ants on highly-visited plants (Freitas & Oliveira 1992, 1996).

Generally we recorded just a small number of *Urbanus esmeraldus* larvae on shrubs of *U. baccifera.* However, in 2004 we registered a fifteen-fold increment on the mean of *U. esmeraldus* infestation levels in relation to 2003. Taking into account all herbivores, we also observed that they were more abundant in 2004. Even with this increase on herbivore abundance ants-visited plants had fewer herbivores compared to ant-excluded ones, suggesting that ant presence may provide a consistent defense system to herbivores. However, our results show that the outcomes of the interaction do not depend only on the reduction of infestation by herbivores. Several authors showed that herbivory can induce leaf abscission (Edwards & Wratten 1983, Pritchard & James 1984, Núnez-Farfán & Dirzo 1989, Stiling & Simberlof 1989). Fonseca (1994) experimentally demonstrated for a myrmecophytic plant that ant-exclusion reduces both herbivore infestation levels and leaf longevity. Similarly our results indicated that higher herbivores infestation levels registered for ant-excluded plants in 2003 induced faster leaf abscission rates. However, we did not detect the same pattern in 2004, and experimental plant groups exhibited similar leaf abscission rates trough time in this year. Variable outcomes in ant plant interactions have commonly been reported for various ant-plant systems (Becerra 1989, Del-Claro & Oliveira 2000, Mody & Linsenmair 2004, see also Bronstein 1998 and references therein).

Temporal variability in the outcomes of ant-herbivore-plant interactions can be caused by fluctuations in herbivores abundance (Bentley 1977, Barton 1986, de la Fuente & Marquis 1999). Protective effects of ants may also be counterbalanced by differences in the susceptibility of distinct herbivores groups to ant predation (Heads & Lawton 1985, Ito & Higashi 1991) and it is possible that foliage-dwelling ants affect herbivores but not necessarily herbivory (Mody & Linsenmair 2004). Changes in herbivore abundance possibly have an effect on the outcomes of the interaction. It is possible high herbivore infestation levels in 2004 in both experimental groups explain the absence of benefits related to leaf abscission in that year. We suggest that ant visitation is translated into slower leaf abscission rates only at intermediate levels of herbivore incidence, as in 2003. On the other hand, when plants face a sharp increase in the herbivore infestation levels, ant visitation is not sufficient to overcome the effects of herbivores. This is also supported by the fact that plants in both experimental groups suffered higher leaf abscission rates in 2004 compared with 2003. Some authors suggest that if herbivore pressure is low, ants are not effective in protecting the plants (Bentley 1977, Barton 1986, de la Fuente & Marquis 1999). Our data reported high herbivore infestation in both years, but especially in 2004. Therefore, we hypothesize that ant derived benefits are probably conditioned by an intermediary herbivore infestation level. Concluding, intense herbivore infestation on *U. baccifera* may reduce the chances of ant-derived benefits. Thus, our study enhances the importance of longer-term studies to understand herbivore-ant interactions.

Ant-plant interactions are extremely diverse (Huxley 1991), and mostly mediated by plant-derived food resources (Bronstein 1998). Recently the role of PBs as ant attractants received more attention (Letourneau 1983, Fiala *et al.*1989, 1994, Fiala & Maschwitz 1990, Vasconcelos 1991, Fonseca 1994, Heil *et al.* 1997, 2001). On the other hand, few studies reported ants climbing onto plants to collect fruits (Pizo 1998, Lu & Mesler 1981, Wheelwright 1985), suggesting that this type of interaction is largely overlooked. This study demonstrates that the simultaneous occurrence of nutritious pearl bodies and fleshy fruits in *U baccifera* induces ant visitation to the plant, and results in both lower herbivore infestation levels and reduced herbivore survival.

Most studies on protective ant-plant mutualisms mediated by pearl bodies involve myrmecophytic plant species (Fiala & Maschwitz 1990, Fiala *et. al.* 1994, Heil *et al.* 1997, Heil *et al.* 2001). Our finding is relevant because it involves a non-symbiotic ant-plant association mediated by dual ant attraction through the offer of pearl bodies and fruits. Although a protective role of PB-harvesting ants have already been documented for facultative systems in Asian forests (Fiala *et al.* 1994, Heil *et al.* 2001), our experimental study with *U baccifera* is the first demonstrate a protective role of PB-harvesting ants in a Neotropical facultative ant-plant system, and the first to show that fleshy fruits increase ant visitation and may add to plant protection.

