

## Selectivity of registered pesticides for the corn crop on immature stages of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae)

### Seletividade de pesticidas registrados para a cultura do milho sobre estágios imaturos de *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae)

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#### ABSTRACT

*Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) is an egg parasitoid of lepidopteran pests in corn, *Zea mays* L. (Poaceae), in Brazil. Chemical products used in agriculture can be toxic to this biocontrol agent. The objective of the present study was to evaluate the longevity, parasitism and emergence of *T. pretiosum* parasitizing Mediterranean flour moth, *Anagasta kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae), eggs treated with herbicide solutions with the active ingredients (chemical group) atrazine (triazines), paraquat dichloride (bipyridylum) and nicosulfuron (sulfonilurea). Pure distilled water was used as a negative control, and a solution of a product with  $\lambda$ -cyhalothrin + thiamethoxam (pyrethroid + neonicotinoid, respectively) was used as the positive control. The experimental design was completely randomized, with five treatments and 10 replicates. *Anagasta kuehniella* eggs were stored for two periods after treatment, 24 and 48 hours. These eggs were subjected to parasitism for two subjecting periods, 24 and 48 hours. The results were analysed statistically and classified according to the International Organization for Biological Control (IOBC). Atrazine and nicosulfuron reduced the longevity of *T. pretiosum* females. Nicosulfuron and the combination  $\lambda$ -cyhalothrin + thiamethoxam reduced the parasitism and emergence of this natural enemy. The paraquat dichloride-based herbicide was the least toxic and therefore represents a potential option for controlling weeds with low toxicity to the *T. pretiosum* parasitoid.

**Index terms:** Biocontrol agent; emergence; herbicide; longevity; parasitism.

#### RESUMO

*Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) é parasitoide de ovos de lepidópteros praga no milho, *Zea mays* L. (Poaceae), no Brasil. Produtos químicos usados na agricultura podem ser tóxicos para este agente de controle biológico. O objetivo do presente estudo foi avaliar a longevidade, parasitismo e emergência de *T. pretiosum* em ovos da traça da farinha, *Anagasta kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) tratados com soluções herbicidas com os ingredientes ativos (grupo químico) atrazina (triazinas), dicloreto de paraquate (bipiridílio) e nicosulfuron (sulfonilureia). Água destilada pura foi usada como controle negativo e a solução de um produto com  $\lambda$ -cicalotrina + tiametoxam (piretróide + neonicotinóide, respectivamente) foi usada como controle positivo. O delineamento experimental foi inteiramente casualizado, com cinco tratamentos e 10 repetições. Ovos de *A. kuehniella* foram armazenados por dois períodos após tratamento, 24 e 48 horas. Esses ovos foram submetidos ao parasitismo por dois períodos de submissão, 24 e 48 horas. Os resultados foram analisados estatisticamente e classificados de acordo com a *International Organization for Biological Control* (IOBC). Atrazina e nicosulfuron reduziram a longevidade das fêmeas de *T. pretiosum*. Nicosulfuron e a combinação  $\lambda$ -cicalotrina + tiametoxam reduziram o parasitismo e a emergência deste inimigo natural. O herbicida à base de dicloreto de paraquate foi o menos tóxico e, por isto, representa uma opção em potencial para o controle de plantas daninhas com baixa toxicidade ao parasitoide *T. pretiosum*.

**Termos para indexação:** Agente de controle biológico; emergência; herbicida; longevidade; parasitismo.

#### INTRODUCTION

Chemical products used in insect pest, disease and weed management can be toxic to natural enemies, aquatic organisms, decomposers, and pollinators (Gonring et al.,

2003; Menezes et al., 2012a; Castilho et al., 2014; Leite et al., 2016). These products are used in several crops, including corn, *Zea mays* L. (Poaceae), which accounts for 70-85% of the grains produced worldwide for animal feed (Couto et al., 2017). Corn crops are protected by chemicals

applied for the control of insects and weeds, which are pests that reduce grain yield (Kozłowski; Koehler; Pitelli, 2009; Menezes et al., 2014).

