

Ant–herbivore interactions in an extrafloral nectaried plant: are ants good plant guards against curculionid beetles?

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Extrafloral nectary drinking ants are known as effective plant guards, but some herbivores may circumvent ant attacks by foraging on different plant parts or presenting adaptations to avoid ant predation. Here we experimentally investigated the effect of *Camponotus blandus* on the florivory of the extrafloral nectaried shrub *Banisteriopsis malifolia*; and a possible spatial segregation between ants and herbivores (leaves and flowers). Flower buds are attacked by *Anthonomus* weevils. Results revealed no significant influence of *C. blandus* on the reduction of florivory. Adult *Anthonomus* are hard-bodied and were immune to ant bites; larvae are endophytic, so protected from ants. Ants and adult beetles were concentrated in different plant parts (leaves and flowers, respectively) so restraining the probability of encounters. Our results indicate that the system *C. blandus*–*Anthonomus*–*B. malifolia* is not stable, as ants receive extrafloral nectar, but are unable to protect the plant against weevils.

Keywords: *Anthonomus*; *Banisteriopsis*; *Camponotus blandus*; Cerrado; extrafloral nectary

Introduction

In general, ant–plant mutualistic relationships are based on reciprocal benefits for both organisms involved. For instance, plants often provide a resource for ants (e.g. extrafloral nectaries, domatia), while ants patrol and defend the plant against herbivore insects (Bronstein et al. 2006). The benefits gained by the plant due to the presence of ants, which are attracted to the nectar-producing structures, have been well documented and include low herbivory rates of both vegetative and reproductive structures, as well as increased vigour, growth and fitness (Fuente and Marquis 1999; Oliveira et al. 1999; Katayama and Suzuki 2004; Kost and Heil 2005). In general, specialized ant–plant interactions, such as those involving myrmecophytic species, provide more benefits to the parties involved, as both ants and plants have close relationships of co-evolution (Longino 1989; Rocha and Bergallo 1992; Brouat et al. 2001). In contrast, interactions involving extrafloral nectaried plants are not so specific (Blüthgen et al. 2007) and one single plant species can harbour many ant

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species and the net effect of each ant species on plant performance can be strikingly different (Mody and Linsenmair 2004).

In a broader consensus, ant–plant interactions based on extrafloral nectaries (EFN) have a positive effect for the plants (Rosumek et al. 2009), but several studies have demonstrated that extrafloral nectaried plants may not benefit from the presence of ants (Rashbrook et al. 1992; Mody and Linsenmair 2004; Nogueira et al. 2012). In fact, aggressive ant behaviour towards herbivores may be ineffective in many scenarios, especially those involving endophytic herbivores and/or large, hard-bodied insects (Heads and Lawton 1985; Koptur and Lawton 1988; Riedel et al. 2013). Also, ant attack towards herbivores can be ineffective if both occupy different plant parts (Bächtold and Alves-Silva 2013). For instance, Del-Claro et al. (1997) showed that florivorous thrips co-occurred with aggressive ants in *Peixotoa tomentosa* Adr. Juss. (Malpighiaceae), but thrips lived inside flowers, where they were inaccessible to all but tiny ants.

In extrafloral nectaried plants, ants most likely spend more time foraging on leaves and not in the plant reproductive structures (Wagner and Kay 2002; Junker and Blüthgen 2008), rendering ant protection generally more effective against leaf herbivores (Ness 2003; Dejean et al. 2009). For instance, Koptur (1979) noted that ants reduced foliar damage in *Vicia* (Fabaceae), but found no indication that ants protected the plant against seed predators. In contrast, Oliveira (1997) demonstrated that ants feeding on EFN were able to remove several florivores from *Caryocar brasiliense* Camb. (Caryocaraceae), which was reflected by increased plant fitness.

A particular scenario of ants visiting both leaves and flowers can be seen in the extrafloral nectaried shrubs of Malpighiaceae. Ants in several species feed on EFN (Alves-Silva 2011; Bächtold et al. 2013), but they can commonly be observed foraging on inflorescences where they hunt down thrips (Del-Claro 1998), chase chrysomelid beetles (Reu and Del-Claro 2005), and may significantly reduce herbivore populations. Fernandes et al. (2005) observed a marked reduction of suckers and chewers in inflorescences of *Byrsonima crassifolia* (Linnaeus) H.B.K. (Malpighiaceae) after ants were experimentally removed, indicating that ants patrolled plant reproductive parts. Therefore, the presence of EFN-feeding ants in Malpighiaceae affects not only folivores (Alves-Silva and Del-Claro 2013), but also florivores. However, ant–plant interactions are highly conditional (Bronstein et al. 2006; Marazzi et al. 2013) as ants are not able to protect the plant against all types of herbivores, especially beetles, due to their hard body and strong sclerotization (Koptur and Lawton 1988). Herbivore beetles are pervasive in Malpighiaceae (Del-Claro 2004; Reu and Del-Claro 2005; Flinte et al. 2006; Torezan-Silingardi 2011; Alves-Silva, Barônio et al. 2013; Ferreira and Torezan-Silingardi 2013), but their interactions with patrolling ants are not well understood and require further investigation to see whether ants can really deter these herbivores.