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Tables

Table 1: Differences in ant visitation between *Urera baccifera* and neighboring plants lacking pearl bodies (PBs).

Variable	*Urera baccifera*	Plants lacking PBs	p
Plants occupied by ants (n=74 plant pairs)	21 (28.4 %)	5 (6.8%)	$p < 0.001$*
Ants per plant (mean ±SE)	0.50 (±0.12)	0.07 (±0.03)	$p < 0.05$**

* Chi-square; ** Wilcoxon

Table 2. Repeated-measure ANOVAs on herbivore infestation levels and leaf survival in *U. baccifera* for the ant exclusion experiment of 2003 and 2004. Analyses were performed on log (x+1) transformed data.

Year Source	SS	DF	MS	F	P
2003					
a. Herbivore infestation					
Ant treatment	0.41	1	0.41	4.34	0.0424
Error	4.87	51	0.10		
Time	0.69	6	0.11	2.65	0.0159
Treatment *X* time	0.17	6	0.03	0.66	0.6842
Error	13.24	306	0.04		
b. Leaf survival					
Ant treatment	496.96	1	496.96	0.40	0.5306
Error	63562.09	51	1246.32		
Time	39223.84	6	6537.31	49.52	<0.0001
Treatment *X* time	2254.26	6	375.71	2.85	0.0103
Error	40392.84	306	132		

2004

a. Herbivore infestation

Ant treatment	1.07	1	1.07	5.54	0.0210
Error	16.19	84	0.19		
Time	0.88	6	0.15	5.50	<0.0001
Treatment *X* time	0.09	6	0.02	0.58	0.0103
Error	13.46	504	0.03		

b. Leaf survival

Ant treatment	312.69	1	312.69	0.17	0.6837
Error	98730.67	53	1862.84		
Time	66173.36	6	11028.89	166.82	<0.0001
Treatment *X* time	5.17.82	6	86.30	1.31	0.2542
Error	21024.06	318	66.11		

Table 3: Infestation levels by three lepidopteran herbivores on control (ants present) and treatment (ants excluded) shrubs of *Urera baccifera* in two years.

Year **Herbivore species**	**No. of control plants** (ants present) Mean (±SE)	**No. of treatment plants** (ants excluded) Mean (±SE)
2003	**29**	**24**
S. blomfildia	0.46 (±0.11)	0.81 (±0.14)
U. esmeraldus	0.03 (±0.01)	0.02 (±0.01)
P. silicalis	0.09 (±0.03)	0.02 (±0.01)
All herbivores	0.49 (±0.11)	0.83 (±0.14)
2004	**30**	**26**
S. blomfildia	0.39 (±0.06)	0.55 (±0.09)
U. esmeraldus	0.30 (±0.06)	0.40 (±0.06)
P. silicalis	0.04 (±0.02)	0.02 (±0.01)
All herbivores	0.73 (±0.10)	0.97 (±0.11)

Figure legends

Figure 1: Interaction between ants and *Urera baccifera*: (A) *Camponotus crassus* feeding on pearl bodies which are scattered over the leaf surface and stem; (B) Scanning electron microscopy of a pearl body; (C) *Pachycondyla villosa* foraging on the infrutescence; (D) *Acromyrmex* sp. acting as primary disperser by removing a fruit directly from the plant.

Figure 2: (a) Ant visitation throughout the year on shrubs of *Urera baccifera*. The censuses were carried out every 15-20 days in the Santa Genebra County Reserve, Campinas, Brazil. Data are means and ±SE (N = 17 plants). (b) Relationship between temperature and ant activity at *U. baccifera* (Linear Regression). (c) Percentage of ants in different vegetative and reproductive plant structures: stem, leaves, flowers and fruits (censuses without ants were excluded).

Figure 3: The role of *Urera baccifera*'s fruits as ant attractants to shrubs of *Piper amalago*. Treatment plants (N = 37) received 10-15 fresh fruits of *U.* baccifera, whereas control plants received only one-leaf of *U. baccifera*. Ant activity on *P. amalago* is affected by fruit presence (Repeated measures ANOVA; $F_{1,72} = 14.70$; $p < 0.0001$). Data are means and ±SE.

