Fitness, acceptance and olfactory responses of Trichogramma pretiosum on eggs of Spodoptera frugiperda fed with Cry1Ac soybean

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Abstract

Spodoptera frugiperda (Smith) (Lepidoptera Noctuidae) is naturally tolerant to MON 87701 × MON 89788 soybean that expresses the Cry1Ac protein from Bacillus thuringiensis (Bt). Therefore, there are reports of outbreaks of this pest in fields where this technology has been cultivated. To support an environmental risk assessment, it is important to investigate the impacts of this technology on non-target organisms like the parasitoids of the genus Trichogramma, which can be used to manage this pest. In this study, we accessed: (i) the biology of a field population of S. frugiperda on MON 87701 × MON 89788 soybean; (ii) the impacts of the eggs produced by S. frugiperda fed with this soybean on the fitness and acceptance of the egg parasitoid Trichogramma pretiosum Riley (Hymenoptera Trichogrammatidae); and, (iii) the olfactory responses of this parasitoid to the volatiles of Bt and non-Bt soybean plants oviposited by S. frugiperda. The results of the biology of a field population of S. frugiperda when fed with Bt and non-Bt soybeans showed a similar total survival and cycle duration. No significant effects of the Bt soybean plants were observed in the life table parameters of S. frugiperda. Fitness and oviposition preference of T. pretiosum on eggs of S. frugiperda that fed with Bt and non-Bt soybeans were not different. Furthermore, the olfactory responses of this parasitoid to volatiles emitted by oviposited Bt and non-Bt soybeans were similar. These results suggest that there are no direct and indirect effects of S. frugiperda eggs fed with Bt soybean on the parasitoid fitness and acceptance, and also that T. pretiosum do not distinguish between Bt and non-Bt soybean plants oviposited by this pest. Therefore, this technology showed no adverse effects on T. pretiosum, which can help mitigate S. frugiperda outbreaks within an integrated pest management context.

Key words: fall armyworm, Glycine max, Bt, non-target, integrated pest management.

Introduction

The fall armyworm, Spodoptera frugiperda (Smith) (Lepidoptera Noctuidae), is native to the (sub)tropical regions of North and South America and has recently invaded the African and Asian continents (Goergen et al., 2016; Shylesha et al., 2018). It is a polyphagous pest species that causes significant damage in several economically important crops, including maize, soybean and cotton (Ashley et al., 1989; Barros et al., 2010; Casmuz et al., 2010). The use of genetically-modified maize and cotton varieties, which expresses Bacillus thuringiensis (Bt) genes, and insecticides are the main control strategies against S. frugiperda in Brazil (Bernardi et al., 2014a; Sorgatto et al., 2015; Leite et al., 2016; Burtet et al., 2017). The Bt soybean (MON 87701 × MON 89788 events), which expresses Cry1Ac protein, is commercially available in Brazil since 2013 (CTNBio, 2019). In the 2016/17 season, Bt soybean cultivation reached 59.8% of the total transgenic soybean cultivated in this country (Céleres, 2017). However, it is not effective against S. frugiperda, due to its natural tolerance to Cry1Ac protein (Bernardi et al., 2014b).

As part of an overall integrated pest management (IPM) strategy, Bt crops can contribute to more efficient biological control of both target and non-target pests. Studies have reported that the adoption of Bt crops leads to a reduction in insecticide use (Hutchison et al., 2010; Kouser and Qaim, 2011; Lu et al., 2012). This may favour non-target pests outbreaks, yet creates an environment supportive for biological control agents (Romeis et al., 2019). Most studies have shown no effects of the Cry1Ac protein on hymenopterans (Liu et al., 2005; Wang et al., 2017; Tian et al., 2018), or only an indirect effect caused by a reduction on the host’s quality (Ding et al., 2009). However, other studies showed that Bt proteins could be transmitted to predators (Meissle and Romeis, 2017) and that adults of S. frugiperda produced eggs containing the Cry1F protein when the larvae fed with a Bt maize that expresses this protein (Souza et al., 2018). Therefore, it is important to understand the direct and indirect impacts of Bt plants on both non-target pest species and their natural enemies.

