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# Pollen Analysis Reveals Plants Foraged by Africanized Honeybees in the Southern Pantanal, Brazil

S Boff[1], CFP Luz[2], AC Araujo[3], A Pott[3]

[1]Programa de Pós-Graduação em Biologia Vegetal, Univ Federal de Mato Grosso do Sul, Campo Grande, MS, Brasil
[2]Secretaria do Meio Ambiente de São Paulo, Instituto de Botânica, Núcleo de Pesquisa em Palinologia, São Paulo, SP, Brasil
[3]Depto de Biologia, Univ Federal de Mato Grosso do Sul, Campo Grande, MS, Brasil

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**Correspondence**
Samuel Boff, Programa de Pós-Graduação em Biologia Vegetal, Univ Federal de Mato Grosso do Sul, CP 549, 79070-900 Campo Grande, MS, Brasil. Current address: Programa de Pós-Graduação em Entomologia FFCLRP-USP, Lab de Abelhas, Rua do Matão 321, Travessa 14, 05508-090, Instituto de Biociências, USP, São Paulo, SP, Brasil; samboff@gmail.com

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## Abstract

The pollen diet of Africanized honeybees *Apis mellifera* L. was studied during seven months (October 2006 to April 2007) in a natural forest fragment in the southern Pantanal, sub-region of Abobral, Mato Grosso do Sul. The analysis of the pollen diet was based on direct observations of the bees visiting flowers as well as through the use of a pollen trap installed in a wild colony in a tree hole in the same forest fragment. The total of 28 species in 15 botanical families were observed as potential sources of pollen for *A. mellifera*, with visits registered in 24 of these species in 13 botanical families. In the pollen trap we recorded 25 pollen types. This study is the first report to use this type of trap for pollen collection in the Neotropical region and aimed to identify the polliniferous bee plants of Brazilian pantanal.

## Introduction

There has been a differentiation regarding source of protein during the evolution of Aculeata Hymenoptera (wasps and bees) (Roubik 1989). Unlike the stinging wasps which fulfill their protein requirements through predation of other arthropods, bees rely on pollen as their only protein source (Roubik 1989, Engels 2001). The pollen is collected at the flowers and transported in a specialized structure to brood cells in the nest (Roubik 1989, Michener 2007).

In spite of a probable relation between bee choice for pollen source and the availability of this resource linked to flower features such as architecture, color and odour, this preference is still under discussion (Bragavan & Smith 1997, Çakmak *et al* 1998, Faegri & van der Pijl 1979). In the case of pollen, the choice of a particular plant species seems to be related to a set of essential aminoacids (Pernal & Currie 2002, Cook *et al* 2003). Pollen grains are utilized by *Apis mellifera* L. as protein source to feed larvae and adults (Zerbo *et at* 2001), also being essential for the development of the hypopharyngeal gland of the nursing bees (Crailsheim 1990).

The composition of pollen loads can vary according to the region or season, indicating patterns and variations of the local flora (Barth 2004). Analysis of pollen loads is a valuable tool for identification of the origin of apicultural products (Barth 2005) and can provide data regarding to the pollen diet of bees (Dimou & Thrasyvoulou 2007). Sampling of pollen loads using traps attached to natural colonies was done with *Trigona collina* (Smith) in Malaysia (Eltz *et al* 2001) and with *A. mellifera* in Europe (Imdorf 1983, Imdorf & Wille 1983). Studies of pollen diets based on pollen loads usually utilize pollen collectors in Langstroth type hives (Luz *et al* 2007, Modro *et al* 2007), direct removal of pollen loads from the corbicula (Fernandes-da-Silva & Serrão 2000, Webby 2004), extracted pollen from colony storage pots (Cortopassi-Laurino & Ramalho 1988, Antonini & Martins 2003) or examination of pollen content in the rectum of honeybees (Dimou & Thrasyvoulou 2009).

