Environmental Entomology, 47(5), 2018, 1165–1172
doi: 10.1093/ee/nvy097
Advance Access Publication Date: 11 July 2018
Research

Natural History of a Sit-and-Wait Dipteran Predator That Uses Extrafloral Nectar as Prey Attractant

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Subject Editor: Heather McAuslane

Received 17 January 2018; Editorial decision 9 June 2018

Abstract

Sit-and-wait predators use different strategies to encounter potential prey. *Rhinoleucophenga myrmecophaga* Vidal et (Vidal et Vilela; Diptera: Drosophilidae) larvae build sticky shelters on top of extrafloral nectaries (EFNs) of *Qualea grandiflora* Mart (Vochysiaceae), a common plant in the Brazilian cerrado savanna. Although larval shelters block the EFNs, nectar production is not obstructed and is used by the larvae to attract and trap nectar-gathering ants that are eventually eaten by the dipteran. Here we describe the natural history of *R. myrmecophaga*, its infestation pattern in *Q. grandiflora*, the ant assemblage at EFNs, and the insects used as prey. We use stable isotope composition ($\delta^{13}C$ and $\delta^{15}N$) of *R. myrmecophaga* and potential food sources to infer its diet, and perform chemical analyses of the droplets found at shelter openings to determine whether nectar is used as a prey attractant. We found that *Rhinoleucophenga* larvae occur on the majority of *Qualea* plants and occupy active EFNs mainly in the rainy season. The two most frequent visiting species were also the most common insects found trapped at larval shelters. The stable isotope analyses confirmed that ants are the main food sources of *R. myrmecophaga*. Chemical analyses and field observations revealed that *Rhinoleucophenga* larvae use extrafloral nectar to attract prey to their shelters by pushing this liquid to the shelter opening where it forms a droplet. This is a rare case of sit-and-wait predator exploiting an ant-plant mutualism through the use of the very food reward produced by the plant to attract and capture potential ant mutualists.

Key words: sit-and-wait predator, cerrado, insect behavior, trophic interaction, Drosophilidae

Intimidation and direct consumption by predators have detrimental effects on prey density (Preisser et al. 2005). However, different types of hunting behavior by predators may have varied impact on lower trophic levels (Schmitz and Suttle 2001). For example, spiders vary in the range of prey used in different food webs depending on whether they are active hunters or passive web-builders (Wimp et al. 2013). Furthermore, spiders with sit-and-wait and sit-and-pursue behaviors can cause a shift on the diet of their prey (Schmitz and Suttle 2001); the prey can also change their foraging paths when threatened by the constant presence of a sit-and-wait predator (i.e., ants as prey, MacKay 1982).

Although sit-and-wait predators usually have lower energetic expenses than active foragers (Huey and Pianka 1981), they have to cope with the uncertainty of encountering potential prey while waiting. Therefore, sit-and-wait predators may use different strategies to increase their chances of prey coming across them, such as traps (e.g., antlions, Griffiths 1980), color attraction (e.g., orb-weaving spiders, Hauber 2002), camouflage in a substrate that will be visited by the prey while foraging (e.g., crab spiders in flowers, Théry and Casas 2002, Heiling et al. 2005), or mimicry of a foraging source (e.g., mantis mimicking flowers, O'Hanlon et al. 2014). Waiting for prey in a constantly visited food source may be an effective strategy to increase the chance of encountering a prey. Additionally, using the food source itself as an attractor could dramatically enhance the chance of capturing a potential prey. However, this type of sit-and-wait strategy has never been shown before.

Extrafloral nectaries (EFNs) are nectar-producing glands not directly involved with the pollination process (Bentley 1977). Due to their high nutritional content, which includes sugars, amino acids, lipids, and other organic compounds, extrafloral nectar may attract a variety of visitors

(Koptur 1992). Ants are usually the main visitors to these glands, including them as part of their territory and behaving aggressively towards other insects feeding on the plant (reviews in Koptur 1992, 2005, Rico-Gray and Oliveira 2007). *Rhinoleucophenga myrmecophaga* Vidal et Vilela (Diptera: Drosophilidae) larvae live on top of EFNs of a common Neotropical savanna plant (*Qualea grandiflora* Mart., Vochysiaceae) and use the extrafloral nectar to attract and trap their prey (Fig. 1a and b, Vidal and Vilela 2015, Vidal et al. 2016). The dipteran predator cannot move from one site to another, so it depends entirely on the chance of trapping EFN visitors to obtain its prey. By using EFNs as a foraging site and the nectar as an attractant, the chance of successfully encountering potential prey is high for *R. myrmecophaga*, which can also exploit the constant source of sugar and water for its own development.

Our previous research has shown that the presence of ant-preying *R. myrmecophaga* larvae reduces ant visitation to foliage and disrupts the mutualism between ants and EFN-bearing *Qualea* plants (Vidal et al. 2016). Here, we present a detailed description of the life strategy and behavior of *R. myrmecophaga*. Specifically, we describe *R. myrmecophaga* oviposition, larval behavior, and interaction with prey. We describe the assembly of ants associated with *Qualea*'s EFNs and the array of prey items used by *R. myrmecophaga* larvae. Using stable isotopes, we provide evidence of the use of ants as prey, as well as chemical evidence of the use of extrafloral nectar as a prey attractant.

