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Floral Biology and Africanized Honeybee Behaviour in Transgenic (Roundup ReadyTM var. BR-245 RR) and Conventional (var. BRS-133) Soybean (Glycine max L. Merrill) Flowers

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1. Introduction

Therefore, this research was carried out to evaluate the Africanized honeybee effect on soybean production, mainly, over genetically modified organisms (GMOs). This chapter presents experimental data about floral biology of soybean *Glycine max* L. Merrill, BR-245 RR (transgenic soy – Roundup Read™) and BRS-133 (conventional soy) with and without application of Glyphosate, as well as the honeybee behaviour in those flowers of these varieties.

Soybean (*Glycine max* L. Merrill) is one of the main commodities in the world. Currently, more than 70% of its production is genetically modified. Brazil has a highlighted place concerning about this crop and is the second place in world production. During the 2010/2011 harvest season, Brazil produced 75 million tons of soybean on 24.2 million hectares of planted area with 3,106 kg.ha^{-1} of productivity. The soybean crop represents the main worldwide oleaginous crop produced consumed by animals and human beings. After several decades of searching for alternatives to control weed pests, genotypes of genetically modified (GM)

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soybean were developed to be resistant to the herbicide glyphosate, of the substituted glycine chemical group.

Brazil increased soybean hectares more than any other country, with an impressive increase of four million hectares in 2009. Brazil is now second in the commercial cultivation of GM plants, 25.4 million hectares, in the whole word [54]. GM soybean, in 2010, occupied 50% of cultivated global area among genetically modified organisms, reaching 73.3 million ha. Since the beginning of commercialization in 1996 until 2010, the tolerance to herbicides is the dominant attribute [54].

The current thought of scientists is increasing the productivity to meet the demand for soybean meal and soybean oil for animal and human consumption, respectively. Despite intensive research in this area with advances in genetically modified genotypes, new techniques in cultivation, irrigation, machine production, and agricultural implements with excellent performance are available. However, pollination, especially that performed by honeybees is little studied.

Around 70% of the world's most produced crop species depends, to some extent, on insect pollination [30], contributing an estimated €153 billion to the global economy, according to [31], and accounting for approximately 6 to 9% of agricultural production [27, 32]. Some authors [30] claim that about one third of the global food production depends on biotic pollination. However, the generally accepted figures are considerably lower [31, 46]. The estimated value of insect pollination for European agriculture is €22 Billion [33].

The scarcity of information about Africanized honeybees in cross-pollination of soybean indicates the need for more research, and for instance, the soybean crop can benefit greatly from biotic pollination with increased profits and production. Therefore, studies in pollination ecology, with special attention to pollinator-flower interaction may contribute to the success in this crop. Although soybean is auto compatible with auto pollination, auto incompatibility occurs too; the increase of production is possible to achieve with pollinator introduction in agricultural areas. A study in floral biology and honeybee behaviour in soybean is necessary to elucidate the effective importance of honeybees for this crop.

Transgenic can aid in genetic improvement of plants, with an aim on food production, fibers and oil, as well as the production of medicines and other industrialized products [1]. One of the main concerns with these plants is that an unanticipated and cumulative effect of contamination from crossing among genetically modified and conventional plants may occur. The instability and risk of propagating one gene to wild species are critical for environmental conservation.

The Monsanto Corporation has developed Roundup Ready™ soybean cultivars with a tolerance to glyphosate, the active ingredient of the weed killer Roundup®, by the production of enolpyruvylshikimate -3- phosphate synthase (EPSPS; EC 2.5.1.19) [2]. This enzyme is a carboxyvinyl transferase that catalyzes the transfer of the enolpyruvyl moiety of phosphoenolpyruvate (PEP) to the 5-hydroxyl of shikimate-3-phosphate (S3P) and forms enolpyruvylshikimate-3-phosphate. It is the sixth key enzyme in the shikimate pathway, which is essential for the production of aromatic amino acids Phe, Tyr, and Trp, as well as chorismate-derived secondary

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metabolites in plants, fungi, and microorganisms [3]. Glyphosate competes with PEP and forms a stable ternary complex with the enzyme and s3p [4, 5]. The safety and efficacy of glyphosate, together with the widely used glyphosate-resistant crop plants containing a transgene for EPSPS, have combined to make glyphosate the most used herbicide in the world [6].

The consequences of what this gene can provoke on plant biology is not known; some studies show that the abortion of conventional soybean flowers can reach up to 80%, depending on the cultivar and from the environmental conditions [6]. [7] found on cultivar BRS-133, an average of 53.31 and 82.90% of flowers aborted on the areas in which Africanized honeybees *A. mellifera* were present, and were not present, respectively.

