Effect of Selectivity of Herbicides and Plant Growth Regulators used in Sugarcane Crops on Immature Stages of *Trichogramma galloi* (Hymenoptera: Trichogrammatidae)[1]

*Seletividade de Herbicidas e Reguladores de Crescimento de Plantas Utilizados na Cultura da Cana-de-Açúcar para Imaturos de **Trichogramma galloi** (Hymenoptera: Trichogrammatidae)*

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ABSTRACT - Herbicides and plant growth regulators are often used in sugarcane management. However, the use of non-selective pesticides can cause adverse effects on the efficiency of beneficial insects in integrated pest management. Within this context, this study aimed to evaluate the effect of such products on the immature stages of the parasitoid *Trichogramma galloi*. Eggs of *Diatraea saccharalis* containing the parasitoid at the egg-larva stage and at the prepupal and pupal stages were immersed in test solutions of the following pesticides (maximum recommended doses for sugarcane): herbicides clomazone and diuron + hexazinone, and plant growth regulators trinexapac-ethyl and sulfometuron-methyl. The biological properties evaluated were emergence (F_1 and F_2) and number of eggs parasitized by *T. galloi* (F_1). The products were classified according to percentage of reduction in emergence and parasitism: harmless (<30%), slightly harmful (30-79%), moderately harmful (80-99%) and harmful (>99%). The pesticides evaluated were considered to be harmless or slightly harmful to immature *T. galloi* and, thus, their use should be preferred for preserving this parasitoid species in sugarcane management programs.

Keywords: *Saccharum* spp., egg parasitoid, *Diatraea saccharalis*, biological control, chemical control, toxicity.

*RESUMO - Herbicidas e reguladores de crescimento de plantas são frequentemente utilizados no manejo da cana-de-açúcar. Entretanto, o uso de compostos não seletivos pode prejudicar a eficiência de insetos benéficos no manejo integrado de pragas. Nesse contexto, avaliou-se o efeito desses produtos sobre as fases imaturas do parasitoide **Trichogramma galloi**. Ovos de **Diatraea saccharalis** contendo o parasitoide no período de ovo-larva e nas fases de pré-pupa e pupa foram imersos em caldas dos produtos avaliados (doses máximas recomendadas para a cana-de-açúcar), sendo eles: os herbicidas clomazone e diuron+hexazinone e os reguladores de crescimento de plantas sulfometuron-methyl e trinexapac-ethyl. As características biológicas avaliadas foram a emergência (gerações F_1 e F_2) e o número de ovos parasitados por **T. galloi** (F_1). Os produtos foram classificados, conforme percentual de redução da emergência e parasitismo, em: inócuo (<30%), levemente prejudicial (30 a 79%), moderadamente prejudicial (80 a 99%) e prejudicial (>99%). Os compostos avaliados foram classificados como inócuos ou levemente prejudiciais aos imaturos de **T. galloi** e, por isso, devem ser preferidos, visando à preservação dessa espécie de parasitoide em programas de manejo da cana-de-açúcar.*

Palavras-chave: *Saccharum* spp., parasitoide de ovos, *Diatraea saccharalis*, controle biológico, controle químico, toxicidade.

INTRODUCTION

Sugarcane plays an important role in the Brazilian economy, mainly because of the production of sugar and ethanol by sugarcane industries: Brazil is the world leader in this sector (Guia ..., 2009; Portela et al., 2011). This success is partly due to the increase of both productive areas and total productivity, combined with good market prospects (Christofolleti et al., 2005). Such prospects are associated with the need to use new methods for producing more sustainable technologies: for instance, provision of credits in the carbon market, or as alternative fuel production aimed at reducing the use of energy produced from non-renewable sources (Santos, 2008).

Sugarcane productivity is often hampered by several factors, including the incidence of borer *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera, Crambidae), which is noteworthy because it causes great economic losses. After mating, females of this insect lay eggs on the leaves of the cane, and after the outbreak of caterpillars, they feed on the leaf parenchyma, penetrating the thatch from the third instar. Within the thatch, caterpillars open galleries as it feeds, thus injuring the plant (Botelho, 1992; Pinto et al. 2006).

