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Fauna and Stratification of Male Orchid Bees (Hymenoptera: Apidae) and their Preference for Odor Baits in a Forest Fragment

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Abstract

This is a study of the population fluctuation of euglossine species, as well as their preferences for scent baits (cineole, eugenol, vanillin and methyl salicylate) in two forest strata (canopy and understory) at the Reserva Florestal do Azulão, a forest fragment located in the municipality of Dourados, MS, Brazil (22°12’S, 54°55’W). We collected a total of 529 males from four genera and eight species. Diversity and equitability for both strata (understory: H’ = 1.195 and J’ = 0.6139; canopy: H’ = 1.193 and J’ = 0.6131) did not show a significant difference and a high similarity index was found (P = 87.5%). On the other hand, abundance was substantially higher in the canopy (n = 358) than in the understory (n = 171). From the scents used, eugenol attracted a larger number of individuals (n = 225), but cineole and vanillin attracted a higher number of species.

Introduction

The euglossine tribe forms a very distinct group within Apidae, comprising five genera (*Euglossa, Eufriesea, Eulaema, Aglae,* and *Exaerete*) with approximately 200 described species (Roubik & Hanson 2004, Ramirez e*t al* 2010). It is broadly distributed throughout the tropical America, occurring from central Argentina to southern United States (Michenner 2007). Its greatest richness and abundance, however, are found in tropical rainforests (Cameron 2004, Roubik 2004).

One of this group’s main characteristics is the males’ practice of collecting aromatic substances, which are floral rewards produced in Orchidaceae, Araceae, Gesneriaceae, Solanaceae, Euphorbiaceae, Amaryllidaceae, and Theaceae osmophores (Sazima *et al* 1993, Melo 1995, Carvalho & Machado 2002, Braga & Garófalo 2003). In face of this characteristic, the use of odor baits has allowed rapid surveys and ecological studies of the group in different Neotropical areas.

In Brazil, systemized studies on euglossine have been concentrated predominantly in tropical forests. However, due to these insects’ great importance as indicators of environmental quality, there is a pressing need for further taxonomic work and the determination of species that occur outside the humid forests region. Otherwise, the use of these bees as indicators of environmental quality will become rather restricted in areas in which data for this group are unavailable (Peruquetti *et al* 1999, Sofia & Suzuki 2004).

There are few studies focusing the fauna and behavior of these bees in open and fragmented areas in Brazil such as the cerrado biome, in the State of Mato Grosso do Sul (Rebelo & Silva 1999, Nemésio & Faria Jr 2004, Alvarenga *at al* 2007). The Brazilian cerrado is characterized by the presence of several forest fragments, and one of the structural factors that greatly influences the maintenance of diversity in these fragments is its spatial heterogeneity

(Hart & Horwitz 1991). This heterogeneity is usually associated to the occurrence of a greater number of microhabitats, therefore allowing the coexistence of a greater number of species (Gonçalves & Lozada 2005). Thus, a new emphasis has been given to studies on the vertical distribution of the physical-chemical and biotic characteristics of these forests (Davies *et al* 1997). In that sense, despite the numerous studies on euglossine bees in Brazil, in the cerrado region its fauna is poorly known (Nemésio & Silveira 2007), and little has been done on the vertical stratification of this group (Oliveira & Campos 1996, Otero & Sallenave 2003, Martins & Souza 2005).

This study is aimed at identifying the fauna and the fluctuations in the population of euglossine males, while also evaluating their preferences for scented baits and their possible vertical stratification in a forest fragment in the cerrado of Mato Grosso do Sul.

Material and Methods

Study area

The present study was undertaken in a 43 ha remnant area of cerrado scrubland in Mato Grosso do Sul, denominated "Reserva Florestal da Fazenda Coqueiro". The remnant is located at 430 m.a.s.l, in the municipality of Dourados (22°12' S, 54°55' W). The area is characterized by its relative isolation from other fragments, which are covered by pastures or plantations. It is also under constant threat, imposed either by real estate speculation, which has been taking over land bordering neighboring areas, or by the timber extraction industry.

The region is represented by continental tropical climate, with two well-defined seasons: rainy summer and dry winter. The average summer temperature is 26.4°C, while the winter one is 19.8°C, and the annual average is 23.7°C (EMBRAPA/CPAO 2008).

