

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

Natália ALVES LEITE, Luiza Rodrigues REDAELLI, Josué SANT'ANA

Department of Crop Protection, Federal University of Rio Grande do Sul, Porto Alegre, Brazil

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 effective 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 insertional mutagenesis, especially as there is currently no control over where genes are inserted into the crop genome (Maessen, 1997). Such changes could interfere with host-habitat location by parasitoids. This is important considering that parasitoids should not distinguish between volatiles emitted by Bt and non-Bt plants.

In this scenario, where MON 87701 × MON 89788 soybean 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 soybean on this egg parasitoid. In this context, we investigated 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 collected 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 Sorghum (Embrapa Milho & Sorgo, Sete Lagoas, MG, Brazil). *T. pretiosum* population was maintained in the laboratory of Biology, Ecology and Biological Control (Bioecolab) 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, $65 \pm 10\%$ RH, and 14L:10D photoperiod). *T. pretiosum* was reared in the Bioecolab according to Parra and Zucchi (1997) under controlled conditions (25 ± 1 °C, $70 \pm 10\%$ RH, and 14L:10D photoperiod).

Biology 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 isoline), were provided by the National Research Center of Maize and Sorghum (Embrapa Milho & Sorgo, Sete Lagoas, MG, Brazil). 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 application and with mechanical weed control. This bioassay was carried out with soybean plants at the V7 developmental stage (Fehr and Caviness, 1977). Soybean leaves were excised, taken to the laboratory, washed with hypochlorite (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 (n° 000). Plates were sealed with plastic lids and placed in climatic chamber (26 ± 2 °C, $65 \pm 10\%$ RH, and 14L:10D photoperiod). The experimental design was completely randomized with eight replicates per treatment (Bt and non-Bt soybeans); 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 isolated 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 paper, and were fed with a solution of 10% honey provided on cotton. To determine the embryonic period and viability, eggs were obtained from the second oviposition of each pair. Eggs were placed into glass tubes with flat bottoms (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 GPLOT) (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 (χ^2) ($p < 0.05$) (Proc FREQ) (SAS, 2011). A life table was calculated by estimating the mean generation time (*T*), the net reproductive rate (*R*₀), the intrinsic rate of increase (*r*_m) and the finite rate of increase (*λ*). The life table parameters were estimated by the "jackknife" method using "Lifetabel.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 without choice. Eggs used in this bioassay came from the couples 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. frugiperda* eggs (< 24 hours old) were offered for each parasitoid 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 GPLOT) (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 (χ^2) ($p < 0.05$) (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 Peñaflor *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 (χ^2) 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%) ($\chi^2 = 9.23$; $df = 1$; $p = 0.0023$) (figure 1b). However, this difference at the larval survival did not affected the total (egg-adult) survival ($\chi^2 = 0.92$; $df = 1$; $p = 0.3359$) (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).

