PLANTA DANINHA

SOCIEDADE BRASILEIRA DA CIÊNCIA DAS PLANTAS DANINHAS

<http://www.sbcpd.org>

ISSN 0100-8358 (print)
1806-9681 (online)

Article

EFFICACY OF *Trichogrammatidae* SPECIES (HYMENOPTERA) SUBMITTED TO THE HERBICIDE GLYPHOSATE

Eficácia de Espécies de Trichogrammatidae (Hymenoptera) Submetidas ao Herbicida Glyphosate

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ABSTRACT - Information on selective herbicide, including glyphosate that does not affect non-target organisms such as natural enemies, are important in integrated pest management programs in maize. The dose 13.94 L ha^{-1} of glyphosate was evaluated in females on 10 Trichogrammatidae species. A female of each Trichogrammatidae species was individually positioned per test tube with a card containing approximately 45 *Anagasta kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) eggs. For 48 h parasitism was allowed, and later the cartons were sprayed with the herbicide or with distilled water, for the control treatment. The glyphosate showed variable effects for parasitoids. The emergence of *T. acacioi* females was lower but that of *T. atopovilia*, *T. demoraesi*, and *T. pretiosum* higher with the glyphosate. The sex ratio of *T. galloi* was lower and that of *T. bruni*, *T. brasiliensis*, *T. demoraesi*, and *T. soaresi* higher with glyphosate. This glyphosate was innocuous to all Trichogrammatidae species females based on the classification adopted internationally.

Keywords: biological control, herbicide Roundup, *Trichogramma*, *Zea mays*, parasitoid.

*RESUMO - Informações sobre herbicidas seletivos, incluindo o glyphosate, que não afetam organismos não alvo, como inimigos naturais, são importantes nos programas de manejo integrado de pragas na cultura do milho. O glyphosate, na dose de 13,94 L ha^{-1}, foi avaliado em fêmeas de 10 espécies de Trichogrammatidae (Hymenoptera). Uma fêmea de cada espécie de Trichogrammatidae foi posicionada individualmente por tubo de ensaio em cartelas contendo aproximadamente 45 ovos de **Anagasta kuehniella** Zeller, 1879 (Lepidoptera: Pyralidae). Durante 48 horas o parasitismo foi permitido e, posteriormente, as cartelas foram pulverizadas com o herbicida ou com água destilada, para o tratamento controle. O herbicida mostrou efeitos variáveis para as espécies de parasitoides. A emergência de fêmeas de **T. acacioi** foi menor, e a daquelas de **T. atopovilia, T. demoraesi** e **T. pretiosum** foi maior com glyphosate. A razão sexual de **T. galloi** foi menor, e a de **T. bruni**, **T. brasiliensis**, **T. demoraesi** e **T. soaresi** maior com glyphosate. Esse herbicida foi pouco tóxico para todas as espécies de Trichogrammatidae com base na classificação adotada internacionalmente.*

Palavras-chave: controle biológico, herbicida Roundup, *Trichogramma, Zea mays*, parasitoide.

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Received: November 13, 2018
Approved: March 18, 2019

Planta Daninha 2019; v37:e019216439

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Doi: 10.1590/S0100-83582019370100147

INTRODUCTION

The herbicide glyphosate is the most used in the world with more than 150 brand names in 119 countries (Bayliss, 2000; Amarante Junior et al., 2002). This herbicide inhibits the shikimate pyruvylenol phosphate synthase (EPSPS) regardless of the salts used in its commercial formulations. In Brazil, glyphosate is formulated with potassium, isopropylamine or ammonium salts (Rodrigues and Almeida, 2018). Glyphosate is non-selective to plants, presents systemic action and molecular weight of 169.1 g mol^{-1} and 228.2 g mol^{-1} formulated with isopropylammonium salt (Amarante Júnior et al., 2002). This herbicide and its salts are highly polar crystalline solids very soluble in water (12 g L^{-1} at 25 °C) but almost insoluble in common organic solvents such as acetone and ethanol at ambient conditions (Amarante Júnior et al., 2002). Glyphosate is indicated to control annual and perennial weeds, dicotyledonous or monocotyledonous in different crops (Amarante Júnior et al., 2002). Glyphosate-tolerant corn plants allow using this herbicide with post-emergence effects without phytotoxicity to crop, but this technique can reduce beneficial desired species - i.e. natural enemies (Ammann, 2005; Bigler and Albajes, 2011).

