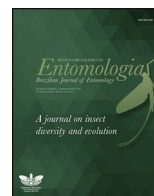




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Biological Control and Crop Protection

Selectivity of different biological products to the egg parasitoid *Telenomus remus* (Hymenoptera: Platygasteridae)



Junio Tavares Amaro^a, Adeney de Freitas Bueno^{b,*}, Pedro Manuel Oliveira Janeiro Neves^a, Débora Mello da Silva^c, Aline Pomari-Fernandes^d, Bruna Magda Favetti^c

^a Universidade Estadual de Londrina, Departamento de Agronomia, Londrina, PR, Brazil

^b Empresa Brasileira de Pesquisa Agropecuária – Embrapa Soja, Londrina, PR, Brazil

^c Instituto Agronômico do Paraná, Londrina, PR, Brazil

^d Universidade Federal da Fronteira Sul, Laranjeiras do Sul, PR, Brazil

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ABSTRACT

The selectivity of five entomopathogens and a chemical insecticide (positive control) to pupae and adults of the egg parasitoid *Telenomus remus* (Nixon) was evaluated in the laboratory under controlled environmental conditions according to protocols established by the International Organization for Biological Control. *Baculovirus anticarsia* (*Baculovirus* AEE[®]), *Bacillus thuringiensis* var. *kurstaki* (*Thuricide*[®]), *Bacillus thuringiensis* var. *aizawai* (*Agree*[®]), *Beauveria bassiana* (*Boveril*[®]), *Metarhizium anisopliae* (*Metarril*[®]) and *Trichoderma harzianum* (*Trichodermil*[®]) are harmless to *T. remus* pupae, and adults. Thus, our results suggest that the insect control strategies applied here are compatible since entomopathogens were classified as harmless to *T. remus* in most examined cases and therefore facilitate a joint application to control different pests. *Bacillus thuringiensis* var. *kurstaki* (*Dipel*[®]), despite being classified as slightly harmful in some of the evaluations, can still be considered compatible for use together with *T. remus*, especially when compared with chemical insecticides such as chlorpyrifos that might be considered harmful to the parasitoid survival.

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Egg parasitoids are biological control agents noteworthy for controlling pests at egg stage, before any injury is caused to infested plants (Figueiredo, 1998). But these biological control agents might suffer from several important undesirable effects when using non-selective pesticides in agriculture (Carmo et al., 2010), including chemical and biological products (Magalhães et al., 1998). In order to avoid these side effects and to maintain medium and long-term agricultural sustainability, the adoption of integrated pest management (IPM) is recommended (Bueno and Bueno, 2012). The most suitable products for IPM are those that combine optimal control of target pests with minimal impact on the activity of natural enemies (Bueno et al., 2017).

Telenomus remus (Nixon, 1937) (Hymenoptera: Platygasteridae) parasitizes eggs of several species of the genus *Spodoptera* such as *Spodoptera frugiperda* (J.E. Smith, 1797), *Spodoptera cosmioides* (Walker, 1858), *Spodoptera eridania* (Cramer, 1782) and *Spodoptera*

albula (Walker, 1857) (Lepidoptera: Noctuidae) (Pomari et al., 2012). However, other pest species may occur together with *Spodoptera* spp. in the field, requiring additional control strategies that might be combined with *T. remus* release. Thus, for the combined use of entomopathogens and parasitoids in IPM strategies (Magalhães et al., 1998) it is of theoretical and practical interest to study the selectivity and/or possible harmful non-target effects of entomopathogens on the efficiency of *T. remus*. Therefore, our study aimed to assess the selectivity of different biological products to the egg parasitoid *T. remus*. Four different bioassays were performed to study the effects of pesticides when applied on host eggs before and after parasitism in order to analyze direct effects as well as a possible repellent effect to parasitism, caused by entomopathogens.

The four bioassays were conducted independently according to protocols established by the International Organization for Biological Control (IOBC) (Hassan, 1992), adapted for *T. remus* (Carmo et al., 2010) in a completely randomized design under controlled laboratory conditions [25 ± 2 °C, 70 ± 10% relative humidity (RH) and a 14-h photoperiod] with five replicates per treatment. *Spodoptera*

* Corresponding author.