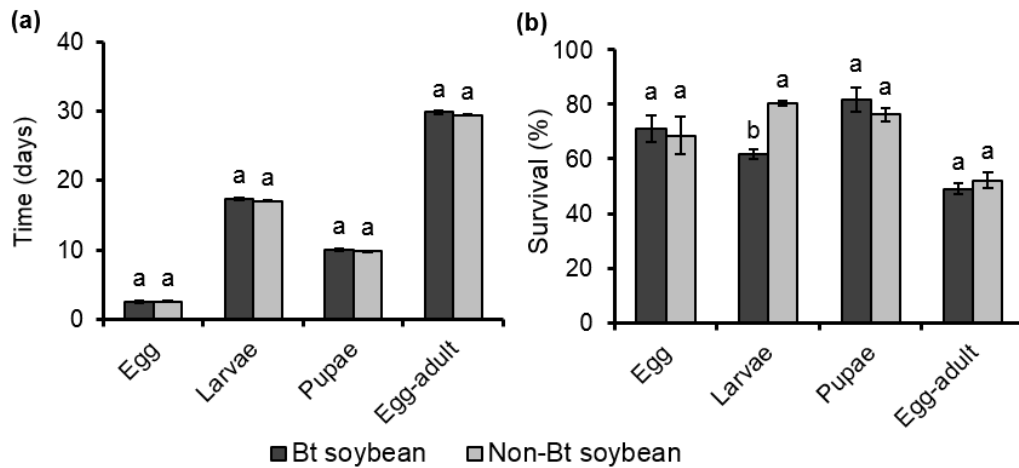


Figure 1. Duration (a) and survival (b) rates of life stages of *S. frugiperda* fed with Bt (MON 87701 × MON 89788) soybean and non-Bt soybean. Pairs of columns with the same letters are not significantly different by *t*-test or Kruskal-Wallis test ($p < 0.05$).

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.

Parameter	Bt soybean	Non-Bt soybean	<i>P</i> -value
Larval weight (mg)	443.94 ± 15.55	481.24 ± 21.07	0.1763
Pupae weight (mg)	198.98 ± 2.72	203.58 ± 2.34	0.2214
Sex ratio	0.46*	0.48*	0.9246
Adult female longevity (days)	12.66 ± 1.16	13.33 ± 1.27	0.7059
Adult male longevity (days)	13.25 ± 1.13	14.16 ± 1.02	0.5558
Fecundity (eggs/female)	1794.25 ± 140.23	1716.16 ± 198.57	0.7511
<i>T</i> (days)	36.79 ± 0.12	36.54 ± 0.13	0.1923
<i>R</i> ₀	404.42 ± 31.60	378.93 ± 43.84	0.6422
<i>r</i> _{<i>m</i>}	0.163 ± 0.002	0.163 ± 0.003	0.8793
λ	1.17 ± 0.002	1.17 ± 0.003	0.8788

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*₀ = net reproductive rate; *r*_{*m*} = intrinsic rate of increase, and λ = finite rate of increase. * Data were not significantly different based on a Chi-square test (χ^2) ($p < 0.05$).

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.

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

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^2) ($p < 0.05$).

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.

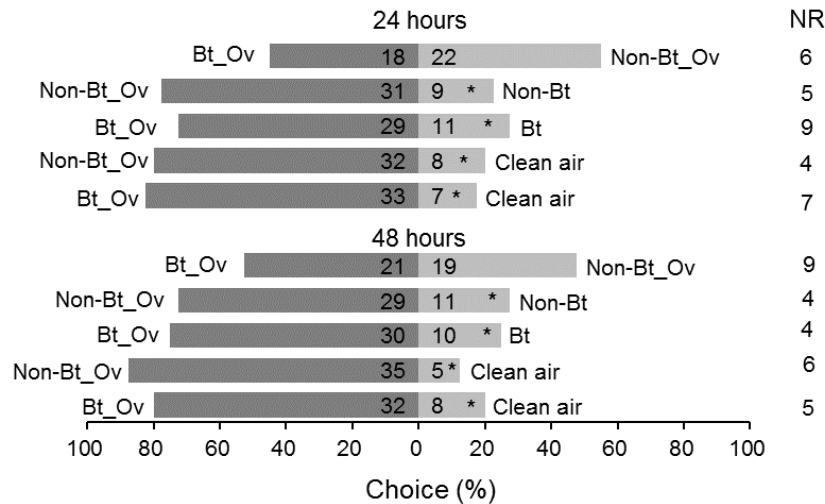


Figure 2. Olfactory response of the egg parasitoid *T. pretiosum* to volatiles emitted by oviposited Bt soybean (Bt_Ov), oviposited non-Bt soybean (Non-Bt_Ov), non-oviposited Bt soybean (Bt), non-oviposited non-Bt soybean (Non-Bt) and clean air after 24 and 48 hours of *S. frugiperda* oviposition. The numbers inside the bars are the total numbers of *T. pretiosum* that responded to each treatment. * Significant at 5% according to Chi-square (χ^2) goodness-of-fit test; NR = non-responsive 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 \times 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; Burtet *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 Platygasteridae) on a 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* (Hübner) (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 high specificity (Jurat-Fuentes and Crickmore, 2017). Cry1 family protein is well known to be Lepidoptera-active (Frankenhuyzen, 2009). Although the Cry1Ac 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 (Aljibory 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 *Euschistus 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 the 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 in 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 discern 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.