Materials and Methods

Study Site and System

We conducted behavioral observations and samplings from December 2009 to March 2012 in an area of cerrado savanna at Itirapina, São Paulo State, Brazil (22° 15′ S, 47° 49′ W). The vegetation consists of a dense scrub of shrubs and trees, which corresponds to the cerrado sensu stricto (Oliveira-Filho and Ratter 2002). The climate of the region is characterized by a dry/cold season (May to September) and warm/rainy season (November to March).

Q. grandiflora is an abundant cerrado plant bearing EFNs (Fig. 1a), which attracts many ant species that protect the plant against herbivores (Oliveira et al. 1987, Costa et al. 1992). *Qualea* plants have two pairs of EFNs next to the insertion of each pair of leaves (node) (Fig. 1a), and each plant has usually 6 or 8 nodes per branch. In addition to ants, many arthropods may use the EFNs of *Qualea* as a food source, including spiders, flies, grasshoppers, hemipterans, beetles, and wasps (Nahas et al. 2012, M.C. Vidal personal observation).

Natural History and Infestation Pattern of *R. myrmecophaga* Associated With *Q. grandiflora*

To investigate the infestation pattern of *R. myrmecophaga* in our study site, during the summer (December and January of 2009) and winter (July and August of 2009), we used 40 *Q. grandiflora* plants along eight transects, each containing five plants. Transects were 50 m apart from one another, and we selected the first five *Q. grandiflora* individuals encountered within each transect as focal plants (transects were not the same length). *Qualea* plants varied from 0.5 to 3.0 m of height. For each plant, we recorded the total number of branches and the total number of *R. myrmecophaga* larvae, and then randomly selected five branches in which we recorded the number of active and nonactive EFNs, and the number and location of larval shelters. We determined whether an EFN was active by visual inspection, focusing on the gland's external

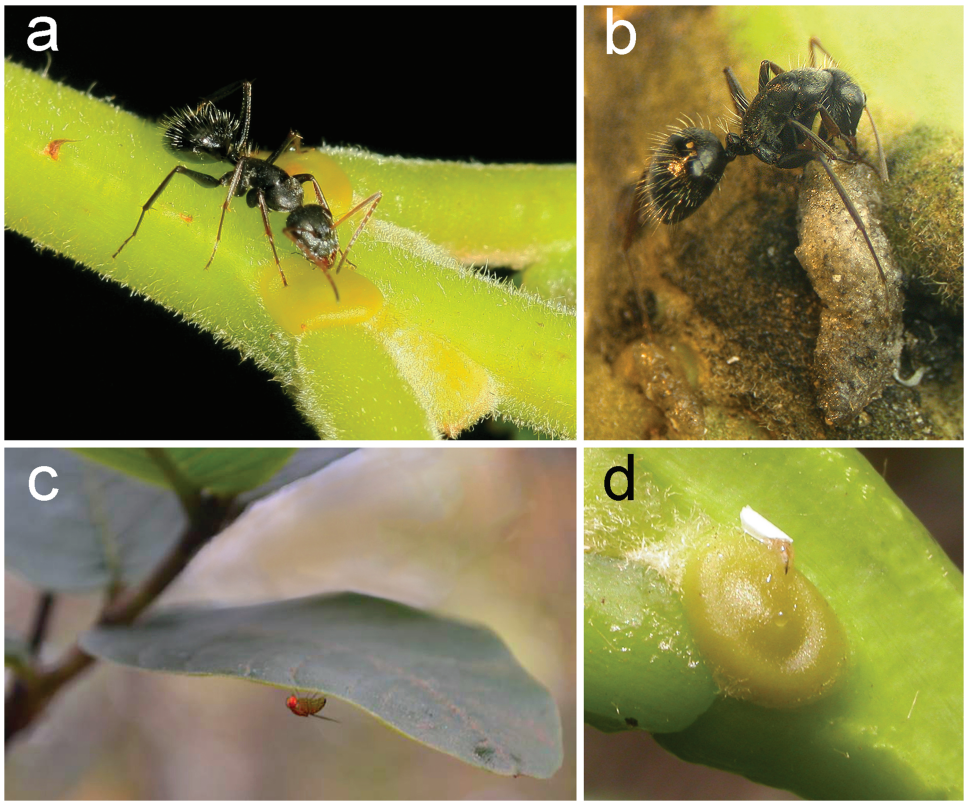

Fig. 1. (a) Worker of *Camponotus* visiting the EFNs of *Q. grandiflora*, (b) worker of *Camponotus* trapped at a larval shelter of *R. myrmecophaga* built on top of an EFN of *Q. grandiflora*; the ant will be sucked empty by the larva hidden in the shelter, (c) female of *R. myrmecophaga* resting under a *Q. grandiflora* leaf, and (d) dipteran larva reaching the EFN right after hatching.

coloration and absence of mold; an active EFN usually has a bright green color. We cannot use presence or absence of nectar droplet as a reliable measure of the EFN activity because EFNs in the area are constantly visited by ants and rarely have accumulated nectar. We also measured the length of larval shelters and their location in the branch: abaxial or adaxial side of the branch, and basal, middle, or apex portion of the branch. We used chi-square to test if infestation of *Q. grandiflora* by *R. myrmecophaga* differed between seasons (summer vs winter) and among different portions of the branches.

