Do ant visitors to extrafloral nectaries of plants repel pollinators and cause an indirect cost of mutualism?

Mariana A. Assunção, Helena Maura Torezan-Silingardi *, Kleber Del-Claro

Universidade Federal de Uberlândia, Instituto de Biologia, Laboratório de Ecologia Comportamental e de Interações (LECI), CP 593, CEP 38400-902 Uberlândia, MG, Brazil

**Article Info**

**Abstract**

Plants and ants have widespread relationships that are commonly mediated by the offer of extrafloral nectar (EFN) to ants that protect plants against herbivores. However, these ant–plant interactions are highly facultative and vary in time and space, mainly depending on the characteristics of the ant species, such as density and aggressiveness. In general, the outcomes of these relationships are positive, but in some cases, the presence of ants is neutral or negative to plants. Some studies suggest that aggressive attacks or merely the presence of ants might reduce the visitation rate of insect pollinators, such as bees, to flowers. We used experimental manipulation in natural conditions to test the hypothesis that ants on flowers of EFN-bearing plants might be recognized as a danger by pollinators (bees) and reduce the plant fitness (fruit-set). Our results show that the avoidance that ant bodyguard species feeding on EFNs of the Malpighiaceae *Heteropterys pteropetala* cause in pollinators, is not enough to decrease plant fruit-set. However, ants were indeed identified as a danger to pollinators as hypothesized and as suggested for other plant–pollinator relationships: flowers with plastic ants placed on the petals produced significantly fewer fruits than other treatments (using instead a plastic circle) or the control (natural condition). Indirect costs of facultative mutualisms are the focus of few studies and have been performed only rarely in the Neotropics; our results show that mutualism must be considered in multitriflor interactions studies for a better understanding of the functioning of the system.

© 2014 Elsevier GmbH. All rights reserved.

**Introduction**

Ants are ubiquitous terrestrial organisms, particularly abundant in shrubs and trees of tropical forests and savannas (Rico-Gray and Oliveira, 2007; Del-Claro and Torezan-Silingardi, 2012). Many ant species use plant surfaces as a foraging substrate to search for both live and dead animal prey, as well as for different types of plant-derived food products (Carroll and Janzen, 1973). Ant activity on foliage can be promoted by the presence of predictable and immediately renewable food sources, such as honeydew from phloem-feeding hemipterans and secretions from lepidopteran larvae (Del-Claro, 2004; Zhang et al., 2012). However, the most common resource offered by plants to ants is extrafloral nectar (EFN), a liquid substance that is rich in carbohydrates, with low concentrations of amino acids, lipids, phenols, alkaloids and volatile organic compounds (González-Teuber and Heil, 2009). Ants feeding on EFNs increase individual survivorship, colony growth rate and reproduction (Byk and Del-Claro, 2011). The main benefit that ants provide to plants is effective protection against natural enemies (Rico-Gray and Oliveira, 2007 and references therein), thereby increasing plant fitness (e.g. Nascimento and Del-Claro, 2010).

Ant–plant interactions are highly facultative and vary in time and space (Bronstein et al., 2006), mainly depending on the characteristics of the ant species, such as density (Rashbrook et al., 1992) and aggressiveness (Byk and Del-Claro, 2010). Herbivore defensive strategies (Floren et al., 2002; Alves-Silva et al., 2013a), and peculiar features of the plant species involved might also affect the final outcomes of these interactions (Blüthgen et al., 2000; Körnhöfer and Del-Claro, 2006; Aguirre et al., 2013). In some cases ants may have a negative impact on pollination by insects, such as bees. Ants were shown to reduce the visitation rate of pollinators to flowers by aggressive attacks or merely by their presence (Altshuler, 1999; Junker et al., 2007). In the cactus with extralfloral nectaries *Ferocactus wislizenii*, flowers were visited by pollinators less frequently and for less time per visit if the plants were exclusively tended by the ant *Solenopsis xyloni* (Ness, 2006). Cacti visited by three different ant species produced fruits with a significantly higher total seed...
mass. Thus, indirect costs can arise from conflicts between distinct mutualistic guilds that simultaneously use the same resource.