Acknowledgements

The financial support and scholarship provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq - process number 150339/2018-6), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES - process number 88887.469188/2019-00) are greatly appreciated. We thank Simone Martins Mendes from the National Research Center of Maize and Sorghum (Embrapa Milho & Sorgo) for providing the soybean seeds and the insect population.

References

- ALJBORY Z., CHEN M. S., 2018.- Indirect plant defense against insect herbivores: a review.- *Insect Science*, 25 (1): 2-23.
- ASHLEY T., WISEMAN B., DAVIS F., ANDREWS K., 1989.- The fall armyworm: a bibliography.- *Florida Entomologist*, 72 (1): 152-202.
- BARROS E. M., TORRES J. B., RUBERSON J. R., OLIVEIRA M. D., 2010.- Development of *Spodoptera frugiperda* on different hosts and damage to reproductive structures in cotton.- *Entomologia Experimentalis et Applicata*, 137 (3): 237-245.
- BERMAN K. H., HARRIGAN G. G., RIORDAN S. G., NEMETH M. A., HANSON C., SMITH M., SORBET R., ZHU E., RIDLEY W. P., 2010.- Compositions of forage and seed from second-generation glyphosate-tolerant soybean MON 89788 and insect-protected soybean MON 87701 from Brazil are equivalent to those of conventional soybean (*Glycine max*).- *Journal of Agricultural and Food Chemistry*, 58 (10): 6270-6276.
- BERNARDI O., AMADO D., SOUSA R. S., SEGATTI F., FATORETTO J., BURD A. D., OMOTO C., 2014a.- Baseline susceptibility and monitoring of Brazilian populations of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and *Diatraea saccharalis* (Lepidoptera: Crambidae) to Vip3Aa20 insecticidal protein.- *Journal of Economic Entomology*, 107 (2): 781-790.
- BERNARDI O., SORGATTO R. J., BARBOSA A. D., DOMINGUES F. A., DOURADO P. M., CARVALHO R. A., MARTINELLI S., HEAD G. P., OMOTO C., 2014b.- Low susceptibility of *Spodoptera cosmioides*, *Spodoptera eridania* and *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to genetically-modified soybean expressing Cry1Ac protein.- *Crop Protection*, 58: 33-40.
- BERNARDI O., SALMERON E., HORIKOSHI R. J., BERNARDI O., DOURADO P. M., CARVALHO R. A., MARTINELLI S., HEAD G. P., OMOTO C., 2015.- Cross-resistance between Cry1 proteins in fall armyworm (*Spodoptera frugiperda*) may affect the durability of current pyramided Bt maize hybrids in Brazil.- *PLoS ONE*, 10 (10): e0140130.
- BORTOLOTO O., SILVA G., DE FREITAS BUENO A., POMARI A., MARTINELLI S., HEAD G., CARVALHO R., BARBOSA G., 2014.- Development and reproduction of *Spodoptera eridania* (Lepidoptera: Noctuidae) and its egg parasitoid *Telenomus remus* (Hymenoptera: Platygastridae) on the genetically modified soybean (Bt) MON 87701 × MON 89788.- *Bulletin of Entomological Research*, 104 (6): 724-730.
- BOTELHO P., PARRA J., ZUCCHI R., 1997.- *Eficiência de Trichogramma em campo*.- FEALQ, Piracicaba, Brazil.
- BRESLOW N., 1970.- A generalized Kruskal-Wallis test for comparing K samples subject to unequal patterns of censorship.- *Biometrika*, 57 (3): 579-594.
- BRUCE T. J., MIDEGA C. A., BIRKETT M. A., PICKETT J. A., KHAN Z. R., 2009.- Is quality more important than quantity? Insect behavioural responses to changes in a volatile blend after stemborer oviposition on an African grass.- *Biology Letters*, 6 (3): 314-317.
- BURTET L. M., BERNARDI O., MELO A. A., PES M. P., STRAHL T. T., GUEDES J. V., 2017.- Managing fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), with Bt maize and insecticides in southern Brazil.- *Pest Management Science*, 73 (12): 2569-2577.
- CASMUZ A., JUÁREZ M. L., SOCIÁS M. G., MURÚA M. G., PRIETO S., MEDINA S., WILLINK E., GASTAMINZA, G., 2010.- Revisión de los hospederos del gusano cogollero del maíz, *Spodoptera frugiperda* (Lepidoptera: Noctuidae).- *Revista de la Sociedad Entomológica Argentina*, 69 (3-4): 209-231.