We observed the oviposition behavior of *R. myrmecophaga* on the EFNs of *Q. grandiflora* in the field site from October to November 2012, focusing on plants that were known to have had ovipositions in the past and that had young and active EFNs. To identify the preferred time for oviposition by the dipteran, we observed *Qualea* plants from 09.00 to 20.00 h. After we saw the first oviposition, we focused our observations from dusk hours up to approximately 20.00 h, after which the adult flies were difficult to see.

To describe the larval behavior, from December 2009 to March 2010, we marked 20 individual larvae at initial stage (first or second instar). For over 30 h of observation, we watched each larva for 10 min during the day (both morning and afternoon) for a total of 9 series of observations per larva; observations of each larva were spaced on multiple days. We paid special attention to interactions between larvae and ants, release of droplets at larval shelters, larval movements of head at shelter opening, and overall shelter characteristics (numbers of openings, presence of prey bodies, and shelter coloration).

Visiting Ants at *Q. grandiflora*, and Prey Items Used by *R. myrmecophaga* Larvae

To identify the visiting ants of *Q. grandiflora*, we used 30 plants from 0.5 to 2.0 m high. In each plant, we conducted four ant samplings in January 2010, at 01.00, 07.00, 13.00, and 19.00 h. We used the key by Fernández (2003) to identify ant genera and grouped the collected ants in morphospecies.

Since *R. myrmecophaga* larvae consume only the internal content of their prey, the exoskeletons of the prey items remain attached to the larval shelter for some time, which makes it possible to sample and identify a good range of prey items used by the larva. We collected 78 prey items from different larval shelters found on 40 different plants from November 2009 to February 2011. Some of the prey items collected were degraded and we could not identify them to species. We compared the spectrum of collected prey at larval shelters by *R. myrmecophaga* with the assembly of ants visiting *Q. grandiflora* plants to see whether there was a specific prey type being used by the dipteran larvae.

Trophic Ecology of *R. myrmecophaga*

To investigate to what extent *R. myrmecophaga* uses visiting ants of *Q. grandiflora* EFNs as a food source, we analyzed the $\delta^{13}C$ and $\delta^{15}N$ of *R. mrymecophaga* larvae ($n = 4$ samples), ants visiting EFNs of *Q. grandiflora* ($n = 4$ species), *Q. grandiflora* leaves ($n = 8$ leaves from different individuals), and common herbivores ($n = 12$ samples from five species) and predators ($n = 4$ samples from three species) that share the habitat. We collected all samples between July and December 2010. *Camponotus rufipes*, *Cephalotes pusillus*, and small Formicinae ants (*Brachymyrmex* or *Myrmelachista*) formed the ant samples, in which we got at least two samples from each species. *Ca. rufipes* are aggressive ants that use animal sources in addition to extrafloral nectar as food (Oliveira et al. 1987), whereas *Ce. pusillus* are passive ants feeding mainly on plant exudates (Sendoya et al. 2009, Byk and Del-Claro 2010). This protocol provided a wide range of the trophic levels occupied by ants that visit the plant, and that likely fall prey to *R. myrmecophaga* larvae. We divided the herbivore samples into two groups: lepidopteran larvae that were feeding exclusively on *Qualea* leaves (Geometridae, $n = 5$ samples), and other herbivores not necessarily feeding exclusively on *Qualea* and collected with entomological net in our study area ($n = 7$ samples). For the latter, we included three grasshoppers (Orthoptera), one cicada (Cicadoidea), two true bugs (Pentatomidae), and one walking stick (Phasmatodea). Predators ($n = 4$ samples) were also collected with entomological net around *Q. grandiflora* plants; we used one dragonfly (Odonata), one predatory hemipteran (Reduvidae), and two praying mantises of the same species (Mantodea).

After collection, we immediately froze our samples at −18°C, and then dried the organisms at 60°C. Considering the small size of the larvae, samples of *R. myrmecophaga* were a combination of several individuals; therefore, we had four isotope samples of multiple individuals, each weighing at least 1 mg. For the other animal samples, all weighed ~2.5 mg (at least 1.5 mg) and plant samples weighed ~3.5 mg. Homogenized materials were weighed in tin-cups and analyzed for $\delta^{13}C$ and $\delta^{15}N$ in an isotope-ratio mass spectrometer (Finnigan-MAT; CA, USA) in line with an Elemental Analyzer. Stable isotope ratios of carbon and nitrogen were measured relative to established international standards, atmospheric N_2, and Vienna Pee Dee Belemnite (VPDB) carbon. The isotope discrimination is expressed in 'delta' notation (‰), where the isotopic composition of a material relative to that of a standard on a per mill deviation basis is given by δ‰ = (R sample/R standard − 1)*1000, where R is the molecular ratio of heavy to light isotope forms (e.g., $^{13}C/^{12}C$). All analyses were performed at the Center of Nuclear Energy in Agriculture (CENA) of the University of São Paulo.

