



Biological Control and Crop Protection

Does *Bacillus thuringiensis* have adverse effects on the host egg location by parasitoid wasps?

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ABSTRACT

This study investigated the interaction between two pest biological control agents, the parasitoid wasp *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) and the entomopathogen *Bacillus thuringiensis* (Bacillales: Bacillaceae) (*Bt*). The aim of this study was to evaluate if the presence of *Bt* (formulated products Agree[®], Dipel[®] and HD1 and HD11 strains) interferes in the oviposition preference of *T. pretiosum* to eggs of *Helicoverpa zea* (Lepidoptera: Noctuidae). Using an olfactometry test, the eggs of *H. zea* were bathed with the commercial formulations, with the *Bt* suspensions or distilled water, and offered to the parasitoid wasps in order to evaluate parasitism. The results showed that *H. zea* eggs sprayed with commercial formulations and *Bt* strains did not interfere in the choice made by the parasitoid. The parasitoid wasp is not able to distinguish between eggs with or without *B. thuringiensis* treatment, independently of strains suspension or commercial formulations. Therefore, these two control agents may be used together without negative interaction.

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Introduction

One of the Integrated Pest Management (IPM) assumptions is the integration of more than one control method (Pedigo and Rice, 2009). The use of parasitoid wasps *Trichogramma* spp. and the bioinsecticide *Bacillus thuringiensis* (Bacillales: Bacillaceae) to improve biological control is among the common alternative components of integrated management of lepidoptera pests in corn.

Trichogramma pretiosum Riley (Hymenoptera: Trichogrammatidae) is an egg parasitoid (Li et al., 2015). This micro-hymenoptera is efficient and widely used, because it controls pest species before the damage occurs (Costa et al., 2014). Mass releases of *Trichogramma* species are frequently used, especially for lepidopteran pest control (Hassan, 1993; Smith, 1996; Khan et al., 2015). However, the use of only the parasitoid agent may not be sufficient for the suppression of a target pest and other control tactics may be necessary (Raymond et al., 2013). As an entomopathogenic agent, *B. thuringiensis* has become one of the most important organisms for insect pests control in agriculture (Tabashnik et al., 2015; Glare and O'Callaghan, 2000). This entomopathogen produces a crystal protein (δ -endotoxin or Cry protein) that has an insecticidal activity

on more than 1000 species of various insect orders (Xu et al., 2014; Sayed et al., 2015).

These two biological control agents (i.e. parasitoid wasps and *Bt*) have been used together to control various agricultural pests worldwide (Vaez et al., 2013). However, little attention has been given to the possible antagonism of these two control agents (Azizoglu et al., 2015). Although there are no reports of direct toxicity of *Trichogramma* species by *Bt*, there is still the possibility that the entomopathogen may adversely affect the next generations of parasitoids (Vianna et al., 2009), besides presenting a negative influence on their choice to parasitize eggs sprayed with *Bt*.

To improve the use of these two tactics in agriculture, it is necessary to investigate whether there is interference of biological products on the foraging behavior of the *Trichogramma* species. Besides the direct toxicity effects, insecticides can act as repellents, inhibitors or olfactory destroyers, which may affect searching and reproductive behavior of adult parasitoid (Amaro et al., 2015). However, little is known about the searching behavior of parasitoids exposed to odors from *Bt* commercial formulations and *Bt* strains. It is known that the stimuli used by parasitoids to locate its host are very important to increase biological control effectiveness (Suverkrupp et al., 2009).

In biological control, parasitoid efficiency is highly related to parasitoid ability to find its host (Greene et al., 1976). Adult parasitoid may use physical cues and, especially, chemical cues

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(semiochemicals) emitted by the plant (Cusumano et al., 2015; Hilker and Fatouros, 2015) and the host (Fatouros et al., 2008) to find the eggs. Semiochemicals emitted by plants are volatile compounds that function as long-range signals. However, they are considered less reliable odors for host location compared to semiochemicals emitted by the host itself (Vet and Dicke, 1992), because the odors emitted by the plant do not guarantee that the parasitoid host is actually in a certain place.

In addition to chemical cues issued by plants and moths (e.g. sex pheromones), wasps detect cues left on or around the eggs at oviposition, originated from the scales or substances used to secure fix the eggs to the substrate (Vinson, 1998). These substances serve as protection for the egg, since it is exposed to parasitoids, predators and adverse weather conditions (Hilker and Fatouros, 2015). Such substances can also serve as kairomones or synomones by parasitoids (Mutiyambai et al., 2015). These substances can be attractant in short distance and they are very important to egg location and recognition, increasing its parasitism rates (Mutiyambai et al., 2015; Xu et al., 2014).

The parasitoids searching capability is directly related to kairomones present in its hosts (Vinson, 1984). Volatiles from the host for egg parasitoid attraction can be derived from fecal pellets, wing scales, egg laying, aggregation, sexual and alarm pheromones and have been the object of study in some research works, (Pires et al., 2001; Fatouros et al., 2005). Therefore, these semiochemicals are extremely important for parasitoids, as the eggs are very small and immobile (Fatouros et al., 2007; Wang et al., 2017).

Thus, is it possible that these products and strains deposited on the eggs can act as a repellent or an attractive to parasitoids. The objective was to investigate if the fact that the *Helicoverpa zea* (Lepidoptera: Noctuidae) eggs, being covered with *Bt* commercial formulations or *Bt* suspensions, interferes with the preference of *Trichogramma pretiosum*.