An alternative to control this pest is through the larval parasitoid *Cotesia flavipes* - Cameron, 1981 (Hymenoptera: Braconidae) and egg parasitoid *Trichogramma galloi* - Zucchi, 1988 (Hymenoptera: Trichogrammatidae) (Lima Filho & Lima, 2003). *T. galloi* has the advantage of controlling this insect pest still at the egg stage, thereby preventing the outbreak of caterpillars and damage to the crop. Furthermore, the parasitoid has also been established in mass production systems for commercialization (Martins et al., 2011), which enhances its use.

Several chemicals are used in sugarcane management, for example herbicides, which are used in weed control (Carvalho et al. 2010a), and plant growth regulators, which are applied in order to anticipate the maturation process, improve the quality of raw material, optimize agribusiness and economic results, and assist in harvest planning (Caputo et al., 2008). However, studies show that the different products used in the management of agricultural crops may adversely affect natural enemies both at the adult and the immature stages, reducing the efficiency of biological control (Cônsoli et al., 2001; Carmo et al., 2010a; Maia et al., 2010; Stefanello Junior et al., 2011).

Selectivity studies with herbicides in maize crops showed detrimental effects on immature *Trichogramma pretiosum* - Riley, 1879 (Hymenoptera: Trichogrammatidae) (Stefanello Junior et al., 2011); evaluations using immature *Telenomus remus* - Nixon, 1937 (Hymenoptera: Scelionidae) in soy have shown adverse effects of herbicides on this parasitoid (Carmo et al., 2010b). For plant growth regulators, there are no studies of their effect on parasitoids.

As the application of phytosanitary products directly affects sugarcane leaves, where also females of *D. saccharalis* lay their eggs, chemicals used in these crops may have contact with the eggs of insect pests; hence the immature stage of *T. galloi* can be affected, as this parasitoid develops at this stage in the host. In this context, the aim of the present study was to evaluate the selectivity of herbicides and plant growth regulators used in sugarcane for immature *T. galloi*, in order to obtain information that may contribute to maintaining the population size of *T. galloi* in this agroecosystem.

MATERIAL AND METHODS

The effects of pesticides used in sugarcane crops on immature parasitoid *T. galloi* were evaluated in the laboratory, using herbicides clomazone (800 g L^{-1}) and hexazinone + diuron (488 g kg^{-1} + 142 g kg^{-1}), and plant growth regulators sulfometuron-methyl (750 g kg^{-1}) and trinexapac-ethyl (250 g L^{-1}). The number in parentheses refers to the concentration of active ingredient in the commercial product; for all products, the evaluation was based on the highest doses recommended for the crop (Agrofit, 2011). Using the maximum dose when testing phytosanitary products for toxicity in laboratory conditions is recommended by the International Organization for Biological and Integrated Control of Noxious Animals and Plants (IOBC) (Hassan et al., 2000).

In order to evaluate the effect of the products on the emergence and parasitism of *T. galloi* of F_1 generation, the newly emerged parasitoids were kept in a plastic container (12.5 cm x 9 cm diameter) containing a trickle of honey for 24 hours for mating purposes. After this period, three females of *T. galloi* by replicate and up to 24 hours of age were sexed based on the characteristics of their antennas and placed in a glass tube (8.5 cm height x 2.5 cm diameter) containing a droplet of honey. In this tube, the females received 60 eggs of *D. saccharalis* aged up to 24 hours which had been oviposited on paper towels. The number of 60 eggs per replicate was used because of previous observations made in the laboratory, where it was found that the maximum number of eggs of *D. saccharalis* parasitized by a female *T. galloi* was 20. After 24 hours, the females were discarded, and the supposedly infected eggs were kept in a environmental chamber at 23 ± 2 ºC with relative humidity 60 ± 10% and photoperiod of 14 hours until the parasitoids could reach the egg-larva stage and the prepupal or pupal stages (0-24h, 72-96h and 168-192h after parasitism, respectively), according to the methodology proposed by Cônsoli et al. (2001) and Carvalho et al. (2010b).