Among the biological control agents of the genus Spodoptera, the egg parasitoid Trichogramma pretiosum Riley (Hymenoptera Trichogrammatidae) stands out for being reared easily (Hassan, 1993), and for the highly parasitic aggressiveness (Botelho et al., 1997). Moreover, in soybeans, this is the most commonly found Trichogramma species. Therefore, its use for applied biological control in this crop is likely to be implemented (Hohmann et al., 1989; Hilbeck and Andow, 2004). Nonetheless, there is no information regarding to the effects on the fitness and acceptance of this parasitoid on S. frugiperda eggs fed with MON 87701 × MON 89788 soybean. Furthermore, the preference of the parasitoid for Bt or non-Bt oviposited soybean plants odours is unknown.

Parasitoids of herbivore eggs have evolved responses to the changes in plant chemistry caused by herbivore oviposition in order to successfully find their hosts (Hilker and Meiners, 2010). Although none of the insect-resistance genes presently employed in transgenic plants expresses volatile compounds, the introduction of a foreign gene construct could conceivably lead to changes in
a plant’s volatile profile by a pleiotropic effect or inser- 
tional mutagenesis, especially as there is currently no 
control over where genes are inserted into the crop ge-
nome (Maessen, 1997). Such changes could interfere 
with host-habitat location by parasitoids. This is im-
portant considering that parasitoids should not distin-
guish between volatiles emitted by Bt and non-Bt plants.

In this scenario, where MON 87701 × MON 89788 soy-
bean is not effective against S. frugiperda, the use of 
T. pretiosum may be a feasible strategy to implement 
IPM programs in this crop. Therefore, it is of theoretical 
and practical interest to understand the impact of Bt soy-
bean on this egg parasitoid. In this context, we inves-
tigated the biology of S. frugiperda on MON 87701 × 
MON 89788 soybean, the impacts of the eggs produced 
by S. frugiperda fed with this soybean on the fitness and 
acceptance of T. pretiosum, and the olfactory responses 
of this parasitoid to the volatiles of oviposited Bt and 
non-Bt soybean plants. Data generated from the study 
should be useful in refining S. frugiperda management 
strategies on soybean crops.

Materials and methods

Insects’ sources and maintenance

S. frugiperda population used in all bioassays was col-
lected in Rondonópolis, Mato Grosso do Sul, Brazil 
(16°28’17”S 54°38’14”W) on February 2019, and was 
provided by the National Research Center of Maize and Sor-
ghum (Embrapa Milho & Sorgo, Sete Lagoas, MG, Bra-
zil). T. pretiosum population was maintained in the labor-
atory of Biology, Ecology and Biological Control (Bio-
colab) at Federal University of Rio Grande do Sul since 
2014. S. frugiperda was reared in the Bioecolab according 
to Parra (2001) under controlled conditions (26 ± 2 °C, 
65 ± 10% RH, and 14L:10D photoperiod). T. pretiosum 
was reared in the Bioecolab according to Parra and Zuc-
chi (1997) under controlled conditions (25 ± 1 °C, 
70 ± 10% RH, and 14L:10D photoperiod).