Material and methods

Host rearing

Adult of corn earworm (CEW), *H. zea*, were confined in special cages made with PVC tubes (30 cm in diameter and 20 cm) for multiplication in laboratory colony maintained at the Embrapa Milho e Sorgo Research Station (Sete Lagoas, MG, Brazil). Inside each cage, napkins were inserted for oviposition. A sugar solution was provided to feed the adults. After four days, the napkins with the eggs were removed, placed in plastic bags and stored in a temperature-controlled room ($25 \pm 2^\circ\text{C}$). After 48 h, the hatched larvae were transferred to individual 50 mL plastic cups containing artificial diet. The larvae developed into adult in the cups. The adults were separated by sex and couples were transferred to a cage, continuing the rearing cycle (Vilela et al., 2014).

Cultivation of bacterial isolates and estimated LC50

The *Bacillus thuringiensis* (*Bt*) isolates, (HD 1) var. *kurstaki*, and (HD 11) var. *aizawai* used came from the Biological Collection of *Bacillus* spp. at Embrapa Milho e Sorgo and the commercially formulated products came from the market, Agree[®] (25,000 $\mu\text{L}/\text{mg}$, var. *aizawai* GC 91), distributed by Biocontrol - Métodos de Controles de Pragas Ltda. and DiPel[®] WP (25,000 IU/mg) var. *kurstaki* (HD-1 strain), manufactured by Abbott Laboratories.

In order to multiply the *Bt* isolates it was used LB – Luria-Bertani medium (yeast extract, peptone, sodium chloride, agar and distilled water) plus MgSO_4 , FeSO_4 , ZnSO_4 and MnSO_4 salts (Valicente and Barreto, 2003) for 72 h at 28°C . After this stage, colonies were

removed from the plates using a spatula and transferred to plastic tubes containing 10 mL of sterile distilled water.

For the spore count, serial dilutions were performed. The dilution was 1 mL of bacteria to 9 mL of water. The spore count was performed in $40\times$ phase contrast optical microscope through a Neubauer chamber using 10 mL of solution on each side of the chamber. The counting was performed on the four lateral fields and one in the central field, averaging the two sides and multiplying by the constant 50,000, thus yielding the number of spores from each isolated dilution. This procedure was conducted for the commercial formulations and the strains.

Bt pathogenicity to *Helicoverpa zea*

As a preliminary test, five suspensions were prepared with different concentrations of each isolate and commercial formulation: HD1 (1.42×10^5 ; 1.42×10^6 ; 1.42×10^7 ; 1.42×10^8 ; 1.42×10^9 spores/mL); HD11 (1.34×10^5 ; 1.34×10^6 ; 1.34×10^7 ; 1.34×10^8 ; 1.34×10^9 spores/mL); Dipel (2.64×10^5 ; 2.64×10^6 ; 2.64×10^7 ; 2.64×10^8 ; 2.64×10^9 spores/mL) and Agree (1.18×10^5 ; 1.18×10^6 ; 1.18×10^7 ; 1.18×10^8 ; 1.18×10^9 spores/mL). As a control, an artificial diet of 1 cm^3 was used in the bioassay which was placed individually in 50 mL plastic cups. For each treatment concentration, twenty-four replications were used.

From each concentration of *Bt* suspensions, 165 μL were disposed on the diet surface that were left at room temperature until all excess of fluid was dried. Three-day-old larvae were placed in the cups, which were closed and maintained at a room temperature ($25 \pm 2^\circ\text{C}$) with a relative humidity of $70 \pm 2\%$, and a 14 h of photoperiod. Twenty-four replicates were used for each concentration of each isolate. For the control, the diet was wet with distilled water plus Tween[®] 80 (U.S.P.) adhesive surfactant. Evaluations of caterpillars were done 3 days later and checked if they were dead with a dry and dark skin appearance (i.e. typical symptom of dead larvae by *B. thuringiensis*).

Data were plotted in graphs and the curves obtained were used to estimate the lethal concentration (LC50). *Bt* commercial formulations and *Bt* strains followed the steps to calculate the mortality and LC50 (Crawley, 2013).

Parasitoids

Trichogramma pretiosum were reared in *Anagasta kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae) eggs, provided from the Koppert Biological Systems Company, and maintained under controlled temperature and relative humidity conditions until the beginning of the experiment.

The sexing of the parasitoids with same age was conducted under stereoscope microscope using a fine brush, distinguishing males and females by the antennae morphology.

Preparation of cards with *Helicoverpa zea* eggs

For the bioassays, 4×4 cm cards with 60 *H. zea* eggs (<24 h old) were prepared using a stereomicroscope ($50\times$) and a fine brush. The cards were prepared as follows: the moths oviposited on soft napkins that were then removed, cut to standard size (4×4 cm), counted the number of eggs and the excess of eggs was eliminated in order to have a total 60 eggs per card. This procedure was performed carefully to reduce possible manipulation interference with the eggs, such as elimination of scales or substances deposited by the moth during the oviposition. Thus, the eggs were offered to the parasitoid as close to reality as possible.

The cards with the eggs were immersed for 5 s in *Bt* solution, freshly prepared, and then left to air-dry. For the control, the cards

were dipped in distilled and sterile water using the same methodology as for the *Bt* isolates and commercial formulation. The LC50 found for the *Bt* isolates and commercial formulations conducted with larva in the preliminary test were used to bathe the eggs offered to the parasitoid.

