

Factors affecting the foraging behavior of bees in different maize hybrids

Darclot Teresinha Malerbo-Souza¹, Thulio Gustavo da Silva¹, Milena Oliveira de Andrade¹,
Lucas Rodrigues de Farias¹, Núbia Maria Guedes Medeiros¹

¹ Universidade Federal Rural de Pernambuco, Departamento de Zootecnia, Recife, PE, Brasil. E-mail: darclot.malerbo@ufrpe.br (ORCID: 0000-0002-3488-4778); thuliogustavo@hotmail.com (ORCID: 0000-0002-5999-2556); miihandrade13@gmail.com (ORCID: 0000-0002-8685-2751); lucasthegreat@gmail.com (ORCID: 0000-0002-5238-8372); nbguedes96@gmail.com (ORCID: 0000-0002-4948-5147)

ABSTRACT: Bees need pollen for their maintenance and development. Maize tassels (*Zea mays* L.) produce large amounts of pollen, which are visited by several bee species. This experiment was conducted in Ribeirão Preto, SP, Brazil, in 2013 with the objective to evaluate the factors affecting the foraging behavior of bees in different maize hybrids. For this purpose, we used the experimental design by randomized blocks. Seven treatments were repeated four times in experimental plots of 4.2 (6 lines) x 6.0 m (25.2 m²), in which borders of 0.70 m were kept clean in two plantings (January and April 2013). The genotypes used were (1) conventional maize 2B587[®] (control); (2) transgenic maize Powercore[®]; (3) YieldGard VT PRO[®]; (4) Viptera[®]; (5) Viptera 3[®]; (6) Herculex[®] and (7) Optimum Intrasect[®]. The frequency of visitations of bees to collect pollen was obtained by counting in the first five minutes each time, from 7:00 am to 11:00 am, with four and five replications (different days) in each hybrid in two plantings, respectively. The crude protein and weight of pollen and the tassels of different maize hybrids were evaluated. We have found the prevalence of Africanized honey bees (*Apis mellifera*) visiting the maize tassels preferably between 7:00 am and 9:00 am exclusively for pollen collection. The most visited hybrids by Africanized honey bees were those with the highest amount of pollen in the collected samples.

Key words: Africanized honey bees; foraging behavior; *Zea mays*

Fatores que afetam o comportamento forrageiro das abelhas em diferentes híbridos do milho

RESUMO: As abelhas precisam de pólen para sua manutenção e desenvolvimento, e o milho (*Zea mays* L.) é uma das espécies que produzem grandes quantidades de pólen que são visitadas por diferentes espécies de abelhas. Este experimento foi conduzido em Ribeirão Preto, SP, em 2013, com o objetivo de avaliar os fatores que afetam o comportamento da forragem de abelhas em diferentes híbridos de milho. Para isso, o delineamento experimental foi blocos casualizados, com sete tratamentos e quatro repetições, em parcelas experimentais de 4,2 (6 linhas) x 6,0 m (25,2 m²), e espaçamento de 0,70 m, em dois plantios (janeiro e abril 2013). Os genótipos utilizados foram (1) milho convencional 2B587[®] (controle); (2) milho transgênico Powercore[®]; (3) YieldGard VT PRO[®]; (4) Viptera[®]; (5) Viptera 3[®]; (6) Herculex[®] e (7) Optimum Intrasect[®]. A frequência de visitação das abelhas para coleta de pólen foi obtida pela contagem nos primeiros cinco minutos de horário, das 7:00 às 11:00 horas, com três repetições (três dias diferentes), em cada híbrido, nos dois plantios. Avaliaram-se a proteína bruta e o peso do pólen dos pendões de diferentes híbridos de milho. Foi observada a prevalência de abelhas africanizadas *Apis mellifera* visitando os pendões do milho, preferencialmente, entre as 7:00 e as 9:00, exclusivamente para coleta de pólen. Os híbridos mais visitados pelas abelhas africanizadas foram os que apresentaram maior quantidade de pólen nas amostras coletadas.

Palavras-chave: abelhas africanizadas; comportamento forrageiro; *Zea mays*

Introduction

The pollination services performed by native and managed bees are indispensable for the maintenance and functioning of ecosystems, reproduction of native plants and agricultural production. The demand for commercial crop pollination services has tripled in the last 50 years and beekeeping is fundamental to guarantee these services and has a great importance in beekeeping production (Goulson et al., 2015). Despite the importance of bees, there has been intense reduction of these pollinators worldwide. Several factors, alone or combined, have been cited as causes of this phenomenon, especially the reduction of flower abundance and diversity, acute and/or chronic exposure to pesticides, increased incidence of parasites and diseases, introduction of exotic species and climate change (Potts et al., 2016).

