Effects of different ant species on the attendance of neighbouring hemipteran colonies and the outcomes for the host plant

Andréa Andrade Vilela & Kleber Del-Claro

To cite this article: Andréa Andrade Vilela & Kleber Del-Claro (2018) Effects of different ant species on the attendance of neighbouring hemipteran colonies and the outcomes for the host plant, Journal of Natural History, 52:7-8, 415-428, DOI: 10.1080/00222933.2018.1432774

To link to this article: https://doi.org/10.1080/00222933.2018.1432774
Effects of different ant species on the attendance of neighbouring hemipteran colonies and the outcomes for the host plant

Andréa Andrade Vilela and Kleber Del-Claro

Laboratório de Ecologia Comportamental e Interações, Instituto de Biologia, Universidade Federal de Uberlândia, Uberlândia, Brasil

ABSTRACT
Ants commonly participate simultaneously in mutualisms with honeydew-producing hemipterans and extrafloral nectar-bearing host plants. These interactions are based on the exchange of resources (honeydew and extrafloral nectar) for ant protection against natural enemies. The benefits may vary according to the species of ant interacting, and the outcomes for the host plant and hemipteran will depend on the results of the ant-plant-hemipteran relationship as a whole. We studied a plant-mealybug association with Camponotus crassus Mayr (Formicinae) and Ectatomma tuberculatum Olivier (Ectatomminae) in a Brazilian tropical savanna. We aimed to elucidate whether the frequency of visitation by different species of ants can be affected by neighbouring colonies of mealybugs, and whether these ant species influence the number of hemipterans in a different way. Furthermore, we intended to find out whether the interaction between ant and hemipteran has any effect on the proportion of fruit production or on the abundance of trophobiont and non-trophobiont herbivores, also influenced by the ant species. Our results show that neighbouring aggregations of mealybugs influenced the frequency of ant visitation and the ants affected the number of nymphs over time, with both positive results only for C. crassus. We also detected an indirect negative effect on proportion of fruit production and an increase in abundance of trophobiont and non-trophobiont herbivores in the presence of C. crassus-mealybug interaction. Thus, we provide further evidence that colonies of hemipterans interacting with ants can be beneficial for these herbivorous insects, but they are quite harmful to the host plant. Nevertheless, the outcomes can be highly conditional in relation to ant species.

ARTICLE HISTORY
Received 19 September 2016
Accepted 16 January 2018
Online 21 February 2018

KEYWORDS
Honeydew; extrafloral nectar; conditional mutualism; Cerrado Biome

Introduction
At the community level, organisms commonly participate in associations with different partners simultaneously, and the outcomes from multiple interactions cannot be predicted by pairwise interactions alone (Savage and Rudgers 2013; Thompson 2013; Stefani et al. 2015). Instead, a group of species act directly or indirectly on each other
to regulate the final outcomes (Grinath et al. 2012; Lange and Del-Claro 2014; Dattilo et al. 2015). In this sense, the relationships among ants, hemipterans and plants bearing extraloral nectar (EFN) provide valuable models for disentangling the complex dynamics of mutualistic interactions exploring the range of outcomes for involved species (Del-Claro and Oliveira 2000; Zhang et al. 2012; Fagundes et al. 2013; Del-Claro et al. 2016).

In ant-hemipteran interactions, ants may be attracted by hemipterans through the release of honeydew, an exudate rich in sugars that also contains amino acids, minerals and secondary compounds, used as a food source by the ants (Völkl et al. 1999; Blüthgen et al. 2004; Woodring et al. 2004; Tena et al. 2013a). In turn, ants protect hemipterans against enemies, resulting in increased survivorship, fecundity and developmental rate (Del-Claro and Oliveira 2000; Moreira and Del-Claro 2005; Fagundes et al. 2013). However, neighbouring hemipteran colonies can reduce ant attendance by reducing the number of ants and, consequently, the benefits for hemipteran colonies. Thus, in the studies of these interactions it is important to consider the presence of other hemipteran colonies nearby, and also the identity, abundance and behaviour of the ant species which can also lead to different outcomes (Bronstein 1994, 1998; Del-Claro and Oliveira 2000; Billick and Tonkel 2003; Stadler and Dixon 2005; Pekas et al. 2011; Tena et al. 2013b).

