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Original Article

Conservation of Nature

Bees Diversity on Flowers of *Eremanthus* spp. (Asteraceae)

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ABSTRACT

Bees are considered important pollinators due to their fidelity to plants, enabling the maintenance of gene flow between plant species. The objective of this study was to recognize the visit of bees in two Candeia species, *Eremanthus erythropappus* and *Eremanthus incanus* (Asteraceae), as well as to analyze the pollen content carried by bees in a regeneration area. Nine samplings were taken in each plant species using entomological nets. The pollen grains were obtained in 338 bees from their legs and bodies. The species *Trigona spinipes*, *Apis mellifera,* and *Bombus pauloensis* were most frequent. Candeias are essential in the study area because they have high adaptive potential, but besides them, other plants are also important for attracting bees searching for pollen and/or nectar.

Keywords: pollen analysis, pollinators, degradation.

1. INTRODUCTION AND OBJECTIVES

Increasing anthropogenic activities through civil construction, mining, deforestation, and pollution is the main cause of fragmentation of natural habitats, leading to biodiversity loss (Hagen et al., 2012). When these practices are inadequate, they promote soil degradation and loss of vegetation cover, resulting in environmental imbalance (Gebremedhin et al., 2018).

Degraded areas lose their capacity of resilience and they require the man's actions to return to their former characteristics or to become as close as possible to the reference ecosystem. A recovery plan is essential in these areas to restore the environment to its basic functions (Andrade & Romeiro, 2011).

In degraded areas where usually there are losses in biodiversity and in the relationships between living organisms, pollinators play an important role in reestablishing these relationships (Oliveira & Engel, 2011). On the other hand, the reduction of plant cover can affect the community of pollinators, making scarce the availability of food and reducing the transfer of pollen between plants (Eckert et al., 2010).

The analysis of the pollen attached to the body of the bees has become an important tool to obtain data about foraging behavior (Silva, Aleixo et al., 2014). By this technique, it is possible to identify the most visited plants in a certain area, pointing out the main food resources used by the bees (Frias et al., 2016; Novais et al., 2009; Silva et al., 2010).

Candeias (*Eremanthus* spp.) are among the most important botanical species in Minas Gerais. *Eremanthus erythropappus* (DC.) McLeish and *Eremanthus incanus* (Less.) Less belong to the Asteraceae family and can be used for the recovery of degraded areas, as they are pioneers in field areas and adapted to nutrient-poor, rocky and sandy soils (Silva, Oliveira et al., 2014).

The wood of candeia can be used in the manufacture of fences by small farmers, and its oil is commercialized in the cosmetic and pharmaceutical industry (Lima et al., 2013). These characteristics are essential because they guarantee the recovery of degraded areas and allow economic return for the small rural producer (Oliveira, Andrade et al., 2010).

Besides its great economic importance, candeia presents a high percentage of viable pollen, although with little availability of nectar for floral visitors, who usually forage all day long with a foraging peak during the hottest hours (Barbosa et al., 2016; Oliveira, Dias et al., 2012; Vieira et al., 2012).

Biribiri State Park, in Diamantina, MG, has an area which has undergone a degradation process where gravel was removed for the construction of a highway bordering the park. Currently, this area undergoes a recovery process, with the planting of candeia, among other species with some of them belonging to the Fabaceae family (Branquinho et al., 2013, Silva, Pereira et al., 2014).

The objective of this work was to verify the main species of bees visiting *Eremanthus erythropappus* and *Eremanthus incanus* found in a gravel area in the plant recovery process and the alternative sources of food for these bees.

2. MATERIALS AND METHODS

The study was carried out in Biribiri State Park, located in the Southern Espinhaço Range, in High Jequitinhonha Valley in Diamantina, MG, between 18°14'53"S and 43°39'57"W and at an altitude approximately 1,300 m above sea level. The climate is typically tropical, Cwb in the Köppen classification. The average annual precipitation varies from 1,250 mm to 1,550 mm and the average annual temperature is between 18 °C and 19 °C. The relative air humidity is almost always high, with annual averages of 75.6% (Neves et al., 2005).

The park has a vegetation characteristic of the Cerrado biome. The predominant plant formations are the savannas and grasslands, and forest formations such as Cerradão and Semidecidual Seasonal Forest are also found, mainly along the streams and rivers slopes (Gonzaga et al., 2014).

