Florivorous myrmecophilous caterpillars exploit an ant–plant mutualism and distract ants from extrafloral nectaries

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Abstract  Females of myrmecophilous butterflies tend to oviposit in plants visited by ant species that engage in stable associations with its larvae. In Banisteriopsis malifolia, caterpillars are attended by the same ants that feed on extrafloral nectaries. A conflict may arise when both the plant and caterpillars compete for ant attention, and ants are assumed to forage on the highest quality resource. By attending caterpillars, ants can be indirectly detrimental to plant fitness because florivorous larvae feed intensively until pupation. In this study, we specifically investigated (i) whether the occurrence of facultative myrmecophilous Synargis calyce (Riodinidae) caterpillars in B. malifolia was based on ant species (Camponotus blandus or Ectatomma tuberculatum) and abundance; (ii) the monopolization of ants by the butterfly larvae and (iii) the florivory rates incurred by the caterpillars on inflorescences. The abundance of S. calyce was six-fold greater in plants with C. blandus, compared to E. tuberculatum treatments. Caterpillars monopolized up to 50% of C. blandus on the plants, indicating that the resources offered by S. calyce were more attractive to ants than extrafloral nectaries. Florivory by riodinids incurred losses of almost 60% of flower buds. Myrmecophilous riodinids exploited an ant–plant mutualism by attracting aggressive ants that become larvae bodyguards. Thus, this ecological interaction is potentially detrimental to B. malifolia, since the ants, which can provide protection against herbivores, shift to provide defence for one of these herbivores.

Key words: Banisteriopsis malifolia, Brazilian savanna, Camponotus, Ectatomma, Synargis calyce.

INTRODUCTION

Many ant species are known for their mutualistic interactions with extrafloral nectary (EFN) plants (Del-Claro et al. 2016). These ants, however, not only feed on plant-derived resources, but also on the secretions of myrmecophilous insects, such as hemipterans and caterpillars (DeVries 1991; Sendoya et al. 2016). For instance, larval stages of Lycaenidae and Riodinidae butterflies have so-called ant-organs (DeVries et al. 1992; Campbell & Pierce 2003), which release a nectar-like solution for ants that, in turn, faithfully attend the larvae (Kaminski & Carvalho-Filho 2012). In this mutualistic interaction, ants receive a valuable sugared food source (Cushman et al. 1994), while butterfly larvae are protected from natural enemies, especially parasitoids (Stadler et al. 2003; Weeks 2003).

In the tropics, florivorous myrmecophilous butterflies occur in a large array of EFN plants, and sometimes during specific periods of EFN activity (Bächtold et al. 2013; Torres & Pomerantz 2016). This timing seems to be important for the natural history of myrmecophilous butterflies because it matches the period when potential specific ant-partners occur on plants (Kaminski 2008). Depending on the ant species, caterpillars can be ignored, tended or preyed upon (Robbins & Aiello 1982; DeVries et al. 1992). Therefore, adult females should be able to evaluate host plants to maximize larval survivorship (Ballmer 2003; Mota & Oliveira 2016) and thus oviposit in plants with certain ant species to achieve this goal (Collier 2007; Bächtold et al. 2017).

A conflict arises when both the plant (with EFNs) and myrmecophilous caterpillars compete for ant attention (DeVries & Baker 1989). Ants can shift from foraging for plants to animal resources, depending on quality (Blüthgen & Fiedler 2004). Thus, a shift from EFN to insect secretions can occur in some scenarios (Katayama et al. 2013). Indeed, it has been recently shown that myrmecophilous caterpillars can chemically (through secretions from ant-organs) manipulate ant behaviour so that ants remain close to larvae (Hojo et al. 2014). In this context, caterpillars can monopolize ant attention (i.e. a high

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abundance of ants are attracted to caterpillars, in comparison to the number of ants that forage on the plant) to get them close and serve as bodyguards.

