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# Effect of increasing rate of insecticides on its selectivity for *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae)

## Efeito do aumento de dose de inseticidas sobre sua seletividade para *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae)

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### Abstract

The present study evaluated the effect of different rates of chlorantraniliprole, chlorantraniliprole + lambda-cyhalothrin, spinosad, and chlorfenapyr on their selectivity for the pupae and adults of *Trichogramma pretiosum*. The negative effect of majority of the insecticides tested on most of the parasitoids was directly proportional to the rate of the insecticide applied. Chlorantraniliprole at a rate of 10 g a.i. ha<sup>-1</sup> was classified as harmless (class 1) to both pupae and adults of the parasitoid. The effect of chlorantraniliprole at a rate of 20 to 50 g a.i. ha<sup>-1</sup> varied from harmless (class 1) to slightly harmful (class 2) to the adults of *T. pretiosum*. Chlorantraniliprole 10 + lambda-cyhalothrin at 5 g a.i. ha<sup>-1</sup> was classified as harmless (class 1) to the pupae and moderately harmful (class 3) to the adults of *T. pretiosum*. On the contrary, the highest rate of chlorantraniliprole 50 + lambda-cyhalothrin tested (i.e., 25 g a.i. ha<sup>-1</sup>) was classified as slightly harmful (class 2) to the pupae and harmful (class 4) to the adults of the parasitoid. Similarly, increasing the application rate of spinosad and chlorfenapyr also exhibited an increase in toxicity. Spinosad at a rate of 24 g a.i. ha<sup>-1</sup> was classified as slightly harmful (class 2) and harmless (class 1) to the pupae and adults of *T. pretiosum*, respectively. Spinosad at 96 g a.i. ha<sup>-1</sup> was classified as both moderately harmful (class 3) and harmful (class 4) to the pupae of the parasitoid. Chlorfenapyr at a rate of 192 g a.i. ha<sup>-1</sup> was classified as both slightly harmful (class 2) and moderately harmful (class 3) to the pupae of *T. pretiosum*, whereas chlorfenapyr at 336 g a.i. ha<sup>-1</sup> was categorized as slightly harmful (class 3) and harmful (class 4) to the pupae. These results indicate that an increase in the rate of insecticide affected *T. pretiosum* negatively. Therefore, higher insecticide rates should only be adopted when strictly necessary to control the target pest, because they might compromise the biological pest control process in the field.

**Key words:** Biological control. Chemical control. IPM. Parasitoid.

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## Resumo

O presente estudo avaliou o efeito de diferentes taxas de clorraniliprole, clorraniliprole + lambda-cialotrina, espinosade, e clorfenapir na seletividade para as pupas e adultos de *Trichogramma pretiosum*. O efeito negativo da maioria dos inseticidas testados nos parasitoides foi diretamente proporcional à dose do inseticida aplicado. O clorraniliprole na dose de 10 g a.i. ha<sup>-1</sup> foi classificado como seletivo (classe 1) para pupas e adultos do parasitoide. O efeito do clorraniliprole na dose de 20 a 50 g a.i. ha<sup>-1</sup> variou de seletivo (classe 1) a levemente prejudicial (classe 2) aos adultos de *T. pretiosum*. Clorraniliprole 10 + lambda-cialotrina a 5 g a.i. ha<sup>-1</sup> foi classificado como seletivo (classe 1) para as pupas e moderadamente prejudicial (classe 3) aos adultos de *T. pretiosum*. Ao contrário, a dose mais alta de clorraniliprole 50 + lambda-cialotrina testada (isto é, 25 g a.i. ha<sup>-1</sup>) foi classificada como moderadamente prejudicial (classe 3) para as pupas e nociva (classe 4) para os adultos do parasitoide. Da mesma forma, o aumento da taxa de aplicação de espinosade e clorfenapir também apresentou aumento na toxicidade. O spinosade na dose de 24 g a.i. ha<sup>-1</sup> foi classificado como levemente prejudicial (classe 2) e seletivo (classe 1) às pupas e adultos de *T. pretiosum*, respectivamente. Espinosade 96 g a.i. ha<sup>-1</sup> foi classificado como moderadamente prejudicial (classe 3) e nocivas (classe 4) para as pupas do parasitoide. Clorfenapir 192 g a.i. ha<sup>-1</sup> foi classificado como levemente prejudicial (classe 2) e moderadamente prejudicial (classe 3) às pupas de *T. pretiosum*, enquanto o clorfenapir a 336 g a.i. ha<sup>-1</sup> foi categorizado como moderadamente prejudicial (classe 3) e nociva (classe 4) às pupas. Estes resultados indicam que um aumento na dose de inseticida afetou negativamente o *T. pretiosum*. Portanto, doses mais altas de inseticidas só devem ser usadas quando estritamente necessários para controlar a praga alvo, pois podem comprometer o controle biológico natural de pragas no campo.