Olfactory response of parasitoids to eggs treated with *Bt* strains and commercial formulations

A blank test was performed in order to evaluate whether the compounds responsible for attracting the parasitoids to *H. zea* eggs are derived from substances deposited on them by the moths during oviposition or from the odors of treatments to which they were submitted (strains and formulations). So, this test assessed whether *T. pretiosum* was able to locate the eggs even in the absence of the host plant. For this purpose, it was used a “Y” olfactometer, made of glass (20 cm in length and 3.5 cm internal diameter), with a flowmeter for the compressed air in each arm adjusted to an air flow of 2.5 L/min. Before entering the olfactometer, the air introduced by a vacuum pump passed through an activated carbon filter and was bubbled in water for humidification. At the air exit of the olfactometer in one arm a card with eggs was placed, supported by a sterilized Petri dish and in the other arm there was just air.

Prior to testing, the wasp females were fed on drops of honey for 30 min. The parasitoid females were individually placed inside the olfactometer using a thin brush. Each observation lasted 600 s and evaluated the choice, control or egg card, by the female. It was considered a choice when a wasp passed the threshold of one of the olfactometer arm and remained for at least 20 s. Each wasp was used once. Females that did not move or did not reach an arm in 300 s were classified as “no response”.

For each odor source pair (treatment × control), 20 adult females with less than 24 h old were tested with 3 replications per treatment. At each repetition, the odor supply position was modified and the olfactometer randomly rotated to eliminate effects of the source position and the choice by the female. The tests were conducted for two days. After finishing each replicate, all whole system was disassembled and washed with water.

The parasitoids attraction to *H. zea* eggs submitted to different treatments was conducted contrasting all the treatments (*Bt* strains and commercial formulations), with the control (eggs without *Bt*, bathed with deionized water).

The treatments of the preference tests were organized as follows: T1 – eggs vs. air (Blank test); T2 – eggs + Agree® vs. distilled water; T3 – eggs + Dipel® vs. eggs + water; T4 – eggs + strain HD1 vs. eggs + water; T5 – eggs + strain HD11 vs. eggs + water.

The experiment was conducted in a completely randomized design with 20 wasps and 3 replicates for each treatment, with a total of 300 observations per response.

Egg parasitism by *Trichogramma pretiosum*

Cardboard strips (3.0 × 0.5 cm) with thirty (30) *H. zea* eggs (about 24 h old) were prepared for testing using a stereoscopic microscope (50×) and a fine brush. The cards were prepared as follows: the moths lay their eggs on soft napkins, which were removed, cut to the standard size (3.0 × 0.5 cm) and counted the eggs and carefully the excess of eggs was eliminated, leaving a total of 30 eggs per card.

Then the cards with the eggs were immersed in the *Bt* solution for 5 s and allowed to air-dry until the liquid completely evaporated (Souza et al., 2014). For the control treatment, the cards were dipped in deionized water, following the same methodology as the other treatments. The solutions were prepared at the same day to be used. The LC50 estimated to CEW larva for each isolate and

commercial formulation in the preliminary test were used to bathe the eggs to be offered to the parasitoid.

For the choice test, two cards with 3.0 × 0.5 cm with 30 eggs each, one containing eggs bathed with distilled water and other eggs bathed with *Bt*, were placed in a 90 × 15 mm Petri dish. The treatments were combined for testing as follows: T1 – eggs + Agree® vs. control; T2 – eggs + Dipel® vs. control; T3 – eggs + Strain HD1 vs. control; T4 – eggs + Strain HD11 vs. control.

To each *T. pretiosum* female, two cards with the appropriate treatments were offered, placed 10 mm apart, allowing the parasitoid a free choice test. A drop of honey was offered as food for the wasps. The eggs were exposed to parasitism for 24 h. After this time, two cycles of each treatment were removed and conditioned in separate glass vials and sealed with plastic film and maintained under controlled conditions of 25 ± 1 °C; 70% RH and 12:12 h L:D photoperiod. The parasitoid females were then discarded.

The evaluations were carried out daily until the death of the individuals. The parameters evaluated were the number of parasitized eggs (i.e. dark eggs), the number of emerged wasps and offspring longevity (in days). The number of parasitized eggs and emerged individuals was expressed as a percentage. The experiment was conducted in a completely randomized design with 20 repetitions for each of the four treatments, totaling 80 experimental plots.

Data analysis

As a preliminary test, *H. zea* was submitted to Agree®, Dipel® and HD1 and HD11 strains, evaluating mortality and then the LC50s were estimated using General Linear Models (GLM) with binomial distribution, appropriated to proportion data (Crawley, 2013).

In the blank test to assess whether there was a significant difference in attracting the wasps to the olfactometer arms with eggs or air, the chi-square test (χ^2) ($\alpha = 5\%$) was conducted. The treatments (eggs + Dipel, eggs + Agree, eggs + HD1 strain, eggs + HD11 strain) were compared to clean eggs (control or eggs immersed in water).