In the current study, ant–herbivore interactions in *Banisteriopsis malifolia* (Nees and Martius) B. Gates (Malpighiaceae), an extrafloral nectaried shrub, were investigated. This plant supports the patrolling ant *Camponotus blandus* (Formicinae) and the florivore weevil *Anthonomus* spp. (Coleoptera: Curculionidae). Adult beetles forage in the plant and are therefore subjected to being found by patrolling *C. blandus*, which is aggressive towards herbivores in general (Oliveira et al. 1987). We specifically examined (1) whether *C. blandus* influenced *Anthonomus* florivory rates; and (2) spatial segregation between ants and beetles (leaves or flowers, respectively).

A positive relationship between ant presence and decreased herbivory might be evidence that ants were protecting the plant against beetles and restraining their oviposition in the plant. Nonetheless we also took into account that *C. blandus* might not be able to deter *Anthonomus*, both because of their hard body and a possible spatial segregation. Hence, the effect of ants on herbivory would not be significant.

Material and methods

Study area

The study was conducted in a *stricto sensu* cerrado area (18°59' S, 48°18' W) in Uberlândia City, Brazil, from March to May 2012. This cerrado area covers 230 hectares and is dominated by herbaceous plants, shrubs and trees ranging from 2 to 4 m tall. The climate is characterized by two well-established seasons, a rainy summer and a dry winter. Average rainfall in the region is approximately 1500 mm per year, of which > 90% occurs during the wet season (October to April). Mean monthly temperatures range from 24.8°C in February to 19.9°C in June, with an annual mean temperature of 23°C (Laboratory of Climatology, Federal University of Uberlândia, Brazil, 2011).

Study organisms

Banisteriopsis malifolia is a shrub (< 2 m in height). Fully expanded leaves may reach up to 15 cm in length and 10 cm in width. The leaf blade has tiny trichomes, and the margin is smooth. Leaves possess a pair of prominent EFN at the base near the petiole on each side of the midrib (Figure 1A). EFN are active during the entire rainy season and are frequently visited by *C. blandus* (Alves-Silva, Barônio et al. 2013), a ground-nesting generalist ant species that forages on shrubs and trees and is very common in cerrado vegetation. Production of *B. malifolia* flower buds usually begins in late February or March and peaks in mid-April, and they grow on inflorescences located at the apex of branches. On average, buds are 7 mm in diameter, pink in colour and surrounded by eight oil glands. Both flower buds and young leaves with active EFN occur close to each other (Figure 1A). Flowers are pink, have five free petals and bear chambers formed by the S-shaped sepals, which curl towards the centre of the flower (Del-Claro et al. 1997). *Anthonomus* (Coleoptera: Curculionidae) is found throughout the reproductive season of *B. malifolia*, feeding preferentially on buds (Figure 1B), but can also be found on flowers and more rarely on fruits. Its larvae are endophytic and develop inside flower buds. *Banisteriopsis malifolia* hosts three *Anthonomus* species: *A. rhinotus*, *A. sulcatus* and *Anthonomus* sp., and all of them have a similar natural history and behaviour (see Torezan-Silingardi 2011). Hence, in the current study, all *Anthonomus* species were considered as a single group, following Alves-Silva, Barônio et al. (2013).

Florivory rates

In March 2012, we tagged 30 *B. malifolia* individuals with approximately the same phenological state (presence of flower buds and leaves with active EFN) and height