- CÉLERES, 2017.- *Informativo Biotecnologia: 3º levantamento de adoção da biotecnologia agrícola no Brasil, safra 2016/17*.- Abrasem, Uberlândia, Brazil.
- CORTEZ H., TRUJILLO J., 1994.- Incidencia del gusano cogollero y sus enemigos naturales en tres agrosistemas de maíz.- *Turrialba*, 44 (1): 1-9.
- CTNBIO, 2019.- *Tabela de plantas aprovadas para comercialização*.- Comissão Técnica Nacional de Biossegurança, [online] URL: <http://ctnbio.mctic.gov.br/>
- DANGAL V., HUANG F., 2015.- Fitness costs of Cry1F resistance in two populations of fall armyworm, *Spodoptera frugiperda* (JE Smith), collected from Puerto Rico and Florida.- *Journal of Invertebrate Pathology*, 127 (1): 81-86.
- DEAN J. M., DE MORAES C. M., 2006.- Effects of genetic modification on herbivore-induced volatiles from maize.- *Journal of Chemical Ecology*, 32 (4): 713-724.
- DING J., LI J., LIU X., ZHANG Q., 2009.- The life parameters of a parasitoid *Microplitis mediator* (Hymenoptera: Braconidae), reared on cotton bollworm *Helicoverpa armigera* (Hübner) with Cry1Ac diet.- *Biocontrol Science and Technology*, 19 (9): 931-941.
- FARIAS J. R., ANDOW D. A., HORIKOSHI R. J., SORGATTO R. J., FRESIA P., DOS SANTOS A. C., OMOTO C., 2014.- Field-evolved resistance to Cry1F maize by *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Brazil.- *Crop Protection*, 64 (1): 150-158.
- FATOUROS N., BUKOVINSZKINE KISS G., KALKERS L., SOLER GAMBORENA R., DICKE M., HILKER M., 2005.- Plant synomone induced by butterfly eggs arrests *Trichogramma* wasps.- *Entomologia Experimentalis et Applicata*, 115 (1): 207-215.
- FEHR W. R., CAVINESS C. E., 1977.- Stages of soybean development.- Ames, University of Science and Technology, *Special Report*, 80: 11.
- FRANKENHUYZEN K. V., 2009.- Insecticidal activity of *Bacillus thuringiensis* crystal proteins.- *Journal of Invertebrate Pathology*, 101 (1): 1-16.
- GOERGEN G., KUMAR P. L., SANKUNG S. B., TOGOLA A., TAMO M., 2016.- First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa.- *PLoS ONE*, 11 (10): e0165632.
- HASSAN S. A., 1993.- The mass rearing and utilization of *Trichogramma* to control lepidopterous pests: achievements and outlook.- *Pest Management Science*, 37 (4): 387-391.
- HILBECK A., ANDOW D. A., 2004.- *Environmental risk assessment of genetically modified organisms, volume 1: a case study of Bt maize in Kenya*.- Cabi Publishing, Wallingford, UK.
- HILKER M., MEINERS T., 2010.- How do plants "notice" attack by herbivorous arthropods?- *Biological Reviews*, 85 (2): 267-280.
- HOHMANN C., SILVA S., SANTOS W. D., 1989.- Lista preliminar de Trichogrammatidae encontrados no Paraná.- *Anais da Sociedade Entomológica do Brasil*, 18 (1): 203-206.
- HUTCHISON W. D., BURKNESS E., MITCHELL P., MOON R., LESLIE T., FLEISCHER S. J., ABRAHAMSON M., HAMILTON K., STEFFEY K. L., GRAY M., 2010.- Areawide suppression of European corn borer with Bt maize reaps savings to non-Bt maize growers.- *Science*, 330 (6001): 222-225.
- JURAT-FUENTES J. L., CRICKMORE N., 2017.- Specificity determinants for Cry insecticidal proteins: insights from their mode of action.- *Journal of Invertebrate Pathology*, 142 (1): 5-10.
- KOUSER S., QAIM M., 2011.- Impact of Bt cotton on pesticide poisoning in smallholder agriculture: a panel data analysis.- *Ecological Economics*, 70 (11): 2105-2113.
- LEITE N. A., MENDES S. M., SANTOS-AMAYA O. F., SANTOS C. A., TEIXEIRA T. P., GUEDES R. N., PEREIRA E. J., 2016.- Rapid selection and characterization of Cry1F resistance in a Brazilian strain of fall armyworm.- *Entomologia Experimentalis et Applicata*, 158 (3): 236-247.