Most studies on the interaction between ants and myrmecophilous larvae have been performed with lycaenids (see Fiedler 1991, 2001; Eastwood & Fraser 1999; Pierce et al. 2002; Bächtold et al. 2014) and little is known about ant associations within myrmecophilous riodinid caterpillars, despite recent efforts in the neotropics (Kaminski et al. 2013). Since many riodinid caterpillars have ant-organisms, they also engage in stable associations, either facultative or obligate, with ants (DeVries 1988; DeVries et al. 2004; Kaminski 2008; Kaminski et al. 2013). Moreover, myrmecophilous riodinids tend to occur in EFN plants, most likely because the same ants that feed on EFNs may also tend the caterpillars (Robbins & Aiello 1982; DeVries 1988; DeVries et al. 1992; Torres & Pomerantz 2016).

In this study, we investigated some aspects of the interaction between a facultative myrmecophilous riodinid caterpillar (Synargis calyce (C. Felder and R. Felder, 1862), Riodinidae) and tending ants (Camponotus blandus (Smith 1858) and Ectatomma tuberculatum (Olivier, 1792)) in the extrafloral nectariarid shrub Banisteriopsis malifolia (Nees and Mart.) B. Gates (Malpighiaceae). We specifically analysed (i) whether riodinid occurrence was based on ant species (C. blandus or E. tuberculatum) and abundance, (ii) a possible monopolization of ants by riodinid larvae and (iii) the florivory rates in inflorescences with and without riodinids. We hypothesized (i) a higher abundance of caterpillars in plants with C. blandus because ants in this genus are known to engage in stable associations with myrmecophilous butterflies, that is, lycaenids (Kaminski et al. 2010), whereas Ectatomma may prey on caterpillars (Robbins 1991); (ii) that ants should be consistently concentrated in inflorescences with riodinids (ant-monopolization) and (iii) that riodinids themselves should inflict high florivory rates in plants (like their sister group Lycaenidae – Badenes-Pérez et al. 2010), compared to other herbivores. In the field, we examined the behaviour of ants towards riodinids to see whether ants consistently attended caterpillars, what would be evidence of stable ant–larvae relationships (i.e. myrmecophily).

**METHODS**

**Study subjects**

*Banisteriopsis malifolia* is an EFN shrub that occurs in the Brazilian savanna (Cerrado biome) including the city where the work was carried out (Uberlândia city, 18°59 S 48°18 W, southeastern Brazil). A pair of round and convex EFNs occur at the base of leaves, near the petiole.

**Sampling and conditions of study**

A total of 60 *B. malifolia* individuals were tagged in the end of January, prior to the onset of the flowering season. These shrubs (1.62 ± 0.02 m tall, mean ± SE) had the same phenological conditions, such as presence of young foliage, absence of flower buds and presence of the target ants. Plants consistently supported either *C. blandus* or *E. tuberculatum* throughout the whole study period (no ant species overlap), and were tagged to account for two equal groups of shrubs (n = 30 shrubs per group) with each ant species. In our study system, *Camponotus* and *Ectatomma* occur consistently in different individual plants, with no overlap of different ant species in the same individual plant, a natural trend noted in other Malpighiaceae (Bächtold et al. 2017). This permitted us to investigate if riodinids occurred more in plants with *Camponotus* or *Ectatomma* because no other ant species were present on tagged plants. Neither ant presence nor species were manipulated in *B. malifolia*.

*Synargis calyce* caterpillars occurred on the plants from February to April. Whenever a larva was found, it was monitored in the field until it reached approximately 10 mm in length, when part of the study was performed (objectives ii, iii). This was done to standardize the data, especially on feeding damage (florivory rate). If more than one larva was found on the same individual plant, one was removed and taken to the laboratory, to maintain only one caterpillar per individual plant. During the study period, plants were visited periodically once or twice per week to make sure that the study system persisted unaltered, without disturbance, and that ants remained on plants. After the fieldwork was completed, caterpillars were collected and reared in the laboratory to investigate parasitism. Adult butterflies obtained from larvae reared in the laboratory were used to confirm species identification and potential

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parasitism. Adult butterflies were eventually released in the field at the study area.