For the analysis of *T. pretiosum* parasitism percentage, we used the method proposed by Luckmann et al. (2014), in which the parasitism in each *Bt* treatment was calculated using the equation $POP_{trat} = (OP_{trat} / (OP_{trat} + OP_{test})) * 100$. Where POP_{trat} = percentage of parasitized eggs in the treatment, OP_{trat} = number of parasitized eggs in the treatment and OP_{test} = number of parasitized eggs in the control. The adult emergence percentage was calculated using the equation $Pe = (Te / To) * 100$, where Pe = emergence percentage, Te = total emerged, To = total of eggs parasitized. The percentage data were transformed using the arcsine function $\times / 100$ in an attempt to comply with the homogeneity of variances, such as ANOVA assumptions. For survival analysis, we used the procedure described by Crawley (2013) for the Kaplan–Meier distribution. All analyses were performed using the R statistical environment (R Development, 2010).

Results

Effect of *Bt* strains and commercial formulations on *Helicoverpa zea*

The concentration required to causing 50% mortality of corn earworm (CEW), *H. zea* population, for different *Bacillus thuringiensis* (*Bt*) source: Agree® and Dipel® commercial formulations, and strains HD 1 and HD 11 were 1.18×10^7 , 2.64×10^8 , 1.42×10^6 and 1.34×10^8 spores/mL, respectively (Fig. 1). These results show the pathogenicity differences between the *Bt* strains and their sources to CEW larvae. The commercial formulation Dipel® showed the highest LC50 and HD 1 strain showed the lowest LC50, indicating

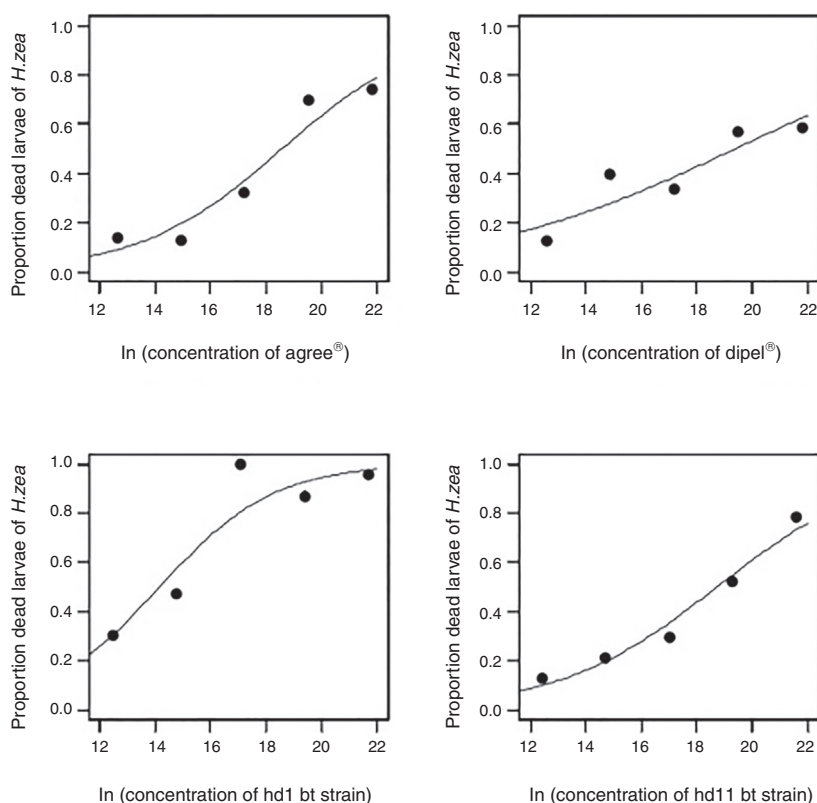


Fig. 1. Proportion of dead *Helicoverpa zea* larvae exposed to a gradient of *Bacillus thuringiensis* concentrations (spores/mL) in the form of Agree®, Dipel®, HD1, or HD11. The lethal concentration was estimated from the model adjusted to observed experimental points. Sete Lagoas, MG, Brazil.

that Dipel formulation was the least toxic to *H. zea* larva and HD1 was the most toxic among the treatments evaluated.

The commercial formulation Agree® showed the lower LC50, being more toxic to *H. zea* than the pure strain preparation. This may be due to a plus of toxicity of the products used in the formulation of Agree®. Comparing the HD1 and HD11, strains of *B. thuringiensis* var. *kurstaki* and var. *aizawai*, respectively, the HD1 strain was more toxic to *H. zea*.

Behavior of wasps in the olfactometer

The results of data analysis ($\chi^2 = 7.2$, $df = 1$, $p < 0.01$; $\chi^2 = 9.8$, $df = 1$, $p < 0.01$ and $\chi^2 = 9.8$, $df = 1$, $p < 0.01$) indicated that more wasp females of *T. pretiosum* (number/repetition – 16, 17 and 17) moved toward the olfactometer arm with *H. zea* eggs than the arm control. These data indicate that parasitoid wasps can locate the *H. zea* eggs even in the absence of the plant host, within a short distance (Fig. 2).

The proportion of *T. pretiosum* that preferred clean eggs, in relation to the other treatments, was significantly different ($\chi^2 = 0.88$, $df = 10$, $p < 0.001$). There was no significant difference between the *Bt* strains and the commercial formulations ($\chi^2 = 5.65$, $df = 10$, $p = 0.156$) when compared within *Bt* strains ($\chi^2 = 8.95$, $df = 10$, $p = 0.463$) and commercial formulations ($\chi^2 = 9.96$, $df = 10$, $p = 0.556$).

