



## Bionomics and Behavior/Bionomia e Comportamento

# Foraging activity of africanized honeybees (*Apis mellifera* L.): A study of nectar and pollen resources on a temporal scale

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**Abstract.** The spatial and temporal distribution of food resources, as well as the type, quantity, and quality of the foods stocked in the hive are the principal regulatory factors of the choice and intensity of floral resource harvesting by bees. We evaluated the annual foraging activity of Africanized honeybees *Apis mellifera* L. (Apidae) on the most abundant natural food resources available. Nineteen abundant plant species susceptible to foraging by bee communities in the interior of a secondary growth forest fragment with a transition physiognomy between Atlantic Forest and Cerradão vegetation were accompanied to estimate the intensity of floral resource collection by Africanized honeybees *A. mellifera* during the year. We determined the productivity of the flowers (the quality and quantity of nectar and/or pollen made available) and floral abundance (the quantities of flowers produced and the duration of flowering) of the 19 plant species selected. Africanized honeybees *A. mellifera* collected floral resources from 11 species. The intensities of visits per flower and per area of floral exposition were greater among plant species visited by Africanized honeybees when bee collecting behavior resulted in pollen transfer to the floral stigmas. It is estimated that 70.5% of all visits by Africanized honeybees *A. mellifera* individuals during the year in the study area occurred on *Senegalia polyphylla* (DC.) Britton & Rose (Fabaceae), *Grazielia cf. dimorpholepis* (Baker) R.M.King & H.Rob (Asteraceae), and *Gouania cf. latifolia* Reissek (Rhamnaceae); those visits demonstrated seasonal patterns, with peaks of activity between January and April. Weak foraging activity was observed in June and between June and November.

**Keywords:** Dominant Species; Ecological Succession; Floral Abundance; Floral Resources; seasonality.

### Atividade de forrageio de abelhas Africanizadas (*Apis mellifera* L.): Um estudo das fontes de néctar e pólen em uma escala temporal

**Resumo.** A distribuição espacial e temporal dos recursos alimentares, bem como o tipo, quantidade e qualidade do alimento estocado na colmeia são os principais fatores reguladores na escolha e intensidade da coleta dos recursos florais pelas abelhas. O objetivo desse trabalho foi avaliar a atividade anual de forrageio de abelhas africanizadas *Apis mellifera* L. (Apidae) nas fontes alimentares naturais mais abundantes. A coleta de dados foi realizada em 19 espécies vegetais abundantes e suscetíveis ao forrageio pela comunidade de abelhas no interior de um fragmento de floresta secundária com fisionomia em transição entre Mata Atlântica e Cerradão, durante um ano. Para estimar a intensidade de coleta de recursos florais pelas abelhas africanizadas *A. mellifera*, foi determinada a produtividade das flores (qualidade e quantidade do néctar e/ou pólen alocada nas flores) e a abundância (quantidade de flores e duração do florescimento) das 19 espécies vegetais selecionadas. As abelhas africanizadas *A. mellifera* coletaram recursos florais em 11 espécies vegetais. As intensidades de visitas por flor e áreas de exposição floral foram superiores nas espécies de plantas que foram visitadas pelas abelhas africanizadas *A. mellifera* cujos comportamentos de coleta resultavam em transferência de pólen aos estigmas das flores. Estima-se que 70,5% de todas as visitas promovidas por *A. mellifera* africanizada no decorrer do ano na região de estudo ocorreram em *Senegalia polyphylla* (DC.) Britton & Rose (Fabaceae), *Grazielia cf. dimorpholepis* (Baker) R.M.King & H.Rob (Asteraceae), e *Gouania cf. latifolia* Reissek (Rhamnaceae), demonstrando, dessa forma, um padrão sazonal, com picos de atividade em janeiro, abril e agosto, respectivamente. Por outro lado, houve fraca atividade de forrageio em junho e entre setembro e novembro.