Herbicides and insecticides are used in corn crops, especially in those genetically modified (GM) to be resistant to herbicides and attacked by insects not targeted by *Bt* (*Bacillus thuringiensis* Berliner, 1915; Bacillales: Bacillaceae) technology (Pellegrino; Bedini; Ercoli, 2018). Genetically modified crops are grown on more than 80% of the area cultivated with corn in Brazil (Bernardi et al., 2017). Atrazine, paraquat dichloride and nicosulfuron are herbicide active ingredients used in corn crops. Atrazine inhibits the weed photosystem and thus prevents electron transport (Chen et al., 2014). Paraquat dichloride increases the production of toxic free radicals and causes weed death by inhibiting photosystem I (Funderburk Jr; Lawrence, 1964). Nicosulfuron prevents the synthesis of branched amino acids (isoleucine, leucine and valine) by inhibiting the acetolactate synthase (ALS) enzyme in weeds (Anderson et al., 1998). Insects attacking corn crops can be managed with insecticides such as those based on the combination of the active ingredients  $\lambda$ -cyhalothrin and thiamethoxam. Pests such as *Frankliniella williamsi* Hood, 1915 (Thysanoptera: Thripidae), *Mahanarva fimbriolata* Stål, 1854 (Hemiptera: Cercopidae) and *Spodoptera frugiperda* J.E. Smith, 1797 (Lepidoptera: Noctuidae) are controlled with  $\lambda$ -cyhalothrin + thiamethoxam, which causes hyperexcitation in their central nervous system (Pasini et al., 2017b).

Weed management with herbicides can reduce the number of flowers used as shelter, a food source and breeding sites by natural enemies, such as parasitoids and predators (Albajes; Lumbierres; Pons, 2009; Menezes; Soares, 2016). In addition, herbicides and the additives used in their formulation, such as adjuvants, can be toxic to such organisms, including *Trichogramma* (Hymenoptera: Trichogrammatidae), a lepidopteran egg parasitoid (Menezes; Soares, 2016; Leite et al., 2016).

*Trichogramma* controls the eggs of lepidopteran pests in corn, such as *S. frugiperda* (Tavares et al., 2009). These biocontrol agents can be reared on a large scale with eggs of alternative hosts, such as *Anagasta* (= *Ephestia*) *kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae), and can prevent the damage caused by hatched caterpillars (Stefanello Júnior et al., 2012; Tavares et al., 2016). Chemical products are usually applied during the day, when *Trichogramma* species exhibit oviposition peaks and are most exposed (Stefanello Júnior et al., 2008). Among the range of products used in corn crops, Sevin 480 SC® (insecticide, a.i. carbaryl,

group: naphthyl methylcarbamate) and Tiomet 400 CE® (insecticide, a.i. dimethoate, group: organophosphate) are toxic, and Orthocide 500® (fungicide, a.i. captan, group: dicarboximide) and Persist SC® (herbicide, a.i. picloran + 2,4-D, groups: pyridine carboxylic acid + aryloxyalkanoic acid, respectively) are selective to *Trichogramma pretiosum* Riley, 1879 (Giolo et al., 2005; Leite et al., 2015).

The environmental persistence of chemical products and their toxicity to non-target organisms should be considered in biological control programmes with parasitoids (Menezes et al., 2012b; Menezes; Soares, 2016; Pasini et al., 2017a). The objective of this study was to evaluate the longevity, parasitism and emergence of *T. pretiosum* on *A. kuehniella* eggs treated with chemical products (i.e., atrazine-, paraquat dichloride- and nicosulfuron-based herbicides and a  $\lambda$ -cyhalothrin + thiamethoxam-based insecticide) used in corn crops.

## MATERIAL AND METHODS

### Experimental site

Insects were reared and the bioassays carried out in the *Laboratório de Entomologia* of the *Instituto Federal do Norte de Minas Gerais* (IFNMG)-campus Januária, in Januária, Minas Gerais state, Brazil, in an air conditioned room at  $25 \pm 1$  °C and  $70 \pm 10\%$  RH and under a 12:12 h (L:D, at 500 lumens) photoperiod.

### *Trichogramma pretiosum* rearing

A *T. pretiosum* parasitoid colony obtained from the *Insetário G.W.G. de Moraes* of the *Universidade Federal de Minas Gerais* (UFMG) in Montes Claros, Minas Gerais state, Brazil, was used to begin the mass rearing of this insect at the IFNMG-campus Januária to obtain individuals for the bioassays. This parasitoid was reared on eggs of the alternative host *A. kuehniella* in 500 mL glass pots sealed with polyvinyl chloride (PVC) plastic after being sterilized in a UV chamber for 60 min.

