Flower mites decrease nectar availability in the rain-forest bromeliad *Neoregelia johannis*

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Flower mites decrease nectar availability in the rain-forest bromeliad *Neoregelia johannis*

Tadeu J. Guerra*,1, Gustavo Q. Romero†‡ and Woodruff W. Benson‡

**Abstract:** Nectarivorous flower mites can reduce the volume of nectar available to pollinators. The effects of the flower mite *Proctolaelaps* sp. on nectar availability in flowers of a melittophilous bromeliad *Neoregelia johannis* (Bromeliaceae) was evaluated in a coastal rain forest in south-eastern Brazil. In a randomized block experiment utilizing 18 flower pairs, one per bromeliad ramet, pollinators (*Bombus morio*) and mites were excluded, and then nectar volume, sugar concentration and sugar mass were quantified over the anthesis period. Mites significantly reduced nectar volume early in the morning (6h00–8h00), but not later (10h00–12h00). Mites decreased total volume of nectar available up to 22%. Sugar concentration in nectar was higher earlier in the morning, and decreased between 10h00–12h00. The pronounced consumption of nectar by mites during the period of higher sugar concentration reduced the total amount of sugar available to pollinators by 31%. This is the first study showing that flower mites decrease nectar rewards in a melittophilous plant. Because nectar volume by itself incompletely describes nectar production rates and the effects of nectar removal by flower mites on the availability of sugar, our study highlights the inclusion of sugar content in future studies assessing the effects of thieves on nectar production rates.

**Key Words:** Atlantic rain forest, Brazil, Bromeliaceae, Melicharidae, nectar thievery, sugar reward

**INTRODUCTION**

Plant-pollinator systems are often targets of exploitation by nectar robbers and thieves (Inouye 1980), which consume nectar without delivering pollen (Maloof & Inouye 2000). The hummingbird-flower mites (Acari: Mesostigmata: Melicharidae), formerly grouped in Ascidae, are common nectar thieves in Neotropical communities (Colwell 1973, Colwell & Naeem 1994, Krantz & Walter 2009). Most species in this group live, mate and feed on ornithophilous host plants, being phoretic on hummingbird pollinators (Colwell 1973). Nymphs and adults are mainly nectarivorous (Dobkin 1984, Heyneman et al. 1991), although they can also consume pollen (Paciorek et al. 1995). They comprise nearly 50 species in four genera formerly included in family Ascidae: *Rhinoseius*, *Tropicoceius*, *Proctolaelaps* and *Lasioseius* (Colwell & Naeem 1994). However, some species live on hosts pollinated by butterflies (Boggs & Gilbert 1987) and by bats (Tschapka & Cunningham 2004), also dispersing on these flower visitors. However, experimental studies evaluating the effect of nectar consumption by flower mites are restricted to hummingbird-pollinated plants, and until now no study has evaluated flower mite effects on nectar of plants pollinated by insects. Few studies have demonstrated that mites can decrease nectar volume in host-plant flowers, thus behaving as competitors of pollinators and as floral parasites (Colwell 1995, Cruz et al. 2007, Lara & Ornelas 2001, 2002a). Although these mites are reported to occur on flowers of ornithophilous bromeliads (Colwell 1979, Naskrecki & Colwell 1998, Siqueira-Filho & Machado 2001), experimental evaluation of their effects on nectar availability of Bromeliaceae is restricted to hummingbird-pollinated *Tillandsia deppeana* (Lara & Ornelas 2002a). However, presence of flower mites and their effects on nectar production rates of melittophilous bromeliads are unexplored.

Bromeliads have evolved a great diversity of floral display, involving colour, shape and scent, related to
pollinator attraction, with nectar being the usual reward (Benzing 2000). Bromeliad nectar is mainly composed of water and sugars, with the proportions of fructose, glucose and sucrose varying among species within three subfamilies, producing insignificant quantities of amino acids and no lipids or proteins (Bernadello et al. 1991). Galetto & Bernadello (1992) observed that nectar removal increased secretion but decreased sugar concentration in some bromeliad species. However, these effects did not alter the total amount of sugar secreted, except for Puya spathacea, in which nectar removal decreased both nectar volume and sugar amount. In contrast, Ordano & Ornelas (2004) found that nectar removal increased both nectar volume and total sugar contents for two Tillandsia species. Thus, the balance of water and sugar secretion varies among bromeliad species. Therefore, evaluating sugar availability may provide a more accurate assessment of pollinator reward than evaluating nectar volume only (Galetto & Bernadello 1992).

