



RESEARCH ARTICLE - WASPS

Wasps are Better Plant-Guards Than Ants in The Extrafloral Nectaried Shrub *Ouratea Spectabilis* (Ochnaceae)

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Abstract

In the Cerrado, many plants are patrolled by ants, but in some cases, predatory wasps may also play an important role in herbivore deterrence. Here we examined the effects of the wasp *Brachygastra lecheguana* and the ant *Pseudomyrmex gracilis* on the predation of endophytic weevils which develop in the flower buds of *Ouratea spectabilis* trees. We (i) compared frequency and the abundance of weevils preyed upon by wasps; (ii) possible synergic effects of ants and wasps in decreasing weevil population; and (iii) whether wasps preyed upon weevils in more visible, easy-access inflorescences, i. e. those located on the edges of canopy. In contrast to ants, wasps were observed significantly more frequently on *O. spectabilis* and were responsible for 88% of the weevil larvae preyed upon. Plants visited by both *B. lecheguana* and *P. gracilis* had a substantial reduction in weevil larvae compared to plants visited by *B. lecheguana* only. This indicates a complementary effect of predators with distinct skills in deterring the weevil population; however, ants were responsible for only a small portion of weevils preyed upon. Weevil larvae occurred evenly in both visible and hidden inflorescences; nonetheless, wasps predominantly visited inflorescences located on the edges of the canopy. In this ecological system, predatory wasps played a substantial part in weevil deterrence and can be considered a better plant-guard than ants.

Introduction

In the Brazilian savanna, many plant species are severely attacked by a wide range of herbivorous insects, which feed and inflict damage on virtually every plant part, especially leaves and flowers (Price et al., 1995; Bächtold et al., 2012; Vilela et al., 2014). However, many plants present biotic protection against herbivores, in the form of aggressive and predatory ant-guards, which feed on extrafloral nectaries (EFNs) and protect the plant against assorted types of insects, especially folivores (Koptur et al., 1998; Bronstein et al., 2006; Alves-Silva, 2011; Heil, 2015). Although many studies provide evidence that ants can suppress herbivore populations and in turn increase plant fitness (Vesprini et al., 2003; Rosumek et al., 2009; Del-Claro et al., 2016), some studies point out that ants are not effective against all types of

herbivores, as some insects occur in structures inaccessible to ants (Mody & Linsenmair, 2004; Nogueira, et al., 2012; Alves-Silva & Del-Claro, 2015). Furthermore, predator behavior can be affected by plant structural complexity, which confers more hiding places for herbivores (Lawton, 1983), or restrains the foraging activity of herbivores' natural enemies (Hopper, 1984; Andow & Prokrym, 1990). As shown by Del-Claro et al., (1997) the presence of natural enemies and/or the likelihood of being preyed upon, influence the behavior of herbivores, and they may seek habitats that confer some type of protection and less risk of being encountered by predators (Bächtold & Alves-Silva, 2013).

As well as ants, wasps are also important natural enemies of herbivores (Pereira & Trigo, 2013; Bächtold et al., 2014), especially endophytic weevils (Toresan-Silingardi, 2011). For instance, the social Polistinae wasp *Brachygastra lecheguana* Latr. (Hymenoptera: Vespidae) is the main predator



of a group of *Anthonomus* species (Coleoptera: Curculionidae) in Malpighiaceae (Alves-Silva et al., 2013), but this wasp is also able to capture a wide range of other herbivores (see Souza & Zanuncio, 2012). In general, predatory insects such as wasps and ants are bounded by the seasonal availability of their prey and as such, variations in herbivore prey are expected to directly affect the occurrence of their natural enemies in the field (see Mooney & Tillberg, 2005; Mody et al., 2011).

Ouratea spectabilis (Mart.) Engl. (Ochnaceae) is an extrafloral nectaried shrub that supports almost 30 ant species; however, studies showed that ants are unable to protect the plant against certain herbivores such as thrips, caterpillars and curculionid beetles whose larvae are endophytic (Byk & Del-Claro, 2010; Bächtold et al., 2012). Nonetheless, in recent observations, a particular ant species, *Pseudomyrmex gracilis* (Fabr.) (Formicidae: Pseudomyrmicinae), was observed lacerating flower buds and preying on endophytic weevil larvae (E.A-S. personal observation). Such behavior is very similar to that of predatory wasps (Alves-Silva et al., 2013) and may indicate that ants and wasps have a cumulative negative effect on the population of weevils, thus ultimately benefiting the plant.

