

Comparative Study of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) Preference for Bt and Non-Bt Soybean Cultivars

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Abstract

Studies on the behavior of *Helicoverpa armigera* in relation to soybean cultivars expressing Bt proteins are fundamentally important for IPM. We determine the no-choice and feeding choice of *H. armigera*, the no-choice and oviposition choice in different Bt and non-Bt soybeans cultivars. In the first step it was carried out the experiment to determine the no-choice and food choice in test for 12 Bt and non-Bt soybean cultivars. Simultaneously the consumption was determined. In a second step it was evaluated the no-choice and oviposition choice in different soybean cultivars. *Helicoverpa armigera* caterpillars showed neither attractiveness and preference for food nor attractiveness and oviposition preference for Bt and non-Bt soybean leaves when simultaneously contrasting the tested cultivars. This study showed that Bt toxin did not influence the oviposition preference, and *H. armigera* adults did not differentiate Bt and non-Bt soybean cultivars for oviposition indicating no behavioral preference. Third instar caterpillars showed no discrimination between Bt and non-Bt soybean leaves. Research in this area must focus on the possibility of widespread planting of genetically modified soybeans containing the Bt protein, which is associated with selection pressure and the behavior of pest species in relation to their hosts, as well as on the adequacy of management tactics that is able to prevent the loss of technology efficiency.

Keywords

Cry1Ac Protein, Genetically Modified Plants, Food Preference, Oviposition

1. Introduction

Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae) causes serious economic

losses to crops such as soybeans corn, cotton, beans and sorghum, white caterpillars feed on leaves, flowers, pods and grains [1]. *Helicoverpa armigera* control in almost every crop system is carried out basically by insecticide spraying [2] [3]. Therefore, populations of this species are under strong selection pressure and resistance to the main chemical groups [4] [5] [6].

Breeders worldwide have focused on identifying soybean cultivars with insect resistance. The resistance mechanisms involved are basically antibiosis and antixenosis with negative effects on insect biology and behavior respectively [7] [8] [9].

Antibiosis is a resistance mechanism in plants. In this mechanism, the mortality rate of insects increases or larval growth and development decreases after insect feeding. By contrast, antixenosis is a resistance mechanism in which the insects are not attracted to the plant. Defoliator resistance in soybean may rely on one or both of these mechanisms [10] [11] [12] [13] [14].

Bacillus thuringiensis can also serve as a source of toxic genes that can be expressed in plants and thus confer toxic property against different species of insect pests. Genetically modified plants (GMPs) that express the Bt genes, such as rice, corn, potato, cotton, and soybean are associated with the control of pests, especially Lepidoptera. The resistant cultivars result in increased productivity, greater economic value, reduction in the use of chemical pesticides, and benefits in the selectivity of the target pest [15] [16].

However, the intensive use of Bt soybean has increased the likelihood of insects developing resistance to Bt-based bioinsecticides and transgenic plants [17]. Considering the little information about the effect of these resistant genotypes against *H. armigera*, information about the behavior of this pest species in relation to soybean cultivars expressing Bt proteins is fundamental for the IPM. Thus, the objective was to determine the attractiveness and the feeding and oviposition preference of *H. armigera* caterpillars for Bt and non-Bt soybeans cultivars.

2. Material and Methods

2.1. Insects

Populations of *H. armigera* used in the experiments were obtained from the institution stock, kept in artificial diet adapted [18] made with beans (500 g), wheat germ (237 g), beer yeast (152 g), ascorbic acid (15 g), sorbic acid (4.45 g), nipagin (9.45 g), Agar-agar (62 g), formaldehyde (12.45 ml), inhibitor solution (12.5 g) (propionic acid 41.8% 4.2% fosphoric acid and 54% water). Neonate caterpillars (<24 hours old) were individualized and transferred to 100 ml plastic containers with lid containing artificial diet until they reached the pupal stage, kept under laboratory conditions (25°C ± 5°C, 60% ± 10% RH, 14:10 L:D). The adults were transferred to PVC cages (40 cm height × 30 cm Ø), internally coated with white standard paper sheets for oviposition, fed with a water-honey solution (9:1), kept under laboratory conditions (25°C ± 5°C, 60% ± 10% RH, 14:10

L:D). The eggs were collected and stored in plastic bags kept in laboratory conditions until the hatch of caterpillars, thus keeping up the complete cycle and constant supply of individuals in the right age to be used in the experiments.

2.2. Plants

Twelve soybean cultivars were used in the experiment: Eight Bt soybean cultivars containing the Bt Cry1Ac gene and four non-Bt (**Table 1**), additional information on the cultivars in **Table S1**. The cultivars were grown in 5 L plastic containers, placing 6 seeds per container, in V5 stage was performed the thinning, leaving only 3 plants per container.

2.3. Attractiveness Index and Larval Preference in Free and No-Choice Tests

The attractiveness indexes and larval preference were evaluated in free and no-choice tests conducted under laboratory conditions ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$, $60\% \pm 10\%$ RH, 14:10 L:D), where leaf discs of different soybean cultivars were used.

In the free choice test, a third instar caterpillars, individualized, were released inside plastic circular arenas (50 cm \varnothing \times 10 cm height), containing a leaf disc (5 cm \varnothing) of each treatment. Before tests, the caterpillars were subjected to a 12 hour fasting. This assay was carried out using randomized blocks design with 24 replications.

In the no-choice test, the leaf disc of each cultivar were isolated in the center of a Petri dish (8 cm \times 2 cm), together with a third instar caterpillars. Each dish is a plot, with 24 repetitions per cultivar in a completely randomized design.

The evaluations for both the free and no-choice tests were performed every 1, 2, 3, 6 and 12 hours after release. At the end of the counting the attractiveness

Table 1. Description of cultivars that were used in the experiments, adapting the name of the genotypes and main observed vegetative characteristics.

