Effects of Atrazine-Based Herbicide on Emergence and Sex Ratio of Trichogrammatidae (Hymenoptera)

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Effects of atrazine-based herbicide on emergence and sex ratio of Trichogrammatidae (Hymenoptera)

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Abstract

The aim of this study was to evaluate the effect of the herbicide atrazine, recommended for weed control in corn, on 10 species of Trichogrammatidae (Hymenoptera). A female of each trichogrammatid was placed individually in a test tube (no-choice) with a card containing approximately 45 *Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae) eggs. Parasitism by these trichogrammatids was allowed for 48 h, and the cards were sprayed with the herbicide atrazine at 6 L/ha, along with a control (distilled water). Atrazine reduced the emergence of *Trichogramma bruni* Nagaraja females, but increased that of *Trichogramma pretiosum* Riley, *Trichogramma demoraesi* Nagaraja, *Trichogramma galloi* Zucchi, and *Trichogramma soaresi* Nagaraja. In addition, atrazine reduced the sex ratio of *T. bruni*, *Trichogramma atopovirilia* Oatman & Platner, and *Trichogramma bennetti* Nagaraja & Nagarkatti, and increased that of *T. demoraesi* and *T. soaresi*. The herbicide was slightly harmful to *T. bennetti* and *T. bruni*, but was relatively harmless to the other species of Trichogrammatidae based on the standards of the International Organization for Biological Control (IOBC).

Key Words: biological control; *Trichogramma*; *Zea mays*; egg parasitoid

Resumo

O objetivo do estudo foi avaliar o efeito do herbicida atrazine, recomendado para a cultura do milho, em 10 espécies de Trichogrammatidae (Hymenoptera). Uma fêmea de cada *Trichogramma* spp. foi individualizada e acondicionada por tubo de ensaio (sem chance de escolha) com uma cartela contendo aproximadamente 45 ovos de *Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae). O parasitismo desses inimigos naturais foi permitido por 48 h e as cartelas foram pulverizadas com o herbicida atrazine (6 L/ha), o controle foi pulverizado com água destilada. Atrazine reduziu a emergência de fêmeas de *Trichogramma bruni* Nagaraja, porém aumentou a de *Trichogramma pretiosum* Riley, *Trichogramma demoraesi* Nagaraja, *Trichogramma galloi* Zucchi e *Trichogramma soaresi* Nagaraja. Além disso, atrazine reduziu a razão sexual de *T. bruni*, *Trichogramma atopovirilia* Oatman & Platner e *Trichogramma bennetti* Nagaraja & Nagarkatti, e aumentou a de *T. demoraesi* e *T. soaresi*. O herbicida foi levemente nocivo para *T. bennetti* e *T. bruni*, mas foi inócuo para as outras espécies de Trichogrammatidae com base nos padrões da "International Organization for Biological Control (IOBC)".

Palavras Chave: controle biológico; *Trichogramma*; *Zea mays*; parasitoides de ovos

Corn (*Zea mays* L.; Poales: Poaceae) is one of the most economically important cereals and is planted on a large scale in Brazil and worldwide. However, weeds can reduce the corn yield by up to 85% (Constantin et al. 2007; Stefanello Júnior et al. 2008). Herbicides based on atrazine are used widely in corn to control dicotyledonous plants at pre- or post-emergence (Menezes et al. 2012a). Atrazine inhibits photosynthesis (photosystem II) causing irreversible damage to plant cells (Chen et al. 2014).

In Brazil, *Spodoptera* species (Lepidoptera: Noctuidae) and weeds are the main pests of corn (Matos-Neto et al. 2004). These insects commonly are controlled using chemical insecticides; however, these products can cause environmental contamination, thus leading to the search of alternative methods for the control of these pests (Céspedes et al. 2004). Natural enemies, especially *Trichogramma* species (Hymenoptera: Trichogrammatidae) egg parasitoids, represent an alternative to control *Spodoptera* (Spínola-Filho et al. 2014). These organisms can reduce the damage caused by pests such as *Spodoptera* in corn crops, and their parasitism of eggs prevents these pests from reaching the adult stage (Gardner et al. 2011).