Caterpillar–ant interactions

To investigate potential variation in ant abundance that might affect the study (objective i), ant abundance was estimated for each plant once per week for 3 weeks in the morning. It was done before plant flowering and the occurrence of riodinid larvae were detected. Nonetheless, no significant temporal variation was noted for any ant species before riodinid occurrence (repeated measures analysis of variance: $F_{2,87} = 0.8133$, d.f. = 2, $P > 0.05$ and $F_{2,87} = 0.1852$, d.f. = 2, $P > 0.05$ for *C. blandus* and *E. tuberculatum*, respectively). Therefore, the abundance of ants at the beginning of the study, just after plants were tagged, was chosen for statistical procedures (objective i) to ease the analyses.

Spatial segregation

To investigate the distribution and the spatial segregation of ants on different stems (objective ii), each plant with a riodinid larva was observed from 08:00 to 19:00 h. The number of ants on inflorescences with riodinids and on other inflorescences within the plant was recorded hourly (Apple & Feener 2001). Thus, we noted the location of ants on plants and the number that were associated with caterpillars or foraging in other stems or inflorescences. Here, we were interested in whether ants would spend more time attending larvae or visiting a plant’s active EFN.

Florivory

To examine the florivory rates (objective iii), we estimated both the number and proportion (number of buds consumed divided by the total number of buds produced) of flower buds consumed by riodinid caterpillars. These larvae eat all the internal flower bud tissues and leave an empty bud shell (Fig. 1c). This type of herbivory is characteristic of caterpillars only. Florivory was examined for a period of 10 days and, during this time, all caterpillars foraged in only two inflorescences, presumably because each plant produces tens of buds per inflorescence. Florivory (%) was compared for inflorescences with and without caterpillars, at the plant level ($n = 2$ inflorescences with and 2 without riodinids, per plant). Here, we intended to investigate whether riodinids inflicted a loss in flower buds, compared to florivory by other herbivores of *B. malifolia*.

Ant behaviour

In the field, we conducted *ad libitum* observations of ants and caterpillars. These observations were made whenever

![Fig. 1. Interactions between ants and caterpillars in *Banisteriopsis malifolia*. (a) Five *Camponotus blandus* individuals attending a *Synargis calyce* larvae. (b) An *Ectatomma tuberculatum* close to a caterpillar. (c) Herbivory mark in a flower bud. (d) *S. calyce* adult butterfly. Scale bars: 5 mm.](image-url)
we went to the field to accompany the development of larvae and confer the plants, and accounted for approximately 40 hours. We were interested to see whether caterpillars were attended, attacked or ignored by ants (Kaminski & Freitas 2010). We also recorded in video the interactions between ants and caterpillars as it could show the eversion of tentacle organs, structures that evert and release sugar-like solution to ants (DeVries et al. 2004; Kaminski et al. 2013).

### Statistical analyses

Quantitative data are presented as the mean and standard error whenever appropriate. The frequency of plants with riodinids according to each ant species was compared using a goodness-of-fit chi-squared test (objective i); the abundance of caterpillars according to each ant species was compared with a Mann–Whitney U (non-parametric data, objective i). We used a Student’s t test to compare the number of ants on plants where riodinids were found and plants where they were absent throughout the study period (objective i). A Student’s t test was also used to investigate the spatial segregation of ants throughout the day (e.g. ants attending riodinids and ants patrolling the plant on other stems, objective ii). The distribution of ants within several plant stems was compared using the Kruskal–Wallis tests (non-parametric data, objective ii). A Student’s t test was performed to compare the florivory (%) (objective iii) incurred by riodinids and other arthropods. Statistical procedures and figures were made in R and GraphPad Prism statistical softwares, respectively.

### RESULTS

A total of 21 *S. calyce* were found and caterpillars were six-fold more abundant on plants with *C. blandus* than on plants with *E. tuberculatum* (*n* = 18; 0.60 ± 0.15; and *n* = 3 larvae, 0.10 ± 0.07 larvae per plant, respectively) (*U* = 243.5; *P* < 0.01). Riodinids were observed on 16 (53%) *B. malifolia* individuals with *C. blandus* and on only two (6.7%) plants with *E. tuberculatum* (*χ²* = 10.889; d.f. = 1; *P* < 0.01). The number of caterpillars on *B. malifolia* ranged between 0 and 3 larvae, and most plants where riodinids were found (*n* = 12 plants) supported only one larva.