The correct use of pesticides can arise the productivity of all crops in the world, mainly because pesticides contain biologically active compounds developed for the purpose of protecting plants. Insecticides control pest insect populations; herbicides control weeds, while fungicides are used to control fungal plant diseases [28]. According to Regulation (EC) No.1107/2009, a plant protection product (pesticides) shall be approved only when pesticides "…has no unacceptable acute or chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behaviour" [29]. Neonicotinoid residues in plants and plant parts only become dangerous for bees once they are exposed. Many lethal and sublethal effects of neonicotinoid insecticides on bees have been described in laboratory studies; however, no effects were observed in field studies [34]. Neurotoxic compounds such as neonicotinoids also interfere with the orientation process of honeybees [35]. The toxicity of neonicotinoids may, however, increase by synergistic effects with other compounds as reported by [36].

The sugar concentration of nectar in the flowers determines the frequency of visitors, while the volume determines the quantity of honeybee foragers that will visit [8]. Besides that, flowers have a period of viability for pollination and fecundation.

In the central region of the USA, the production of nectar and the visit of honeybees to soybean flowers occur among 9h00 and 15h00 every day. The peak of these activities and the time in which the flowers will remain open vary between the cultivar and the local environmental condition. The contents of total sugar in nectar varied from 37 to 45%, and the sugar of soybean flowers increased and the volume decreased according to the time of day and temperature [9, 46]. Most likely, the sugar concentration in nectar is associated with the intensity of foraging by honeybees, which is directly related to the nectar quantity and quality [47, 48] or to its sugar composition [49, 50, 51].

The volume of nectar on each flower, which is increased in warm climates, varied significantly between cultivars from 0.2 to 0.5 μL. In another experiment, performed by [10], in Haiti observing the same parameters, the authors verified that the most significant change occurred on the output rate of nectar per flower, which varied from 0.022 to 0.127 μL between cultivars.

A study in state of Parana (Brazil), with cultivar BRS-133 [7] showed that the Africanized honeybees searched the soybean culture to collect nectar and pollen. Just a small amount of pollen was from some other areas; however, more than 50% of the total amount of collected pollen by some colonies could have been from soybean [11].

The structure of the soybean flower assured the foraging of *A. mellifera,* favoring the pollen transfer [12, 46]. The soybean autogamy and that the self-pollination would guarantee good productivity to the agriculturists, not needing insects to do the pollination [13].

Based on the lack of information of the effect of honeybees on soybean production and, mainly, on genetically modified organisms, this research was carried out to study the floral biology of the *Glycine max*, cultivar BR-245 RR (transgenic soy - Roundup Read™) and BRS-133 (conventional soybean) and to evaluate the Africanized honeybee *A. mellifera* behaviour in the flowers of these cultivars.

2. Materials and methods

This research was carried out in the experimental area of Empresa Brasileira de Pesquisa Agropecuária (Embrapa Soja), located in Londrina city (23° 08'47" S and 51º 19'11" W), which is situated in the North region of state of Paraná, Brazil. The planting season, the cultivation, and management of the culture and crop occurred in appropriated time, and followed technical recommendations for soybean plants [27].

A completely randomized design was used with three treatments and six replications each. Three treatments were evaluated: covered soybean area with a colony of honeybees inside during the flowering; covered soybean area without a honeybee colony; and an uncovered soybean area, free for insect visitation. In each area, of 24 m^2 each, soybean planting was in eight lines, of 6 m, interlaced two by two, with cultivars BR-245 RR and BRS-133. The stand used was 0.5 m between lines and 30 seeds by linear metre (Figure 1).

For covered areas, pollination cages were installed, made with nylon screen (two mm), supported by PVC tubes (¾ inch), and iron (3/8 inch), forming cages in a semi-arch four metres wide, six metres long and two metres high, covering an area of 24 m^2 (Figure 2) to prohibit the passage of insects [14].

Each treatment was six parcels, with 24 m^2 for each pollination cage and the planting was carried through in eight intercalated lines, two by two, with cultivars BR-245 RR (transgenic soybean - Roundup Ready™), with and without an application of glyphosate (32 days after the germination) and BRS-133 (conventional soybean). Therefore, in each treatment, there were three parcels with an application of herbicide and three parcels without an application. The culture of soybean was monitored during all periods, with particular attention at bloom, which began on December 31 2003 and extended up to January 28 2004. Harvesting was carried out separately line-by-line on the 18 parcels (Figure 2).