The active ingredients of herbicides can be toxic by penetrating the tegument or the insect cuticle (Menezes and Soares, 2016) and thus impacting natural enemies, especially the egg parasitoids, *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) (Giolo et al., 2005). Herbicides effect on parasitoids depends on the quantity and components present in commercial formulations (Giolo et al., 2005; Stefanello Júnior et al., 2011). The egg parasitoids *Trichogramma* represent an alternative to control lepidopteran pests (Soares et al., 2007, 2012, 2014; Pratissoli et al., 2008; Spínola-Filho et al., 2014) and they can be used to determine the selectivity of agrochemicals (Hassan and Abdelgader, 2001). These organisms can reduce the damage by pests including *Spodoptera* spp. in corn crops by preventing this pest to reach the adult stage (Soares et al., 2007; Gardner et al., 2011). *Spodoptera* spp. (Lepidoptera: Noctuidae) (Matos Neto et al., 2004) and weeds are the primary pests of the corn crop. *Spodoptera* spp. are controlled with insecticides; however, these products can cause environmental pollution (Zanuncio et al., 1998), thus leading to searching alternative methods to control this insect pest (Céspedes et al., 2004).

Considering the widespread use of glyphosate worldwide and the registration of this molecule for most crops in Brazil, mainly in transgenic corn crops, to understand the effect of this herbicidal molecule on Trichogrammatidae species becomes essential for integrated pest management. The objective of this research is to evaluate the compatibility of glyphosate to 10 species of Trichogrammatidae.

MATERIAL AND METHODS

The herbicide glyphosate was tested on 10 species of parasitoids in a completely randomized design with 10 replicates. The experimental plots were composed of white paper fragments measuring 0.4 cm wide by 2.0 cm long, containing 45 *Anagasta kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) eggs.

A total of 10 Trichogrammatidae species, commonly found in the crops in Brazil, being nine of the genus *Trichogramma* - *T. acacioi*; *T. atopovirilia*; *T. bennetti*; *T. brasiliensis*; *T. bruni*; *T. demoraesi*; *T. galloi*; *T. pretiosum; T. soaresi*, and one of the genus *Trichogrammatoidea* - *T. annulata*. The treatments comprised the dose 13.94 L ha^{-1} of glyphosate (isopropylamine salt, Commercial Product: Roundup Original DI®, Monsanto of Brazil LTDA, toxicological classification – CLASS II – Highly Toxic, classification of the potential of environmental hazard - Dangerous Product to the Environment – CLASS III) and distilled water (150 L ha^{-1}).

A total of 45 *A. kuehniella* eggs were glued per paper leaf with 10% Arabic gum, exposed to ultraviolet radiation (UV) for 60 min, placed in glass vials (7.5 cm × 13.0 cm), sealed with plastic polyvinyl chloride (PVC) and an elastic film, and stored in a refrigerator at 5 °C and 80% R.H. for one day. After this period, each card was placed in a transparent glass tube (9.0 cm × 1.0 cm) with a female parasitoid for 48 h at 12 h photoperiod at 24.39 ± 0.01 °C (Soares et al., 2007, 2012, 2014). The herbicide was diluted in distilled water. After 48 h, the cards with parasitized *A. kuehniella* eggs were sprayed with the herbicide Roundup Original DI at 13.94 L ha^{-1}, which corresponds to 0.06 µL cm^{-2} of commercial product/paperboard, 0.03 µL cm^{-2}

of glyphosate/paperboard). The herbicide solution was sprayed with a Guarany® hand sprayer (Itú, São Paulo, Brazil) until runoff began. For comparison purposes, a control treatment was maintained in which the experimental plots were sprayed with distilled water. The cards were subsequently reserved at shade outdoors for 2 h to disperse water excess and immediately packaged in sealed tubes as described.