Treatment	Cultivar	Technology	Relative maturation	Type of growth	Flower color	Requirement fertility	Lodging
Bt1	TMG 2183	I PRO	8.3	Determined	Purple	High	Tolerant
Bt2	M8210	I PRO	8.2	Determined	White	High	Resistant
Bt3	M 8644	I PRO	8.6	Determined	Purple	High	Resistant
Bt4	M 8372	I PRO	8.3	Determined	White	High	Resistant
Bt5	AS 3820	I PRO	8.2	Determined	Purple	High	Moderate
Bt6	M 8330	I PRO	8.3	Determined	Purple	High	Resistant
Bt7	HK 8314	I PRO	8.3	Determined	White	High	Moderate
Bt8	HK 8514	I PRO	8.5	Determined	White	High	Moderate
RR1	M 9144	RR	9.1	Determined	Purple	Average	Moderate
RR2	FTS Campo novo	RR	8.3	Undetermined	White	High	Resistant
RR3	FTS Paragominas	RR	9.3	Determined	Purple	Average	Moderate
CONV	M 9350	Conventional	9.3	Determined	Purple	Average	Moderate

Conv = Conventional: No event; RR = RR has events of tolerance to the herbicide glyphosate; Bt = I PRO (Cry1Ac and RR): two events, tolerance to glyphosate and protection against caterpillars.

index was estimated, by the formula: $AI = 2T/(T + P)$ where: AI = Attractiveness Index, T = number of insects attracted to the evaluated cultivar, P = number of insects attracted to the standard cultivar. AI values range between zero and two, as follows: AI = 1 indicates similar attraction between the evaluated and standard cultivars, IA < 1 corresponds to less attraction to cultivar, IA > 1 indicates greater attraction to evaluated cultivar in relation to the standard one [19] [20] [21]. The cultivars were classified by comparing the ratios obtained in the evaluated cultivars and the standard cultivar.

Simultaneously to the attractiveness free and no-choice tests, evaluated the consumption of leaf discs by third instar caterpillars of *H. armigera*. The discs were measured, with the aid of leaf area meter LI 3000 A (Li-Cor®) to determine the area consumed by the caterpillars.

For both attractiveness free and no-choice tests and consumption of leaf discs, the data were submitted to analysis of variance by the F test and the means compared by the Tukey test, at 5% probability, using the SAS statistical software [22].

2.4. Attractiveness Index and Preference for Oviposition

The attractiveness and oviposition preference were evaluated in free-choice tests in a greenhouse, coated with screened. For assembly, four vessels/cultivars were placed with three soybean plants each, in stages R3, the different cultivars in 30 cm spacings between vessels and 40 cm between rows of vessels. In the center of the greenhouse, 50 cm above the plants, it was released a *H. armigera* moth couple per soybean vessel, *i.e.*, 48 couples were released at 6 am, and the evaluations of attractiveness were performed 6, 12, 24 and 48 hours after the release. At the end of the attractiveness test the attractiveness index was estimated. Oviposition was evaluated 72 hours after the release of insects, counting the number of eggs per soybean cultivars. The preference index was also calculated for oviposition by the equation: $POI = [(TP)/(T + P)] * 100$ where: POI = preference for oviposition index, T = number of eggs counted in cultivars, P = number of eggs counted in conventional cultivars, the index ranges from: +100 (very stimulating), -100 (total deterrence) and 0 indicates neutrality [21] [23] [24]. The cultivar used as a standard in the bioassays showed the most attractiveness and consumption in the free-choice test. The design was a randomized block with 12 treatments, with four replications. Each replication consists of a vase with three soybean plants.

After the evaluation the analysis of variance was carried out, and when there was a significant difference between cultivars, the Tukey test ($P < 0.05$) was performed for the comparison of means, using the SAS statistical software [22].

3. Results

3.1. Attractiveness and Attractiveness Index of Caterpillars in Free-Choice Test

The time effect was significant, at all times of evaluations. For each time it was possible to find great variation among the cultivars with higher averages. How-

ever, the best results were obtained by the cultivars Bt5 and Bt7, which maintained among the highest averages for all times evaluated, while the cultivars that presented lower means of attracted individuals were Bt8 and Conv (**Table 2**).

The attractiveness indexes varied between the evaluation times, with the most cultivars attracted at the 30 min, 1 and 3 hours evaluation, while at 2, 6 and 12 hours were less attractive for most cultivars. The indexes varied among treatments, where Bt5 and RR3 treatments were more attractive in the first four evaluations while Bt8, RR1 and Conv were more repellent (**Figure 1**).

For the attractiveness no-choice bioassay, there was a variation among the evaluation times, with a more noticeable effect in the first evaluations. At the same time, there was a variation among treatments, where Bt2, Bt3 and Bt8 had higher averages of attracted caterpillars and RR1 and RR3, in contrast, had lower averages of attracted caterpillars (**Table 3**).

The indices of attractiveness without choice differ between the evaluations times according to the formula used for evaluation. The best results for attractiveness were obtained in the first hours (**Figures 2(a)-(c)**) and more repellents in the latter (**Figures 2(d)-(f)**). Treatments Bt1, Bt5, RR1, RR3 and Conv showed higher repellency index when compared with treatments Bt2, Bt3, Bt7 and Bt8.

3.2. Consumption with and without Choice

The leaf area consumed in the free-choice test varied among treatments, being treatment RR2 the one that had the largest consumed area, while Bt1, Bt3 and

Table 2. Average number (\pm SE) third instar *Helicoverpa armigera* caterpillar attracted in free-choice tests 30 min, 1, 2, 3, 6 and 12 hours after the release, for the different soybean cultivars.

Cultivars	Time (hours)					
	30 min	1	2	3	6	12
Bt1	0.7 \pm 0.1 ABCab	1 \pm 0 Aa	0.7 \pm 0.1 ABab	0.4 \pm 0.1 BCbc	0.2 \pm 0.01 Cc	0.6 \pm 0.07 ABCb
Bt2	0.6 \pm 0.1 ABCbc	0.7 \pm 0.1 ABCab	0.4 \pm 0.07 ABCbc	0.3 \pm 0.04 Cc	0.3 \pm 0.03 BCc	1 \pm 0.1 Aa
Bt3	0.7 \pm 0.1 ABCab	0.5 \pm 0.01 BCDab	0.4 \pm 0.06 ABCb	0.8 \pm 0.09 Aba	0.5 \pm 0.07 ABCab	0.5 \pm 0.09 BCab
Bt4	0.5 \pm 0.04 BCb	0.5 \pm 0.04 BCDb	0.4 \pm 0.06 ABCb	0.4 \pm 0.04 BCb	0.2 \pm 0.03 Cb	0.5 \pm 0.02 BCb
Bt5	0.9 \pm 0.7 ABab	0.8 \pm 0.1 ABab	0.8 \pm 0.1 Aab	1 \pm 0.03 Aa	0.7 \pm 0.1 ABab	0.6 \pm 0.2 ABCb
Bt6	0.4 \pm 0.08 Cbc	0.5 \pm 0.09 BCDab	0.6 \pm 0.1 ABab	0.8 \pm 0.08 Aba	0.1 \pm 0.01 Cc	0.3 \pm 0.03 Cbc
Bt7	0.6 \pm 0.1 ABCab	0.6 \pm 0.1 ABCDab	0.7 \pm 0.1 ABab	0.9 \pm 0.07 Aa	0.5 \pm 0.09 ABCb	0.7 \pm 0.1 ABCab
Bt8	0.6 \pm 0.07 ABCa	0.2 \pm 0.03 Db	0.4 \pm 0.03 ABCab	0.6 \pm 0.1 ABCa	0.4 \pm 0.06 ABCab	0.5 \pm 0.07 BCab
RR1	0.4 \pm 0.1 Cbc	0.3 \pm 0.03 CDc	0.4 \pm 0.1 ABCbc	0.7 \pm 0.1 ABCab	0.8 \pm 0.1 Aa	0.8 \pm 0.1 Aba
RR2	0.4 \pm 0.08 Cbc	0.5 \pm 0.1 BCDabc	0.5 \pm 0.1 ABCbc	0.3 \pm 0.04 Cc	0.7 \pm 0.1 ABab	0.8 \pm 0.1 Aba
RR3	1 \pm 0.07 Aa	0.8 \pm 0.08 ABab	0.5 \pm 0.07 ABCc	0.4 \pm 0.08 BCc	0.4 \pm 0.04 ABCc	0.3 \pm 0.03 Cc
Conv	0.3 \pm 0.03 Cab	0.6 \pm 0.07 ABCDa	0.4 \pm 0.08 BCab	0.3 \pm 0.04 Cab	0.5 \pm 0.06 ABCab	0.5 \pm 0.08 BCab