Herbicides can affect the parasitism by *Trichogramma* species (Giolo et al. 2005). These products could be ingested by parasitoids or penetrate the insect cuticle, resulting in toxicity (Malkones 2000). The effects of herbicides on parasitoids may vary with the quantity and type of active ingredient, salt, and adjuvants, or their mixture (Giolo et al. 2005; Stefanello Júnior et al. 2011). *Trichogramma* species can be used to determine the selectivity of agrochemicals to natural en-

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emies (Hassan & Abdelgader 2001). The aim of the present study was to evaluate the compatibility of the herbicide atrazine with 10 species of Trichogrammatidae during the immature stage.

Materials and Methods

This study was conducted at the Laboratory of Entomology and in the G.W.G. de Moraes Insectarium of the Institute of Agricultural Sciences (ICA) of the Federal University of Minas Gerais (UFMG), in Montes Claros, Minas Gerais State, Brazil. The experiment had a completely randomized design with 10 parasitoid species and 1 herbicide. There were 10 replicates for each species. A water control was included for each replicate.

A total of 10 species of Trichogrammatidae were obtained from the Insectarium of the ICA/UFMG, with 9 in the genus *Trichogramma* — *T. acacioi* Brun, Moraes & Soares; *T. atopovirilia* Oatman & Platner; *T. bennetti* Nagaraja & Nagarkatti; *T. brasiliensis* Ashmead; *T. bruni* Nagaraja; *T. demoraesi* Nagaraja; *T. galloi* Zucchi; *T. pretiosum* Riley; and *T. soaresi* Nagaraja — and 1 in the genus *Trichogrammatoidea* — *T. annulata* De Santis. The treatments consisted of the application of the herbicide atrazine — Gesaprim 500 Ciba Geisy (6 L/ha) — or distilled water (control). This concentration of Gesaprim 500 is slightly higher (1 L) than recommended for the corn crop (5 L/ha), to simulate excessive use in the field due to errors in the application, successive applications (Aladesanwa & Akinbobola 2008; Dornelles et al. 2009), and use of higher doses for effective weed control in organic soils (Mudhoo & Garg 2011).

Cards with 45 eggs of *Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae) were obtained in the manner described by Soares et al. (2012, 2014). Each card was placed in a transparent glass test tube (9.0 × 1.0 cm) with a female parasitoid for 48 h at a 12:12 h L:D photoperiod and 24.4 ± 0.01 °C (Soares et al. 2014) (200 total cards). The herbicide was diluted in distilled water (150 L/ha) to a concentration 3.0 kg a.i. ha^{-1}. After the 48 h of parasitism, the cards with parasitized *A. kuehniella* eggs were sprayed with the herbicide atrazine (0.06 $\mu L/cm^2$ of commercial product per card, 0.03 $\mu g/cm^2$ of active ingredient per card). The control was sprayed with distilled water. The cards were held until the water evaporated, and were placed in sealed test tubes (with cotton plugs) as described.

Herbicide toxicity was classified based on the percentage of parasitism reduction as follows: I = harmless (< 30% reduction), II = slightly harmful (30–79% reduction), III = moderately harmful (80–99% reduction), and IV = harmful (> 99% reduction); this was based on the standards of the International Organization for Biological Control (IOBC) (Sterk et al. 1999). The reduction in the emergence and sex ratio of the parasitoid species were calculated as follows: % reduction = 100 − mean [(% mean of the treatment ÷ % mean of the control) × 100] (Carvalho et al. 2010).

The percentage of adult emergence (males and females) and sex ratio (female ÷ [male + female]) of the parasitoids after 20 d were evaluated under a binocular microscope with 40 × magnification. The data were arcsine transformed and evaluated with analyses of variance (ANOVA), and the means were examined using the Tukey test at 1% or 5% probability.

Results

Atrazine produced the highest reduction in the emergence of *T. bruni* females, followed by *T. bennetti*. However, this herbicide did not significantly affect (*P* > 0.05) *T. acacioi* and *T. atopovirilia* female emergence. Conversely, the emergence of *T. pretiosum*, *T. demoraesi*, *T. galloi*, and *T. soaresi* females was higher following atrazine application. Thus, this herbicide was classified as slightly harmful (class II, 30–79% reduction) to *T. bennetti* and *T. bruni*, and harmless (class I, < 30% reduction) to the other trichogrammatid species (Table 1).