- LIU X. X., SUN C. G., ZHANG Q. W., 2005.- Effects of transgenic Cry1A+ CpTI cotton and Cry1Ac toxin on the parasitoid, *Campoketis chloridae* (Hymenoptera: Ichneumonidae).- *Insect Science*, 12 (2): 101-107.
- LU Y., WU K., JIANG Y., GUO Y., DESNEUX N., 2012.- Widespread adoption of Bt cotton and insecticide decrease promotes biocontrol services.- *Nature*, 487 (7407): 362-365.
- MAESSEN G., 1997.- Genomic stability and stability of expression in genetically modified plants.- *Acta Botanica Neerlandica*, 46 (1): 3-24.
- MAIA A. D. N., LUIZ A. J. B., CAMPANHOLA C., 2000.- Statistical inference on associated fertility life table parameters using jack-knife technique: computational aspects.- *Journal of Economic Entomology*, 93 (2): 511-518.
- MEISSLE M., ROMEIS J., 2017.- Transfer of Cry1Ac and Cry2Ab proteins from genetically engineered Bt cotton to herbivores and predators.- *Insect Science*, 25 (5): 823-832.
- MICHEREFF M. F. F., LAUMANN R. A., BORGES M., MICHEREFF-FILHO M., DINIZ I. R., NETO A. L. F., MORAES M. C. B., 2011.- Volatiles mediating a plant-herbivore-natural enemy interaction in resistant and susceptible soybean cultivars.- *Journal of Chemical Ecology*, 37 (3): 273-285.
- MOTAVALLI P. P., KREMER R. J., FANG M., MEANS N. E., 2004.- Impact of genetically modified crops and their management on soil microbially mediated plant nutrient transformations.- *Journal of Environmental Quality*, 33 (3): 816-824.
- OMOTO C., BERNARDI O., SALMERON E., SORGATTO R. J., DOURADO P. M., CRIVELLARI A., CARVALHO R. A., WILLSE A., MARTINELLI S., HEAD G. P., 2016.- Field-evolved resistance to Cry1Ab maize by *Spodoptera frugiperda* in Brazil.- *Pest Management Science*, 72 (9): 1727-1736.
- PARRA J. R. P. 2001.- Técnicas de criação de insetos para programas de controle biológico.- FEALQ, Piracicaba, Brazil.
- PARRA J. R. P., ZUCCHI R. A. 1997.- *Trichogramma* e o controle biológico aplicado.- FEALQ, Piracicaba, Brazil.
- PEÑAFLOR M. F. G. V., ERB M., MIRANDA L., WERNEBURG A., BENTO J. M. S., 2011a.- Herbivore-induced plant volatiles can serve as host location cues for a generalist and a specialist egg parasitoid.- *Journal of Chemical Ecology*, 37 (12): 1304-1313.
- PEÑAFLOR M. F. G. V., ERB M., ROBERT C. A. M., MIRANDA L. A., WERNEBURG A. G., DOSSI F. C. A., TURLINGS T. C., BENTO J. M. S., 2011b.- Oviposition by a moth suppresses constitutive and herbivore-induced plant volatiles in maize.- *Planta*, 234 (1): 207-215.