In each line, five floral buttons were randomly marked with labels numbered and followed by comments made periodically, during all anthesis period. The stigma receptivity to the pollen grains was evaluated in five flowers collected at 8h00, 11h00 and at 17h00 in five days during the flowering, from January 6 to January 10 2004, in the transgenic and conventional lines of all parcels, following the method of [15].

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Figure 1. Pollination cage model used in the experiment with dimensions of 24 m^2, below the Africanized honeybee colony inside the cage.

The stigma of each flower was separated and immersed in hydrogen peroxide (20 volumes) and the air bubbles detached observed and evaluated with scores that varied from zero for non-receptive, one for moderate receptivity and two for high receptivity [7, 40]. For the verification of pollen grain viability, withdrawal of the pollen grains was made from the same flowers used to evaluate receptivity; these were deposited in blades, stained with acetic carmine and stored for posterior analysis, following the technique of [16, 17]. The viability of

Figure 2. The soybean harvesting carried out separately line-by-line on the 18 parcels in the experimental area of Embrapa – Soja in Londrina-PR, Brazil.

the pollen grains removed from the honeybee forager corbicula was also verified using this same technique.

The percentage of abortion in the soybean flowers was measured by counting all floral blossoms of three marked plants of each line with ribbons of different colors, in all parcels. In

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the maturation phase, the string beans of each plant were counted to get the percentage of the aborted flowers (Figure 3).

The frequency of the insect visits, during the day, was obtained by counting them, observing during 10 minutes in each schedule (8h00 to 17h00) in the transgenic and conventional lines of the covered area by pollination cages with honeybees and in the area of free insect visitation. The insects most frequent were photographed, filmed, collected with an entomological net, fixed and later, identified by specialists.

The time of nectar and/or pollen collection was evaluated with a chronometer, having followed the honeybee forager in its activity. In addition, the time of permanence of the honeybee in the flower and the number of visits per minute were counted and recorded.

Figure 3. A transgenic soybean plant in flowering, and in zoom showing the abortion of the flowers.

To observe the type of collection (nectar and/or pollen) carried through by the *A. mellifera*, six honeybees were collected each hour (9h00 to 16h00) in all the parcels for three days in the area with honeybees and in the area of free insect visitation. Corbicula content and honey stomach content was recorded with scores that varied from zero for empty (honey stomach and corbicula), one for moderate, two for full and three for maximum load. Evaluation of the total sugar concentration was performed by placing the crop content in a manual refractometer and recording the brix value.

The statistical analyses of the variables were carried out with Statistical Analysis System [18] software using the following model:

Y_{ijklm}= μ + B_i + H_j + T_k + C_l + $(BH)_{ij}$ + $(BT)_{ik}$ + $(BC)_{jl}$ + $(HT)_{jk}$ + $(HC)_{jl}$ + $(TC)_{kl}$ + and $_{ijklm}$, where,

Y_{ijklm}= Observed in reference to variants of Block i, Herbicide j, Treatment k, Cultivar l

μ is the effect of general average;

B_i is the effect of Block (i = 1, 2... 6);

H_j is the effect of herbicide (j = 1, 2);

T_k is the effect of treatment (1, 2, 3);

C_l is the effect of cultivar;

$(BH)_{ij}$ is the interaction of Block i and Herbicide J;

$(BT)_{ik}$ is the interaction of Block i and Treatment k;

$(BC)_{jl}$ is the interaction of Block i and Cultivar l;

$(HT)_{jk}$ is the interaction of Herbicide j and the Treatment k;

$(HC)_{jl}$ is the interaction of Herbicide i and the Cultivar l;

eijklm is the mistake associated to the observation ijklm.

The delineation used was of completely randomized blocks and the data submitted to the variance analysis. Tukey's test was used for comparison of the averages.

Frequency of stigma receptivity was analyzed by the NPAR1WAY Wilcoxon procedure and the viability was estimated by the Kruskal-Wallis test.

Data without normal distributions were analyzed using generalized linear models [19], assuming Poisson distribution with logarithmic link function.

For analysis of the type of collection, the model considers the treatment effect as a fixed variable, random for the effect of the interaction treatment versus day and the linear effect, squared and cubical for the hour collection.

3. Results

There was no effect (*P*> 0.05) of cultivar, from the application of the herbicide and the interactions of these variables, therefore floral biology and behaviour of the insects in two cultivars were similar (*P*> 0.05), independent of the herbicide used, but the presence of insects influenced these parameters (Table 1).

