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BIOLOGICAL CONTROL

Selectivity of Organic Products to *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae)

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Abstract
The selectivity of various entomopathogens and one insecticide (chlorpyrifos = positive control) to *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) was evaluated in the laboratory, using the protocol established by the Working Group on "Pesticides and Beneficial Organisms" of the IOBC. The evaluated parameters were parasitism (%), adult emergence (%), and product repellency to the parasitoid when sprayed on host eggs prior to parasitism (free-choice and no-choice tests). Most of the studied entomopathogens (*Bacillus thuringiensis* var. *kurstaki*, *Bacillus thuringiensis* var. *aizawai*, *Beauveria bassiana*, *Metarhizium anisopliae*, and *Trichoderma harzianum*) had no effects on biological parameters and were classified as harmless to *T. pretiosum*. Emergence of parasitoids (progeny viability) was reduced, but remained above 90%, when host eggs were sprayed with *Baculovirus anticarsia* prior to parasitism in the free-choice test, and *B. anticarsia* was therefore considered harmless. Chlorpyrifos (positive control) caused high adult parasitoid mortality in all bioassays. While *T. pretiosum* and the tested entomopathogens may be used simultaneously in integrated pest management programs, the use of chlorpyrifos should be avoided.

Introduction

Egg parasitoids of the genus *Trichogramma* are used as biological control agents in agriculture worldwide due to the simplicity of production and their efficient control of pests (Parra & Zucchi 2004). They have been used in Latin America to fight pests which damage crops such as cotton, sorghum, soybean, and sugarcane. In Mexico, for example, about 1.5 million hectares is treated with *Trichogramma* spp. They have also been applied to large areas of Colombia and Cuba but limited in other Latin American countries partially due to the lack of selective pesticides that ensure their preservation (Van Lenteren & Bueno 2003). It is important to emphasize that *Trichogramma* is a biological control agent primarily of eggs of lepidopterans, but as agroecosystems are represented by a number of different pests and that damage can still be induced by immatures escaping egg parasitization, the use of integrated strategies for efficient pest control is still mandatory (Monnerat *et al* 2007).

The spraying of synthetic insecticides is the most common control method for Lepidoptera management in agriculture worldwide. It may have various unwanted effects on the agroecosystem and may reduce or disable biological control by egg parasitoids (Bueno *et al* 2011). However, a combination of various control methods within integrated pest management is possible and has been recommended. The integration of different control methods in sustainable agriculture allows the suppression of agricultural pest populations below the threshold of economic damage, thereby preserving the environment and benefiting beneficial arthropods (Van Lenteren & Bueno 2003).

Entomopathogens are another control method that may be used in the management of various agricultural pests (Magalhães *et al* 1998). Entomopathogens of agricultural importance include *Baculovirus anticarsia* (AgMNPV), sprayed to control the velvetbean caterpillar *Anticarsia gemmatalis* Hübner (Lepidoptera: Eribidae) in soybean (Hoffmann-Campo *et al* 2003); *Bacillus thuringiensis*, which causes mortality in more than 1000 species of insects from various orders (Glare & O'Callaghan 2000); the fungus *Beauveria bassiana,* which has a wide geographic distribution and causes disease in orders including Lepidoptera, Coleoptera, Hemiptera, Diptera, Hymenoptera, and Orthoptera (Alves 1998); *Metarhizium anisopliae*, one of the most important species of entomopathogenic fungi that may infect more than 300 species of insects from various orders (Alves 1998); and *Trichoderma harzianum*, a necrotrophic microorganism that is effective in controlling some phytopathogenic fungi (Melo 1998), but that may also have adverse effects on insects as previously reported for *Myzus persicae* Sulzer (Homoptera: Aphididae) (Ganassi *et al* 2009), *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) (El-Katatny 2010), and *Gryllotalpa gryllotalpa* L. (Orthoptera: Gryllotalpidae) (Veena-Bhamrah 2007).

Notwithstanding the efficiency of these entomopathogens, they may also negatively impact the performance and/or efficiency of egg parasitoids, or even infect them directly (Magalhães *et al* 1998). Therefore, selectivity is crucial for a harmonious integration of these different management tools in integrated pest management (IPM) approaches aimed at reducing the population of harmful insects with the fewest possible changes in other components of the agroecosystem and the environment in general. Therefore, only insecticides with some degree of selectivity may be used together with other adopted IPM methods (Van Den Bosch *et al* 1982), including biological control by *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae).