### *Anagasta kuehniella* rearing

The alternative host was reared with wheat bran (*Triticum aestivum* L.; Poaceae) and beer yeast at a ratio of 97:3. The mixture obtained from these two products was uniformly distributed in plastic trays (29.5 cm long  $\times$  20.0 cm wide  $\times$  6.5 cm tall) and covered with a snap-on plastic cap. The lids were cut in their upper part, where a fine white mesh fabric was fixed with acrylic glue on the inner surface.

*Anagasta kuehniella* adults were collected daily over a 20-day period. These individuals were transferred to round plastic containers (25.0 cm diameter × 19.0 cm tall) covered with fabric and placed upside down on a plastic dish (30.0 cm diameter) for egg collection. *Anagasta kuehniella* eggs were collected, separated from the residues of this insect and stored at 1.8 °C.

### **Anagasta kuehniella egg treatment**

A 10% nontoxic glue solution diluted in distilled water was used to fix forty-five 24-hour-old *A. kuehniella* eggs onto a piece of white paperboard (2 cm wide × 4 cm long) previously sterilized with UV light for 60 min. and then immersed in a solution of each commercial product (Atrazina Atanor 50 SC<sup>®</sup>, Paradox<sup>®</sup> and Sanson 40 SC<sup>®</sup>) (Table 1) for two seconds. An Engeo Pleno<sup>®</sup> solution (Table 1) was used as a positive control, and pure distilled water was used as a negative control. Solutions of the products were prepared using distilled water, and their concentrations followed the recommendation of the respective manufacturers for utilization in corn crops. The two-second period was used because it was sufficient for complete egg immersion according to preliminary tests. The treated pieces of paperboard were left at 25 ± 2 °C for 30 min. for excess water evaporation (Menezes et al., 2014). A paperboard piece with eggs that were treated 24 and 48 hours before and a 24-hour-old mated female of *T. pretiosum* were placed into individual glass test tubes (2.5 cm wide × 8.5 cm

long). These tubes were sealed with a cotton swab without food for the parasitoid. Parasitism was allowed for up to 48 hours. Engeo Pleno<sup>®</sup>, with λ-cyhalothrin and thiamethoxam as the main active ingredients, was selected as a positive control due to its solution toxicity to *Trichogramma galloi* Zucchi, 1988 larvae on *Diatraea saccharalis* F., 1794 (Lepidoptera: Crambidae) eggs; treatment was performed by immersion for five seconds after parasitism (Antigo et al., 2013).

The experimental design was completely randomized, with five treatments consisting of the chemical products and the positive and negative control. *Anagasta kuehniella* eggs that were treated with the chemical solutions were stored for two storage periods (i.e., 24 and 48 hours). These eggs were offered to the parasitoids using two periods of egg offering (i.e., 24 and 48 hours). Ten replicates (each with one *T. pretiosum* female and 45 *A. kuehniella* eggs) were used per treatment.

### **Bioassay with *Trichogramma pretiosum***

*Trichogramma pretiosum* longevity on alternative host eggs that had been treated with the chemical products 24 and 48 hours before parasitism was evaluated every 60 min for 48 hours. The dead females of this parasitoid were removed from the tubes at each evaluation.

Parasitized eggs were characterized by black colouration on the fifth day after exposure to parasitism. The percentage of alternative host eggs per sampling unit showing parasitoid emergence was evaluated three days after emergence.

**Table 1:** The commercial names (Com.) of the herbicides Atrazina Atanor 50 SC<sup>®</sup> (A.A.), Paradox<sup>®</sup> (P.), Sanson 40 SC<sup>®</sup> (S.), and the insecticide Engeo Pleno<sup>®</sup> (E.P.) as the positive control (PC), active ingredient (a.i.; g L<sup>-1</sup>), chemical group (Group), dose of the commercial product per hectare (He.; L ha<sup>-1</sup>), dose of the commercial product per paperboard (Pa.; μL paperboard<sup>-1</sup>), and toxicology and formulation (Tox., Form.) of the chemical products used in the treatment of *Anagasta kuehniella* (Lepidoptera: Pyralidae) eggs pre-offered to parasitism by *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae).

