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Nota científica / Scientific note

Toxicological and behavioral impacts of atrazine on Trichogrammatidae (Hymenoptera) in choice tests

Impactos toxicológicos y conductuales de la atrazina en Trichogrammatidae (Hymenoptera) en la prueba de elección

GERMANO LEÃO DEMOLIN LEITE[1]; EDILSON PAULO RODRIGUES BISPO[2]; ANARELLY COSTA ALVARENGA[3]; PAULA DAIANA DE PAULO[4]; MARCUS ALVARENGA SOARES[5]; PEDRO GUILHERME LEMES[6]

[1] D. Sc., Insetário G. W. G. Moraes, Instituto de Ciências Agrárias, Universidade Federal de Minas Gerais, Caixa Postal 135, 39404-547 - Montes Claros, Minas Gerais State, Brazil, *germano.demolin@gmail.com*, https://orcid.org/0000-0002-2928-3193. [2] Agronomist, Insetário G. W. G. Moraes, Instituto de Ciências Agrárias, Universidade Federal de Minas Gerais, Caixa Postal 135, 39404-547 - Montes Claros, Minas Gerais State, Brazil, *ed-agro@hotmail.com,* https://orcid.org/0000-0003-4514-1395. [3] D. Sc., Insetário G. W. G. Moraes, Instituto de Ciências Agrárias, Universidade Federal de Minas Gerais, Caixa Postal 135, 39404-547 - Montes Claros, Minas Gerais State, Brazil, *aacosta@yahoo.com.br*, https://orcid.org/0000-0002-0487-881X. [4] D. Sc., Insetário G. W. G. Moraes, Instituto de Ciências Agrárias, Universidade Federal de Minas Gerais, Caixa Postal 135, 39404-547 - Montes Claros, Minas Gerais State, Brazil, *ppaula@gmail.com*, https://orcid.org/0000-0002-6540-8969. [5] D. Sc., Departamento de Agronomia, Universidade Federal dos Vales do Jequitinhonha e Mucuri, 39.100-000, Diamantina, State of Minas Gerais, Brazil, *marcusasoares@yahoo.com.br*, https://orcid.org/0000-0002-8725-3697. [6] D. Sc., Insetário G. W. G. Moraes, Instituto de Ciências Agrárias, Universidade Federal de Minas Gerais, Caixa Postal 135, 39404-547 - Montes Claros, Minas Gerais State, Brazil, *pedroglemes@hotmail.com*, https://orcid.org/0000-0002-1364-0424.

Abstract: Weeds should be controlled with low impact methods and selective agrochemicals that have little or no effect on non-target organisms. This study aimed to evaluate the effect of the herbicide atrazine (triazine class) on 10 Trichogrammatidae (Hymenoptera) species. A female of 10 species of these natural enemies was individually placed in a glass test tube (free-choice test) with two paper cards containing 45 *Anagasta* (*Ephestia*) *kuehniella* (Lepidoptera: Pyralidae) eggs (treatment and control), with 10 replications. The cards were sprayed with the herbicide atrazine at 8.07 L/ha; the control was sprayed with distilled water. Parasitism by these natural enemies was allowed for 48 h. Atrazine changes the behavior of female parasitoids, reducing the parasitism (≈ 71 %) and emergence (≈ 74 %) rates and sex ratio (≈ 74 %) of the Trichogrammatidae species, except for *Trichogramma galloi* and *T. bennetti*. No females emerged from eggs parasitized by *T. acacioi*, *Trichogrammatoidea annulata*, *T. atopovirilia*, *T. bruni*, *T. brasiliensis*, *T. demoraesi*, and *T. soaresi* with atrazine. The results revealed that atrazine herbicide is harmless to *T. bennetti* and *T. galloi*, but it was moderately harmful (80 – 99 % reduction) to the other Trichogrammatidae species based on the parasitism and emergence rates.