Biological of S. frugiperda on Bt and non-Bt soybeans

Soybean cultivars used in this study, Syn13671 IPRO 
(MON 87701 × MON 897788), which express the Cry1Ac 
protein, and BRS 7380 RR (a non-Bt isolate), were pro-
vided by the National Research Center of Maize and Sor-
ghum (Embrapa Milho & Sorgo, Sete Lagoas, MG, Bra-
zil). Bt and non-Bt soybean seeds were sown weekly in 
11 litres plastic pots in a greenhouse. The cultivation 
practices used were as recommended for soybean in the 
region (Santos et al., 2008) without any pesticide appli-
cation and with mechanical weed control. This bioassay 
was carried out with soybean plants at the V7 develop-
mental stage (Fehr and Caviness, 1977). Soybean leaves 
were excised, taken to the laboratory, washed with hypo-
chlorite (5%) for 15 minutes and, after drying, cut into 
pieces of approximately 4 cm². Afterwards, soybean 
pieces were placed on a non-gelled mixture of water-agar 
2.0% in plastic plates with 32 cells (Advento do Brasil, 
São Paulo, Brazil). Leaf pieces were separated from the 
water-agar layer by a piece of filter paper. One neonate 
larvae (< 24 hours old) was placed per cell on each 
soybean leaf-piece using a fine brush ( nº 000). Plates 
were sealed with plastic lids and placed in climatic cham-
ber (26 ± 2 °C, 65 ± 10% RH, and 14L:10D photoperiod). 
The experimental design was completely randomized 
with eight replicates per treatment (Bt and non-Bt soy-
beans); each replicate consisted of 16 neonate larvae for 
a total of 128 neonate larvae tested per treatment. The 
soybean leaves were replaced every two days. Pupae 
were collected, placed on trays with filter paper, and iso-
lated using plastic cups (50 mL). To evaluate longevity 
of adults and female fecundity, when adults emerged, 20 
couples from each treatment were formed and 12 that 
were fertile and that adults did not escape were selected 
for statistical analysis. These couples were individualized 
in 500 mL plastic cups, turned upside down on filter pa-
per, and were fed with a solution of 10% honey provided 
on cotton. To determine the embryonic period and viabil-
ity, eggs were obtained from the second oviposition of 
each pair. Eggs were placed into glass tubes with flat bot-
toms (8.5 × 2.5 cm). A piece of filter paper (2 × 1 cm) 
moistened with distilled water daily was placed inside 
the tube, which was closed at the top with plastic film. Eggs 
and number of larvae hatched were counted daily. For 
each treatment, the following biological parameters were 
evaluated: duration and survival rates of egg, larval and 
pupal periods; total cycle duration and survival (egg to 
adult); larval weight 14 days after infestation; pupae 
weight (< 24 hours old); sex ratio; adults longevity; and 
female fecundity (eggs/female) and fertility. Eggs, egg 
viability and duration of egg, larval and pupal periods 
and total cycle were determined in daily observations. Data 
were assessed for normality and homogeneity of variance 
(Proc MIXED followed by Proc UNIVARIATE and Proc 
GPILOT) (SAS, 2011). Data on Bt and non-Bt soybeans, 
when normally distributed were compared by t-test 
( p < 0.05) (Proc TTEST) (SAS, 2011). Nonparametric 
data were submitted to Kruskal-Wallis test ( p < 0.05) 
(Breslow, 1970) in R 2.15.1 (R Development Core Team, 
2012). The putative deviation in the sex ratio was com-
pared using the Chi-square test (χ²) ( p < 0.05) (Proc 
FREQ) (SAS, 2011). A life table was calculated by esti-
mating the mean generation time (T), the net reproductive 
rate (R₀), the intrinsic rate of increase (rᵢ₀) and the finite 
rate of increase (λ). The life table parameters were esti-
ated by the “jackknife” method using “Lifetable.sas” 
(Maia et al., 2000) and compared using a bilateral t-test 
( p < 0.05) (SAS, 2011).

T. pretiosum fitness and acceptance on eggs of 
S. frugiperda fed with Bt and non-Bt soybeans

This study was conducted in a completely randomized 
experimental design with two treatments (eggs from 
S. frugiperda that were fed with Bt soybean and non-Bt 
soybean) and five replicates of five female parasitoids 
totaling 25 parasitoids tested/treatment), with and with-
out choice. Eggs used in this bioassay came from the cou-
ples of the previous bioassay. T. pretiosum mated females 
(1 day old) were individualized in glass tubes with flat 
bottoms (8.5 × 2.5 cm) containing a droplet of pure honey 
as food source. For the test without choice, 40 S. frugi-
perda eggs (< 24 hours old) were offered for each parasi-
toid for 3 hours (Vargas et al., 2017) from each treatment
separately. Eggs offered to the parasitoids were fixed on blue sulphite paper cards (8.0 × 2.0 cm) using water-diluted Arabic gum (10%). After exposure to the parasitoids, the cards were transferred to new glass tubes and kept in climatic chambers (25 ± 2 °C, 70 ± 10% RH, and 14L:10D photoperiod) until the emergence of T. pretiosum adults or eclosion of S. frugiperda larvae. Larvae that emerged were removed daily. Parental females were also kept in the same climate chamber for daily observation and longevity record. The biological parameters assessed were: longevity of parental females; total cycle duration (egg to adult); percentage of S. frugiperda parasitized eggs; parasitoid viability (from the parasitized eggs) and sex ratio. For the test with choice, 40 S. frugiperda eggs (< 24 hours old) were offered for each parasitoid. However, 20 eggs were from S. frugiperda that were fed Bt soybean, and the other 20 eggs were from S. frugiperda that were fed non-Bt soybean. The protocol used was the same described above for the without choice test, and the two cards with the eggs were placed opposing each other. Although, only percentage of S. frugiperda parasitized eggs and parasitoid viability (from the parasitized eggs) were evaluated. Data were assessed for normality and homogeneity of variance (Proc MIXED followed by Proc UNIVARIATE and Proc GLOG) (SAS, 2011). Data on Bt and non-Bt soybeans, when normally distributed were compared by t-test (p < 0.05) (Proc TTEST) (SAS, 2011). Nonparametric data were submitted to Kruskal-Wallis test (p < 0.05) (Breslow, 1970) in R 2.15.1 (R Development Core Team, 2012). The putative deviation in the sex ratio was compared using the Chi-square test (χ²) (p < 0.05) (Proc FREQ) (SAS, 2011).