In Brazil, knowledge on the flora visited by bees is partly empirical and is limited to certain regions (Barth 2004). Many plant species of the Pantanal wetlands are visited by bees (Pott & Pott, 1999, Pott & Pott unpubl data), but little is known about the phenology of these plants, especially in Mato Grosso do Sul (Reis 2003). For the southern Pantanal region, Pott & Pott (unpubl data) listed the bee plants by observation of visits (either *Apis* or native bees) to flowers, but did not evaluate which plants were the sources of pollen and/or nectar. The pantanal of Mato Grosso do Sul presents a wide diversity of habitats, with predominance of floodable areas, with herbaceous species more frequent than woody ones (Pott & Pott 1999). One of the distinct vegetation types are the natural forested fragments of semideciduous forest, locally known as "capões".

Since bees are the main pollinators of angiosperm species (Michener 2007), the exotic species *Apis mellifera* might compete with native bees for resources offered by these plants (Butz Huryn 1997). Considering the incentives to expand areas designated to apiculture in the Pantanal (Reis & Comastri 2003), as well as to the fact that studies regarding these aspects are scarce, our objective was to survey the poliniferous flora utilized by *A. mellifera* in the pantanal of Mato Grosso do Sul by carrying field observations and pollen analysis, and to test a new technique of pollen trap on a wild colony of Africanized bees.

## Material and Methods

Field observations were performed on the edge of a natural forested patch (capão) on the Fazenda São Bento (19° 34' 36''S/ 57° 01' 08''W), sub-region of Abobral, township of Corumbá, in the Brazilian pantanal wetland, Mato Grosso do Sul. The climate is the tropical sub-humid ("Aw") as defined by Köppen (1948), with distinct rainy (October-March) and dry (April-September) seasons. Average annual rainfall is 1100 mm, and mean annual temperature is 26°C.

The study was performed between October 2006 and April 2007, covering one rainy season in a natural forested patch of 0.62 ha. These forested patches are islets of woody vegetation generally circular or elliptical in shape, on a terrain up to 5 m above the floodable grassland. The vegetation on its edge is flood tolerant, while dry forest species occur in the interior (Damasceno Jr *et al* 1999).

The flowering species present at the edge of the studied forested patch were recorded monthly in four plots of 5 x 20 m (100 $m^2$). Plots were oriented as follows: one to north, one to south, one to east and one to the west of the studied forested patch. Only exclusively polliniferous and polliniferous/nectariferous plants were recorded in these plots. To obtain data on the probable pollen diet, direct observations on the honeybees foraging for pollen in flowers were monthly made in the studied plots (October 2006 - April 2007). Each flowering species in a given plot was visually observed for a period of 15 min regarding *A. mellifera* visits.

In addition, to include in our sample species that were not registered in the censuses at the studied plots, we installed a pollen trap attached to the entrance of a wild colony of *A. mellifera* in a tree hole inside the forested patch.Only one pollen trap was included in our sample since the monitored nest was the only one found in the forested patchs of the studied area. This trap is a perforated metal plate with a collector underneath it. Some holes in the plate had a diameter similar to the pollen collectors for Langstroth hives (4 mm), retaining the pollen load of the corbicula; other holes were slightly larger (5 mm), allowing the bee's entrance to the hive with the pollen load. The pollen trap was used for one day every month, except in the rainy period, when it remained on the hive for three months (December-February) due to difficulties of access. The pollen collected in the trap in this three-month period represented one sample.

The samples of pollen loads were kept in glycerin 50% and were later analyzed with the classic European method (Maurizio & Louveaux 1965). Pollen was mixed in 10 ml of 70% ethanol, centrifuged, and the sediment was washed in distilled water, left for 30 min in 1:1 glycerol:$H_2O$, and centrifuged again. For light microscopy observations, slides were prepared using glycerin-jelly and sealed with paraffin. The identification of pollen types was based on the floristic survey and on pollen catalogues (Salgado-Labouriau 1973, Roubik & Moreno 1991). A pollen type is named after an enclosed genus or species, establishing proximity with a taxonomic group (Salgado-Labouriau 1973, Lorscheitter 1989). For calculation of frequency classes of each pollen type a total of 300 pollen grains were counted per sample. Frequency classes were defined as: dominant pollen (D) > 45% of the total of counted pollen grains; accessory pollen (A) = 15% to 45%; important isolated pollen (I) = 3% a 15%; and occasional isolated pollen (O) < 3% (Zander 1935).