**Palavras-chave:** Abundância Floral; Espécies Dominantes; Recursos Florais; Sucessão Ecológica; Sazonalidade.

One of the advantages of bee social organization is that colony individuals have efficient mechanisms of communication that allow them to explore their habitat and collect information about floral alternative resources (possibly better than those currently used) and recruit additional foragers for harvesting them (BEEKMAN *et al.* 2007; DÍAZ *et al.*

2007). That situation allows the colony to selectively exploit the most rewarding resources in a frequently unstable environment (FRISCH 1967; SEELEY 1995), once appropriate natural food resources are ephemeral, dispersed, and highly diverse.

The nutritional state of the colony – that is the quantities of food stocks stored in the hive – is also a preponderant regulatory

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factor of colony foraging activity (SEELEY 1989; PÍREZ & FARINA 2004; GRÜTER & FARINA 2007). As such, the foraging strategies of social bees will depend on the conditions of their surrounding habitat, including the spatial and temporal distributions of food resources (SHERMAN & VISSCHER 2002; DORNHAUS & CHITTKA 2004; PASQUET *et al.* 2008), and the types, quantities, and qualities of stocked supplies in the hive (FREE 1980).

Two principal methodologies can be used to identify the floral resources collected by bees: pollen traps installed at the hive entrance that can remove pollen grains adhering to the legs of the bees (KOPPLER *et al.* 2007; TAHA 2015); and direct observations of the floral resources collected from flowers (HAALAND *et al.* 2011). Those two methods produce independent results and in different ecological levels: the colonies themselves and local bee populations respectively (KLEINERT *et al.* 2009). Although the first method is highly efficient in terms of recording the diversity and abundance of the pollen load, it does not allow verifying the nectar sources (DONKERSLEY *et al.* 2017; SMART *et al.* 2017). The second method allows to verify the floral resources used by bees (including nectar and oils, and not just pollen), although the results are limited to just the set of floral sources directly observed by the researcher (POLATTO & CHAUD-NETTO 2013). The present study employed the second method to define the floral resources used by Africanized honeybees (a hybrid resulting from crosses between the African subspecies *Apis mellifera scutellata* Lepelletier and other European subspecies of *Apis mellifera* L.) on a temporal scale.

The efficient communication system of Africanized honeybee, together with other factors (*e.g.*, large population, foraging strategies and rapid population growth), makes it the dominant floral visitor in many natural and agricultural areas. That situation can result in negative impacts on the reproduction of native plants that cannot be adequately pollinated by those bees – but whose floral resources are collected by them – resulting in the reduction of the native pollinator activities (TRAVERSE & RICHARDSON 2006; CARBONARI *et al.* 2009). Other native plant species, on the other hand, will presumably have their reproductive efficiencies maximized as a result of the intense pollination activities of Africanized honeybee.

Many studies focused on pollen and nectar collection by Africanized honeybee at both the plant species and plant community levels, however, there has been little effort yet to

examine the temporal scale of that harvesting. As such, the present study sought to evaluate the annual foraging activity of Africanized honeybee on the most abundant natural food resources in a forest fragment.

## MATERIAL AND METHODS

**Study area and the plant species investigated.** This study was carried out in a fragment of secondary forest (approximately 355 hectares) in the municipality of Ivinhema, Mato Grosso do Sul State, Brazil (22°15'42"S; 53°48'10"W), for 12 consecutive months (July 2010 to June 2011). The regional climate is classified as humid to subhumid (ZAVATTINI 1992).

The forest fragment was composed of plants at different successional stages, in a transition zone between Atlantic Forest and Cerradão (Forested Neotropical Savanna). The vegetation of the study site is classified in three successional stages according to the criteria proposed by BUDOWSKI (1965): pioneer vegetation, corresponding to approximately 20% of the total area; initial secondary vegetation, corresponding to approximately 35% of the total area; and late secondary vegetation, corresponding to the balance of the vegetation (approximately 45% of the total area) (Figure 1).

The most abundant plant species that demonstrated high flowering rates in the study area were observed during 12 months. Bee communities visited those plant species to collect resources independent of whether they were foraged on by Africanized honeybees. During the selection of the plant species to be accompanied, we did not take into consideration the necessity of the flower and the bee communities having reciprocal adaptive features (so that all of the plant species with attractive flowers were included in the sample, even if the collection strategies of the bees did not result in successful pollination).