Thus, selectivity and/or possible harmful nontarget effects of entomopathogens on the efficiency of egg parasitoids must be taken into account when defining the best biological control strategies. In this context, our study aimed to assess the selectivity of different entomopathogens to the egg parasitoid *T. pretiosum*. Four different bioassays were performed to study pesticide effects when applied on host eggs before and after parasitism with the aim to analyze the direct effects as well as possible repellence to parasitism, triggered by entomopathogens.

Material and Methods

Four different bioassays were conducted in the laboratory to assess the impact of different entomopathogens (Table 1) on pupae and adults of *T. pretiosum*, as well on parasitization of treated host eggs under free-choice and no-choice conditions. Trials were carried out under controlled conditions (25±2°C; 70±10% RH; 14L:10D h photoperiod) with five replicates in a completely randomized design, in accordance with the protocols proposed by the "International Organization for Biological Control" (IOBC) (Hassan 1992, Hassan *et al* 1985, Manzoni *et al* 2007). Each replicate consisted of a cage with more than 200 adults for which all evaluations were performed.

Parasitoid and host colonies

Eggs of *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) used as hosts and specimens of the egg parasitoid *Tg. pretiosum* were obtained from insect colonies of Embrapa Soybean (Brazilian Agricultural Research Corporation—Soybean), where they had been reared according to Parra (1997) for nearly 6 years.

Bioassay 1: impact of pupal exposure to entomopathogens

The selectivity of entomopathogens to *T. pretiosum* pupae was tested according to the standard protocols established by the IOBC (Hassan 1992, Hassan *et al* 1995, Manzoni *et al* 2007). Briefly, 3-cm^2 cards (1 card per replicate) holding approximately 500 0–24-h-old eggs of *A. kuehniella* were exposed to newly emerged (0–24-h-old females), mated *T. pretiosum* females. Parasitization was allowed for 24 h. Subsequently, the cards were transferred to vials and kept under controlled conditions until parasitoid pupation, as indicated by the darkening of the host egg (nearly 192 h after parasitization) (Cônsoli *et al* 1999). Parasitized host egg containing the parasitoid pupae was then sprayed with solutions of the different entomopathogens tested (Table 1) as specified in Manzoni *et al* (2007). Treatment with chlorpyrifos was chosen as the positive control according to Bueno *et al* (2008).

Egg spraying was performed using a Potter spray tower calibrated to deposit a volume to correspond to 1.25± 0.25 mg cm^{-2}, in accordance with the norms established by the IOBC (Hassan 1992, Hassan *et al* 1995, Manzoni *et al* 2007). This volume was controlled by weighing the cards with the parasitoid pupae before and after the application of treatments. The sprayed eggs with *Tg. pretiosum* pupae were kept at 25±2°C and 70±10% RH for approximately 2 h to remove excessive moisture. Subsequently, the treated cards were placed into cages made of glass plates (13×13 cm) prepared according to the method proposed by Hassan (1992) where they remained until the emergence of adults, which were then fed with honey.

Table 1 Commercial products and commercial doses evaluated for selectivity to the egg parasitoid *Trichogramma pretiosum* under controlled laboratory conditions.

Commercial product (c.p.)	Formulation	Active ingredient (a.i.)	a.i. 100 L^{-1} H_2O
Water	–	Distilled water	–
Baculovirus AEE®	0.6 WP	AgMNPV	1.4×10^{11} PIB
Thuricide®	3.2 WP	*Bacillus thuringiensis* var. *kurstaki*	9.6×10^{9} IU
Agree®	50 WP	*Bacillus thuringiensis* var. *aizawai*	5×10^{9} IU
Dipel®	3.2 WP	*Bacillus thuringiensis* var. *kurstaki*	6.2×10^{9} IU
Boveril®	5 WP	*Beauveria bassiana*	1×10^{13} conidia
Metarril®	5 WP	*Metarhizium anisopliae*	1.6×10^{12} conidia
Trichodermil®	48 SC	*Trichoderma harzianum*	5×10^{12} conidia
Lorsban®	480 EC	Chlorpyrifos	240 g

PIB polyhedral inclusion bodies, *IU* international units, *WP* wettable powder.