The Brazilian tropical Savanna (Cerrado) is the second-largest biome in Brazil. It is dominated by small trees and shrubs, where plants with EFNs are very common and can comprise up to 25% of the woody species (in some sites between 30 and 40% of individual trees possess EFN glands; see Rico-Gray and Oliveira, 2007). Such plants are commonly visited by ants, but no studies have been performed in this area to explore the indirect costs of ant tending, such as a reduction in pollinator activity that might result in a decrease in fruit-set in EFNs-bearing plants (Del-Claro and Torezan-Silingardi, 2009). By performing experimental manipulations in natural conditions, we tested the hypothesis that ants on flowers of EFN-bearing plants in the Cerrado are identified as a danger by pollinators (bees), causing them to avoid visiting these flowers and, leading to a reduction in fruit-set. Consequently, we intended to show that the indirect costs of facultative mutualisms must also be considered in multitrophic interactions studies involving ants and plants.

**Material and methods**

**Study site**

Field work was carried out from January to March of 2011 and 2012, in the Cerrado area sensu stricto inside the ecological reserve (628 ha) of the Clube de Caça e Pesca Itororó, Uberlândia (CCPIU), State of Minas Gerais, southeast Brazil (18°59′ S, 48°18′ W). The study site is surrounded by farms that have patches of savanna connected to the CCPIU. The Cerrado vegetation (Oliveira-Filho and Ratter, 2002) consists of a scrub of shrubs and trees with additional herbaceous plants. The climate of the region is the Aw type of Köppen system, consisting of well-defined rainy and dry seasons. A wet/warm season occurs from October to March (rainfall 270 ± 50 mm; temperature 23 ± 5 °C), and a dry/cold season from April to September (22 ± 20 mm rainfall; 19 ± 3 °C temperature; daily data from the climatological station of the Universidade Federal de Uberlândia; additional details in Bächtold et al., 2012).

**Plant species**

The Malpighiaceae species Heteropterys pteropetala A. Juss. is a common shrub (1.5–2.5 m tall) at the study site (Réu and Del-Claro, 2005). The plants have two extrafloral nectaries (EFNs) at the base of each leaf, in the bracts of inflorescences and also at the base of the bud pedicels (Fig. 1a), which attract ants that commonly climb and crawl on buds and flowers.

The inflorescences (racemes) bear dozens to hundreds of bisexual and slightly zygomorphic flowers (approximately 1.0 cm in diameter) with five pink, clawed petals and five sepals with oil glands (elaiophores), totaling 8–10 glands per flower (Réu and Del-Claro, 2005; Torezan-Silingardi, 2011). Heteropterys pteropetala flourishes between January and March and its reproductive system suggests that it is a self-compatible species, but without spontaneous self-pollination, i.e., the plant requires pollinator services. The species is melittophilous, and is pollinated exclusively by bees (H.M. Torezan-Silingardi, in preparation).

**Stigmatic receptivity and pollen availability**

To know whether flowers were visited by pollinators and/or ants at the time that they were ready for pollination, the timing of floral anthesis, stigmatic receptivity and pollen availability was investigated at the end of January 2011. We chose one large flowering individual of *H. pteropetala* and observed its floral structures for 12 h (6:30–18:30) following the methodology proposed by Faegri and Van der Pijl (1976). The inflorescences were protected from visitors with voile bags the day before the experiments. Recently opened flowers were tagged and numbered. The order of numbering followed the end of all anthesis events. We considered open flowers to be those with turned-back petals. After the end of flower opening we waited for 5 min before starting tests for stigmatic receptivity and pollen availability. To test stigmatic receptivity, the stigmatic surface was carefully placed in a glass tube filled with hydrogen peroxide (3%). If bubbles were produced inside the tube over the stigma, it was considered to be receptive (sensu Faegri and Van der Pijl, 1976). To verify pollen availability, we positioned the flower over a black surface and softly brushed the anthers with a brush. When pollen was available to pollinators, it detached from the stamens and spread onto the black surface. When no pollen was recorded, tests were repeated during the following time every 30 min.