**Palavras-chave:** Controle biológico. Controle químico. MIP. Parasitoide.

## Introduction

For a long time, the effect of selective insecticides on biological control agents has been studied globally (DESNEUX et al., 2007). It has been highlighted as a key strategy for the success of integrated pest management (IPM). However, the selectivity of an insecticide for an organism is directly related to the application rate of the insecticide (DESNEUX et al., 2004). Therefore, when the application rate of an insecticide in field is modified, new studies are necessary to re-evaluate the selectivity of these insecticides for different beneficial organisms. The application rate of an insecticide might vary according to the pest species or crop. For instance, chlorantraniliprole at a rate of 10 g a.i. ha<sup>-1</sup> has been recommended to control *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in soybean crop. However, an increased rate of chlorantraniliprole 30 g a.i. ha<sup>-1</sup> has been suggested to control the same pest species in cotton crop. Similarly, chlorantraniliprole + lambda-cyhalothrin 15+7.5 g a.i. ha<sup>-1</sup> should be doubled to 30+15 g a.i. ha<sup>-1</sup> to control *Helicoverpa*

spp. in maize and cotton, respectively. Spinosad is recommended to control *H. armigera* at the rate of 24 g a.i. ha<sup>-1</sup> in soybean but this rate must be increased to 60 g a.i. ha<sup>-1</sup> in cotton to control the same pest species (AGROFIT, 2017). Therefore, the selectivity of an insecticide might vary according to the rate of application (BUENO et al., 2017). Furthermore, growers usually increase the application rate under different situations, such as: 1) when the current rate of application is no longer effective due to the selection of resistant pests or 2) when a new species that is naturally more tolerant to insecticides becomes a significant pest to a specific crop or region (QUINTELA, 2001). Because of this dynamic scenario, studies evaluating the effect of different rates of insecticide applied on the biological control agents are of theoretical and practical interest.

Among different crops, soybean [*Glycine max* (L.) Merrill] is one of the largest and most important crops worldwide in which insecticides have been overused to control pest outbreaks

(OERKE, 2006; FAOSTAT, 2013). Brazil is the second largest producer of soybean in the world (USDA, 2016). The Brazilian use of insecticides increased considerably in 2013 due to the detection of new pest species in the country (POMARI-FERNANDES et al., 2015). *Helicoverpa armigera*, which is considered one of the most prominent pests worldwide (TAY et al., 2013), was detected in Brazil and found to attack different crops, mainly soybean, maize, and cotton (SPECHT et al., 2013). This triggered a more frequent application of insecticides, and also an increase in the application rate. Sometimes the application rate was beyond the rate recommended in IPM, which worsened the pest problem (POMARI-FERNANDES et al., 2015). Since it is important to adopt various control strategies in IPM, studies on selectivity are important to formulate compatible chemical and biological control strategies depending on the application rate of insecticides (BUENO et al., 2017). Among the IPM strategies, the use of the egg parasitoid *Trichogramma* sp. is widely adopted owing to its ease of rearing and aggressiveness toward pests (PARRA; ZUCCHI, 2004) including *H. armigera*.

In this sense, the present study aimed to evaluate the selectivity of several insecticides, commonly recommended to manage *H. armigera*, at different rates in the pupae and adults of *T. pretiosum* Riley (Hymenoptera: Trichogrammatidae) in order to determine the most harmless treatments.

## Material and Methods

The experiment was divided into different bioassays (Table 1) and carried out in a completely randomized design with five replicates under controlled conditions [ $25 \pm 2^\circ\text{C}$ ,  $70 \pm 10\%$  relative humidity (RH), 14:10 h light/ dark (L/D) photoperiod] according to the protocol proposed by the International Organization for Biological Control (IOBC) (HASSAN, 1992), which has been briefly described below. The treatments (Table 1) included different rates of the insecticides used to control *H. armigera* in different crops to evaluate selectivity differences at different application rates. The treatments were diluted in water to a spray volume of  $200 \text{ L ha}^{-1}$ .

**Table 1.** Insecticides tested against the egg parasitoid *Trichogramma pretiosum* under controlled conditions in the laboratory.

Bioassay	Active ingredient (a.i.)	Commercial product (CP)	Chemical group	C <sup>1</sup>	mL of CP ha <sup>-1</sup>	g a.i. ha <sup>-1</sup>
Bioassay 1 ( <i>T. pretiosum</i> pupae) and bio-assay 3 ( <i>T. pretiosum</i> adults)	Chlorantraniliprole	Premio	Diamide	200	50	10
	Chlorantraniliprole	Premio	Diamide	200	75	15
	Chlorantraniliprole	Premio	Diamide	200	100	20
	Chlorantraniliprole	Premio	Diamide	200	150	30
	Chlorantraniliprole	Premio	Diamide	200	250	50
	Chlorantraniliprole + lambda-cyhalothrin	Ampligo	Diamide + pyrethroid	100 + 50	100	10 + 5
	Chlorantraniliprole + lambda-cyhalothrin	Ampligo	Diamide + pyrethroid	100 + 50	300	30 + 15
	Chlorantraniliprole + lambda-cyhalothrin	Ampligo	Diamide + pyrethroid	100 + 50	500	50 + 25

continue

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Bioassay 2 ( <i>T. pretiosum</i> pupae) and bioassay 4 ( <i>T. pretiosum</i> adults)	Spinosad	Tracer 480 SC	Spinosyns	480	50	24
	Spinosad	Tracer 480 SC	Spinosyns	480	125	60
	Spinosad	Tracer 480 SC	Spinosyns	480	200	96
	Chlorfenapyr	Pirate	Pirazol	240	800	192
	Chlorfenapyr	Pirate	Pirazol	240	1000	240
	Chlorfenapyr	Pirate	Pirazol	240	1200	288
	Chlorfenapyr	Pirate	Pirazol	240	1400	336