Keywords: Biological control, egg parasitoid, hormesis, *Trichogramma*, *Trichogrammatoidea*, *Zea mays.*

Resumen: Las malezas deben ser controladas con métodos de bajo impacto y agroquímicos selectivos que afectan poco o nada a organismos no blanco. El objetivo de este estudio fue evaluar el efecto del herbicida atrazina (clase triazina) en 10 especies de Trichogrammatidae (Hymenoptera). Una hembra de 10 especies de estos enemigos naturales se colocó individualmente en un tubo de ensayo de vidrio (prueba de libre elección) con dos tarjetas de papel que contenían 45 huevos de *Anagasta* (*Ephestia*) *kuehniella* (Lepidoptera: Pyralidae) (tratamiento y control), con 10 repeticiones. Las tarjetas se rociaron con el herbicida atrazina a 8,07 L/ha y las del control con agua destilada. Durante 48 h se permitió el parasitismo de estos enemigos naturales. La atrazina cambia el comportamiento de los parasitoides hembra al reducir las tasas de parasitismo (≈ 71 %) y de emergencia (≈ 74 %) y la proporción de sexos (≈ 74 %) de las especies de Trichogrammatidae, excepto para *Trichogramma galloi* y *T. bennetti*. Ninguna hembra emergió de huevos parasitados por *T. acacioi*, *Trichogrammatoidea annulata*, *T. atopovirilia*, *T. bruni*, *T. brasiliensis*, *T. demoraesi* y *T. soaresi* en el tratamiento con atrazina. Los resultados de esta investigación revelan que el herbicida atrazina es inofensivo para *T. bennetti* y *T. galloi*, pero moderadamente dañino (80 - 99 % reducción) para las otras especies de Trichogrammatidae.

Palabras clave: Control biológico, parasitoide del huevo, hormesis, *Trichogramma*, *Trichogrammatoidea*, *Zea mays.*

Corresponding author
D. Sc., Marcus Alvarenga Soares. Departamento de Agronomia, Universidade Federal dos Vales do Jequitinhonha e Mucuri, 39.100-000, Diamantina, State of Minas Gerais, Brazil. *marcusasoares@yahoo.com.br*, https://orcid.org/0000-0002-8725-3697.

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Introduction

Corn (*Zea mays* L., Poaceae) is the most important cereal originating in the Americas and planted on large scale worldwide. Caterpillars and weeds are the main pests of the corn crop in Brazil (Zanuncio *et al.* 2013; Menezes *et al.* 2014). The caterpillars are controlled using insecticides, however, these products can cause environmental contamination and alternative methods are necessary to manage these insect pests (Tavares 2010). Natural enemies, especially egg parasitoids such as *Trichogramma* spp. (Hymenoptera: Trichogrammatidae), parasitize *Spodoptera* spp. (Lepidoptera: Noctuidae) and other caterpillars (Spínola-Filho *et al.* 2014). These organisms can reduce damage by caterpillar pests in corn crops and their parasitism on eggs prevents larvae from hatching (Gardner *et al.* 2011).

Weeds can reduce corn yield by up to 85 % (Fickett *et al.* 2013; Pascoaloto *et al.* 2017) and herbicides are exclusively used for their control. Herbicides with atrazine (triazine class), which inhibits the photosystem II causing irreversible damage to plant cells (Chen *et al.* 2014), are the most widely used to control dicotyledonous plants at pre- or post-emergence in this crop (Das *et al*. 2010).

Herbicides can affect *Trichogramma* spp. parasitism because their active ingredient can penetrate the insects' cuticle (Leite *et al.* 2015). The herbicide's effects on parasitoids may vary with the salt quantity and type and adjuvants or their mixture, which is usually done in the field (Stefanello Júnior *et al.* 2011). *Trichogramma* spp. can be used as a model to determine the selectivity of agrochemicals to natural enemies (Menezes and Soares 2016).

This study aimed to evaluate the compatibility of parasitoids with atrazine herbicide by toxicological and behavioral impacts of parasitoid females from 10 species of Trichogrammatidae (9 *Trichogramma* spp. + 1 *Trichogrammatoidea* sp.).