When *H. zea* eggs were bathed with the Agree®, 12, 6 and 7 of the wasps went toward the olfactometer arm with eggs, while 12, 11 and 8 wasps were attracted to the eggs treated with Dipel®, and 9, 8 and 8 went toward the arm with eggs treated with the strain HD1, and 14, 9 and 6 went in the direction of the treatment with the HD11 strain (Fig. 2). However, none of these treatments were significantly different.

Egg parasitism by *Trichogramma pretiosum*

The treatment of *H. zea* eggs with the formulated Agree®, Dipel® and *B. thuringiensis* strains HD1 and HD11 did not affect parasitism ($F_{(3,76)} = 1.13$; $p = 0.34$) and adult emergence ($F_{(7,100)} = 1.43$; $p = 0.20$) of *T. pretiosum* compared to the control (Fig. 3). Although there was not observed significant difference, even presenting higher LC 50, 2.64×10^8 spores/mL.

The treatment of *H. zea* eggs with the formulated Agree®, Dipel® and *B. thuringiensis* strains HD1 and HD11 did not affect the survival of *T. pretiosum* when the number of adult emergences from these treatments were compared to the control.

It was observed that, although without significant difference, the control wasps, i.e., those from eggs bathed with water, showed decline in survival compared to the Agree® treatment. The opposite occurred in comparison with the HD1 treatment, with parasitoids derived from eggs bathed with this strain, which showed a decline in survival, but also presented no significant difference.

Discussion

The blank test in the olfactometer showed that the *T. pretiosum* female wasps were able to locate the clean eggs. This suggests that the olfactometer methodology to study the parasitoids behavior is appropriated to test the hypothesis and indicated that besides the wasps use the compounds from the plant and sex pheromone to locate the host, also they identify the moth eggs by the released semiochemicals (Noldus and van Lenteren, 1985; Gazit et al., 1996). It is known that the semiochemicals from host plant have great importance for the parasitoids (Fatouros et al., 2007). The presence of commercial formulated (Agree® and Dipel®) and *Bt* strains (HD1 and HD11) on *H. zea* eggs did not affect the parasitism, larval survival and adult emergence of *T. pretiosum*. These results suggest

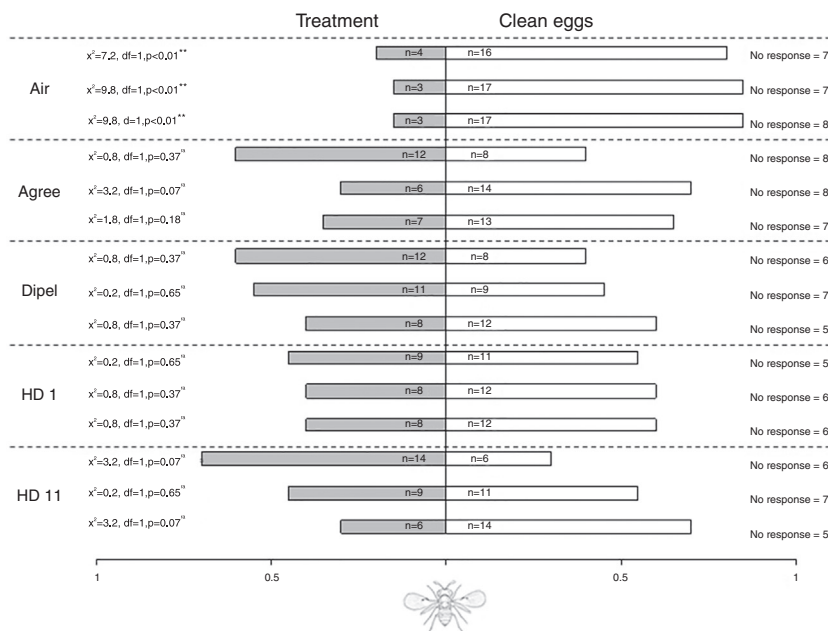


Fig. 2. Response of *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) females in Y-tube olfactometer: air (control), Agree[®], Dipel[®], HD 1, or HD 11 versus clean eggs of *Helicoverpa zea*. Each bar represents a single replicate experiment involving liberation of 20 wasps with response (Chi-square Test, $^{**}p < 0.01$, see text for more explanation). Sete Lagoas, MG, Brazil.