The stable isotope of nitrogen ($\delta^{15}N$) can be used to infer trophic position, since an enrichment in the heavier isotopes from source to consumer is expected (Vanderklift and Ponsard 2003). The amount of increment might vary for different organisms; however, the meta-analysis by Vanderklift and Ponsard (2003) showed an enrichment of 1.43–1.82‰ for the carnivorous fly *Calliphora vicina* R.-D. (Diptera: Calliphoridae). The stable carbon isotope composition ($\delta^{13}C$) changes little among trophic levels, and in terrestrial ecosystem, it differs mainly due to different photosynthetic pathways of plants (Peterson and Fry 1987). With this information and comparison among samples, we can infer which trophic level *R. myrmecophaga* larvae occupy. For instance, when we compare lepidopteran larvae and the *Qualea* leaves that they were feeding on, we would expect an increment on the nitrogen stable isotope of larvae relative to leaves, but no difference in the carbon isotope. Samples of each group (ants, predators, herbivores, lepidopteran herbivores, *Qualea*, and dipteran larvae) were investigated using analyses of variance and posterior Tukey's HSD test for both nitrogen and carbon isotopes, all were performed using R environment 3.4.1 (R Development Core Team 2011). We did not calculate a mixing model (Phillips and Gregg 2001) because of small sample sizes and the lack of direct measure of stable isotopes of extrafloral nectar.

Chemical Analyses of *Q. grandiflora* EFN, and of Droplets Released by *R. myrmecophaga*

To determine whether *R. myrmecophaga* larvae are using the nectar that accumulates inside their shelters as an attractant to potential prey, we compared the chemical composition of *Qualea* extrafloral nectar and the droplets released by *R. myrmecophaga* at the shelter openings. To collect the nectar, we isolated branches of *Q. grandiflora* with Tanglefoot (Grand Rapids, MI, USA) at 19.00 h, to impede nectar feeding by nocturnal ants and other walking arthropods. In

the beginning of the next morning (07.00 h), we collected the extrafloral nectar using microcapillary tubes. To collect the larval droplets, we observed larvae for 20 min and collected the released liquid with microcapillary tubes. We collected extrafloral nectar from nine different *Qualea* plants in the wet (January) and dry season (April), and three droplet samples from different larvae (March).

We analyzed the samples using mass spectrometry (micrOTOF-Q II Bruker, Billerica, MA, USA) with direct injection. We used the following parameters: capillary voltage of 4500 V; nebulizer and dry gas (N_2) were 300.03 mm Hg and 4 l/min, respectively; collision and quadrupole energy were 12 and 6 eV, respectively; and water flow at 180 µl/min as mobile phase. The chemical analyses were performed at the Laboratory of Chemistry of Natural Products, University of São Paulo. We also measured the sugar concentration of *Qualea* extrafloral nectar in the field using sucrose hand-held refractometer HSR-500 (Atago Inc., Bellevue, WA, USA).

Results

Natural History of *R. myrmecophaga* Associated With *Q. grandiflora*

Our survey revealed that 85% of the *Qualea* trees sampled were infested with *R. myrmecophaga* larvae. Infestation was higher in the summer than in the winter (X^2 = 4.92, df = 1, P = 0.026); 82% of the 234 recorded shelters were found in the summer. Only one larva was alive in the winter (n = 42 shelter remains found), and it is thus possible that the shelters found in the winter were residuals of the summer infestation. In the summer, live larvae were found mostly at the abaxial EFNs located on the apex of the branches (95%, n = 138 larvae). Overall, we found 22 more larval shelters on the apex than on the middle or basal portions of the branches (X^2 = 67.58, df = 2, P < 0.001), and four times more shelters in the abaxial than in the adaxial face (X^2 = 60.45, df = 1, P < 0.001). EFNs on the apex of branches tend to be more active than on the base (M.C. Vidal personal observation). The larval shelters measured on average 4.2 ± 1.63 (st. dev.) cm in length.

We observed three oviposition events by *R. myrmecophaga* in the twilight, around 19.00 h, in October and November 2012. The eggs were laid near the opening of the EFNs of *Q. grandiflora*, a pattern also observed on *Qualea multiflora* and *Bauhinia rufa* (Vidal and Vilela 2015). Females typically lay one egg per EFN, but more than one egg on the same plant. During the oviposition process, females tend to visit more than one branch, and apparently taste the nectar before laying the eggs (Fig. 1c). Each oviposition lasts around 5 s. The plants that we observed the females ovipositing were visited by ants, mainly *Camponotus crassus*, *Ce. pusillus*, *Pseudomyrmex gracilis* and *Crematogaster* sp. Eggs usually hatch in 3 d, and after hatching, the larva goes straight to the extrafloral nectary opening (Fig. 1d), on top of which it constructs its shelter, possibly using nectar as the main component. The dipteran larvae develop entirely inside their shelters. We did not see any pupa, but observations from a few specimens taken to the laboratory suggest that larvae may detach from the plant when fully developed to pupate elsewhere.

The larval shelter is used to trap prey and possibly also as a refuge from natural enemies and abiotic stress factors. Shelters are usually a translucent white and have a very sticky consistency; they are cylindrical with only one opening or rarely two (Fig. 1b) from which the larvae project their cephalic segment. Larvae seem to expand the shelter openings with their heads as they grow larger. Due to the translucent nature of the shelters, we could see most larvae pushing liquid from inside the shelter so as to form a droplet at the opening. The release of droplets at shelter openings was seen on 36 occasions (out of 164 total records). The majority of the *Rhinoleucophenga* larvae had their shelters on top of active young EFNs (83% of 164 records).