Nutrition is a key factor for the development, maintenance and healthiness of the bees. In this sense, all the nutrients needed to maintain their colonies come from nectar and pollen. The nectar is converted into honey as the main source of energy for the colony's individuals; while pollen is transformed into beebread by an acid-lactic fermentation process and provides proteins, amino acids, lipids, carbohydrates, vitamins and minerals (Brodtschneider & Crailsheim, 2010).

Pollen is essential for larval development, growth, immunity and longevity of bees and may increase the resistance of these insect to pesticides (Vaudo et al., 2015; Frias et al., 2016). The amino acids provided by pollen are indispensable for the synthesis of antimicrobial peptides responsible for the immune response of the bees and enzymes that act on detoxification (Yi et al., 2014). Without quantity and quality of pollen protein, the development of hypopharyngeal glands, responsible for training of food for the brood, is not complete, and the production and reproduction cycle of the colony is affected (Pereira et al., 2006).

The chemical composition of the pollen varies with the plant species, environmental conditions, age and nutritional status of the plant, in different locations, between seasons of the year and in different years. The protein content of the pollen varies according to the plant of origin and may have 8 to 40% of crude protein, 1 to 18% of carbohydrates and 0.7 to 7% of minerals. Chemical analysis of pollen has shown that it contains lipids, free amino acids, as well as vitamins, such as pantothenic and nicotinic acid, thiamine, riboflavin, ascorbic acid and small amounts of vitamins D and E, enzymes and coenzymes and pigments (Couto & Couto, 1996).

For honey bees, pollen represents the main source of proteins, fats (lipids), vitamins and minerals and has fundamental functions in the development of brood and adults, in the functioning of the glands and ovaries and in the formation of body fat, besides serving as a source of nitrogen. Thus, the importance of pollen to the colony is unquestionable. Honey bees resort to their own source of reserve, metabolizing tissues of their bodies, to prolong their existence in the absence of pollen (Marchini et al., 2006).

A populous hive consumes 35 kg of pollen to feed the brood. Honey bees need 17,000 flights to collect 250g of pollen. The daily need of a bee would be 145mg and it is necessary to visit 84 flowers for a bee to complement a load of pollen, which weighs up to 15mg (Breyer, 2017).

The composition of the beebread varies according to the plant of origin and may have 2.5 to 61% of crude protein, 1 to 18% of carbohydrates and 0.7 to 7% of minerals. Chemical analysis of pollen has shown that it contains lipids, free amino acids, as well as vitamins, such as pantothenic and nicotinic acid, thiamine, riboflavin, ascorbic acid and small amounts of vitamins D and E, enzymes and coenzymes and pigments (Modro, 2006).

Among the vegetal species that produce pollen, the maize (*Zea mays* L.) stands out with large areas of planting. Maize is a monoecious species, with male and female unisexual flowers in the same individual, with anemophilous (wind) pollination (Malerbo-Souza et al., 2008). According to these authors, species pollinated by the wind can benefit from pollinating insects, which visit their flowers to collect pollen and possibly nectar. The maize tassels are intensely visited by the Africanized honey bees (Malerbo-Souza, 2011).

So, this experiment was designed to evaluate characteristics of pollen and the tassels in different maize hybrids to analyze the foraging behavior of bees under field conditions in two plantings in 2013.

Material and Methods

This experiment was conducted at the experimental area of the campus of Moura Lacerda University Center (CUML) in Ribeirão Preto, SP, Brazil, whose height was 620 meters, with the following geographic coordinates: 21°10'04" South latitude (S) and 47°46'23" West longitude (W), temperate and subtropical climate with average annual temperature around 21°C and average annual rainfall of 1,500 mm.

In the studied area, every year the maize (*Zea mays*) crop was planted in two periods: October to November and January to April. The field experiment was sown on November 23, 2012 and January 18, 2013 with spacing of 0.7 m between rows and 0.3 m between plants. In two plantings the experimental design was a randomized block design, in which seven treatments were repeated four times in experimental plots of 4.2 (6 rows) x 6.0 m (25.2 m²), with 0.70 meters between rows, maintaining five plants per meter. A cover fertilization with ammonium sulfate equivalent to 500 kg ha⁻¹ (01/06/2013) was carried out on the first planting date and the weeds were controlled using 2,4-dichlorophenoxyacetic herbicide (2,4-D) at the planting date.

The genotypes used were (1) conventional maize 2B587 (control) and transgenic maize expressing the (2) Cry1F + pat + Cry1A105 + Cry2Ab2 + EPSPS genes (2B587, PowerCore®); (3) Cry1A105 + Cry2Ab2 (DKB 390, YieldGard VT PRO®); (4) Vip3A + pmi (Status, AgrisureViptera®); (5) Vip3A + Cry1Ab + EPSPS + pmi (Maximus, AgrisureViptera 3®); (6) Cry1F + pat

(2B587, Herculex®);(7) Cry1Ab + Cry1F + EPSPS + Pat (30F53, Optimum Intrasect®). All hybrids were early and bloomed on the same days.