<sup>1</sup>Concentration of the active ingredient in g L<sup>-1</sup>.*Bioassays 1 and 2 (T. pretiosum pupae)*

Tests were conducted using a square cardboard (1 cm<sup>2</sup>) containing about 400 eggs of *Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae) with the pupae of *T. pretiosum*. Initially, this cardboard containing unparasitized eggs of *A. kuehniella* was offered to the freshly emerged females of the parasitoid for 24 h for oviposition. The card was then transferred to a tube until the pupae stage (168-192 h after parasitism) (CÔNSOLI et al., 1999). The pupae were then sprayed directly with the respective treatments (Table 1) using a potter tower calibrated to deposit  $1.25 \pm 0.25$  mg cm<sup>2</sup> (HASSAN, 1992). The applied volume was then verified according to Carmo et al. (2010). The card was placed under ambient conditions for a period of 1-2 h for drying. Subsequently, the card was inserted in a study cage (HASSAN, 1992). On 1-, 2-, and 3-day(s) after emergence (DAE) of first adults, the cardboard with approximately 200 *A. kuehniella* eggs was offered to the parasitoids with droplets of honey. The card was removed from the cage 4-DAE of parasitoids, and stored in a transparent plastic bag under controlled conditions ( $25 \pm 2^\circ\text{C}$ ,  $70 \pm 10\%$  RH, 14:10 h L/D photoperiod) to evaluate parasitism and parasitoid emergence.

*Bioassays 3 and 4 (T. pretiosum adults)*

The treatment was sprayed directly on a glass plate (2 mm thick and with an area of 13 cm × 13 cm), similar to the procedure described for the

pupae. After the treatment, the plate was placed at an ambient temperature for 1-2 h for drying. The plate was then coated with a dried film of insecticide and placed in the study cage (HASSAN, 1992). Adults were introduced into the cage via a connection in the emergence tube. Each tube contained a circular white cardboard (1 cm radius) with  $200 \pm 50$  eggs of *A. kuehniella* previously parasitized by *T. pretiosum*. The tube with active, 24 h-old parasitoid adults, previously fed with honey, was connected to the contact cage allowing the parasitoids to enter. On 1-, 2-, and 3-day(s) after the release of parasitoids, the cardboard holding approximately 200 unparasitized *A. kuehniella* eggs was offered to the parasitoids inside the cage. The card was removed from the cage 4-days after the release of parasitoids and stored in a transparent plastic bag under controlled conditions ( $25 \pm 2^\circ\text{C}$ ,  $70 \pm 10\%$  RH, 14:10 h L/D photoperiod) until parasitoid emergence to evaluate parasitism and parasitoid emergence later.

*Statistical analysis*

The assumption of normality (SHAPIRO; WILK, 1965) and homogeneity of variance of all the treatments (BURR; FOSTER, 1972) were tested. When the data did not follow normality or homogeneity of variance, they were transformed prior analysis of variance (ANOVA). Means were compared by the Tukey's test ( $p \leq 0.05$ ) for statistical significance (SAS INSTITUTE, 2009). Furthermore, a decrease in emergence or

parasitism of *T. pretiosum* was compared with the that of the control treatment and was computed by the equations: 1) Percent of decrease in adult emergence (EP) =  $(1 - \text{adult emergence in treatment} / \text{adult emergence in control}) \times 100$  (CARMO et al., 2010); and 2) Percent of decrease of parasitism (E) =  $(1 - \text{parasitism in treatment} / \text{parasitism in control}) \times 100$  (HASSAN, 1992). The chemicals were classified according to the IOBC standards as: class 1, harmless ( $EP/E < 30\%$ ); class 2, slightly harmful ( $30\% \leq EP/E \leq 79\%$ ); class 3, moderately harmful ( $80\% \leq EP/E \leq 99\%$ ); and class 4, harmful ( $EP/E > 99\%$ ) (HASSAN, 1992).

## Results

*Trichogramma pretiosum* emergence from the pupae treated with chlorantraniliprole was

comparable to that of the negative control (water) despite of the application rate, which ranged from 10 to 50 g a.i. ha<sup>-1</sup> (Table 2). Furthermore, the parasitism capacity of the adults was similar to that of the negative control (water) on 1-, 2-, and 3-DAE. The parasitism and emergence of parasitoids of the following generation (P<sub>1</sub>) did not differ from that of the control (Table 2). Therefore, the treatment with chlorantraniliprole was classified as class 1 to *T. pretiosum* on all the days evaluated (Table 3). However, parasitoid emergence was affected in pupae treated with chlorantraniliprole + lambda-cyhalothrin (Table 2). The highest evaluated rate of chlorantraniliprole + lambda-cyhalothrin (50 + 25 g a.i. ha<sup>-1</sup>) reduced the emergence of *T. pretiosum* from the pupae, and also the parasitism capacity of its progeny (second generation P<sub>0</sub>) 1- and 3-DAE. Therefore, it was classified as class 2 (Table 3).

**Table 2.** *Trichogramma pretiosum* emergence from the pupae treated with different insecticides and the parasitism capacity of the emerged adults (second generation) 1-, 2-, and 3-day(s) after emergence (DAE) (25 ± 2°C, 70 ± 10% relative humidity, 14:10 h L/D photoperiod).