The study area called gravel area comprises 8.4 hectares and it is in the process of recovery. The process of degradation started with the removal of gravel for the construction of the highway BR-367, bordering the park. In 2002, the process of recovery began by planting species adapted to the region. *E. erythropappus* and *E. incanus* stand out among them, as they have shown great adaptive potential.

The samplings were done in two stages, beginning with the appearance of the first flowering plants and ending with the end of each flowering period.

The first stage was carried out in 41 flowering plants of *E. erythropappus* between July 23rd and August 24th, 2012. The samplings were done once a week, totaling five days of samplings (samplings 1 to 5), with effort of 42 hours. The second stage occurred after the flowering period of *E. erythropappus*, which coincided with the appearance of the first flowers of *E. incanus*. Its flowering period occurred between August 30th and September 19th, 2012, with weekly samplings totaling four days of samplings (6 to 9), with an effort of 35 hours, in 18 plants. The collection period was from 8 a.m. to 5 p.m. for both species. The method used to capture the bees was the "sweep" type, using entomological nets. The collections were performed simultaneously by two collectors, in different plants, for five minutes each, following the methodology used by Viana et al. (2002).

The captured individuals were sacrificed and taken to the Bee Genetics Laboratory of Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM). The identification was based on dichotomous keys (Silveira et al., 2002), done by specialists or by comparison with species already identified. The specimens were deposited in the "Coleção de Abelhas" of this University.

When present, the pollen was removed from the legs and body of the bees. The pollen samples were submitted to the acetolysis process. Three slides were prepared with glycerinated gelatin for each sample after the process (Barth & Duarte, 2008). The pollen grains were photomicrographed and identified in types, following a pollen catalog of cerrado plants (Bastos et al., 2008). The quantitative analysis was performed counting 500 pollen grains per slide. The pollen types were classified according to the frequency in the slide, being dominant those with representation above 45%, accessories between 16% and 45%, and occasional with up to 15%.

The characterization of the community of bees was performed through the abundance, richness, constancy, dominance, and frequency of faunal indexes according to D'Avila & Marchini (2008).

The climatic data were obtained through the Climatological Normals maintained by INMET. Spearman's correlation (Rs) was performed using the Bioestat 5.0 software to correlate bee abundance with mean temperatures on each day of sampling.

3. RESULTS AND DISCUSSION

Thirteen species of bees belonging to three families were collected: Apidae, Andrenidae, and Halictidae. Collected bees from Apidae belonged to two subfamilies (Apinae and Xylocopinae) and from Andrenidae and Halictidae to only one subfamily each (Panurginae and Halictinae, respectively) (Table 1).

A total of 338 individuals were collected in both species of candeia. The Apidae family was the most representative with 99.1% of the individuals (Table 2).

Table 1. Bees visiting flowers of *Eremanthus erythropappus* and *Eremanthus incanus* in a gravel pit area located at Biribiri State Park, Diamantina, MG, 2012.

Family	Subfamily	Species
Apidae	Apinae	*Apis mellifera* Linnaeus, 1758
		Bombus (*Fervidobombus*) *pauloensis* Friese, 1913
		Bombus (*Fervidobombus*) *morio* (Swederus, 1787)
		Geotrigona mombuca (Smith, 1863)
		Melipona (*Melikerria*) *quinquefasciata* Lepeletier, 1836
		Melipona (*Melipona*) *quadrifasciata* Lepeletier, 1836
		Trigona fulviventris Guérin Méneville, 1845
		Trigona spinipes (Fabricius, 1793)
		Centris sp.
	Xylocopinae	*Xylocopa* (*Neoxylocopa*) sp.
		Xylocopa sp.
Adrenidae	Panurginae	Morphospecies
Halictidae	Halictinae	Morphospecies

Table 2. Bees visitors (%) in flowers of *Eremanthus erythropappus* and *Eremanthus incanus* in the area of gravel located in Biribiri State Park, Diamantina, MG, 2012.