**Numbers of visits per flower.** Our observations of bee activities in the field followed the methods described by POLATTO & ALVES-JR (2008). Three plants of each species were closely observed during their high-frequency flowering period (76% to 100% of the plants in flower) for visitation by Africanized honeybee workers, following the classification system of FOURNIER (1974). An area of 1 m<sup>2</sup> with flowering branches near ground level (the focal area) was selected on each plant, in which we recorded the numbers of flowers at the beginning of

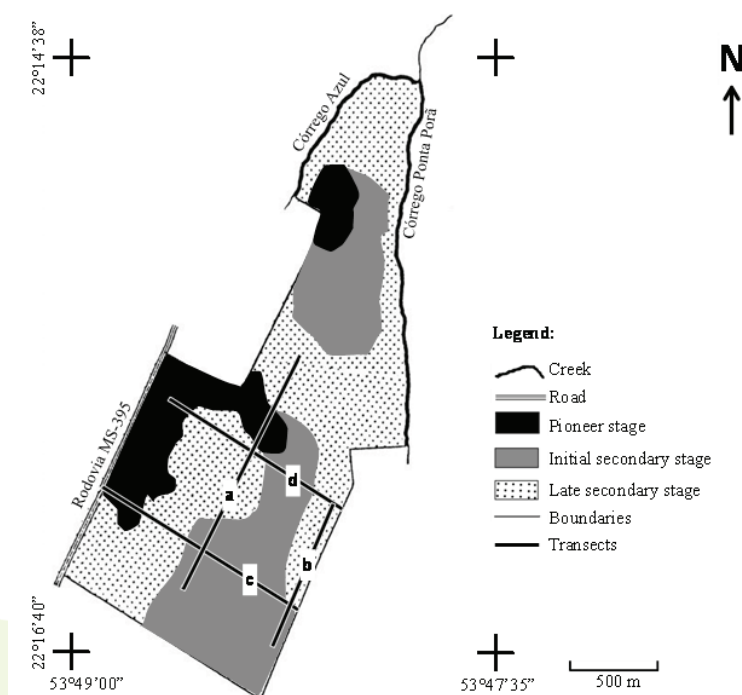


Figure 1. Localization of the study area and the transects (lines a, b, c, d).

the day and the numbers of visiting foraging bees appearing in an interval of 20 minutes during each hour of the day (between 06:00 and 17:00). The numbers of foraging bees as well as the type of resources they collected on those plants were recorded. Each foraging event was defined as the presence of a bee in the focal area, independent of the number of flowers it visited before returning to the hive.

In a second subsequent interval of 10 minutes in each hour, we observed the numbers of flowers visited by each bee during a single visit. Among those plant species that offered both pollen and nectar, we recorded the numbers of flowers visited for each type of resource collected.

During our observations (during both time intervals) the observer remained immobile at a distance of 0.5 to 3 m from the focal area, therefore avoiding (to a maximum degree possible) any type of disturbance of the bees' foraging activities.

The following equation was used (POLATTO & ALVES-JR 2008; with some modifications) to estimate the numbers of floral visits of Africanized honeybee to each focal plant species.

$$\text{Number of visits per flower} = \frac{\text{Foragers} \times \text{Visits} \times \text{Anthesis}}{\text{Flowers}}$$

(equation 1)

where, *Foragers* correspond to the numbers of foraging events undertaken in the focal area by the bees ( $n^{\circ}$  of observations of bee visits in the 1st interval of 20 min, multiplied by 3 – thereby amplifying the period to 1 h and extending the foraging period to include the entire day); *Visits* represents the numbers of flowers visited during each bee foraging event within the focal area (the mean observed in the 2<sup>nd</sup> 10 min interval); *Anthesis* refers to the duration of anthesis of the flower, measured in days; *Flowers* indicates the number of flowers in anthesis in the focal area (counts of the flowers in the focal area undertaken at the beginning of the day).

The plant species selected were grouped into three classes based on the estimated numbers of visits to each flower by a honeybee. That classification established a confidence interval (CI) of a 95% probability of success, with: (1) high numbers of visits = number of visits per flower greater than the upper limit of the 95% CI; (2) moderate numbers of visits = number of visits per flower situated within the 95% CI; (3) low numbers of visits = number of visits per flower less than the lower limit of the 95% CI.