Sampling

The collection of euglossine males was conducted from April 2004 to March 2005 in two strata of a cerrado fragment: understory and canopy. In the understory, the traps were placed at a height of up to 2 m, and at least 5 m apart. Traps in the canopy were installed at heights of 10-15 m, and also 5 m apart from each other. The captured individuals were fixed in 70% ethanol and stored for later identification.

Fifty-two collections were conducted on a weekly basis. Modified scent traps manufactured with 2-liter PET bottles (Fig 1) had an increased internal area and a reduced number of entrances in order to avoid the escape of captured individuals. In addition, bottles were used with stoppers and wicks to prolong the attractiveness of the scents used. A total of eight traps were distributed throughout the forest, with four traps per stratus. The maintenance (insect removal and replacement of scents) of the traps was performed once a week. The following scents were used: cineole, eugenol, methyl salicylate, and vanillin.

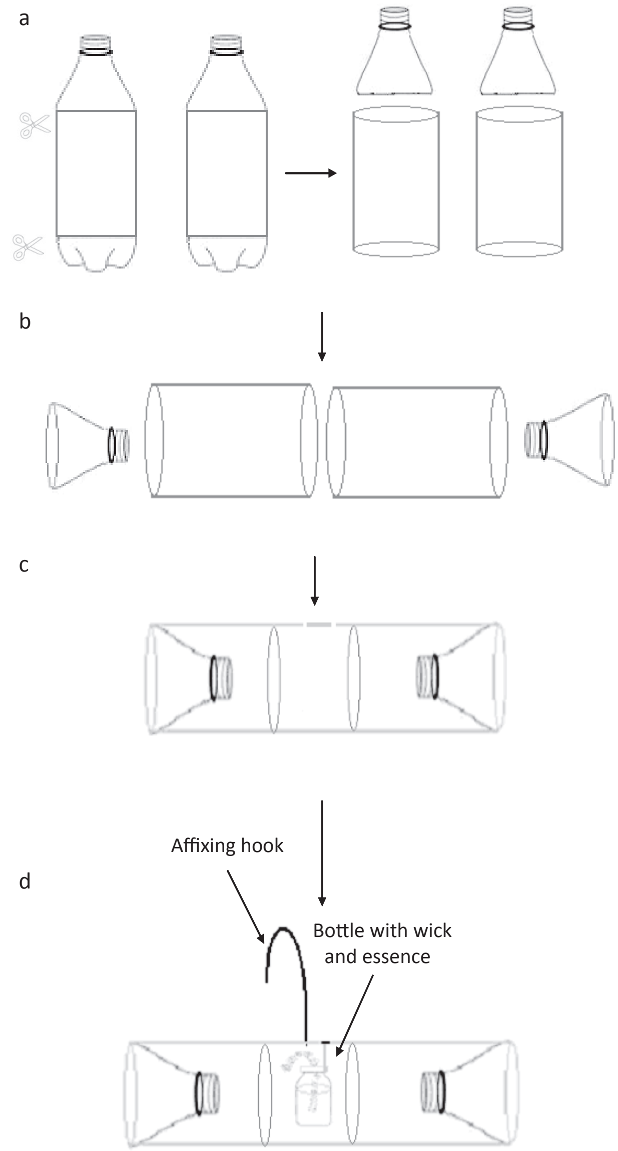

Fig 1 Construction of the odor bait trap: a) cutting of bottlenecks and bottoms of two Pet bottles; b) discard of bottoms and use of cylinders and bottlenecks; c) union and fixation of the cylinders and inverted bottlenecks at the ends; d) hook insertion for setting the trap and fixing the gallipot containing the essence with the wick.

The weekly averages of climatic data, relative humidity, and temperature, were supplied by the meteorological station of the Universidade Federal da Grande Dourados (Dourados, MS).

Data analysis

Analyses of the bee species captured in the traps were based on the indexes of frequency, constancy, abundance and dominance (Silveira Neto *et al* 1976). Once the percentage of constancy was obtained, species were grouped into the following categories: constant (w) when present in more than 50% of the collections; accessories (y) when present in 25-50% of the collections; and accidental (z) when present in less than 25% of the collections.