Riodinids oviposited predominantly in plants where *C. blandus* were more abundant. Data showed that the average initial abundance of *C. blandus* was 33.4% higher in plants where riodinids were later found (*t* = 3.7710; *P* < 0.001) (Fig. 2). The abundance of *E. tuberculatum* was three and four ants on the two plants that supported riodinids; the other plants hosted three to five *E. tuberculatum* ants. Due to the low frequency/abundance of riodinids in plants with *E. tuberculatum*, we were unable to run statistical tests.

In plants without riodinids, *C. blandus* ants were distributed evenly on up to four stems within plants (Fig. 3) (*H* = 0.8758; *P* > 0.05) and each stem was visited by no more than three individual ants. In contrast, for plants with riodinids, ants were markedly concentrated on inflorescences with caterpillars (*H* = 49.1379; *P* < 0.0001). Thus, a pattern of spatial segregation of ants was observed, as most ants remained on inflorescences upon which riodinids were feeding on, and attending caterpillars (Fig. 4) (*t* = 8.4801; *P* < 0.0001) throughout the whole day. Furthermore, a single riodinid larva could be

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**Fig. 2.** Abundance (median, quartiles and range) of *Camponotus blandus* in plants of *Banisteriopsis malifolia* where caterpillars were found and where they were absent. The symbol ‘***’ denotes *P* < 0.001, according to a Students’ *t* test.

**Fig. 3.** Percentage of *Camponotus blandus* ants attending caterpillars and foraging on other stems or inflorescences within plants. The symbols ‘***’ and ‘n.s.’ denote *P* < 0.0001 and non-significant, respectively, according to Kruskal–Wallis tests.

attended by up to five *C. blandus* individuals simultaneously, all of which walked over the caterpillar body and took turns to attend larvae (Fig. 1a).

Caterpillars everted the tentacle organs in the end of the abdomen very often in response to antennation by *C. blandus*. *Ectatomma tuberculatum* ants also attended riodinids, but these ants occasionally abandoned the caterpillars and foraged on other plant parts. No more than two individuals of *E. tuberculatum* attended a larva simultaneously in the field. No larvae reared in the laboratory were parasitized.

Among inflorescences containing riodinids, 57.55% (± 3.64%; range: 38–86%) of buds had herbivory signs and all of these (29.25 ± 1.0 buds; range: 22–37 buds) displayed the characteristic marks of riodinid feeding damage, such as consumption of the internal flower bud parts (Fig. 1c). In contrast, inflorescences without riodinids showed only 4.29% (± 0.51%; range: 1.4–8%; abundance = 2.21 ± 0.24 buds) of buds with herbivory marks (*t*14 = 16.1673; *P* < 0.0001), such as necrosis and lacerations, none of which was caused by caterpillars.

### DISCUSSION

All the hypotheses established for this work were corroborated: (i) riodinids occurred mostly in association with *C. blandus*, and were present in the plants where these ants were more abundant; (ii) ant recruitment increased in response to riodinid occurrence since most ants within plants engaged in interactions with caterpillars and a few foraged in other stems and (iii) florivory by riodinids incurred losses of up to half the number of flower buds per inflorescence.

*Synargis calyce* caterpillars were found predominantly in shrubs visited by *C. blandus* and these ants attended the caterpillars very often and intensively. The association between *Camponotus* ants (i.e. *crassus, blandus* and *rufipes*) and myrmecophilous caterpillars (especially lycaenids) is very common in several plant species (Ross 1966; Fiedler 2001; Kaminski et al. 2010). Species of *Ectatomma* also engage in stable associations with riodinid larvae (DeVries 1988; Torres & Pomerantz 2016), but in our study, the abundance and frequency of riodinids in plants with *E. tuberculatum* was almost negligible. Similar results were also found for tropical myrmecophilous lycaenids, which presented a strong tendency to associate with *Camponotus* than with *Ectatomma* (Bächtold et al. 2016). For myrmecophilous butterflies, associating with *E. tuberculatum* may be risky because these ants can either attend or prey on caterpillars (Ross 1966; Robbins 1991), especially the first stages when larvae are small (Bächtold & Alves-Silva 2013). In contrast, *Camponotus* might either tend or ignore larvae (Bächtold & Alves-Silva 2013). Therefore, in this case, the risk for caterpillars is lower and might be counterbalanced by the positive effect of ants in luring away natural enemies of the larvae (Kaminski et al. 2010).