The Institution has an apiary with eight colonies of Africanized honey bees *Apis mellifera* located close to the area of maize cultivation, about 1 km away.

The most frequent insects were collected and preserved in alcohol, properly labeled, and subsequently identified by comparison with the entomological collection of the Moura Lacerda Institution.

The frequency of visitations of these insects to collect pollen on maize tassels was obtained by counting the species and the number ones in the first five minutes of each hour, from 7:00 am to 11:00 am with four and five replications in each hybrid in the two plantings, respectively. The constancy (C) of these insects was obtained by the formula: $C = (P \times 100) / N$, where P is the number of samples containing this species and N is the total number of collections performed (Silveira-Neto et al., 1976).

To evaluate the crude protein content of the pollen, 10 tassels were collected with two replicates, totaling 20 tassels for each hybrid and planting. The tassels were taken to the Moura Lacerda Institution's laboratory and the pollen was separated from the spikelets in individual sieves. Then, the pollen was weighted, labeled and kept refrigerated for further analysis. Two slides of each hybrid were made to identify differences in the structure of the pollen grains. Later, the pollen samples were analyzed for crude protein at the Laboratory of Food Analysis of the Department of Animal Science of the Faculty of Agrarian and Veterinary Sciences of Jaboticabal, UNESP, according to the Weende method, described by Salman et al. (2010).

The length and width of the tassels of each hybrid were measured with a graduated ruler and the structural shape of these tassels was also evaluated with 10 replicates for each hybrid.

Data were analyzed using analysis of variance in randomized SAS (2012) program, which includes the Tukey test to compare the means of all variables and regression analysis for orthogonal polynomials to test each variable at a time. The data were considered at 5% significance level.

Results a Discussion

Data were collected between 24 to 28 January, 2013 and 15 to 18 April, 2013 and we have observed mainly Africanized honey bees (*Apis mellifera*) in maize tassels (88.9%, on average) (Figure 1), however, other species of native bees were observed (11.1%), such as stingless bees *Trigona spinipes* (Hymenoptera, Apidae) and *Tetragonisca angustula* (Hymenoptera, Apidae), and bumblebees *Xylocopa frontalis* (Hymenoptera, Apidae). Others insects were also observed, such as coleopterous *Diabrotica speciosa* (Coleoptera, Chrysomelidae) and butterflies.

Experiments carried out in the same experimental area in previous years (Malerbo-Souza, 2011) also reported the



Figure 1. Africanized honey bees *Apis mellifera*(A), bumblebees *Xylocopa frontalis* (B), stingless bees *Trigona spinipes* (C) and *Tetragonisca angustula* (D) collecting pollen in maize tassels (*Zea mays*) in Ribeirão Preto, Brazil, in 2013.

predominance of these bees collecting pollen in the maize tassels, being 97.13% in 2009 and 94.66% in 2010 of total insects observed. Other insects have also visited maize tassels, such as stingless bees *T. spinipes* and *T. angustula*, coleopterous *D. speciosa*, and insects of the order Diptera and Lepidoptera.

In agreement with Malerbo-Souza (2011), stingless bees *Trigona* sp. and *T. angustula* have also been observed collecting pollen on maize tassels, however, the frequency of these stingless bees was very low. These bees have visited the maize tassels from 12:00 am to 5:00 pm with a peak frequency between 2:00 pm and 4:00 pm. Coleopterous *D. speciosa* feeding pollen from 1:00 pm to 6:00 pm, with oscillation frequency in this period. Other beetles have been seen collecting a sweet secretion released by the anus of aphids (honeydew) existing in the maize tassels. Lepidopterous and Dipterous have visited the flowers, however, they have not been seen feeding pollen.

According to the Index of Constance, only the Africanized honey bee was a constant species in the maize tassels (88.25%, on average). The other insect species have been accidental: lepidopterous (15.88%), dipterous (11.52%), coleopterous (17.64%), stingless bees *Trigona* sp. (18.78%) and *T. angustula* (5.02%, on average) in the maize tassels.