## Results

Plants identified as pollen sources were defined as flowering species that offered pollen or both nectar and pollen as resources. During the focal observations and in the vegetation survey a total of 28 species, in 15 families, were observed as pollen sources, with Malvaceae, Fabaceae and Rubiaceae as the most representative (Table 1). These plants were mainly herbs or shrubs, and nine species were exclusively polliniferous, while 19 species offered both pollen and nectar (Table 1). Among all these plants, 24 species were visited by *A. mellifera*.

A total of 25 types of pollen (Fig 1) were found in the pollen trap at the entrance of the wild nest (Table 2). The families with the largest number of pollen types were: Fabaceae (4), Asteraceae (3), Rubiaceae (3), Euphorbiaceae (2) and Arecaceae (2). Other families were represented by a single pollen type. Pollen types varied both in time, frequency and species richness (Table 2). The highest number of pollen types was recorded (n = 13) during October, followed by December-February, when the pollen trap was continuously present (n = 10). The other three samples, in November, March and April, showed lower richness, with six, one and three pollen types, respectively (Table 2). The Piperaceae and Scrophulariaceae pollen types had less than three

Table 1 Plant families, species names, potential resource (Res) offered by the plant (p = pollen, n = nectar) and flowering period from October 2007 to April 2007 for species occurring on the edge of the forest fragment (capão) in southern pantanal, MS, Brazil.

| Families | Species | Res | Months | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Oct | Nov | Dec | Jan | Feb | Mar | Apr |
| Alismataceae | *Echinodorus paniculatus* | P+n | | | | | X | | |
| | *Echinodorus tenellus* | P | | | | | | | X |
| Asteraceae | *Centratherum punctatum* | P+n | | X | | X | | | |
| | *Wedelia brachycarpa* | P+n | X | X | X | X | | | |
| Commelinaceae | *Commelina erecta* | P | X | X | X | | X | | X |
| Euphorbiaceae | *Croton densiflorus* | P+n | | X | | X | | | |
| | *Sebastiania hispida* | P | X | X | X | | X | X | X |
| Fabaceae | *Senna occidentalis*[1] | P | | | | | | | X |
| | *Senna spectabilis*[1] | P | | | | | | | X |
| | *Senna splendida*[1] | P | | X | X | X | X | X | |
| Hippocrateaceae | *Hippocratea volubilis* | P+n | | X | | | X | | X |
| Lamiaceae | *Hyptis suaveolens* | P+n | X | X | X | X | X | X | X |
| Lythraceae | *Adenaria floribunda* | P+n | | X | X | X | X | X | X |
| Malvaceae | *Melochia parvifolia* | P+n | | X | X | X | X | X | X |
| | *Melochia pyramidata* | P+n | X | | | | | | |
| | *Melochia simplex* | P+n | | | | | | | X |
| | *Sida linifolia* | P+n | X | | | | | | |
| | *Sida santaremensis* | P+n | | | | X | | | |
| | *Sida viarum* | P+n | | | | | | | X |
| Onagraceae | *Ludwigia octovalvis* | P+n | | | | | X | | X |
| Portulacaceae | *Portulaca fluvialis* | P | | | | X | | | |
| Rubiaceae | *Psychotria carthagenensis* | P+n | X | | | | | | |
| | *Richardia grandiflora* | P+n | X | X | X | X | X | | |
| | *Staëlia thymoides* | P+n | X | | | | | | |
| Salicaceae | *Casearia aculeata* | P+n | | | | | X | | |
| Scrophulariacae | *Bacopa australis* | P+n | | | | | X | | X |
| | *Scoparia montevidensis* | P | X | | X | X | X | | |
| Solanaceae | *Solanum viarum*[1] | P | X | X | | X | X | | X |

[1] Species in which visits of *Apis mellifera* were not observed.