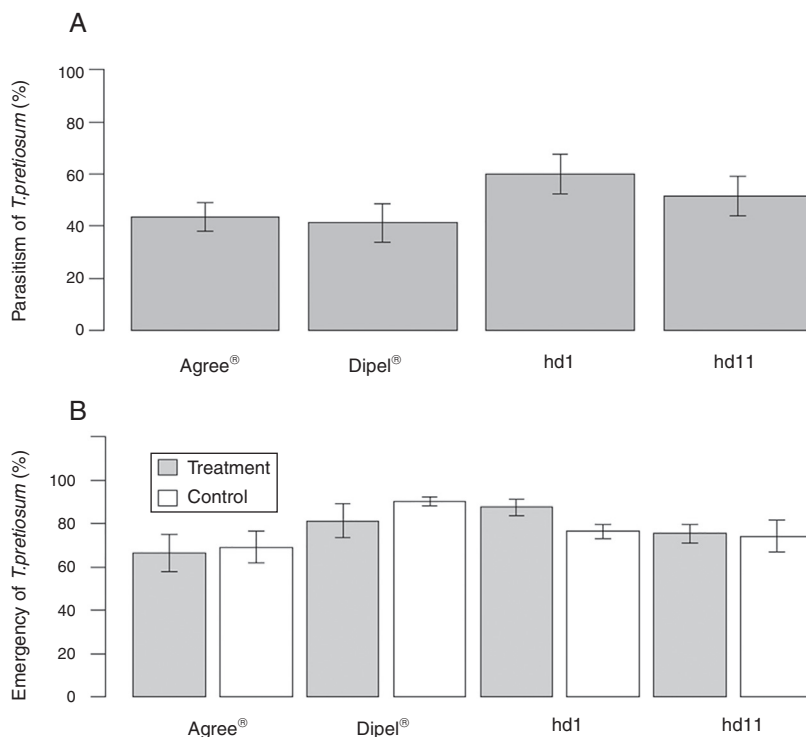


Fig. 3. Parasitism percentage (A) and emergence (B) (mean \pm standard error) of *Trichogramma pretiosum* from *Helicoverpa zea* eggs treated with two formulated (Agree[®] and Dipel[®]) and two strains (HD1 and HD11) of *Bacillus thuringiensis*. Sete Lagoas, MG, Brazil.

that the biological control tactics combining the microbial control with Bt and the parasitoid *T. pretiosum* can be compatible.

The results show that *T. pretiosum* females realize and locate their host without the presence of the plant, leading us to realize that this parasitoid can find the eggs at short distances, detecting the location of its host on the plant, for example. Thus, we noted the importance to the parasitoids of the specific chemicals deposited on the egg by the moth, which act as kairomones

since these substances allow the parasitoid to restrict its search area, increasing the chance of finding the host and reproductive success.

As result of the bioassays with the two *Bt* strains and commercial formulations, was observed that *T. pretiosum* can locate the eggs even when covered with *Bt* solutions. Besides being able to perceive their host, the wasps' preference for these eggs was not altered in comparison with the control.

The compounds deposited on the moth eggs are primarily responsible for protection, but the attraction of the parasitoids toward their host (Wang et al., 2014) was a result of the coevolution. Our results show that the presence of the formulations and *Bt* strains on the eggs did not influence the attraction, as there was no significant difference between the attraction of parasitoids by the eggs bathed with *Bt* and eggs without the presence of *Bt*.

This study indicates that the use of *Bt* and *T. pretiosum* can be combined for the integrated management of *H. zea* without losses of efficacy of the parasitoid. Amaro et al. (2015) also found positive results of compatibility between these two biological control agents. The parasitism of *A. kuehniella* eggs sprayed with two *Bt* based bioinsecticides, *B. thuringiensis* var. *kurstaki* (Dipel®) and *B. thuringiensis* var. *aizawai* (Agree®), the same formulas used in this study, were similar to the control without *Bt*, concluding that the bacteria did not affect the performance of parasitoids.

Azizoglu et al. (2015) found that the strain of *B. thuringiensis* subsp. *kurstaki* (HD1) mixed with pure honey and offered to the parasitoid did not reduce parasitism performance or longevity of *Trichogramma evanescens* (Hymenoptera: Trichogrammatidae) adults. Although the methodology used in this study is different from ours, the results are similar and indicate the harmonious use of *Bt* and parasitoid wasps in an integrated pest management program. Our study, although not having evaluated parameters such as longevity, parasitism, emergence and lethal and sub-lethal effects, provides us with the information of extreme importance that the *Bt* does not alter the olfactory response of parasitoids to find the *H. zea* eggs.

Furthermore, the short-range detection of eggs by the parasitoid can be affected by agrochemicals, resulting in a parasitism decrease. The parasitoids often rely on chemical or color cues, so it is believed that chemicals may alter these cues (Vet and Dicke, 1992). However, various authors argue that organosynthetic insecticides cause a high degree of damage in the physiological and behavioral processes of wasps and they also can affect longevity, fertility and sex ratio of offspring (Khan et al., 2015; Delpuech et al., 2015; Li, 1994). As already discussed, certain microbial insecticides can affect negatively the parasitoid performance (Tounou et al., 2003; Potrich et al., 2009), indicating the need for more joint research looking on microorganisms and wasp parasitoid interactions.

From that, it is concluded that not all pest control tactics are compatible within the IPM and require specific studies of the wasp–biological product–host system, aiming conclusive answers to the use of control practices carried out in the field, since these tactics are used together and are not compatible, the biodiversity of non-target organisms may suffer serious further damage.

In conclusion, *T. pretiosum* is able to find the eggs of *H. zea* within short distances independently of some biological treatments. The fact that the eggs are bathed with the *Bt* strains or formulated products does not affect wasps' ability to locate them. It suggests that inundative programs including both *Bt* and *T. pretiosum* are compatible and could be used together without negative interference on their effectiveness.

The overall results of this study clarify some aspects about the simultaneous use of two biological control agents; *Bt* and *T. pretiosum*, in the management of *H. zea*, but that can be used to another pest species. However, more studies are needed to assess whether the wasp exhibits the same behavior when coming into direct contact with eggs of different species. Also, it is necessary to evaluate parameters such as emergence percentage and survival of the parasitoid offspring derived from eggs submitted to treatments with the strains and formulations used in this study. The results obtained can contribute to the pest management because they show the compatibility of two important biological control agents of *H. zea* in corn fields.