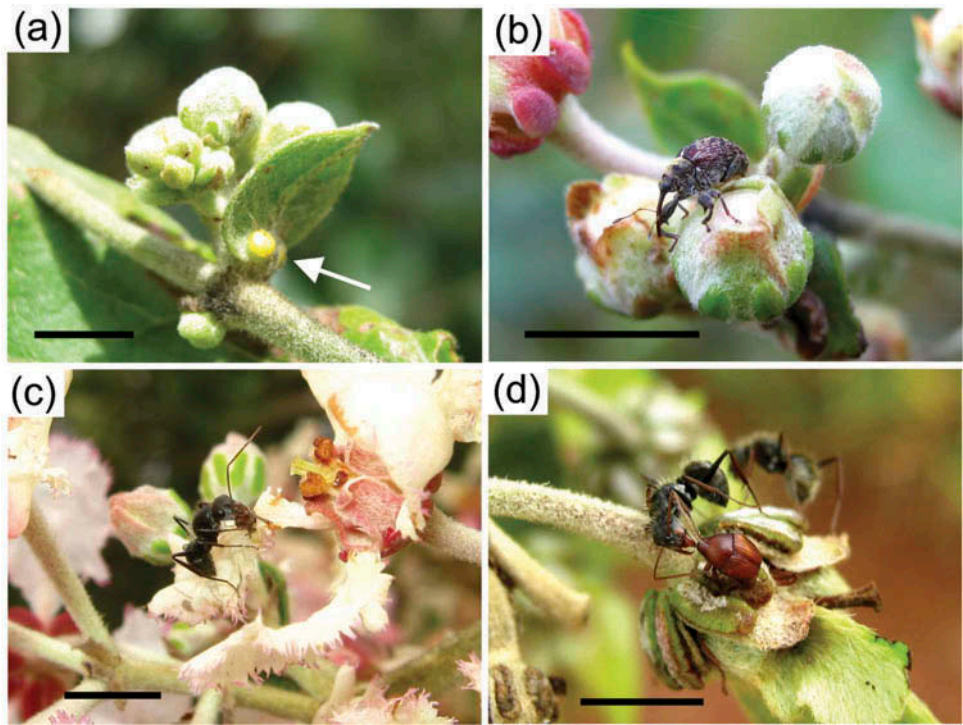


Figure 1. Ant–plant–herbivore interactions in *Banisteriopsis malifolia*. (A) Leaf with active extrafloral nectaries, growing close to flower buds. (B) Adult *Anthonomus*. (C) *Camponotus blandus* foraging on a flower. (D) *C. blandus* attacking an *Anthonomus*. Scale: A, B – 10 mm; C, D, – 5 mm.

(~ 1.60 m). All study plants supported only *C. blandus* and no other EFN-feeding ant was observed on the plants during the study. Plant specimens were distributed evenly over 10 ha within the study area. In each plant, a control and a treatment branch containing flower buds and leaves with active EFN were selected. At the base of each treatment branch, atoxic wax (Tanglefoot™) was applied to prevent access of *C. blandus* (Apple and Feener 2001). The control stem was left unaltered, allowing free access of ants to the entire branch. Leaves and other branches that could be used by ants as bridges to climb on experimental branches were removed. Tagged branches were also deprived of other insect herbivores. Before application of wax, the number of ants and adult beetles on each individual plant was counted.

We initially counted the total number of buds and the ones with signs of *Anthonomus* florivory (i.e. small punctures on bud surface in control and experimental branches). Florivory rate (%) was calculated as the number of damaged flower buds divided by the total number of buds. At the beginning of the study there was no statistical difference in florivory between branches (control = $7.23 \pm 2.32\%$; $M \pm SE$, $n = 4881$ buds; treatment $7.00 \pm 1.69\%$ of damaged buds, $n = 5321$ buds; Wilcoxon test = 110.0; $p = 0.8484$), so discarding study bias. Counting of flower buds (damaged and undamaged) and calculation of the florivory rate were performed in mid-April, just before flowering.

Insect spatial segregation

Spatial segregation of *C. blandus* and *Anthonomus* was examined on control branches from a subsample of 10 *B. malifolia* specimens. The number of ants and weevils in flowers and leaves was counted every 60 min from 08h00 to 17h00. In the field, a total of 60 h of observations (*ad libitum*) were devoted to the behaviour of *C. blandus* towards adult *Anthonomus*. Each plant was observed once for 3 hours (20 individual plants, 08h00 to 11h00) on sunny days. Whenever ants encountered beetles, we recorded whether they were attacked or presented any strategy/behaviour against *C. blandus* attack.

Statistical analyses

Quantitative data are shown as mean \pm SE. In those cases where data did not satisfy the assumptions of a normal distribution ($p < 0.05$) and transformations were unable to achieve data normality, non-parametric statistical tests were used. Florivory rate (%) in control and treatment branches was compared using a Wilcoxon test. The abundance of ants and beetles per plant (assessed in the beginning of the study before application of wax) was compared using a Student's *t*-test (\log_{10} transformed data). The relationship between *C. blandus* and *Anthonomus* abundances was examined using a Pearson correlation test (\log_{10} transformed data). The difference in spatial segregation (flowers or leaves) of ants and beetles was examined using a Student's *t*-test. The same test was used to examine which insect was more abundant in flowers during daytime. Statistical tests were performed in SYSTAT 12[®] and GRAPHPAD PRISM 5.0 softwares.

Results

Florivory by *Anthonomus* in ant-excluded branches was 14% higher than branches with freely roaming ants; however, this difference was not statistically significant (Wilcoxon test = 152.0; $p = 0.3676$) (Figure 2). *Anthonomus* and *C. blandus* abundance per plant was 4.2 ± 0.83 ($n = 126$) and 17.23 ± 2.01 ($n = 517$), respectively ($t = 6.4885$; $df = 58$; $p < 0.0001$). There was no relationship between the abundance of beetles and ants ($r = -0.0430$; $df = 28$; $p = 0.7355$). Both *C. blandus* and *Anthonomus* foraged during the daytime in *B. malifolia*. Ants showed a marked preference for foraging on leaves ($t = 6.3420$; $df = 18$; $p < 0.0001$) (Figure 3A), but they were also observed patrolling flowers (Figure 1C). In contrast, *Anthonomus* foraging activity was concentrated on flowers ($t = 13.1738$; $df = 18$; $p < 0.0001$) (Figure 3B). Even so the abundance of ants on flowers was higher than the abundance of beetles ($t = 2.8271$; $df = 18$; $p < 0.05$; comparing Figure 3A and 3B).