The anthesis period in hours was, on average, 8h04min greater (*P*=0.0001) in the pollination cages without honeybee colonies than in the pollination cages with honeybee colonies and then in the uncovered area for free insect visitation (*P*>0.05). In Table 1, there is a summary of the variance analysis and the average comparison of the anthesis period in the three treatments, and two cultivars.

The receptivity of the stigma was not influenced by cultivar (*P*>0.05) with a general average of 87.43%, and was not influenced by treatments either (P>0.05) which had a general mean of 88.71%. There was no difference (*P*> 0.05) in the percentage of viable pollen grains in the soybean flowers between treatments and the general average was 89.82% ±7.27 (Table 2).

The percentages of viable pollen grains removed from corbicula honeybee forager in the pollination cages with honeybee colonies and in the areas of free insect visitation were not different (*P*> 0.05) and the average was 99.95% (±0.14).

In the analysis of the removed pollen grains from corbicula of honeybee forager, 100% were of soybean, showing a high constancy of the honeybees to this crop.

Variation source	Anthesis period in hours	
Cultivar	0.13	*P* = 0.7243
CV % of cultivar	10.24	
Treatments	3.26	*P*= 0.0104
CV % of treatments	15.27	
Transgenic soy	42.37	a* (±6.83)
Conventional soy	42.89	a (±6.36)
Covered area with honeybees	37.90	b (±4.05)
Covered area without honeybees	48.00	a (±3.72)
Uncovered area	41.98	b (±6.95)

Means followed by different letters, in the same column, are different by Tukey's test (*P*< 0.05)

Table 1. F values with respective probability (*P*), coefficient of variation (CV%), means and their standard deviation of anthesis period, in hours, for soybean flowers, *Glycine max*, var. BR-245 RR (transgenic soybean - Roundup Ready™) and BRS-133 (conventional soybean), in experimental area of EMBRAPA Soja, in Londrina-PR, Brazil.

Variation source	Stigma receptivity (%)		Viable pollen grains (%)	
Cultivar	0.52	*P* = 0.9100	0.53	*P* = 0.4668
CV % of cultivar	8.48		8.19	
Treatments	0.87	*P* = 0.4816	0.62	*P* = 0.6167
CV % of treatments	7.17		8.11	
Transgenic soy	87.65	a (±6.19)	90.14	a (±7.52)
Conventional soy	87.20	a (±6.52)	89.51	a (±7.07)
Covered area with honeybees	89.81	a* (±7.04)	89.22	a (±7.70)

Variation source	Stigma receptivity (%)		Viable pollen grains (%)	
Covered area whithout honeybees	87.50	a (±4.81)	90.38	a (±6.64)
Uncovered area	84.72	a (±6.28)	89.86	a (±7.46)

Means followed by different letters, in the same column, are different by Tukey's test ($P< 0.05$)

Table 2. F values with respective probability (*P*), coefficient of variation (CV%), means and their standard deviation of stigma receptivity and viable pollen grains (%), in soybean flowers *Glycine max*, var. BR-245 RR (transgenic soybean - Roundup Ready™), and BRS-133 (conventional soybean) in experimental area of EMBRAPA Soja, in Londrina-PR, Brazil.

The percentage of aborted flowers was greater (P=0.0001) in the covered area by cages without honeybees in relation to the covered areas by cages with honeybee colony and of free insect visitation; these last treatments did not differ (P>0.05). The averages and the standard deviation of the abortion percentage in the soybean flowers in the treatments are presented in Table 3.

Variation source	Abortion percentage of flowers	
Cultivar	2.55	P=0.1302
CV % of cultivar	6.55	
Treatments	13.21	$P< 0.0001$
CV % of treatments	18.08	
Transgenic soy	73.51	a* (±9.54)
Conventional soy	80.21	a (±7.03)
Covered area with honeybees	50.78	b* (±8.30)
Covered area without honeybees	71.10	A (±3.05)
Uncovered area	55.12	B (±5.84)

Means followed by different letters, in the same column, are different by Tukey's test ($P< 0.05$)

Table 3. F values with respective probability (*P*), coefficient of variation (CV%), means and their standard deviation of abortion percentage of soybean flowers *Glycine max*, var. BR-245 RR (transgenic soybean - Roundup Ready™), and BRS-133 (conventional soybean) in experimental area of EMBRAPA Soja, in Londrina-PR, Brazil.

In the area of free insect visitation, *A. mellifera* was the species most abundant with 97.02% frequency. Other bee species with 1.65%10 frequency had been observed also, and, Lepidoptera with 1.33% (Figure 4). Amongst the collected bees, there were nine species, six from the *Apidae* family, two from *Megachilidae* and one from *Halictidae*.