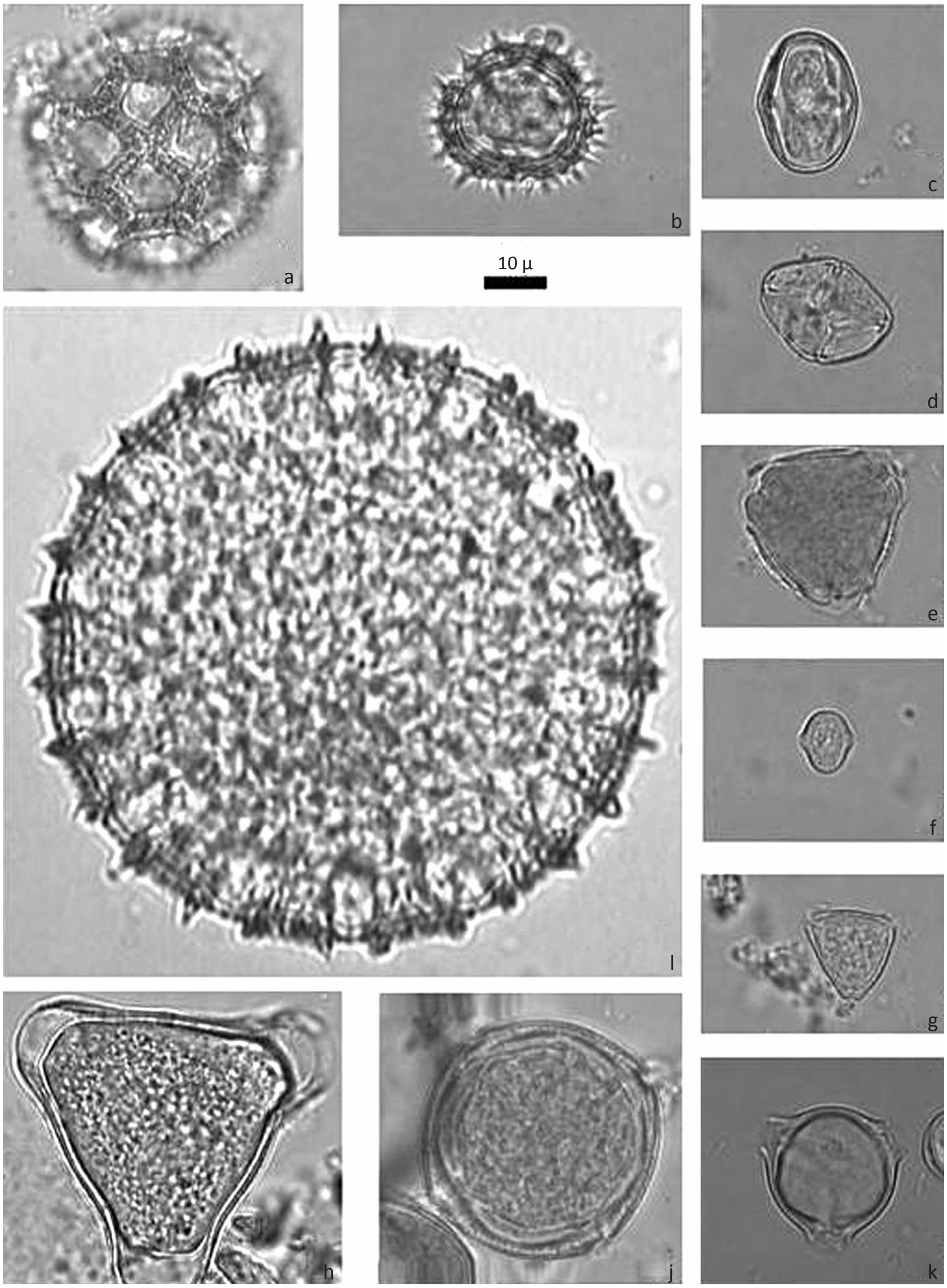

Fig 1 Light microscopy (LM) pollen micrographs observed in the pollen load samples of wild Africanized *Apis mellifera* 0n the edge of a forest fragment (capão) in southern Pantanal, MS, Brazil. a-b) Asteraceae. a. *Elephantopus*; b) *Wedelia*; c) Euphorbiaceae; d-e) Fabaceae; d) *Mimosa selloi*; e) Fabaceae; f) Moraceae, *Cecropia*; g) Myrtaceae, *Myrcia*; h) Onagraceae; i-j) Rubiaceae; i) *Richardia*; j) Rubiaceae; k) Solanaceae, *Solanum*. Scale = 10 µm.

grains per sample, and were therefore considered to be contaminants.