Figure 4: Infestation levels by *Smyrna blomfildia*, *Urbanus esmeraldus*, and *Pleuroptya silicalis* lepidopteran larvae on experimental plant groups of *Urera*

baccifera through time in two years, 2003 and 2004. Herbivore activity is affected by ant treatment in both years. Data are means and ±SE. See also Table 2.

Figure 5. Survivorship leaf curves of *Urera baccifera* in experimental plant groups of *U. baccifera* in two years, 2003 and 2004. Ant treatment had a positive impact on leaf survival in 2003, but not in 2004. Data are means and ±SE. See also Table 2.

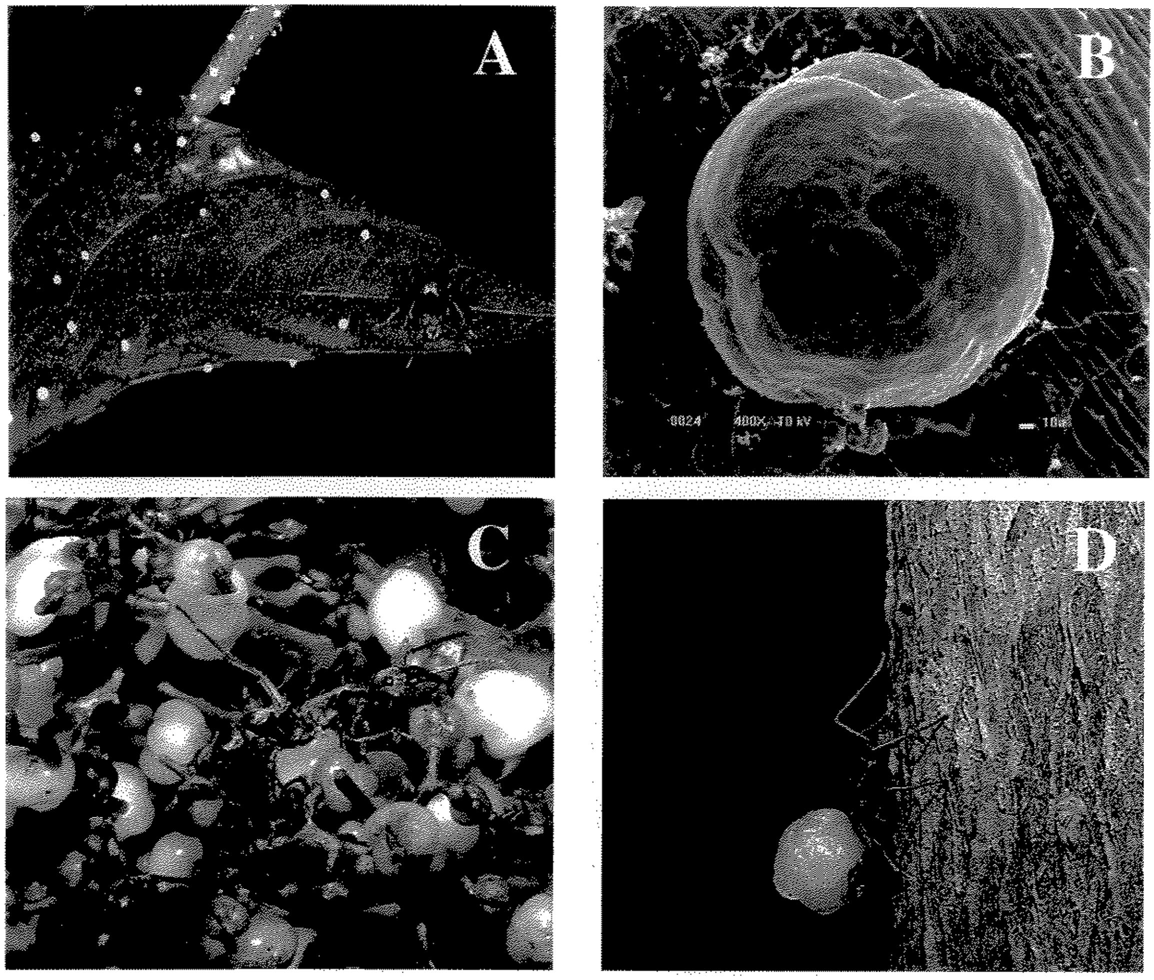