Averages followed by the same capital letter in the column and small letter in the row do not differ statistically among each other by Tukey test [$P > 0.05$, $F = 4.8$, $CV = 50.7$ (SAS Institute, 2002)].

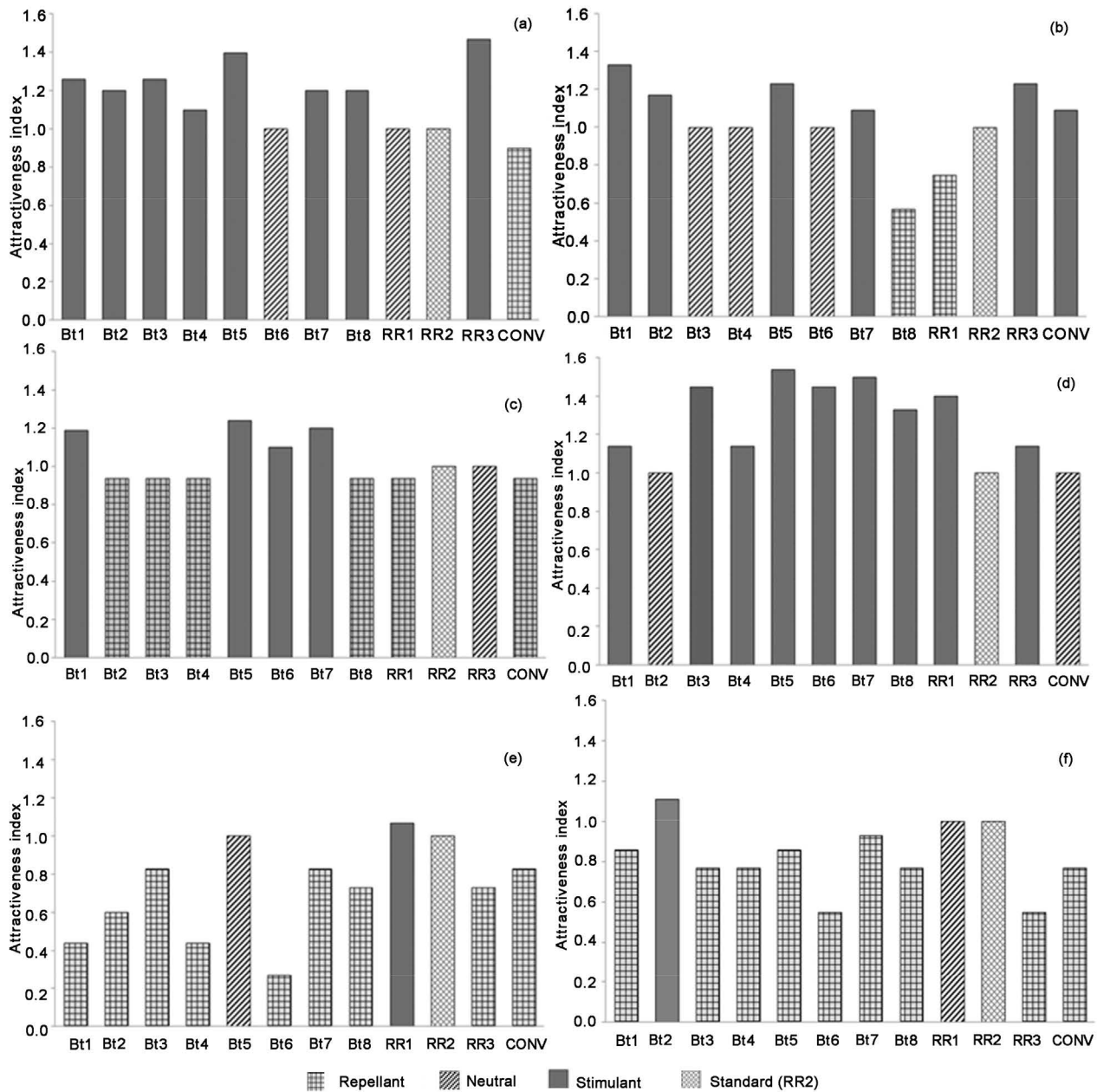


Figure 1. Attractiveness index of third instar *Helicoverpa armigera* caterpillars and soybean cultivars rating (a) 30 min; (b) 1 hour; (c) 2 hours; (d) 3 hours; (e) 6 hours; and (f) 12 hours after the start of free-choice test.

Bt7 were the ones with smaller consumed areas (Figure 3(a)). For the no-choice test, there was a difference in the consumed area by the treatments, with RR1 standing out for the highest consumption and with smaller consumed areas, treatments Bt2, Bt4, Bt5, Bt6, Bt7, Bt8 and Conv (Figure 3(b)).

3.3. Attractiveness and Attractiveness Index of Adult *Helicoverpa armigera*

The time effect was significant showing that the moths have different behavior in exposure times. There was difference for moths' landing preference among cultivars

Table 3. Average number (\pm SE) third instar *Helicoverpa armigera* caterpillar attracted in no-choice tests 30 min, 1, 2, 3, 6 and 12 hours after the release, for the different soybean cultivars.