Anthonomus individuals were very mobile and often migrated from buds to flowers and occasionally fruits. *Camponotus blandus* attacked *Anthonomus* on 17 occasions, with most attacks taking place on the main stem of branches and in one instance on a fruit (Figure 1D). During contact with ants, beetles shrank their body and clung strongly to the branch. Ants walked rapidly around and over the *Anthonomus* body, biting and rubbing their abdomen against the beetle to release formic acid. Beetles, however, remained unharmed and attached to the stem. No ant recruiting was noticed. Ants eventually stopped their attack on beetles and backed off

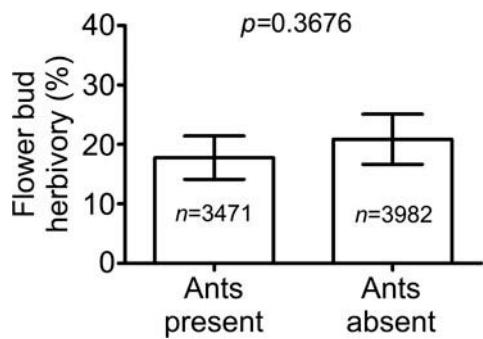


Figure 2. Florivory rates (mean \pm SE) in *Banisteriopsis malifolia* based on the presence or absence of *Camponotus blandus*. *Anthonomus* florivory rates were higher in branches without ants, but this difference was not statistically significant. The number of flower buds analysed in each treatment is given inside bars. $p = 0.3676$ (Wilcoxon test) indicates no statistical significant differences between treatments.

in less than a minute. On a single occasion, we observed an *Anthonomus* individual dropping from one inflorescence as it was touched by a *C. blandus*. However, the beetle fell on a flower and continued to forage in the plant. *Anthonomus* individuals were frequently observed in flower chambers. As such, only the dorsal portion of their bodies was exposed to the exterior of the chamber. A single flower was capable of supporting up to three adult *Anthonomus*, but often only one beetle was found in any given flower.

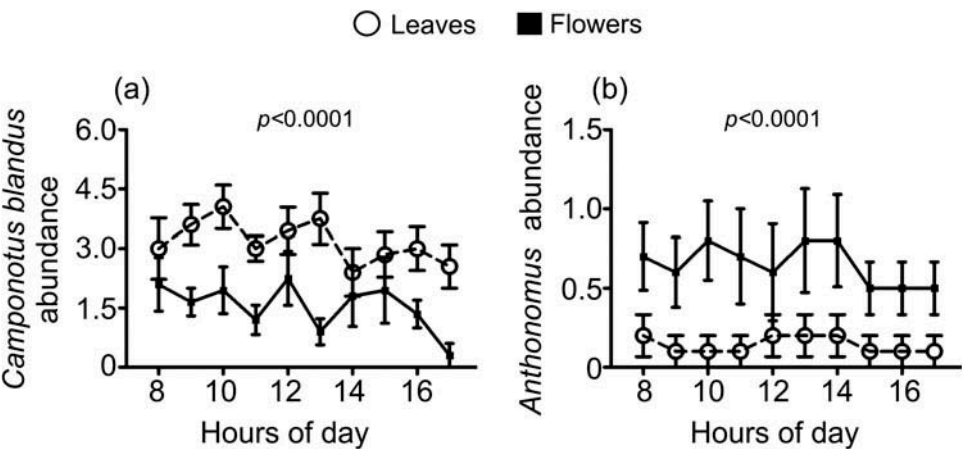


Figure 3. Spatial segregation (mean \pm SE) of *Camponotus blandus* and *Anthonomus* in *Banisteriopsis malifolia*. Ants (A) were more abundant on leaves (circles and dotted lines), while beetles (B) were concentrated on flowers (squares and continuous lines). $p < 0.0001$ (Student's t -tests) indicates statistical significant differences.