After adult emergence, cards containing approximately 200 *A. kuehniella* eggs (less than 24 h after oviposition) were introduced into the cages (one card on the first day and a second card on the fifth day). A drop of honey was provided to the parasitoids on the first and the fifth day after the emergence of adults. Both cards remained in the cages for 6 days after adult emergence (one card remained for 5 days and the other for 1 day). On the sixth day after adult emergence, both cards were removed and stored in air-filled plastic bags under controlled conditions (25±2°C; 70±0% RH; 14L:10D h photoperiod) until assessment of the parasitoid biological parameters. Parasitoid host egg ratio used (1 female per 40 host eggs) results in egg parasitization in between 80 and 100% in the control treatment (water), essential to evaluate the impact of the pesticide on the parasitoid (Hassan *et al* 1985).

Adult emergence from sprayed parasitized host eggs was calculated by dividing the number of *A. kuehnilla* eggs with emerged parasitoid adults by the total number of parasitized eggs, multiplied by 100. The parasitization capacity of the F_o generation (adults originating from treated eggs containing the parasitoid at the pupal stage) and the transgenerational effects on the percentage of emergence of the F_1 generation (progeny of the treated generation) were determined using a stereomicroscope.

Bioassay 2: impact of adult exposure to the dry residue of entomopathogens

Duran tubes (emergence vials, 0.6 cm diameter×6 cm height) containing a droplet of honey and approximately 200 *A. kuehniella* eggs parasitized by newly emerged, mated *T. pretiosum* females were sealed with a plastic film and stored under controlled conditions (25±2°C; 70 ±10% RH; 14L:10D h photoperiod) until parasitoids emerged. After emergence, the glass plates (13×13 cm) used to make the cages were sprayed with suspensions of entomopathogens (a single entomopathogen per treatment, Table 1), using the Potter spray tower set to deposit a suspension volume to correspond to 1.25± 0.25 mg cm^{-2}. Following the application, plates were dried for 2 h at 25±2°C and 70±10% RH and subsequently fixed in aluminum frames in a flow of circulating air according to the method proposed by Hassan (1992). In the next step, 200 parasitoids were released into the cages with the treated glass plates (Hassan 1992).

Cards containing *A. kuehniella* eggs (approximately 200 eggs, less than 24 h after oviposition) with trickles of honey were provided 1 and 5 days after the release of adults into the cages. Both cards were removed 6 days after adult emergence (see also bioassay 1), placed in transparent air-filled plastic bags and stored under the controlled conditions earlier mentioned until the emergence of parasitoids for the subsequent evaluation of parasitism and progeny viability, as described in bioassay 1.

Bioassay 3: impact of host egg exposure to entomopathogens on parasitism by T. pretiosum *(free-choice test)*

Cards (3 cm^2) holding approximately 200 *A. kuehniella* eggs (less than 24 h after oviposition) were sprayed with entomopathogens as earlier described. Subsequently, individual cards with treated eggs were introduced into the cages using circulating airflow as proposed by Hassan (1992). Each cage was supplied with a card sprayed with a single entomopathogen and a card sprayed with water (control card), placed next to each other, and exposed to approximately 300 parasitoids (newly emerged, mated *T. pretiosum*). On the fifth day after the start of the bioassay, a second card with the same amount of eggs and recently sprayed with the same products was introduced into the test cage. Both cards were removed on the sixth day (see also bioassays 1 and 2) and stored for later evaluation as before (bioassay 1).

Bioassay 4: impact of host egg exposure to entomopathogens on parasitism by T. pretiosum *(no-choice test)*

No-choice tests were carried as in bioassay 3 with the exception that parasitoids were exposed to a single treatment in each cage.

Statistical analysis

Data obtained were subjected to exploratory analysis to evaluate normality assumptions for the residuals (Shapiro & Wilk 1965), homogeneity of variance between treatments (Burr & Foster 1972), and additivity of the model in order to be subjected to analysis of variance (ANOVA). Data not following normality assumptions or homogeneity of variance were transformed. Data on adult emergence (%) from treatments in which the egg host containing the parasitoid pupae was sprayed were arcsine $\sqrt{X/100}$ transformed before ANOVA. Means were compared using Tukey's HSD test (5% error probability) implemented in SAS (SAS Institute 2001).