Olfactory response of T. pretiosum

Olfactory bioassays were performed with T. pretiosum females (1 day old) individuals without experience on hosts’ eggs or plants volatiles. We used the methodology described by Pehaflor et al. (2011a) with some modifications. A Y-tube olfactometer was used to determine the parasitoid preference, either between two different odour sources or one source and a blank (clean air). Bioassays with the following combinations were carried out: (i) oviposited Bt soybean plants versus oviposited non-Bt soybean plants; (ii) oviposited Bt soybean plants versus non-oviposited Bt soybean plants (positive control); (iii) oviposited non-Bt soybean plants versus non-oviposited non-Bt soybean plants (positive control); (iv) oviposited Bt soybean plants versus clean air (negative control); (v) oviposited non-Bt soybean plants versus clean air (negative control). The soybean cultivars used were the same as those described above, however, in stages V1-V2 (Fehr and Caviness, 1977). The plants were sown weekly in 400 mL plastic cups (1 plant/cup) and were maintained in climatic chamber (26 ± 2 °C, 65 ± 10% RH, and 14L:10D photoperiod). The cultivation practices used were as recommended for soybean in the region (Santos et al., 2008) without any pesticide application. The plants (Bt soybean and non-Bt soybean) were offered separately to 10 pairs of S. frugiperda, inside cages made with voile fabric (50 × 30 × 30 cm). Those that have one to two postures (at least 200 eggs each posture) on the day after exposure were removed from the cage. After 24 or 48 hours of contact with the eggs, the plants were used in the bioassays. For this purpose, the soil of each plant was covered with aluminum foil. All bioassays were conducted in the laboratory at the olfactometer room (25 ± 1 °C, 70 ± 10% RH, and incandescent light on) during day time, between 10:00 and 17:00. The Y-tube olfactometer consisted of a bifurcated glass tube (2 cm internal diameter, 10 cm stem length, 8 cm arms length and 50° angle). The odours sources were placed inside glass bottles of 3 L (12.5 cm diameter × 29.5 cm height), which were connected to the extremities of the olfactometer. From the main arm of the olfactometer, a tube from a vacuum pump was connected, and the air from the environment was humidified and purified with the use of activated carbon before pulling it through the system. The air flow was adjusted to 600 mL/min using a calibrated flowmeter connected to the vacuum pump. Insects then were positioned individually at the beginning of the central arm of the Y-tube and observed for 5 minutes. When the parasitoids crossed the threshold line (located in the middle of each arm) and stayed in the arm for at least 1 minute, this was considered as “choice”. Only insects that made a choice for one arm within the 5 minutes were considered for statistical analysis. An insect that did not choose either of the arms within 5 minutes was recorded as non-responsive. Each parasitoid was used only a single time to prevent associative learning. To avoid any bias, the Y-tube was alternated, and odour sources were connected to the opposite arm after every four parasitoids tested, while the respective plant materials were replaced after eight parasitoids tested. Also, when plants were replaced, the olfactometer was disassembled and all glassware was washed with neutral dishwashing soap (v/v 5%), distilled water, and alcohol (v/v 70%). Forty responsive female parasitoids were tested per treatment. Frequency count data were subjected to Chi-square (χ²) goodness-of-fit test (p < 0.05) (Proc FREQ) (SAS, 2011).