Discussion

In general, ants play a decisive role in defending extrafloral nectaried plants against herbivores (Marazzi et al. 2013). Nonetheless, beetles may disrupt ant–plant mutualism, as these hard-bodied insects are rarely preyed upon by ants in natural systems, both because they are usually larger than ants and because of their strong sclerotization (Messina 1981; Koptur and Lawton 1988; Fiala et al. 1989). In the current study, despite the frequent presence of *C. blandus* in the plants, these ants failed to significantly protect *B. malifolia* from *Anthonomus*. In economically important crops, small-sized weevils can be controlled by predatory ants (Peng and Christian 2007; Abera-Kalibata et al. 2008), but this trend was not observed in *B. malifolia*. *Camponotus blandus* (~5 mm) are larger than *Anthonomus* (~2.5 mm), but in contact with ants, no beetle was injured or preyed upon. *Camponotus blandus* aggressive behaviour towards herbivores includes frequent bites, release of acids from the acidopore and the removal of the prey from the plant, so rendering plant protection (Oliveira et al. 1987; Guimaraes et al. 2006). Nonetheless here we showed that a particular herbivore (*Anthonomus*) was not affected by ants, as no adult beetle was injured by *C. blandus*. The population of *B. malifolia* at the study area supports other patrolling ants, such as *Ectatomma* and *Cephalotes* (Alves-Silva 2011), but we have never observed these ants attacking and/or preying upon *Anthonomus*. In fact *Cephalotes* was shown to be an ineffective plant guard in *Ouratea spectabilis* Engl. (Ochnaceae), as it did not protect the plant against curculionids (Byk and Del-Claro 2010).

In *B. malifolia*, as in other extrafloral nectaried Malpighiaceae, EFN are functional during the plant reproductive season, and leaves with EFN grow next to inflorescences, which might indicate that the foraging behaviour of ants would extend to flowers (Del-Claro et al. 1997; Reu and Del-Claro 2005). In fact, Possobom et al. (2010) observed *Camponotus* ants patrolling both vegetative and reproductive structures of *Diplopterys pubipetala* (A. Juss.) W.R. Anderson & C. Cav. Davis (Malpighiaceae), and also exhibiting aggressive behaviour. However, in the current study, *C. blandus* foraged mostly on leaves. *Camponotus blandus* is a sugar-dependent ant species (Guimarães et al. 2006), and in *B. malifolia*, this ant species does not visit the inflorescences very often, except when it tends myrmecophilous insects (Alves-Silva, Bächtold et al. 2013). Hence the flower-dwelling behaviour of *Anthonomus* in *B. malifolia* flowers is advantageous for beetles, as they are seldom disturbed by ants in this structure.

Anthonomus in *B. malifolia* can damage up to 20% of flower buds, greatly influencing plant reproductive outputs (Alves-Silva, Barônio et al. 2013). Nonetheless, these weevils are preyed upon by the nectar-drinking social Polistinae wasp, *Brachygastra lecheguana*. As shown by Alves-Silva, Barônio et al. (2013) this wasp can reduce up to 50% of the beetle population in *B. malifolia*, acting as an effective plant guard. Nonetheless the authors demonstrated that *C. blandus* may sometimes attack and displace *B. lecheguana* from the plant. Hence, in addition to not protecting *B. malifolia* from *Anthonomus*, *C. blandus* may also interrupt the predatory behaviour of wasps, which may otherwise control the weevil population. Antagonistic behaviour of ants on predatory wasps was also reported by Cuautle and Rico-Gray (2003), who demonstrated that ant-excluded plants were more frequently visited by wasps, which controlled herbivore populations (see also Mody et al. 2011;

Pereira and Trigo 2013). Other studies have also suggested that ants may indirectly benefit herbivore populations by preying on or interfering with the predatory behaviour of parasitoids (Pierce and Mead 1981) and spiders (Mody and Linsenmair 2004; Nahas et al. 2012).

Three specific instances in which *C. blandus* was not beneficial to *B. malifolia* were described in the current study. First, no adult *Anthonomus* was preyed upon or expelled from the plant by ants. Second, *Anthonomus* larvae are endophytic, so are protected from ants during the entire immature stage. Third, adult weevils and ants usually occupied different regions of the plant, restraining the probability of encounters. We can also list that *C. blandus* may negatively interfere with the predatory behaviour of *B. lecheguana*, which is the main natural enemy of *Anthonomus* (Alves-Silva, Barônio et al. 2013). In summary, in this specific system, it can be suggested that the presence of *C. blandus* was not beneficial for the plant.

Ant–herbivore systems can provide new and unexpected situations to enrich the knowledge of biotic interactions. Asymmetries in ant–plant mutualism (where ants receive nectar but do not protect the plant against herbivores) are still poorly understood (Byk and Del-Claro 2010; Riedel et al. 2013), but can contribute to the advances of ant–plant interactions, especially in the tropics where extrafloral nectar-ied plants are abundant and diverse (Machado et al. 2008).