In addition, the effect of each pesticide on *T. pretiosum* 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), and class 4 = harmful (E/EP≥99%).

Results

Bioassay 1: impact of pupal exposure to entomopathogens

The entomopathogens applied to host eggs containing *T. pretiosum* pupae inside had no effect on parasitoid emergence. Furthermore, parasitism and progeny viability of F_1 individuals sprayed as pupae with entomopathogens did not differ between treatments and control (water) on any evaluated day (Table 2). Therefore, all tested entomopathogens (Table 3) were classified as harmless (class 1, Hassan *et al* 1985) to the pupal stage of parasitoid development. Only chlorpyrifos (positive control) negatively affected parasitism and adult emergence with significant differences to the control and other treatments (Table 2). Therefore, this chemical was classified as moderately harmful (class 3) or harmful (class 4) to *T. pretiosum* pupae (Hassan 1985) (Table 3).

Bioassay 2: impact of adult exposure to the dry residue of entomopathogens

Parasitism and progeny viability of *T. pretiosum* on *A. kuehniella* eggs were assessed on the first and fifth days after the emergence of adults exposed to treatments by walking on the sprayed surface. None of the entomopathogen treatments differed from the control (Table 4) and, therefore, were all classified as harmless (class 1, Hassan 1985) on both evaluation days and for both variables analyzed (Table 3). Furthermore, parasitism did not occur in the chlorpyrifos treatment (positive control) at both periods analyzed (Table 2) as a consequence of the high mortality of adults caused by the insecticide. Therefore, this chemical was classified as harmful (class 4, Hassan 1985) (Table 3).

Bioassay 3: impact of host egg exposure to entomopathogens on parasitism by T. pretiosum *(free-choice test)*

When *T. pretiosum* adults were allowed to choose between cards sprayed either with a treatment or with water, we detected a significantly lower progeny viability (91.8%) on the first day after spraying (Table 5) only in the treatment with AgMNPV at 1.4×10^{11} polyhedral inclusion bodies (PIB). However, since this value (91.8%) was still higher than 90%, it can be considered satisfactory (Navarro 1998). Progeny viability and parasitism did not differ between the other evaluated entomopathogens and their controls (Table 5). Considering that the entomopathogens did not affect the parasitoid's choice of host eggs, they were all classified as harmless (class 1, Hassan 1985) (Table 3). Similar to the results of bioassays 1 and 2, when host eggs were treated with chlorpyrifos (positive control), the levels of parasitism and progeny viability were significantly lower than in the controls (Table 5) due to the high mortality of both adults and larvae caused by the insecticide. They were thus classified as moderately harmful (class 3, Hassan 1985) (Table 3).

Bioassay 4: impact of host egg exposure to entomopathogens on parasitism by T. pretiosum *(no-choice test)*

Parasitism by *T. pretiosum* was also evaluated in no-choice tests in which cards sprayed with each treatment (Table 1) were placed in separate cages. Progeny viability and parasitism did not differ significantly between the entomopathogens and control in the no-choice test on the first and fifth days after spraying (Table 6). According to these results, tested entomopathogens were classified as harmless (class 1), as in the free-choice test (Hassan 1985) (Table 3). Only chlorpyrifos (positive control) reduced

Table 2 Effects of exposure of parasitized host eggs at the pupal stage (bioassay 1) to entomopathogens on *Trichogramma pretiosum* emergence (%) and on the parasitism (%) and progeny survival (%) of adults emerged from exposed eggs at 1 and 5 days after emergence (DAE).