Results

Biology of S. frugiperda on Bt and non-Bt soybeans

There was no significant difference in the duration of all life stages of S. frugiperda fed with Bt soybean and non-Bt soybean (p > 0.05) (figure 1a). In addition, there was no difference in the survival of the stages evaluated, except for larval survival, which was significantly higher in non-Bt soybean (80%) than in Bt soybean (62%) (χ² = 9.23; df = 1; p = 0.0023) (figure 1b). However, this difference at the larval survival did not affect the total (egg-adult) survival (χ² = 0.92; df = 1; p = 0.3359) (figure 1b).

Feeding on Bt soybean caused no reduction in the 14-day larval weight compared to the weight of larvae that fed with non-Bt soybean (table 1). This reflected on the mean pupal weight, which also did not differ. The sex ratio, female and male longevity and female fecundity did not differ between treatments. Furthermore, feeding on Bt soybean did not affect any life table parameters compared to non-Bt soybean (table 1).
Figure 1. Duration (a) and survival (b) rates of life stages of *S. frugiperda* fed with Bt (MON 87701 × MON 89788) soybean and non-Bt soybean. Pairs of columns with the same letters are not significantly different by *t*-test or Kruskal-Wallis test (*p* < 0.05).

Table 1. Biological parameters and fertility life table (means ± SE) of *S. frugiperda* fed on Bt (MON 87701 × MON 89788) soybean and a non-Bt soybean.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bt soybean</th>
<th>Non-Bt soybean</th>
<th><em>P</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larval weight (mg)</td>
<td>443.94 ± 15.55</td>
<td>481.24 ± 21.07</td>
<td>0.1763</td>
</tr>
<tr>
<td>Pupae weight (mg)</td>
<td>198.98 ± 2.72</td>
<td>203.58 ± 2.34</td>
<td>0.2214</td>
</tr>
<tr>
<td>Sex ratio</td>
<td>0.46*</td>
<td>0.48*</td>
<td>0.9246</td>
</tr>
<tr>
<td>Adult female longevity (days)</td>
<td>12.66 ± 1.16</td>
<td>13.33 ± 1.27</td>
<td>0.7059</td>
</tr>
<tr>
<td>Adult male longevity (days)</td>
<td>13.25 ± 1.13</td>
<td>14.16 ± 1.02</td>
<td>0.5558</td>
</tr>
<tr>
<td>Fecundity (eggs/female)</td>
<td>1794.25 ± 140.23</td>
<td>1716.16 ± 198.57</td>
<td>0.7511</td>
</tr>
<tr>
<td><em>T</em> (days)</td>
<td>36.79 ± 0.12</td>
<td>36.54 ± 0.13</td>
<td>0.1923</td>
</tr>
<tr>
<td><em>R</em>&lt;sub&gt;0&lt;/sub&gt;</td>
<td>404.42 ± 31.60</td>
<td>378.93 ± 43.84</td>
<td>0.6422</td>
</tr>
<tr>
<td><em>r</em>&lt;sub&gt;m&lt;/sub&gt;</td>
<td>0.163 ± 0.002</td>
<td>0.163 ± 0.003</td>
<td>0.8793</td>
</tr>
<tr>
<td><em>λ</em></td>
<td>1.17 ± 0.002</td>
<td>1.17 ± 0.003</td>
<td>0.8878</td>
</tr>
</tbody>
</table>

A separate *t*-test or Kruskal-Wallis test (*p* < 0.05) was conducted between Bt and non-Bt soybeans for each biological parameter. *T* = mean generation time; *R*<sub>0</sub> = net reproductive rate; *r*<sub>m</sub> = intrinsic rate of increase, and *λ* = finite rate of increase. * Data were not significantly different based on a Chi-square test (*χ*<sup>2</sup>) (*p* < 0.05).