Com.	a.i.	Group	He.	Pa.	Tox. <sup>2</sup> , Form. <sup>3</sup>
A.A. <sup>1</sup>	Atrazine (500)	Triazines	5.0	0.3	TC: III, EC: III and CS
P. <sup>1</sup>	Paraquat dichloride (200)	Bipyridylum	2.0	0.12	TC: I, EC: II and CS
S. <sup>1</sup>	Nicosulfuron (40)	Sulfonylurea	1.5	0.09	TC: III, EC: III and CS
E.P. <sup>1</sup> (PC)	λ-Cyhalothrin (106) + Thiamethoxam (141)	Pyrethroid + Neonicotinoid	0.25	0.015	TC: III, EC: I and CS

<sup>1</sup>Manufacturer (local): Atanor S.A. (Buenos Aires, Argentina) (website: [http://www.adapar.pr.gov.br/arquivos/File/defis/DFI/Bulas/Herbicidas/atrazinaatanor50\\_SC.pdf](http://www.adapar.pr.gov.br/arquivos/File/defis/DFI/Bulas/Herbicidas/atrazinaatanor50_SC.pdf)), Arysta LifeScience do Brasil Indústria Química e Agropecuária Ltda. (Salto de Pirapora, São Paulo state, Brazil) (website: <http://www.adapar.pr.gov.br/arquivos/File/defis/DFI/Bulas/Herbicidas/paradox160318.pdf>), Sinon Corporation (Taichung City, Taiwan) (website: [http://www.adapar.pr.gov.br/arquivos/File/defis/DFI/Bulas/Herbicidas/Sanson\\_40SC\\_2017.pdf](http://www.adapar.pr.gov.br/arquivos/File/defis/DFI/Bulas/Herbicidas/Sanson_40SC_2017.pdf)), and Syngenta Proteção de Cultivos Ltda. (São Paulo, São Paulo state, Brazil) (website: <http://www.adapar.pr.gov.br/arquivos/File/defis/DFI/Bulas/Inseticidas/ENGEOPENOS010318.pdf>), respectively. <sup>2</sup>TC: toxicological class (I: extremely toxic, II: highly toxic, III: moderately toxic, and IV: slightly toxic). EC: environmental classification (I: highly dangerous, II: very dangerous, III: dangerous, and IV: slightly dangerous) according to the *Ministério da Agricultura, Pecuária e Abastecimento* (MAPA) of Brazil (MAPA, 2017). <sup>3</sup>CS: concentrated suspension.

## Data analysis

Three bioassays were carried out to evaluate the longevity, parasitism and emergence of *T. pretiosum*. Every bioassay was repeated three times, and the results are presented as the means of the replicates. The data on *T. pretiosum* longevity, parasitism and emergence when presented with *A. kuehniella* eggs in the different treatments were corrected (Abbott, 1925) and classified as 1- harmless (lower than 30% reduction), 2- slightly harmful (between 30 and 79% reduction), 3- moderately harmful (between 80 and 99% reduction), and 4- harmful (higher than 99% reduction) according to the International Organization for Biological Control (IOBC) (Sterk et al., 1999). These data were also submitted to analysis of variance (ANOVA), and the means were compared with the Scott-Knott test at 5% probability (Scott; Knott, 1974) using the statistical program SISVAR, version 5.6 (Supplier: *Universidade Federal de Lavras*, Lavras, Minas Gerais state, Brazil) (Ferreira, 2011).

## RESULTS AND DISCUSSION

### *Trichogramma pretiosum* longevity after parasitism on *A. kuehniella* eggs

#### Longevity at 24 hours post-treatment

The longevity of *T. pretiosum* females submitted to parasitism of *A. kuehniella* eggs at 24 hours post-treatment with the products was similar between the herbicides and the controls, ranging from  $18.00 \pm 3.22$  to  $27.60 \pm 6.20$  hours for the treatments with nicosulfuron and paraquat dichloride, respectively (Table 2). The similar longevity of *T. pretiosum* on *A. kuehniella* eggs at 24 hours post-treatment suggests selectivity of the tested chemical products. However, this differs from reports of nicosulfuron being the most toxic herbicide in terms of adult survival and longevity of *Trichogramma ostrinae* Pang & Chen, 1974 ( $LC_{50} = 0.266$  mg L<sup>-1</sup>) and *Trichogramma dendrolimi* Matsumura, 1926 ( $LC_{50} = 1.78$  mg L<sup>-1</sup>) after 24 hours of exposure to the dry residues of this herbicide, although there were some variations in toxicity between parasitoid species (Xu et al., 2013).