Treatment (g a.i. ha <sup>-1</sup> )	Parasitoid emergence (%) from treated pupae <sup>2</sup>	Parasitoid progeny P <sub>1</sub> (second generation)					
		1-DAE <sup>1</sup>		2-DAE <sup>1</sup>		3-DAE <sup>1</sup>	
		Parasitism (%) <sup>2</sup>	Parasitoid emergence (%) <sup>2</sup>	Parasitism (%) <sup>2</sup>	Parasitoid emergence (%) <sup>2</sup>	Parasitism (%) <sup>2</sup>	Parasitoid emergence (%) <sup>2</sup>
Bioassay 1							
Chlorantraniliprole 10	93.7 ± 0.7ab <sup>3</sup>	87.1 ± 5.2 a	98.7 ± 0.4 <sup>ns;3</sup>	82.5 ± 5.8 <sup>ns;3</sup>	95.5 ± 1.9 <sup>ns;3</sup>	62.3±9.9ab <sup>3</sup>	86.6±12.2 <sup>ns</sup>
Chlorantraniliprole 15	97.7 ± 0.8a	85.3 ± 7.5 a	98.0 ± 1.7	90.2 ± 2.6	98.9 ± 0.9	64.0 ± 6.8ab	99.8 ± 0.2
Chlorantraniliprole 20	88.9 ± 4.8ab	86.1 ± 4.1 a	97.7 ± 2.1	69.3 ± 8.7	98.2 ± 1.8	79.7 ± 4.5ab	100.0 ± 0.0
Chlorantraniliprole 30	96.8 ± 0.9ab	78.1 ± 5.9 ab	99.6 ± 0.3	68.1 ± 9.9	99.4 ± 0.4	67.5 ± 9.1ab	99.1 ± 0.6
Chlorantraniliprole 50	95.8 ± 1.4ab	83.7 ± 2.8 a	98.9 ± 0.8	78.8 ± 3.7	99.6 ± 0.4	84.6 ± 0.5a	97.1 ± 2.1
Chlorantraniliprole 10 + lambda-cyha- lothrins 5	91.0 ± 2.7ab	85.3 ± 4.1 a	96.4 ± 2.1	81.0 ± 4.2	93.8 ± 4.3	66.0 ± 7.1ab	92.8 ± 5.2

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Chlorantraniliprole 30+ lambda-cyhalothrin 15	88.3 ± 3.3b	88.4 ± 1.9 a	94.4 ± 2.8	82.1 ± 7.5	91.1 ± 2.8	72.1 ± 10.6ab	86.6 ± 6.7
Chlorantraniliprole 50+ lambda-cyhalothrin 25	65.6 ± 5.5c	62.6 ± 3.6 b	85.8 ± 7.5	62.0 ± 14.3	98.0 ± 1.8	47.7 ± 9.2b	86.4 ± 7.5
Negative control (water)	95.8 ± 0.9b	91.6 ± 1.9 a	91.1 ± 5.6	80.4 ± 7.4	98.1 ± 1.7	86.4 ± 2.9a	88.7 ± 7.0
Positive control (chlorpyrifos 480)	0.0 ± 0.0c	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>
F	118.83	4.38	0.84	1.15	2.10	2.82	1.37
p	< 0.0001	0.0013	0.5750	0.3572	0.0659	0.0185	0.2478
CV (%)	7.64	10.92	10.88	22.97	8.75	16.98	15.46
Bioassay 2							
Spinosad 24	32.5 ± 6.8bc <sup>3</sup>	58.9 ± 5.1bc	97.6 ± 1.5 <sup>ns,3</sup>	43.3 ± 5.1b	93.9 ± 4.0 <sup>ns,3</sup>	17.8 ± 2.1b	95.9 ± 4.1a <sup>3</sup>
Spinosad 60	22.8 ± 3.8bc	9.1 ± 3.6e	92.0 ± 8.0	6.1 ± 3.8cd	100.0 ± 0.0	3.7 ± 2.2cd	36.8 ± 0.0 b
Spinosad 96	15.7 ± 1.5c	5.6 ± 3.2e	97.1 ± 2.9	0.4 ± 0.4d	100.0 ± 0.0	0.8 ± 0.8d	80.0 ± 0.0ab
Chlorfenapyr 192	35.4 ± 4.6b	88.2 ± 2.2a	100.0 ± 0.0	18.7 ± 3.4c	98.6 ± 1.4	5.6 ± 3.3cd	100.0 ± 0.0a
Chlorfenapyr 240	29.3 ± 4.1bc	72.5 ± 5.2ab	97.6 ± 1.7	0.0 ± 0.0d	- <sup>4</sup>	11.1 ± 0.9bc	100.0 ± 0.0a
Chlorfenapyr 288	24.8 ± 2.1bc	52.7 ± 4.6cd	100.0 ± 0.0	2.8 ± 1.8d	100.0 ± 0.0	0.0 ± 0.0d	- <sup>4</sup>
Chlorfenapyr 336	40.4 ± 3.7b	38.4 ± 1.1d	99.1 ± 0.5	0.6 ± 0.6d	96.4 ± 3.6	0.9 ± 0.9d	100.0 ± 0.0a
Negative control (water)	84.4 ± 3.0a	85.5 ± 3.9a	98.2 ± 1.8	77.4 ± 3.9a	99.6 ± 0.4	76.4 ± 1.4a	93.4 ± 4.3ab
Positive control (chlorpyrifos 480)	0.0 ± 0.0 d	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>
F	49.02	64.06	1.06	84.36	0.67	219.47	3.48
p	< 0.0001	< 0.0001	0.4180	< 0.0001	0.6762	< 0.0001	0.0401
CV (%)	16.76	16.40	7.84	35.35	9.84	26.74	14.02