R. myrmecophaga larvae are not active predators since they do not leave their shelters during larval development. Instead, they wait for EFN visitors to interact with their shelters and get trapped (Fig. 1b). When insects get trapped, *R. myrmecophaga* larvae apparently wait for them to die or stop moving. Then the larvae use their mouth hooks to open the prey's exoskeleton, and eat the inside content of the victim. Even at early development, *R. myrmecophaga* larvae are able to trap prey at their sticky shelters. However, only small prey are captured at initial larval stages. In 27.4% of the observations (45 out of 164 records), we found entire or fragmented bodies of prey attached to the larval shelters. Ants seem to be especially attracted to the larval shelters, since we repeatedly observed (26 out of 164 records) them visiting shelter openings and touching them with their mouth parts and antennae. Sometimes ants were stuck in the shelter while visiting but managed to break free after some struggling. Ants failing to escape were eventually consumed by the larvae.

Visiting Ants on *Q. grandiflora*, and Prey Items Consumed by *R. myrmecophaga* Larvae

We found 28 different morphospecies of ants visiting *Q. grandiflora*, mainly in the subfamilies Formicinae, Myrmicinae, and Pseudomyrmecinae. The plants were visited more frequently by *Brachymyrmex* sp. 1 and *Ca. crassus* (Fig. 2). *Wasmannia* sp. 1 was abundant when present (58 individuals on average), but this ant species occurred on only 20% of the plants. There was variation in the species found during the day (07.00 and 13.00 h) and night (19.00 and 01.00 h). During the day, plants were visited mostly by *Ca. crassus* and *P. gracilis*, each occurring on 30% of the plants. At night, the most frequent visitors were *Camponotus* sp. 6 (27%) and *Brachymyrmex* sp. 1 (24%).

All the prey items collected in the larval shelters appeared to have been consumed by the larvae, since the exoskeletons were empty or broken apart. Ants were by far the most common (93.6%) prey items consumed by *R. myrmecophaga*, followed by Diptera (3.84%), Coleoptera, and Vespidae (1.28% each) (Fig. 3). Ants in the genus *Brachymyrmex* were the most common (26.9%) prey item captured by dipteran larvae, followed by *Ca. crassus* (14.1%).

Trophic Ecology of *R. myrmecophaga*

Values of $\delta^{15}N$ differ among samples ($F_{5,21}$ =8.43, P = 0.0002, Fig. 4) and were significantly higher for predators compared with *Qualea* leaves, ants, and nonlepidopteran herbivores (Tukey–Kramer HSD test, P < 0.01), but *R. myrmecophaga* larvae, lepidopteran larvae, and predators had similar $\delta^{15}N$ (Fig. 4). Values of $\delta^{13}C$ differ among samples ($F_{5,21}$=26.16, P < 0.0001, Fig. 4) and were significantly higher for predators, ants, nonlepidopteran herbivores, and *R. myrmecophaga* larvae in comparison with *Qualea* leaves and lepidopteran larvae (Fig. 4, Tukey's HSD test, P < 0.01). The average $\delta^{15}N$ for ants was –0.31‰ (±0.7 SE) and 1.1‰ (±0.33 SE) for *R. myrmecophaga*, which corresponds to an ~1.4‰ enrichment in ^{15}N for the larvae. When we removed the ant sample that was not found in the prey item survey (*Ca. rufipes*), there was an ~2‰ enrichment in $\delta^{15}N$ for the dipteran larvae compared with the remaining ants. In fact, *Ca. rufipes* had the highest value of $\delta^{15}N$ (1.34) among the ant species, especially *Ce. pusillus* ($\delta^{15}N$ = –2.1). Furthermore, both ants and *R. myrmecophaga* larvae had similar $\delta^{13}C$ to each other but different $\delta^{13}C$ from *Qualea* leaves, differing in more than 4‰. Lepidopteran larvae that were feeding on *Q. grandiflora* had similar $\delta^{13}C$ compared with *Qualea* leaves and a 1.7‰ enrichment in $\delta^{15}N$.