Materials and methods

This study was conducted at the Laboratory of Entomology and in the G.W.G. de Morais Insectarium of the Institute of Agricultural Sciences (ICA) of the "Universidade Federal de Minas Gerais (UFMG)" in Montes Claros, Minas Gerais state, Brazil, in 2014.

The experiment had a completely randomized design with 10 parasitoid species and one herbicide, besides the control, with 10 replications. Each parcel had two white paper cards (0.4 cm width × 2.0 cm length) with 45 *Anagasta (Ephestia) kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) eggs (200 total cards). Ten Trichogrammatidae species, commonly found in the crops in Brazil, were obtained from the Insectarium of the ICA/UFMG, with nine species of the genus *Trichogramma* - *T. acacioi* Brun, Moraes and Soares, 1984; *T. atopovirilia* Oatman and Platner, 1983; *T. bennetti* Nagaraja and Nagarkatti, 1973; *T. brasiliensis* Ashrnead, 1904; *T. bruni* Nagaraja, 1983; *T. demoraesi* Nagaraja, 1983; *T. galloi* Zucchi, 1988; *T. pretiosum* Riley, 1879, and *T. soaresi* Nagaraja, 1983, and one of the genus *Trichogrammatoidea* - *T. annulata* De Santis, 1972. The treatments were carried out with the herbicide atrazine - Gesaprim 500 Ciba-Geigy® (recommended commercial dose for corn) and distilled water (control) (Stefanello Júnior *et al.* 2008; 2011).

A total of 45 *A. kuehniella* eggs were glued per white paper card with 10 % Arabic gum, exposed to ultraviolet radiation (UV) for 60 min, placed in glass vials (7.5 cm diameter × 13.0 cm height), sealed with plastic polyvinyl chloride (PVC) and an elastic film, and stored in a refrigerator at 5 °C and 80 % R.H. for 24 h. After this period, each card was sprayed with atrazine using a Guarany® hand sprayer (Itú, São Paulo, Brazil) until runoff began (Gesaprim 500 Ciba-Geigy®) at 8.07 $L.ha^{-1}$ (20.16 × 10^{-5} mL of commercial product/card, 10.08 × 10^{-2} mg of active ingredient/card). The control eggs were sprayed with distilled water. The hand sprayer used was measured and had its conformity evaluated by the "Instituto Nacional de Metrologia, Qualidade e Tecnologia (INMETRO)" of the "Ministério do Desenvolvimento, Indústria e Comércio Exterior (MDIC)" of Brazil. The hand sprayer was tested three times (replicates) with a 30 min calibration process before its use. The cards were subsequently kept in the shade outdoors for 2 h to evaporate water excess and placed in sealed transparent glass test tubes (1.0 cm diameter × 9.0 cm height) with a newly emerged female parasitoid (< 24 h old, without food) for 48 h at 12:12 h (light:dark) photoperiod and 24.39 ± 0.01 °C (Soares *et al.* 2012; 2014). Each test tube received two cards with treatment and control. The parasitism was allowed until Trichogrammatidae female death (after about six days). The experimental plot was repeated if females died before 48 h of parasitism.

The parasitized eggs, male and female emergence and the female-biased sex ratio (number of females ÷ number of males + number of females) were evaluated. Parasitized eggs were those with dark colour after 20 days of subjection to parasitism, and the non-parasitized of yellow colour (Pratissoli *et al.* 2004; Prezotti *et al.* 2004). Male and female Trichogrammatidae were identified according to the antenna dimorphism (males have feathery antennae and females nailed ones) (Zuim *et al.* 2017), using a binocular microscope with 40 × magnification.