To predict whether an Africanized honeybee worker could be considered a pollinator of a given plant species, or not, we also recorded the exploitation technique used in harvesting the floral resources (INOUE 1980), determining the occurrence, or not, of contact by the bee's body with the anthers and stigma of the flower, the numbers of flowers visited, and the occurrence, or not, of foraging events between plants.

The Mann-Whitney (U) test was used to determine if there was a relationship between the two variables (spatial occupation and the numbers of visits per flower of the focal plant species) and their probability (or not) of being pollinated by Africanized honeybees. Pollination was considered to be probable when the bees touched the reproductive organs of the flowers during most of their visits. That test is a nonparametric alternative to the Student t test, which was chosen due to the fact that the variable "their probability (or not) of being pollinated by Africanized honeybee" demonstrates a nominal qualitative distribution (DAWSON & TRAPP 2003). The values of the spatial occupations of the populations of each plant species were obtained from transects laid out during their flowering periods that estimated their total leaf areas.

**Rates of resource collection throughout the year.** In addition to the estimated numbers of visits by Africanized honeybees to each flower, the quantities of flowers produced and the durations of flowering were preponderant variables for determining the rates of resource collection in a given environment, as well as the distributions of resources collection throughout the year.

Equation 2 was developed to determine the rate of resources collection by Africanized honeybee in each of the focal plant populations.

$$\text{Rate of resource collection} = \frac{N. \text{ visits per flower} \times \text{Quantity} \times \text{Flowering}}{\text{Anthesis}}$$

(equation 2)

where, *N. visits per flower* represents the number of floral visits that Africanized honeybees undertook on each focal plant species, which was obtained in equation 1; *Quantity* indicates the estimated quantities of flowers in the transects, derived by multiplying the mean number of flowers in anthesis in each 1 m<sup>2</sup> of plant area by the total flowering area within the transect; *Flowering* corresponds to the duration (in days) that a given plant population flowered at high-frequency; *Anthesis* refers to the duration (in days) of the anthesis of those flowers.

The rates of resource collection by Africanized honeybee among all of the plant populations accompanied were divided into three classes (again established by a 95% probability CI) (1) high rate of resource collection = foraging frequency greater than the upper limit of the 95% CI; (2) moderate rate of resource collection = foraging frequency situated within the 95% CI; (3) low rate of resource collection = foraging frequency less than the lower limit of the 95% CI.

## RESULTS

The key plant populations present in the forest fragment where especially well-represented by the families Bignoniaceae and Malpighiaceae, with five and four species respectively; Asteraceae and Fabaceae were represented by three species, Sapindaceae by two, and Lamiaceae and Rhamnaceae by one species each. In terms of the habits of the key plant species, only two were arboreal; the remaining species were vines or shrubs (with 10 and seven representatives respectively).

Although 19 key plant species were identified in the forest fragment, not all of them were exploited by Africanized honeybee (Figure 2). Nonetheless, the exploitation of 11 key plant species (collecting only nectar from three species, and both nectar and pollen from eight) shows that Africanized honeybee demonstrates wide plasticity, and can therefore be considered a highly generalist exploiter of the floral resources available in that environment. Three of the plant species in the forest fragment (two members of Asteraceae and one of Fabaceae) experienced high numbers of visits per flower. Eight plant species, on the other hand, were not visited by Africanized honeybee, while another eight plant species received only moderate numbers of visits (Figure 2).

The rates of floral resource collection were greater on plant species visited by Africanized honeybees showing collection behaviors that resulted in pollen transferred to the stigmas of their flowers ( $U = 0$ ;  $p < 0.001$ ; Figure 3A). As such, there appeared to be a stimulation of the exploitation of the floral resources of plant species on which bee behavior was adapted for effective pollination. Those plant species demonstrated greater areas of floral exposition than other plant species that were rarely successfully pollinated by Africanized honeybees (in those cases, the bees acted as thieves or robbers of the floral resources) ( $U = 13$ ;  $p = 0.016$ ; Figure 3B).