Figure 1

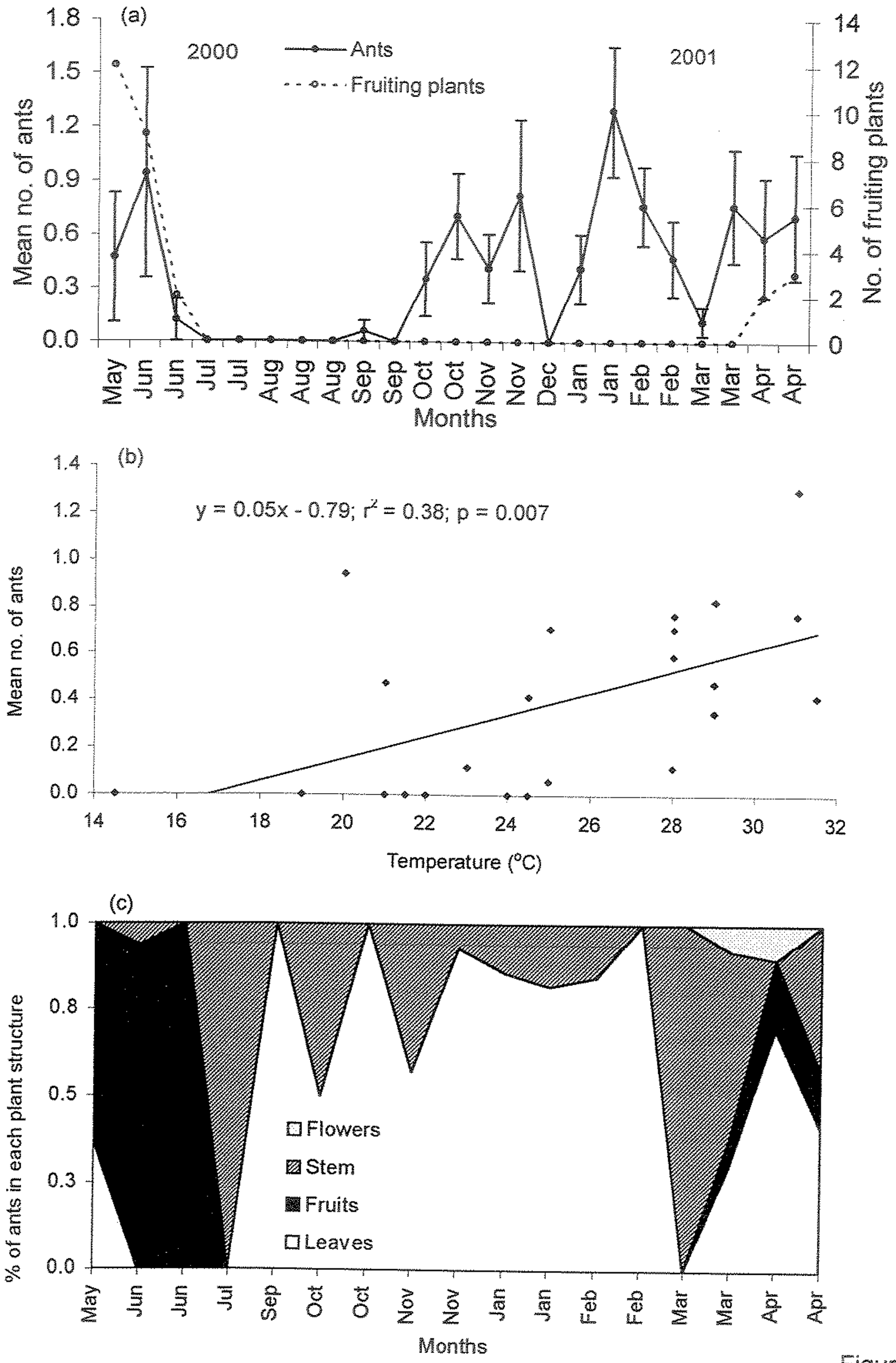

(a)
2000
2001
Ants
Fruiting plants
Mean no. of ants
No. of fruiting plants
0.0
0.3
0.6
0.9
1.2
1.5
1.8
0
2
4
6
8
10
12
14
May
Jun
Jun
Jul
Jul
Aug
Aug
Aug
Sep
Sep
Oct
Oct
Nov
Nov
Dec
Jan
Jan
Feb
Feb
Mar
Mar
Apr
Apr
Months
(b)
y = 0.05x - 0.79; r2 = 0.38; p = 0.007
Mean no. of ants
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
14
16
18
20
22
24
26
28
30
32
Temperature (°C)
(c)
% of ants in each plant structure
0.0
0.3
0.5
0.8
1.0
Flowers
Stem
Fruits
Leaves
May
Jun
Jun
Jul
Sep
Oct
Oct
Nov
Nov
Jan
Jan
Feb
Feb
Mar
Mar
Apr
Apr
Months