The eggs of *D. saccharalis* containing *T. galloi* in each of the abovementioned stages of development were immersed for five seconds in aqueous dilutions and / or distilled water (control), according to methodology by Carvalho et al. (2003) and Moura et al. (2005). To remove the excess of liquid of the dilutions from the surface of the eggs, they were kept for one hour at room temperature and thereafter transferred to glass tubes (8.5 cm x 2.5 cm diameter) and kept under the same conditions described above, until the emergence of the parasitoid.

The effects of the compounds on the specimens of F_1 generation of *T. galloi* were assessed for percentage of emergence = (number of eggs of the parasitoid with exit holes/total number of parasitized eggs) x 100) and number of parasitized eggs per female in 24 hours = (number of parasitized eggs/3).

To assess the effects of the compounds on the specimens of F_1 generation, surviving adults of *T. galloi* from each treatment were previously fed with droplets of honey; subsequently, three females per replicate aged up to 24 hours received 60 eggs of *D. saccharalis* (not exposed to chemicals). The parasitoids remained in contact with the eggs for 24 hours; after that, they were removed and kept at 23 ± 2 ºC, with relative humidity of 60 ± 10% and photoperiod of 14 hours until the emergence of parasitoids. The percentage of emergence of insects of F_2 generation was evaluated.

The products evaluated were classified as for the percentage of reduction (PR) in the beneficial capacity of parasitoids (parasitism and emergence): harmless, class 1 (<30%), slightly harmful, class 2 (30-79%), moderately harmful, class 3 (80 to 99%) and harmful, class 4 (> 99%), as recommended by IOBC (Sterk et al. 1999). The following formula was used to calculate PR:

$$PR = 100 - (\frac{\text{overall mean percentage of treatment with product}}{\text{overall mean percentage of control}} \text{x}100)$$

The experiments used a completely randomized factorial 3 x 5 design (three periods of development x four chemicals and control), with 15 replicates. The data were submitted to analysis of variance (ANOVA) and the means of the treatments were compared at the 5% level of significance (p <0.05) by the Scott-Knott test, using the statistical software SISVAR 5.3 (Ferreira, 2010).

RESULTS AND DISCUSSION

It was found that all the compounds applied on eggs of *D. saccharalis* containing immature *T. galloi* during egg-larva and prepupal stages reduced the emergence of parasitoids of F1 generation. Diuron + hexazinone and trinexapac-ethyl were the most harmful to the parasitoid at the egg-larva stage, and clomazone, at the prepupal stage. At the pupal stage, only diuron + hexazinone reduced this biological property (Table 1).

Differences between the stages of development as for the emergence of insects of F1 generation were observed for diuron + hexazinone and trinexapac-ethyl, and the egg-larva stage was the most susceptible to their effects (Table 1). At the egg-larva stage, the parasitoids tend to be more sensitive, probably

Tabela 1 - Percentage of emergence (± PE) of *Trichogramma galloi* (F$_1$ generation) originated from eggs of *Diatraea saccharalis* treated with chemicals containing parasitoids during egg-larva, prepupal and pupal stages

Treatment	Stages of development		
	Egg-larva	Prepupal	Pupal
Control	64.58 ± 4.62 aA	62.53 ± 5.05 aA	62.65 ± 4.92 aA
Clomazone	43.78 ± 7.10 bA	38.67 ± 7.56 cA	50.73 ± 6.10 aA
Diuron+hexazinone	28.85 ± 5.98 cB	48.95 ± 7.79 bA	27.36 ± 4.35 bB
Trinexapac-ethyl	19.33 ± 5.69 cB	39.77 ± 4.71 bA	50.40 ± 4.49 aA
Sulfometuron-methyl	42.78 ± 5.68 bA	44.65 ± 6.85 bA	51.65 ± 7.01 aA

Means followed by the same letter, lowercase in the column and uppercase on the line, do not differ by the Scott-Knott test (p>0.05).

because of the longer period of penetration of the product through the chorion of the host egg until emergence and also increased activity of the larvae, when compared to the prepupal and pupal stages, which corroborates the results obtained by Carvalho et al. (2001). Furthermore, the penetration ability of the products depends on their physicochemical properties, such as their lipophilicity (Crespo et al. 2002), and also the properties of the host, which vary depending on the species, age and stage of development of the egg (Cônsoli et al. 1999a, b).