In this study we aimed to investigate (i) the temporal variation of *P. gracilis*, *B. lecheguana* and *Anthonomus* in *O. spectabilis* according to the plant's flowering season; (ii) the abundance of weevils preyed upon by wasps and ants; (iii) possible synergic effects of ants and wasps in decreasing weevil population; and (iv) the influence of inflorescence location on the abundance of *Anthonomus* and on the prey finding ability of wasps. We hypothesized that wasps and ants would be more abundant following an increase in weevil larvae availability; plants supporting both wasps and ants would present higher reductions in weevil population, but wasps would play a major role in weevil predation; and easy-access inflorescences (i.e. those on the edges of the plant canopy) would be more visited by wasps.

Material and Methods

Study area

Fieldwork was carried out in a *sensu stricto* Cerrado area (18°59' S, 48°18' W) in Uberlândia City, Brazil, from August to September 2012 and for the same period of 2013, which corresponds to the flowering period of *O. spectabilis*. The Cerrado covers about 230 ha and is dominated by herbaceous plants; the height of shrubs and trees ranges between 2 and 4 m. The climate is markedly seasonal with two well established seasons: a dry winter (May to September) and a rainy summer (October to April) (Réu & Del-Claro, 2005).

Study species

Ouratea spectabilis is a common sclerophyllous tree species (2-5 m in height) in the Cerrado savanna. Its leaves

may reach up to 80 mm in length and 50 mm in width and the EFNs occur at the stipules, close to the leaf base (Bächtold et al., 2012). Flowers are pentamerous and yellow, and blooming starts in August and lasts until October. Flower buds (~0.7 mm in length) are greenish and conical with an acute apex (Fig 1A), and are attacked by species of *Anthonomus* beetles (weevils), whose larvae develop inside the flower buds (Byk & Del-Claro, 2010). These beetles are a complex of three species with very similar behavior and natural history (Torezan-Silingardi, 2011), but their recognition in the field conditions is unpractical, thus we studied *Anthonomus* as a group, for the sake of clarity.

Wasp predation towards beetles

In order to examine the temporal abundance of wasps, ants and beetles in the field, in 2012 we tagged 20 *O. spectabilis* plants with approximately the same phenological status (i.e. presence of flower buds in abundance). We also took care to select short plants (< 2 m in height) to enhance the observers' acuity. Fieldwork was conducted for three weeks, at the peak of flower bud production. In the first week of observation, adult weevils were frequently observed engaged in mating behavior in inflorescences of *O. spectabilis*, so we considered that the following week was the period in which weevil larvae might be under development inside flower buds. This second week also coincided with the peak of flowering in *O. spectabilis*. From the third week onwards, the plants started to cease production of flower buds and blooming prevailed, so we ended our fieldwork, as these weevils develop exclusively in flower buds (Torezan-Silingardi, 2011).

During each field trip, we stood carefully in front of each tagged plant for 20 minutes and performed focal observations to register the number of visits of *B. lecheguana* to the plant and the frequency with which weevil larvae were successfully captured and preyed upon by wasps (adapted from Pereira & Trigo, 2013). 'Visits' were considered as whenever a given individual wasp landed on the plant; 'successful predation' was the act of capturing and preying upon a weevil larva (Alves-Silva et al., 2013). We also scanned the plant for *P. gracilis* individuals, and their predation upon weevils was also registered. All the observations were conducted once a week on sunny days between 08:00h and 13:00h, since in preliminary observations wasps were commonly noted visiting the plants during this period. Our total sampling effort together with fieldwork accounted for approximately 65 hours.

An experimental factorial procedure, examining 'ant-present/excluded' and 'wasp-present/excluded' and its variations, is often impractical since the exclusion of wasps from plants is a major issue (Pereira & Trigo, 2013). So we attained to compare weevil larvae preyed upon in plants where wasps and ants were seen together with plants where only wasps were observed. This might give us a good estimate of the role of wasps alone and wasps+ants in deterring the weevil population in *O. spectabilis*.