In ant-plant interactions, some plant species produce extraloral nectar secreted by extraloral nectaries (EFNs) and promote a protective interaction with ants in periods of high vulnerability to herbivores (Nahas et al. 2012; Del-Claro et al. 2013). In these cases, ants use the extraloral nectar as a profitable resource and in turn protect the plant against herbivores (Byk and Del-Claro 2011; Vilela et al. 2014). Experimental ant-exclusion studies have shown that the absence of ants can increase leaf-area loss in plants possessing EFNs (Komdörfer and Del-Claro 2006), and can reduce fruit set (Nascimento and Del-Claro 2010), seed production and viability (Moreira and Del-Claro 2005; Zhang et al. 2015). However, data concerning the effectiveness of ant-plant mutualisms are sometimes controversial (Nahas et al. 2012; Del-Claro et al. 2016). In addition, ants interacting with honeydew-producing hemipterans can affect host plants (Moreira and Del-Claro 2005; Oliveira and Del-Claro 2005).

Ant-hemipteran interactions may have a variety of indirect ecological effects on plants (Styrsky and Eubanks 2007; Zhang et al. 2012, 2015). The consequences of these interactions represent a trade-off between the indirect benefit by ant preying and reducing the non-honeydew-producing herbivores and the direct costs of herbivory by the ant-tended hemipterans (Lach 2003; Trager et al. 2010). In this sense, the interaction can be beneficial to the host plant from the standpoint of herbivory reduction and increased fitness (e.g. Del-Claro 2004; Moreira and Del-Claro 2005; Zhang et al. 2012, 2015). However, some studies were unable to detect these positive effects (e.g Buckley 1983; Rico-Gray and Thien 1989; Rashbrook et al. 1992; Itino et al. 2001a). Population density of hemipterans, their developmental stage, the abundance of natural enemies and the ability of ant species to dissuade herbivores and/or their natural enemies can determine how beneficial or detrimental the interaction will be for the host plant (Del-Claro and Oliveira 2000; Rico-Gray and Oliveira 2007).

Ant-plant-hemipteran relationships are particularly pervasive in cerrado (the Brazilian Tropical Savanna) due to the high incidence of exudates of insects and plants on foliage (e.g. Del-Claro et al. 2013; Vilela et al. 2014; Fagundes et al. 2015). In this study, we sought to
understand the effect of two ant species, *Ectatomma tuberculatum* (Ectatomminae) and *Camponotus crassus* (Formicinae), in interactions with mealybugs of the family Ortheziidae in a typical EFN-bearing plant of cerrado, *Banisteriopsis campestris* (A. Juss.) Little (Malpighiaceae). Therefore, we intended to: (1) elucidate whether the frequency of visitation by different species of ants can be affected by neighbouring colonies of mealybugs, and whether these ant species influence the number of mealybugs per colony in a different way; and (2) find out whether the interaction between ant and hemipteran has any effect on the proportion of fruit production or on the abundance of trophobiont and non-trophobiont herbivores, also influenced by the ant species. We hypothesised that the presence of neighbouring colonies of mealybugs will affect the attendance of both ant species, but we expected different influences on the number of mealybugs due to species’ distinct biological features. We also hypothesised that the ant-hemipteran interactions influence the proportion of fruit production and insect abundance with differences for each ant species interacting.

**Materials and methods**

**Site and studied groups**

Fieldwork was carried out from November 2013 to April 2014 at the Reserva Ecológica do Clube Caça e Pesca Itororó de Uberlândia (CCPIU; 18.590°S, 48.180°W), Uberlândia, Minas Gerais State, Brazil. We used a 640-ha cerrado site consisting of a dense scrub of shrubs and trees, known as Cerrado sensu stricto (Oliveira-Filho and Ratter 2002). The climate is markedly seasonal, with a dry winter (April to September) and a rainy summer (October to March) (for additional details, see Réu and Del-Claro 2005; Ferreira and Torezan-Silingardi 2013).

The Malpighiaceae species selected for the study, *Banisteriopsis campestris* (A. Juss.) Little, was chosen because it is a very common small shrub in the cerrado with paired EFNs on the leaf base (Gates 1982; Anderson 1990; Torezan-Silingardi 2011). This plant species has close relationships with protective ants and experiences intensive interactions with ant-honeydew-producing hemipterans, especially mealybugs of the family Ortheziidae (Torezan-Silingardi 2007; Vilela et al. 2014).