Cultivars	Time (hours)					
	30 min	1	2	3	6	12
Bt1	0.7 \pm 0.1 BCa	0.3 \pm 0.07 DEb	0.5 \pm 0.09 Aab	0.6 \pm 0.08 ABab	0.4 \pm 0.05 ABab	0.5 \pm 0.09 ABab
Bt2	1.1 \pm 0.6 Aa	0.8 \pm 0.08 ABab	0.5 \pm 0.09 Abcd	0.3 \pm 0.06 Bd	0.4 \pm 0.08 ABcd	0.6 \pm 0.1 ABbcd
Bt3	1 \pm 0.02 ABab	1.1 \pm 0.07 Aa	0.6 \pm 0.07 Ac	0.6 \pm 0.09 ABc	0.6 \pm 0.05 Ac	0.4 \pm 0.07 ABCc
Bt4	0.6 \pm 0.07 Cab	0.8 \pm 0.08 ABa	0.7 \pm 0.09 Aa	0.3 \pm 0.06 Bb	0.6 \pm 0.1 Aab	0.6 \pm 0.1 ABab
Bt5	0.6 \pm 0.1 Ca	0.7 \pm 0.1 BCa	0.5 \pm 0.1 Aa	0.5 \pm 0.1 ABa	0.5 \pm 0.09 Aba	0.1 \pm 0.02 Cb
Bt6	0.7 \pm 0.08 BCab	0.6 \pm 0.1 BCDbc	0.5 \pm 0.09 Abc	0.4 \pm 0.07 Bbc	0.6 \pm 0.07 Abc	0.3 \pm 0.07 BCc
Bt7	0.7 \pm 0.1 BCa	0.7 \pm 0.1 BCa	0.6 \pm 0.1 Aab	0.8 \pm 0.08 Aa	0.5 \pm 0.09 ABab	0.3 \pm 0.04 BCb
Bt8	0.8 \pm 0.08 ABCb	0.7 \pm 0.1 BCb	0.6 \pm 0.2 Abc	0.3 \pm 0.08 Bc	0.7 \pm 0.08 Ab	0.5 \pm 0.07 ABbc
RR1	0.2 \pm 0.05 DEb	0.1 \pm 0.02 Eb	0.4 \pm 0.08 Aab	0.6 \pm 0.1 ABa	0.7 \pm 0.08 Aa	0.7 \pm 0.1 Aa
RR2	0.6 \pm 0.07 Ca	0.4 \pm 0.04 CDEa	0.5 \pm 0.09 Aa	0.5 \pm 0.09 ABa	0.6 \pm 0.1 Aa	0.7 \pm 0.08 Aa
RR3	0.1 \pm 0.2 Eb	0.3 \pm 0.02 DEab	0.5 \pm 0.07 Aa	0.3 \pm 0.05 Bab	0.2 \pm 0.03 Bab	0.3 \pm 0.04 BCab
Conv	0.5 \pm 0.1 CDab	0.4 \pm 0.1 CDEab	0.7 \pm 0.1 Aa	0.5 \pm 0.1 ABab	0.4 \pm 0.1 ABab	0.3 \pm 0.03 BCb

Averages followed by the same capital letter in the column do not differ statistically among each other by Tukey test [$P > 0.05$, $F = 6$, $CV = 49.71$ (SAS Institute, 2002)].

depending on the evaluation time (Table 4). Evaluations between 12 and 24 hours of exposure showed greater preference for *H. armigera* moths' landing. Treatments Bt7, Bt8 and Conv had lower averages of attracted individuals, while Bt6 was had the highest landing preference.

Through the attractiveness index of adult *H. armigera* obtained 6 hours after the release (Figure 4(a)) it was found attractiveness to most treatments, where only treatments Bt4, Bt7 and Bt8 were classified as repellents. After 12 hours the attractiveness index dropped, and only treatment Bt7 remained attractive when compared to the standard genotype (RR2) (Figure 4(b)).

After 24 hours after the release, treatments Bt5, Bt6, RR1, RR3 and Conv were rated as attractive, especially Bt6 and RR1 with a higher attractiveness index, being the other treatments classified as repellents (Figure 4(c)). For evaluation of the attractiveness in 48 hours, treatments Bt7, Bt8 and Conv were classified as repellents, while the others were attractive, especially treatment Bt3, due to its higher attractiveness index (Figure 4(d)).

3.4. Preference and Preference Index for Oviposition

There were significant differences among cultivars in all thirds of the evaluated plant for *H. armigera* oviposition (Table 5). For most of the studied cultivars, oviposition behavior was more concentrated on the upper third of the plant, except for Bt1 and Bt5 which obtained the highest average number of eggs on the middle third of the plant.

For treatments Bt1, Bt6 and RR3, the oviposition did not distinguish the parts of the plant, with no difference in the average among the lower, middle and