Temporal variation in weevil larvae

The temporal abundance of weevil larvae in *O. spectabilis* was examined in a further 10 individual plants in the same weeks of the observations described above. Weevil larvae could not be estimated in the tagged *O. spectabilis* plants, as we might affect the visitations of predatory wasps and ants. From each of the 10 trees we randomly collected 20 flower buds, which were taken to the laboratory and dissected to look for weevil larvae.

Plant phenology

The phenology and intensity of flower bud production was examined in two inflorescences for each individual plant ($n = 20$ plants). Inflorescences were taken from opposite sides of the canopy and we also took care to select structures which had no previous signs of herbivore attack, fungus or other type of damage. The percentage of flower buds per plant was obtained by counting all buds and dividing the total by the number of flowers. Therefore, the intensity of bud production is presented in percent values (see Alves-Silva & Del-Claro, 2015).

Wasp visitation and inflorescence location

In order to examine whether inflorescence location influenced wasp visitation to *O. spectabilis*, we randomly chose one pair of inflorescences per plant ($n = 10$ plants) in 2013. Inflorescences were located ~1 m from each other and, whenever possible, located in the same horizontal plane of plant symmetry. Each pair consisted of an inflorescence located and/or growing towards the edge of the plant canopy, and another inflorescence at the core or growing towards the interior of the canopy. The former inflorescences were characterized by constant sun exposure, lack of surrounding leaves and high visibility; while the latter received little or no sunlight, were surrounded by leaves from neighboring branches and were less conspicuous (Fig 1A, B). For the sake of clarity, each inflorescence will hereafter be referred to as a “visible inflorescence” or “hidden inflorescence”.

Wasp visitation to these inflorescences was examined on two non-consecutive days; each plant was examined for 30 minutes between 08:00h and 12:00h, and we recorded the frequency with which weevils in each inflorescence type were captured. At the same period, other pairs of inflorescences with the same characteristics described above were collected from a further 10 individual plants, taken to the laboratory and dissected to investigate the weevil infestation. We intended to examine whether weevil larvae were more abundant in visible or hidden inflorescences and relate it to the visits of wasps.

Statistical analysis

Quantitative data are presented as the mean \pm SE. Parametrical statistical tests were given priority whenever data fitted the assumptions of normality (bell-shaped distribution and $P > 0.05$ in the normality test). Otherwise, non-parametrical

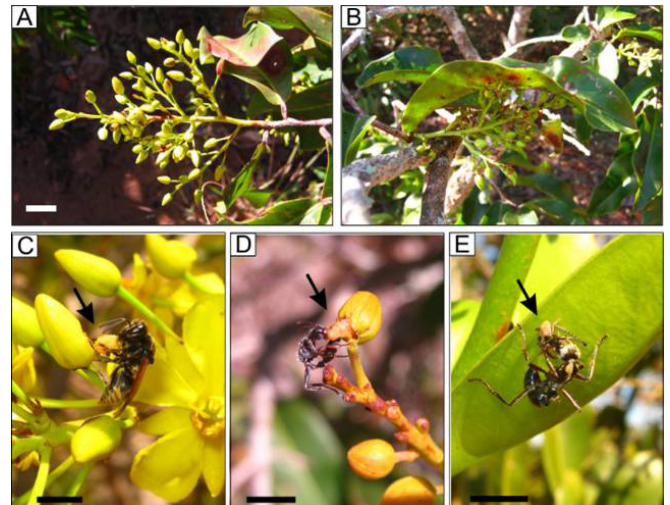


Fig 1. Predator-prey interactions in *Ouratea spectabilis* in a Brazilian tropical savanna. (A) a visible inflorescence; (B) hidden inflorescence; (C) *Brachygastra lecheguana* and (D) *Pseudomyrmex gracilis* capturing weevil larvae (arrows); (E) *Pachycondyla villosa* biting an adult weevil. (Photo E by Alexandra Bächtold). Scale bars = 10 mm.