Treatment a.i. 100 L^{-1} H_2O	Sprayed pupae[a]	1 DAE[a]		5 DAE[a]	
	Adult emergence (%)	Parasitism (%)	Progeny viability (%)	Parasitism (%)	Progeny viability (%)
Water	92.54±1.11 a[b]	87.91±2.10 a	97.64±1.70 a	84.10±2.06 a	97.56±0.86 a
AgMNPV 1.4×10^{11} PIB	92.87±1.10 a	89.74±1.55 a	96.48±1.34 a	85.35±2.58 a	95.80±0.94 a
B. thuringiensis var. *kurstaki* 9.6×10^{9} IU	94.26±0.72 a	88.22±2.90 a	96.57±0.23 a	91.02±2.02 a	96.80±0.80 a
B. thuringiensis var. *aizawai* 5×10^{9} IU	94.21±1.40 a	87.48±3.85 a	94.45±0.47 a	88.28±3.02 a	95.92±1.86 a
B. thuringiensis var. *kurstaki* 6.2×10^{9} IU	92.83±0.47 a	88.67±2.90 a	96.93±1.27 a	87.03±1.35 a	97.10±0.83 a
B. bassiana 1×10^{13} conidia	93.13±1.60 a	92.95±2.56 a	96.67±0.58 a	80.60±4.63 a	95.80±1.43 a
M. anisopliae 1.6×10^{12} conidia	94.64±1.30 a	86.32±4.57 a	96.52±1.05 a	87.81±3.15 a	95.63±1.13 a
T. harzianum 5×10^{12} conidia	95.31±1.30 a	85.05±3.17 a	96.80±0.98 a	85.04±5.44 a	93.21±2.66 a
Chlorpyrifos 240 g	8.10±3.03 b	0.00±0.00 b	–	0.00±0.00 b	–
CV (%)	5.49	8.29	2.45	8.74	3.37
F	140.35	102.85	0.75	93.11	0.84
P	<0.0001	<0.0001	0.6361	<0.0001	0.56
$DF_{residual}$	36	36	32	35	31

CV coefficient of variation

[a] Means±standard error of means (SEM) in each column followed by the same letter did not differ from each other according to the Tukey test (5% probability).

[b] The original analyses were followed by analyses performed using arcsine $\sqrt{X/100}$ transformed data.

parasitism to 0% and was therefore classified as harmful (class 4, Hassan 1985) (Table 3).

Discussion

Emergence of *T. pretiosum* adults was not affected by exposure of parasitized hosts with the parasitoid at the pupal stage to the different entomopathogens tested (Table 1, bioassay 1), different to what has been reported by others for entomopathogens such as *B. bassiana* and *M. anisopliae* (Samuels *et al* 2002, Rampelotti *et al* 2007). The absence of an effect of entomopathogens on *T. pretiosum* pupae in our study may have resulted from the short time period (~24 h) between exposure to entomopathogens and parasitoid adult emergence, since the time required for fungi germination is at least 12 h for deuteromycetes, 18 h for *B. bassiana*, and 16 h for *M. anisopliae* (Alves 1998, Lomer *et al* 2001). Colonization requires additional time for appressorium formation and their penetration into the eggs (Alves 1998), which together with germination time might be surpassing the time period of 24 h between parasitoid pupal exposure and *T. pretiosum* adult emergence. This short time tested was important to evaluate any possible negative impact of entomopathogens on pupae, which is usually massive released close to the last 24 h before adult emergence. However, a negative effect of these entomopathogens on parasitism by *T. pretiosum* might be observed when entomopathogens are applied to parasitized host eggs at earlier stages of parasitoid development (natural occurrence), allowing enough time for infection and fungal appressorium formation and penetration into the infected egg, possibly killing any parasitoid developing inside that egg. Therefore, pupae (less than 24 h of adult emergence) and entomopathogens can be simultaneously used as applied biological control in IPM.

The parasitization capacity of females emerged from exposure of host eggs at their pupal stage may be reduced directly by pupae mortality or indirectly by adult-induced physiological and behavioral responses (Carmo *et al* 2010). However, the products did not cause a sufficient reduction of the *T. pretiosum* population required to trigger a reduction in parasitism, probably because there was not enough time for fungi to infect host eggs. Each *Trichogramma* sp. female may parasitize 20 to 120 eggs, depending on the host (Pinto 1997), which helps to explain the high parasitism observed in the F_1 generation.