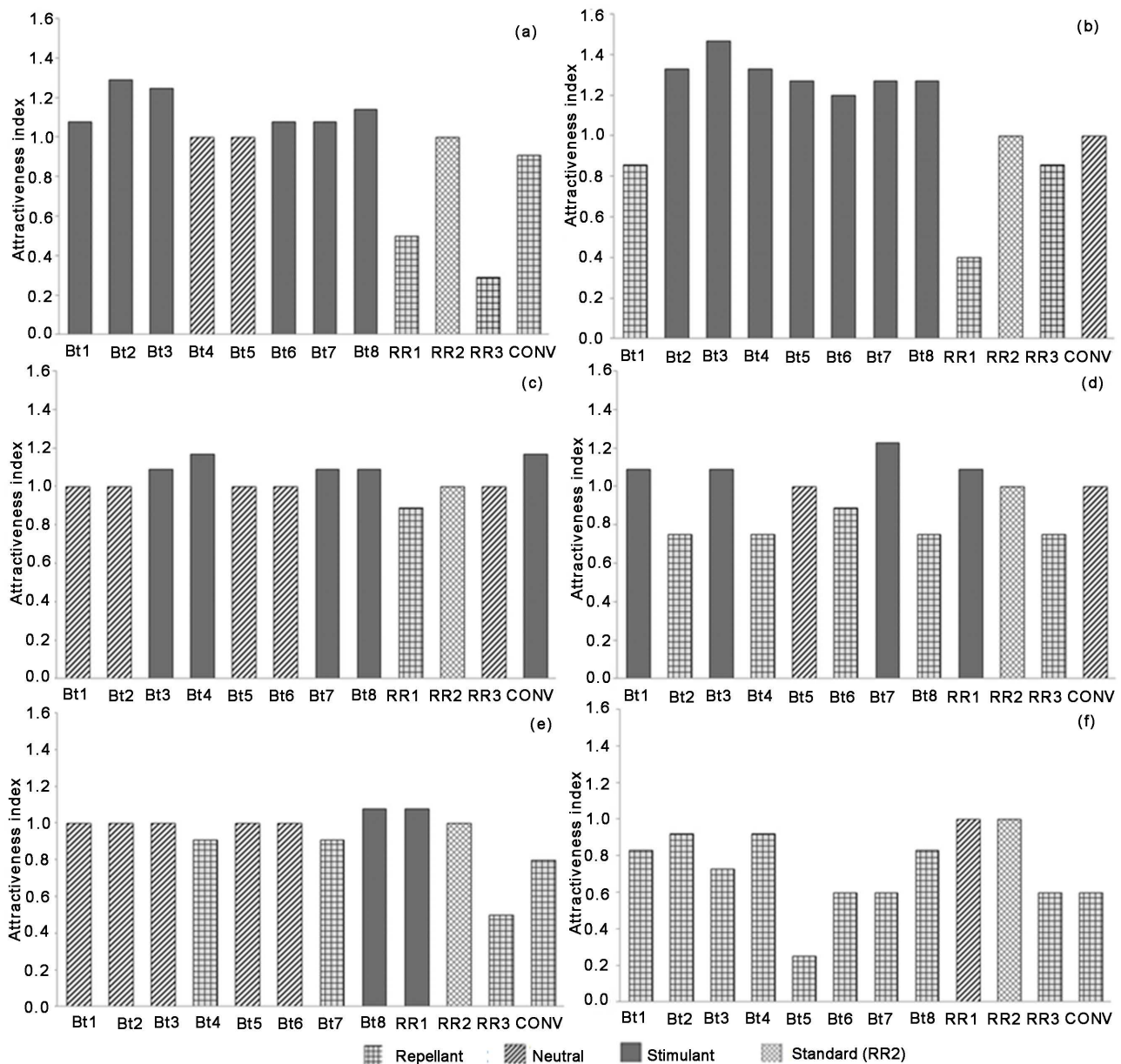


Figure 2. Attractiveness index of third instar *Helicoverpa armigera* caterpillars and soybean cultivars rating (a) 30 min; (b) 1 hour; (c) 2 hours; (d) 3 hours; (e) 6 hours; and (f) 12 hours after the start of no-choice test.

upper third of the plant. In contrast, Bt4, RR1 and RR2 were the treatments with the lowest egg average (Table 5).

The preference index for free-choice oviposition on the lower third of the plant (Figure 5(a)) reveals a contrast among the standard treatment (RR2) and treatments Bt3, Bt4, Bt5, RR1 and Conv, which were classified as deterrents. On the other hand, Bt1, Bt2, Bt6, Bt7, Bt8 and RR3 were considered stimulants to the oviposition of *H. armigera*, especially for Bt1, Bt2 and Bt6 which had higher preference indexes.

On the middle third, only treatment RR1 was classified as a deterrent, with the others considered stimulants when compared to the standard one (Figure 5(b)).

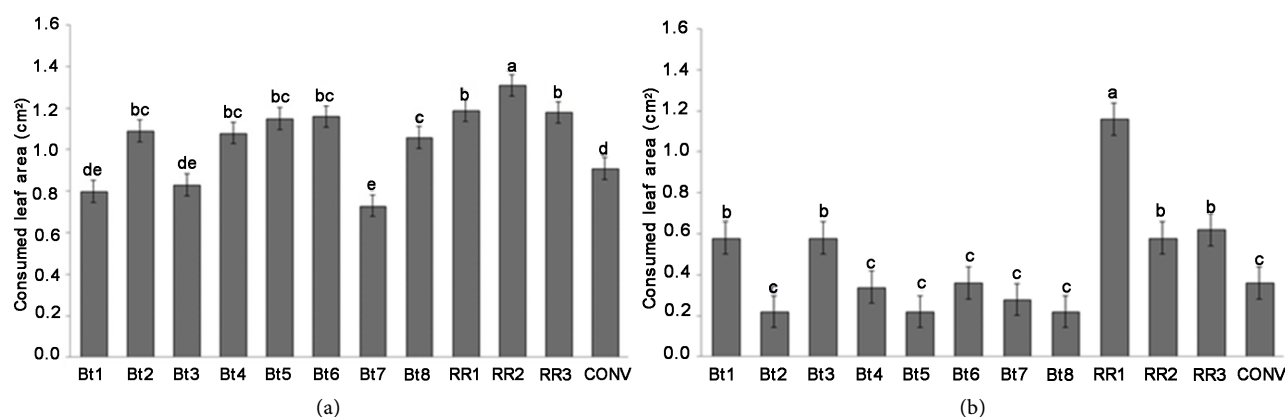


Figure 3. Consumed leaf area (cm²) by third instar *Helicoverpa armigera* caterpillars (a) Obtained in free-choice test; and (b) Obtained in no-choice test, in different soybean cultivars. Averages followed by the same letter do not differ statistically by Tukey test at 5% significance level.

Table 4. Average number (\pm SE) adult *Helicoverpa armigera* attracted in free-choice tests 30 min, 1, 6, 12, 24 and 48 hours after the release, for the different soybean cultivars in greenhouse.

CULTIVAR	Time (hours)				AVERAGE
	6	12	24	48	
Bt1	1 \pm 0 CD _b	1.5 \pm 0.04 BC _a	0.5 \pm 0.1 EF _c	0.5 \pm 0.04 B _c	0.88 \pm 0.11 CD
Bt2	1 \pm 0 CD _a	0.75 \pm 0.14 D _a	0.25 \pm 0.05 FG _b	0.25 \pm 0.03 BC _b	0.56 \pm 0.09 E
Bt3	1.75 \pm 0.14 A _a	1.25 \pm 0.1 BC _D _b	0 \pm 0 G _c	1.25 \pm 0.1 A _b	1.06 \pm 0.17 BC
Bt4	0.25 \pm 0.02 E _b	1 \pm 0 CD _a	0.5 \pm 0.1 EF _b	0.5 \pm 0.06 B _b	0.56 \pm 0.08 E
Bt5	0.75 \pm 0.1 D _b	1.25 \pm 0.10 BC _D _a	1 \pm 0 CD _a _b	0.25 \pm 0.05 BC _c	0.81 \pm 0.1 D
Bt6	1.25 \pm 0.03 BC _c	2.5 \pm 0.29 A _a	1.75 \pm 0.14 A _b	0.25 \pm 0.03 BC _d	1.44 \pm 0.22 A
Bt7	0.25 \pm 0.03 E _b	0.75 \pm 0.14 D _a	0 \pm 0 G _b	0 \pm 0 C _b	0.25 \pm 0.09 F
Bt8	0.25 \pm 0.02 E _a _b	0.25 \pm 0.05 E _a _b	0.5 \pm 0.1 EF _a	0 \pm 0 C _b	0.25 \pm 0.05 F
RR1	1.25 \pm 0.03 BC _b	1.75 \pm 0.25 B _a	1.5 \pm 0.10 AB _a _b	0.25 \pm 0.05 BC _c	1.19 \pm 0.16 B
RR2	0.75 \pm 0.10 D _b	1.75 \pm 0.14 B _a	0.75 \pm 0.14 DE _b	0 \pm 0 C _c	0.81 \pm 0.17 D
RR3	1.25 \pm 0.03 BC _a	1.25 \pm 0.14 BC _D _a	1 \pm 0.1 CD _a	0.25 \pm 0.05 BC _b	0.94 \pm 0.11 CD
Conv	1.5 \pm 0.04 A _a _b	1 \pm 0.2 CD _b	1.25 \pm 0.14 BC _a _b	0 \pm 0 C _c	0.94 \pm 0.16 CD