tests were used instead. The variation of weevils (adult and larvae) and wasps during the study was examined with Kruskal-Wallis tests. The relationship between the abundance of adult *Anthonomus* and parasitism in flower buds (per plant) was examined with a linear regression test. In this test, we used the mean abundance of flower bud parasitism during the three weeks of the study. The relationship between weevil larvae consumed and wasp visits to *O. spectabilis* was also examined with a linear regression test. In this test, we used the cumulative abundance of wasp visitations and *Anthonomus* larvae consumed during the study. The difference in the number of weevils captured by wasps and ants was examined with a Wilcoxon test. Individuals of *P. gracilis* were not observed on *O. spectabilis* as frequently as *B. lecheguana*, so we decided to compare the number of weevil larvae preyed upon in plants where only wasps were observed and in plants where both ants and wasps were registered. This comparison was made with a Student's *t* test. The visits of wasps to visible and hidden inflorescences, as well as the abundance of weevil larvae in such inflorescences, were compared with *G*-tests.

Results

The number of adult weevils was higher at the beginning of the study, during the weevil reproductive season (Fig 2A), but as the season progressed and flower buds became scarce (Fig 2B), the abundance of adult *Anthonomus* gradually decreased ($H_3 = 37.2098$; $P < 0.0001$). Weevil larvae, on the other hand, were much more abundant at the end of the study ($H_3 = 9.1386$; $P < 0.05$) (Fig 2A). The infestation of flower buds was on average 11.25% ($n = 45$ of the 400 buds examined). A positive and significant relationship between the abundance of adult *Anthonomus* and the infestation of larvae (per plant) was found ($F_{18} = 12.1572$; $R^2 = 0.4031$; $P < 0.01$; regression coefficients: constant = 2.1708; slope = 0.7177).

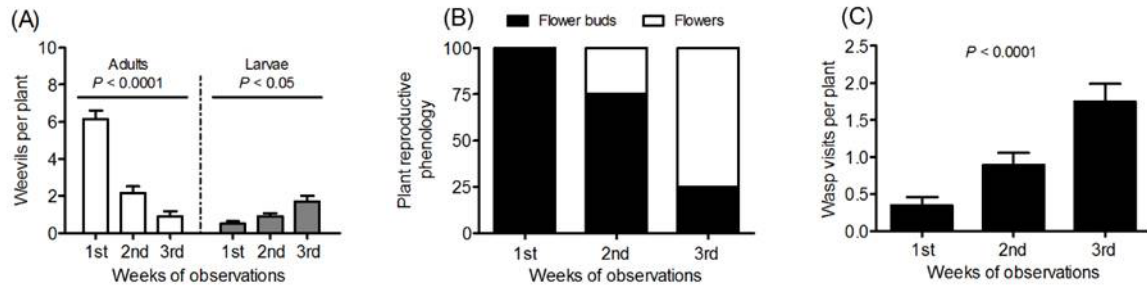


Fig 2. (A) Abundance of adult and larvae of *Anthonomus* (Curculionidae) in *Ouratea spectabilis* in a Brazilian tropical savanna; (B) phenological events of *O. spectabilis*; (C) temporal variation in the abundance of *Brachygastra lecheguana*. Figures A and C – mean \pm SE; $P < 0.0001$ and $P < 0.05$ indicate statistical significant differences (Kruskal-Wallis test).

The abundance of *B. lecheguana* followed the same trend as weevil larvae (Fig 2C). For instance, wasp visits to the plants at the end of the study were five times higher than at the beginning of the study ($H_3 = 19.6225$; $P < 0.0001$). As shown in Fig 2A-C, there was an inverse association between the abundance of adult *Anthonomus* and wasps, and a positive increase in wasp visits to the plants following the high levels of weevil larvae on flower buds. *P. gracilis* was observed only at the end of the study and its abundance was low ($n = 6$ observations).

The relationship between the number of weevil larvae consumed (2.25 ± 0.29 ; $n = 45$ larvae preyed upon) and wasp visits to the plants (3.0 ± 0.31 ; $n = 60$ visits to 20 plants) was positive and statistically significant ($F_{1,8} = 21.7181$; $R^2 = 0.5468$; $P < 0.001$; constant = 0.1667; slope = 0.6944). Wasps (Fig 1C) landed on inflorescences and wandered on flower buds, very often migrating from buds. Antennation on flower buds was commonplace and whenever an endophytic weevil larva was found, the wasp started to lacerate the external layers of the flower bud with its mandibles to reach the larva inside. As soon as the flower bud was opened, the wasp pulled the weevil larvae out with the mandibles and started to eat it immediately (complete behavior described in Alves-Silva et al., 2013).