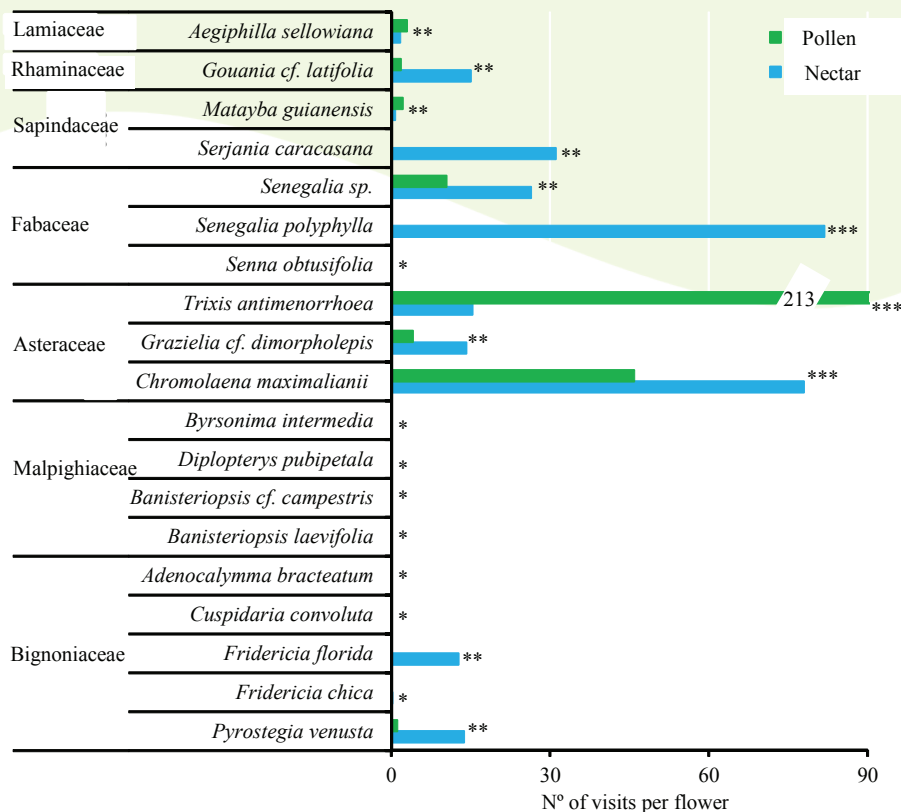


Figure 2. Estimated numbers of visits by Africanized honeybee workers to each flower of the key plant species. Values obtain using equation 1. Values followed by \* indicate low numbers of visits (n° of visits per flower < 95% CI); \*\* indicates moderate numbers of visits (n° of visits per flower within the 95% CI); \*\*\* indicates high numbers of visits (n° of visits per flower > 95% CI). A 95% CI = between 2.2 and 58.0 visits per flower.