- POMARI A. F., BUENO A. F., BUENO R. C. O. F., MENEZES A. O. JR., 2012.- Biological characteristics and thermal requirements of the biological control agent *Telenomus remus* (Hymenoptera: Platygastridae) reared on eggs of different species of the genus *Spodoptera* (Lepidoptera: Noctuidae).- *Annals of the Entomological Society of America*, 105 (1): 73-81.
- PRADO J. R., SEGERS G., VOELKER T., CARSON D., DOBERT R., PHILLIPS J., COOK K., CORNEJO C., MONKEN J., GRAPES L., 2014.- Genetically engineered crops: from idea to product.- *Annual Review of Plant Biology*, 65 (1): 769-790.
- R DEVELOPMENT CORE TEAM, 2012.- *R: a language and environment for statistical computing*.- R Foundation for Statistical Computing, Vienna, Austria.
- ROMEIS J., NARANJO S. E., MEISSLE M., SHELTON A. M., 2019.- Genetically engineered crops help support conservation biological control.- *Biological Control*, 130 (1): 136-154.
- SANTOS F. C. D., LIMA NEVES J. C., FERREIRA NOVAIS R., ALVAREZ V. V. H., SEDIYAMA C. S., 2008.- Modelagem da recomendação de corretivos e fertilizantes para a cultura da soja.- *Revista Brasileira de Ciência do Solo*, 32 (4): 1661-1674.
- SANTOS H. J. JR, MARQUES E. J., PRATISSOLI D., KLOSS T. G., MACHADO L. C., ANDRADE G. S., 2011.- Efeito de *Bacillus thuringiensis* (Bacillaceae) sobre parâmetros biológicos do parasitoide *Trichogramma pretiosum* (Trichogrammatidae).- *Natureza on line*, 9 (1): 1-6.
- SANTOS-AMAYA O. F., RODRIGUES J. V., SOUZA T. C., TAVARES C. S., CAMPOS S. O., GUEDES R. N., PEREIRA E. J., 2015.- Resistance to dual-gene Bt maize in *Spodoptera frugiperda*: selection, inheritance, and cross-resistance to other transgenic events.- *Scientific Reports*, 5 (1): 18243.
- SAS, 2011.- *Base SAS® 9.3 Procedures Guide*.- SAS Institute Inc., Cary, NC, USA.
- SCHULER T. H., POTTING R. P. J., DENHOLM I., POPPY G. M., 1999.- Parasitoid behaviour and Bt plants.- *Nature*, 400 (6747): 825-826.
- SHELTON A. M., ROMEIS J., NARANJO S., TIAN J., HELLMICH R., 2016.- Use of Bt-resistant caterpillars to assess the effect of Cry proteins on beneficial natural enemies.- *IOBC/wprs Bulletin*, 114: 51-55.
- SHYLESHA A., JALALI S., GUPTA A., VARSHNEY R., VENKATESAN T., SHETTY P., OJHA R., GANIGER P. C., NAVIK O., SUBAHARAN K., 2018.- Studies on new invasive pest *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) and its natural enemies.- *Journal of Biological Control*, 32 (3): 1-7.
- SORGATTO R. J., BERNARDI O., OMOTO C., 2015.- Survival and development of *Spodoptera frugiperda* and *Chrysodeixis includens* (Lepidoptera: Noctuidae) on Bt cotton and implications for resistance management strategies in Brazil.- *Environmental Entomology*, 44 (1): 186-192.