Averages followed by the same capital letter in the column and small letter in the row do not differ statistically among each other by Tukey test [$P > 0.05$, $F = 13$, $CV = 25$ (SAS Institute, 2002)].

On the upper third, all treatments were classified as stimulants in relation to the standard cultivar, with Bt6 and RR3 standing out due to their higher preference for oviposition index (**Figure 5(c)**).

In the preference index, considering the overall average in the whole plant, all treatments were classified as stimulants for oviposition, with Bt1, Bt6 and RR3 (**Figure 5(d)**) standing out for their higher preference indexes, while RR1 was the less stimulating treatment on the upper third of the plant and deterrent on the lower and middle thirds.

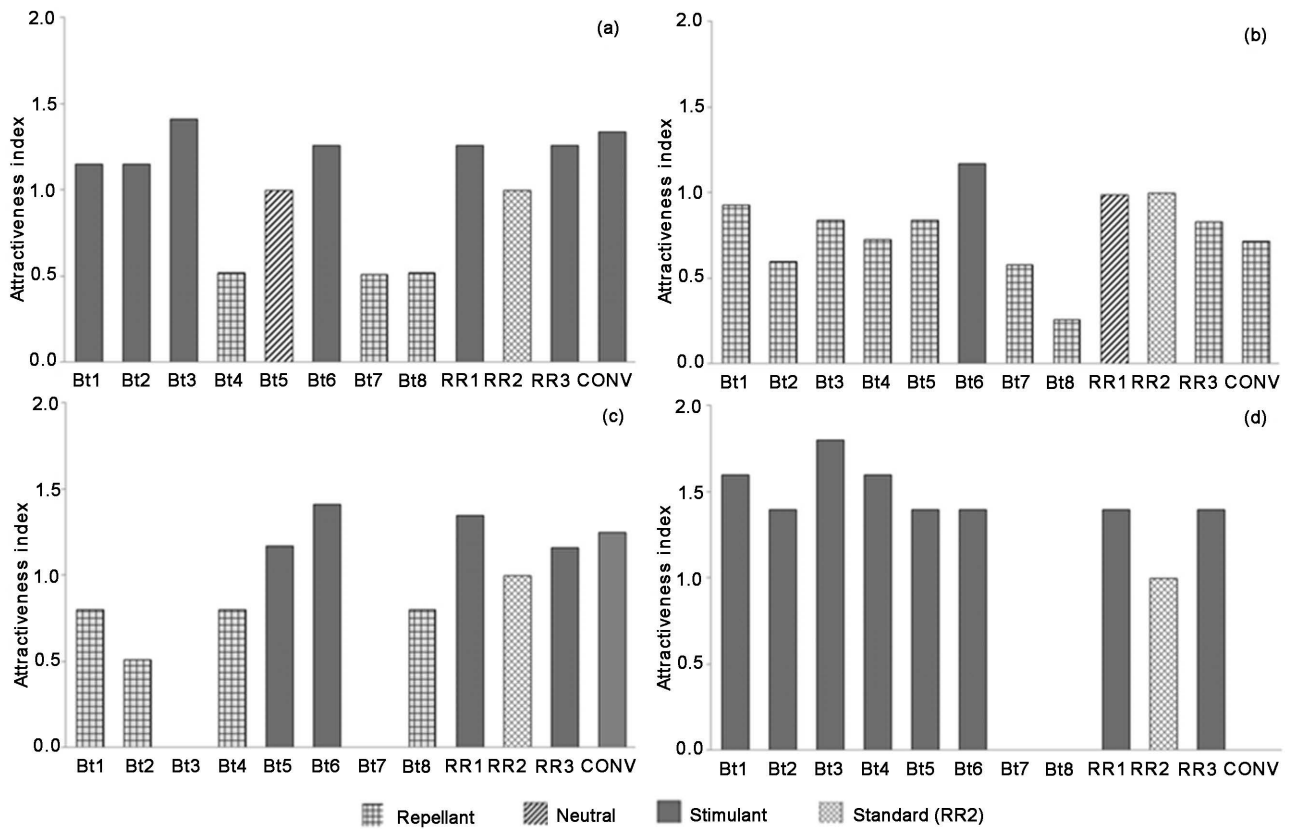


Figure 4. Adult *Helicoverpa armigera* attractiveness index and soybean cultivars rating, (a) 6 hour; (b) 12 hours; (c) 24 hours; and (d) 48 hours in free-choice tests in greenhouse.

Table 5. Average number (\pm SE) *Helicoverpa armigera* eggs obtained in free-choice tests after 72 hours from the release, for the different soybean cultivars in greenhouse.

CULTIVAR	Plant third			
	Lower	Middle	Higher	Total
Bt1	8.75 \pm 0.25 Ab	22 \pm 0.82 Aa	11.50 \pm 1.19 BCb	42.25 \pm 1.38 A
Bt2	5.5 \pm 0.28 Bb	6.25 \pm 0.48 Bb	12 \pm 0.41 BCa	23.75 \pm 0.63 BC
Bt3	0.5 \pm 0.04 Fc	4.75 \pm 0.48 Bb	12.75 \pm 1.03 Ba	18 \pm 1.47 C
Bt4	0.25 \pm 0.05 Fc	5.5 \pm 0.29 Bb	12.75 \pm 0.85 Ba	18.5 \pm 0.62 D
Bt5	0.25 \pm 0.02 Fc	16.25 \pm 0.48 Aa	12.75 \pm 0.48 Bb	29.25 \pm 0.81 B
Bt6	3.25 \pm 0.14 CDb	4.25 \pm 0.25 Bb	34.25 \pm 1.65 Aa	41.75 \pm 1.96 A
Bt7	1.75 \pm 0.14 DEFc	8.25 \pm 0.63 Bb	12 \pm 0.71 BCa	22 \pm 0.54 BC
Bt8	4 \pm 0.2 BCb	6.25 \pm 0.25 Bb	9.75 \pm 0.48 BCDa	20 \pm 0.68 C
RR1	0 \pm 0 Fb	2.25 \pm 0.25 Bb	7.75 \pm 0.25 CDa	9.25 \pm 0.41 D
RR2	1 \pm 0 EFb	2.75 \pm 0.25 Bab	5.75 \pm 0.48 Da	9.5 \pm 0.29 D
RR3	275 \pm 0.25 CDEb	5 \pm 0.41 Bb	34 \pm 0.82 Aa	41.75 \pm 0.63 A
CONV	0.75 \pm 0.14 EFc	6.75 \pm 0.48 Bb	11.75 \pm 0.95 BCa	19.25 \pm 0.92 C