Family	Subfamily	Number of genera	Number of species	Number of individuals	*Eremanthus erythropappus* (%)	*Eremanthus incanus* (%)
Apidae	Apinae	6	9	331	68.28	31.72
	Xylocopinae	1	2	4	0.00	100.00
Adrenidae	Panurginae	1	1	2	0.00	100.00
Halictidae	Halictinae	1	1	1	0.00	100.00
Total		9	13	338		

There are five bee families in Brazil: Andrenidae, Apidae, Halictidae, Colletidae, and Megachilidae (Silveira et al., 2002). This study verified the presence of bees belonging only to the first three. The distribution of bees in Brazil is related to the type of vegetation, with some endemic species. For example, in tropical rainforests in the south of the country, higher species richness of Halictidae family is observed (Milet-Pinheiro & Schlindwein, 2008). In areas of cerrado, the family Apidae is the most representative, followed by Megachilidae. Andena et al. (2005), studying a Cerrado area in a country town of São Paulo, observed that the number of species can vary between 103 to 189 and the number of individuals collected can reach 3,010. Because the present work was carried out on only two species of plants and during its flowering period, the number of bee species observed was low.

A. mellifera, *T. spinipes* and *B. pauloensis* were constant and dominant species; the others were accidental or accessory. In the first stage, that is, in the flowering period of *E. erythropappus*, five collections were performed and a total of 226 individuals were observed, distributed in one family, five genera and six species. Only individuals belonging to Apidae family were collected. *B. pauloensis* was the dominant species with 79.73% of the individuals collected, followed by *A. mellifera* with 14.98%. In the second stage, i.e., the flowering period of *E. incanus*, 112 individuals were collected, distributed in three families, seven genera and 13 species. The Apidae family was again the most abundant with 97.32%, followed by Andrenidae with 1.78% and Halictidae with 0.89% of the individuals. *T. spinipes* was the most abundant species with 44.64%, followed by *B. pauloensis* with 35.71% of the individuals. *T. fulviventris, Centris* sp., *M. quadrifasciata, Xylocopa* sp. and one species of the subfamily Halictinae were occasional with only one individual collected (Table 3).

In the present study, *B. pauloensis* and *T. spinipes* were considered constant and dominant in *E. erythropappus* and *E. incanus*, respectively. These species belong to the Apidae family and are characterized by presenting colonies with a communication system and generalist foraging behavior (Castillo et al., 2015; Gonzalez et al., 2004). A similar pattern was found by Vieira et al. (2012), when studying the floral biology of *E. incanus*. In general, bees prefer to visit Asteraceae flowers in surveys conducted in areas of cerrado or rupestrian fields. This is due to the large number of species of this family in this biome that supply pollen and nectar (Andena et al., 2005). The flowering period of *E. erythropappus* and *E. incanus* occurred in the dry and cold season, as observed by Vieira et al. (2012); thus, these two species are food resources for bees during a period of two months. One of the striking characteristics of Cerrado is the fact that the flowering of many plant species is synchronized with the seasons. In this biome, it is possible to observe the flowering of some species during the dry and cold season and others during the beginning of the rainy season (Lenza & Klink, 2006). *E. erythropappus* and *E. incanus* flowering occur in the cold and dry season, where there is usually a scarcity of resources, making this species an important source of food for bees. Candeia presents dense inflorescences with an average of 29 open flowers, increasing the supply of pollen and nectar to its visitors (Vieira et al., 2012). A large supply of flowers in a given environment contributes to the maintenance of bee populations due to the greater supply of food resources (Dick et al., 2003). This is important in an area where degradation processes are still visible and available food resources are scarce. The five collections performed in *E. erythropappus* were characterized by the great abundance of bees, especially *B. pauloensis*, which were collected during the warmer hours of the day (close to 1 p.m.). In this period of collection, days

Table 3. Species richness and faunal indices of bees collected in flowers of *Eremanthus erythropappus* and *Eremanthus incanus* in a gravel pit area located at Biribiri State Park, Diamantina, MG, 2012.