Neoregelia johannis (Carri`ere) L. B. Smith. (Bromelioideae) is a common bromeliad found in a coastal rain forest in south-eastern Brazil. Flowers produce nectar as a reward to pollinators, especially bumblebees, that transport the flower mite Proctolaelaps sp. among host plants. We hypothesized that nectar consumption by this flower mite could negatively affect nectar rewards available to pollinators. To test this prediction, we conducted a randomized block experiment with mite and pollinator exclusion to address the following questions: (1) Do flower mites affect availability of nectar to pollinators? (2) Do experimental results vary if nectar measurements are obtained in terms of volume or sugar content?

METHODS

Study area and species

We conducted this study during January–February 2007 at Praia da Fazenda in the Parque Estadual da Serra do Mar (Núcleo Picinguaba), São Paulo, south-eastern Brazil (23°22′S, 44°50′W). The climate is wet subtropical, with annual rainfall c. 2600 mm and average temperature c. 22°C, with no well-defined wet and dry seasons (Canela & Sazima 2005). The study site was characterized by lowland forest growing on an old sandy beach (‘restinga’ forest, 100–200 m from the ocean) with trees approximately 15 m tall with a heavy growth of epiphytes, especially bromeliads.

Neoregelia johannis is a large tank bromeliad with leaves that may be 1 m long, or more, in shady sites. Cogliatti-Carvalho & Rocha (2001) observed that in mature forest, this bromeliad commonly grows epiphytically in tree canopies 30 m above the ground, but in secondary forest, it forms aggregations and commonly occurs on rock outcrops where nearly 50% of new rosettes recruit vegetatively.

In the study area, N. johannis grows on the forest floor and on tree trunks from near ground level up to 10 m into the canopy. Inflorescences vary from 10 to 15 cm in diameter and can produce up to 100 flowers over its life span. All but the flat upper surface of the inflorescence is immersed in the water stored in the tank formed by the rosette. Tanks are inhabited by a rich arthropod fauna comprising adult and larval insects, copepods, spiders, harvestmen and at least one species of crab. In many plants, litter fallen into the tank may form bridges that allow access to inflorescences by ants and other terrestrial arthropods.

This bromeliad has inconspicuous greenish inflorescence from which may open up to seven flowers each day. They are white and actinomorphic with tubular corollas, 20.6 ± 1.8 mm (range = 16.4–23.5 mm, N = 18) from the internal base of corolla tube to its mouth. Mouth diameter at another height is 4.4 ± 0.5 mm (range = 3.5–5.2 mm, N = 14). Flowers, which last a single day, emerge completely closed from inside the inflorescences around 4h00, well before sunrise. Shortly afterwards, each flower starts to open with a slow outward bending of its petal tips. The flower is fully open by 9h00 and then flowers start to close slowly until 16h00, when most flowers are completely closed. Then late in the afternoon closed flowers start to withdraw into the inflorescence (Guerra et al. in press).

The bumblebee Bombus (Fervidobombus) morio (Swederus, 1787) (Apidae) was the most frequent flower visitor; female workers were responsible for 99% of visits during observations over 2 y at Praia da Fazenda. Three hummingbird species, Ramphodon naevius Dummont, Thalurania glaucopsis Gmelin and Amazilia sp. (Trochilidae) were occasional flower visitors. The bee Trigona fulviventris Guérin, 1835 (Apidae) was also infrequently observed in small groups foraging on pollen. In contrast to hummingbirds that visit plants quickly while hovering, bumblebees always land on inflorescences, and insert their heads into flowers, using their long tongues to access nectar inside flowers. The mite Proctolaelaps sp. (Ascidae) lives on flowers of N. johannis and is phoretic on the bumblebees. They are abundant on inflorescences during late afternoon until the pre-dawn when flowers are closed. Groups of mites enter flowers as soon as they open and leave just before they close. However, during the day flower mites stay inside flowers most of the time, although they can also move between flowers within an inflorescence, or disperse to other plants using bumblebees (Guerra et al. in press). These flower mites were deposited in the Acari Collection of Departamento de Zoologia e Botânica (DZSJR-Pacari, No 8147–8168), Universidade Estadual Paulista (UNESP), Campus de São José do Rio Preto, São Paulo, Brazil (http://splink.cria.org.br).
Field experiment