- SOUZA C. S., SILVEIRA L. C., PAULA D. P., ANDOW D. A., MENDES S. M., 2018.- Transfer of Cry1F from Bt maize to eggs of resistant *Spodoptera frugiperda*.- *PLoS ONE*, 13 (9): e0203791.
- STEINBRECHER I. 2004.- Effects of Bt transgenes on herbivorous insect-parasitoid interactions, pp. 81. *PhD thesis*, Georg-August-Universität Göttingen, Germany.
- TAMIRU A., BRUCE T. J., WOODCOCK C. M., CAULFIELD J. C., MIDEGA C. A., OGOL C. K., MAYON P., BIRKETT M. A., PICKETT J. A., KHAN Z. R., 2011.- Maize landraces recruit egg and larval parasitoids in response to egg deposition by a herbivore.- *Ecology Letters*, 14 (11): 1075-1083.
- TIAN J. C., WANG X. P., CHEN Y., ROMEIS J., NARANJO S. E., HELLMICH R. L., WANG P., SHELTON A. M., 2018.- Bt cotton producing Cry1Ac and Cry2Ab does not harm two parasitoids, *Cotesia marginiventris* and *Copidosoma floridanum*.- *Scientific Reports*, 8 (1): 307.
- TOONDERS T. J., SANCHEZ J. L. C., 1987.- Evaluación de la efectividad de *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) en el combate de *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae). Recomendaciones para su uso.- *Agrocien-cia*, 67 (1): 75-84.
- VARGAS C. C., REDAELLI L. R., SANT'ANA J., MORAIS R. M. D., PADILHA P., 2017.- Influência da idade do hospedeiro e da aprendizagem no comportamento quimiotático e no parasitismo de *Trichogramma pretiosum*.- *Iheringia*, 107 (1): e2017015.
- VINSON S. B., 1998.- The general host selection behavior of parasitoid Hymenoptera and a comparison of initial strategies utilized by larvaphagous and oophagous species.- *Biological Control*, 11 (2): 79-96.
- WANG Z. Y., WU Y., HE K. L., BAI S. X., 2007.- Effects of transgenic Bt maize pollen on longevity and fecundity of *Trichogramma ostriniae* in laboratory conditions.- *Bulletin of Insectology*, 60 (1): 49-55.
- WANG Z. X., LI Y. H., HE K. L., BAI S. X., ZHANG T. T., CAI W. Z., WANG Z. Y., 2017.- Does Bt maize expressing Cry1Ac protein have adverse effects on the parasitoid *Macrocentrus cingulum* (Hymenoptera: Braconidae)?- *Insect Science*, 24 (4): 599-612.
- ZURBRÜGG C., HÖNEMANN L., MEISSLE M., ROMEIS J., NENTWIG W., 2010.- Decomposition dynamics and structural plant components of genetically modified Bt maize leaves do not differ from leaves of conventional hybrids.- *Transgenic Research*, 19 (2): 257-267.

Authors' addresses: Natália ALVES LEITE (corresponding author, alvesnat@gmail.com), Luiza Rodrigues REDAELLI, Josué SANT'ANA, Department of Crop Protection, Federal University of Rio Grande do Sul, Porto Alegre, 91550-000, Brazil.

Received February 18, 2020. Accepted June 5, 2020.