In this experiment, the release of pollen grains occurred in the early morning between 7:00 am and 10:00 am in two plantings. The Africanized honey bees preferred to collect pollen between 7:00 am and 8:00 am in January (Table 1 and Figure 2) and the frequency of these bees did not present significant differences between observed times in April (Table 2). This occurred because the temperature in January was higher in the early morning than in April, causing the pollen to dehydrate faster and anticipating the bee visitation. In addition, in January the days dawn earlier and the honey bees begin their activities as soon as the sun rises. The native bees began their activities later compared to the Africanized

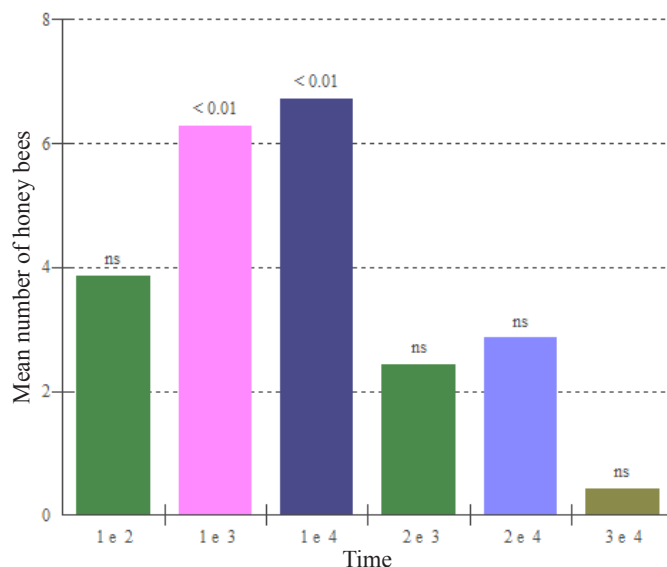


Figure 2. Mean number of Africanized honey bees (*Apis mellifera*) collecting pollen from 7:00 am to 11:00 am in the tassels of different maize hybrids (*Zea mays*), in Ribeirão Preto, Brazil, in January 2013 (Tukey test, $p < 0.01$, 1 = 7:00, 2 = 8:00, 3 = 9:00 and 4 = 10:00).

honey bees so much so that in this experiment they have been observed always in the afternoon.

In the first flowering period (January), the Africanized honey bees have visited the maize tassels only for pollen collection from 7:00 am to 10:00 am with a peak frequency between 7:00 am and 8:00 am. Through polynomial regression in time was observed that these bees decreased their frequency to 7:00 to 10:00 pm, according the following equation: $Y = -14.1X + 62.3$ ($R^2 = 0.837$), where Y is the number of honey bees in tassels and X is the time of day in first planting. In the second flowering period (April), the frequency increased at 8:00 am, decreasing later, according the equation: $Y = -21.35X^2 + 99.64X + 32$ ($R^2 = 0.817$) (Figure 3).

Table 1. Mean number of Africanized honey bees (*Apis mellifera*) collecting pollen from 7:00 am to 11:00 am in the tassels of different maize hybrids (*Zea mays*), in Ribeirão Preto, Brazil, in January 2013.

Time	Powercore	Viptera	Optimum	Herculex	YG VT PRO	Viptera 3	Conventional	Mean
7:00 am	0	10	6	6	2	10	15	7.0a
8:00 am	4	4	0	2.5	0	0.5	11	3.1ab
9:00 am	1.5	1.5	0.5	0.5	0	0	1	0.7b
10:00 am	0	1	0	0	0	0	0	0.1b
Mean	1.38ns	4.13.3ns	1.63ns	2.25ns	0.5ns	2.63ns	6.75ns	

Means followed by the same lower letter in the same line or column do not differ significantly by Tukey test (1%); Between hybrids ($F = 1.0562$, $p = 0.4205$); Between times ($F = 6.3121$, $p = 0.0029$).

Table 2. Mean number of Africanized honey bees (*Apis mellifera*) collecting pollen from 7:00 am to 11:00 am in the tassels of different maize hybrids (*Zea mays*), in Ribeirão Preto, Brazil, in April 2013.

Time	Powercore	Viptera	Optimum	Herculex	YG VT PRO	Viptera 3	Conventional	Mean
7:00 am	10	35	4	6	5	25	10	13.6ns
8:00 am	18	53	10	17	7	36	47	26.9ns
9:00 am	10	42	9	13	9	11	10	14.9ns
10:00 am	7	38	8	12	9	11	8	13.3ns
Mean	11.25bc	42.00ab	7.75c	12.00bc	7.50c	20.75bc	18.75bc	

Means followed by the same lower letter in the same line or column do not differ significantly by Tukey test (1%); Between hybrids ($F = 6.6132$, $p = 0.0007$); Between times ($F = 1.5950$, $p = 0.2158$).

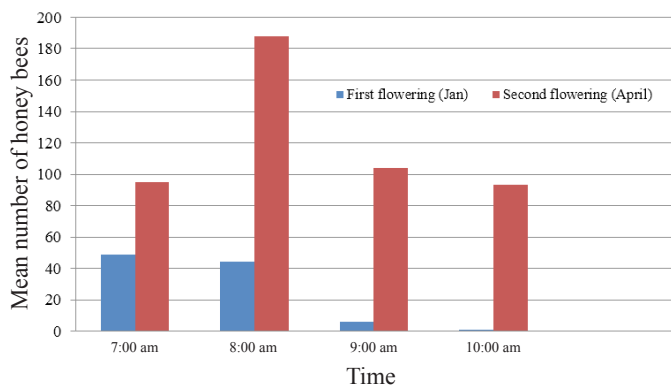


Figure 3. Mean number of Africanized honey bees (*Apis mellifera*) collecting pollen from 7:00 am to 11:00 am in the tassels of different maize hybrids (*Zea mays*) in two plantings in Ribeirão Preto, Brazil, in 2013.