The Ortheziidae are a morphologically well-defined family of scale insects (Vea and Grimaldi 2012) known for their adornment with thick wax patches or plates that cover most of their body, and a thick waxy ovisac that is attached to the body of the adult female (Miller and Kozár 2002). Females are apterous with little movement in their host plants, while males are winged and rarely seen (Kozár 2004). The life history of these scale insects is not well described but, in general, they show four female and most likely five male instars. They complete their life cycle in 30 days, with offspring being deposited over 24 days and 80–102 nymphs being produced per female (Shivakumar and Lakshmikanth 2001). The taxonomy of Brazilian species in this group is poorly known and several new species are expected.
**Control and treatment groups**

In November 2013, a total of 42 individuals of *B. campestris* of similar phenological state (without leaves), similar size and architecture (0.5–1 m tall with 2–5 stems) and at least 5 m apart from each other were tagged. These plants were randomly divided into four groups: in the ‘control’ group, plants were not manipulated in any way and ants and mealybugs had free access to all plant parts (seven plants with *C. crassus* and mealybugs; seven plants with *E. tuberculatum* and mealybugs). In the second group, called ‘ants’, the mealybugs were removed and only ants had access to the plants (seven plants with *C. crassus*; seven plants with *E. tuberculatum*). In the third group, named ‘mealybugs’, ants were removed, leaving only the mealybugs (n = 7 plants); and in the fourth group, called ‘exclusion’, all ants and mealybugs were excluded from plants (n = 7 plants). Ants were excluded from plants manually, and subsequently we applied an adhesive paper strip with a layer of Tanglefoot® resin on the main plant stem. All plants of groups without mealybugs (‘exclusion’ and ‘ant’) were treated with tobacco diluted in water and sprayed on the plant surface at 2-week intervals to prevent any mealybugs on plants (Renault et al. 2005).

**Effects of neighbouring colonies of mealybugs on frequency of ant visitation**

We performed this experiment in January with only the ‘control’ group. We randomly selected two branches side by side from the same individual plant (n = 7) and defined them as control or treatment, to assess whether the presence of one mealybug aggregation affects the frequency of ant visitation (represented by ants touching using their antennas; Del-Claro and Oliveira 1996) on a neighbouring aggregation. During all experiments the abundance of mealybugs was kept unchanged and equal for all branches (n = 10). The individuals were followed for 9 days (14–22 January 2014) and the experiment was divided into three periods. In a pre-manipulation period (days 1, 2 and 3), we counted the number of ants tending mealybug populations for 10 minutes for both control and treatment groups of each plant. At the end of day 3, we excluded tending ants from group treatment by placing an adhesive paper strip with a layer of Tanglefoot® around the base of the branches. During the manipulation period (days 4, 5 and 6), we counted the frequency of ants tending mealybugs in the control group for 10 minutes. At the end of day 6, we removed the ant barriers from treatment branches and monitored subsequent ant visitation frequency for 10 minutes on days 7, 8 and 9 in both groups (postmanipulation period).

Considering that variations in ant frequency can be promoted by environmental disturbances occurring during the experiment, we observed other plants with mealybugs and *C. crassus* (n = 7) and mealybugs and *E. tuberculatum* (n = 7) to verify the frequency of ant visitation without manipulation, in an attempt to exclude any local environmental variations and validate our experimental results. We also separated two branches that were side by side from the same individual plant and defined them as control or treatment, in the same way. These individuals were followed during the same 9 days to record the frequency of visitation by ants, but they only received an adhesive paper strip without a layer of Tanglefoot®.

A repeated-measures analysis of variance (one-way ANOVA) were performed using the frequency of ant visitation on the group with ant access at all times considering the
premanipulation (days 1, 2 and 3) and manipulation periods (days 4, 5 and 6) to verify whether the frequency of ant attendance on control aggregation increased when prevented from visiting the neighbouring aggregation on the treatment branch. The data were log-transformed.