Glyphosate toxicity was classified based on the emergence and parasitism reduction: I= harmless (<30% reduction), II= slightly harmful (30-79% reduction), III= moderately toxic (80-99% reduction), and IV= harmful (>99% reduction) according to the International Organization for Biological Control (IOBC) (Sterk et al., 1999). The decrease in the emergence of the parasitoid species was calculated: % reduction= 100 - mean [(% mean of the treatment ÷ % mean of the control) × 100] (Carvalho et al., 2010).

The proportion (%) of adult emergence (males and females) and sex ratio ((female/(male + female)) of the parasitoids after 20 days were evaluated under a binocular microscope with 40× magnification. The data were transformed to arcsine, tested with Variance Analyses (ANOVA), and the means were examined using the Tukey test at 1% or 5% probability.

RESULTS AND DISCUSSION

The emergence of *T. acacioi* females had a higher reduction with glyphosate, followed by *T. bruni* and the other Trichogrammatidae species, but did not affect that of *T. annulata*, *T. bennetti*, *T. brasiliensis*, *T. galloi*, and *T. soaresi* females. The emergence of *T. atopovilia*, *T. demoraesi*, and *T. pretiosum* females was higher following glyphosate application. This herbicide was classified as harmless (class 1, <30% reduction) for all Trichogrammatidae species (Table 1).

Table 1 - Percentage of emergence (mean and standard error = SE), reduction (%) (Redu.), and IOBC classification (Cl) of *Trichogrammatoidea annulata* and nine *Trichogramma* spp. females from eggs parasitized after treatment with glyphosate

Species	Glyphosate		Control		IOBC		ANOVA (*df* = 9)	
	Mean	SE	Mean	SE	Redu.	Cl	*F*	*P*
*T. acacioi***	64.89 b	8.61	83.37 a	2.09	22.17	I	5.136	0.04967
T. annulata ns	76.94 a	8.47	93.51 a	1.80	17.72	I	3.224	0.10612
*T. atopovirilia**	94.39 a	1.30	82.38 b	2.23	-14.58	I	39.255	0.00015
T. bennetti ns	95.64 a	0.99	101.10 a	5.04	5.40	I	1.259	0.29094
*T. bruni***	91.12 b	2.42	98.54 a	2.65	7.53	I	6.177	0.03468
*T. brasiliensis*ns	95.31 a	0.99	96.53 a	1.06	1.26	I	0.594	***
*T. demoraesi***	97.83 a	0.66	91.79 b	1.77	-6.58	I	8.579	0.01678
T. galloi ns	94.57 a	1.21	92.20 a	0.49	-2.57	I	4.553	0.06165
*T. pretiosum**	95.68 a	1.37	81.69 b	1.88	-17.13	I	34.443	0.00024
T. soaresi ns	94.49 a	1.11	93.34 a	1.30	-1.23	I	0.619	***

Means followed by the same small letter per line do not differ by the Tukey test (* P<0.01,** P<0.05). ns not significant by ANOVA (P>0.05). *** highly not significant.

The emergence of *T. acacioi* and *T. bruni* females showed that species of this genus have different susceptibilities to glyphosate. The toxic and repellent effects on non-target organisms by glyphosate, surfactant formulations, and different concentrations of salts (i.e., isopropylamine) of herbicides may explain the impact on these parasitoids (i.e. *T. pretiosum*) (Tsui and Chu, 2003; Stefanello Júnior et al., 2008; Menezes and Soares, 2016). The lower emergence and parasitism by *T. pretiosum* females with the glyphosate (1.44% of equivalent acid) show that this effect depends on the salt used to formulate the herbicide and of inert ingredients of Commercial Product [(e.g. Roundup Original DI® of Monsanto, isopropylamine salt, parasitism reduction (PR) H"87%)] (Giolo et al., 2005). Agrisato® 480 SL of Alkagro (isopropylamine salt, PR =75.6%), Glifos® of Adapar (isopropylamine salt, PR =63.9%), Gliz® 480 SL of Adapar (isopropylamine salt, PR =72.1%), Glyphosate Nortox® of Nortox (isopropylamine salt, PR =73.5%), Polaris® of Du Pont (isopropylamine salt, PR =75.3%), Trop® of Milênia (isopropylamine salt, PR =79.9%), and Zapp Qi®