To evaluate the role of mites as nectar thieves, we conducted an experiment using a randomized block design. We selected plants in the forest without conscious bias, utilizing those plants in accessible locations to allow experimental manipulation. Because we utilized some reproductive ramets from the same genet, paired design was necessary to avoid pseudoreplication (Hurlbert 1984). Daily nectar secretion was evaluated only once for each ramet, and ramets of the same genet were observed on different days. We recovered nectar samples on pairs of flowers from the same ramet (inflorescence) after excluding flower visitors. On four sunny mornings during January and February 2007 we measured nectar availability on around six plants per day (N = 24), 3–100 m apart. We randomly selected one flower of each inflorescence to experimentally exclude mites; the other flower was kept accessible to mites (control). To exclude flying visitors prior to flower opening, we protected flowers using 7-cm-diameter plastic cups with the bottoms removed and replaced with fine nylon netting. To exclude flower mites we carefully cut away surrounding bracts and sepals to prevent mites using them as access routes and then applied sticky resin (The Tanglefoot Corn., USA) around the base of the corolla to block mite access. We also cut neighbouring bracts and sepals in control flowers, but applied resin to just one side of the corolla. The experiment was set up at 3h00–4h00, when flowers were still closed and in the process of, or had just finished, emerging from an inflorescence.

Nectar samples were taken four times from each flower during anthesis, at 2-h intervals. We recovered nectar from each flower from 6h00 until 12h00. Total amount of nectar produced corresponds to the sum of the four samples taken. We stopped sampling at 12h00, before flowers began closing and mites started to leave them. We collected nectar with microcapillary tubes and then estimated nectar volume. We obtained sugar concentrations as mass percentages (% mass sugar/total mass solution) for nectar samples using a pocket refractometer (Atago N1 hand refractometer; Brix 32%). It was found during preliminary observations that most samples had sugar concentrations outside the measurement range. Thus, to evaluate sugar concentration we diluted nectar by 1/2 or 1/3 with fresh water, depending on the nectar volume. In order to estimate energy values it was necessary to convert mass percentages to mass-based concentrations. We utilized the formula:

\[ Y = 0.00226 + (0.00937X) + (0.000585X^2) \]

according to Galetto & Bernadello (2005), where \( Y \) represents the nectar sugar concentration (mg µl⁻¹) for a given sugar mass percentage \( X \) determined with the refractometer. The amount of sugar available from a flower could then be calculated by multiplying the nectar sugar concentration by the volume of nectar. After nectar sampling at 12h00, flowers were cut and stored in 70% ethanol in glass vials. We counted the number of mites in each flower under a stereomicroscope to evaluate effectiveness of exclusion procedures. On six plants inside the dissected flowers we found one or more ant individuals, including three species Conomyrma sp., Azteca sp. and Solenopsis sp. Because ants may consume nectar and disturb mites, we excluded these plants from our analysis. Therefore, we utilized only 18 plants in our analyses of nectar volume and sugar mass. However, six individuals produced no nectar on at least one sampling period. Because considering absence of nectar as zero sugar concentration could bias our results, for statistical analyses evaluating variation in sugar concentration among treatments through flower anthesis, we utilized 12 plants that produced nectar on both treatments in all sampling periods.

Statistical analyses

We evaluated the effects of mites on nectar availability with a repeated-measures randomized block ANOVA. In the model, mite exclusion treatment was a fixed factor, plant was the block factor, and time of day was the repeated measure. Along with the main effects, we included in the model the time of day \( \times \) treatment, and the time of day \( \times \) block interactions. We utilized the Greenhouse–Geisser procedure to correct probability values (Zar 1996). To compare sugar concentration among flowers with and without mite exclusion we restricted the sample to 12 individuals in which nectar could be sampled in all four sampling periods for both control and mite-excluded flowers. We also evaluated the effect of mites on total accumulated nectar and total sugar using randomized block ANOVAs. Prior to data analysis, nectar volumes and sugar content were log₁₀(n + 1)-transformed. Sugar concentrations (%) were arcsine square root-transformed (Zar 1996), all figures show untransformed values. Differences among treatments over the time for distinct sources of variation were compared using Fisher LSD post hoc tests with \( \alpha = 0.05 \).