These data are in accordance with Malerbo-Souza & Silva (2011) that cited the Africanized honey bees preferred to collect pollen from 7:00 am to 11:00 am. The majority of plant species has pollen production concentrated in the morning and nectar throughout day (Pierrot & Schindwein, 2003).

The fact that Africanized honey bees have visited intensively the maize tassels in all the occasions studied disagrees with Sabugosa-Madeira et al. (2007), who mentioned that honey bees do not show great interest in the fields of maize plants when there are other good sources of pollen close to ensure their livelihoods. According these authors, the honey bees come to feed almost exclusively on maize pollen in case of famine or when apiaries are located in areas with large plantings of maize.

Near the experimental area, there was an experimental orchard with several species of fruit trees, as well as a riparian forest area, in which there were other flowering species that may have attracted more bees. However, in the summer period (December to January), in the southeastern region of Brazil there are more blooming species compared to autumn (February to April). This explains the greater activity of the Africanized honey bees in the maize tassels in April, since it is a month with fewer blooming species and, therefore, with less availability of food sources for these bees.

In January 2013 the air temperature ranged from 20.0 to 30.5°C, with a mean of 24.0°C, mean relative humidity of 80% and 380 mm of precipitation. In April 2013 the temperature ranged from 16.5 to 28.5°C, with a mean of 21.5°C, relative humidity of 79% and precipitation of 60 mm, according to data provided by the Agroclimatological station from FCAV, UNESP, Jaboticabal, SP, Brazil (<http://www.fcav.unesp.br/#!/estacao-agroclimatologica/dados/estacao-convencional/>).

In the same experimental area, Malerbo-Souza & Silva (2011) observed that, in January 2008, the Africanized honey bees collected more nectar and pollen compared to April 2008, as there were more blooming species

In January no significant differences were observed between the hybrids, however, in April the Viptera® and Viptera 3® hybrids stood out (April) (Figure 4), showing

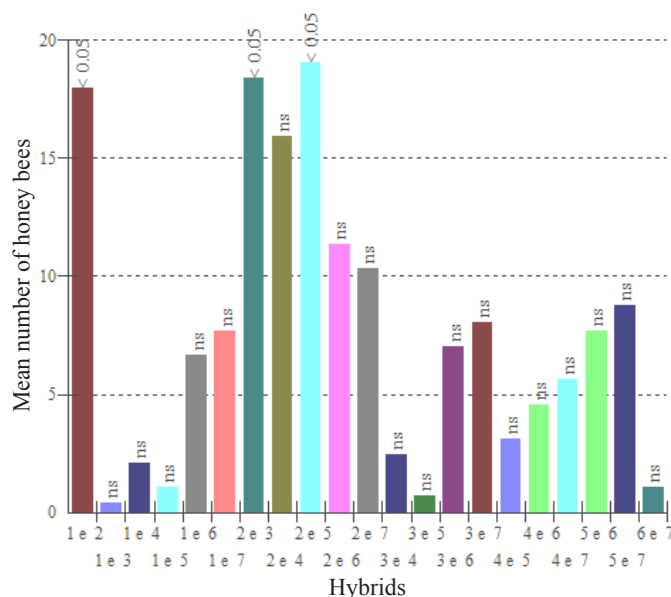


Figure 4. Mean number of Africanized honey bees (*Apis mellifera*) collecting pollen in the tassels of different maize hybrids (*Zea mays*), in Ribeirão Preto, Brazil, in April 2013 (Tukey test, $F=6.6138$, $p=0.0007$; 1=Powercore, 2=Viptera, 3=Optimum, 4=Herculex, 5=YG, 6=Viptera3 and 7=Conventional hibrids).

that the Africanized honey bees make no difference between conventional and transgenic maize and showed no preferences.

When comparing the total values of the two plantings, we observed that the second planting (April) was significantly more visited than the first one (January) (Figure 5).