Taxon	*Eremanthus erythropappus*	*Eremanthus incanus*	Ab	Co[1]	Fr	Do[2]
Apis mellifera	34	3	37	C	0.109	D
Bombus pauloensis	181	40	221	C	0.653	D
Bombus morio	2	2	4	AC	0.011	ND
Geotriona mombuca	1	3	4	AD	0.011	ND
Melipona quinquefasciata	3	4	7	AC	0.020	ND
Melipona quadrifasciata	0	1	1	AD	0.002	ND
Trigona fulviventris	0	1	1	AD	0.002	ND
Trigona spinipes	5	50	55	C	0.162	D
Centris sp.	0	1	1	AD	0.002	ND
Xylocopa (Neoxylocopa) sp.	0	3	3	AD	0.008	ND
Xylocopa sp.	0	1	1	AD	0.002	ND
Panurginae	0	2	2	AD	0.006	ND
Halictinae	0	1	1	AD	0.002	ND
Total	226	112	338			

Ab: abundance; Co: constancy; Fr: frequency; Do: dominance; [1]C: constant; AC: accessory; AD: accidental; [2]D: dominant; ND: non-dominant.

were marked by low temperatures, strong winds, and sporadic rain, mainly in the morning. However, from the sixth to the ninth days temperatures were higher and there was a reduction of the wind intensity and rain amount. This stage was marked by increased richness with the presence of seven other species: *T. fulviventris*, Panurginae, *Centris* sp.1, Halictinae, *M. quadrifasciata*, *Xylocopa* (*Neoxylocopa*) sp. and *Xylocopa* sp. There was also a prevalence of *T. spinipes* as one of the most abundant species. There was no significant correlation between bees' richness and mean temperatures on collection days (Rs = 0.4767; p = 0.1494) (Figure 1).

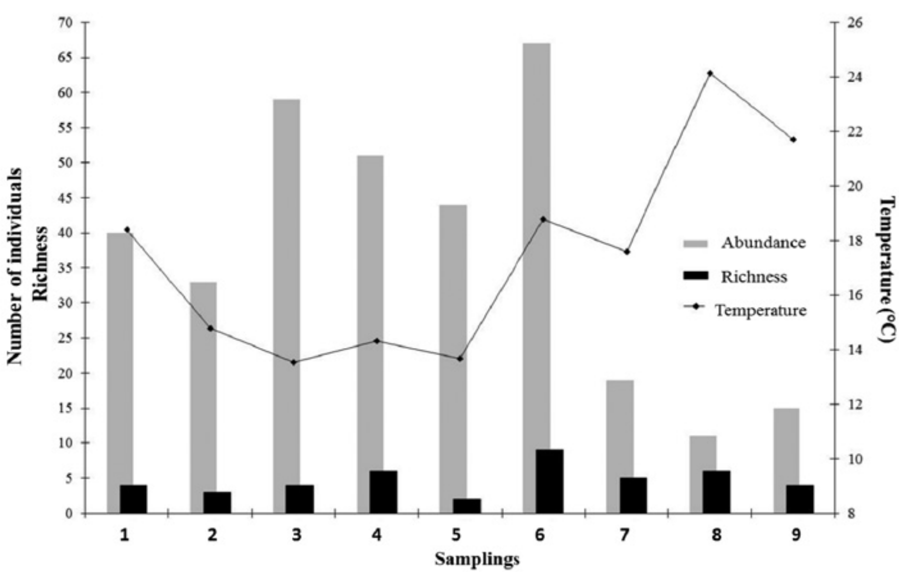

Figure 1. Relationship between abundance and richness of bees with average temperature in nine days of samplings. From the first to the fifth day, sampling was performed in flowers of *Eremanthus erythropappus*, and from the sixth to the ninth, in flowers of *Eremanthus incanus*. Diamantina, MG, 2012.

The number of visiting species increased during the flowering period of *E. incanus* when compared to *E. erythropappus*. Seven new species were collected in the flowers of *E. incanus*, although with a lower abundance. The two candeia species flourished one after the other and synchronously. Synchronized production of flowers of different species for a short period of time could attract the attention of generalist pollinators, while asynchronous flowering species would attract specialized pollinators (Janzen, 1974). Despite the increase in species richness in the second stage, only one individual was collected in some species. These bees remained for a short time in the flowers, and can be considered as occasional or pillage, having low contribution in the process of pollination of the species. Therefore, bees considered occasional in this work did not present pollen adhered to the body, which limited the pollen analysis of these bees.