As for toxicity, according to criteria of IOBC, herbicides and plant growth regulators were considered to be harmless or slightly harmful to the emergence of parasitoids of F1 generation of *T. galloi*, regardless of stage of development (Table 2). At the egg-larva stage, all products were considered to be slightly harmful and showed reductions in emergency ranging between 32 and 70%.

This selectivity of the evaluated products can be associated with two hypotheses: the absence or low toxicity of these compounds, or their low penetrating capacity into the membranes of the chorion of the host egg, as described by Carvalho et al. (2001), who studied the effects of some pesticides on *T. pretiosum.*

Studies on the effect of diuron + hexazinone, trinexapac-ethyl and methyl-sulfometuron on immature parasitoids of the genus *Trichogramma* were not found in the literature. Carmo et al. (2010a) reported that clomazone was harmeless to *T. pretiosum* on eggs of Anagasta kuehniella (Zeller, 1879) (Lepidoptera: Pyralidae) when used at the recommended dose for soybean crops, a smaller

dose than the one assessed in the present study (maximum dose for sugarcane crops).

As for parasitism, only trinexapac-ethyl reduced the number of eggs parasitized by females of F1 generation, when the eggs of *D. saccharalis* were treated with this herbicide containing *T. galloi* during egg-larva stage (Table 3), with a reduction of 44.5% (Table 4). At the prepupal stage, all products were harmless to parasitism (Tables 3 and 4).

At the pupal stage, the products did not have hamper parasitism capacity (Table 3). However, the classification of products at this stage varied between harmless for hexazinone + diuron and sulfometuron-methyl and slightly harmless for clomazone and trinexapac-ethyl. Treatments based on clomazone and trinexapac-ethyl caused reductions in parasitism of *T. galloi*, compared with the control (Table 4). These reductions probably occurred because precisely at this stage, there was emergence of adults with deformed wings. Deformed insects may have limitations, such as difficulty in feeding, mating and parasiting, thus affecting their parasitism capacity. It is noteworthy that reductions in biological properties of insects (e.g. fertility), as a result of the action of sublethal pesticides, have been reported in the literature (Carvalho et al., 1999; Foerster, 2002).

An evaluation of the effects of compounds on parasitism capacity between the stages of development of *T. galloi* showed no significant differences (Table 3).

For the emergence of the specimens of F$_2$ generation of *T. galloi*, originated from eggs of *D. saccharalis* containing the parasitoid during

Table 2 - Reduction in emergence (RE) of *Trichogramma galloi* (F_1 generation) originated from eggs of *Diatraea saccharalis* treated with chemicals containing parasitoids during egg-larva, prepupal and pupal stages, and toxicity classes of compounds

Treatment	Development					
	Egg-larva		Prepupa		Pupa	
	RE[1]	Class[2]	RE[1]	Class[2]	RE[1]	Class[2]
Clomazone	32.20	2	38.15	2	19.02	1
Diuron+hexazinone	55.32	2	21.71	1	56.32	2
Trinexapac-ethyl	70.06	2	36.39	2	19.55	1
Sulfometuron-methyl	33.75	2	28.59	1	17.55	1

[1] Mean percentage of reduction in emergency. [2] Toxicity index as recommended by IOBC (Sterk et al., 1999).