of Syngenta (potassium salt, PR =36.4%) were slightly harmful, and Roundup Original® of Monsanto (isopropylamine salt, PR =81.1%), Roundup Transorb® of Monsanto (isopropylamine salt, PR =85.0%), and Roundup WG® of Monsanto (Ammonium salt, PR =82.6%) (glycine class) moderately harmful to *T. pretiosum* adults (i.e. reduction on parasitism/emergence) (Stefanello Júnior et al., 2008).

The lack of the impact of glyphosate on emergence of *T. annulata*, *T. bennetti*, *T. brasiliensis*, *T. galloi*, and *T. soaresi* females may be due to the slower penetration of the herbicide in host eggs (Stefanello Júnior et al., 2011) probably since this herbicide is hydrophilic and with molecular weight of 169.1 g mol^{-1}, with low capacity of penetration in the egg chorion, what explains the lower toxicity to immature compared to adult *T. pretiosum* (Nörnberg et al., 2008; Stefanello Júnior et al., 2008, 2011). Another possibility is the detoxification capacity of these parasitoids species due to enzymes cytochrome P450 monooxygenases, larval glutathione S-transferases, and catalase as observed in *Aedes aegypti* (L. 1762) (Diptera: Culicidae) larvae and *Aporrectodea caliginosa* Orley, 1885 (Haplotaxida: Lumbricidae) exposed to glyphosate (Riaz et al., 2009; Givaudan et al., 2014). Agrisato 480 SL, Glifos, Glifosato 480 Agripec, Glifosato Nortox, Gliz 480 CS, Polaris, Roundup, Roundup Transorb, Roundup WG, Trop, and Zapp Qi were classified as harmless (class 1, <30% reduction) to immature *T. pretiosum*, but females of this parasitoid showed high emergence with Roundup Ready (Nörnberg et al., 2008; Stefanello Júnior et al., 2011). The low impact of glyphosate on emergence, on penetration of this herbicide in host eggs and on lower toxicity to immature Trichogrammatidae species is not dependent on glyphosate salt and commercial formulation.

The higher female emergence of *T. atopovilia*, *T. demoraesi*, and *T. pretiosum* with glyphosate (Roundup Ready) may be related to the "hormesis" phenomenon - sublethal quantities (Guedes and Cutler, 2014) as noted for *Palmistichus elaeisis* Delvare and LaSalle, 1993 (Hymenoptera: Eulophidae) with higher production of females produced per female with glyphosate higher (Menezes et al. 2012). The herbicide 2,4-D also showed potential elicitor, in low doses, on *Chilo suppressalis* Walker, 1863 (Lepidoptera: Crambidae) on *Oryza sativa* L. (Poaceae) and was highly attractive to *Anagrus nilaparvatae* Pang and Wang, 1985 (Hymenoptera: Mymaridae) - egg parasitoid - and at low doses it increased the trypsin proteinase inhibitor activity and volatile production of rice plants (Xin et al., 2012). In this assay, the commercial herbicide was applied in the host already eggs parasitized, thus, the herbicide needs through the chorion of the host egg to reach the immature *Trichogramma* probably at lower doses, suggesting the occurrence of "hormesis" (Zanuncio et al., 2003).

The sex ratio of *T. galloi* was lower with glyphosate, but this herbicide did not affect that of *T. acacioi*, *T. annulata*, *T. atopovilia*, *T. bennetti*, and *T. pretiosum*. On the other hand, it increased the sex ratio of *T. bruni*, *T. brasiliensis*, *T. demoraesi*, and *T. soaresi*. The glyphosate was classified as harmless (class 1, <30% reduction) for all Trichogrammatidae species (Table 2).