Averages followed by the same capital letter in the column and small letter in the row do not differ statistically among each other by Tukey test at 5% significance level [$P > 0.05$, $F = 129$, $CV = 13.67$ (SAS Institute, 2002)].

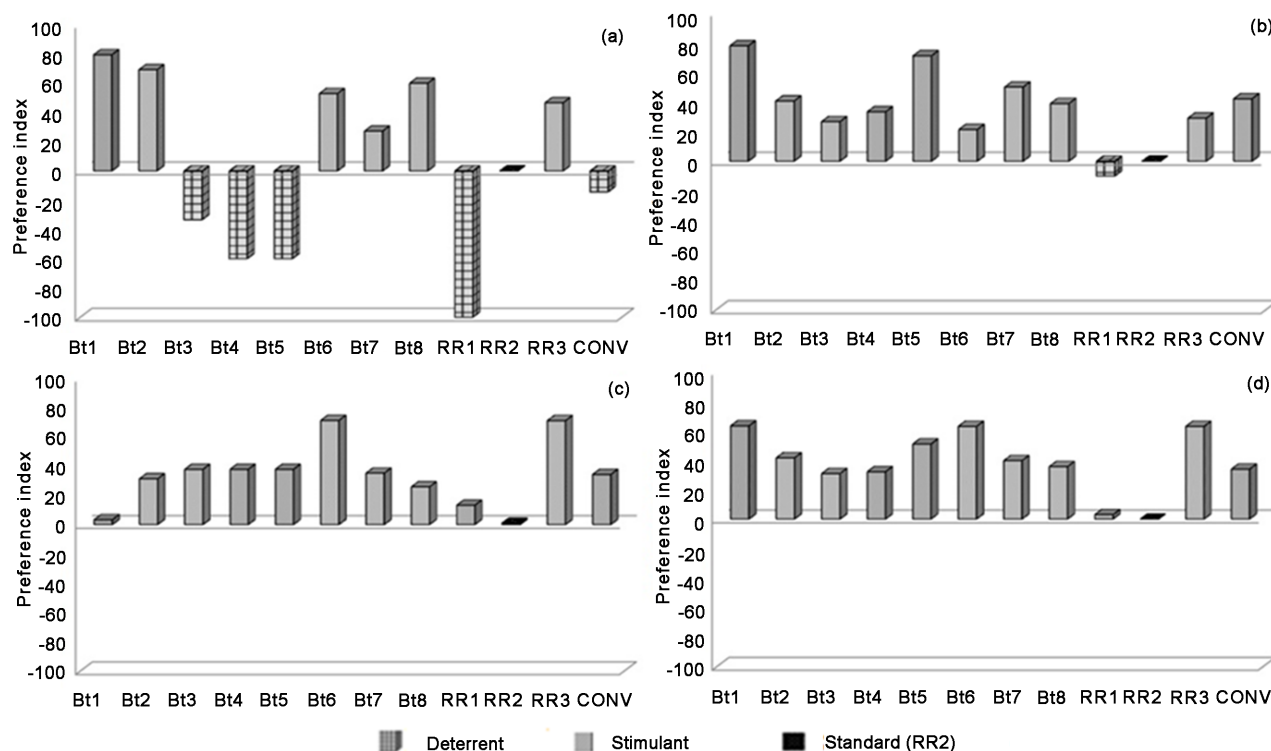


Figure 5. Preference index for *Helicoverpa armigera* and soybean cultivars classification on the (a) Lower third; (b) Middle third; (c) Upper thirds; and (d) All parts of the plant after 72 hours in free-choice test in the greenhouse.

4. Discussion

Third instar *H. armigera* caterpillars showed attractiveness in free and no-choice test for food between Bt and non-Bt soybean leaves when simultaneously contrasting the tested cultivars. With respect to an insect's feeding preference for a particular plant genotype, the responses vary in relation to the stimuli coming from the plant, which may be positive or negative, and of chemical (allelochemicals), physical (color) or morphological (hairiness, hardness, texture, thickness, structures dimensions) nature. It is important to highlight that both stimuli (positive and negative) are present in the plant, in a way that the insect response depends on what stimulus overpowers the other [25].

The attractiveness and feeding preference found for some cultivars for *H. armigera* caterpillars demonstrate that Cry1Ac toxin had no effect on the preference and feeding and that these results are related to the physical and nutritional factors of cultivars. Kumar and Saini [26] reported that total phenols, gossypol, tannin and potassium are negatively correlated, while total sugars, proteins and nitrogen are positively correlated with the infestation of *H. armigera* caterpillars on different cotton cultivars. Loung *et al.* [27] reported similar results for *H. armigera* first instar caterpillars on Bt and non-Bt cotton. However, in this same work, they found that after six days there was a low percentage of survival of caterpillars susceptible to Bt plants. As a practical implication, in spite of proving the efficiency of Bt plants, their use without due care regarding the rotation of Bt

cultivars, as well as the use of shelters can favor the resistance in the field due to the high selection pressure due to non-destination between Bt and non-Bt plants [28].

There was no difference between the attractiveness index for Bt and non-Bt treatments, demonstrating the plant resistance to *H. armigera*, and emphasizing the nutritional status of plants as a factor capable of influencing the attractiveness [29].

Yamasaki and Fujisaki [30] in studies on the *H. armigera* feeding preference and performance in *Cosmos bipinnatus* Cav. (Asteraceae) suggest that nutritional quality as well as substances such as secondary metabolites may have negative effects on larval development, which can influence the larval preference. It is also important to note that the presence of the compounds induced by herbivory becomes a constant in the chemical composition of the plant, insects can, over the generations, adapt to adverse conditions, developing defense mechanisms [25].