Herbicide toxicity was classified based on the parasitism and emergence rate reduction as follows: I = harmless (< 30 % reduction), II = slightly harmful (30 - 79 % reduction), III = moderately harmful (80 - 99 % reduction), and IV= harmful (> 99 % reduction) (Sterk *et al.* 1999). The reduction in the emergence rate of the parasitoid species was calculated as follows: % reduction = 100 - mean [(% mean of the treatment ÷ % mean of the control) × 100] (Carvalho *et al.* 2010). The female parasitism and emergence reduction were calculated with the formula: % reduction = 100 - mean [(% overall of the treatment with insecticide ÷ % overall of the control treatment) × 100]. The data were transformed to arcsine, also submitted to analysis of variance (one-way ANOVA) and the means examined using the Tukey's HSD (honest significant difference) test at 1 % or 5 % probability. The "sistema para análises estatísticas (SAEG)", version 9.1 (Supplier: UFV) was the program used.

Results

Atrazine changes the behavior of parasitoid females by reducing the parasitism rate (≈ 71 %) of the Trichogrammatidae species, except for *T. galloi* and *T. bennetti*. This herbicide was harmless (< 30 % reduction) to these two natural enemies but it was moderately harmful (80 - 99 % reduction) to the other parasitoid species (Table 1).

The emergence rate (≈ 74 %) of Trichogrammatidae females, except those of *T. galloi* and *T. bennetti*, was lower with atrazine. No parasitoid female emerged from eggs parasitized by *T. acacioi*, *T. annulata*, *T. atopovirilia*, *T. bruni*, *T. brasiliensis*, *T. demoraesi*, and *T. soaresi* that had been previously sprayed with atrazine. This herbicide was harmless to *T. bennetti* and *T. galloi*, but moderately harmful to *T. pretiosum* and harmful (> 99 % reduction) to the other Trichogrammatidae species (Table 1).

The sex ratio (≈ 74 %) of ten Trichogrammatidae species, except for *T. galloi* and *T. bennetti*, was lower with atrazine. This herbicide was harmless to these two *Trichograma* species but moderately harmful to *T. pretiosum*, and harmful to the other Trichogrammatidae species (Table 1).

Discussion

Trichogrammatidae species showed a reduction in the parasitism behavior and the rates of emergence of females in eggs sprayed with atrazine, probably due to the effect of repellency to oviposition and also to the mortality of immatures inside the host eggs (Leite *et al.* 2015). The effects of atrazine-based herbicides on Trichogrammatidae species depend on the doses used and formulations of this chemical (Stefanello Júnior *et al.* 2008; Menezes and Soares 2016). The lower parasitism rate by the Trichogrammatidae species (repellence

Table 1. Percentage of parasitism, emergence of female and sex ratio (mean and standard error), reduction (%) (Redu.), and classification (Class.) of *Trichogrammatoidea annulata* (Hymenoptera: Trichogrammatidae), and nine *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) after treatment with atrazine. Montes Claros, Minas Gerais state, Brazil

