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Research note

Nectar robbing in *Collaea cipoensis* (Fabaceae), an endemic shrub of the Brazilian rupestrian grasslands

Robo de néctar en Collaea cipoensis *(Fabaceae), una especie endémica de los campos rupestres, Brasil*

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Abstract

Nectar robbery is common in flowering plants with tubular corollas and can affect plant reproductive success. Our study characterized the interaction between potential pollinators and nectar robbers, and assessed the effects on flower abortion in a population of the restricted endemic *Collaea cipoensis* (Fabaceae) at Serra do Cipó, Brazil. We conducted observations of floral visitors to identify potential pollinators from nectar robbers and described visitor behavior. We also analyzed the frequency of robbery and its relationship with flower visitation. *Trigona spinipes* and *Apis mellifera* were identified as the main nectar robbers and two hummingbird species *Colibri serrirostris* and *Eupetomena macroura*, as potential pollinators. Overall, 570 sampled flowers presented robber damage while 716 were intact. Intact flowers received more visits than flowers with damage. Mean flower abortion ratio did not vary between damaged and intact flowers. Accordingly, a survey of developed fruits indicated that 86% of fruits came from intact flowers. Our results suggest that the damage by nectar robbers in *C. cipoensis* flowers could affect the reproductive success of this species, interfering with fruit production.

Keywords: Nectar robberies; *Collaea cipoensis*; Hummingbirds; *Trigona*; Pollination

Resumen

El robo de néctar es común en plantas con flores de corolas tubulares y puede afectar su éxito reproductivo. Se caracteriza la interacción entre robadores de néctar y polinizadores, así como sus consecuencias en la aborción de flores en una especie endémica, *Collaea cipoensis* (Fabaceae) en la Serra do Cipó, Brasil. Se realizaron observaciones de visitantes florales para identificar a los polinizadores, así como a los robadores de néctar basados en su conducta. Se analizó la frecuencia de robo y su relación con los visitantes florales. *Trigona spinipes* y *Apis mellifera* fueron los principales ladrones de néctar y 2 especies de colibrí *Colibri serrirostris* y *Eupetomena macroura*, como polinizadores potenciales. Se presentó

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daño en 570 flores y 716 estuvieron intactas. Las flores sin daño recibieron más visitas vs. las flores dañadas. El promedio de flores abortadas no difirió entre flores dañadas vs. intactas. El 86% de los frutos proviene de flores intactas. Los resultados sugieren que el robo de néctar en *C. cipoensis* podría afectar negativamente el éxito reproductivo de esta especie interfiriendo con la producción de frutos.

Palabras clave: Robadores de néctar; *Collaea cipoensis*; Colibríes; *Trigona*; Polinización

Flowering plants involve mutualistic (e.g., pollinators, seed dispersers) and antagonistic interactions (e.g., herbivores, seed predators, nectar robbers) simultaneously (Bronstein, 2001; Karban & Baldwin, 1997). Nectar robbery is a common antagonistic interaction in species with tubular corollas (Irwin & Maloof, 2002; Milet-Pinheiro & Schlindwein, 2009), which occurs when robbers consume nectar through holes made at the base of the corolla, without transferring pollen among flowers (Irwin, Adler, & Brody, 2004). Impacts of nectar robbers affect, directly or indirectly, the plant reproduction performance (Milet-Pinheiro & Schlindwein, 2009). Direct impacts are functional damage of reproductive female flowers (e.g., ovaries and style; Maloof & Inouye, 2000; Roubik, 1982) and male (e.g., less pollen availability; Irwin, Bronstein, Manson, & Richardson, 2010) structures. Indirect impacts include interference with legitimate pollinators by changing their foraging behavior or causing flower avoidance (Irwin, 2003; Irwin et al., 2010). Nectar robbers can have negative effects on endemic species fitness. In *Polygala vayredae* flowers that were robbed produced fewer seeds and had less pollen transfer than intact flowers, spotlighting its relevance for conservation and management (Castro, Silveira, & Navarro, 2008).

The aim of this study was to document the intensity of nectar robbers and potential pollinators in the Brazilian endemic *Collaea cipoensis.* We addressed the following questions: (1) Which are the floral visitors and potential pollinators of *C. cipoensis*? (2) Is nectar robbing a frequent phenomenon in *C. cipoensis* flowers? (3) Does nectar robbery affect floral abortion?

Our study was conducted in an area under a recovery process belonging to Vellozia Private Reserve (19°16′54″ S, 43°35′45″ W) at Serra do Cipó, Minas Gerais, Brazil. The region has an average annual temperature of 21.3 °C and an average annual rainfall of 1247 mm (Inmet, 2015). Typically there is a cold, dry season from May to September and a hot rainy season from November to March (Madeira & Fernandes, 1999). The rupestrian grasslands region comprises 7000 km^2 characterized by rocky outcrops and sandy fields with soils poor in nutrients (Furley & Ratter, 1988; Oliveira et al., 2015). The flora comprises ca. 4000 plant species with the highest levels of plant endemism of Brazil (Giulietti, Pirani, & Harley, 1997). Despite the high biodiversity and conservation importance of this region, many species with restricted distributions are endangered due to strong human disturbance (Menezes & Giulietti, 2000).

C. cipoensis (Fabaceae) is a restricted endemic species limited geographically to Serra do Cipó, Minas Gerais, Brazil (Rapini, Ribeiro, Lambert, & Pirani, 2008). It is known from only a few populations, so it is considered extremely rare (Fortunato, 1995). *C. cipoensis* is a perennial shrub with 2–3 m in height, with red, tubular flowers disposed in inflorescences with 2–6 flowers and is hummingbird pollinated, flowering and fruiting continuously throughout the year with peaks from May to January (Fortunato, 1995). It is distributed along watercourses, associated with acidic, poor in nutrients and high aluminum saturation soils (Negreiros, Borges-Moraes, & Fernandes, 2008).