For Baghery *et al.* [31], nutritional indices are important factors that can determine plant resistance to insects. The analysis of feeding parameters, as well as their conversion, can help to understand the behavior of populations in the field [32], favoring the use of effective management.

According to the feeding behavior of *H. armigera*, there is no preference in leaf consumption between Bt plants and Bt not comparing bioassays with and without choice. It shows that *H. armigera* has no choice behavior between soybeans with and without Bt toxin, probably due to the rapid evolution of the resistance of *H. armigera* to *Bacillus thuringiensis* (Bt), this rapid evolution of resistance to Bt crops in several pests has reduced these benefits [33]. Rao and Rao [34] reported that third stage *H. armigera* larvae were repelled after the first bite of Bt plant.

For attractiveness and attractiveness for oviposition index of *Helicoverpa armigera*, there was variation in the choice for landing in time, however, there was no difference between Bt and non-Bt soybean treatments. Torres and Ruberson [35] found no difference in the landing preference of *Heliothis virescens* (Fabricius) and *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) between Bt and non-Bt cotton. Thus, maintaining the attractiveness to Bt plants favors the greatest number of caterpillars in the area, which demonstrates the potential use of genetically modified plants to keep balanced the insect-pest population.

Treatments Bt7 and Bt8 were considered repellants for *H. armigera*, suggesting that the physical and chemical factors act in different ways among cultivars and these factors are fundamentally important for the behavioral analysis of insects [36].

Chemical factors such as the volatile compounds are related to the location mechanisms of some species of moths. Tingle *et al.* [37] in studies using *H. virescens* moths, reported that they responded positively to the extracts of appropriate host plants and did not fly against the wind in response to the odor of a resistant tobacco cultivar and extracts of non-host plants, suggesting that specific

volatile compounds play a role in the positioning for landing and oviposition. Furthermore, after landing on a host plant, *H. virescens* was able to discriminate between host plants by chemicals on the leaf surface using chemoreceptors in the tarsal [30] [38].

The major volatile compounds capable of triggering antennal response in *H. armigera*, are alpha-pinene and beta-pinene, which are 5.5 and 2.85 times higher in Bt cotton than in non-Bt cotton, respectively. These compounds may induce greater preference in Bt plants than in non-Bt cotton in the field. In addition, tannins, which play an important role in resistance to arthropod pests in cotton, are also lower in Bt plants [39] [40].

For the study of oviposition, *H. armigera* moths were not able to differentiate the treatments containing the Bt protein, where treatment RR1 (non-Bt) showed less preference for oviposition. The preference of *H. armigera* for a specific host can be influenced by the physiological state of the plant, including age, type of feeding of the larvae and adults mating that can perceive differences in the quality of the plant by the nutrient requirements of the pest or differences in the levels of secondary compounds. The presence of certain phytochemicals in soybean cultivars as antixenotic agents or the absence of primary nutrients essential for growth and development of *H. armigera* can compromise their development, keeping the population below the economic damage level [41].

The high number of eggs in Bt cultivars may be related to the fact that plants containing the Bt protein promote the attractiveness produced by volatile compounds. The insertion of genes in Bt cotton plants is known for inducing significant changes in relevant secondary compounds related to plant-herbivores interactions [42]. Thus, it is important to study and know whether there is a change in the composition of secondary compounds capable of triggering a positive response in the plant-herbivore interaction.

The highest number of eggs in both Bt and non-Bt soy treatments occurred on the middle and upper thirds of the plant, justifying the habit of this species that prefers buds, flowers and pods. *H. armigera* eggs are generally concentrated on the upper third of cotton plants and most eggs are usually laid near plants terminals [43].

As time passes the physiological behavior of pests may change in relation to Bt cultivars, but it's also possible to occur a genetic evolution where the pest species can avoid the main host and prefer the non-Bt host which until then were alternative hosts, such as other crops or weeds [27] [44]. As resistance mechanism, female moths can also change their behavior and select parts of plants without or with low expression of toxins for oviposition. This change in behavior may allow them to avoid caterpillars to hatch in direct contact with high concentrations of toxins and provides greater survival and dispersal of species [45].

This study showed that Bt toxin did not influence the oviposition preference, *H. armigera* adults did not differentiate Bt and non-Bt soybean cultivars for oviposition indicating no behavioral preference. Third instar caterpillars showed no discrimination between Bt and non-Bt soybean leaves. Research in this area

must focus on the possibility of widespread planting of genetically modified soybeans containing the Bt protein, which is associated with selection pressure and the behavior of pest species in relation to their hosts, as well as on the adequacy of management tactics that are able to prevent the loss of technology efficiency.

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Appendix

Table S1. Description of soybean cultivars used for the study of preference of *Helicoverpa armigera*.

Cultivar	Genetic Transformation Event	Common Name	Scientific Name	N° Record
TMG 2183IPRO	MON 87701 × 89788	Soybean	<i>Glycinemax</i> (L.) Merr.	28,139
M 8210 IPRO	MON 87701 × 89788	Soybean	<i>Glycinemax</i> (L.) Merr.	28,137
M 8644 IPRO	MON 87701 × 89788	Soybean	<i>Glycinemax</i> (L.) Merr.	31,180
M 8372 IPRO	MON 87701 × 89788	Soybean	<i>Glycinemax</i> (L.) Merr.	31,185
AS 3820 IPRO	MON 87701 × 89788	Soybean	<i>Glycinemax</i> (L.) Merr.	29,751
M 8330 IPRO	MON 87701 × 89788	Soybean	<i>Glycinemax</i> (L.) Merr.	29,690
HK 8314 IPRO	MON 87701 × 89788	Soybean	<i>Glycinemax</i> (L.) Merr.	32,208
HK 8514 IPRO	MON 87701 × 89788	Soybean	<i>Glycinemax</i> (L.) Merr.	32,207
M 9144 RR	GTS-40-3-2	Soybean	<i>Glycinemax</i> (L.) Merr.	19,393
FTS CAMPO NOVORR	GTS-40-3-2	Soybean	<i>Glycinemax</i> (L.) Merr.	27,083
FTS PARAGOMINAS RR	GTS-40-3-2	Soybean	<i>Glycinemax</i> (L.) Merr.	29,772
M-SOY 9350	Convencional	Soybean	<i>Glycinemax</i> (L.) Merr.	02,675

Fonte: CTNBIO =

http://ctnbio.mcti.gov.br/liberacao-comercial/-/document_library_display/SqhWdohU4BvU/view/678023?_110_INSTANCE_SqhWdohU4BvU_redirect=http%3A%2F%2Fctnbio.mcti.gov.br%2Fliberacao-comercial



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