Atrazine reduced the sex ratio of *T. bruni*, *T. atopovirilia*, and *T. bennetti*, but did not significantly affect (*P* > 0.05) that of *T. annulata*, *T. acacioi*, *T. brasiliensis*, *T. galloi*, and *T. pretiosum*. In contrast, it increased the sex ratio of *T. demoraesi* and *T. soaresi*. Thus, based on its effect on these parasitoid sex ratios, atrazine was classified as slightly harmful (class II, 30–79% reduction) to *T. bruni* and harmless (class I, < 30% reduction) to the other trichogrammatid species (Table 2).

Discussion

The effects of atrazine herbicides on insects are variable due to the doses used and different types of formulations. In this study, atrazine was used at a dose higher than recommended by the herbicide label because such use is common in agriculture due to errors in the application (Dornelles et al. 2009) and the need for successive applications after weed control failures (Aladesanwa & Akinbobola 2008). In organic soils with high clay concentrations, the effect of post-emergence control by the product is lowered (Mudhoo & Garg 2011), forcing the use of higher doses for effective weed control.

Table 1. Percentage of host eggs producing female parasitoids (mean and SE), adjusted % reduction in female emergence (Redu.), and International Organization for Biological Control (IOBC) classification (Class.) of *Trichogrammatoidea annulata* and 9 *Trichogramma* species (Hymenoptera: Trichogrammatidae) females from eggs parasitized after treatment with atrazine (Montes Claros, Minas Gerais State, Brazil).

	Atrazine		Control		IOBC		ANOVA (df = 9)	
Species	Average	SE	Average	SE	Redu.	Class.	*F*	*P*
T. annulata **	78.39 b	4.08	93.51 a	1.80	16.2	I	10.291	0.01069
T. acacioi n.s.	77.19 a	2.87	83.37 a	2.09	7.4	I	4.875	0.05464
T. atopovirilia n.s.	75.97 a	1.93	82.38 a	2.23	7.8	I	2.997	0.11747
T. bennetti *	69.78 b	2.01	101.10 a	5.04	31.0	II	36.026	0.00020
T. bruni *	25.77 b	13.18	98.54 a	2.65	73.8	II	36.228	0.00020
T. brasiliensis **	92.71 b	1.36	96.53 a	1.06	4.0	I	6.987	0.02676
T. demoraesi **	98.46 a	0.56	91.79 b	1.77	−7.3	I	8.979	0.01504
T. galloi *	98.99 a	0.56	92.20 b	0.49	−7.4	I	78.603	0.00000
T. pretiosum *	98.72 a	0.56	81.69 b	1.88	−20.8	I	79.298	0.00000
T. soaresi *	98.28 a	0.53	93.34 b	1.30	−5.3	I	12.283	0.00667

Means within the same row followed by the same small letter do not differ by the Tukey test (* *P* < 0.01, ** *P* < 0.05). n.s. = not significant by 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).

Table 2. Sex ratio (mean and SE), adjusted % reduction (Redu.), and International Organization for Biological Control (IOBC) classification (Class.) of *Trichogrammatoidea annulata* and 9 *Trichogramma* species (Hymenoptera: Trichogrammatidae) from eggs parasitized after treatment with atrazine (Montes Claros, Minas Gerais State, Brazil).

	Atrazine		Control		IOBC		ANOVA (df = 9)	
Species	Average	SE	Average	SE	Redu.	Class.	*F*	*P*
T. annulata n.s.	0.88 a	0.01	0.88 a	0.01	0	I	0.002	****
T. acacioi n.s.	0.88 a	0.01	0.84 a	0.04	−4.8	I	0.609	****
T. atopovirilia *	0.85 b	0.02	1.03 a	0.02	17.5	I	31.297	0.00034
T. bennetti *	0.85 b	0.01	1.00 a	0.00	15.0	I	110.446	0.00000
T. bruni *	0.28 b	0.13	0.84 a	0.02	66.7	II	17.920	0.00220
T. brasiliensis n.s.	0.95 a	0.01	0.91 a	0.01	−4.4	I	3.108	0.11177
T. demoraesi *	0.99 a	0.00	0.87 b	0.02	−13.8	I	22.012	0.00113
T. galloi n.s.	1.00 a	0.00	1.00 a	0.00	0	I	****	****
T. pretiosum n.s.	0.99 a	0.00	1.00 a	0.00	1.0	I	5.971	0.03714
T. soaresi *	1.00 a	0.00	0.88 b	0.01	−13.6	I	98.824	0.00000