Figure 2

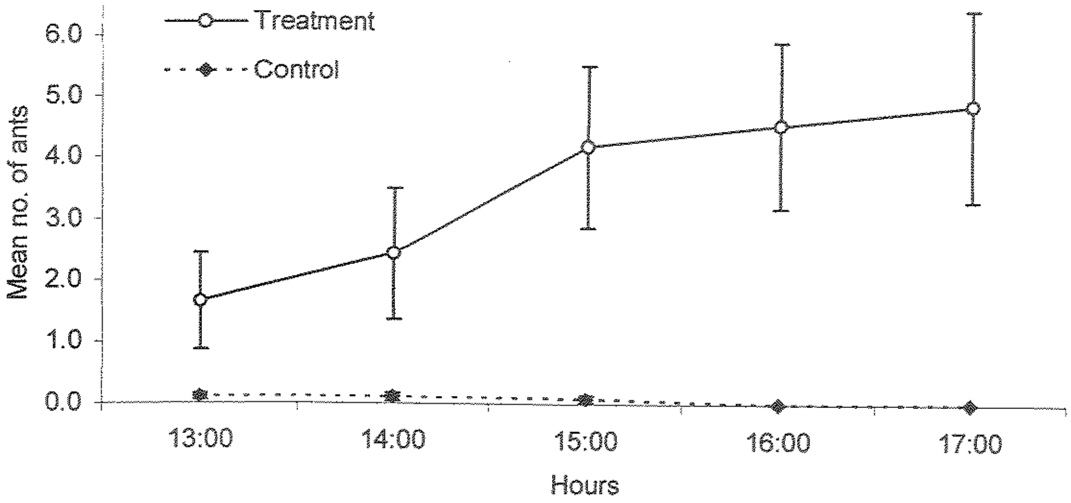
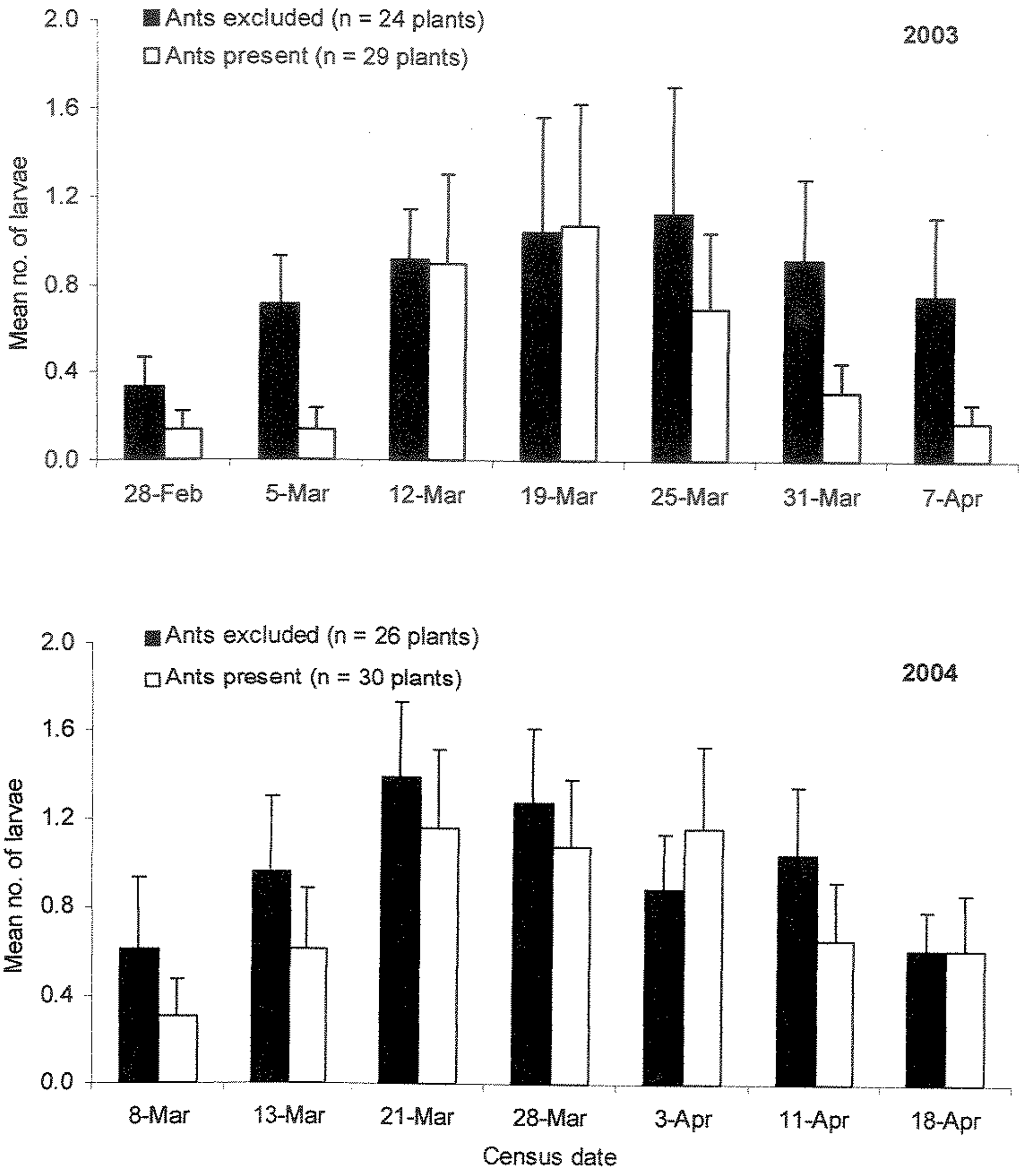