Conflicts of interest

The authors declare no conflicts of interest.

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References

- Amaro, J.T., Bueno, A.F., Poari-Fernandes, A.F., Neves, P.M.O.J., 2015. Selectivity of organic products to *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae). *Neotrop. Entomol.* 44, 489–497.
- Azizoglu, U., Yilmaz, S., Ayvaz, A., Karabörklü, S., 2015. Effects of *Bacillus thuringiensis* subsp. *kurstaki* HD1 spore–crystal mixture on the adults of egg parasitoid *Trichogramma evanescens* (Hymenoptera: Trichogrammatidae). *Biotechnol. Biotechnol. Equip.* 29, 653–658.
- Costa, M.A., Moscardini, V.F., Gontijo, P.C., Carvalho, G.A., Oliveira, R.L., Oliveira, H.N., 2014. Sublethal and transgenerational effects of insecticides in developing *Trichogramma galloi* (Hymenoptera: Trichogrammatidae). *Ecotoxicology* 23, 1399–1408.
- Crawley, J.M., 2013. *The R Book*, second ed. John Wiley & Sons, Chichester.
- Cusumano, A., Weldegergis, B.T., Colazza, S., Dicke, M., Fatouros, N.E., 2015. Attraction of egg-killing parasitoids toward induced plant volatiles in a multi-herbivore context. *Oecologia* 179, 163–174.
- Delpuech, J.M., Bussod, S., Amar, A., 2015. The sublethal effects of endosulfan on the circadian rhythms and locomotor activity of two sympatric parasitoid species. *Chemosphere* 132, 200–205.
- Fatouros, N., Bukovinszki, K.G., Dicke, M., Hilker, M., 2007. The response specificity of *Trichogramma* egg parasitoids towards infochemicals during host location. *J. Insect. Behav.* 20, 53–65.
- Fatouros, N.E., Dicke, M., Mumm, R., Meiners, T., Hilker, M., 2008. Foraging behavior of egg parasitoids exploiting chemical information. *Behav. Ecol.* 19, 677–689.
- Fatouros, N.E., Kiss, G.B., Kalkers, L.A., Gamborena, R.S., Dicke, M., Hilker, M., 2005. Oviposition-induced plant cues: do they arrest *Trichogramma* wasps during host location. *Entomol. Exp. Appl.* 115, 207–215.
- Gazit, Y., Lewis, W.J., Tumlinson, J.H., 1996. Arrestment of *Telenomus remus* (Hymenoptera: Scelionidae) by a kairomone associated with eggs of its host *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Biolog. Control.* 6, 283–290.
- Glare, T.R., O'Callaghan, M., 2000. *Bacillus thuringiensis* Biology, Ecology and Safety. John Wiley & Sons, Chichester, pp. 350.
- Greene, G.L., Leppla, N.C., Dickerson, W.A., 1976. Velvetbean caterpillar: a rearing procedure and artificial medium. *J. Chem. Ecol.* 69, 487–488.
- Hassan, S.A., 1993. The mass rearing and utilization of *Trichogramma* to control lepidopterous pests: achievements and outlook. *J. Pest. Sci.* 37, 387–391.
- Hilker, M., Fatouros, N.E., 2015. Plant responses to insect egg deposition. *Annu. Rev. Entomol. Annu. Rev. Entomol.* 60, 493–515.
- Khan, M.A., Khan, H., Robertson, J.R., 2015. Lethal and behavioral effects of selected novel pesticides on adults of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae). *Pest Manag. Sci.* 71, 1640–1648.
- Li, L.Y., 1994. Worldwide use of trichogramma for biological control on different crops: a survey. In: Wajnberg, E., Hassan, S.A. (Eds.), Reference to a Chapter in an Edited Book: Biological Control with Egg Parasitoids. CAB International, Wallingford, UK, pp. 37–53.
- Li, W., Zhang, P., Zhang, J., Lina, W., Lua, Y., Gaob, Y., 2015. Acute and sublethal effects of neonicotinoids and pymetrozine on an important egg parasitoid *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae). *Biocontrol. Sci. Technol.* 25, 121–131.
- Luckmann, D., Gouveia, A., Potrich, M., Silva, E.R.L., Puret, B., Dallacort, S., Gonçalves, T.E., 2014. Seletividade de produtos naturais comerciais a *Trichogramma pretiosum* (Riley, 1879) (Hymenoptera: Trichogrammatidae). *Rev. Ceres* 61, 924–931.
- Mutyambai, D.M., Bruce, T.J., Midega, C.A., Woodcock, C.M., Caulfield, J.C., Van Den, B.J., Pickett, J.A., Khan, Z.R., 2015. Responses of parasitoids to volatiles induced by chilo partellus oviposition on teosinte, a wild ancestor of maize. *J. Chem. Ecol.* 41, 323–329.
- Noldus, L.P.J., van Lenteren, J.C., 1985. Kairomones for the egg parasite *Trichogramma evanescens* Westwood. Effect of volatile substances produced by two of its hosts, *Pieris brassicae* L. and *Mamestra brassicae* L. *J. Chem. Ecol.* 11, 192–781.
- Pedigo, L.P., Rice, M.E., 2009. *Entomology and Pest Management*, sixth ed. Pearson, Columbus, Ohio.
- Pires, C., Sujii, E.R., Borges, M., Schmidt, F.V.G., Zarbin, P.H.G., Azevedo, V.C.R., Lacerda, A.L., Pantaleão, D., 2001. Ação caioromonal de componentes do feromônio de alarme do percevejo verde pequeno da soja *Piezodorus guildinii* sobre o parasitoide de ovos *Telenomus podisi*. *EMBRAPA*, Brasília, pp. 22.