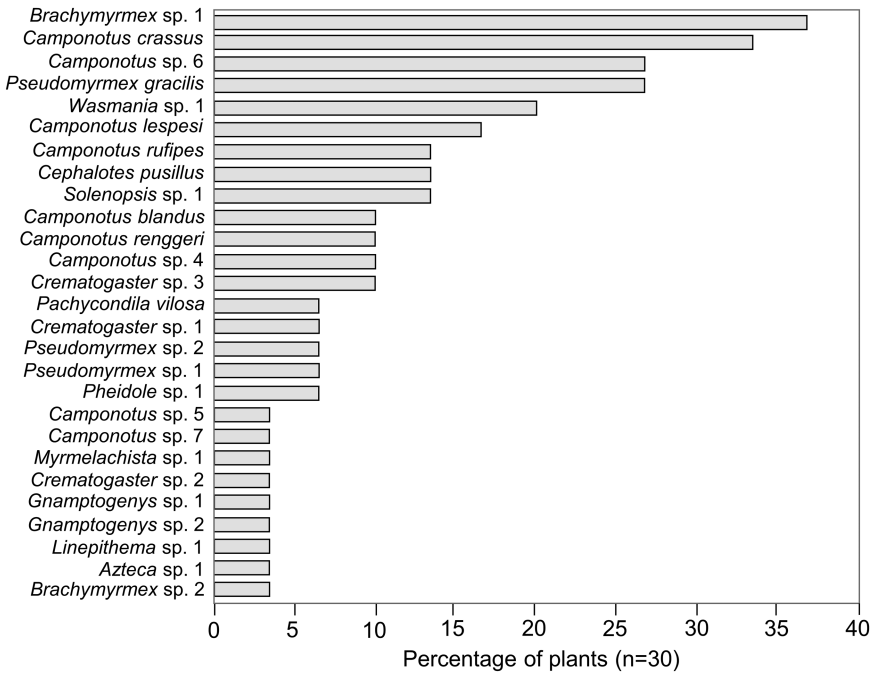

Fig. 2. Percentage of *Q. grandiflora* plants ($N = 40$) visited by different ant species in a cerrado area at Itirapina, southeast Brazil.

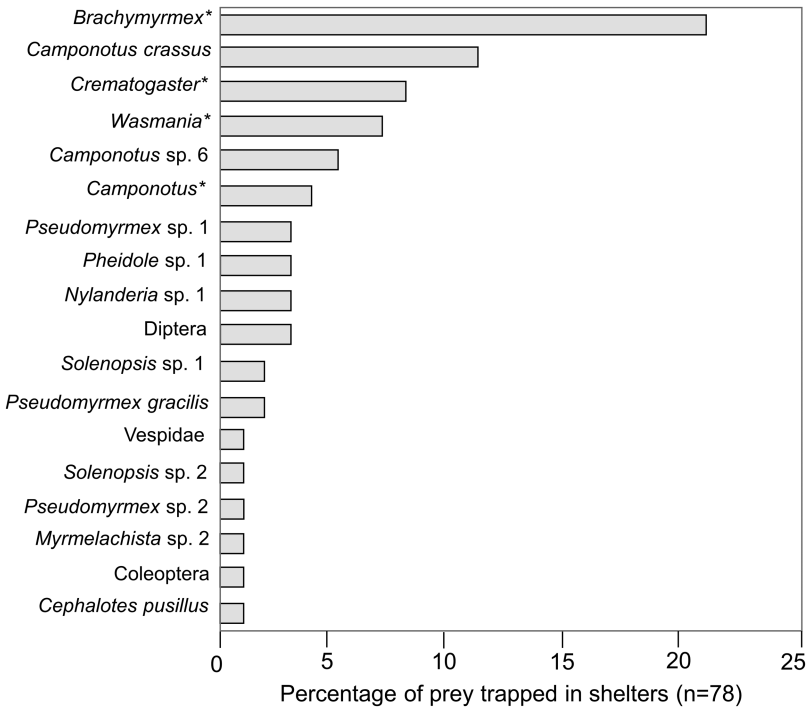

Fig. 3. Percentage of different insect exoskeletons found stuck to the shelters of *R. myrmecophaga* larvae (* indicate ants not identifiable to a morphospecies level). Non-ant prey items are denoted by the family only.

Chemical Analysis of *Q. grandiflora* EFN, and of Droplets Released by *R. myrmecophaga*

The extrafloral nectar collected from *Q. grandiflora* and the liquid released at the opening of larval shelters presented similar patterns of molecular mass (Fig. 5). We found the predominant presence of the sugars glucose and trehalose detected as sodium adducts $[M+Na]^+$ 203.05 and 365.105 m/z, which corresponds to the molecular mass of $C_6H_{12}O_6$ and $C_{12}H_{22}O_{11}$, respectively. The profile of mass spectrophotometry was more complex than just these two sugars; however, the identification of the other peaks (not all shown in Fig. 5) was not viable due to small amounts of the sample available. It is most probable that the other components present are also sugars, since

the molecular masses were lower than what would be expected for lipids and proteins. Although the small molecular mass can indicate secondary metabolites, our measure in the field using the hand refractometer found that EFN of *Q. grandiflora* was composed by 87% of sugars.

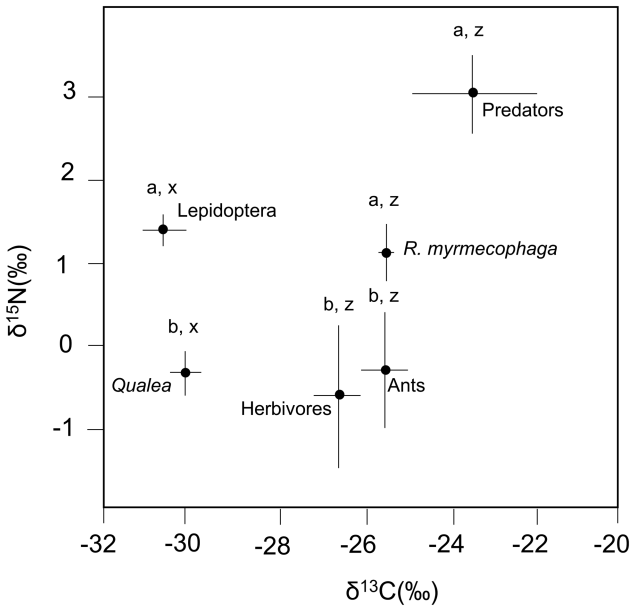

Fig. 4. Isotope signatures of *R. myrmecophaga*, ants visiting EFNs of *Q. grandiflora*, lepidopteran larvae that fed on *Q. grandiflora*, leaves of *Q. grandiflora*, different herbivores, and predators. Data are means and standard error bars. Letters a and b represent differences found with pairwise Tukey's HSD tests for nitrogen isotopes, whereas letters z and x show pairwise differences for carbon isotopes.