Treatment
Control
Mean no. of ants
6.0
5.0
4.0
3.0
2.0
1.0
0.0
13:00
14:00
15:00
16:00
17:00
Hours

Figure 3



■ Ants excluded (n = 24 plants)
□ Ants present (n = 29 plants)
2003
Mean no. of larvae
2.0
1.6
1.2
0.8
0.4
0.0
28-Feb
5-Mar
12-Mar
19-Mar
25-Mar
31-Mar
7-Apr
■ Ants excluded (n = 26 plants)
□ Ants present (n = 30 plants)
2004
Mean no. of larvae
2.0
1.6
1.2
0.8
0.4
0.0
8-Mar
13-Mar
21-Mar
28-Mar
3-Apr
11-Apr
18-Apr
Census date

Figure 4

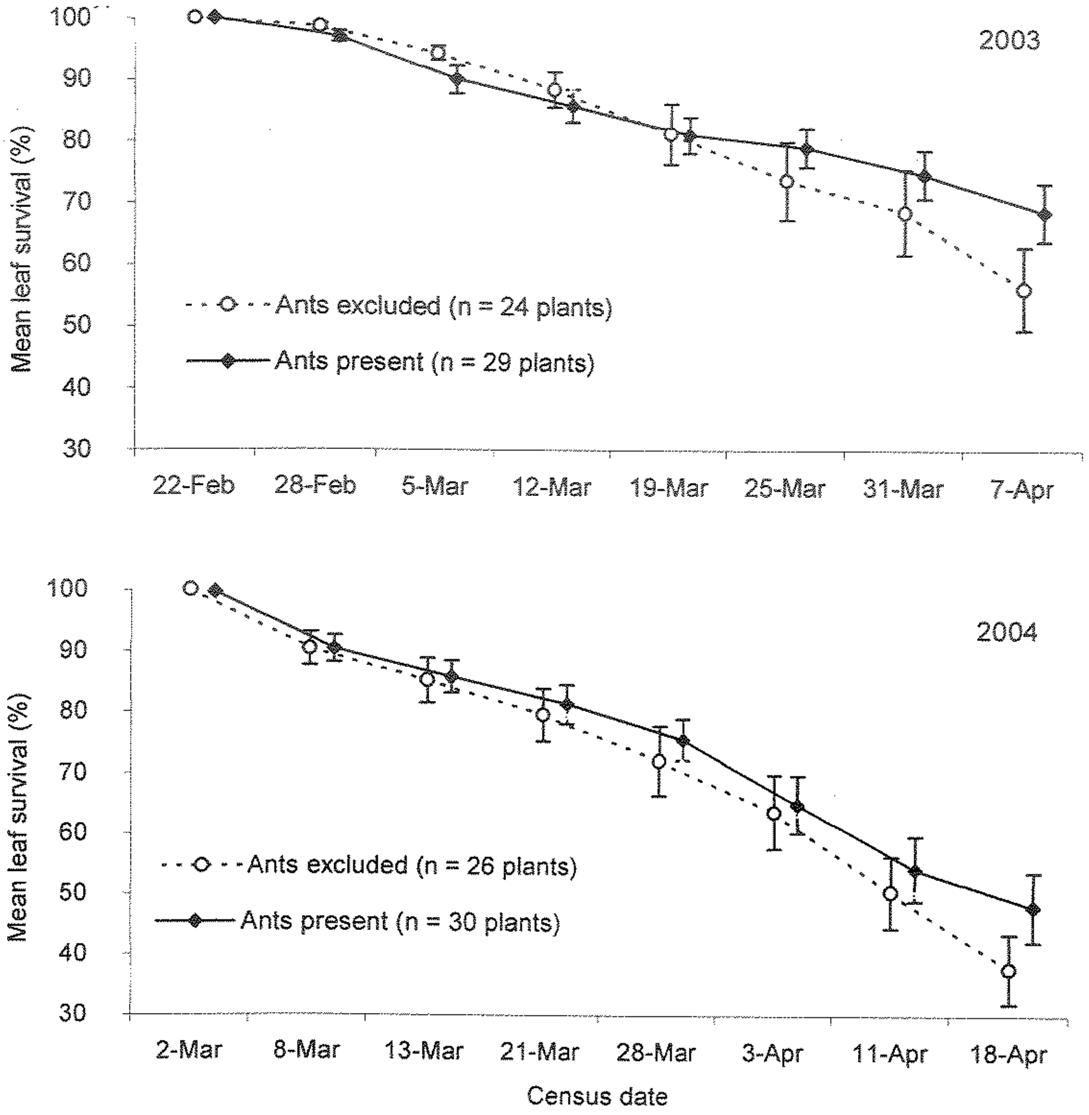

2003
Mean leaf survival (%)
100
90
80
70
60
50
40
30
- - ○ - - Ants excluded (n = 24 plants)
—◆— Ants present (n = 29 plants)
22-Feb
28-Feb
5-Mar
12-Mar
19-Mar
25-Mar
31-Mar
7-Apr
2004
Mean leaf survival (%)
100
90
80
70
60
50
40
30
- - ○ - - Ants excluded (n = 26 plants)
—◆— Ants present (n = 30 plants)
2-Mar
8-Mar
13-Mar
21-Mar
28-Mar
3-Apr
11-Apr
18-Apr
Census date

Figure 5

Conclusão Geral

Os dados obtidos mostram que a associação entre *Urera baccifera* e formigas é mediada por duas recompensas alimentares de natureza distintas, corpos perolados e frutos. A presença de corpos perolados em *U. baccifera* aumenta a densidade de formigas na planta em relação a plantas vizinhas que não possuem atrativos para formigas. Além disso, os frutos da planta também possuem um papel na atração de formigas. Os frutos de *U. baccifera* promovem o aumento da densidade e diversidade de formigas que visitam a planta. Estas duas recompensas atraem toda uma comunidade formigas à planta, de modo que durante o ano todo, exceto durante o período de deciduidade da planta, é possível observar formigas forrageando nas partes áreas de *U. baccifera*.

O forrageamento das formigas na folhagem confere um benefício à planta na medida em que reduz a sobrevivência e incidência de 3 espécies herbívoros que utilizam *U. baccifera* como planta hospedeira. O interessante é que há uma variação temporal nos resultados da interação entre *U. baccifera* e formigas. Observamos que nem sempre a redução dos níveis de infestação por herbívoros é traduzida em uma diminuição das taxas de abscisão foliar. Aparentemente as formigas não conseguem promover uma proteção efetiva da planta quando o nível de infestação por herbívoros é muito alto. Assim sugerimos que o caráter mutualístico da interação entre formigas e *U. baccifera* pode ser condicionado pela abundância de herbívoros.