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References

- Abera-Kalibata AM, Gold CS, Van Driesche R. 2008. Experimental evaluation of the impacts of two ant species on banana weevil in Uganda. *Biol Control*. 46:147–157. doi:10.1016/j.biocontrol.2008.04.017
- Alves-Silva E. 2011. Post fire resprouting of *Banisteriopsis malifolia* (Malpighiaceae) and the role of extrafloral nectaries on the associated ant fauna in a Brazilian savanna. *Sociobiology*. 58:327–339.
- Alves-Silva E, Bächtold A, Barônio GJ, Del-Claro K. 2013. Influence of *Camponotus blandus* (Formicinae) and flower buds on the occurrence of *Parrhasius polibetes* (Lepidoptera: Lycaenidae) in *Banisteriopsis malifolia* (Malpighiaceae). *Sociobiology*. 60:30–34. doi:10.13102/sociobiology.v60i1.30-34
- Alves-Silva E, Barônio GJ, Torezan-Silingardi HM, Del-Claro K. 2013. Foraging behavior of *Brachygastra lecheguana* (Hymenoptera: Vespidae) on *Banisteriopsis malifolia* (Malpighiaceae): Extrafloral nectar consumption and herbivore predation in a tending ant system. *Entomol Sci*. 16:162–169. doi:10.1111/ens.12004

- Alves-Silva E, Del-Claro K. 2013. Effect of post-fire resprouting on leaf fluctuating asymmetry, extrafloral nectar quality, and ant–plant–herbivore interactions. *Naturwissenschaften*. 100:525–532. doi:[10.1007/s00114-013-1048-z](https://doi.org/10.1007/s00114-013-1048-z)
- Apple JA, Feener Jr DF. 2001. Ant visitation of extrafloral nectaries of *Passiflora*: The effects of nectary attributes and ant behavior on patterns in facultative ant–plant mutualisms. *Oecologia*. 127:409–416. doi:[10.1007/s004420000605](https://doi.org/10.1007/s004420000605)
- Bächtold A, Alves-Silva E. 2013. Behavioral strategy of a lycaenid (Lepidoptera) caterpillar against aggressive ants in a Brazilian savanna. *Acta Ethol*. 16:83–90. doi:[10.1007/s10211-012-0140-2](https://doi.org/10.1007/s10211-012-0140-2)
- Bächtold A, Alves-Silva E, Del-Claro K. 2013. Lycaenidae larvae feeding on *Peixotoa parviflora* (Malpighiaceae) in a semi-deciduous forest in southeastern Brazil. *J Lep Soc*. 67:65–67.
- Blüthgen N, Menzel F, Hovestadt T, Fiala B, Blüthgen N. 2007. Specialization, constraints, and conflicting interests in mutualistic networks. *Curr Biol*. 17:341–346. doi:[10.1016/j.cub.2006.12.039](https://doi.org/10.1016/j.cub.2006.12.039)
- Bronstein JL, Alarcón R, Geber M. 2006. The evolution of plant–insect mutualisms. *New Phytol*. 172:412–428. doi:[10.1111/j.1469-8137.2006.01864.x](https://doi.org/10.1111/j.1469-8137.2006.01864.x)
- Brouat C, Garcia N, Andary C, McKey D. 2001. Plant lock and ant key: pairwise coevolution of an exclusion filter in an ant–plant mutualism. *Proc Royal Soc B: Biol Sci*. 268:2131–2141. doi:[10.1098/rspb.2001.1763](https://doi.org/10.1098/rspb.2001.1763)
- Byk J, Del-Claro K. 2010. Nectar- and pollen-gathering *Cephalotes* ants provide no protection against herbivory: a new manipulative experiment to test ant protective capabilities. *Acta Ethol*. 13:33–38. doi:[10.1007/s10211-010-0071-8](https://doi.org/10.1007/s10211-010-0071-8)
- Cuautle M, Rico-Gray V. 2003. The effect of wasps and ants on the reproductive success of the extrafloral nectaried plant *Turnera ulmifolia* (Turneraceae). *Funct Ecol*. 17:417–423. doi:[10.1046/j.1365-2435.2003.00732.x](https://doi.org/10.1046/j.1365-2435.2003.00732.x)
- Dejean A, Grangier J, Leroy C, Orivel J. 2009. Predation and aggressiveness in host plant protection: a generalization using ants from the genus *Azteca*. *Naturwissenschaften*. 96:57–63. doi:[10.1007/s00114-008-0448-y](https://doi.org/10.1007/s00114-008-0448-y)
- Del-Claro K. 1998. A Importância do comportamento de formigas em interações: Um exemplo no Cerrado: Formigas e tripses em *Peixotoa tomentosa* (Malpighiaceae). *J Ethol*. 1:3–10.
- Del-Claro K. 2004. Multitrophic relationships, conditional mutualisms, and the study of interaction biodiversity in tropical savannas. *Neotrop Entomol*. 33:665–672. doi:[10.1590/S1519-566X2004000600002](https://doi.org/10.1590/S1519-566X2004000600002)
- Del-Claro K, Marullo R, Mound LA. 1997. A new Brazilian species of *Heterothrips* (Insecta: Thysanoptera) co-existing with ants in the flowers of *Peixotoa tomentosa* (Malpighiaceae). *J Nat Hist*. 31:1307–1312. doi:[10.1080/00222939700770731](https://doi.org/10.1080/00222939700770731)
- Fernandes GW, Fagundes M, Greco MKB, Barbeitos MS, Santos JC. 2005. Ants and their effects on an insect herbivore community associated with the inflorescences of *Byrsonima crassifolia* (Linnaeus) H.B.K. (Malpighiaceae). *Rev Bras Entomol*. 49:264–269. doi:[10.1590/S0085-56262005000200011](https://doi.org/10.1590/S0085-56262005000200011)
- Ferreira CA, Torezan-Silingardi HM. 2013. Implications of the floral herbivory on malpighiacea plant fitness: visual aspect of the flower affects the attractiveness to pollinators. *Sociobiology*. 60:323–328. doi:[10.13102/sociobiology.v60i3.323-328](https://doi.org/10.13102/sociobiology.v60i3.323-328)
- Fiala B, Maschwitz U, Pong TY, Helbig AJ. 1989. Studies of a South East Asian ant–plant association – protection of *Macaranga* trees by *Crematogaster borneensis*. *Oecologia*. 79:463–470. doi:[10.1007/BF00378662](https://doi.org/10.1007/BF00378662)
- Flinte V, Araujo CO, Macedo MV, Monteiro RF. 2006. Insetos fitófagos associados ao murici da praia, *Byrsonima sericea* (Malpighiaceae), na Restinga de Jurubatiba (RJ). *Rev Bras Entomol*. 50:512–523. doi:[10.1590/S0085-56262006000400012](https://doi.org/10.1590/S0085-56262006000400012)