Discussion

Our field observations confirm that *R. myrmecophaga* larvae are sit-and-wait predators that live inside shelters built on top of EFNs and use the extrafloral nectar to attract and trap the glands' visitors. These predaceous larvae apparently do not select their prey; they rather depend upon visitations of insects that may get stuck to their sticky shelters. Our behavioral observations are corroborated by the trophic ecology analyses showing that *R. myrmecophga* occupies a higher trophic position than other nonlepidopteran herbivores and ants, suggesting that ants visiting EFNs of *Qualea* are the main components of *R. myrmecophaga* diet. In addition to blocking the direct access of other arthropods to the EFNs, our observations and chemical analysis confirm that *R. myrmecophaga* larvae actively use the continuous nectar flow to attract, trap, and prey on potential ant mutualists that visit the plant for its secretions (Vidal et al. 2016).

The ant fauna visiting *Q. grandiflora* in this study is similar to that recorded by Oliveira et al. (1987) and Nahas et al. (2012) on *Q. grandiflora* and *Q. multiflora*, respectively. Since ants are the most frequent visitors of EFNs in cerrado (Oliveira and Brandão 1991, Schoereder et al. 2010), they are also more frequently taken as prey by *R. myrmecophga* larvae compared with other less common visitors. As expected, the two ants most commonly found trapped at larval shelters, *Brachymyrmex* and *Ca. crassus*, were also the two most common visitors to EFNs. Because larvae of *R. myrmecophaga* do not leave their shelters during development, they depend on the chance of visitors to get trapped to their shelters. Therefore, the more often members of a particular species visit the plant, the higher the chance they will get trapped to a larval shelter.

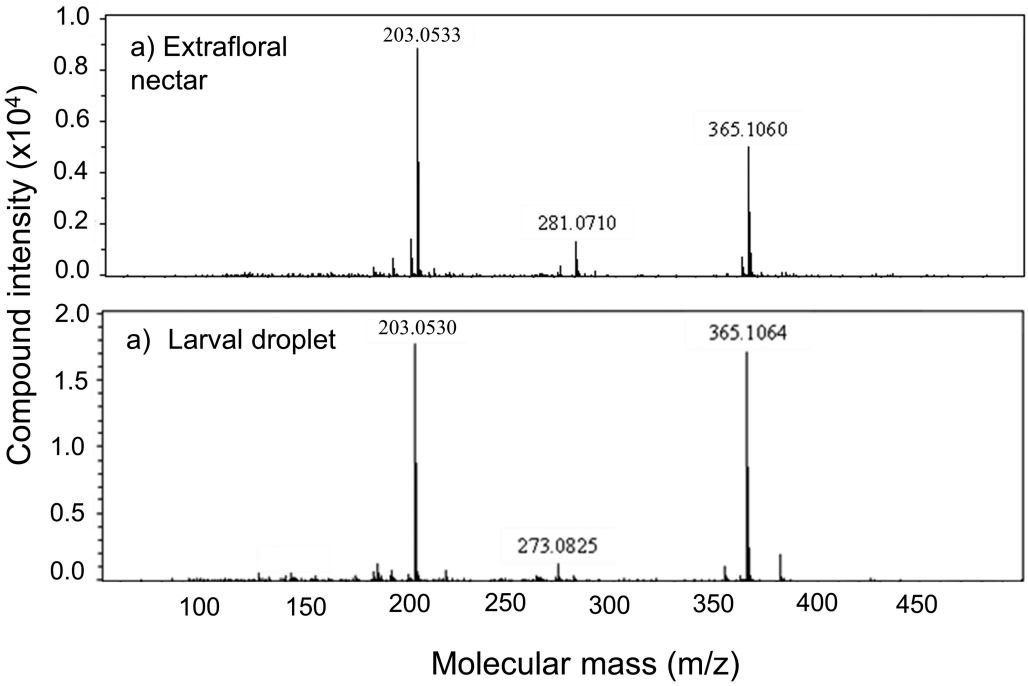

Fig. 5. Chemical compounds found in the analyses of extrafloral nectar of *Q. grandiflora* (a) and droplets released by *R. myrmecophaga* larvae (b). Numbers on top of peaks represent the exact molecular mass (m/z) of the compounds with higher intensity on the samples: trehalose (365.106 m/z) and glucose (203.05 m/z). The graphs represent one of the results for each liquid obtained through positive injection of the nine samples of EFN and three samples of droplets released by larvae (in the summer and winter).