**Table 2:** Longevity in hours (mean  $\pm$  SE) of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) females, percentage of parasitized *Anagasta kuehniella* (Lepidoptera: Pyralidae) eggs (N= 45) and their emergence (mean  $\pm$  SE) after 24 and 48 hours of treatment (HT) with the following treatments (T): Atrazina Atanor 50 SC® (atrazine, triazines) (A.A.), Paradox® (paraquat dichloride, bipyridylum) (P.), Sanson 40 SC® (nicosulfuron, sulfonyleurea) (S.), and Engeo Pleno® ( $\lambda$ -cyhalothrin + thiamethoxam, pyrethroid + neonicotinoid, respectively) (E.P.) as a positive control (PC) and distilled water (D.W.) as a negative control (NC), their mean with chemical products, reduction of parasitism (R, %) and toxicological class (Class).

T	HT	Variables											
		Longevity			Parasitized eggs			Emergence					
		Mean	R.	Class	Mean	R.	Class	Mean	R.	Class	Mean	R.	Class
A.A.	24	24.00 $\pm$ 3.09aA	16.80	28.20	1	19.70 $\pm$ 2.78aA	12.90	0	1	76.91 $\pm$ 9.04aA	56.42	19.3	1
	48	9.60 $\pm$ 3.48bB				6.10 $\pm$ 2.57bB				35.94 $\pm$ 14.80bB			
P.	24	27.60 $\pm$ 6.20aA	24.00	0	1	18.20 $\pm$ 2.83aA	17.75	0	1	74.01 $\pm$ 9.21aA	72.22	0	1
	48	20.40 $\pm$ 2.56aA				17.30 $\pm$ 3.14aA				70.44 $\pm$ 11.97aA			
S.	24	18.00 $\pm$ 3.22aA	16.80	28.20	1	0.10 $\pm$ 0.01bA	0.05	99.6	4	10.00 $\pm$ 10.00bA	5.00	92.8	3
	48	15.60 $\pm$ 3.60bA				0.00 $\pm$ 0.00cA				0.00 $\pm$ 0.00cA			
E.P. (PC)	24	20.40 $\pm$ 5.38aA	23.40	0	1	0.42 $\pm$ 0.01bA	0.44	96.5	3	20.00 $\pm$ 13.33bA	28.48	59.3	2
	48	26.40 $\pm$ 3.91aA				0.47 $\pm$ 0.01cA				36.97 $\pm$ 15.27bA			
D.W. (NC)	24	20.40 $\pm$ 2.56aA	23.40	-	-	16.70 $\pm$ 2.75aA	12.75	-	-	75.73 $\pm$ 10.03aA	69.93	-	-
	48	26.40 $\pm$ 5.30aA				8.80 $\pm$ 2.30bB				64.13 $\pm$ 14.21aA			

\*Means followed by the same lowercase (hours) or capital letter (treatments) in a column did not differ according to the Scott-Knott test ( $P < 0.05$ ) (Scott; Knott, 1974); reduction (%) =  $1 - (\text{general mean of the product} \div \text{general mean of negative control})$  (Abbott, 1925); classification of toxicity according to the International Organization for Biological Control (IOBC) (Sterk, 1999), class 1= harmless (<30% reduction), class 2= slightly harmful (30 to 79% reduction), class 3= moderately harmful (80 to 99% reduction), and class 4= harmful (> 99% reduction).

### Longevity at 48 hours post-treatment

The longevity of *T. pretiosum* females submitted to parasitism of *A. kuehniella* eggs at 48 hours post-treatment with the products was lowest with atrazine and nicosulfuron, with values of  $9.60 \pm 3.48$  and  $26.40 \pm 5.30$  hours for atrazine and the negative control, respectively (Table 2). The shorter longevity of *T. pretiosum* females at 48 hours post-treatment with atrazine and nicosulfuron demonstrates the persistence of these herbicides. Atrazine is highly toxic and persistent in the environment, as reported in the field, where it adversely affected sunflower, *Helianthus annuus* L. (Asteraceae), in areas previously cultivated with corn (Brighenti et al., 2002). However, this disagrees with the low toxicity of atrazine after 144 hours of exposure of *T. pretiosum* adults to the herbicide Primóleo® (herbicide, a.i. atrazine, group: triazine) (Stefanello Júnior et al., 2008) and of the effects of Sanson 40 SC® and Primóleo® on the longevity of females of the gregarious endoparasitoid *Palmistichus elaeisis* Delvare & La Salle, 1993 (Hymenoptera: Eulophidae) 48 hours after the parasitism of treated mealworms, *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) pupae (Menezes et al., 2014).