**Effect of ant exclusion on the number of hemipterans per colony**

After the ninth day of the previous experiment, we used the same plants to verify whether the number of mealybugs per colony was influenced by the presence of ants. The control branches (with ants) were kept unmanipulated while the treatment branches (without ants) received an adhesive paper strip with a layer of Tanglefoot® around their base again, on 23 January 2014. The abundance of mealybugs (nymphs and adults) was monitored every 3 weeks (5 February, 26 February, 19 March and 9 April 2014). We considered their life cycle to be around 30 days and offspring to be deposited over 24 days (sensu Shivakumar and Lakshmikanth 2001). We analysed the effects of ant visitation on the abundance of mealybugs throughout the monitoring period. Initially we performed a paired t-test to verify there were no significant differences in the number of mealybugs between the branches marked as control and treatment for both ant species. Afterward, we performed a Friedman test with the abundance of nymphs and adults of mealybugs throughout the time period for plants with C. crassus and E. tuberculatum. The Friedman test is a nonparametric counterpart of repeated-measures ANOVA for paired groups (Zimmerman and Zumbo 1993; Gonzaga and Leiner 2012).

**Effect of ant-mealybug interaction on proportion of fruit production**

The factorial designs use the combined effects of multispecies interactions to reveal whether the effects of species together differ from those we would expect on the basis of their separate effects (Savage and Rudgers 2013). All groups of B. campestris marked in November were used for the factorial experiment with ‘control’, ‘ants’, ‘mealybugs’ and ‘exclusion’ groups. Each plant was monitored every 2 weeks during its reproductive period (12 December 2013 to 2 April 2014). The plants were always inspected by the same observer, and the numbers of floral buds and fruits produced were counted. The proportion of fruit was expressed as the proportion of fruits formed per buds produced (e.g. Del-Claro et al. 1996; Vilela et al. 2014). The proportion of fruit production was compared using two-way ANOVA with the presence/absence of ants and mealybugs treated as separate factors. A significant interaction would indicate the occurrence of an interactive effect of ants and mealybugs on the proportion of fruit production (see Nahas et al. 2012). The data were arcsine square root transformed, and Levene’s test was also done for equality of variances.

**Effects of ant-plant-mealybug interaction on abundance of trophobiont and non-trophobiont herbivores**

We also quantified the abundance of ants, mealybugs, and herbivores that do not produce honeydew (non-trophobiont). Each plant was monitored every 2 weeks during its reproductive period (12 December 2013 to 2 April 2014) and the number of insects (ants, mealybugs and non-trophobiont herbivores) was counted by the same observer. We compared the abundance of mealybugs between plants with ant-hemipteran association and plants with only mealybugs using t-test for each species of ants (Zar 1996).
The abundances of non-trophobionts were also compared between plants with hemipteran-ant association, and plants with only ants, using non-parametric Mann-Whitney U-tests for each species of ants. This test was used because the data were not normally distributed even after transformation (Zar 1996).

See the Supplementary Table for complementary information about each assay.

**Results**

**Effects of neighbouring colonies of mealybugs on frequency of ant visitation**

The presence of neighbouring colonies affected the frequency of ant visitation in each one; however, this result was obtained only for *C. crassus*. The frequency of visitation by *C. crassus* increased in control aggregation when prevented from tending the treatment aggregation, considering the premanipulation (days 1, 2 and 3) and manipulation period (days 4, 5 and 6; \( F = 3.53, \text{df} = 6, p = 0.012; \) Figure 1a). On the other hand, the frequency of *E. tuberculatum* visitation did not increase when prevented from tending a neighbouring aggregation (\( F = 1.51, \text{df} = 6, p = 0.220; \) Figure 1b). For the other plants examined only to verify the frequency of ant visitation without manipulation and to exclude any local environmental variations (i.e. with only adhesive paper strap without Tanglefoot®), the mean number of *C. crassus* and *E. tuberculatum* visits remained the same during all experiments (\( F = 1.53, \text{df} = 6, p = 0.213 \) for *C. crassus* and \( F = 2.107, \text{df} = 6, p = 0.098 \) for *E. tuberculatum*), which validated our experimental results, ruling out any possibility of influence of environmental variations on results.