For the estimation of abundance, the limits established by the confidence interval (CI) at 5% and 1% of probability were evaluated, and the following classes were determined: rare (r), when the number of individuals from a given species was less than the lower limit of the CI at 1% of probability; disperse (d), if the number of individuals was between the lower limits of the CI at 1% and 5% of probability; common (c), when the number of individuals was maintained between the limits of 5% and 95%; abundant (a), if the number of individuals was between the limits of 95% and 99%; and very abundant (ma), when the number of individuals was greater than 99% of probability. Regarding dominance, a species was considered dominant when it presented relative frequency superior to 1/S, where S is the total number of species in each environment. In order to compare the average number of individuals among the environments, the non-parametric U-test by Mann-Whitney was applied.

The diversity in the environments was calculated by using the Shannon-Wiener Index (Poole 1974), and differences were checked using the *t* test ($P < 0.05$). Based on the diversity index, the evenness (J') of Pielou (1977) was also calculated, where $J' = H'/H'_{Max}$; $H'_{Max} = \ln(S)$; and S = number of species, its value ranging from 0 to 1, where 1 corresponds to the situation in which all species are equally abundant.

The similarity between strata was obtained using the Renkonen index (Wolda 1981), given by $P = \sum$ minimum ($p1i$, $p2i$), where, $p1i$ is the percentage of species in stratum 1, and $p2i$ is the percentage of species in stratum 2. The percentage can range from 0 (no shared species) to 100 (completely shared).

The Past statistical program, 1.37 Hammer version *et al* (2001), was used to calculate diversity (H' and J'). The remaining tests were performed with the Spss program for Windows, version 12.0 (Spss Inc. 2005).

Results

Fauna and stratification

A total of 529 euglossine males, belonging to eight species of *Euglossa, Eufriesea, Eulaema*, and *Exaerete* were captured. *Euglossa* was the most abundant genus totalizing 59.7% of all the individuals collected, and it was represented by four species: *Euglossa cordata* (L.), with six individuals (1.1%) showing discreet occurrences in June, July, September, December, and January; *Euglossa pleosticta* Dressler, with 25 individuals (4.7%) showing greater occurrence in April, and discrete presence in May, September, October, December, January, February, and March; *Euglossa fimbriata* Rebêlo & Moure, with only one individual (0.2%) captured in December; and *Euglossa stellfeldi* Moure, with 284 individuals (53.7%) collected throughout the whole sampling period (Fig 2).

Eufriesea was represented by 154 individuals (29.1%) that belonged all to the *Eufriesea violacea* (Blanchard) species, most frequent from November to January. The 38 collected specimens (7.2%) of *Eulaema nigrita* Lepeletier, on the other hand, were distributed throughout April, May, September, November, December, January, and February. The least abundant genus of *Exaerete*, on its turn, was represented by 20 (3.8%) specimens of *Exaerete smaragdina* Guérin collected mostly in November, February and March, and by a single specimen (0.2%) of *Exaerete dentata* L., captured in December. Altogether, *E. stellfeldi, E. violacea* and *E. nigrita* represented 90% of all specimens captured (Fig 2).

Besides being highly frequent during the sampling

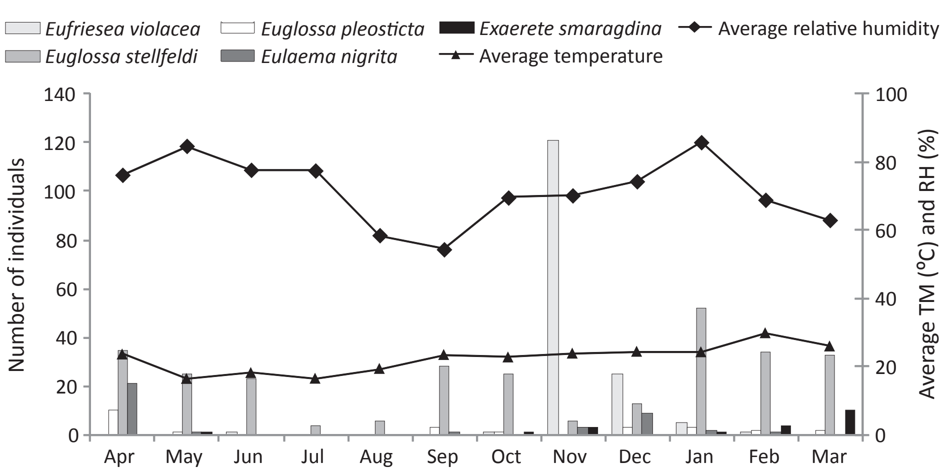

Fig 2 Distribution of males of the more abundant euglossine and its relationship with temperature (TM) and relative humidity (RH).

period, *E. stellfeldi* was also observed in the two studied strata. While 88 specimens of *E. stellfeldi* were found in the understory, 196 individuals were collected from the canopy. This species was therefore classified as very abundant (ma) and dominant (s), although it was constant (w) only in the canopy stratum (Table 1). *Euglossa stellfeldi* was also less affected by climatic conditions, showing little variation in its frequency throughout the collection period. Other species such as *E. nigrita* and *E. violacea* on their turn, showed greater abundance in April and in the period between November and December, respectively (Fig 2).