<sup>1</sup>Days after parasitoid emergence (DAE); <sup>2</sup>Means ± SE followed by the same letter in each column are not statistically different according to the Tukey's test ( $p \geq 0.05$ ); <sup>3</sup>Original data followed by statistics done on the data converted to  $\arcsin \sqrt{X/100}$  to perform ANOVA according to Burr and Foster (1972); <sup>4</sup>Value nonexistent; <sup>ns</sup>ANOVA non-significant.

The effects of pesticide on the adults of *T. pretiosum* (bioassays 3 and 4) were different from those on the pupae. Parasitism of the adults that had contact with chlorantraniliprole was similar to the control 1-DAE (Table 4), therefore this treatment was classified as class 1 (Table 3). Parasitism was reduced by chlorantraniliprole at rates of 15, 20, 30,

and 50 g a.i. ha<sup>-1</sup> 2-DAE, and by chlorantraniliprole 20, 30, and 50 g a.i. ha<sup>-1</sup> 3-DAE (Table 4). These treatments were classified as slightly class 2 (Table 3). The effect of chlorantraniliprole + lambda-cyhalothrin on the parasitoid adults was high (Table 4). The treatments with this mixture were classified as class 3 to class 4 (Table 3).

**Table 3.** Classification of insecticide selectivity for *Trichogramma pretiosum* according to the International Organization for Biological Control (IOBC) in different bioassays and days after emergence (DAE) of adults or days after spraying (DAS) ( $25 \pm 2^\circ\text{C}$ ,  $70 \pm 10\%$  relative humidity, 14:10 h L/D photoperiod).

Treatment (g a.i. ha <sup>-1</sup> )	Bioassays with pupae (bioassay 1 and 2)								Bioassays with adults (bioassay 3 and 4)							
	Sprayed pupae		1-DAE		2-DAE		3-DAE		1-DAS		2-DAS		3-DAS			
			EP% <sup>1</sup>	C <sup>3</sup>	E% <sup>2</sup>	C <sup>3</sup>	E% <sup>2</sup>	C <sup>3</sup>	E% <sup>1</sup>	C <sup>2</sup>	E% <sup>2</sup>	C <sup>3</sup>	E% <sup>2</sup>	C <sup>3</sup>	E% <sup>2</sup>	C <sup>3</sup>
	Bioassay 1								Bioassay 3							
Chlorantraniliprole 10	2.1	1	4.9	1	0	1	27.9	1	0	1	14.5	1	17.2	1		
Chlorantraniliprole 15	0	1	6.8	1	0	1	26.0	1	27.9	1	35.2	2	11.0	1		
Chlorantraniliprole 20	7.2	1	6.0	1	13.8	1	7.7	1	14.8	1	44.0	2	50.6	2		
Chlorantraniliprole 30	0	1	14.8	1	15.3	1	21.9	1	7.3	1	40.9	2	73.1	2		
Chlorantraniliprole 50	0	1	8.6	1	1.9	1	2.1	1	2.3	1	32.8	2	32.9	2		
Chlorantraniliprole 10 + lambda-cyhalothrin 5	5.0	1	6.9	1	0	1	23.7	1	83.7	3	85.9	3	97.8	3		
Chlorantraniliprole 30 + lambda-cyhalothrin 15	7.8	1	3.4	1	0	1	16.6	1	97.3	3	99.4	4	83.3	3		
Chlorantraniliprole 50 + lambda-cyhalothrin 25	31.5	2	31.7	2	22.8	1	44.8	2	93.6	3	99.3	4	100	4		
Positive control (chlorpyrifos 480)	100	4	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	100	4	100	4	100	4
	Bioassay 2								Bioassay 4							
Spinosad 24	61.5	2	31.1	2	44.1	2	76.7	2	2.6	1	3.0	1	9.5	1		
Spinosad 60	73.0	2	89.4	3	92.1	3	95.2	3	11.2	1	17.6	1	26.3	1		
Spinosad 96	81.3	3	93.5	3	99.5	4	99.0	3	4.3	1	9.8	1	9.4	1		
Chlorfenapyr 192	58.0	2	0	1	75.8	2	92.7	3	0	1	12.5	1	22.4	1		
Chlorfenapyr 240	65.3	2	15.2	1	100	4	85.5	3	0	1	12.1	1	25.5	1		
Chlorfenapyr 288	70.6	2	38.4	2	96.3	3	100	4	6.6	1	10.8	1	26.5	1		
Chlorfenapyr 336	52.2	2	55.1	2	99.2	4	98.8	3	14.1	1	25.5	1	59.7	2		
Positive control (chlorpyrifos 480)	100	4	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	100	4	100	4	100	4

<sup>1</sup>EP (Percent of reduction in adult emergence) =  $(1 - \text{Treatment adult emergence} / \text{Control adult emergence}) \times 100$  (CARMO et al., 2010); <sup>2</sup>E (Percent of reduction in parasitism) =  $(1 - \text{Treatment parasitism} / \text{Control parasitism}) \times 100$  (HASSAN, 1992); <sup>3</sup>Classification: class 1 = harmless ( $E/EP < 30\%$ ), class 2 = slightly harmful ( $30\% \leq E/EP < 80$ ), class 3 = moderately harmful ( $80\% \leq E/EP < 99$ ), class 4 = harmful ( $E/EP \geq 99\%$ ) (HASSAN, 1992); <sup>4</sup>Value nonexistent.