Pseudomyrmex gracilis was also observed walking on *O. spectabilis* inflorescences and lacerating flower buds to capture *Anthonomus* larvae (Fig 1D). However, unlike *B.*

lecheguana, which consumed the larvae on the plant, the ants took the larvae to their nest. The number of weevils captured by *P. gracilis* ($n = 6$ larvae preyed upon) was much lower than the abundance of weevils captured by *B. lecheguana* ($n = 45$) (Wilcoxon test = 7.0648; $P < 0.0001$).

Plants that were visited by both *B. lecheguana* and *P. gracilis* ($n = 6$ plants) had a substantial reduction of weevil larvae compared to plants visited by *B. lecheguana* only ($n = 14$ plants) ($t_{18} = 2.9686$; $P < 0.01$) (Fig 3A). In these plants, however, each ant preyed upon only one weevil larvae, while wasps were responsible for the major part of weevils captured (2.83 ± 0.48 individuals). It is also worth mentioning that on one single occasion, in a sporadic observation in the field, an individual of *Pachycondyla villosa* (Fabr.) (Ponerinae) managed to capture and bite an adult weevil (Fig 1E).

Wasps predominately visited inflorescences located on the edges of the canopy ($G = 9.7891$, $df = 1$, $P < 0.01$). Nonetheless, weevil larvae occurred evenly in visible and hidden inflorescences ($G = 0.3620$, $df = 1$, $P > 0.05$) (Fig 3B).

Discussion

Main findings

Confirming our main hypothesis, wasps and ants reduced the infestation of endophytic weevils in *O. spectabilis*, so both predators can be considered as important biotic defenses for the plant. *Brachygastra lecheguana* was the main predator of *Anthonomus* larvae, while *P. gracilis* was less abundant and was responsible for 12% of the total weevil larvae preyed upon. Moreover, weevil's abundance was bounded by plant reproductive phenology, which ultimately affected the whole ecological system (trophic cascade), because as bud production ceased, so did weevil reproduction and wasps, which fed on weevil's larvae.

Weevil infestation

Weevil infestation in *O. spectabilis* (11.25%) was lower compared to another Cerrado plant, *Banisteriopsis malifolia* (20.7%) (Alves-Silva et al., 2013), but regardless of the level of parasitism, weevil development in flower buds is negatively related to plant fitness, as larvae consume the whole

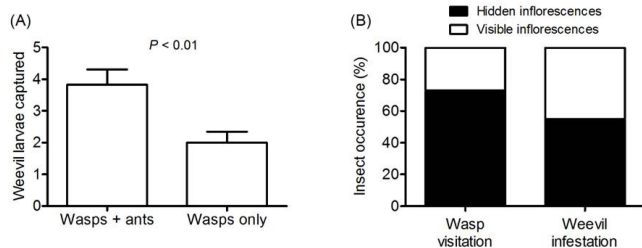


Fig 3. (A) Abundance (mean \pm SE) of weevil larvae preyed upon in plants where only *Brachygastra lecheguana* were observed and in plants where both wasps and *Pseudomyrmex gracilis* were noted. $P < 0.01$ indicates statistical significant differences, Student's *t* test; (B) Wasps visited predominantly inflorescences located on the surrounding of canopy, but weevils occurred evenly in visible and hidden inflorescences.

of the internal part of buds. Thus, the predatory behavior of both *B. lecheguana* and *P. gracilis* towards weevils can be reflected in higher flower abundance in future events of *O. spectabilis* reproductive phenology.