**Effect of ant exclusion on the number of hemipterans per colony**

There was no significant difference in the number of hemipterans between the branches marked as control and treatment at the beginning of the experiment for both ant species (\( t = 1.214; \text{df} = 6; p = 0.27 \) for *C. crassus* and \( t = 0.568; \text{df} = 6; p = 0.59 \) for *E. tuberculatum*). The number of nymphs per colony was influenced by the presence of *C. crassus*, but not the number of adults per colony (Friedman ANOVA, Chi = 4, df = 1, \( p < 0.05 \) for nymphs; Chi = 1, df = 1, \( p > 0.05 \) for adults; Figure 2a). The presence of

![Figure 1](image-url)
E. tuberculatum did not affect the number of either nymphal or adult mealybugs (Friedman ANOVA, Chi = 1, df = 1, p < 0.05 for nymphs and adults; Figure 2b).

**Effect of ant-mealybug interaction on proportion of fruit production**

The factorial experiment showed a significant effect of the C. crassus-mealybug interaction on plants, with a greater negative effect on proportion of fruit production than in the groups with the presence of this ant and mealybugs considered separately (F = 6.752, df = 1, p = 0.019; Figure 3a; Table 1). The result was not significant for plants with E. tuberculatum-mealybug interaction (F = 3.403, df = 1, p = 0.08; Figure 3b; Table 1).
The abundance of mealybugs on plants with *C. crassus* increased by 40.8% (t = 2.5, df = 12, p = 0.024; Figure 4a) while the abundance of mealybugs on plants with *E. tuberculatum* increased only by 9.6%, with no significant difference between plants with ants and mealybugs and plants with only mealybugs (t = 1.65, df = 11, p = 0.126; Figure 4b). The abundance of non-trophobiont herbivores (herbivores that do not produce honeydew) was greater in plants with *C. crassus* and mealybug associations in relation to plants with only ants (58.6%; U = 9.000, df = 1, p = 0.038; Figure 5a). In plants with *E. tuberculatum* there was no difference in the abundance of non-trophobiont herbivores in plants with and without this ant-mealybug interaction (U = 22.000, df = 1, p = 0.876; Figure 5b).

**Discussion**

Our results show that the presence of neighbouring aggregations of mealybugs affected their interactions with ants, influencing the frequency of *C. crassus* visitation in each...
colony. Also, this ant species was the only one to influence the number of mealybug nymphs, confirming our first hypothesis. We also detected an indirect negative effect on proportion of fruit production and an increase in abundance of trophobiont and non-trophobiont herbivores influenced by the interaction between C. crassus and mealybugs, corroborating our second hypothesis.

The presence of ants can result in increased reproductive rate (Fagundes et al. 2013), longevity (Flatt and Weisser 2000), survivorship (Morales and Beal 2006), development rate (Flatt and Weisser 2000) and size of hemipterans (Morales and Beal 2006). In this sense, Cushman and Addicott (1989) postulated that hemipterans can compete intraspecifically by the services of ants when neighbouring hemipteran aggregations are able to reduce the number of ants that each attracts, and when the number of tending ants, not just their presence, is important to the fitness of hemipterans. We demonstrated the influence of neighbouring colonies of mealybugs on frequency of visitation by C. crassus. Thus, our results were crucial to demonstrate this important aspect influencing the interaction between ants and hemipterans, considering the implications of this relationship for an entire network of interactions.

Camponotus crassus was able to positively interact with mealybugs. The presence of these ants resulted in a positive effect on the number of mealybugs per colony, and their frequency of visitation was influenced by the presence of neighbouring mealybug aggregations. On the other hand, the species E. tuberculatum did not have any significant effect on the mealybugs. Therefore, we reinforced the conditionality of ant-mealybug mutualism since two different species of ant revealed different outcomes in the benefits provided to the mealybugs. Other authors have demonstrated the positive effects of ants on hemipteran numbers, and the factors that influenced these results, highlighting the ecological and physiological features of ants (see Morales 2000; Renault et al. 2005; Herbert and Horn 2008).