*Synargis calyce* caterpillars monopolized ants that might otherwise patrol the plant and exert their role as plant guards. One of the early alleged functions of EFNs was to distract ants from myrmecophilous insects (Del-Claro et al. 2016), but in this study, we may assume that riodinids represented a better resource to ants than EFNs because caterpillars gathered several attending individuals of *C. blandus* that remained in close contact with larvae throughout the whole day. The quality of resources is important for the decisions of foraging ants (Blüthgen & Fiedler 2004), and secretion from riodinids may contain more amino acids than EFNs (DeVries 1988). Our results also suggest that riodinids are a better resource for ants, which potentially allowed the caterpillars to monopolize ant attention and gather up to 50% of ants from plants. Studies with lycaenids indicate that tended larvae are less parasitized (Weeks 2003). In our study, no immature was parasitized during the fieldwork.

Florivory by *S. calyce* larvae incurred losses of up to 50% of flower buds of *B. malifolia*. In comparison, inflorescences without riodinids displayed low florivory rates. Caterpillars consumed flower buds that otherwise could turn into seeds or seedlings. Therefore, the presence of this myrmecophilous riodinid species was detrimental to *B. malifolia* because larvae both monopolize patrolling ants and feed intensively on plant reproductive structures. In similar studies

*Fig. 4.* Abundance (mean ± standard error) of *Camponotus blandus* ants attending *Synargis calyce* caterpillars, and ants foraging on inflorescences without riodinids.

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with riodinid–ant interactions, authors found losses of 33% in seed production and up to 38% of foliage (Horvitz & Schemske 1984; DeVries & Baker 1989). This asymmetric ant–plant mutualism, that is, the ants feed on EFNs, but fail to protect the plant against herbivores, is quite common in facultative systems (Del-Claro et al. 2016). Nonetheless, the exploitation of ant–plant mutualisms by myrmecophilous insects, with consequent disadvantages for the host plant as shown in the present study, has scarcely been studied, but show severe decrements in plant fitness when a myrmecophilous insect exploit the ant–plant mutualism (Buckley 1983; Horvitz & Schemske 1984). For instance, Ferreira and Torezan-Silingardi (2013) showed that florivory in B. malifolia renders flowers less attractive to pollinating bees, which negatively affects the fruiting rate and the reproductive success of plants.

Attendance to caterpillars appeared to boost the aggressive behaviour of C. blanda because sudden approaches near (~1 m) plants during observation triggered marked changes in ant behaviour: they became very agitated near the caterpillar, turned their abdomens downward (Yamamoto & Del-Claro 2008) and also displayed co-specific aggressive behaviour. Such behaviour was not noticed when ants were foraging on EFNs. This behaviour is characteristic of the liberation of alarm pheromones that trigger a fast-running behaviour of ants associated with enemy hunting and recruitment (Ali & Morgan 1990; Fujiwara-Tsuji et al. 2006). In addition, this ant behaviour can be labelled as ‘enticement and binding’ (DeVries 1988), whereby caterpillars affect ant behaviour so that ants remain close to larvae and display aggressive behaviour. This excitation can be attributed to the discovery of a new potential food resource and result in the recruitment of conspecifics.

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CONCLUSION
The evidence presented here indicates that myrmecophilous riodinid caterpillars exploit an ant–plant mutualism to their own advantage, by attracting aggressive ants (C. blanda) that become larvae body-guards and by feeding on flower buds that would otherwise be defended by ants from herbivores. From the plant’s perspective, the association between ants and caterpillars results in losses of flower buds and reproductive output. Taken together, the evidence suggests that caterpillars of S. calyce might be acting as parasites of its host plant, by stealing a direct and important resource of plants – in this case, the EFN feeding ants (which are responsible for herbivore deterrence). Larvae of S. calyce then disrupt the ant–plant mutualism. Given the pervasiveness of myrmecophilous caterpillars (especially lycaenids) and ants in EFN (tropical) plants, such outcomes may be common and largely detrimental to plants, as ants attend the herbivores that they should be protecting the plants from.