The highest species richness of bees collected in *E. incanus* flowers can also be attributed to the increase in temperature during the collection days. Although there was no correlation between the average temperature in the days of collection and the bees' richness, the first stage was highlighted by low temperatures with a great abundance of individuals, mainly of *B. pauloensis*, while in the second stage, the collections were performed on hot days, without strong winds or rain. The temperature may be a limiting factor in the flight activities of some bee species, especially for small ones (Gouw & Gimenes, 2013; Oliveira et al., 2012). *Bombus* is a robust bee and, for the study area, this species seems to play an important role since they perform their activities even on cold days and with strong winds where no other species is observed foraging (Araújo et al., 2006). In addition, *B. pauloensis* presents a wide diversity of habitats used for nesting, including forests, savannas, pastures, and even disturbed environments (Gonzalez et al., 2004). The study area offers adequate nesting sites for this species, often observed entering holes in the soil covered by dry grass.

Due to the great similarity between *E. erythropappus* and *E. incanus* pollen and the difficulty in distinguishing between the two pollen types, both were considered as *Eremanthus* spp. (Figure 2A-B). Pollen was removed from those bees with a large amount of it in their body. The pollen found in the bees' bodies was distributed in five plant families and six pollen types (Figure 2).

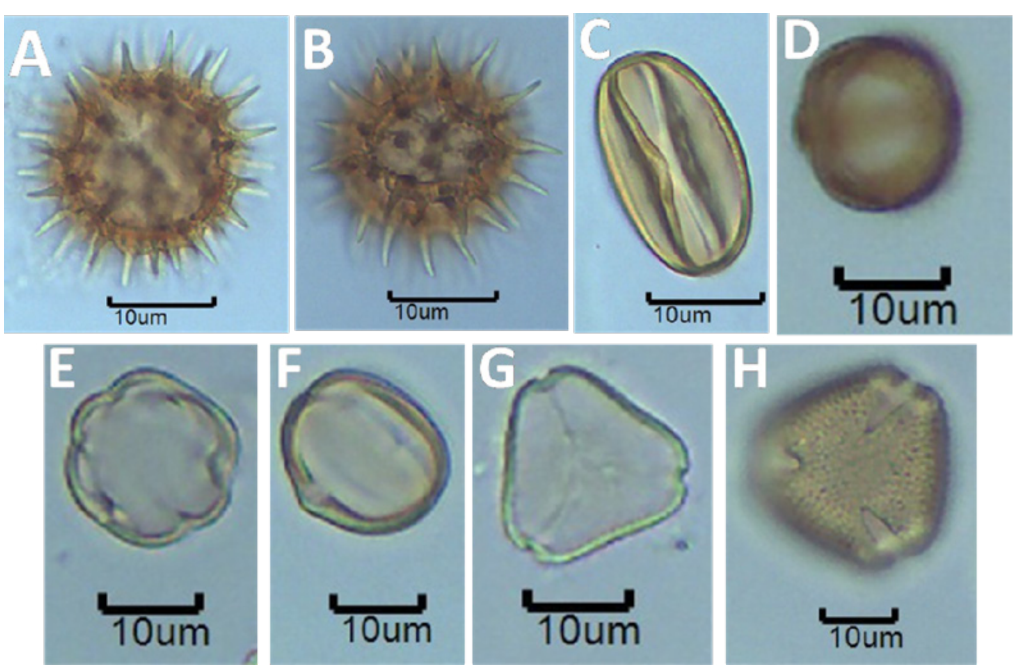

Figure 2. Pollen types adhered to bees' bodies. The bees were collected in flowers of *Eremanthus erythropappus* and *Eremanthus incanus* in a gravel pit area located at Biribiri State Park, Diamantina, MG, 2012. A-B: *Eremanthus* sp.; C: Fabaceae *Chamaecrista* sp.1; D: Lamiaceae type 1; E-F: Melastomataceae *Tibouchina* sp.; G: Myrtaceae type 1; H: Myrtaceae type 2.

In the samples collected in the first stage, the pollen from *Eremanthus* sp. was dominant only in *T. spinipes* with 73.15%. In *B. pauloensis,* the largest variety of types was observed, and *Tibouchina* sp. (Figure 2E-F) and Myrtaceae type 1 (25.96%) (Figure 2G) were the most representative (Table 4).