Unlike fungi, viruses and bacteria must be ingested to have any effect on insects (Castro *et al* 1999, Copping & Menn 2000). *Trichogramma pretiosum* adults may ingest viruses or bacteria sprayed onto the host egg during emergence as the adult chews the host egg chorion on its way out. However, the failure to observe any lethal effects of the applied viruses and bacteria on the

Table 3 Classification of entomopathogen selectivity to *Trichogramma pretiosum* according to the "International Organization for Biological Control" (IOBC) in different bioassays and 6 days after emergence (DAE) of adults or days after spraying (DAS).

Treatment a.i. 100 L^{-1} H_2O	Pupae		DAE/DAS							
			1		5		1		5	
	EP[a]	C[b]	E[c]	C	E	C	E	C	E	C
	Bioassay 1[d]						Bioassay 2[d]			
AgMNPV 1.4×10^{11} PIB	0.4	1	0	1	0	1	0	1	4.8	1
B. thuringiensis var. *kurstaki* 9.6×10^{9} IU	1.8	1	0	1	0	1	1.3	1	6.3	1
B. thuringiensis var. *aizawai* 5×10^{9} IU	1.8	1	0.5	1	0	1	0	1	5.3	1
B. thuringiensis var. *kurstaki* 6.2×10^{9} IU	0.3	1	0	1	0	1	3.5	1	6.3	1
B. bassiana 1×10^{13} conidia	0.6	1	0	1	4.2	1	0	1	8.8	1
M. anisopliae 1.6×10^{12} conidia	2.3	1	1.8	1	0	1	2.5	1	6.1	1
T. harzianum 5×10^{12} conidia	3.0	1	3.2	1	0	1	1.8	1	10.3	1
Chlorpyrifos 240 g	91.3	3	100	4	100	4	100	4	100	4
	–	–	Bioassay 3[e]				Bioassay 4[e]			
AgMNPV 1.4×10^{11} PIB	–	–	1.9	1	12.9	1	5	1	25	1
B. thuringiensis var. *kurstaki* 9.6×10^{9} IU	–	–	2.4	1	6.8	1	0	1	6	1
B. thuringiensis var. *aizawai* 5×10^{9} IU	–	–	2.4	1	1.9	1	4	1	0	1
B. thuringiensis var. *kurstaki* 6.2×10^{9} IU	–	–	0.0	1	9.2	1	0	1	77	2
B. bassiana 1×10^{13} conidia	–	–	0.1	1	0.0	1	0	1	0	1
M. anisopliae 1.6×10^{12} conidia	–	–	4.4	1	0.9	1	0	1	0	1
T. harzianum 5×10^{12} conidia	–	–	2.7	1	3.9	1	0	1	0	1
Chlorpyrifos 240 g	–	–	97.8	3	–	–	100	4	100	4

[a] EP (Percent of reduction of adult emergence)=(1−Treatment adult emergence/Control adult emergence)×100).

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

[c] E (Percentage of reduction of parasitism)=(1−Treatment parasitism/Control parasitism)×100 (Hassan 1985).

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

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

Table 4 Effects of different entomopathogens on adults (bioassay 2) of *Trichogramma pretiosum* 1 and 5 days after the emergence (DAE) from treated eggs of the host *Anagasta kuehniella*.

Treatment a.i. 100 L^{-1} H_2O	1 DAE[a]		5 DAE[a]	
	Parasitism (%)	Progeny viability (%)	Parasitism (%)	Progeny viability (%)
Water	89.65±2.51 a	97.70±0.73 a	94.20±5.80 a	96.81±1.90 a
AgMNPV 1.4×10^{11} PIB	94.67±2.20 a	94.20±1.20 a	89.64±7.20 a	96.23±1.50 a
B. thuringiensis var. *kurstaki* 9.6×10^{9} IU	88.56±3.57 a	94.80±0.60 a	88.24±4.66 a	98.80±0.85 a
B. thuringiensis var. *aizawai* 5×10^{9} IU	93.20±3.82 a	95.26±2.08 a	89.17±5.03 a	97.82±1.12 a
B. thuringiensis var. *kurstaki* 6.2×10^{9} IU	86.54±2.36 a	96.24±1.41 a	88.31±4.30 a	96.75±2.08 a
B. bassiana 1×10^{13} conidia	91.36±3.12 a	94.14±1.89 a	85.91±6.16 a	97.22±0.80 a
M. anisopliae 1.6×10^{12} conidia	87.40±1.07 a	95.12±0.87 a	88.50±3.08 a	96.31±0.77 a
T. harzianum 5×10^{12} conidia	88.04±4.22 a	95.36±2.04 a	84.45±3.82 a	95.93±1.53 a
Chlorpyrifos 240 g	0.00±0.00 b	–	0.00±0.00 b	–
CV (%)	8.1	3.46	13.01	3.08
F	108.59	0.62	42.16	0.49
P	<0.0001	0.7349	<0.0001	0.83
$DF_{residual}$	34	31	33	30

[a] Means±SEM in each column followed by the same letter did not differ from each other according to the Tukey test (5% probability).