E-mail: adeney.bueno@embrapa.br (A.F. Bueno).

Table 1
Classification of selectivity of different biological products to *Telenomus remus* according to the “International Organisation for Biological Control” (IOBC) in different bioassays one and two days after emergence (DAE) of adults or days after spraying (DAS).

Treatment a.i. 100L ⁻¹ H ₂ O	Pupae		DAE/DAS									
	EP ^a	C ^c	1		2		1		2			
			E ^b	C	E	C	E	C	E	C		
	Bioassay 1 ^d				Bioassay 2 ^d							
AgMNPV 1.4 × 10 ¹¹ PIB	0	1	3	1	0	1	0	1	21	1		
<i>Bt var. kurstaki</i> 9.6 × 10 ⁹ IU	0	1	11	1	10	1	0	1	17	1		
<i>Bt var. aizawai</i> 5 × 10 ⁹ IU	0	1	16	1	23	1	13	1	19	1		
<i>Bt var. kurstaki</i> 6.2 × 10 ⁹ IU	1	1	6	1	55	2	0	1	12	1		
<i>B. bassiana</i> 1 × 10 ¹³ conidia	0	1	2	1	2	1	21	1	6	1		
<i>M. anisopliae</i> 1.6 × 10 ¹² conidia	1	1	6	1	0	1	0	1	11	1		
<i>T. harzianum</i> 5 × 10 ¹² conidia	2	1	28	1	0	1	0	1	9	1		
Chlorpyrifos 240 g	99	4	100	4	100	4	100	4	100	4		
	Bioassay 3 ^e				Bioassay 4 ^e							
AgMNPV 1.4 × 10 ¹¹ PIB	0	1	6	1	5	1	25	1				
<i>Bt var. kurstaki</i> 9.6 × 10 ⁹ IU	0	1	0	1	0	1	6	1				
<i>Bt var. aizawai</i> 5 × 10 ⁹ IU	22	1	0	1	4	1	0	1				
<i>Bt var. kurstaki</i> 6.2 × 10 ⁹ IU	5	1	0	1	0	1	77	2				
<i>B. bassiana</i> 1 × 10 ¹³ conidia	0	1	0	1	0	1	0	1				
<i>M. anisopliae</i> 1.6 × 10 ¹² conidia	0	1	6	1	0	1	0	1				
<i>T. harzianum</i> 5 × 10 ¹² conidia	0	1	0	1	0	1	0	1				
Chlorpyrifos 240 g	75	2	99.8	4	89	3	100	4				

^a EP (percentage of reduction of adult emergence) = (1 – treatment adult emergence/control adult emergence) × 100.

^b E (percentage of reduction of parasitism) = (1 – treatment parasitism/control parasitism) × 100 (Hassan et al., 1985).

^c Classification: class 1 = harmless (E/EP < 30%), class 2 = slightly harmful (30% ≤ E/EP < 80), class 3 = moderately harmful (80% ≤ E/EP < 99), class 4 = harmful (E/EP ≥ 99%).

^d Bioassays 1 (sprayed pupae) and 2 (sprayed contact surface).

^e Bioassays 3 (choice) and 4 (no-choice) with egg spraying.

frugiperda eggs and *T. remus* specimens used in this study were reared at Embrapa Soybean laboratories according to the methodology described by Pomari et al. (2012).

Bioassay 1 evaluates the selectivity of entomopathogens sprayed on *T. remus* pupae. Cards (3 cm²) with approximately 150 *S. frugiperda* eggs parasitized by *T. remus* at pupal stage [11 days after parasitism at 25 °C under a 14/10 photoperiod (Pomari et al., 2012)] were sprayed with entomopathogen suspensions or with the positive (chlorpyrifos) or negative control (water) treatments. Afterwards the sprayed eggs were maintained at 25 ± 2 °C and 70 ± 10% relative humidity (RH) for approximately 2 h under constant illumination to remove excess moisture. Next, the cards were kept in cages until the emergence of adults, which were fed honey. Cards with unsprayed *S. frugiperda* eggs (±400 eggs, 24 h old at most) as well as a drop of honey were provided on the first and second day after adult emergence. On the third day, the cards were stored in plastic bags until evaluation.