The study was conducted in October and November of 2014 in plants located in a regeneration plantation inside to the Vellozia Reserve during peak flowering: (1) to evaluate the effects of nectar robbers on flowers with and without damage of *C. cipoensis.* We selected 6 plots of 5 m × 5 m (25 m^2) each separated by 10 m throughout the study area and sampled all reproductive individuals; (2) to identify nectar robbers and flower visitors we observed 17 plants and 350 flowers; and (3) we performed a paired design considering robber and intact flowers to quantify visitation events (separated no more 50 cm), and we conducted focal observations (by 6 persons) during 07:00 to 18:00 h, totalizing 132 h in 2 days.

In total we found 44 flowering plants, and quantified the number of flowers and developing fruits with and without damage by nectar robbers. We consider nectar robbing as holes at the base of the corolla, easily distinguished in the calyx of flowers and in newly formed fruits (Fig. 1A). We assumed a floral abortion occurred when a viable flower fell from the plant when touched lightly. We calculated flower abortion ratio as the number of aborted flowers divided by the total number of flowers produced by each plant. We tested for differences in flower abortion ratio per plant between damaged and intact flowers using a Generalized Linear Mixed Model using SAS v. 9.2 (SAS Institute, 2008). A binomial distribution was specified for abortion ratio. Since damaged and non-damaged flowers were found in the same individual, the plant was included as a random factor in the analysis.

Our results showed that the main floral visitors of *C. cipoensis* were bees and hummingbirds. The most common bee visitor was *Apis mellifera* (N = 99 flowers visited), followed by *Trigona spinipes* (N = 22), *Bombus* sp. (N = 13) and wasps (N = 4). The main nectar robber was *T. spinipes*, which chewed a hole through the calyx and corolla to access the nectar chamber. *A. mellifera* was second in robbery visits, and less frequently consumed pollen and made legitimate visits. We recorded two hummingbird species visiting the flowers: *Colibri serrirostris* (Fig. 1B) and *Eupetomena macroura* (Fig. 1C). Based on the frequency of visits and the observation that both made contact with the reproductive organs of the flower, we

Figure 1. (A) Floral bud with *Trigona spinipes* causing damage at the base of corolla of *C. cipoensis*; (B) 2 species of hummingbirds were considered as potential pollinators *Colibri serrirostris*, and (C) *Eupetomena macroura* visiting flowers of *C. cipoensis*.

consider these species as potential pollinators. Our results show that hummingbirds preferentially visit flowers without damage (mean visits flower 10 ± s.e. = 17.11 ± 2.7, $N = 237$) than flowers with damage (3.44 ± 1.05, $N = 44$) (Wilcoxon test $Z = 3.97$, $p < 0.0001$).

To assess the damage by nectar robbers we sampled 1286 flowers in 44 individuals of *C. cipoensis*; of these flowers, 716 (56%) were intact and 570 (44%) showed robbery. Flower abortion ratio did not statistically differ between damaged and intact flowers within plants ($F_{1,80} = 2.6$, $p = 0.11$); however, there was a trend indicating mean flower abortion ratios were higher for robbed flowers (0.38 ± 0.061) than for intact flowers (0.22 ± 0.057) (mean ± s.e.). Additionally, sampling of 544 unripe and mature fruits of 44 plants showed that only 14% of fruits had signs of robbery as indicated by damage in the remnant calyx.

Our results show that the primary robber is *T. spinipes*, which agrees with findings reported for other plant species where major nectar robbers correspond to bees (Irwin et al., 2010; Navarro, 2000). As in the case of bumblebees, *T. spinipes* uses its proboscis to make holes at the base of the flowers (Irwin et al., 2010; Macior, 1966). This interaction may decrease fruit or seed set by damaging plant reproductive structures (McDade & Kinsman, 1980). Our results suggest that damage caused by robbers to *C. cipoensis* flowers could lead to flower abortion, which may reduce overall fruit production.

Nectar robbing leads to negative effects on plant reproductive success when it exists, for example, destruction of ovaries or ovules (Navarro & Medel, 2009), selective seed abortion (Zhang et al., 2011), or by inducing behavioral changes in pollinators with the ability to detect robbed flowers (Irwin & Brody, 1999). In *C. cipoensis* we observed *T. spinipes* making a large hole at the base of the corolla and this damage could lead to ovary injuries triggering flower abortion. Nonetheless, other variables, like pollen availability, must be considered. A similar scenario, although we did not record any other bird visiting *C. cipoensis* flowers, was found in *Puya coerulea* where robbing by the blackbird *Curaeus curaeus* on hummingbird pollinated flowers; negatively affected the flowers by causing intense damage androecium (González-Gómez & Valdivia, 2005). This study documented that almost 50% of the flowers in the population showed evidence of nectar robbing; hence the phenomenon is likely a significant determinant of plant reproductive success. A small proportion of fruits (14%) have evidence of damage, however, it could indicate that if injury does not affect the ovary the fruits can develop. Additionally, this suggests that nectar robbing by *T. spinipes* could have adverse effects on reproductive success of this restricted endemic Brazilian species. Further studies should address the intensity and cost of nectar robbing, interactions between robbers and pollinators, and consequences of floral abortion to the plant present and future reproductive success.

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