The treatment of pupae with spinosad and chlorfenapyr individually reduced parasitoid emergence at all the rates evaluated (Table 2) and were classified as class 2 to class 3 (Table 3). Furthermore, the lower parasitism capacity of the parasitoid progeny in all the treatments, except for chlorfenapyr at rates of 192 and 240 g a.i. ha<sup>-1</sup>, was similar to that of the negative control 1-DAE

(Table 2). The negative effect of spinosad on the parasitoid was directly proportional to the evaluated rate, ranging from 24 g a.i. ha<sup>-1</sup> (class 2) to 60 g a.i. ha<sup>-1</sup> (between class 2 and class 3) to 96 g a.i. ha<sup>-1</sup> (between class 3 and class 4) (Table 3). Chlorfenapyr was classified as class 2 when applied on the pupae of the parasitoid (Table 3). Considering its effect on parasitoid progeny that emerged from the treated



pupae, chlorfenapyr was classified as class 1 to class 2 one-DAE, class 2 to class 3 two-DAE, and class 3 to class 4 three-DAE, with a more harmful effect at higher rates and later evaluations (3-DAE) compared with that of the lower rates and earlier evaluations (1-DAE) (Table 3).

On the contrary, spinosad tested on adults

exhibited effects similar to those of the negative control (Table 4), therefore was classified as class 1 for all the treatments (Table 3). Chlorfenapyr was also classified as harmless (class 1) to the adults of *T. pretiosum*, except at the highest evaluated rate of 336 g a.i. ha<sup>-1</sup>, which reduced parasitism 3-DAE (Table 4) and was classified as class 2 (Table 3).

**Table 4.** *Trichogramma pretiosum* parasitism (%) after parasitoid emergence (%) on different days after insecticide spraying (DAS) (25 ± 2°C, 70 ± 10% relative humidity, 14:10 h L/D photoperiod).

Treatment (g i a ha <sup>-1</sup> )	1-DAS <sup>1</sup>		2-DAS <sup>1</sup>		3-DAS <sup>1</sup>	
	Parasitism (%) <sup>2</sup>	Parasitoid emergence (%) <sup>2</sup>	Parasitism (%) <sup>2</sup>	Parasitoid emergence (%) <sup>2</sup>	Parasitism (%) <sup>2</sup>	Parasitoid emer- gence (%) <sup>2</sup>
Bioassay 3						
Chlorantra- niliprole 10	81.4 ± 3.7 a	95.7 ± 1.7 a <sup>3</sup>	79.9 ± 7.1 ab <sup>3</sup>	86.5 ± 6.7 a	59.3 ± 6.3 ab <sup>3</sup>	86.0 ± 6.8 ab <sup>3</sup>
Chlorantra- niliprole 15	78.2 ± 9.0 a	98.3 ± 1.0 a	60.5 ± 8.6 b	96.4 ± 3.6 a	63.7 ± 5.1 ab	94.1 ± 5.7 ab
Chlorantra- niliprole 20	68.0 ± 6.6 a	99.3 ± 0.7 a	52.3 ± 11.3 b	93.3 ± 6.7 a	35.3 ± 6.5 bc	98.7 ± 1.3 a
Chlorantra- niliprole 30	74.0 ± 1.8 a	96.6 ± 2.2 a	55.2 ± 11.0 b	94.3 ± 5.7 a	19.2 ± 2.9 cd	98.2 ± 1.8 a
Chlorantra- niliprole 50	78.0 ± 6.7 a	96.7 ± 2.5 a	62.8 ± 10.1 b	99.4 ± 0.5 a	48.1 ± 6.8 ab	87.7 ± 7.5 ab
Chlorantra- niliprole 10 + lambda-cyha- lothrins 5	13.0 ± 2.3 b	90.5 ± 9.5 a	13.1 ± 4.2 c	53.3 ± 9.6 b	1.6 ± 1.6 e	56.2 ± 22.9 b
Chlorantra- niliprole 30 + lambda-cyha- lothrins 15	2.1 ± 1.1 b	100.0 ± 0.0 a	0.6 ± 0.4 c	100.0 ± 0.0 a	11.9 ± 4.9 de	100.0 ± 0.0 a
Chlorantra- niliprole 50 + lambda-cyha- lothrins 25	5.1 ± 2.6 b	43.7 ± 6.3 b	0.6 ± 0.4 c	100.0 ± 0.0 a	0.0 ± 0.0 e	- <sup>4</sup>
Negative con- trol (water)	79.8 ± 4.8 a	92.2 ± 4.4 a	93.4 ± 1.9 a	89.7 ± 6.7 a	71.6 ± 5.4 a	90.9 ± 6.4 ab
Positive con- trol (chlorpyri- fos 480)	0.0 ± 0.0 b	- <sup>4</sup>	0.0 ± 0.0 c	- <sup>4</sup>	0.0 ± 0.0 e	- <sup>4</sup>
F	74.07	7.39	39.69	3.55	38.68	1.97
p	< 0.0001	< 0.0001	< 0.0001	0.0071	< 0.0001	0.1017
CV (%)	20.53	11.70	27.90	12.65	27.83	17.94