**Impact of ants on pollinator visitation**

In both study years in the first week of January, we selected 60 individuals of *H. pteropetala* with similar phenological features: the same height (approximately 1 m tall), possessing young leaves, active EFNs and reproductive stems (3–4) at an early developmental stage. Individuals chosen were separated from each other at least 5 m. Each plant was tagged and numbered from 1 to 60. The plants were sorted and divided into four groups of 15 individuals, which received the following treatments: Ant access; ants excluded; placement of plastic ants; placement of plastic circles. During the initial flowering periods, we selected five flowers on each plant to observe and perform the manipulations, totaling 75 flowers per group and year, i.e., 150 flowers per group in total. In the “ant access” group, the plants received an adhesive paper covered with the ant barrier resin tanglefoot (The Tanglefoot Company®, Rapids, MI, USA) at the base of the stems, about 15 cm from the soil level and encircling half of the plants’ circumference. Thus, despite the presence of the resin, ants could freely climb onto these individuals. The selected flowers did not receive any other treatment. In the “ant excluded” group, we performed the same procedure, with the exception that the resin completely encircled the whole circumference of stems and the ants present on the plants were manually removed. Furthermore, all structures were removed that could be used by ants as a bridge or climbing device in order to get access to the plants. The flowers also did not receive any manipulation. The other two groups received the same manipulation as performed for the “ant excluded” group. However, in the “plastic ants” group, one small black plastic ant (1 cm long) was glued to the petals of the flowers. In the “plastic circle” group, instead of plastic ants, one 0.5 cm-diameter black plastic circle was glued to the petals of the flowers.

The procedure to choose flowers to be observed and/or manipulated in each group was always the same. At dawn (06:30–07:00), in previously bagged inflorescences (as explained in the “stigmatic receptivity and pollen availability” section), a recently opened flower (with yellow anthers full of pollen and petals turned back) was tagged with a small piece of green cotton line. Only five flowers that could be easily and directly observed without any chance of a misidentification in relation to floral visitors were tagged per day and were included in the experiment. The observations began at 07:00 and finished at 18:00, when all observed or manipulated flowers were protected again with the same voile bag to avoid subsequent visitation or damage. The flowers were inspected weekly for 30 days when the bags were removed and the fruits that were produced were quantified. If possible, the visiting ants in the “ant access” group were observed and the visitor species were identified. We also recorded the plant part that ants visited, the time of day and duration that each ant spent visiting EFNs in the bracts.
of inflorescences and flowers. In all groups, the exact time that bees visited the flowers was also recorded. A contingency table of the number of bees visiting the treatments (“plastic ants” versus “plastic circle”) was performed and the data were compared by Chi-squared tests with Yates’ correction (Sokal and Rohlf, 1995). Fruit production among treatments was compared by performing a 4 × 2 contingency table and applying a G-test of independence.

Results

Heteropterys pteropetala flower opening started at about 06:30 and the whole process was completed by 07:05 (7:05 ± 25 min; x ± SD; N = 50 flowers of 10 different plants observed), when the petals were fully expanded. The latest flowers (N = 3) had completed their anthesis by 08:28. The same set of flowers showed that anthers were open and pollen was available from between 07:40 and 08:53 onwards (Fig. 2), but no positive stigmatic receptivity was observed, except for two cases in which Centridini bees visited the flowers as soon as the voile bags were removed (08:49 and 09:33). The bee activity on flowers began just after peak floral anthesis (07:00) with a peak of visitations between 09:00 and 11:00 (Fig. 2), when the stigma became receptive (Fig. 1a). Ants visited EFNs in the bracts of inflorescences (Fig. 1b), between 06:00 and 07:00, with a peak at 10:00–11:00 (Fig. 2).

We observed 10 ant species from five sub-families. The most common ants visiting EFNs in inflorescences and/or inspecting flowers were Ectatomma tuberculatum, Camponotus crassus and Cephalotes pusillus (Table 1). These same ant species were also the most frequent species observed on the tagged plants.

Five bee species, all Apidae, visited flowers of H. pteropetala during the study. The most numerous visits were by Centris (Ptilotopus) scopipes, Epicharis (Epicharana) flav,a, Lophopedia sp., Ceratalictus sp. and Epicharis (Epicharoides) sp.

The field experiment using plastic ants versus plastic circles as a control, showed that bees were able to distinguish ant shape and avoid these flowers in a significant manner (χ² test with
Table 1
List of ants that visited Heteropterys pteropetala (Malpighiaceae) shrubs of the "Ant access" group in the Cerrado reserve of CCPIU, Uberlândia, MG, Brazil. The symbols in "Plant structures visited" column mean: S – stems; L – leaves; F – flowers; N – extrafloral nectaries; O – oil glands.