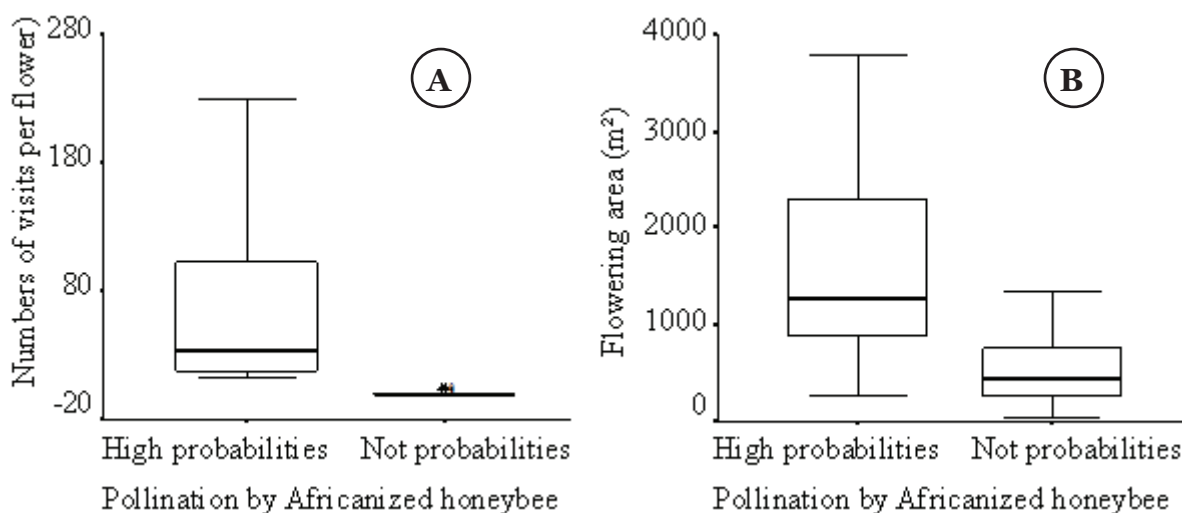


Figure 3. Relationship between the numbers of visits by Africanized honeybee workers (A) and the spatial areas occupied (B) by plant species demonstrating high probabilities, or not, of pollination by Africanized honeybee. The closed rectangle represents 50% of the central spread of the data; the central line indicates the mean; the upper line represents the greater 25% of the values, and the lower line the lower 25%.

In terms of the rates of resource collection by Africanized honeybee, just three plant species (classified as having high rates of resources collection) were responsible for 70.5% of all harvested resources. The eight plant species that demonstrated the lowest rates of resource collection, on the other hand, were practically ignored by those bees (Figure 4).

Figure 5 demonstrates that the foraging activities of Africanized honeybees followed a seasonal pattern. The intensities of visits per flower and per area of floral exposition were greater among plant species visited by Africanized *A. mellifera* when bee collecting behavior resulted in pollen transfer to the floral stigmas. It is estimated that 70.5% of all visits by Africanized *A.*

*mellifera* individuals during the year in the study area occurred on *Senegalia polyphylla* (DC.) Britton & Rose (Fabaceae), *Grazielia cf. dimorpholepis* (Baker) R.M.King & H.Rob (Asteraceae), and *Gouania cf. latifolia* Reissek (Rhaminaceae); those visits demonstrated seasonal patterns, with peaks of activity between January and April. Weak foraging activity was observed in June and between June and November.

### DISCUSSION

As the research site was in a regenerating forest fragment, it was predominantly populated by vines and shrubs (BUDOWSKI 1965; HORA & SOARES 2002), as edge effects, a greater incidence of