Bioassay 2 evaluates the selectivity of entomopathogens sprayed on *T. remus* adults. *Spodoptera frugiperda* eggs in Duran tubes (emergence tubes) (±250 eggs) were parasitized by *T. remus* and then sealed with plastic film and stored in a controlled environment until parasitoid emergence. After adult emergence, glass Petri dishes (13 cm × 13 cm) were sprayed with the treatments to be tested (Table 1). Next, these Petri dishes were fixed in aluminum frames, applying a circulating air flow forced by an exhaust according to adopted IOBC protocol (Hassan, 1992). Then the emergence tubes were connected to the emergence holes (Carmo et al., 2010). On the first and second day after releasing the adults from the cages, they were provided with *S. frugiperda* eggs (cards with ±400 eggs) and droplets of honey. These cards were removed from the cages on the third day, placed in plastic bags and stored in a controlled environment until parasitoid emergence for later evaluation.

Bioassay 3 evaluates the selectivity of entomopathogens sprayed on host eggs in choice tests for *T. remus* parasitism. Cards (3 cm²) with approximately 150 *S. frugiperda* eggs were sprayed with the tested treatments (Table 1). Next, the cards with treated

eggs were inserted inside cages with circulating air flow (Hassan, 1992). Afterwards, emergence tubes containing approximately 250 parasitoids were wrapped in aluminum foil and connected to the emergence holes of the cages (Carmo et al., 2010), thus providing a choice between treated and untreated (double choice) *S. frugiperda* eggs. After 24 h, two new cards with the same quantity of recently sprayed eggs and the same treatments were also inserted into the test cages. These cards were removed from the cages on the third experimental day, placed in transparent plastic bags and stored in a controlled environment until parasitoid emergence for later evaluation.

Bioassay 4 evaluates the selectivity of entomopathogens sprayed on host eggs in no-choice tests for *T. remus* parasitism. Similar to bioassay 3, the cards containing the eggs were inserted into cages, but without adding a control to the cages. Each treatment was inserted into independent cages. The final steps of this bioassay were the same as in bioassay 3.

In all four bioassays, adult emergence from pupae and adult parasitism were evaluated. The effect of each treatment on *T. remus* was determined by comparison with a negative control (distilled water) and calculated using the formula proposed by Hassan et al. (1985): E% = (1 – parasitism in the treatment/parasitism in the control) × 100 for adult assays and EP% = (1 – adult emergence from sprayed pupae/adult emergence from pupae treated with the control) × 100 for pupa assays. Treatments were classified as follows: class 1 = harmless (E/EP < 30%), class 2 = slightly harmful (30% ≤ E/EP < 80%), class 3 = moderately harmful (80% ≤ E/EP < 99%), class 4 = harmful (E/EP ≥ 99%).

In bioassay 1, adult emergence from pupae sprayed with the selected entomopathogens indicated that all biological products tested on *T. remus* pupae were classified as harmless (class 1), while the chlorpyrifos treatment was classified as harmful (class 4) (Table 1). On the first day after adults emerged from the sprayed pupae, none of the biological products had a discernible effect on parasitism of adults emerged from sprayed pupae. Different results were observed on the second day after adult emergence

from the sprayed pupae (2 DAE). Of all treatments involving entomopathogens, only the treatment with *B. thuringiensis* var. *kurstaki* 6.2×10^9 IU 100L^{-1} of water reduced parasitism to a level sufficient to be classified as slightly harmful (class 2). All other biological products were classified as harmless (class 1) (Table 1). Despite the emergence of a few adults from the chlorpyrifos-sprayed pupae, parasitism was not observed (1 or 2 DAE). This treatment was therefore classified as harmful (class 4) (Table 1).