The behaviour for the type of collection observed in workers of *A. mellifera* foraging the soybean flowers was typical of nectar collecting, although, workers with unique behavior for pollen collection was observed on occasion (Figure 5).

In an accurate evaluation of the honey stomach contents and corbicula, it was observed that, throughout the day, 69.39% of the foragers had collected nectar, 37.05% had collected nectar and pollen and 2.56% had only collected pollen.

The nectar collection throughout the day varied and differed in the amount of honeybees in the different schedules (P=0.0001), but did not differ (P> 0.05) between the covered area by cages with honeybees present and of free insect visitation. In these two areas, the moment of greatest visitation of the *A. mellifera* was at 12h34min. The frequency of honeybees with a larger amount of nectar in the covered areas by cages with honeybees and of free insect visitation is represented in Figure 6.

In the area of free insect visitation, the time that *A. mellifera* spent collecting nectar was 2.59 ±0.52 seconds/flower which was less (P=0.0001) than the observed time in the pollination cages with honeybee colonies (2.99 ±0.54 seconds/flower). Regardless of treatment, honeybee *A. mellifera* spent, on average, 2.74 ±0.56 seconds/flower in nectar collection and 4.37 ±0.62 seconds/flower in pollen collection and this difference was significant (P=0.0001). *A. mellifera* visited 7.14 (±0.26) flowers/min. collecting nectar and 3.75 (±0.30) flowers/min in pollen collection.

The average concentration of total sugar removed from the honey stomach (called crop) of the honeybees that collected nectar throughout the day was of 41.19% ±3.72, in pollination cages with honeybee colonies inside; this was greater (P=0.0008) than the 38.22% ±3.37 measured from the area of free insect visitation (Figure 7). In these two areas there were also a difference (P=0.0001) between the collecting schedule.

Figure 4. Lepidoptera in soybean flower

Figure 5. The honeybee searching a reward in soybean flower

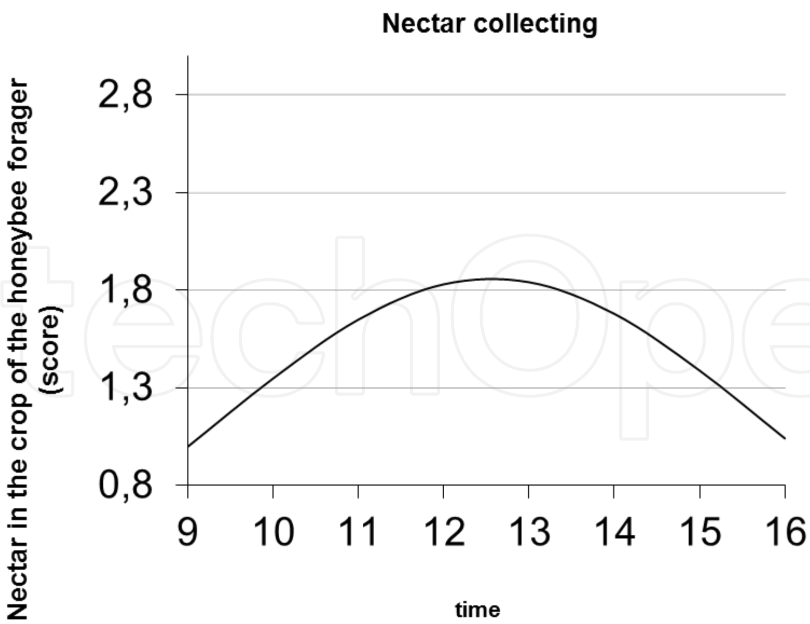

Figure 6. Regression curve obtained through the equation: $Y=\exp^{(-7.111+1.231xh-0.049xh2)}$ of number of worker honeybees collecting nectar during the day in covered area with honeybee colony inside, and $Y=\exp^{(-7.111+1.318xh-0.049xh2)}$ in the area for free insect visitation, in soybean *Glycine max*, var. BR-245 RR (transgenic soybean - Roundup Ready™) and BRS-133 (conventional soybean) in experimental area of EMBRAPA Soja, in Londrina-PR, Brazil.