### Longevity at 24 and 48 hours post-treatment

The longevity of *T. pretiosum* females was similar at 24 and 48 hours post-treatment with the products and lowest with atrazine 48 hours post-treatment ( $9.60 \pm 3.48$  hours), with all the others and the controls classified as harmless (lower than 30% reduction) (Table 2). The reduction in parasitism and emergence of *T. pretiosum* under the positive control (product containing  $\lambda$ -cyhalothrin + thiamethoxam) and the low impact on the longevity of this parasitoid agree with reports of low  $\lambda$ -cyhalothrin toxicity to *T. pretiosum* adults in terms of longevity 48 hours after parasitism on treated *A. kuehniella* eggs (Souza et al., 2013). Cyhalothrin sprayed onto the leaves of upland cotton, *Gossypium hirsutum* L. (Malvaceae) plants was innocuous to *Trichogramma exiguum* Pinto & Platner, 1983 females four days after treatment (Suh; Orr; Van Duyn, 2000). On the other hand, of the chemicals tested, this insecticide was the most harmful to *T. galloi* larvae parasitizing *D. saccharalis* eggs immersed for five seconds after 24 hours of parasitism (Oliveira et al., 2013). The toxicity of  $\lambda$ -cyhalothrin to egg parasitoids depends on host susceptibility and the developmental stage of the target insect's nervous system because this pyrethroid acts by interrupting this system (Cônsoли; Parra; Hassan, 1998).

### Parasitism of *A. kuehniella* eggs treated with *T. pretiosum*

#### Parasitism of eggs at 24 hours post-treatment

The number of eggs parasitized by *T. pretiosum* at 24 hours post-treatment with the chemical products was lowest with nicosulfuron and the positive control ( $\lambda$ -cyhalothrin + thiamethoxam), with values of  $0.10 \pm 0.01$  and  $19.70 \pm 2.78$  for nicosulfuron and atrazine, respectively (Table 2). The lowest parasitism rate of *T. pretiosum* under the positive control with  $\lambda$ -cyhalothrin + thiamethoxam and nicosulfuron when submitted to parasitism after 24 hours post-treatment of *A. kuehniella* eggs agrees with the reduction of greater than 30% of this parameter for this natural enemy when treated with  $\lambda$ -cyhalothrin (Stefanello Júnior et al., 2008). The parasitism of *A. kuehniella* eggs in the laboratory was lower for *T. pretiosum* adults after contact with the dry residues of Sanson 40 SC® on glass plates (Stefanello Júnior et al., 2012). In addition, Sanson 40 SC® reduced parasitism by *P. elaeisis* on *T. molitor* pupae after immersion for two seconds in a solution of this product (Menezes et al., 2014). However, nicosulfuron at the recommended dose had a low effect on the mortality and parasitism of *T. pretiosum* on eggs of the alternative host after immersion in the herbicide solution (Khan; Ruberson, 2017).

#### Parasitism of eggs at 48 hours post-treatment

The number of eggs parasitized by *T. pretiosum* at 48 hours post-treatment with the chemical products was lowest with nicosulfuron and the positive control ( $\lambda$ -cyhalothrin + thiamethoxam) and intermediate with atrazine and the negative control, with values ranging from  $0.00 \pm 0.00$  to  $17.30 \pm 3.14$  with nicosulfuron and paraquat dichloride, respectively (Table 2). The highest impact of nicosulfuron and the positive control based on  $\lambda$ -cyhalothrin + thiamethoxam on the parasitism of *T. pretiosum* on the eggs of the alternative host *A. kuehniella* at 48 hours post-treatment was due to the toxicity of these compounds to Trichogrammatidae. However, the effects of chemical products on *T. pretiosum* and other parasitoids of this group depend on their toxicity and may be enhanced by adjuvants and other compounds in their formulation (Menezes et al., 2014). This was shown by the lack of impact on the mortality of pupae and parasitism of subsequent generations of *Trichogramma chilonis* Ishii, 1941 when parasitizing rice moth, *Corcyra cephalonica* Stainton, 1866 (Lepidoptera: Pyralidae) eggs treated with the herbicides 2,4-DEE 38 EC® (group: aryloxyalkanoic acid), imazosulfuron 10 SC® (group: sulfonyleureas), paraquat

dichloride 24 SL<sup>®</sup> (group: bipyridylum), pretilachlor 50 EC<sup>®</sup> (group: chloroacetamide), sulfosulfuron 75 WP<sup>®</sup> (group: sulfonyleureas), and sumisoya 50 WG<sup>®</sup> (group: cyclohexene dicarboximide). This suggests that adjuvants and other compounds added to the herbicide solution enhance negative effects on Trichogrammatidae (Mandal; Debnath; Panja, 2006).