No difference in species diversity was observed between the understory (H' = 1.195) and the canopy (H' = 1.193). The evenness of the understory (J' = 0.6139) and canopy (J' = 0.6131) allowed us to conclude that the two strata were similar regarding uniformity in the collection of bees (Table 1). The Renkonen similarity-index was high (P = 8.75%), showing that six of the eight species found in this study were shared by the two strata. However, a higher abundance of specimens was found in the canopy (n = 358) as compared to the understory (n = 171). *Euglossa stellfeldi, E. violacea* and *E. nigrita* were considerably more abundant in the canopy than in the understory (Table 1).

Essences

Cineole and vanillin (H' = 1.05) attracted a larger number of species than eugenol (H' = 0.38) and methyl salicylate (H' = 0.60) (Table 2). However, eugenol-scented traps were by far the most attractive ones, collecting an average of 2.4 bees/trap. They were followed by cineol and vanillin, which collected 1.3 bees/trap each. The least attractive trap was the one scented with methyl salicylate (0.1 bee/trap) (Table 4). Bee attractiveness to scented traps was also species dependent. While nearly 80% of the *E. stellfeldi* specimens were attracted to eugenol-scented traps, most individuals of *E. smaragdina* were attracted to methyl salicylate-scented traps. Cineole and vanillin-scented traps were most attractive to *E. violacea*. Cineole-scented traps were also attractive to *E. stellfeldi* and *E. nigrita*, whereas those with vanillin were attractive to *E. pleosticta* and *E. nigrita* (Table 3).

Discussion

Although the diversity and similarity indexes found in the two studied strata did not differ, the abundance of specimens was substantially greater in the upper stratum, which is contrary to what had been previously reported (Oliveira & Campos 1996, Otero & Sallenave 2003, Martins & Souza 2005).

Several hypotheses have been presented to explain the possible causes of the euglossine stratification. In one of these hypotheses, Otero & Sallenave (2003) suggested that lower wind velocity, and consequently the slower dispersion of odor baits in the undergrowth, would difficult bait detection by bees and, therefore, explain the higher abundance in the canopy. For Ramalho (2004), the bees' vertical stratification could be related to the

Table 1 Faunistic parameters of euglossine bee populations collected in two strata in the region of Dourados, MS, from April 2004 to March 2005.

Species	Undergrowth					Canopy					Total				
	N	F	C	A	D	N	F	C	A	D	N	F	C	A	D
Eufriesea violacea	55	32.2	z	a	s	99	27.7	z	c	s	154	29.1	z	a	s
Euglossa cordata	1	0.6	z	c	n	5	1.4	z	c	n	6	1.1	z	c	n
Euglossa fimbriata	0	.	.	.	.	1	0.3	z	c	n	1	0.2	z	c	n
Euglossa pleosticta	8	4.7	z	c	n	17	4.7	z	c	n	25	4.7	y	c	n
Euglossa stellfeldi	88	51.5	y	ma	s	196	54.7	w	ma	S	284	53.7	w	ma	s
Eulaema nigrita	3	1.8	z	c	n	35	9.8	z	c	n	38	7.2	z	c	n
Exaerete dentata	1	0.6	z	c	n	0	.	.	.	.	1	0.2	z	c	n
Exaerete smaragdina	15	8.8	z	c	n	5	1.4	z	c	n	20	3.8	z	c	n
H'(Shannon)	1.195a	.	.	.	.	1.193a	.	.	.	.	.	.	.	.	.
Individuals	171	.	.	.	.	358	.	.	.	.	.	.	.	.	.
J'(Pielou)	0.6139	.	.	.	.	0.6131	.	.	.	.	.	.	.	.	.
Species	7	.	.	.	.	7	.	.	.	.	.	.	.	.	.