In bioassay 2, all entomopathogen products sprayed on *T. remus* adults were classified as harmless on both days of the evaluation and for both variables analyzed (Table 1). No parasitism occurred in the chlorpyrifos treatment on any day of the experiment. Therefore, this chemical was classified as harmful (class 4) (Table 1).

In bioassay 3, given that the entomopathogens impacted *T. remus* parasitism only slightly, all of the entomopathogens were still classified as harmless (class 1) 1 and 2 days after spraying (DAS) (Table 1). Chlorpyrifos treatment was classified as slightly harmful (class 2) 1 DAS, and as harmful (class 4) 2 DAS (Table 1).

In bioassay 4, all of the entomopathogens sprayed on host eggs in no-choice tests for *T. remus* parasitism were classified as harmless except for *B. thuringiensis* var. *kurstaki* (6.2×10^9 IU), which was classified as slightly harmful (class 2) (Table 1). Chlorpyrifos was considered as moderately harmful (class 3) and harmful (class 4) 1 DAS and 2 DAS, respectively (Table 1).

Adult emergence from the pupae sprayed with different treatments was unaffected by the evaluated entomopathogens. In addition, the biological products did not alter the ability of the offspring of sprayed pupae to parasitize host eggs two days after parasitoid emergence, with the exception of those treated with *B. thuringiensis* var. *kurstaki* (6.2×10^9 IU). This entomopathogen was slightly harmful 2 DAE, directly affecting the number of parasitized eggs. This reduced value may be due to contamination of the female parasitoids' ovipositors during parasitism. By introducing their ovipositors into treated eggs, the female parasitoids were most likely contaminated with the product. Because *Bacillus thuringiensis* is a rapidly growing pathogen *in vitro* (Habib and Andrade, 1998), it may have become attached to the ovipositors. This most likely prevented parasitism on the second day of parasitism in this bioassay.

It is important to note that at the pupal stage, the parasitoid is protected within the host egg, and therefore this developmental phase is considered more resistant to toxic action than the free-living adult phase, which is usually more sensitive to agricultural chemicals (Hassan, 1992).

When adult parasitoids were exposed to the treatments sprayed on the walking surface, parasitism and viability of *S. frugiperda* eggs exposed to the parasitoids did not differ from the control. These results indicate that either the entomopathogens are unable to cause disease in *T. remus* adults via contact when walking on a treated surface, or that the resultant mortality was not sufficient to significantly reduce parasitism, considering that each female can parasitize up to 270 eggs (Morales et al., 2000).

In bioassay 4 (no-choice), *B. thuringiensis* var. *kurstaki* (6.2×10^9 IU) negatively impacted parasitism. The fact that this treatment had a negative effect on parasitism in two of the experiments may be explained by penetration of the egg by a contaminated ovipositor of the parasitoid and the bacterium's ability to grow *in vitro*. Although the parasitism index in this treatment was lower compared with the other tested entomopathogens, egg viability remained unaltered, exhibiting values higher than 80% in all bioassays. Thus, it is likely that *B. thuringiensis* var. *kurstaki* (6.2×10^9 IU) acts directly on *T. remus* adults, reducing their potential for parasitism.

These results are important because under the field conditions in which the biological product would be sprayed (tested together with the mass release of *T. remus* in the present work), the entomopathogens would control the caterpillars, and the parasitoid would prevent new *S. frugiperda* caterpillars from hatching. Thus, there is no problem of incompatibility between these control agents employed for different biological targets in the field when integrating control strategies under the concept of IPM. Our results suggest that the insect control strategies applied here are compatible since entomopathogens were classified as harmless to *T. remus* in most examined cases and therefore facilitate a joint application to control different pests. *Bacillus thuringiensis* var. *kurstaki* (Dipel®), despite being classified as slightly harmful in some of the evaluations, can still be considered compatible for use together with *T. remus*, especially when compared with chemical insecticides such as chlorpyrifos that might be considered harmful to the parasitoid survival.

Conflicts of interest

The authors declare no conflicts of interest.

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