### Parasitism of eggs at 24 and 48 hours post-treatment

The number of *A. kuehniella* eggs parasitized by *T. pretiosum* was lower at 48 hours post-treatment with atrazine and under the negative control. Nicosulfuron and the positive control ( $\lambda$ -cyhalothrin + thiamethoxam) were classified as class 4 (harmful, >99% reduction) and class 3 (moderately harmful, 80 to 99% reduction), respectively (Table 2). A low impact of the paraquat dichloride herbicide on *T. pretiosum* parasitism was also reported for this herbicide when applied to *C. cephalonica* eggs after 96 hours of parasitism by *T. chilonis*, without affecting this parameter for this parasitoid (Mandal et al., 2006). This is likely due to the tolerance of this biocontrol agent to the active ingredient because paraquat dichloride was the most toxic to the parasitoid *Telenomus remus* Nixon, 1937 (Hymenoptera: Platygastridae) among the tested fungicides, herbicides and insecticides (Carmo; Bueno; Bueno, 2010). The parasitism of *A. kuehniella* eggs by *T. pretiosum* after contact with dry chemical product residues was lowest with Gramoxone 200<sup>®</sup> (herbicide, a.i. paraquat dichloride, group: bipyridylum) (Stefanello Júnior et al., 2008), and Gramocil<sup>®</sup> (herbicide, a.i. paraquat dichloride, group: bipyridylum) was innocuous to larvae of the endoparasitoid *T. remus* after treatment of *S. frugiperda* eggs submitted to parasitism (Carmo et al., 2009). The paraquat dichloride selectivity for *T. pretiosum* disagrees with the act from 2008 of the Agência Nacional de Vigilância Sanitária (ANVISA) of the Ministério da Saúde, Brasília, Brazil, which directed the toxicological re-evaluation of products formulated with this active ingredient via the Resolução da Diretoria Colegiada (RDC) No. 10. This was justified given that this active ingredient shows highly acute and chronic toxicity to farmers. In addition, RDC No. 190, dated November 30, 2017, published in the Diário Oficial da União No. 230 on December 1, 2017, declared that the importation of all products and formulations based on this active ingredient will be prohibited in Brazil beginning on September 22, 2020 (ANVISA, 2017). Paraquat dichloride is a non-selective herbicide with contact action and is recommended for use in weed control in exportation crops, such as *Digitaria sanguinalis* (L.) Scop. (Poaceae) control in corn, in addition to the control of *Euphorbia heterophylla*

(Euphorbiaceae) and *Lolium multiflorum* Lam. (Poaceae) in upland cotton, *Cenchrus echinatus* L. (Poaceae) and *Portulaca oleracea* L. (Caryophyllales) in coffee (*Coffea*; Rubiaceae), *Brachiaria plantaginea* (Link) Hitchc. (Poales) in soybean (*Glycine max* L. Merrill; Fabaceae), and *Bidens pilosa* L. (Asteraceae) and *Galinsoga parviflora* Cav. (Asteraceae) in wheat (ANVISA, 2017).

### Emergence of *T. pretiosum* from treated *A. kuehniella* eggs

#### Emergence from eggs at 24 hours post-treatment

The emergence of *T. pretiosum* from eggs at 24 hours post-treatment was lowest with nicosulfuron and in the positive control ( $\lambda$ -cyhalothrin + thiamethoxam), with values ranging from  $10.00 \pm 10.00$  to  $76.91 \pm 9.04\%$  for nicosulfuron and atrazine, respectively (Table 2). The lowest emergence of *T. pretiosum* from *A. kuehniella* eggs submitted to parasitism at 24 hours post-treatment with the products containing nicosulfuron and  $\lambda$ -cyhalothrin + thiamethoxam differed from results showing the highest female emergence of this biocontrol agent and *Trichogramma acacioi* Brun, Moraes & Soares, 1984 and *Trichogrammatoidea annulata* de Santis, 1972 from *A. kuehniella* eggs treated with nicosulfuron at  $1.50 \text{ L ha}^{-1}$  than in the control with only distilled water but reduced female emergence of *Trichogramma bruni* Nagaraja, 1983 (Leite et al., 2016). Sanson 40 SC<sup>®</sup> applied to glass plates in rearing cages with *T. pretiosum* adults was classified as selective for the emergence of this parasitoid from *A. kuehniella* eggs (Stefanello Júnior et al., 2008).