RESULTS

Although a few flower mites got past the resin barrier, our procedure was very successful in generating pairs of flowers with high and low mite densities. Flowers accessible to mites (control) had significantly more mites
Table 1. Randomized blocks, repeated-measures ANOVA examining the effects of flower-mite exclusion treatment on nectar volume, sugar concentration of nectar and sugar content. Plants were considered as blocks and time of the day was the repeated factor. G-G are Greenhouse–Geisser corrected P values.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>F</th>
<th>P</th>
<th>G-G</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nectar volume</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>1</td>
<td>6.02</td>
<td>0.020</td>
<td></td>
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<tr>
<td>Plant</td>
<td>17</td>
<td>3.46</td>
<td>0.007</td>
<td></td>
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<tr>
<td>Error</td>
<td>17</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Time of day</td>
<td>3</td>
<td>5.45</td>
<td>0.002</td>
<td>0.003</td>
</tr>
<tr>
<td>Time × Plant</td>
<td>51</td>
<td>2.52</td>
<td>0.000</td>
<td>0.001</td>
</tr>
<tr>
<td>Time × Treatment</td>
<td>3</td>
<td>1.40</td>
<td>0.253</td>
<td>0.255</td>
</tr>
<tr>
<td>Error</td>
<td>51</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sugar concentration</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>1</td>
<td>1.17</td>
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<td></td>
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<tr>
<td>Plant</td>
<td>11</td>
<td>1.16</td>
<td>0.407</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time of day</td>
<td>3</td>
<td>59.5</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Time × Plant</td>
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<td>1.59</td>
<td>0.099</td>
<td>0.182</td>
</tr>
<tr>
<td>Time × Treatment</td>
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<td>3.00</td>
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<tr>
<td>Error</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sugar content</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
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<td>15.6</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Plant</td>
<td>17</td>
<td>5.42</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time of day</td>
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<td>9.78</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time × Plant</td>
<td>51</td>
<td>2.78</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time × Treatment</td>
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<td>2.87</td>
<td>0.045</td>
<td>0.048</td>
</tr>
<tr>
<td>Error</td>
<td>51</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

(mean ± SD, 61.0 ± 81.1) than flowers of the exclusion treatment (1.1 ± 1.7; paired t-test, t = 3.04, df = 17, P = 0.007). The number of mites on flowers in the exclusion treatment was so small that we can assume that their effect on nectar availability was negligible.

Nectar volume in flowers inaccessible to pollinators varied both with mite access and with time of morning, we found less nectar in flowers with mites and less nectar at 6h00 than later (Figure 1a, Table 1). The interaction terms time of day × mite exclusion for nectar volume was not significant (Table 1), indicating that the negative effects of mites on nectar volume did not change significantly over time. However, pairwise comparisons showed that mite negative effects on nectar volume differed statistically only at 6h00 and 8h00, when volume diminished by 34% and 25% on average, respectively, in the presence of mites (Figure 1a).

Sugar concentration (%) was highly variable and decreased significantly over the morning. Although its average was lower in mite-excluded flowers at 6h00 and 8h00 (Figure 1b), statistical results indicated that sugar concentration was not affected by mites (Table 1). We pooled samples of mite-excluded and control flowers to evaluate changes in sugar concentration over the morning. Results were highly significant (F(3,116) = 47.8, P < 0.0001) and pairwise comparisons showed that mean sugar concentration gradually decreased between 6h00 and 10h00 (42.0% ± 5.9% to 34.1% ± 4.9% to 27.3% ± 5.7%) and thereafter remained relatively constant, at least until noon (27.0% ± 6.4%).

Nectar consumption by mites reduced nectar volume earlier in the morning when nectar sugar concentration was much higher. The interaction terms time of
DISCUSSION

Nectar consumption by Proctolaelaps sp. significantly reduced the volume of nectar available in N. johannis flowers when pollinators are excluded, supporting results of some previous studies. For instance, Colwell (1995) excluded pollinators and mites from flowers of Hamelia patens (Rubiaceae) and found that Proctolaelaps kirmsei decreased nectar volume by 41%. Lara & Ornelas (2001) excluded Tropidoseius sp. from flowers of Moussonia deppeana (Gesneriaceae) and found that mites reduced total nectar volume in up to 50% when pollinators are excluded. Similarly, Cruz et al. (2007) found that mites reduced the accumulated volume of nectar by 49% and 30% in Heliconia spathocircinata and H. laneana, respectively. The reduction of nectar volume in flowers of N. johannis by Proctolaelaps sp. was comparatively more moderate (22%) when compared with these other species. It is important to note that N. johannis can secrete much more nectar (up to 50 μL) and higher sugar concentrations (up to 54%) than H. patens (15 μL, 16%) and M. deppeana (<10 μL, concentration not provided), indicating that net nectar rewards consumed by Proctolaelaps sp. can be higher in N. johannis. It could be due to a larger average number of mites found on flowers of this bromeliad (61 mites per flower) when compared to H. patens (47 mites per flower) and M. deppeana (16.5 mites per flower).