Table 3 - Number (± PE) of eggs parasitized by females of *Trichogramma galloi* of F_1 generation, derived from eggs of *Diatraea saccharalis* treated with chemicals containing parasitoids during egg-larva, prepupal and pupal stages

Treatment	Development		
	Egg-larva[1]	Prepupa[1]	Pupa[1]
Control	16.67 ± 2.49 aA	11.44 ± 2.57 aA	10.60 ± 2.08 bA
Clomazone	12.06 ± 3.14 aA	14.66 ± 3.56 aA	05.06 ± 2.01 bA
Diuron+hexazinone	14.93 ± 3.31 aA	12.25 ± 2.88 aA	17.20 ± 5.96 aA
Trinexapac-ethyl	09.26 ± 2.91 bA	16.66 ± 4.93 aA	07.13 ± 2.88 bA
Sulfometuron-methyl	11.40 ± 2.79 aA	14.33 ± 4.15 aA	13.40 ± 2.81 aA

[1] Means followed by the same letter, lowercase and uppercase in the column on the line, do not differ by the Scott-Knott test (p>0.05).

Table 4 - Percentage of reduction (PR) in the parasitism of female Tric*hogramma galloi* of F_1 generation, derived from eggs of *Diatraea saccharalis* treated with chemicals containing parasitoids during egg-larva and prepupal and pupal stages, and toxicity classes of compounds

Treatment	Development					
	Egg-larva		Prepupa		Pupa	
	PR[1]	Class[2]	PR[1]	Class[2]	PR[1]	Class[2]
Clomazone	27.65	1	0	1	52.26	2
Diuron+hexazinone	10.43	1	0	1	0	1
Trinexapac-ethyl	44.45	2	0	1	32.73	2
Sulfometuron-methyl	31.61	2	0	1	0	1

[1] Mean percentage of reduction in parasitism. [2] Toxicity index as recommended by IOBC (Sterk et al., 1999).

the egg-larva and pupal stages, no significant differences were found (Table 5). For the prepupal stage, clomazone and sulfometuron-methyl reduced the emergence of F_2 generation, compared with the control, and the products were classified as slightly harmful, with a percentage of reduction of 30.3 and 36.0%, respectively (Tables 5 and 6).

When comparing the influence of the products on the emergence of F_2 at different immature stages of *T. galloi*, only trinexapac-ethyl showed no change (Table 5).

In general, herbicides and plant growth regulators used in this study were selective for immature *T. galloi* and, therefore, can be used in sugarcane crops without compromising the integrated pest management of the sugarcane borer, thus preserving this species of parasitoid.

Table 5 - Emergence (%) (± PE) of *Trichogramma galloi* of F_2 generation derived from females (F_1) that emerged from eggs of *Diatraea saccharalis* treated with chemicals containing parasitoids during egg-larva and prepupal and pupal stages.

Treatment	Development		
	Egg-larva[1]	Prepupa[1]	Pupa[1]
Control	69.96 ± 05.33 aA	75.90 ± 3.80 aA	57.12 ±07.61 aA
Clomazone	76.39 ± 05.15 aA	52.92 ± 6.09 bB	49.66 ± 11.27 aB
Diuron+hexazinone	87.41 ± 04.45 aA	71.25 ± 5.90 aA	46.06 ± 11.31 aB
Trinexapac-ethyl	80.80 ± 03.75 aA	72.06 ± 7.41 aA	67.64 ± 14.88 aA
Sulfometuron-methyl	87.07 ± 02.38 aA	48.56 ± 8.68 bC	70.21 ± 04.56 aB

[1] Means followed by the same letter, lowercase and uppercase in the column on the line, do not differ by the Scott-Knott test (p> 0.05).

Table 6 - Percentage of reduction (PR) in the emergence of *Trichogramma galloi* of F_2 generation originated from females of F_1 generation that emerged from eggs of *Diatraea saccharalis* treated with chemicals containing parasitoids during egg-larva, prepupal and pupal stages and toxicity classes of compounds

Treatment	Development					
	Egg-larva		Prepupa		Pupa	
	PR[1]	Class[2]	PR[1]	Class[2]	PR[1]	Class[2]
Clomazone	0	1	30.27	2	13.06	1
Diuron+hexazinone	0	1	06.12	1	19.36	1
Trinexapac-ethyl	0	1	05.05	1	0	1
Sulfometuron-methyl	0	1	36.02	2	0	1

[1] Mean percentage of emergence reduction. [2] Toxicity index as recommended by IOBC (Sterk et al., 1999).

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