<table>
<thead>
<tr>
<th>Subfamily</th>
<th>Ant species</th>
<th>Plants visited* % (N)</th>
<th>Plant structures visited</th>
<th>Time spent visiting each extrafloral nectary X ± 1DP (N)</th>
<th>Time spent visiting each flower X ± 1DP (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ectatommineae</td>
<td>Ectatomma tuberculatum Oliver 1792</td>
<td>73.3 (11)</td>
<td>SLFNO</td>
<td>13 ± 9s (37)</td>
<td>23 ± 11s (30)</td>
</tr>
<tr>
<td></td>
<td>Ectatomma sp</td>
<td>13.3 (2)</td>
<td>SLFN</td>
<td>11 ± 7s (7)</td>
<td>17 ± 8s (13)</td>
</tr>
<tr>
<td></td>
<td>Gnaptogenys semiferox Brown 1958</td>
<td>6.6 (1)</td>
<td>SLFN</td>
<td>8 ± 7s (6)</td>
<td>13 ± 7s (7)</td>
</tr>
<tr>
<td>Ponerinae</td>
<td>Pachycondyla villosa Fabricius 1804</td>
<td>20(3)</td>
<td>SLFNO</td>
<td>10 ± 8s (8)</td>
<td>15 ± 9s (6)</td>
</tr>
<tr>
<td>Formicinae</td>
<td>Camponotus crassus Mayr 1862</td>
<td>53.3 (8)</td>
<td>SLFN</td>
<td>1 ± 1s (23)</td>
<td>2 ± 1s (17)</td>
</tr>
<tr>
<td></td>
<td>Camponotus islandus Fr Smith 1858</td>
<td>20(3)</td>
<td>SLFN</td>
<td>1 ± 1s (11)</td>
<td>2 ± 2s (5)</td>
</tr>
<tr>
<td>Myrmicineae</td>
<td>Cephalotes pusillus Klug 1824</td>
<td>46.6 (7)</td>
<td>SLFNO</td>
<td>3 ± 2s (20)</td>
<td>4 ± 2s (12)</td>
</tr>
<tr>
<td></td>
<td>Cephalotes sp</td>
<td>13.3 (2)</td>
<td>SLN</td>
<td>1 ± 2s (7)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Crematogaster sp</td>
<td>6.6 (1)</td>
<td>SLN</td>
<td>3 ± 2s (11)</td>
<td>–</td>
</tr>
<tr>
<td>Dolichoderineae</td>
<td>Azteca sp1</td>
<td>6.6 (1)</td>
<td>SLFN</td>
<td>1 ± 2s (5)</td>
<td>2 ± 2s (5)</td>
</tr>
</tbody>
</table>

* Some shrubs may have been visited by two or more ant species simultaneously.

Yates’ correction, χ² = 31.23; df = 1; p < 0.0001; Fig. 3). The results also showed that flowers with a plastic ant on the petals produced significantly fewer fruits than other treatments or the control (G-test of independence (contingence table 4 x 2); G = 134.7388; p < 0.0001, df = 3; Fig. 4). Field observations also revealed that bees avoided inflorescences only when Ectatommineae and Ponerinae ants were present. These ants remained for much longer on inflorescences and fed on EFNs more than other species (Table 1).

Discussion

Multitrophic interaction studies must consider the indirect costs of mutualisms to provide a more realistic view of these systems as a whole. This study, involving plant pollinators and plant defensive bodyguards (ants), corroborates this view. Our main hypothesis that ants on the flowers of EFN-bearing plants in the Cerrado are identified as a danger by pollinators (bees) was confirmed. The ant bodyguard species feeding on EFNs of H. pteropetala did not cause a reduction in fruit-set production. The avoidance response that these ant species cause in pollinators is not enough to affect negatively the plant’s reproduction, as it was observed in other ecosystems (e.g. Bronstein, 1991; Ness, 2006). However, ants might be perceived as a danger for pollinators of H. pteropetala, as we

Fig. 3. Bees avoid visiting flowers with an ant-shaped plastic figure on the petals (χ² test with Yates’ correction).