continue

continuation

Bioassay 4						
Spinosad 24	90.8 ± 1.4 a <sup>3</sup>	93.1 ± 4.2 <sup>ns</sup>	81.8 ± 6.2 a	98.9 ± 0.8 <sup>ns</sup>	74.6 ± 6.8 a <sup>3</sup>	89.5 ± 2.5 b
Spinosad 60	82.7 ± 7.0 a	98.6 ± 0.7	69.5 ± 7.1 a	99.2 ± 0.6	60.8 ± 11.3 ab	98.5 ± 0.9 a
Spinosad 96	89.2 ± 3.6 a	98.6 ± 1.2	76.1 ± 8.9 a	98.6 ± 0.9	74.6 ± 8.4 a	96.1 ± 1.6 ab
Chlorfenapyr 192	94.4 ± 1.7 a	99.6 ± 0.4	73.8 ± 9.3 a	99.1 ± 0.9	63.9 ± 8.9 ab	98.5 ± 1.5 a
Chlorfenapyr 240	93.5 ± 2.6 a	95.2 ± 4.0	74.2 ± 5.6 a	99.1 ± 0.9	61.4 ± 12.5 ab	98.0 ± 1.2 a
Chlorfenapyr 288	87.0 ± 1.6 a	99.5 ± 0.5	75.2 ± 9.7 a	94.5 ± 4.6	60.5 ± 7.4 ab	97.8 ± 1.0 a
Chlorfenapyr 336	80.1 ± 8.3 a	97.1 ± 2.0	62.9 ± 4.3 a	96.6 ± 2.1	33.2 ± 0.8 b	95.5 ± 2.6 ab
Negative control (water)	93.2 ± 1.5 a	92.4 ± 4.8	84.3 ± 3.4 a	96.3 ± 2.4	82.4 ± 3.5 a	99.2 ± 0.8 a
Positive control (chlorpyrifos 480)	0.0 ± 0.0 b	- <sup>4</sup>	0.0 ± 0.0 b	- <sup>4</sup>	0.0 ± 0.0 c	- <sup>4</sup>
F	54.26	1.04	14.44	0.69	13.79	3.73
P	< 0.0001	0.4215	< 0.0001	0.6798	< 0.0001	0.0060
CV (%)	11.55	6.49	22.75	4.82	24.99	3.43

<sup>1</sup>Days after insecticide spraying (DAS); <sup>2</sup>Means ± SE followed by the same letter in each column are not statistically different according to the Tukey's test ( $p \geq 0.05$ ); <sup>3</sup>Original data followed by statistics done on the data converted to arcsin  $\sqrt{X/100}$  to perform ANOVA according to Burr and Foster (1972); <sup>4</sup>Value nonexistent; <sup>ns</sup>ANOVA non-significant.

## Discussion and Conclusion

All the evaluated rates of chlorantraniliprole from 10 to 50 g a.i. ha<sup>-1</sup> were classified as harmless to the pupae of *T. pretiosum* indicating that it is appropriate for use in IPM programs owing to its negligible effect on the parasitoid at the tested rates. Similar results have been reported in different beneficial arthropods, including the egg parasitoid *Trichogramma dendrolimi* Matsumura (Hymenoptera: Trichogrammatidae), predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae), and parasitoid *Aphidius rhopalosiphii* De Stefani-Perez (Hymenoptera: Aphidiinae) (DINTER et al., 2008; BRUGGER et al., 2010). This suggests that chlorantraniliprole is harmless not only to *T. pretiosum* pupae, but also to other biological control agents.

The selectivity observed for chlorantraniliprole might be because chlorantraniliprole acts mainly

after ingestion (LAHM et al., 2007), therefore limiting its action to phytophagous insects. Chlorantraniliprole acts on the nervous system of an organism by activating the receptors of ryanodine by releasing calcium from the sarcoplasmic reticulum of muscle cells causing paralysis and death of the insect (CORDOVA et al., 2006). However, it does not affect the biological control agent due to the limited ingestion of the insecticide by the beneficial arthropods. Similar results have been observed in *Podisus nigrispinus* (Hemiptera: Pentatomidae) (STECCA et al., 2017). In the present study, the mixture of chlorantraniliprole and lambda-cyhalothrin affected *T. pretiosum* more than chlorantraniliprole alone. This is due to the action of lambda-cyhalothrin—an insecticide belonging to the pyrethroid group—which has been reported to be harmful to most beneficial arthropods (CAÑETE, 2005).