Same letters in the Shannon index means non-significant difference to a level of 5% of significance: (N) number of bees captured in the traps; (F) relative frequency (%); (C) constancy: (w) constant, (y) accessories, (z) accidental; (A) abundance: (ma) very abundant, (a) abundant, (c) common, (d) disperse, (r) rare; (D) dominance: (s) dominant, (n) not dominant.

Table 2 Number of euglossine species collected in the two strata with each of the odor baits tested in Dourados, MS, from April 2004 to March 2005.

Species	Cineole			Eugenol			Vanillin			Methyl salicylate		
	UG	C	Total	UG	C	Total	UG	C	Total	UG	C	Total
Eufriesea violacea	34	32	66	0	0	0	21	67	88	0	0	0
Euglossa cordata	0	0	0	0	5	5	1	0	1	0	0	0
Euglossa fimbriata	0	0	0	0	1	1	0	0	0	0	0	0
Euglossa pleosticta	0	0	0	3	1	4	5	16	21	0	0	0
Euglossa stellfeldi	13	35	48	71	154	225	4	6	10	0	1	1
Eulaema nigrita	2	20	22	0	0	0	1	15	16	0	0	0
Exaerete dentata	0	0	0	0	0	0	0	0	0	1	0	1
Exaerete smaragdina	1	0	1	5	5	10	0	0	0	9	0	9
Shannon_H[1]	0.82	1.07	1.05a	0.39	0.34	0.38b	1.04	1.02	1.05a	0.33	0	0.60b

UG = undergrowth; C = canopy; [1]Same letters in the line mean non-significant Sameity to 10% of significance and different letters, significant difference in the Shannon index. The formula used in the test includes correction for small samples.

seasonality of resources and body size of the species. Or, perhaps, as suggested in this study, the activities of the euglossine may be related to the climatic oscillations during the year, which would act in the different strata. By doing so, these variations would create micro-habitats where, for some species, there would be greater occurrence of individuals. In that sense, the hypothesis proposed by Tonhasca *et al* (2002a), which states that within the same habitat, priority micro-habitats with greater abundance and richness of euglossine can exist, is here supported.

Considering the seasonal extremes, we highlight *E. stellfeldi* as the species found with the least variation during the collection period. *Eufriesea violacea,* on the other hand, had considerable peaks of occurrence in the rainy period, revealing a more seasonal behavior than the other studied species (Fig 2). These findings corroborate studies conducted in other locations that also indicated an accentuated seasonality for this species (Peruquetti & Campos 1997, Sofia & Suzuki 2004).

Eufriesea violacea was considered endemic to the Atlantic rain forest (Nemésio & Silveira 2007), being well represented mainly in the Southern and Southeastern Brazil (Wittmann *et al* 1987, Peruquetti & Campos 1997, Rebêlo & Garófalo 1991, Sofia & Suzuki 2004). Although the presence of this species had also been recorded by Kimsey (1982) in Central Brazil, more exactly in the Mato Grosso State region, doubts were left about its occurrence in the cerrado of Mato Grosso do Sul. These doubts were cleared in this study, where *E. violacea* was

Table 3 Faunistic parameters of bee populations collected with the odor baits in Dourados, MS, from April 2004 to March 2005.

Species	Cineole			Eugenol			Vanillin			Methyl salicylate		
	C	A	D	C	A	D	C	A	D	C	A	D
Eufriesea violacea	z	ma	s	.	.	.	z	ma	s	.	.	.
Euglossa cordata	.	.	.	z	c	n	z	c	n	z	.	.
Euglossa fimbriata	.	.	.	z	c	n	.	.	.	.	.	.
Euglossa pleosticta	.	.	.	z	c	n	z	c	n	.	.	.
Euglossa stellfeldi	z	a	s	w	ma	s	z	c	n	z	c	n
Eulaema nigrita	z	c	n	.	.	.	z	c	n	.	.	.
Exaerete dentata	.	.	.	.	.	.	.	.	.	.	c	n
Exaerete smaragdina	z	c	N	z	c	n	.	.	.	Z	ma	s
Shannon	1.05a	.	.	0.38b	.	.	1.05a	.	.	0.60b	.	.

Same letters in the line mean non-significant difference to 10% of significance, and difference letters indicate significant difference in the Shannon index; (C) constancy: (w) constant, (y) accessory (z) accidental; (A) abundance: (ma) very abundant, (a) abundant, (c) common, (d) disperse, (r) rare; (D) dominance: (s) dominant, (n) not dominant.