The role of ants in plant defence

Pseudomyrmex gracilis forages in a diverse array of plants, regardless of the presence of other ants (Stefani et al., 2000; Santos & Del-Claro, 2001) and is pointed out as one common ant species in ant-plant ecological networks in Cerrado vegetation (Lange et al., 2013). Unfortunately, the natural history of this ant species is not well understood, most likely because of the low number of individuals found on plants compared to other ants (Rodrigues et al., 2008). Despite that, some studies (Dansa et al., 1989; Fagundes et al., 2013) found that *P. gracilis* has a wide feeding flexibility, consuming both arthropods and plant exudates (extrafloral nectar). In the study by Byk and Del-Claro (2010), the authors did not record interactions between ants and *Anthonomus*, but *P. gracilis* was not the focus of their investigation. In this context, our findings in the present research are important by showing that an overlooked ant species acts as an herbivore (weevil) deterrent for *O. spectabilis*. Nonetheless, its role in weevil predation was low compared to the quantity of weevils captured by wasps. To our knowledge, this is the first record of *P. gracilis* preying on endophytic weevils, and given the widespread frequency of both *Anthonomus* and *P. gracilis* in the Cerrado (Torezan-Silingardi, 2007), the potential interaction between these parties could be pervasive.

Predatory wasps and weevil predation

Brachygastra lecheguana predation upon weevils was intense, but wasps were more abundant in the last two weeks of the study, during the period of weevil development. According to the data presented in Fig 2, there was an inverse association between the abundance of flower buds in the field, and the abundance of both wasps and weevil larvae. That might have occurred because as the phenology of *O. spectabilis* advanced, fewer buds were available in the field, thus leading to the densification of weevil larvae in the remaining buds. Hence, the higher abundance of wasps at the end of the study might be a reflection of the high density of weevil larvae in the flower buds. This temporal variation in wasp behavior is expected, as new evidence (unpublished data) indicates that *B. lecheguana* visits several plant species according to the flowering phenology and availability of prey (see also Torezan-Silingardi, 2007; Alves-Silva et al., 2013). In crops, where *B. lecheguana* attacks leaf-miners, the presence of wasps can also be related to the abundance of its prey, as pests occur at the specific time of leaf maturation (see Perioto, et al. 2011). Our observations also revealed that adult weevils can be preyed upon by *P. villosa*, but these ants are infrequent visitors to *O. spectabilis* (Byk & Del-Claro, 2010), so their role in weevil deterrence is negligible.

Plant architecture, weevils and wasps

Predatory wasps can use chemical and olfactory cues to locate their prey (Richter & Jeane, 1985), as damaged plants metabolize blends of volatile compounds which attract herbivores' natural enemies, especially wasps (Turlings et al., 1995; Moraes et al., 1998; Paré & Tumlinson, 1999). Furthermore, environmental cues can also play an important role in the prey finding ability of wasps (Turlings et al., 1993). The foraging activity of *B. lecheguana* was concentrated on the inflorescences located predominantly on the edges of *O. spectabilis* canopy. These inflorescences were not shaded by leaves or in contact with any other structure of the plant, in contrast to the hidden inflorescences. Previous evidence (Alves-Silva et al., 2013) suggests that *B. lecheguana* may use visual and mechanical/olfactory cues to find their weevil prey as plants with plentiful inflorescences were significantly more visited; and antennation on parasitized flower buds was frequent. In *O. spectabilis*, the weevil parasitism in hidden and visible inflorescences was similar, thus the higher visit rates of wasps to the latter might indicate that *B. lecheguana* is guided by visual cues. However, chemical signals emitted by the plant in response to weevil herbivory cannot be ruled out yet, as in our case, hidden inflorescences may restrict the range of such chemicals, in contrast to buds located on the edges of the canopy.

Conclusion

As shown in other studies, *B. lecheguana* has a wide feeding flexibility, as it can consume beetles, lepidopterans, extrafloral and floral nectar, and honey (Gusmão et al., 2000; Mussury et al., 2003; Aguiar & Santos, 2007; Alves-Silva et al., 2013). Therefore, its actual host range may be enormous and this wasp can visit several plant species, acting as an herbivore deterrent. Further studies may reveal that this wasp species has an important impact on herbivore population while indirectly benefiting plants. *O. spectabilis* is patrolled by a diverse community of predators (e.g. ants, wasps and spiders, see Byk & Del-Claro, 2010; Bächtold et al., 2012; Nahas et al., 2012), but only *P. gracilis* and (especially) *B. lecheguana* are able to capture the endophytic weevils. This reveals that the outcomes of ant-herbivore-plant systems are highly conditional, and that wasps might play a more important role in herbivore deterrence than extrafloral nectar-drinking ants.

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