Table 2. Biological parameters (means ± SE) of *T. pretiosum* in eggs from *S. frugiperda* that fed with Bt (MON 87701 × MON 89788) soybean and non-Bt soybean, in tests with and without choice.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bt soybean</th>
<th>Non-Bt soybean</th>
<th><em>P</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without choice test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parental female longevity (days)</td>
<td>9.44 ± 0.70</td>
<td>8.35 ± 0.78</td>
<td>0.1820</td>
</tr>
<tr>
<td>Egg-adult (days)</td>
<td>9.48 ± 0.14</td>
<td>9.72 ± 0.13</td>
<td>0.2961</td>
</tr>
<tr>
<td>Parasitized eggs (%)</td>
<td>77.40 ± 2.87</td>
<td>75.90 ± 2.55</td>
<td>0.8542</td>
</tr>
<tr>
<td>Parasitoid viability (%)</td>
<td>98.06 ± 0.58</td>
<td>98.04 ± 0.73</td>
<td>0.7221</td>
</tr>
<tr>
<td>Sex ratio</td>
<td>0.62*</td>
<td>0.66*</td>
<td>0.8957</td>
</tr>
<tr>
<td>With choice test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parasitized eggs (%)</td>
<td>73.80 ± 4.20</td>
<td>75.20 ± 4.12</td>
<td>0.8130</td>
</tr>
<tr>
<td>Parasitoid viability (%)</td>
<td>96.68 ± 0.64</td>
<td>97.89 ± 1.09</td>
<td>0.5401</td>
</tr>
</tbody>
</table>

A separate *t*-test or Kruskal-Wallis test (*p* < 0.05) was conducted between Bt and non-Bt soybeans for each biological parameter. * Data were not significantly different based on a Chi-square test (*χ*<sup>2</sup>) (*p* < 0.05).

*T. pretiosum* fitness and acceptance on eggs of *S. frugiperda* fed with Bt and non-Bt soybeans

The percentage of *S. frugiperda* eggs parasitized by *T. pretiosum* and the parasitoids viability did not differ between Bt and non-Bt soybeans, in both with and without choice tests (table 2). Similarly, there was no difference between treatments for the sex ratio and parental longevity and total cycle (egg-adult) duration evaluated in the without choice test.
Figure 2. Olfactory response of the egg parasitoid *T. pretiosum* to volatiles emitted by oviposited Bt soybean (Bt_Ov), oviposited non-Bt soybean (Non-Bt_Ov), non-oviposited Bt soybean (Bt), non-oviposited non-Bt soybean (Non-Bt) and clean air after 24 and 48 hours of *S. frugiperda* oviposition. The numbers inside the bars are the total numbers of *T. pretiosum* that responded to each treatment. * Significant at 5% according to Chi-square ($\chi^2$) goodness-of-fit test; NR = non-respondive parasitoids.

Olfactory response of *T. pretiosum*

Female *T. pretiosum* parasitoids showed a significant preference for odours emitted by oviposited Bt soybean (24 hours: $\chi^2 = 33.8$, df = 1; $p < 0.0001$; 48 hours: $\chi^2 = 28.8$, df = 1; $p < 0.0001$) and oviposited non-Bt soybean (24 hours: $\chi^2 = 28.8$, df = 1; $p < 0.0001$; 48 hours: $\chi^2 = 45.0$, df = 1; $p < 0.0001$) in contrast with clean air after 24 hours and 48 hours of oviposition contact (figure 2). *T. pretiosum* preferred oviposited Bt soybean over non-oviposited Bt soybean - 24 hours: $\chi^2 = 24.2$, df = 1; $p < 0.0001$; 48 hours: $\chi^2 = 20.0$, df = 1; $p < 0.0001$; and oviposited non-Bt soybean over non-oviposited non-Bt soybean - 24 hours: $\chi^2 = 24.2$, df = 1; $p < 0.0001$; 48 hours: $\chi^2 = 16.2$, df = 1; $p < 0.0001$. However, *T. pretiosum* did not distinguish between the odours of oviposited Bt soybean or oviposited non-Bt soybean (24 hours: $\chi^2 = 0.8$, df = 1; $p = 0.3711$; 48 hours: $\chi^2 = 0.2$, df = 1; $p = 0.6547$).