Fig. 4. Results of field experiments to test fruit production in flowers (N = 150 per group) exposed to four distinct treatments. Flowers with a fake (plastic) ant glued onto one petal produced significantly fewer fruits than the others (G-test of independence). Numbers above the bars indicate the number of flowers that did or did not develop into fruits.
The visitation of flowers by bees differs among plants that are tended by different ant bodyguards (Ness, 2006). Several cues can aid bees in their decision to visit a flower that is occupied or was recently visited by an ant, most commonly scent marks (Junker et al., 2007). However, a recent study showed that bumblebees are not inherently repelled by ants; they can learn with experience and ant-deposited scent marks can be ignored (e.g. Ballantyne and Willmer, 2012). Therefore, the question is, what can be a clear signal of ant presence that might represent danger to a bee. Sendoya et al. (2009) experimentally showed that butterflies of the Brazilian Cerrado recognize and avoid a specific ant shape from that of others. Here, we show for the first time, that also bees use ant shapes as a cue to identify and avoid undesirable flowers. The plastic ants we used resemble the Ectatomminae ants that occur in the study site in shape and size and, therefore, might represent a real risk of getting attacked for pollinators in the Cerrado (see Fig. 1c). Ectatomma and Pachycondyla are aggressive and predatory ants that are abundant and common in H. pteropetala shrubs in the study site (Réu and Del-Claro, 2005). These ants remain for several seconds on flowers and visit the plants at the same time as pollinator bees (Table 1) and can disturb some pollination events (pers. observ.). This might result in a cost for the plants that support these mutualisms, as is suggested in other systems (Sutte, 2003; Altschuler, 1999; Junker et al., 2007). Considering that the peaks of visitation of ants and bees have an overlap that coincides with the period of H. pteropetala pollen availability and stigmatic receptivity and, that the plant is not self-pollinated and therefore requires the services of pollinator bees, there is a potential cost behind the ant–plant mutualism. However, since both ants and bees move from one inflorescence to another in quick sequence, we suggest rather an energetic cost for the bees in the predator-pollinator interaction, whereas costs for the plant in terms of a reduction in fruit-set production will only occur if the ants remain on flowers for a longer period of time. Indeed, there is no reason for this to occur, because these flowers have no nectar to offer to ants.

Predators that deter floral visitors can have positive or negative effects on plant fitness, depending on whether they deter all or a subset only of visitors (Del-Claro et al., 2013). For example, if they deter pollen thieves and not the effective pollinators, they can have a positive impact (Torezan-Silingardi, 2011; González et al., 2013). Field observation suggests that only large bees such as Centris and Epicharis effectively pollinate the flowers of H. pteropetala, whereas small bees are probably pollen thieves. Future studies should investigate whether ants effectively disturb these small visitors more than the larger ones.

Studies in tropical savannas that investigate predator costs and the indirect costs of plant bodyguard mutualists are still relatively few and recent. For example, ants might catch fig pollinators on Ficus pertusa as they arrive at the receptive syconia (Bronstein, 1991), but the wasp Banchylidra lechequana (Vespidae) can reduce the floral herbivory in Banisteriopsis maliformia (Malpighiaceae) without interfering with the visits of plant pollinators (Alves-Silva et al., 2013b). Ants and spiders can have a complementary effect against the herbivores of Qualea multiflora (Vocysceaceae) without affecting plant fruit-set (Nahas et al., 2012). Particularly, studies that not only take the pollinator’s perspective into account, but also explore the consequences of these multitrifit interactions on plant fitness are rare (Sutte, 2003; Ness, 2006). Surprisingly, we did not find any previous studies on this topic in South America. In summary, our results neither support nor refute the hypothesis that the visiting of flowers by ants causes a reduction in plant fitness, because we showed clearly that only the constant presence of ants deters pollinators. Similar to other systems (González et al., 2013), only certain ant species will remain for sufficient time on the same flower preventing by this way its pollination. We strongly encourage more experimental studies (maintaining and removing predators, such as ants from plants) that investigate all aspects of these interactions (predators/pollinators; identity, abundance and behavior; plant resources and fruit–set). This was also discussed by Suttle (2003): “predation on pollination is widespread, as is pollen limitation of fruit and seed production in flowering plants. We may begin to discern the extent to which the former influences the latter by bridging gaps in our thinking on top–down effects and pollination ecology” (I.c., p. 693).

Acknowledgements

We express our thanks to Christoph Grueter and Marcelo O. Gonzaga for English correction and valuable suggestions in the original version of the manuscript, for research grants provided from CNPq to KDC (301248/2009-5; 472046/2011-0), HMTS (476074/2008-8) and MAA (Undergraduate Scientific Initiation 502063/2010-6), and to CCPIU staff for permission to work in the Cerrado reserve.

References


Alves-Silva, E., Bächold, A., Barónio, G.J., Del-Claro, K., 2013a. Influence of Camponotus blandus (Formicidae) and flower buds on the occurrence of Parhasius polibetes (Lepidoptera: Lycanidae) in Banisteriopsis maliformia (Malpighiaceae). Insect Soc. 60, 30–34.


Del-Claro, K., Guillerme-Ferreira, R., Almeda, E.M., Zardini, H., Torezan-Silingardi, H.M., 2013. Ants visiting the post-floral secones of pericarpial nectaries in Polycarpos rigidos (Rubiaceae) provide protection against leaf herbivores but not against seed parasitoids. Sociobiology 60, 217–221.