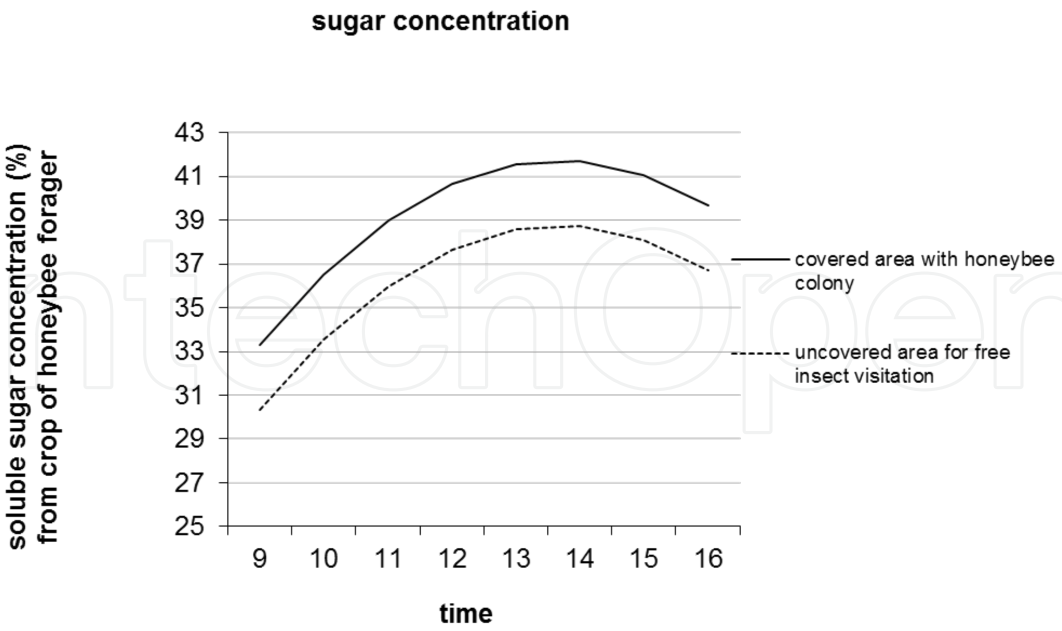

Figure 7. Regression curve obtained through the equation $Y=41.19+0.9127(h-12.5)-0.3836(h-12.5)^2$ in covered area with honeybee colony and $Y=38.22+0.9127(h-12.5)-0.3836(h-12.5)^2$ in uncovered area for free insect visitation showing the total sugar, in Brix, measured from worker crop content of captured honeybees, foraging soybean flowers *Glycine max*, var. BR-245 RR (transgenic soybean - Roundup Read™) and BRS-133 (conventional soybean) in experimental area of EMBRAPA Soja, in Londrina-PR, Brazil.

4. Discussion

The results showed that the genetically modified cultivar, regardless of receiving an application of the herbicide had a similar pattern as the conventional cultivar for the analyzed parameters. This showed that the transgenes did not interfere in the analyzed parameters, like floral biology, and the Africanized honeybee behaviour. The bees, mainly honeybees, are considered the most effective pollinator in nature, principally, in cultivated plants like soybean [39]. The honeybee has become a domesticated animal; they can be managed, moved to a new site, divided, mature, and stimulated to go to a specific crop for a specific time.

When evaluating the outcome of Africanized honeybee presence on soybean culture, the parameters of anthesis and the percentage of aborted flowers decreased just as much on the transgenic cultivar as the conventional one. [37] describes an excellent protocol to be applied to focal crops at the farm-scale level to assess pollinator density and diversity for comparison purposes among different sites. It becomes an important tool for researchers and professors who work in pollination because in several areas, one of the most difficult things is to compare results that are obtained by different methods.

The anthesis period was, on average, 20.49% longer in the area where insects had been prevented from visiting the flowers than in the pollination cages with honeybee colonies and the area for free insect visitation. Similar results have been observed by [7] on BRS-133, which verified an average increase of 20.38% into the anthesis period in the area that insects were prevented from visiting in relation to the areas where the insects had access. In agriculture and horticulture pollination management, a good pollenizer is a plant that provides compatible, viable and plentiful pollen and blooms at the same time as the plant that is to be pollinated or has pollen that can be used and/or stored when needed to pollinate the desired flowers.

An increase in the anthesis period in areas that insects were prevented from visiting has also occurred with other species of plants. [18] observed an increase of 43.5% into the anthesis period in flowers of siratro (*Macroptilium atropurpureum* Urb.) when they had been prevented from having insect visitation. The outcomes of the above mentioned authors show that the Africanized honeybees have been efficient in the pollination process, since that anthesis period was smaller in areas for free insect visitation than flowers protected from them, which presented a greater anthesis period and when the Africanized honeybees were present, the anthesis period of soybean and *M. atropurpureum* was smaller.

The stigma receptivity was similar between treatments. Similar results had been observed by [18] in the *M. atropurpureum* flowers which had 76.9% of the blossoms and 91.95% of the flowers opened in the receptive stigma to pollen grains.