Discussion and conclusions

Our results showed a low level of activity of the Cry1Ac protein against a field population of *S. frugiperda*. The population used in our study was collected in the same geographic region, in the central region of Brazil (Rondonópolis, MS, Brazil), as the one collected by Bernardi et al. (2014b). However, these authors collected their *S. frugiperda* population in 2008, while we collected ours in 2019, six years after the MON 87701 × MON 89788 soybean was commercially available (CTNBio, 2019). Our population had a larval survival of 62%, while Bernardi et al. (2014b) population had a larval survival of 37% at that time, a 25% increment. Moreover, these authors found a lower total survival (egg to adult) on Bt soybean compared to non-Bt soybean, with less than 27% of the insects reaching the adult stage in the former. In our study, despite the higher larval survival, overall survival was similar between *S. frugiperda* fed with Bt (48%) and non-Bt (52%) soybeans. The other parameters evaluated in our study for *S. frugiperda* were not different between Bt and non-Bt. It is worthy to notice that we observed higher fecundity in our population on Bt and non-Bt soybeans, compared to the ones observed by Bernardi et al. (2014b). In addition, the biological parameters of the life table were similar to those reported by these authors in non-Bt soybean, except for the net reproduction rate ($R_0$) which was 7.5-fold higher on Bt soybean and 1.3-fold on non-Bt soybean in our study. Therefore, population growth on Bt soybean plants could be similar to non-Bt soybean plants in the field.

Studies have shown that there is cross-resistance among Cry1F, Cry1Ab, Cry1Ac and Cry1A.105 proteins in *S. frugiperda* (Bernardi et al., 2015; Santos-Amaya et al., 2015; Burret et al., 2017). In fact, *S. frugiperda* is the first target pest that has developed field-evolved resistance with control problems to Bt crops in multiple areas across different countries and continents (Dangal and Huang, 2015). In Brazil, field-evolved resistance in this species is reported for Cry1Ab and Cry1F proteins (Leite et al., 2016; Omoto et al., 2016). *S. frugiperda* is constantly being exposed to Cry1 proteins expressed in maize (Cry1Ab, Cry1A.105 and Cry1F), soybean (Cry1Ac) and cotton (Cry1Ac and Cry1Ab) (Bernardi et al., 2014b). In the central region in Brazil the winter season is dry and hot, but the use of irrigation has allowed maize, cotton and soybean production during the entire year without a break. This has enabled *S. frugiperda* to have overlapping generations throughout the year and exacerbated this pest problem (Farias et al., 2014). Thus, the increased survival observed in our population may be caused by cross-resistance among Cry1 proteins and constantly selection pressure.

Within the IPM context, alternative strategies are necessary to control *S. frugiperda*. Our results showed that *T. pretiosum* could be an excellent candidate to be used for applied biological control in Bt and non-Bt soybean.
areas. No impact was observed in our study with regard to this parasitoid. Similar results were obtained by Bortolotto et al. (2014), when they evaluated biological parameters of Telenomus remus Nixon (Hymenoptera Platygastriidae) on an non-susceptible Bt soybean host, Spodoptera eridania (Cramer) (Lepidoptera Noctuidae). Indirect effects could have been observed caused by the host (eggs) quality, because we used a host that was not highly tolerant (survival of 62%) (Shelton et al., 2016). Lower quality of the eggs of Helicoverpa armigera (Hubner) (Lepidoptera Noctuidae), that survived to the exposure to Bt maize, reduced the parasitism success of Trichogramma brassicae Bezdenko (Hymenoptera Trichogrammatidae) (Steinbrecher, 2004). However, it seems that the Cry1Ac soybean ingested by S. frugiperda did not change the quality of its eggs, which did not harm the fitness and acceptance of the parasitoid.

Although Souza et al. (2018) found the Cry1F protein in eggs of S. frugiperda, when this species fed with Cry1F maize, the detection may have occurred of traces of the processed protein, which might have no effect on a natural enemy. No studies of Cry1Ac detection on eggs have yet been done, but protein traces are likely to be detected as well. Therefore, T. pretiosum exposure to Cry1Ac would be zero or very low in our study and direct effects can be excluded. Also, no direct effect on the biology of parasitoids of the genus Trichogramma was detected when feeding of pollen suspensions containing Bt proteins and Bt isolates suspensions (Wang et al., 2007; Santos et al., 2011). One of the most relevant attributes of the Bt protein-based insecticidal technologies is their specificity (Jurat-Fuentes and Critchmone, 2017). Cry1 family protein is well known to be Lepidoptera-active (Frankenhuyzen, 2009). Although the Cry1Aa protein also has activity against some Diptera, there is none against Hymenoptera (Frankenhuyzen, 2009). This may be the main reason why there was a lack of a direct detrimental effect on T. pretiosum if the Cry1Ac or its traces are present into S. frugiperda eggs.