Del-Claro and Oliveira (2000) was the first study to simultaneously demonstrate the conditionality in ant-derived benefits to hemipterans. These authors showed that when their mandibles are loaded with honeydew, ant species from more primitive lineages, such as Ectatomma edentatum Roger (Ectatomminae), cannot store liquid in
the stomach, which makes them less prompt in chasing the hemipterans’ enemies. In contrast, *Camponotus rufipes* Fabricius, (Formicinae), whose mandibles are always free for aggression due to its capability to store liquid in its stomach (‘social stomach’, sensu Hölldobler and Wilson 1990), was able to effectively dissuade the herbivores. Therefore, ant partners can differ greatly in their direct protective effectiveness to hemipterans (see Buckley and Gullan 1991; Itioka and Inoue 1999) and also in their effects on host plants (see Messina 1981; Byk and Del-Claro 2010; Del Claro and Marquis 2015). Formicinae species are equipped with an advanced proventriculus and are able to carry a greater amount of sugary liquid in relation to Ectatomminae species. We suggest that the presence of a specialised proventriculus, a large crop capacity and mandibles free for defence in *C. crassus* contribute to greater protection of the mealybugs.

The genus *Camponotus* is intensively recorded as avid consumers of plant and insect exudates in tropical forests (Davidson et al. 2003), and is also the most common genus in ant-hemipteran interactions in cerrado vegetation (Sendoya et al. 2009; Nascimento and Del-Claro 2010; Lange and Del-Claro 2014). Studies that demonstrated positive effects of ants on hemipterans and host plants (e.g. Moreira and Del-Claro 2005; Fagundes et al. 2013) presented the influence of several ant species, especially species of *Camponotus*, reinforcing the importance of these species as worldwide attendants of trophobiont insects. In these studies the ants attacked and drove away the natural enemies of hemipterans, and even the herbivores, from the host plant. In our study, *C. crassus* defended the mealybug nymphs, based on the positive effects on number of nymphs per colony, but they did not protect the plants against other herbivores.

The outcomes of ant-hemipteran interactions for host plants represent a trade-off between the indirect benefit of increased ant suppression of non-trophobiont herbivores and the direct cost of herbivory by ant-tended hemipterans (Lach 2003; Del-Claro et al. 2016). The factorial experiment demonstrated a negative effect of *C. crassus* and mealybug interaction on proportion of fruit production. The abundance of trophobiont mealybugs was higher in plants visited by *C. crassus* (40.8%), as well as the abundance of other herbivores (58.6%). In plants with mealybugs and *E. tuberculatum*, differences were not detected. Thus, *C. crassus* increased the number of mealybugs but did not attack and drive away other herbivores, which may have resulted in the lower proportion of fruits produced. Some studies that demonstrated negative effects on plant fitness also detected outbreaks of hemipterans promoted by ant protection (Holway et al. 2002), which can impose tremendous costs on plant fitness via consumption of plant sap and transmission of plant pathogens (Delabie 2001; Cooper 2005). Renault et al. (2005), for example, reported that the aphid *Aphis coreopsidis* (Thomas) (Hemiptera, Aphididae) was 34% more abundant in the presence of tending *Camponotus* ants than in their absence, and that related plants produced fewer viable seeds than did plants without the interaction.

This study pointed out the importance of neighbouring colonies of hemipterans conditioning the ant-hemipteran interaction, the influence of ant species on positive effects for hemipterans and the consequent results for ant-plant relationships. Thus, we reinforced the conditionality of these important interactions and also complemented the
core of interaction studies suggesting other conditional aspects, such as the influence of physiological and morphological traits of different ant species.

Acknowledgements

We thank Karsten Mody and two anonymous reviewers for reviewing the manuscript. We also thank CNPq for funding this research (KDC) and FAPEMIG for awarding fellowships to AAV and KDC.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico [301605/2013-0].

Geolocation information

18.9751°S, 48.2908°W

References

Cooper LB 2005. The potential effects of red imported fire ants (Solenopsis invicta) on arthropod abundance and Cucumber Mosaic Virus [MSc thesis]. Alabama: AuburnUniversity.


Torezan-Silingardi HM 2007. A influência dos herbívoros florais, dos polinizadores e das características fenológicas sobre a frutificação das espécies da família Malpighiaceae em um cerrado de Minas Gerais [The influence of floral herbivores, pollinators and the phenological characteristics of the fruit of the family Malpighiaceae species in a Minas Gerais cerrado] [Doc thesis]. Ribeirão Preto: Universidade de São Paulo.