Table 4 Average number of the most abundant bees captured (standard deviation) per odor bait in Dourados, MS, from April 2004 to March 2005 (N = 104).

Essences	Total	*Eufriesea violacea*	*Euglossa pleosticta*	*Euglossa stellfeldi*	*Eulaema nigrita*	*Exaerete smaragdina*
Cineole	1.32(2.98)a	0.63(2.54)a	0.00(0.00)a	0.46(1.47)a	0.21(0.99)a	0.01(0.10)ab
Eugenol	2.36(3.99)b	0.00(0.00)b	0.04(0.19)ab	2.16(3.85)b	0.00(0.00)b	0.10(0.41)a
Vanillin	1.31(3.62)a	0.85(3.42)a	0.20(0.74)b	0.10(0.38)a	0.15(1.03)a	0.00(0.00)b
Methyl Salicylate	0.11(0.39)c	0.00(0.00)b	0.00(0.00)a	0.01(0.10)a	0.00(0.00)b	0.09(0.37)ab

Different letters in the columns indicate significant difference between the averages through the Mann-Whitney multiple comparison U test, with P < 0.05/6.

well represented. Furthermore, despite being described as sensitive to fragmentation (Giangarelli *et al* 2009), *E. violacea* abundance was greater than would be expected for the size of the studied fragment.

Isolation and fragmentation are always followed by physical modifications that are responsible for large reductions in plant and animal communities. In this sense, the occurrence and maintenance of diverse species can be directly or indirectly affected by the resulting micro-climatic modifications (Morato & Campos 2000). Some studies have sought to enlighten the possible impacts caused by deforestation and fragmentation of forests on the euglossine communities (Tonhasca *et al* 2003, Brosi 2009). Bee abundance has shown to be affected mainly by the size of the fragment and surrounding area, whereas species richness has been mainly related to the surrounding area. Although our data indicate that the decrease in abundance and species richness is related to the size of the fragment and to the degree of disturbance, we have to acknowledge that these data may have been influenced in part by the sampling method. Although the entomological nets have been shown to be more efficient than baited traps in bees sampling (Nemésio & Morato 2004, 2006), a recent report demonstrated differently (Storck-Tonon *et al* 2009).

Baited traps were selected as a sampling tool in this study due to the difficulties in collecting bees with entomological nets in the forest canopy. In addition, the chosen method was partly justified by the higher abundance of individuals registered in the canopy, which demonstrated that working only with the entomological net would have increased the risk of underestimating the abundance of euglossine in this fragment. Furthermore, the modifications undertaken in the traps guaranteed that the essences remained attractive for longer periods of time, which perhaps allowed the only representative of the rare *E. dentata* to be collected in baited traps (Silva & Rebelo 1999, Storck-Tonon *et al* 2009).

The indication that the euglossine abundance would depend on the scent used is not conclusive, since scents different to those used here are shown to be the most attractive in similar studies (Morato *et al* 1992, Rebelo & Garófalo 1997, Storck-Tonon *et al* 2009). Thus, the preference for a particular scent appears to be related to the existing local flora (availability and floral diversity) or even to the climate of the region where the study is undertaken. In that sense, the higher the number of essences used as baits in the traps, the higher would be the number of individuals and species collected (Oliveira & Campos 1995, 1996, Oliveira 1999).

The abundance of euglossine, unlike its richness, was significantly greater in the canopy, which provides evidence of their stratification when it comes to the number of specimens. Such fact may be related to the climatic oscillations during the year, which are capable of influencing the climate within the fragment in distinct ways by creating microclimates that may affect the activities of the different euglossine species in the forest strata.

Despite the low values in abundance and richness, our data highlighted the accentuated seasonality and the marked presence of *E. violacea* in the region of Mato Grosso do Sul, in addition to the record of *E. dentata,* a rare euglossine. Furthermore, the reduced number of species and specimens collected may be related to the size and degree of disturbance of the studied fragment, despite the methods used for collection. Nevertheless, eugenol was by far the most attractive essence, attracting the greatest number of specimens, while cineole and vanillin attracted the greatest number of species. Methyl salicylate was the least attractive essence, collecting one single representative of *E. dentata*. Therefore, the use of a greater number of essences is required in studies of diversity of euglossine bees.

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