#### Emergence from eggs at 48 hours post-treatment

The emergence of *T. pretiosum* from eggs at 48 hours post-treatment was lowest with nicosulfuron and intermediate with atrazine and the positive control ( $\lambda$ -cyhalothrin + thiamethoxam), with values ranging from  $0.00 \pm 0.00$  to  $70.44 \pm 11.97$  for nicosulfuron and paraquat dichloride, respectively (Table 2). The lowest emergence of *T. pretiosum* from *A. kuehniella* eggs submitted to parasitism at 48 hours post-treatment with the product containing nicosulfuron differed from results showing the highest adult emergence of this parasitoid after contact with nicosulfuron applied to glass plates in rearing cages and their pre-submission to parasitism on eggs of this alternative host (Stefanello Júnior et al., 2008) and the larvae of this parasitoid on eggs of this moth treated with the same product after submission to parasitism (Leite et al., 2016), possibly due to the different forms of herbicide application. The first study evaluated the emergence of

offspring whose host eggs were treated pre-parasitism with the herbicide, which may have slowly penetration into them (Hassan; Abdelgader, 2001), causing toxicity and reducing offspring emergence. The second and third studies, with lower risk to emergence, involved glass plates treated with the herbicide in adult rearing cages and eggs of the host treated post-parasitism, respectively. The parasitoid may have been protected by its own eggs in the third study. The mixture of Sanson 40 SC<sup>®</sup> and Gesaprim 500 Ciba Geigy<sup>®</sup> (herbicide, a.i. atrazine, group: triazines) also showed no reduction in the emergence of *T. pretiosum* adults on post-treated *A. kuehniella* eggs (Leite et al., 2017). The classification of nicosulfuron as harmless (class 1, <30% reduction), having a low capacity to penetrate into the chorion of eggs of the alternative host, *A. kuehniella*, with low acidity (pH= 6.09), a hydrophilic quality and a molecular weight of 410.4 mg mol<sup>-1</sup> (Leite et al., 2016), is therefore questionable.

### Emergence from eggs at 24 and 48 hours post-treatment

*Trichogramma pretiosum* emergence was lowest under atrazine at 48 hours post-treatment. Nicosulfuron was classified as class 3= moderately harmful (80 to 99% reduction), the positive control ( $\lambda$ -cyhalothrin + thiamethoxam) was classified as class 2= slightly harmful (30 to 79% reduction), and atrazine was classified as class 1= harmless (lower than 30% reduction) (Table 2). The lack of impact of atrazine on *T. pretiosum* emergence was similar to that reported for the herbicide Gesaprim 500 Ciba Geigy<sup>®</sup> when sprayed onto *A. kuehniella* eggs subjected to parasitism (Leite et al., 2015). The higher emergence of *T. pretiosum* and *T. remus* from *A. kuehniella* and *S. frugiperda* eggs treated with the herbicide Paradox<sup>®</sup> than with Gramoxone 200<sup>®</sup> classified them as slightly harmful and selective, respectively (Carmo et al., 2009, 2010).

### CONCLUSIONS

The products were classified as harmless to the longevity of *T. pretiosum*. On the other hand, the nicosulfuron-based product and the positive control with  $\lambda$ -cyhalothrin + thiamethoxam were classified as moderately harmful and harmful to parasitism, respectively, and as moderately harmful and slightly harmful to the emergence of *T. pretiosum* from *A. kuehniella* when offered for parasitism after 48 hours of egg treatment, respectively. Paraquat dichloride showed compatibility for the management of weeds in corn

crops with biological control using *T. pretiosum*. This parasitoid coexists with several other natural enemies and non-target arthropod species in corn crops that could be affected negatively by herbicides. Even considering the low toxicity to the parasitoid species used in the present test, paraquat dichloride should be used carefully for the control of weeds in integrated pest management programmes in corn crops.

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