	Atrazine		**Control**				**ANOVA (*gl* = 9)**	
Species	**Average**	**SE**	**Average**	**SE**	**Redu.**	**Class**	**F**	**P**
Percentage of parasitism								
*Trichogramma acacioi***	7.78 b	5.25	41.78 a	12.30	81.4	3	9.128	0.01445
*Trichogrammatoidea annulata**	6.44 b	4.54	52.44 a	6.66	87.7	3	50.736	0.00006
*Trichogramma atopovirilia***	6.00 b	6.00	31.56 a	4.59	81.0	3	17.080	0.00255
Trichogramma bennetti n.s.	28.00 a	3.73	33.33 a	9.39	16.0	1	0.237	*****
*Trichogramma bruni***	3.11 b	3.11	41.78 a	10.42	92.6	3	10.158	0.01106
*Trichogramma brasiliensis**	7.78 b	4.04	54.00 a	9.70	85.6	3	21.565	0.00121
*Trichogramma demoraesi**	2.22 b	2.22	43.56 a	9.55	94.9	3	20.669	0.00139
Trichogramma galloi n.s.	39.11 a	7.33	35.87 a	6.50	- 9.03	1	0.098	*****
*Trichogramma pretiosum***	2.89 b	2.88	23.11 a	6.86	87.6	3	7.904	0.02033
*Trichogramma soaresi**	2.44 b	2.66	35.56 a	6.73	92.5	3	20.639	0.00140
Emergence of female								
*Trichogramma acacioi**	0.00 b	0.00	49.17 a	13.57	100.0	4	13.113	0.00556
*Trichogramma annulata**	0.00 b	0.00	74.29 a	9.10	100.0	4	66.544	0.00002
*Trichogramma atopovirilia**	0.00 b	0.00	65.56 a	8.99	100.0	4	53.132	0.00005
Trichogramma bennetti n.s.	85.49 a	10.01	55.50 a	15.19	- 54.0	1	2.275	0.16571
*Trichogramma bruni**	0.00 b	0.00	68.83 a	15.04	100.0	4	20.943	0.00133
*Trichogramma brasiliensis**	0.00 b	0.00	72.27 a	12.08	100.0	4	35.747	0.00021
*Trichogramma demoraesi**	0.00 b	0.00	83.91 a	6.66	100.0	4	75.424	0.00001
Trichogramma galloi n.s.	52.43 a	8.30	56.00 a	7.86	6.38	1	0.097	*****
*Trichogramma pretiosum***	10.00 b	10.00	60.00 a	16.32	83.3	3	9.00	0.01496
*Trichogramma soaresi**	0.00 b	0.00	66.53 a	11.49	100.0	4	33.520	0.00026
Sex ratio								
*Trichogramma acacioi**	0.00 b	0.00	0.51 a	0.14	100.0	4	13.123	0.00555
*Trichogramma annulata**	0.00 b	0.00	0.90 a	0.10	100.0	4	81.000	0.00060
*Trichogramma atopovirilia**	0.00 b	0.00	0.72 a	0.08	100.0	4	63.728	0.00002
Trichogramma bennetti n.s.	0.90 a	0.10	0.60 a	0.16	- 50.0	1	1.976	0.19342
*Trichogramma bruni**	0.00 b	0.00	0.69 a	0.15	100.0	4	20.948	0.00133
*Trichogramma brasiliensis**	0.00 b	0.00	0.80 a	0.13	100.0	4	36.000	0.00020
*Trichogramma demoraesi**	0.00 b	0.00	0.90 a	0.10	100.0	4	81.000	0.00000
Trichogramma galloi n.s.	0.75 a	0.09	0.82 a	0.09	8.54	1	0.209	*****
*Trichogramma pretiosum***	0.10 b	0.10	0.60 a	0.16	83.3	3	9.000	0.01495
*Trichogramma soaresi**	0.00 b	0.00	0.80 a	0.13	100.0	4	36.000	0.00020

Means followed by a lower letter per line do not differ by the Tukey's HSD (honest significant difference) test (* P < 0.01, ** P < 0.05). n.s.= not significant by Variance Analyses (ANOVA) (P > 0.05). Classification of toxicity index: class I = harmless (< 30 % reduction), class II = slightly harmful (30 % - 79 % reduction), class III = moderately harmful (80 % - 99 % reduction), and class IV = harmful (> 99 % reduction).

effect of atrazine), except in *T. galloi* and *T. bennetti*, can be explained by the ability of these species to detect nocive substances, rejecting hosts and, consequently, reducing parasitism. *Trichogramma galloi* may be more resistant to pesticides as chlorantraniliprole (insecticide, anthranilic diamides chemical group), sulfometruron methyl (plant growth regulator, sulfonylurea chemical group), and triflumuron (insecticide, benzoylurea chemical group) that were demonstrated to not repel or reduced its parasitism rate in a previous Brazilian study (Antigo *et al.* 2013). Tolfenpyrad (insecticide, pyrazole chemical group) reduced the foraging behavior of *T. pretiosum* but this was not found with 11 other pesticides, indicating that parasitoids could successfully forage on eggs treated with many pesticides in a Pakistani study (Khan *et al.* 2015).