Our results support the conclusion that mites can reduce availability of nectar in plants with short-lived (1 d) flowers. Similar results were also obtained by Cruz et al. (2007) for two Heliconia species, which also have 1-d flowers. In contrast, Lara & Ornelas (2002a) showed negative effects of flower mites on nectar volume only in plants with long-lived flowers, and that produced copious nectar, such as Lobelia laxiflora, Lobelia cardinalis (Lobeliateae) and Bouvardia ternifolia (Rubiaceae), but not in Palicourea padifolia, Hamelia patens (Rubiaceae) and the bromeliad Tillandsia deppeana, which have 1-d flowers. Lara & Ornelas (2002a) suggested that the volume of nectar produced in flowers may be more important than flower longevity as a determinant of the role of mites in nectar availability. In addition, they argued that effects of mites could be proportionately higher in those plants that produce larger volumes of nectar. However, we found more striking negative effects of mites on N. johannis flowers (up to 34% less nectar volume and 43% lower sugar content) early in the morning, when nectar volume secreted was lower. Weak effects of mites on nectar secretion at 10h00 and 12h00 could be related to responses to repeated removal. Both sugar concentration and amount of sugar decreased through the morning indicating that N. johannis responded to repeated nectar removals by producing diluted nectar, a pattern also found for other bromeliad species (Ordano & Ornelas 2004). Besides variable effects of mites among distinct host plants, specific features of nectar, such as viscosity or amounts of distinct types of sugar (Bernadello et al. 1991) combined with population dynamics and rates of nectar production and consumption by different flower-mite species (Colwell & Naeem 1994) should also be taken into account for conclusive results.

Proctolaelaps sp. reduced sugar content available to pollinators in up to 31%. Unfortunately, most studies that evaluate the effects of nectar thieves and robbers on nectar availability have measured differences in volume of nectar (McDade & Kinsman 1980, Morris 1996,
Roubik 1982, Roubik et al. 1985), and the same approach is provided by studies investigating the role of flower mites as nectar thieves (Colwell 1995, Cruz et al. 2007, Lara & Ornelas 2001, 2002b). The problem in measuring only nectar volume and sugar concentration is that these variables vary greatly with period of the day (as in the present study), with nectar removal and flower age (Galetto & Bernadello 1992), but also with abiotic factors such as temperature and atmospheric relative humidity (Galetto & Bernadello 2005). Thus, for a better assessment of nectar production costs and energetic content, it is necessary to transform samples with volume and concentration in sugar biomass (Galetto & Bernadello 2005). Indeed, whereas Proctolaelaps sp. mites did not influence nectar concentration and consumed 22% of nectar volume, their negative effect on sugar content was actually higher (31%). Therefore, data on nectar volume alone may underestimate the effects of flower mites on energy availability to reward pollinators, and consequently misjudge their potential as competitors of flower visitors as well as floral parasites.

Our field experiment showed for the first time that flower mites decrease nectar rewards in a melittophilous plant. Nonetheless, the effects of robbers and thieves are complex and not necessarily negative to their host plants. It depends on the identity of the robbers and legitimate pollinators, how much nectar is left by robbers and the variety of flower resources available in the environment (Malof & Inouye 2000). Indeed, the effect of nectar thievery by flower mites can even be positive to host plants. Lara & Ornelas (2002b) found that reduction of nectar volume in nearly 50% altered hummingbird probing behaviour, increasing seed set in experimentally robbed plants. Reduction of nectar rewards by Proctolaelaps sp. could affect N. johannis reproduction by changing foraging decisions of bumblebees, and this awaits further investigations. We agree with Colwell (1995), who suggested that the effect of flower mites on nectar secretion patterns of host plants could be underestimated if they are not appropriately excluded. The effect of flower mites on the amount of sugar available to pollinators was considerably more pronounced than their effect on nectar volume. Therefore, we recommend that future studies on the effects of flower mites and other nectar thieves should also consider the amount of sugar produced in addition to the nectar volume as usually reported.

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LITERATURE CITED


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