Table 2 - Sex ratio (mean and standard error = SE), reduction (%) (Redu.), and IOBC classification (Cl) of *Trichogrammatoidea annulata* and nine *Trichogramma* spp. from eggs parasitized after treatment with glyphosate

Species	Glyphosate		Control		IOBC	Cl	ANOVA (*df* = 9)	
	Mean	SE	Mean	SE	Redu.		*F*	*P*
T. acacioi ns	0.90 a	0.03	0.84 a	0.04	-7.14	I	4.281	0.06848
T. annulata ns	0.87 a	0.09	0.88 a	0.01	1.14	I	0.001	***
*T. atopovirilia*ns	0.98 a	0.01	1.03 a	0.02	4.85	I	2.676	0.13630
T. bennetti ns	1.00 a	0.00	1.00 a	0.00	0.00	I	1.000	0.34344
*T. bruni**	0.98 a	0.02	0.84 b	0.02	-16.67	I	26.891	0.00058
*T. brasiliensis***	0.98 a	0.01	0.91 b	0.01	-7.69	I	8.497	0.01717
*T. demoraesi**	0.98 a	0.01	0.87 b	0.02	-12.64	I	13.627	0.00499
*T. galloi**	0.95 b	0.01	1.00 a	0.00	5.00	I	15.609	0.00335
T. pretiosum ns	0.99 a	0.01	1.00 a	0.00	1.00	I	4.750	0.05722
*T. soaresi**	0.95 Aa	0.01	0.88 Cb	0.01	-7.95	I	20.197	0.00150

Means followed by the same small letter per line do not differ by the Tukey test (* P<0.01, ** P<0.05). ns not significant by ANOVA (P>0.05). *** highly not significant.Means followed by the same small letter per line do not differ by the Tukey test (* P<0.01, ** P<0.05). ns not significant by ANOVA (P>0.05). *** highly not significant.

The sex ratio of *T. galloi* was lower with glyphosate, but the sex ratio of *T. bruni*, *T. brasiliensis*, *T. demoraesi*, and *T. soaresi* was increased, maybe due to being owing to the reduction in the bacterium *Wolbachia* sp. (Rickettsiales: Rickettsiales), changing sexual rates, as observed to that of heat and antibiotic treatments of *Trichogramma* spp. (Stouthamer et al., 1990; Russell and Stouthamer, 2011; Tulgetske and Stouthamer, 2012). This bacterium can reverse the sex and produce offspring by thelolitic parthenogenesis (Stouthamer et al., 1990; Russell and Stouthamer, 2011), reducing the efficiency of these natural enemies due to only females exert biological control. *Wolbachia* sp. can cause unidirectional and/or bidirectional cytoplasmic incompatibility in mites (Breeuwer, 1997; Van Opijnen and Breeuwer, 1999) and feminization in Crustacea (Van Meer et al., 1999). The reduction of the sex proportion of the parasitoid *P. elaeisis* by glyphosate was related to formulation of this herbicide with surfactants, which can be toxic or repellent (Menezes et al., 2012). The lack of impact of the glyphosate on *T. acacioi*, *T. annulata*, *T. atopovilia*, *T. bennetti*, and *T. pretiosum* sex rates agrees with reports for the herbicides Finale, Gliz CS 480, Nortox glyphosate, Roundup Original, and Roundup WG on *Malus* spp. (Rosaceae), being safe to *T. pretiosum* immature and those of the glycine class to adults of this parasitoid (Manzoni et al., 2006; Nörnberg et al., 2008). The protection within the host egg may help to explain the lack of impact of this herbicide on the Trichogrammatidae species as observed for *T. pretiosum* (Stefanello Júnior et al., 2011) or to their increased detoxification capacity.

It was concluded that the herbicide glyphosate (isopropylamine salt, Commercial Product: Roundup Original DI®) was harmless to females of all Trichogrammatidae species based on the IOBC classification, probably due to the hydrophilic type and molecular weight of this herbicide with low penetration capacity through the egg chorion. The higher emergence of *T. atopovilia*, *T. demoraesi*, and *T. pretiosum* females shows that glyphosate (isopropylamine salt, Commercial Product: Roundup Original DI®) can improve the biological control with these natural enemies.

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