Our stable isotope analyses give further evidence that *R. myrmecophaga* occupies an upper trophic level and feeds on visiting ants. The $\delta^{15}N$ can be used to infer trophic position and was shown to increase 1.4–1.8‰ for blow flies who were fed horse and pig meat (Vanderklift and Ponsard 2003). The increase of nitrogen ratio of larvae was within this range and it was similar to the increase observed for lepidopteran larvae compared with *Qualea* leaves that they were feeding on. Other research found a similar range of increase in nitrogen in the upper trophic level. For instance, *Pardosa* spiders feeding on collembola had an increase of 1.8‰ (± 0.6‰) of their $\delta^{15}N$ compared with their prey (Wise et al. 2006). The ~1.4‰ enrichment of *R. myrmecophaga* $\delta^{15}N$ in comparison to the ants' nitrogen isotope, shown together with no or very little change in $\delta^{13}C$, reliably confirms the trophic interaction between *R. myrmecophaga* and ants. We found that the predacious *R. myrmecophaga* larvae had similar nitrogen isotope to the herbivorous lepidopteran larvae, whereas other herbivores had lower nitrogen isotope values. However, the increment of nitrogen isotope value and the similar value of carbon isotope between lepidopteran larvae and *Qualea* leaves were as expected to confirm that the lepidopteran larvae were feeding on *Qualea* leaves. It is possible that other plants used as food by the nonlepidopteran herbivores have lower values of nitrogen than *Qualea* plants and/or that these herbivores have lower increment in nitrogen isotope than the lepidopteran larvae.

Ants had on average very low $\delta^{15}N$, similar to *Qualea* leaves and nonlepidopteran herbivores. It is possible that the nitrogen isotope of extrafloral nectar is very low, which would explain the low values of nitrogen isotope of the ants. Blüthgen et al. (2003) report that $\delta^{13}C$ of canopy ants and their mutualistic plants usually do not match, but $\delta^{15}N$ of ants increased significantly compared with that of the plants. This pattern is probably due to differences in isotopic composition among plant products and tissues with different isotopic compositions that ants may use. The ants' $\delta^{15}N$ in our study overlapped with the nitrogen isotope composition of *Qualea*; however, the ants used in our study do not forage exclusively on vegetation and might use a wider range of food sources, including resources from other plant species and carbon sources from animal origin (Sendoya et al. 2009, Schoereder et al. 2010). Furthermore, ants and *R. myrmecophaga* larvae had similar values of $\delta^{13}C$, as is expected if ants are the main food source of the dipteran larvae.

Although we did not have a direct measure of the extrafloral nectar isotope profile, we found from our chemical analysis that the nectar is mostly composed of sugars. Considering the similar chemical composition between the EFN and the droplets pushed out by larvae, we propose that these predacious larvae are using the nectar accumulated inside their shelters to attract potential ant prey. The two most abundant compounds found in EFN and larval droplets were glucose and trehalose. Trehalose is very commonly found in insect hemolymph and can also be found in hemipteran honeydew (Wäckers 2001). We further suggest that the dipteran larvae use the extrafloral nectar to construct their shelters, and that the nectar is possibly responsible for their sticky consistency. However, more detailed chemical analyses of shelter composition should be performed in order to better understand the process of shelter construction.

In this study, we showed that ant-preying *R. myrmecophaga* larvae use the EFNs of *Q. grandiflora* as a place to construct their shelters and to attract and prey on nectar-feeding insects, particularly ants that are the most common visitors. This is the first reported case of an exploiter of ant-plant mutualism using the extrafloral nectar and feeding on visiting ants. Other exploiters of ant-plant mutualism usually use the extrafloral nectar or other plant-provided food, but do not prey on ants (e.g., jumping spider on ant-acacia interactions, Meehan et al. 2009; myrmecophilous butterfly larvae feeding on extrafloral nectar, DeVries and Baker 1989). A rare case of exploiters using both the plant resource and feeding on ants has been reported for *Tasobaenus* beetles that exploit the *Cecropia-Pheidole* mutualism and attack ants (Letourneau 1990).

Ants and plants bearing EFNs may maintain mutualistic interactions in which nectar-gathering aggressive ants may defend the plant against herbivores (Bentley 1977, Koptur 2005, Rico-Gray and Oliveira 2007). Indeed, intense foraging by ants has already been demonstrated to decrease levels of herbivory to *Q. grandiflora* and *Q. multiflora* in cerrado vegetation (Costa et al. 1992, Del-Claro et al. 1996, Nahas et al. 2012). We reported on a rare case of sit-and-wait predator behavior that exploits this ant-plant mutualistic relationship (Vidal et al. 2016) by employing a peculiar predatory strategy: the use of the very food reward produced by the plant (extrafloral nectar) to attract and capture potential ant mutualists.

Acknowledgments

We thank Luisa Mota and André Tacioli for help in the field, André Freitas and Hélio Soares Jr. for help with the photographs, and Shannon Murphy for comments on the manuscript. M.C.V. was supported by the São Paulo Research Foundation (FAPESP) (2010/13619-0, 2011/18580–8), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and by a PhD grant by Science without Borders (CAPES, 1198-13-0). S.F.S. was sponsored by a fellowship from FAPESP (2012/23399-3). P.S.O. was supported by research grants from FAPESP (BIOTA Program 2014/23141-1, 2017/16645-1) and the Brazilian Research Council (CNPq) (306115/2013-1, 302219/2017-0).

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