It is noteworthy that the effect of an insecticide can range from harmless to harmful depending on the developmental stage of the parasitoid (SANTOS et al., 2006), as demonstrated in the present study. Chlorantraniliprole at relatively high rates (20, 30, and 50 g a.i. ha<sup>-1</sup>) was marginally more toxic to the adults than to the pupae. The higher tolerance of the pupae of *T. pretiosum* to the insecticide when compared with that of the adults is synonymous with earlier reports (STECCA et al., 2016). This might be due to the presence of the parasitoid within the host egg where the chorion protects the parasitoid from the insecticide (STECCA et al., 2016). It is also important to note that the ability of an insecticide to penetrate the chorion of an insect egg might vary depending on the physicochemical properties of the tested insecticide. Chemicals with a relatively high molecular weight have a greater difficulty in crossing the chorion (STOCK; HOLLOWAY, 1993). In the present study, an increased tolerance to insecticides was also observed in pupae treated with chlorantraniliprole + lambda-cyhalothrin. On the contrary, the effect of spinosad and chlorfenapyr was higher on the pupae than on the adults. The difference in selectivity of an insecticide during different developmental stages of an insect might be related to the site of action, which can change during development. Thus, affecting the rate of penetration through the integument of an insect and/or rate of metabolization of the insecticide within the insect (BUENO et al., 2017). The rate of insecticide penetrating the integument depends on the relationship between insecticide affinity, cuticle thickness, and chemical composition (FERNANDES et al., 2010), which might differ among the pupae and adults of the same parasitoid species. The lipophilicity of insecticides is inversely proportional to their solubility in water (FERNANDES et al., 2010). Therefore, compounds with a relatively high lipophilicity can penetrate the body of an insect at a relatively high rate due to their similarity with the cuticle that can differ among the pupae and adults of the parasitoid.

Spinosad, a mixture of compounds produced by the bacterium *Saccharopolyspora spinosa*, is often classified as low risk and less harmful to predators (WILLIAMS et al., 2003). However, parasitoids belonging to the order hymenoptera are significantly susceptible to spinosad (WILLIAMS et al., 2003). Spinosad acts mainly as a stomach poison (BRET et al., 1997) with low contact activity. This might have been responsible for its higher effect on the pupae than on the adults of *T. pretiosum*. In the present study, the adult parasitoids came in contact only with the dry spinosad residue on the cardboard plates inside the study cages (tarsal contamination). Whereas, the parasitoids in pupae test usually eat the chorion of the egg during adult emergence, thus they might have ingested the spinosad residues during emergence.

The effect on *T. pretiosum* varied not only for chlorantraniliprole, but also for the other insecticides tested. In general, an increase in the application rate was directly proportional to the increase in the negative effect of the insecticide on the biological control agent. Chlorantraniliprole at a rate of 10 g a.i. ha<sup>-1</sup> was classified as harmless to both the pupae and adults of the parasitoid. However, chlorantraniliprole at rates of 20 to 50 g a.i. ha<sup>-1</sup> was harmless only to the pupae, while it was classified as slightly harmful to the adults of *T. pretiosum* adults. Chlorantraniliprole + lambda-cyhalothrin at a rate of 10 + 5 g a.i. ha<sup>-1</sup> was classified as harmless to the pupae and moderately harmful to the adults of *T. pretiosum*. On the contrary, the highest tested rate of chlorantraniliprole 50 + lambda-cyhalothrin (25 g a.i. ha<sup>-1</sup>) was categorized as slightly harmful to the pupae and harmful to the adults of the parasitoid. A similar increase in toxicity was observed with the increasing rates of spinosad and chlorfenapyr. Spinosad at 24 g a.i. ha<sup>-1</sup> was slightly harmful and harmless to the pupae and adults of *T. pretiosum*, respectively. Spinosad at a rate of 96 g a.i. ha<sup>-1</sup> was classified as moderately harmful and harmful to the pupae of the parasitoid. Chlorfenapyr at 192 g a.i. ha<sup>-1</sup> was categorized as slightly harmful and

moderately harmful to the pupae of *T. pretiosum*, while chlorfenapyr at 336 g a.i. ha<sup>-1</sup> was classified as slightly harmful and harmful to the same parasitoid stage.

Chlorantraniliprole at a rate of 10 g a.i. ha<sup>-1</sup> was harmless (class 1) to both the pupae and adults of *T. pretiosum*. The effect of chlorantraniliprole ranging from 20 to 50 g a.i. ha<sup>-1</sup> varied from harmless (class 1) to slightly harmful (class 2) to *T. pretiosum* adults. Chlorantraniliprole + lambda-cyhalothrin 10 + 5 g a.i. ha<sup>-1</sup> was harmless (class 1) to the pupae and moderately harmful (class 3) to the adults of *T. pretiosum*. On the contrary, the highest tested rate of chlorantraniliprole + lambda-cyhalothrin (50 + 25 g a.i. ha<sup>-1</sup>) was categorized as slightly harmful (class 2) to the pupae and harmful (class 4) to the adults of the parasitoid.

Spinosad at a rate of 24 g a.i. ha<sup>-1</sup> was slightly harmful (class 2) and harmless (class 1) to the pupae and adults of *T. pretiosum*, respectively. However, spinosad at a rate of 96 g a.i. ha<sup>-1</sup> was classified as moderately harmful (class 3) and harmful (class 4) to the pupae of the parasitoid.

Chlorfenapyr at a rate of 192 g a.i. ha<sup>-1</sup> was classified as slightly harmful (class 2) and moderately harmful (class 3) to the pupae of *T. pretiosum*, whereas chlorfenapyr at 336 g a.i. ha<sup>-1</sup> was classified as slightly harmful (class 2) and harmful (class 4) to the same parasitoid stage. An increase in the rate of tested insecticides increased their negative effects on *T. pretiosum*. Therefore, these insecticide rate increases should only be applied when strictly necessary to control the target pest, because they might compromise the biological pest control process in the field.

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