The lack of impact of atrazine on the emergence of *T. galloi* and *T. bennetti* females may be due to its detoxification capacity (Stefanello Júnior *et al.* 2011; Oliveira *et al.* 2014). This indicates greater resistance of these parasitoid species to atrazine. Primoleo® (herbicide, triazine chemical group) and Siptran 500 SC® (herbicide, triazine chemical group) were classified as class I (harmless, < 30 % reduction) and the Gesaprim GrDA® (herbicide, triazine chemical group) as class II (slightly harmful, 30 - 79 % reduction) for the mortality of *T. pretiosum* adults in a previous Brazilian study (Stefanello Júnior *et al*. 2008). The survival of the predator *Podisus nigrispinus* Dallas, 1851 (Hemiptera: Pentatomidae), the parasitoids *T. atopovirilia*, *T. bennetti* and *T. brunni*, and soil arthropods such as Collembola, mites (Acari), and ants (Hymenoptera: Formicidae) was lower than 50 % when submitted to herbicides with atrazine in three different Brazilian studies (Pereira *et al.* 2005; Lins *et al.* 2007; Menezes *et al.* 2012). The higher emergence rate of *T. pretiosum*, *T. demoraesi*, *T. galloi*, and *T. soaresi* with atrazine in a no-choice test in a Brazilian study may be related to the "hormesis" phenomenon (Leite *et al.* 2015) where in sublethal quantities of a stressor benefit an organism (Pratissoli *et al.* 2010). This hypothesis is possible, especially considering that the herbicide quantity reaching the parasitoid inside the host egg is very low (Leite *et al.* 2015). However, the "hormesis" phenomenon with these four *Trichogramma* species was not detected in this work (free-choice test), possibly due to the reduction of the parasitism and, consequently, female emergence rate.

No impact of atrazine on *T. galloi* and *T. bennetti* sex ratio may show, as explained before, host egg protection and detoxification capacity (Stefanello Júnior *et al.* 2011; Oliveira *et al.* 2014), but it needs to be better studied to understand the responses of both parasitoids to the herbicide. The emergence of *Aedes* (= *Stegomyia*) *aegypti* L., 1762 (Diptera: Culicidae) and *Aedes* (= *Stegomyia*) *albopictus* Skuse, 1894 (Diptera: Culicidae) were higher with atrazine than with glyphosate (herbicide, glycine chemical class) in a North American study. The sex ratio was distorted with male bias observed in the control and glyphosate treatments, but not with atrazine (Bara *et al.* 2014). The emergence period for both sexes of these two mosquito species was longer with atrazine than with glyphosate and in the control (Bara *et al.* 2014). The reduced sex ratio of *T. bruni*, *T. atopovirilia* and *T. bennetti* and higher values for *T. demoraesi* and *T. soaresi* in no-choice tests in a Brazilian study (Leite *et al.* 2015) shows that the impact of atrazine, a widely used herbicide, on life-history traits as reported for mosquitoes (Bara *et al.* 2014) depends on the species of insect.

Conclusions

The parasitoids *T. bennetti* and *T. galloi* showed adequate values of parasitism and emergence in eggs previously sprayed with atrazine and can be used simultaneously with the application of this herbicide.

Trichogramma acacioi, T. annulata, T. atopovirilia, T. bruni, T. brasiliensis, T. demoraesi, and *T. soaresi* species should be released, after the residual toxicity period of the herbicide, to avoid reducing its efficiency in biological control.

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Author contribution

Edilson Paulo Rodrigues, Germano Leão Demolin Leite, Anarelly Costa Alvarenga and Paula Daiana De Paulo carried out the experiments.

Germano Leão Demolin Leite, Pedro Guilherme Lemes and Marcus Alvarenga Soares analyzed the data and performed the interpretation of the results.

Germano Leão Demolin Leite and Marcus Alvarenga Soares wrote the manuscript.

Germano Leão Demolin Leite supervised the project.