Table 5 Parasitism (%) of eggs of the host *Anagasta kuehniella* treated with entomopathogens by *Trichogramma pretiosum* 1 and 5 days after host treatment and parasitoid progeny viability (%) in free-choice tests (bioassay 3).

Treatment a.i. 100 L^{-1} H_2O	1 DAS		5 DAS	
	Parasitism (%)[a]	Progeny viability (%)[a]	Parasitism (%)[a]	Progeny viability (%)[a]
Water	90.1±0.9 a	95.0±0.6 a	81.0±4.2 a	95.0±0.7 a
AgMNPV 1.4×10^{11} PIB	88.4±1.3 a	91.8±1.1 b	70.5±3.3 a	95.3±1.0 a
CV	2.84	2.06	10.05	1.87
Water	87.9±2.6 a	92.4±2.1 a	80.8±4.3 a	93.2±2.3 a
B. thuringiensis var. *kurstaki* 9.6×10^{9} IU	85.8±1.1 a	90.9±2.0 a	75.3±0.5 a	94.1±4.6 a
CV	5.16	4.98	8.63	6.66
Water	89.3±2.4 a	93.7±1.1 a	83.7±1.8 a	95.2±2.0 a
B. thuringiensis var. *aizawai* 5×10^{9} IU	87.2±2.1 a	93.2±0.4 a	82.1±2.6 a	96.0±2.4 a
CV	5.74	1.67	5.35	4.64
Water	87.2±2.2 a	94.3±1.1 a	79.6±5.5 a	94.1±0.9 a
B. thuringiensis var. *kurstaki* 6.2×10^{9} IU	88.3±2.8 a	94.0±1.4 a	72.3±7.6 a	88.4±4.2 a
CV	6.54	2.98	17.55	6.69
Water	91.9±1.0 a	94.5±0.8 a	78.5±3.8 a	95.5±1.5 a
B. bassiana 1×10^{13} conidia	91.9±2.0 a	93.8±0.5 a	82.1±6.3 a	90.5±2.2 a
CV	3.88	1.63	11.30	3.48
Water	87.1±2.0 a	92.8±0.8 a	82.2±2.5 a	95.0±1.1 a
M. anisopliae 1.6×10^{12} conidia	83.2±1.4 a	91.4±1.2 a	81.4±1.7 a	95.1±0.9 a
CV	4.66	2.20	5.90	2.30
Water	87.7±3.3 a	91.0±2.0 a	85.2±1.5 a	93.9±1.1 a
T. harzianum 5×10^{12} conidia	85.3±2.6 a	90.4±2.9 a	81.8±2.7 a	96.4±0.3 a
CV	7.74	6.30	5.37	1.42
Water	78.8±7.6 a	54.2±2.6 a	–	–
Chlorpyrifos 240 g	1.7±1.7 b	0.0±0.0 b	–	–
CV	26.80	6.52	–	–

[a] Means±SEM in each column followed by the same letter for each comparison between sprayed and nonsprayed cards did not significantly differ from each other according to the *F* test (5% probability).

Table 6 Parasitism (%) of eggs of the host *Anagasta kuehniella* treated with entomopathogens by *Trichogramma pretiosum* 1 and 5 days after host treatment and parasitoid progeny viability (%) in no-choice tests (bioassay 4).