The high viability of the pollen grains in all treatments, 99.6% (±0.02) on average, suggest that these do not depend on the conditions imposed on the flowers in this cultivar. [18] found 100% of viability in the pollen grains in *M. atropurpureum*. [7] on cultivar BRS-133 obtained 99.86% of viable pollen grains.

In the analysis of the removed pollen grains from corbicula of foragers in the area of free insect visitation, no pollen from other flowers was found. These results are in accordance with [7] that observed 93 to 100% of honeybee foragers collected pollen in one single species. These results have shown that the alimentary resource offered by the soybean flower satisfies the requirements of *A. mellifera* and they did not need to visit other cultures while the samples were being collected.

The number of aborted flowers was 40.02% greater in the covered area by cages without honeybees in relation to the covered area by cages with honeybees, and 28.99% greater than in the area of free insect visitation. These results match the results of [20] that observed high rates of abortion in soybean, varying from 43% to 87%, depending on the cultivar. [9] had 75% of the flowers aborted in some cultivars, [11] had superior rates of more than 75% of abortion and [7] found a rate of abortion 53.66% greater in areas protected of insect visitation to flowers compared with covered area of cages with *A. mellifera*. This biological response of the flower shows that, even if soybean is considered to have autogamy by researchers such as [13], [21], it probably also possesses a mechanism of genetic auto-incompatibility to prevent 100% of self-fertilization, as observed on plants of the genus *Brassica* by [22]. This evidences the importance of honeybees for the reduction in the abortion percentage and, consequently, increases the amount of string beans and seeds in soybean.

The largest frequency of *A. mellifera* (97.02%) in soybean flowers in the area for free insect visitation was similar to the results obtained by [7], which found 95.18% of this species visiting soybean flowers on cultivar BRS-133. Therefore, in this cultivar of soybean, the presence of *A. mellifera* is important not only for honey production but also for pollination, and mainly for an increase of productivity. The larger part of agricultural land consists of cultivated areas like fields. However, an amount of space remains that is often quantitatively underestimated, and could be managed to promote plant and biotope diversity. Natural or semi-natural habitat remnants provide nesting sites and reliable food sources for pollinators. Conserving these areas can benefit biodiversity and offer potential for improved crop productivity [28]. Soybean is largely used in Brazilian agriculture in extensive systems called monoculture. Therefore, in these areas it is easier to implant management programs for rational use of pesticides focusing on the reduction of negative effects on the bees [38].

The comments regarding the collecting behavior have shown that *A. mellifera* visits the soybean flowers intensely, mainly, for nectar collection and, in part of the morning, from 9h00 to 11h30, the nectar and pollen collection was intense. On occasion, the behavior of foragers collecting pollen was observed. These results are in accordance with [23] and [24], which observed a great number of worker honeybees visiting soybean flowers. Even with nectar collecting behavior, *A. mellifera* was efficient as a pollinator, strengthening the affirmations of [11] that the honeybee does not necessarily need to be pollen collectors to make an efficient pollinator since they are able to accomplish that as they collect nectar. [7] observed intense visits of *A. mellifera* in the cv. BRS-133 collecting, predominantly, nectar and had an increase of 58.86% in the areas with *A. mellifera* in relation to the area with insect restriction. Honeybee nectar foragers were more frequent (2.28 bees per chapter) than pollen foragers (0.40 bees per chapter) in sunflowers and seed yield was 43% higher ($P < 0.05$) from sunflower plants that were visited by pollinator-insects than plants restricted to pollinators [25].

The evaluation of amount of total sugars present in the crop content of the foragers showed that the harvested nectar awakened the interest of the *A. mellifera* for the visitation of these cultivars of soybean. The consistency of the honeybees to the soybean showed that the flowers of this species satisfies its nutritional requirements and they do not need to visit other species, even if there are other flowers available and some very attractive, such as sunflower (*Helianthus annuus*).

In the research of [41] and [42], the means of sucrose.hexose^{-1} (S/H) per flower for all treatments were: 0.91 μg.μL^{-1}, for covered areas with Africanized honeybee colonies – rich in sucrose; 0.74 μg.μL^{-1}, semi-covered areas with free insect visitation – rich in sucrose; 0.86 μg.μL^{-1}, uncovered areas with free insect visitation – rich in sucrose; and 3.05 μg.μL^{-1}, for covered areas without Africanized honeybee colonies – sucrose dominant. According to [43], this range suggests that sugar concentration in soybean nectar is influenced by other environmental factors independently of pollinator action. [44] reported that edaphic and climatic factors affect the number of flowers and other floral characteristics during soybean growing. Therefore, the environmental conditions that generate an increase in number and size of flowers, longer anthesis period, more intense coloring, and greater nectar production are the factors responsible for making flowers more attractive to honeybees [42]. [45] suggested that the maximum nectar accumulation occurs before or at the beginning of pollination activity.