- Fuente MAS, Marquis RJ. 1999. The role of ant-tended extrafloral nectaries in the protection and benefit of a Neotropical rainforest tree. *Oecologia*. 118:192–202. doi:10.1007/s004420050718
- Guimarães Jr PR, Raimundo RLG, Bottcher C, Silva RR, Trigo JR. 2006. Extrafloral nectaries as a deterrent mechanism against seed predators in the chemically protected weed *Crotalaria pallida* (Leguminosae). *Austral Ecol.* 31:776–782. doi:10.1111/j.1442-9993.2006.01639.x
- Hedges PA, Lawton JH. 1985. Bracken, ants and extrafloral nectaries. III. How insect herbivores avoid ant predation. *Ecol Entomol.* 10:29–42. doi:10.1111/j.1365-2311.1985.tb00532.x
- Junker RR, Blüthgen N. 2008. Floral scents repel potentially nectar-thieving ants. *Evol Ecol Res.* 10:295–308.
- Katayama N, Suzuki N. 2004. Role of extrafloral nectaries of *Vicia faba* in attraction of ants and herbivore exclusion by ants. *Entomol Sci.* 7:119–124. doi:10.1111/j.1479-8298.2004.00057.x
- Koptur S. 1979. Facultative mutualism between weedy vetches bearing extrafloral nectaries and weedy ants in California. *Am J Bot.* 66:1016–1020. doi:10.2307/2442565
- Koptur S, Lawton JH. 1988. Interactions among vetches bearing extrafloral nectaries, their biotic protective agents, and herbivores. *Ecology*. 69:278–283. doi:10.2307/1943183
- Kost C, Heil M. 2005. Increased availability of extrafloral nectar reduces herbivory in Lima bean plants (*Phaseolus lunatus*, Fabaceae). *Basic Appl Ecol.* 6:237–248. doi:10.1016/j.baae.2004.11.002
- Longino JT. 1989. Geographic variation and community structure in an ant–plant mutualism: *Azteca* and *Cecropia* in Costa Rica. *Biotropica*. 21:126–132. doi:10.2307/2388703
- Machado SR, Morellato LPC, Sajo MG, Oliveira PS. 2008. Morphological patterns of extrafloral nectaries in woody plant species of the Brazilian cerrado. *Plant Biol.* 10:660–673. doi:10.1111/j.1438-8677.2008.00068.x
- Marazzi B, Bronstein JL, Koptur S. 2013. The diversity, ecology and evolution of extrafloral nectaries: current perspectives and future challenges. *Ann Botany*. 111:1243–1250. doi:10.1093/aob/mct109
- Messina FJ. 1981. Plant protection as a consequence of an ant–membracid mutualism: Interactions on goldenrod (*Solidago* Sp.). *Ecology*. 62:1433–1440. doi:10.2307/1941499
- Mody K, Linsenmair KE. 2004. Plant-attracted ants affect arthropod community structure but not necessarily herbivory. *Ecol Entomol.* 29:217–225. doi:10.1111/j.1365-2311.2004.0588.x
- Mody K, Spoerndli C, Dorn S. 2011. Within-orchard variability of the ecosystem service ‘parasitism’: effects of cultivars, ants and tree location. *Basic Appl Ecol.* 12:456–465. doi:10.1016/j.baae.2011.05.005
- Nahas L, Gonzaga MO, Del-Claro K. 2012. Emergent impacts of ant and spider interactions: Herbivory reduction in a tropical savanna tree. *Biotropica*. 44:498–505. doi:10.1111/j.1744-7429.2011.00850.x
- Ness JH. 2003. *Catalpa bignonioides* alters extrafloral nectar production after herbivory and attracts ant bodyguards. *Oecologia*. 134:210–218.
- Nogueira A, Guimarães E, Machado S, Lohmann L. 2012. Do extrafloral nectaries present a defensive role against herbivores in two species of the family Bignoniaceae in a Neotropical savannas? *Plant Ecol.* 213:289–301. doi:10.1007/s11258-011-9974-3
- Oliveira P, Da Silva A, Martins A. 1987. Ant foraging on extrafloral nectaries of *Qualea grandiflora* (Vochysiaceae) in cerrado vegetation: ants as potential antiherbivore agents. *Oecologia*. 74:228–230. doi:10.1007/BF00379363
- Oliveira PS. 1997. The ecological function of extrafloral nectaries: Herbivore deterrence by visiting ants and reproductive output in *Caryocar brasiliense* (Caryocaraceae). *Funct Ecol.* 11:323–330. doi:10.1046/j.1365-2435.1997.00087.x