Our dates disagreed with those of Ramirez-Romero et al. (2008), who observed changes in the behavior of bees, as it took longer for the bees to consume the contaminated solution with transgenic pollen and they have reduced the number of visits to artificial flowers with a sugar solution containing the proteins synthesized by Bt corn, keeping this reduction even after they were presented with the same uncontaminated flowers, concluding that the Cry1Ab toxin has an anti-nutritional or repulsive factor, with sub-lethal

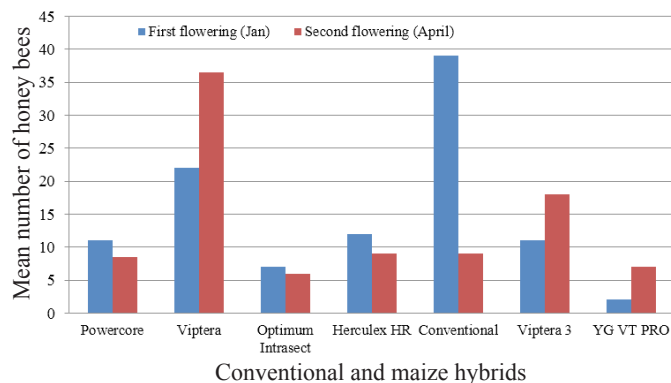


Figure 5. Mean number of Africanized honey bees (*Apis mellifera*) collecting pollen in the tassels of different maize hybrids (*Zea mays*), in two plantings in Ribeirão Preto, Brazil, in 2013 ($F = 26.83$, $p < 0.0001$, DMS = 14.35).

effects that have been stored by bees. We believe, like Huang et al. (2004), that the honey bees are unable to distinguish transgenic from non-transgenic flowers.

In relation to the crude protein content of the pollen, we have observed that the Viptera® hybrid had significantly higher (24.79%, on average) content, not differing only from the Optimum® hybrid (23.16%, on average), but differing from the others (Table 3). These data were higher compared to those obtained by others authors, like 16.7% (Babendreier et al. 2004) and 15.0% (Wiese 2005), who considered the crude protein content of maize pollen as weak. Frias et al. (2016) showed that the amount of protein consumption and the pollen type influenced bee survival and physiology.

Another important aspect is that the tassels release the pollen for only one or two days, in which on the initial day of release of the pollen, the pollen grains are in the foliage, in the hair of the ears and, in the end, the pollen grains fly over the area. Many pollen grains have even been seen between the maize lines and nearby.

When comparing the frequency of Africanized honey bees in the tassels and the crude protein content from pollen, we observed that Viptera® hybrid in second planting was the most visited and had the highest level of crude protein (24.79%, on average). However, the hybrid Optimum® also showed high levels of crude protein (23.16%, on average) and was one of hybrids least visited by bees. So, hybrids differ concerning the attractiveness of bees in different plantations, which sometimes prefer conventional hybrids, other times, transgenic hybrids, and the crude protein content was not considered responsible for this attractiveness.

These data agree with Roulston et al. (2000), who stated that the protein variation may not be directly related to the attraction of pollinators, since zoophilous species of pollen is not as rich in protein than anemophilous species, such as maize and coconut.

Possibly, the amount of pollen produced by maize tassels, in the different hybrids, affected bee visitation. With less pollen in the banners, the bees need to visit more flowers with higher energy expenditure.

With respect to foraging behavior, the Africanized honey bee approached the tassel, sat on it and with the help of mouthparts and legs removed the pollen. Subsequently,

Table 3. Average crude protein content (%) and overall average of conventional and transgenic maize hybrids (*Zea mays*), in Ribeirão Preto in 2013.

Maize hybrids	Average crude protein content (%)
Viptera	24.79a
Optimum	23.16ab
Powercore	21.79 bc
Convencional	21.29 bc
Viptera3	20.92 bc
YG VT PRO	20.13c
Herculex	20.11c
Overall average	21.74

Means followed by the same letter in the same column do not differ significantly, Tukey test at the level of 1%.

these bees transferred the appeal to the collected pollen basket. Moreover, we observed during the visit, the bee's behavior of cleansing the body, legs and wings, storing the pollen in the pollen basket. At the end of collection, the pollen basket had to be filled with ball-shaped yellowish pollen. Another observed behavior was that the Africanized honey bee, while collecting the pollen, moved the wings, forming a pollen trail (cloud) that could help the dispersal of the pollen of the maize in days of little wind (Figure 6). So, when the honey bee moved its wings it helped in pollen dispersal in maize.

Regarding the structure of banners, differences were observed between width and height of hybrids (Table 4), among which the Optimum® presented more open format (Figure 5). In this hybrid, we often observed that honey bees reached the tassels but did not land to make pollen collection. When weighing the pollen samples, the one that presented the lowest weight was exactly the hybrid Optimum® (0.0039 mg, on average). We believe this may be an important factor in the choice of the bees for tested hybrids.

The most visited maize hybrids (Conventional and Viptera®) were those with the highest amount of pollen in the samples collected (0.0186 and 0.0152 mg, on average, respectively) (Tables 1, 2 and 4).

Regarding the spikes, we observed that the hybrids Powercore® and Herculex® had totally yellowish hair; the conventional hybrid had slightly reddish hair at the tips and



Figure 6. Africanized honey bee (*Apis mellifera*) leaving trail while collecting pollen on maize tassels.