Means within the same row followed by the same small letter do not differ by the Tukey test (* $P < 0.01$, ** $P < 0.05$). n.s. = not significant by ANOVA ($P > 0.05$). **** = highly not significant. 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).

The highest reductions in female emergence occurred in *T. bruni* and *T. bennetti*. The level of reduction was consistent with what has been reported for the atrazine herbicides Primóleo and Siptran 500 SC (which have been classified as class I), and Gesaprim GrDA (class II), for *T. pretiosum* adults (Stefanello Júnior et al. 2008). Reduction in insect survival also was reported for the predator *Podisus nigrispinus* Dallas (Hemiptera: Pentatomidae) when exposed to herbicides containing atrazine; in this case, survival was less than 50% (Menezes et al. 2012a). This herbicide also was reported to affect the population dynamics of the soil entomofauna under a corn crop (Pereira et al. 2005). Differences in atrazine product selectivity may be caused mainly by formulation (Menezes et al. 2012b). Commercial formulations may contain salts and adjuvants that can cause poisoning in non-target organisms.

Not all of the trichogrammatids were affected by the atrazine. The lack of a significant impact of atrazine on the sex ratio of *T. annulata*, *T acacioi*, *T. brasiliensis*, *T. galloi*, and *T. pretiosum* may be due to the protection these natural enemies obtain within the host egg (Stefanello Júnior et al. 2011), though if this is the case, it is not apparent why *T. bruni* and *T. bennetti* would be affected. An alternative hypothesis is that the species differ in their detoxification capacity. Clearly, it is difficult to generalize about the effects of atrazine. For example, the emergence rates of *Aedes* (*Stegomyia*) *aegypti* (L.) (Diptera: Culicidae) were higher with atrazine application than with glyphosate (systemic herbicide) or in the control treatments, and those of *Aedes* (*Stegomyia*) *albopictus* Skuse (Diptera: Culicidae) were higher with atrazine application than with glyphosate (Bara et al. 2014). For both mosquito species, a sex ratio distortion with male bias was observed in the control and glyphosate treatments, but not with atrazine, and the emergence period for both sexes was longer in the atrazine treatment than in the glyphosate or control treatment (Bara et al. 2014). Thus, this widely used herbicide can influence the life history traits of insects, but not in an entirely predictable manner.

The increased female emergence of *T. pretiosum*, *T. demoraesi*, *T. galloi*, and *T. soaresi* with atrazine application may be related to the "hormesis" phenomenon wherein sublethal quantities of a stressor benefit an organism (Morse 1998). This hypothesis is possible, especially considering that the herbicide would be present in low doses for the parasitoid inside the host egg. This was noted in *Palmistichus elaeisis* Delvare and LaSalle (Hymenoptera: Eulophidae), which produced an increased number of females produced per female with glyphosate application (Menezes et al. 2012b). Furthermore, the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) was found to affect *Chilo suppressalis* Walker (Lepidoptera: Crambidae), which attacks Asian rice, *Oryza sativa* L. (Poales: Poaceae). This compound was highly attractive to the egg parasitoid *Anagrus nilaparvatae* Pang & Wang (Hymenoptera: Mymaridae), and low doses increased trypsin proteinase inhibitor activity and volatile production by rice plants (Xin et al. 2012).

The herbicide atrazine was slightly harmful to *T. bennetti* and *T. bruni*, but harmless to the other trichogrammatid species based on the IOBC classification. Probably, this fact is due to this herbicide being hydrophilic, weekly basic, and having a molecular weight of 215.69 g/mol, with low capacity of penetration through the chorion of the egg. The increased emergence of *T. pretiosum* females with the application of this herbicide suggests that atrazine can improve the biological control with a natural enemy, though the data do not permit a generalization to all trichogrammatids.

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