Treatment a.i. 100 L^{-1} H_2O	1 DAS		5 DAS	
	Parasitism (%)[a]	Progeny viability (%)[a]	Parasitism (%)[a]	Progeny viability (%)[a]
Water	85.5±1.1 a	95.0±1.0 a	80.7±2.8 a	95.4±0.3 a
AgMNPV 1.4×10^{11} PIB	87.2±0.8 a	97.6±0.8 a	81.2±5.1 a	97.6±0.6 a
B. thuringiensis var. *kurstaki* 9.6×10^{9} IU	84.5±2.4 a	95.1±1.0 a	87.0±3.0 a	96.5±1.7 a
B. thuringiensis var. *aizawai* 5×10^{9} IU	86.8±1.0 a	95.0±1.7 a	79.9±3.6 a	96.5±1.0 a
B. thuringiensis var. *kurstaki* 6.2×10^{9} IU	85.8±2.0 a	95.0±0.8 a	85.9±2.1 a	96.0±0.8 a
B. bassiana 1×10^{13} conidia	86.1±1.8 a	93.6±0.9 a	81.0±3.5 a	94.8±2.0 a
M. anisopliae 1.6×10^{12} conidia	84.5±2.3 a	93.4±1.4 a	83.7±2.3 a	94.9±1.7 a
T. harzianum 5×10^{12} conidia	89.5±1.6 a	94.5±0.6 a	77.0±5.3 a	96.5±1.6 a
Chlorpyrifos 240 g	0.0±0.0 b	–	0.0±0.0 b	–
CV	4.82	2.60	10.18	2.74
F	302.75	1.41	69.44	0.60
P	<0.0001	0.24	<0.0001	0.75
$DF_{residual}$	36	31	32	28

[a] Means±SEM in each column followed by the same letter did not differ from each other according to the Tukey test (5% probability).

parasitism obtained by F_1 descendants of treated pupae indicates that ingestion may not have occurred or that it occurred at a nonlethal dose. Furthermore, viruses in general are very specific (Moscardi 1998), explaining the noted selectivity.

Parasitism and progeny viability (= emergence of adults) were unaffected by entomopathogens when parasitoids entered in direct contact by exposure to treated surfaces (bioassay 2). The observed bacterial and viral selectivity may be explained because the tested entomopathogens are only harmful to insects when ingested (Copping & Menn 2000, Castro *et al* 1999). Ingestion of viruses or bacteria was unlikely because *T. pretiosum* adults walked on dry residues of the sprayed products. From all of the evaluated entomopathogens, only fungi can contaminate and infect insects by contact via conidia penetration through the insect cuticle (Alves 1998). Potrich *et al* (2009) observed mortality of *T. pretiosum* caused by *B. bassiana* and *M. anisopliae* by contact, although not at a significant rate, thus indicating that contamination and/or infection did not occur in our study or was insufficient to cause mortality of *T. pretiosum* adults.

Bioinsecticides may also influence the parasitoid host choice by modifying color, shape, odor, or behavior of the infected host, in addition to directly impact the insects (Magalhães *et al* 1998). In our study, parasitism and parasitoid progeny viability were not affected by the entomopathogens tested when evaluated in free- or no-choice tests. Only eggs sprayed with 1.4×10^{11} PIB AgMNPV in free-choice tests showed a reduced emergence of parasitoids than the control, although still higher than 90%, which is above the minimum quality standard (85%) of progeny viability (Navarro 1998). Therefore, this entomopathogen may still be considered selective to the parasitoid. These results indicate that host egg infection did not occur, even with a longer time period between spraying with an entomopathogen and parasitoid emergence, and therefore, no negative effect on *T. pretiosum* was found, similar to earlier reports (Potrich *et al* 2009).

The bioinsecticides *B. anticarsia* (Baculovirus AEE®), *B. thuringiensis* var. *kurstaki* (Thruricide®), *B. thuringiensis* var. *aizawai* (Agree®), *B. thuringiensis* var. *kurstaki* (Dipel®), *B. bassiana* (Boveril®), *M. anisopliae* (Metarril®), and *T. harzianum* (Trichodermil®) can be classified as selective to *T. pretiosum* pupae and adults, which is an indication that they may be used in conjunction with egg parasitoids without major negative effects on the studied parasitoid. In contrast, the insecticide chlorpyrifos is harmful, as it causes high mortality and it negatively affects the behavior of *T. pretiosum* in free-choice tests. Therefore, its use in IPM should be avoided and should be replaced by a more selective product to natural enemies whenever possible.

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