In the second stage, the pollen from *Eremanthus* sp., *Tibouchina* sp. and Myrtaceae type 1 were dominant in the samples of *A. mellifera*, *B. pauloensis,* and *B. morio*, with 84.68%, 95.41% and 93.34%, respectively. All samples had *Eremanthus* sp. pollen, although it was considered occasional in some bee species (Table 5).

Other plant species showed flowering in the same period of *Eremanthus* spp. During the collection period, plants from other botanical families such as Amarantaceae, Melastomataceae and Myrtaceae were blooming and also provided food resources for the bees. The analysis of the pollen carried by the bees in the two stages revealed the presence of six pollen types. This result shows that bees do not have a broad spectrum of pollen resources used in the area, perhaps due to the reduced number of plants in the area. *Eremanthus* pollen was dominant only in the eusocial species *T. spinipes* and *A. mellifera*. The behavior of these bees as floral visitors is because they are opportunists rather than specialists, exploiting those available resources to the maximum with high density (Kleinert & Giannini, 2012). *A. mellifera*, for example, shows preference for Asteraceae plants and especially those that show mass flowering as candeia species (Maruyama et al., 2018). The preference for Asteraceae flowers in Cerrado biome has already been observed and may be related to the great diversity of species of this family in different areas (Mota et al., 2018). The largest variety of pollen types was observed in the *B. pauloensis* bees. These bees usually have large niche amplitude (Cortopassi-Laurino et al., 2003), which may vary according to the characteristics of each area, such as the diversity and availability of plants in the area (Franco et al., 2009).

Among the pollen types carried by *B. pauloensis,* those of *Tibouchina* sp. (Melastomataceae) found in the first and second stages deserves attention. In the studies carried out in rupestrian fields, relationships between bees of the genus *Bombus* and flowers of poricidal anthers are reported (Cortopassi-Laurino et al., 2003). These bees are large and capable of performing pollination by vibration, which is restricted to certain bee groups (Silva, Ballesteros et al., 2010). In the study area, the species of

Table 4. Average frequency (%) of pollen types found in the body of bees visiting *Eremanthus erythropappus* in a gravel area located at Biribiri State Park, Diamantina, MG, 2012.

Family	Pollen types	*Bombus pauloensis*	*Trigona spinipes*
Asteraceae	*Eremanthus* sp.	3.77	73.15*
	Vernonanthura sp.	2.14	26.85
Melastomataceae	*Tibouchina* sp.	55.99*	-
	Melastomataceae type	5.25	-
Myrtaceae	Myrtaceae type 1	25.96	-
	Myrtaceae type 2	6.89	-

* Dominant pollen (> 45%).

Table 5. Average frequency (%) of pollen types found in the body of bees visiting *Eremanthus incanus* in a gravel area located at Biribiri State Park, Diamantina, MG, 2012.

Family	Pollen types	*Bombus morio*	*Bombus pauloensis*	*Apis mellifera*
Asteraceae	*Eremanthus* sp.	6.03	11.08	95.41*
	Vernonanthura sp.	0.63	0.36	3.46
Fabaceae	*Chamaecrista* sp.	-	3.81	-
Lamiaceae	Lamiaceae type	-	0.05	-
Melastomataceae	*Tibouchina* sp.	-	84.68*	1.13
Myrtaceae	Myrtaceae type 1	93.34*	0.02	-

* Dominant pollen (> 45%).

Melastomataceae, mainly *Tibouchina* sp., appear to be important sources of pollen. The presence, in greater quantity, of *Bombus* spp. in *Eremanthus* flowers probably occurs more by the demand for nectar, since the pollen frequency of other families such as Melastomataceae and Myrtaceae was higher. However, the use of *Eremanthus* spp. pollen is more frequent by *A. mellifera* and *T. spinipes*.

4. CONCLUSIONS

- The eusocial bees *Bombus pauloensis*, *Trigona spinipes,* and *Apis mellifera* were the ones with the highest occurrence in the two species of *Eremanthus*.
- The pollen of *Eremanthus* spp. showed higher frequency in *Trigona spinipes* and *Apis mellifera*.
- *Bombus pauloensis* was responsible for the greater variety of pollen, demonstrating that, in addition to candeia species, other families such as Melastomataceae and Myrtaceae are important sources of food resources for these bees in the study area.

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