- Oliveira PS, Rico-Gray V, Castillo-Guevara C, Díaz-Castelazo C. 1999. Interaction between ants, extrafloral nectaries and insect herbivores in Neotropical coastal sand dunes: Herbivore deterrence by visiting ants increases fruit set in *Opuntia stricta* (Cactaceae). *Funct Ecol.* 13:623–631. doi:[10.1046/j.1365-2435.1999.00360.x](https://doi.org/10.1046/j.1365-2435.1999.00360.x)
- Peng R, Christian K. 2007. The effect of the weaver ant, *Oecophylla smaragdina* (Hymenoptera: Formicidae), on the mango seed weevil, *Sternuchus mangiferae* (Coleoptera: Curculionidae), in mango orchards in the Northern Territory of Australia. *Int J Pest Manage.* 53:15–24. doi:[10.1080/09670870600968859](https://doi.org/10.1080/09670870600968859)
- Pereira MF, Trigo JR. 2013. Ants have a negative rather than a positive effect on extrafloral nectaried *Crotalaria pallida* performance. *Acta Oecol.* 51:49–53. doi:[10.1016/j.actao.2013.05.012](https://doi.org/10.1016/j.actao.2013.05.012)
- Pierce NE, Mead PS. 1981. Parasitoids as selective agents in the symbiosis between lycaenid butterfly larvae and ants. *Science.* 211:1185–1187. doi:[10.1126/science.211.4487.1185](https://doi.org/10.1126/science.211.4487.1185)
- Possobom C, Guimarães E, Machado S. 2010. Leaf glands act as nectaries in *Diplopterys pubipetala* (Malpighiaceae). *Plant Biol.* 12:863–870. doi:[10.1111/j.1438-8677.2009.00304.x](https://doi.org/10.1111/j.1438-8677.2009.00304.x)
- Rashbrook VK, Compton SG, Lawton JH. 1992. Ant–herbivore interactions: reasons for the absence of benefits to a fern with foliar Nectaries. *Ecology.* 73:2167–2174. doi:[10.2307/1941464](https://doi.org/10.2307/1941464)
- Reu Jr WF, Del-Claro K. 2005. Natural history and biology of *Chlamisus minax* Lacordaire (Chrysomelidae: Chlamisinae). *Neotrop Entomol.* 34:357–362. doi:[10.1590/S1519-566X2005000300001](https://doi.org/10.1590/S1519-566X2005000300001)
- Riedel J, Dorn S, Brand G, Barrios H, Mody K. 2013. Effects of ants on arthropod assemblages of a native timber tree in a tropical reforestation plantation. *J Appl Entomol.* 137:418–428. doi:[10.1111/jen.12009](https://doi.org/10.1111/jen.12009)
- Rocha CFD, Bergallo HG. 1992. Bigger ant colonies reduce herbivory and herbivore residence time on leaves of an ant–plant: *Azteca muelleri* vs. *Coelomera ruficornis* on *Cecropia pachystachya*. *Oecologia.* 91:249–252. doi:[10.1007/BF00317792](https://doi.org/10.1007/BF00317792)
- Rosumek F, Silveira FS, Neves FU, Barbosa N, Diniz L, Oki Y, Pezzini F, Fernandes G, Cornelissen T. 2009. Ants on plants: a meta-analysis of the role of ants as plant biotic defenses. *Oecologia.* 160:537–549. doi:[10.1007/s00442-009-1309-x](https://doi.org/10.1007/s00442-009-1309-x)
- Torezan-Silingardi HM. 2011. Predatory behavior of *Pachodynerus brevithorax* (Hymenoptera: Vespidae, Eumeninae) on endophytic herbivore beetles in the Brazilian Tropical Savanna. *Sociobiology.* 57:181–189.
- Wagner D, Kay A. 2002. Do extrafloral nectaries distract ants from visiting flowers? An experimental test of an overlooked hypothesis. *Evol Ecol Res.* 4:293–305.