- Potrich, M., Alves, L.F.A., Haas, J., Silva, E.R.L., Daros, A., Pietrowski, V., Neves, P.M.O.J., 2009. Seletividade de *Beauveria bassiana* e *Metarhizium anisopliae* a *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae). *Neotrop. Entomol.* 38, 822–826.
- R Development Core Team, 2010. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, URL <http://www.Rproject.org/>.
- Raymond, B., Wright, D.J., Crickmore, N., Bonsall, M.B., 2013. The impact of strain diversity and mixed infections on the evolution of resistance to *Bacillus thuringiensis*. *Proc. R. Soc. B* 280, 20131497.
- Sayed, S.M., Elsayed, G., Mahmoud, S.F., Elzahrany, O.M., 2015. Efficacy of *Bacillus thuringiensis* and indigenous *Trichogramma turkistanica* for controlling lepidopterous pests on Taify Pomegranate Fruits. *Afr. Entomol.* 23, 443–450.
- Smith, S.M., 1996. Biological control with *Trichogramma*: advances, successes, and potential of their use. *Annu. Rev. Entomol.* 41, 375–406.
- Souza, J.R., Carvalho, G.A., Moura, A.P., Couto, M.H.G., Maia, J.B., 2014. Toxicity of some insecticides used in maize crop on *Trichogramma pretiosum* (Hymenoptera Trichogrammatidae) immature stages. *Chilean J. Agric. Res.* 74, 234–239.
- Suverkropp, B.P., Bigler, F., van Lenteren, J.C., 2009. Dispersal behaviour of *Trichogramma brassicae* in maize fields. *Bull. Insectol.* 62, 113–120.
- Tabashnik, B.E., Zhang, M., Fabrick, J.A., Wu, Y., Gao, M., Huang, F., Wei, J., Zhang, J., Yelich, A., Unnithan, G.C., Bravo, A., Soberón, M., Carrière, Y., Li, X., 2015. Dual mode of action of Bt proteins: protoxin efficacy against resistant insects. *Nature* 5, 15107.
- Tounou, A.K., Agboka, K., Poehling, H.M., Raupach, K., Langewald, J., Zimmermann, G., Borgemeister, C., 2003. Evaluation of the entomopathogenic fungi *Metarhiziumanisopliae* and *Paecilomycesfumosoroseus* (Deuteromycotina: Hyphomycetes) for control of the green leafhopper *Empoasca decipiens* (Homoptera: Cicadellidae) and potential side effects on the egg parasitoid *Anagrusatomus* (Hymenoptera: Mymaridae). *Environ. Sci. Technol.* 13, 715–728.
- Vaez, N., Iranipour, S., Hejaz, M.J., 2013. Effect of treating eggs of cotton bollworm with *Bacillus thuringiensis* Berliner on functional response of *Trichogramma brassicae* Bezdenko. *Arch. Phytopathology Plant Protect.* 46, 2501–2511.
- Valicente, F.H., Barreto, M.R., 2003. *Bacillus thuringiensis* survey in Brazil: geographical distribution and insecticidal activity against *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). *Neotrop. Entomol.* 32, 639–644.
- Vet, L.E.M., Dicke, M., 1992. Ecology of infochemical use by natural enemies in a tritrophic context. *Annu. Rev. Entomol.* 37, 141–172.
- Vianna, U.R., Pratisoli, D., Zanuncio, J.C., Lima, E.R., Brunner, J., Pereira, F.F., Serrão, J.E., 2009. Insecticide toxicity to *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) females and effect on descendant generation. *Ecotoxicology* 18, 180–186.
- Vilela, M., Mendes, S.M., Valicente, F.H., Carvalho, S.S.S., Santos, A.E., Santos, C.A., Araújo, O.G., Barbosa, T.A.N., Carvalho, E.A.R., Costa, V.H.D., 2014. Metodologia para Criação e Manutenção de *Helicoverpa armigera* em Laboratório. *Circular técnica*, ISSN 1679-1150.
- Vinson, S.B., 1984. Parasitoid–host relationships. In: Bell, W.J., Cardé, R.T. (Eds.), *Chemical Ecology of Insects*. Chapman and Hall, London, pp. 205–233.
- Vinson, S.B., 1998. The general host selection behavior of parasitoid Hymenoptera and a comparison of initial strategies utilized by larvaphagous and oophagous species. *Biol. Control.* 11, 79–96.
- Wang, Y., Wu, C., Cang, T., Yang, L., Yu, W., Zhao, X., Wang, Q., Cai, L., 2014. Toxicity risk of insecticides to the insect egg parasitoid *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae). *Pest Manag. Sci.* 70, 398–404.
- 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 Sci.* 24, 599–612.
- Xu, J., Huigens, M.E., Orr, D., Groot, A.T., 2014. Differential response of *Trichogramma* wasps to extreme sex pheromone types of the noctuid moth *Heliothis virescens*. *Ecol. Entomol.* 39, 627–636.