Table 4. Weight, width and height mean of different maize hybrids in Ribeirão Preto, SP, Brazil in 2013.

Maize hybrids	Weight (mg)	Width (cm)		Height (cm)
Viptera	0.0152a	12.2b	29.8b	
Viptera 3	0.0085b	12.0b	26.4b	
YG VT PRO	0.0078b	14.0b	34.2b	
Herculex HR	0.0064b	17.2b	34.2b	
Powercore	0.0073b	19.6b	35.0b	
Conventional	0.0186a	24.2b	34.8b	
Optimum Intrasect	0.0039c	47.6a	40.8a	
Mean	0,0097	20.98	33.6	

Means followed by the same letter in the same column do not differ significantly, Tukey test at the level of 1%.

the Optimum®, Viptera®, Viptera3® and YG VT PRO® hybrids showed reddish hair from the tips to the ears. Pollen grains showed differences in relation to staining but not in shape in hybrids. With regard to the maize cobs, the Powercore® and Herculex® hybrids showed totally yellowish hair; the conventional hybrid showed slightly reddish hair on the tips and the Optimum®, Viptera®, Viptera 3® and YGVT PRO® hybrids had reddish hair from the tips to the ears (Figure 7).

As the bees seem to be unable to distinguish transgenic from non-transgenic flowers, bee colonies have been confronted with increasing amount of pollen containing new and foreign proteins to the colony room. Some works indicate

that GM crops do not directly influence the development of bees (Huang et al. 2004).

Before and after this experiment, other data were collected in the same conditions and the honey bees showed different preferences for hybrids. Despite the Optimun® hybrid have high crude protein content in pollen, it was one of the least visited for Africanized honey bees and showed tassels with lower amount of spikelets and lower amount of pollen to offer to these bees. So, we can conclude that one of the factors that had the greatest impact on Africanized honey bees' choice was the amount of pollen offered to them.



Figure 7. Format of tassels of different maize hybrids (*Zea mays*) in Ribeirão Preto, Brazil, in 2013.

Conclusion

The male flowers of the tassels have been widely visited by Africanized honey bees *Apis mellifera* for pollen collection, and they have visited the tassels preferably from 7:00 am to 9:00 pm. Maize pollen has around 21.74% of crude protein, being an important source of food for bees. Bees have visited the hybrids that presented the heaviest pollens and consequently collected more protein (Conventional, Viptera and Viptera 3). Other factors, such as width and height, pollen coloration, stem coloration and tassel shape may have an effect on bees' preference.