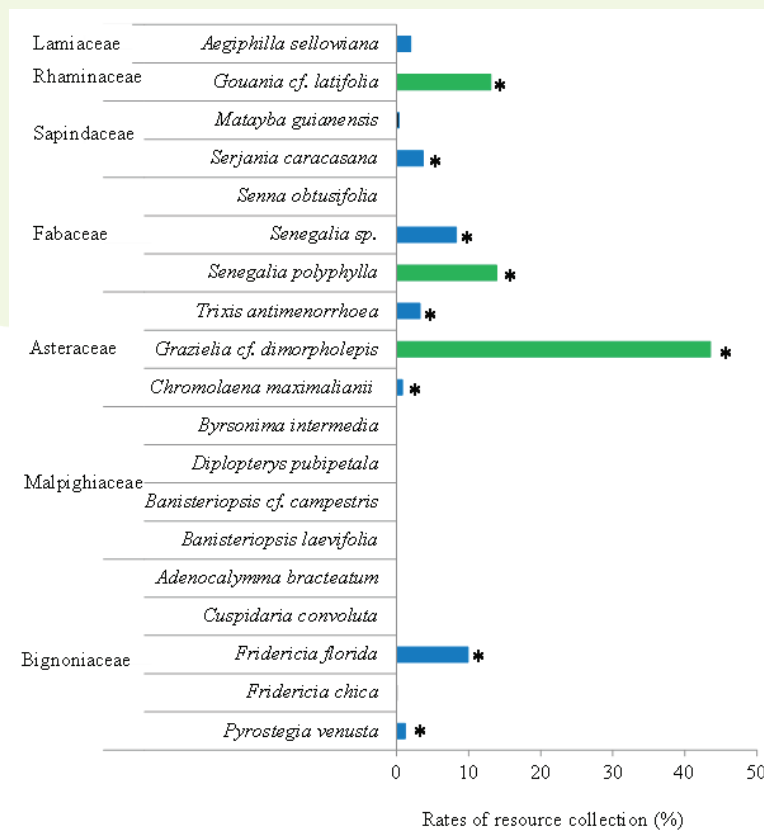


Figure 4. Rates of resource collection from the key plant species extracted by Africanized honeybees. Values obtained from equation 2. Legend: green bars = high rates of resource collection; blue bars = moderate rates of resource collection; absence of bars = low rates, or absence, of resource collection; \* = plant species from which floral resource collection would result in probable pollination.

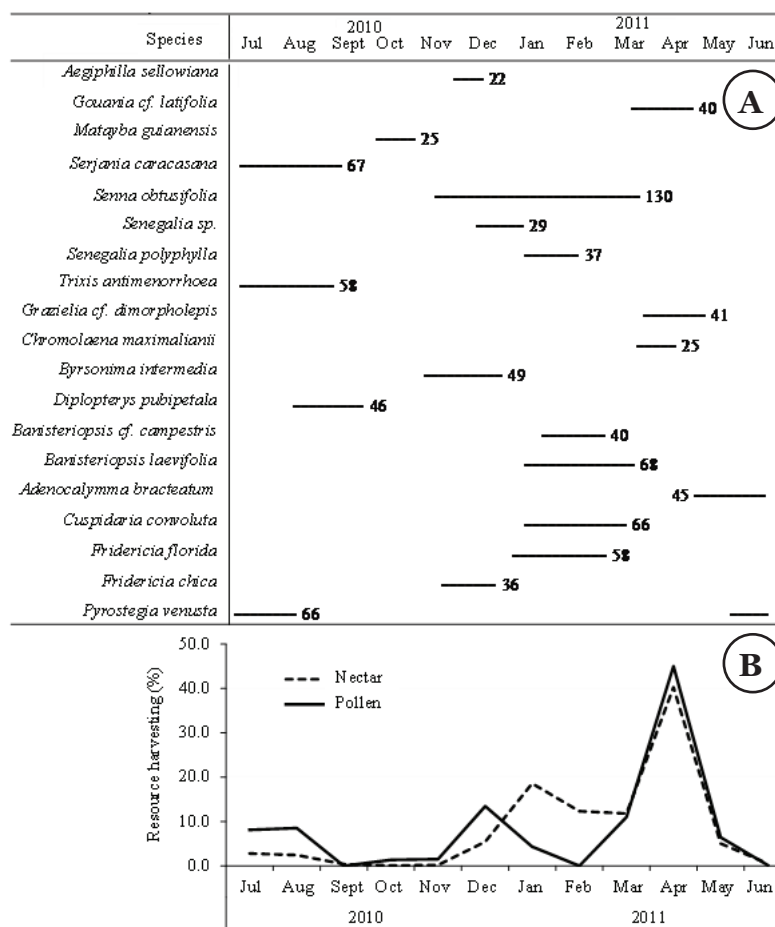


Figure 5. Phenology of high-frequency flowering (76% to 100% of the plants flowering) of the key species (A) and proportions of floral resources collected by Africanized honeybee from the key plant species during the year (B). In Graph A, the values accompanying the horizontal lines indicate the numbers of days that the plants demonstrate high-frequency flowering.

sunlight, and the formation of clearings all favor occupation by those types of plants (HEGARTY & CABALLÉ 1991). Bignoniaceae and Asteraceae were the most diverse families, with all of their key species demonstrating vine and shrub habits.