Technological innovations play an important role in pollinator protection. Application technologies allow for reductions in spray drift; this helps prevent pesticide residues in non-target areas. This is achieved with application nozzles that create spray droplets large enough to be less affected by wind [28].

One of the greatest concerns of Brazilian farmers in relation to GM plants is the unexpected and cumulative effect of cross-contamination between GM plants and conventional plants. The instability and risk of propagation of a gene to wild species are critical to maintenance of the environment [52]. Genetic pollution is inevitable and the transgenic pollen may contaminate conventional or biological fields located several kilometres from GM plantations [55].

In another study [52] in transgenic and conventional soybean, it was reported that the estimate of grain production increased 37.84% in the area where honeybee visits were permitted. However, the cv. BRS-133, not GM [7, 14] was intensively visited by *A. mellifera* Africanized honeybees. The researchers reported an increase of 61.38% in number of pods, and 58.86% in seed production, when compared with plants not visited by insects. [26] reported that Africanized honeybees provided a considerable increase of gene flow from transgenic cv. BR-245 RR to conventional cv. BRS-133 soybean (1.57%). Since these cultivars of soybean were attractive to the honeybees, they performed the cross-pollination in the tested varieties.

The economic value of honeybee service in the USA reported by [57, 58, 59, and 60] was about US $15 billion, and specifically for soybean, the value was US $754 million. [53] using the same table of [57, 58, and 59], estimated the economic value of Africanized honeybee service for soybean culture, using data from [7, 14, and 52] obtained as medium value US $3.561,2 million. These estimates are considerable in Brazil and in other parts of the world, and must not be disregarded.

The Green Revolution has reduced the percentage of the word-wide population that suffers from hunger from 50% in sixty years to 20% at present. In plantations free of pesticides, the loss of production is 10% to 40%. Without this technology, about two billion of the seven billion inhabitants of the planet would be starving. The use of transgenic crops may help to increase the productivity, avoiding more deforestation, and more erosion of the soils [56].

Floral biology and the behaviour of the honeybees in transgenic soybean cv. BR-245 RR did not depend on the application of herbicide and was similar to the verified one in the conventional cv. BRS-133. This implies that it is possible to have cross-pollination between transgenic and conventional soybean and gene flow between them like reported by [26].

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General summary

The experiment was carried out to study the floral biology of the *Glycine max* L. Merrill, cultivar BR-245 RR (transgenic - Roundup Ready™) and BRS-133 (conventional) and to evaluate the behavior of the Africanized honeybee *A. mellifera* in the flowers of these cultivars. Three treatments established included: a covered area with an Africanized honeybee colony, a covered area without a honeybee colony and area of free insect visitation. Each treatment was six parcels of 24 m^2 each and the planting of soybean was carried through in this area in eight pared and intercalated lines with tested cultivars, being applied herbicide (glyphosate) in the plants of transgenic cultivars in half of the parcels in the three treatments. The anthesis period was 8h04min longer in the covered area without a honeybee colony than in the covered area with a honeybee colony and in the area of free insect visitation. The average of the analyzed stigma receptivity was 87.35% and there was no difference between treatments. The average of viable pollen was 89.82% with no difference between treatments. In the analysis of the removed pollen grains from the honeybee forager corbicula, 100% observed belonging to soybean. The flower abortion rate was 71.10% in covered areas without honeybee colonies, which was greater by 50.78% and 55.12%, respectively, than the covered area with a honeybee colony and area of free insect visitation. *A. mellifera* was the insect that most frequently (97.02%) visited the soybean flowers. The time that *A. mellifera* spent to collect nectar was greater in the covered area with a honeybee colony than in the area of free insect visitation. The average time foragers spent for nectar collection was 2.74 second/flower and 4.37 second/flower for the pollen collection. *A. mellifera* visited, on average, 7.14 flowers/min, collecting nectar, and 3.75 flowers/min for pollen collection. The total sugar concentration in the honey stomach content was 41.19% in the covered area with a honeybee colony, greater than the 38.22% observed for the area of free insect visitation. In the uncovered areas for free insect visitation and covered areas with Africanized honeybee colony, the results were different from the covered area without honeybee colony. There was no difference in the evaluations of floral biology and behaviour of insects between the transgenic and the conventional soybean, independent of the application of the herbicide glyphosate in transgenic soybean.

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