Literature Cited

- Babendreier, D.; Kalberer, N.; Romeis, J.; Fluri, P.; Bigler, F. Pollen consumption in honey bee larvae: a step forward in the risk assessment of transgenic plants. *Apidologie*, v. 35, n. 3, p. 293-300, 2004. <https://doi.org/10.1051/apido:2004016>.
- Breyer, O. O pólen apícola. http://www.breyer.ind.br/apicultura/apicultura_polen.htm. 25 Nov. 2017.
- Brodshneider, R.; Crailsheim, K. Nutrition and health in honey bees. *Apidologie*, v. 41, n. 3, p. 278-294, 2010. <https://doi.org/10.1051/apido/2010012>.
- Couto, R.H.N.; Couto, L.A. Apicultura: manejo e produtos. Jaboticabal: FUNEP, 2006. 191p.
- Frias, B.E.D.; Barbosa, C.D.; Lourenço, A.P. Pollen nutrition in honey bees (*Apis mellifera*): impact on adult health. *Apidologie*, v. 47, n. 1, p. 15-25, 2016. <https://doi.org/10.1007/s13592-015-0373-y>
- Goulson, D.; Nicholls, E.; Botías, C.; Rotheray, E.L. Bee declines driven by combined stress from parasites, pesticides and lack of flowers. *Science*, v. 347, n. 6229, 1255957, 2015. <https://doi.org/10.1126/science.1255957>.
- Huang, Z.; Hanley, A.; Pett, W.; Langenberger, M.; Duan, J. Field and semifield evaluation of impacts of transgenic canola pollen on survival and development of worker bees. *Journal Economy Entomology*, v. 97, n. 5, p. 1517-1523, 2004. <https://doi.org/10.1603/0022-0493-97.5.1517>.
- Malerbo-Souza, D.T. The corn pollen as a food source for honeybees. *Acta Scientiarum.Agronomy*, v. 33, n. 4, p. 701-704, 2011. <https://doi.org/10.4025/actasciagr.v33i4.10533>.
- Malerbo-Souza, D.T.; Silva, F.A.S. Comportamento forrageiro da abelha africanizada *Apis mellifera* L. no decorrer do ano. *Acta Scientiarum.Animal Science*, v. 33, n. 2, p. 183-190, 2011. <https://doi.org/10.4025/actascianimsci.v33i2.9252>.
- Malerbo-Souza, D.T.; Toledo, V.A.A.; Pinto, A.S. Ecologia da polinização. Piracicaba: CP2, 2008. 31p.
- Marchini, L.C.; Reis, V.D.A.; Moreti, A.C.C.C. Composição físico-química de amostras de pólen coletado por abelhas africanizadas *Apis mellifera* (Hymenoptera: Apidae) em Piracicaba, estado de São Paulo. *Ciência Rural*, v. 36, n. 3, p. 949-953, 2006. <https://doi.org/10.1590/S0103-84782006000300034>.
- Modro, A.F.H. Flora e caracterização poliníferas para abelhas *Apis mellifera* na região de Viçosa, MG. Viçosa: UFV, 2006. 98p. Dissertação Mestrado. <http://locus.ufv.br/handle/123456789/4000>. 05 Nov. 2017.
- Pereira, F.M.; Freitas, B.M.; Vieira Neto, J.M.; Lopes, M.T.R.; Barbosa, A.L.; Camargo, R.C.R. Desenvolvimento de colônias de abelhas com diferentes alimentos proteicos. *Pesquisa Agropecuária Brasileira*, v. 41, n.1, p. 1-7, 2006. <https://doi.org/10.1590/S0100-204X2006000100001>.
- Pierrot, L. M.; Schindwein, C. Variation in daily flight activity and foraging patterns in colonies of urucu – *Melipona scutellaris* Latreille (Apidae, Meliponini). *Revista Brasileira de Zoologia*, v. 20, n. 4, p. 565-571, 2003. <https://doi.org/10.1590/S0101-81752003000400001>.
- Potts, S. G.; ImperatrizFonseca, V.; Ngo, H. T.; Aizen, M. A.; Biesmeijer, J. C.; Breeze, T. D.; Dicks, L. V.; Garibaldi, L. A.; Hill, R.; Settele, J.; Vanbergen, A. J. Safeguarding pollinators and their values to human well-being. *Nature*, v. 540, p. 220-229, 2016. <https://doi.org/10.1038/nature20588>.
- Ramirez-Romero, R.; Desneux, N.; Decourtye, A.; Chaffiol, A.; Pham-Delegue, M. Does Cry 1Ab protein affect learning performances of the honey bee *Apis mellifera* L. (Hymenoptera, Apidae)? *Ecotoxicology. Environment Safety*, v. 70, n. 2, p. 327-333, 2008. <https://doi.org/10.1016/j.ecoenv.2007.12.002>.
- Roulston, T.H.; Cane, J.H.; Buchmann, S.L. What governs protein content of pollen: pollinator preferences, pollen-pistil interactions, or phylogeny? *Ecological Monographs*, v.70, n. 4, p.617-627, 2000. [https://doi.org/10.1890/0012-9615\(2000\)070\[0617:WGPCOP\]2.0.CO;2](https://doi.org/10.1890/0012-9615(2000)070[0617:WGPCOP]2.0.CO;2).
- Sabugosa-Madeira, J. B.; Abreu, I.; Ribeiro, H.; Cunha, M. Bt transgenic maize pollen and the silent poisoning of the hive. *Journal of Apicultural Research*, v. 46, n. 1, p. 57-58, 2007. <https://doi.org/10.1080/00218839.2007.11101367>.
- Salman, A. K. D.; Ferreira, A. C. D.; Soares, J. P. G.; Souza, J. P. Metodologia para avaliação de ruminantes, Porto Velho, RO: Embrapa Rondônia, 2010. 21p. <https://www.infoteca.cnptia.embrapa.br/bitstream/doc/884369/1/doc136alimentacaoderuminantes.pdf>. 10 Nov. 2017.
- Statistical Analysis System - SAS. System for microsoft windows. OnlineDoc® for Windows 9.3. Cary: Statistical Analysis System Institute, 2012.
- Silveira-Neto, S.; Nakano, O.; Barbin, D.; Villa Nova, N.A. Manual de ecologia dos insetos. Piracicaba: Agronômica Ceres, 1976. 419p.
- Vaudo, A.D.; Grozinger, T.J.; Patch, C.M.H.M. Bee nutrition and floral resource restoration. *Current Opinion in Insect Science*, v. 10, p. 133-141, 2015. <https://doi.org/10.1016/j.cois.2015.05.008>.
- Wiese, H. Apicultura: novos tempos. 2.ed. Guaíba: Agrolivros, 2005. 378p.
- Yi, H.Y.; Chowdhury, M.; Huang, Y.D.; Yu, X.G. Insect antimicrobial peptides and their applications. *Applied Microbiology and Biotechnology*, v. 98, n. 13, p. 5807-5822, 2014. <https://doi.org/10.1007/s00253-014-5792-6>.