The ample trophic niche observed to be occupied by Africanized honeybees in the present study corroborated other reports (e.g., WILMS *et al.* 1996; SANTOS *et al.* 2004; ANDENA *et al.* 2005; GONÇALVES & MELO 2005; BOFF *et al.* 2013; POLATTO & CHAUDNETTO 2013), and reflects the typical feeding strategy of that bee. Most of the areas studied by researchers showing Africanized honeybees as the principal floral visitors demonstrated a combination of degraded environments and/or those composed of sparse vegetation (similar to African savannas – the region of origin *A. m. scutellata* [one of the original genitors of the hybrid studied here]). PEDRO & CAMARGO (1991), however, reported that Africanized honeybee visited only a small proportion of the floral resources available in a natural Cerrado (neotropical savanna) ecosystem in Brazil. As such, Africanized honeybee may only have a predominant adaptive advantage over native bees in exploiting floral resources in degraded environments. POLATTO & CHAUDNETTO (2013) argued that the adaptive advantages of native bees diminish when the environment becomes degraded, and they hypothesize that degraded environments in the Neotropical region acquire physiognomic characteristics similar to African savannas – the original habitat of Africanized honeybee.

Savanna habitats appear to be particularly adequate for optimal foraging by Africanized lines of *A. mellifera* (POLATTO & CHAUDNETTO 2013). That hybrid lineage was the most common representative of *A. mellifera* in the study area (VITAL *et al.* 2012), demonstrating the capacity to rapidly re-colonize environments after a disturbance. Additionally, although Africanized honeybee and other eusocial bee species constitute just a small fraction of the bee diversity present in an ecosystem, they retain large numbers of individuals and their colonies are perennial – making generalized collections of floral resources necessary to satisfy their metabolic needs (ROUBIK 1989).

*Chromolaena maximalianii* (Schrad.) R. M. King & Rob and *Trixis antimenorrhoea* (Schrank) Kuntze (Asteraceae) and *S. polyphylla* (Fabaceae) are grouped here as they demonstrate large numbers of small flowers displayed in compact inflorescences (called paintbrush flowers) that allow easy access to their abundant floral resources (PROCTOR *et al.* 1996), and visual and odor characteristics attractive to insects (FAEGRI & PULJ 1979). The plant family Malpighiaceae is not exploited by Africanized honeybee, but being an important source of oils for many native bees in the Neotropical region (VOGEL 1990), but only minimally attractive to other bees that do not utilize those oils, especially Africanized honeybee (BARÔNIO & TOREZAN-SILINGARDI 2017). Africanized honeybee colonies have the ability to stock large quantities of honey in their hives (KLEINERT *et al.* 2009), which stimulates intensive searches for nectar more than for pollen during the year, as honey is the end product of the bulk of the nectar collected (ROUBIK 1989).

The interactions of Africanized honeybee with large numbers of plant species can lead to alterations in the composition of plant communities (ASLAN *et al.* 2016). Those bees can directly promote the reproduction of many native plants through their pollination activities and consequently reduce the fitness of other native plants (KATO *et al.* 1999; ASLAN *et al.* 2016). Hypothetically, the greater foraging intensity on plants with flowers that can be adequately pollinated by Africanized honeybee will result in slight increases in their pollination rates and maximize their reproductive efficiencies – helping to make them predominant in that locality and therefore provide even more resources for the bees. Plant species not pollinated by Africanized honeybee, on the other hand, will suffer from reproductive disadvantages, as that altered environment will sustain progressively fewer numbers and varieties of other animal pollinators (ABROL 2012). As such,

according to the theory put forth by KATO *et al.* (1952), the results of the present study represent a typical example of the action of a dominant species in modifying the environment to its benefit, by provoking alterations in plant species compositions (either directly or indirectly) to favor those that interact with it.

Abiotic variables (e.g., luminosity, relative air humidity, and temperature) were not accompanied to be able to confirm, or not, their influence on the seasonal activities of Africanized honeybees, although climatic variations in the study region are rarely severe enough to inhibit foraging activity (e.g., ZAVATTINI 2009). A positive correlation has been shown to exist, however, between offspring production and nectar and pollen foraging intensity (FEWELL & WINSTON 1992). As such, colony success is intimately associated with the capacity to collect food resources in the field in light of the consistently high rate of egg production by the queen throughout the entire year (CARBONARI *et al.* 2016). As such, the predominance of floral resource harvesting in April reflected the abundant flowering of the highly productive plant species *G. cf. dimorpholepis*, as well as the presence of strong colonies of *A. mellifera* in the study area.

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