Among species which offered exclusively pollen, *Scoparia montevidensis* was observed being visited only by *A. mellifera*. In addition, flowers adapted for buzz-pollination, which have poricide anthers, (*e.g. Solanum* and Melastomataceae types) were also visited by *A. mellifera*. In occasional observations, other exclusively polliniferous species (*e.g. Solanum viarum* and *Senna splendida* - Table 1) were recorded being visited by buzz-pollinating bees such as *Bombus pauloensis* (Friese), *Xylocopa frontalis* (Olivier) and *Augochloropsis*

Table 2 Dominance of pollen types in pollen loads foraged by wild Africanized *Apis mellifera* during seven months from October 2007 to April 2007 on the edge of a forest fragment (capão) in the southern pantanal, MS, Brazil.

| Pollen type | Months | | | | |
|---|---|---|---|---|---|
| | Oct | Nov | Dec-feb | Mar | Apr |
| *Bactris* | I | | | | |
| Arecaceae | I | | | | O |
| *Cecropia* | A | A | | | |
| *Elephantopus* | O | | A | | |
| *Wedelia* | A | I | O | | |
| *Sorocea* | O | | | | |
| *Richardia* | O | D | I | | |
| *Hyptis suaveolens* | O | | | | |
| Euphorbiaceae | O | I | | | |
| *Myrcia* | O | O | | | |
| Melastomataceae/ Combretaceae | O | | | | |
| *Croton* | O | | | | |
| Poaceae | O | | | | |
| *Acacia* | O | | | | |
| *Borreria* | | I | I | | |
| Sterculiaceae | | | O | | |
| *Mimosa scabrella* | | | I | | |
| Alismataceae | | | I | | |
| Sapindaceae | | | O | | |
| *Mimosa selloi* | | | A | | |
| Asteraceae | | | O | | |
| Onagraceae | | | | D | |
| Fabaceae | | | | | A |
| *Solanum* | | | | | A |
| Rubiaceae | | | | | A |

Frequency classes applied were: dominant pollen (D) ≥ 45% of the total of counted pollen grains; accessory pollen (A) = 15% to 45%; important isolated pollen (I) = 3% a 15%; and occasional isolated pollen (O) < 3%.

sp. Although *Trigona fuscipennis* (Friese) is not adapted for buzz-pollination, it was also observed harvesting pollen from *S. splendida*, forcing the anther pores with its mandibles.

## Discussion

Fabaceae, Asteraceae, Rubiaceae, Euphorbiaceae and Arecaceae are among the families whose flowers were most used by *A. mellifera* bees in the area studied. Studies in areas outside pantanal also reported similar patterns to those reported here. A survey based on pollen analysis of pollen loads from *A. mellifera* and *Melipona scutellaris* (Latreille) showed that Arecaceae, Asteraceae, Fabaceae, Malvaceae, Poaceae and Rubiaceae were among the most important families for these bees (Carvalho *et al* 1999, Ramalho *et al* 2007). Although the habitats in which these studies were conducted are quite different from Pantanal, the results are similar to the present survey. The most attractive species for honeybees in the present study were herbs and shrubs, which frequently grow on the edges of the studied forested patch (Damasceno Jr *et al* 1999).

Some of the observed species and genera considered melittophilous had already been indicated as potential bee plants in pantanal (Araujo & Sazima 2003, Pott & Pott unpubl data). However, since our study encompassed only part of the year, the number of bee plants in the forested patches is probably higher than the number reported here. In addition, due to the dynamics of the flooding regime in the pantanal wetland, the number of flowering species can also vary throughout the year (Pott & Pott unpubl data).

In a study using artificial flowers, Çakmak *et al* (1998) verified that *A. mellifera* can concentrate foraging efforts on a single type of flower, a fidelity that is important for the reproductive success of the involved species, including cultivated plants (Basualdo *et al* 2000). As a consequence of floral fidelity during a certain period, almost all the pollen load of a colony is uniformly derived from a single species and the proportion of mixed loads can vary from 0 to 3% (Deodikar 1965).