Our study demonstrated that T. pretiosum preferred soybean plants that had been oviposited regardless of whether they were Bt or not. According to Peñaflor et al. (2011b), egg deposition should be investigated prior to herbivory in studies on induced plant volatiles, because in a natural situation, oviposition usually precedes feeding. Deposition of insect eggs can induce the production of volatiles or change leaf chemistry in a way that the plants attract and/or arrest certain egg parasitoids (Fatouros et al., 2005; Bruce et al., 2009; Tamiru et al., 2011). Furthermore, emissions of induced plant volatiles can change over time (Aljbory and Chen, 2018). For soybean plants, Michereff et al. (2011) showed that the amount of two main volatile compounds were higher after 48 hours of Eusichistes heros (F.) (Hemiptera Pentatomidae) oviposition compared to 24 hours. However, in our tests, the responses of T. pretiosum were similar in both times tested (24 and 48 hours after oviposition), showing that volatiles’ emissions by S. frugiperda oviposition on Bt and non-Bt soybean plants might be similar in quantity and quality even after 48 hours of oviposition contact.

Apart from oviposition-induced plant volatiles, the orientation of egg parasitoids towards egg-derived odours might be an effective alternative strategy for host location (Vinson, 1998). However, our results showed that the chemical composition of the eggs might not have changed due to the feeding of S. frugiperda on Bt and non-Bt soybeans. This could have happened because de composition of a transgenic Bt plant and the corresponding non-transformed plant are likely to differ to some extent due to genetic differences between them (Motavalli et al., 2004). Several steps of conventional breeding are required to introduce the Bt trait into the non-Bt plant after transformation (Zurbrügg et al., 2010). As a consequence, transgenic (MON 8701 × MON 89788) soybeans had higher levels of carbohydrates and lower levels of proteins (Berman et al., 2010). Though, when a transgene is inserted into a plant, the inserted gene and the regions that flank the insertion site are sequenced and characterized to avoid that host genes or regulatory elements are present is close proximity to the transgene (Prado et al., 2014). Studies with Bt plants showed that their volatiles emissions seem to be not different to non-Bt plants’ volatiles, corroborating to our results. For example, Dean and De Moraes (2006) compared herbivore-induced volatiles emissions from Bt to non-Bt maize by Helicoverpa zea Boddie (Lepidoptera Noctuidae) damage. These authors found that changes in the volatile profiles of Bt maize were due to altered larval feeding behaviour rather than of changes in biochemical plant defense pathways. Similarly, the parasitic wasp Cotesia plutellae (Kurdjumov) (Hymenoptera Braconidae) was found to be equally attracted to Bt oilseed rape plants equally damaged by Bt-resistant herbivores, suggesting no change in the composition of volatiles produced by Bt and non-Bt plants (Schuler et al., 1999).

In summary, we conclude that MON 87701 × MON 89788 soybean effects on a field population of S. frugiperda biology are small, and that there is no adverse effects of this technology on the egg parasitoid T. pretiosum. In addition, this parasitoid does not discriminate between eggs from S. frugiperda fed with Bt and non-Bt soybeans and oviposited Bt and non-Bt soybean plants. Our results are promising, since there are evidences that the biological control of S. frugiperda by T. pretiosum in Bt soybean crops can be as effective as in non-Bt soybeans. This is important, since the use of Bt plants facilitates the integration of biological control into IPM programs and favours more sustainable farming practices. T. pretiosum has difficulties in parasitizing S. frugiperda egg masses because they are covered in scales and the eggs are deposited in layers (Toonders and Sánchez, 1987; Cortez and Trujillo, 1994). However, it can parasitize the eggs on the top, edge, and also single layer egg masses. This parasitoid can also mitigate other pests of the genus Spodoptera in soybean fields, like S. eridania, which only lays its eggs in a single layer (Pomeri et al., 2012). Furthermore, it can be used with selective insecticides, promoting an optimal pest control. Therefore, this parasitoid may assist in mitigating S. frugiperda outbreaks, while helps preventing its resistance evolution to Bt plants and insecticides. It is important to point out that future studies with other Bt crops (i.e. maize and cotton) and other parasitoids, especially larvae parasitoids, are important to assess their responses to the Bt technologies.
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