In our study this fact could explain the presence of Onagraceae as a single pollen type in March. Many Onagraceae species occur in pantanal, mainly on flooded areas around the forested patches, remaining until the soil dries after flooding. Therefore, the reported fidelity in the studied area could be related to the scarcity of other flowering species, due to seasonal inundation of the floodplain (Pott & Pott 1999). This fact could force honeybees to visit flowers of a limited number of plants. Also, floral fidelity could be related to the distance of the hives to floral sources, sometimes being more advantageous for bees to forage on a single flowering species that occurs in proximity rather than search for different flowers (Krebs & Davies 1996). In addition, our data were gathered from only one nest, and differences regarding flower resources between hives could occur (Ramalho *et al* 2007). Nevertheless, in most of the sampled months, the bees harvested pollen from different floral sources. According to Schmidt & Buchmann (1993), a colony of *A. mellifera* harvests many plant species, thus receiving a good nutritional balance that is reflected by the highest richness of the "occasional important" pollen group found in our study. This is expected when many plant species flower in the same period.

Seasonal variation in collection of pollen by honeybees could also contribute to the conservation of plant species in pantanal, if the pollination efficiency of visiting bees ensures their pollination. On the other hand, if honeybees are not efficient pollinators, both reproduction of visited plants and populations of native bees that depend upon these resources are affected. However, this would mainly occur with oligoletic bees that are strictly dependent upon one or a few plant species (Alves dos Santos 2003, Schlindwein 2004). The plants preferred by oligoletic bees are mostly herbaceous or shrubs, like the species of Onagraceae and Asteraceae (Schlindwein 2004), both recorded as visited by *A. mellifera* in this study.

Some resources utilized by honeybees were not observed in the plots, such as *Acacia*, Arecaceae, *Cecropia,* and *Myrcia*. However, they do occur in the forested patches (Damasceno Jr *et al* 1999, Araujo 2001) and have been observed nearby, indicating that the bees of the monitored nest also gathered resources in adjacent forested patchs. The analysis of pollen loads also allowed us to conclude that certain plants known for supplying nectar, also offer pollen to *A. mellifera* (*e.g*. *Wedelia brachycarpa*). This also indicates that the trap used for pollen sampling is an efficient method to study pollen diet of bees in natural colonies. The observed pollen types in the pollen loads collected in the trap are to be considered typical of the habitats and vegetation forms of the study area (natural forested islands and herbaceous surroundings), allowing the identification of the floral origin of the pollen supply during the analyzed period. *Apis mellifera* fed mainly on herbs and shrubs, the predominant growth habit of species occurring on the edges of the forested patchs, as well as in the floodable grasslands. Meanwhile, these bees also forage on flowers of trees and lianas, mainly during the beginning of the wet period in October-November.

The physiognomy of pantanal wetlands composed of open grasslands with forested areas restricted to the natural forested patchs, ancient levee and gallery forests (Pott & Pott 2004) favors the expansion of populations of Africanized honeybees (Gonçalves 1994). A fundamental aspect to be focused in future research regarding conservation in pantanal is the relation between the fauna of native bees and plants they pollinate, analyzing the effects of the presence of *A. mellifera* on this process. An alternative proposal is meliponiculture, which is the management of colonies of native stingless bees. This activity is important also for the development of human local communities, being an economic alternative (Cortopassi-Laurino *et al* 2006).

In conclusion, our data support an apicultural calendar for the Abobral sub-region. However, we suggest that the evaluation of the flora of the pantanal as pollen resource for *A. mellifera* should include a larger number of the forested patches and landscapes, as well as year-round studies, since species richness tends to increase with sample size. Finally, *Apis mellifera* feeding on the same flower resources as the native bees may be a possible competitor. Hence, intensive apiculture in this environment should be thoroughly discussed, evaluating the hypothesis of resource overlap between *A. mellifera* and native bees, as well as verifying the effect of pollination by honeybees on the reproductive success of the native plants of the pantanal wetland. Studies comparing pollination efficiencies of native bees and honeybees, focusing on the number of fruits and seeds produced by plant species recorded here, as well as monitoring nests of native bees are encouraged.

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