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Pesticide selectivity for the insect egg parasitoid *Telenomus remus*

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Abstract We evaluated the side-effects of insecticides, herbicides and fungicides on adults of the egg parasitoid *Telenomus remus* (Nixon) under laboratory conditions. The protocol was adapted from that proposed by the Pesticides and Beneficial Organisms Working Group of the International Organization for Biological Control (IOBC) for *Trichogramma cacoeciae* (Marchal). Chlorpyrifos, acephate, beta-cyfluthrin + imidacloprid, spinosad, and pyrethroids were harmful to the parasitoid, whereas methoxyfenozide, diflubenzuron, and flufenoxuron had no effect. Of the herbicides examined, only glyphosate + imazethapyr and 2,4-D amine were classified as harmless on the first and second days of parasitism; paraquat was the most harmful. Other herbicides were harmless on the first day of parasitism, but caused various levels of reduction of *T. remus* parasitism on the second day. The fungicides were harmless or only slightly harmful.

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Introduction

Soybean is an important crop for many countries, and from Argentina to the Southeast United States some caterpillars have adversely affected yields (Panizzi and Corrêa-Ferreira 1997; Hoffmann-Campo et al. 2003). Among these, some species of the genus *Spodoptera*, such as the southern armyworm, *Spodoptera eridania* (Cramer), as well as *Spodoptera cosmioides* (Walk.) (Lepidoptera: Noctuidae), are now considered as key pests by some soybean growers, mainly in Brazil (Bueno et al. 2008a).

An important practice for addressing this problem is the use of biological control methods, which have been emphasized by researchers as a promising alternative to insecticide application. It is economically and ecologically feasible for soybean farmers (Bueno et al. 2009), in addition to helping to reduce negative impacts of intensive agriculture on the environment (Van Lenteren and Bueno 2003).

Among various biological control agents, *Telenomus remus* (Nixon, 1937) (Hymenoptera: Scelionidae) has been reported to parasitize the eggs of five *Spodoptera* species. Although *T. remus* females each produce about 270 offspring, superparasitism is rare (Cave 2000). Furthermore, *T. remus* is able to parasitize even *Spodoptera* eggs located in the lower layers (Bueno et al. 2008b). Parasitism of 90% was reported by Ferrer (2001) in *Spodoptera frugiperda* eggs in Venezuela, emphasizing the potential of this parasitoid as a biological control agent.

Despite the importance of biological methods for insect control, the use of pesticides is still necessary within the current agricultural system. In this context, multiple tactics have been stressed within the integrated pest management (IPM) concept, demonstrating that when pesticides are used in a compatible manner, the effectiveness of biological control methods may be improved (Croft 1990). Thus, selective pesticides are of great value. A significant advantage of these products is their effectiveness with minimal side-effects on natural enemies of the pests (Bacci et al. 2007; Broadbent and Pree 1984; Bueno and Freitas 2004; Theiling and Croft 1988). Consequently, knowledge of the effects of chemicals, commonly used on soybean crops, upon population densities of natural enemies of insect pests, such as *T. remus*, becomes extremely important when pesticide application is necessary.

Pesticide impact on non-target insects includes lethal and sub-lethal effects (Desneux et al. 2007; Stark and Banks 2003). Therefore, evaluation of pesticide selectivity must involve not only effects on the viability of the biological control agents but also on their fecundity. In this context, the Pesticides and Beneficial Organisms Group of the International Organization for Biological Control (IOBC) has developed methods for standardized study of side effects of insecticides on natural enemies (Hassan et al. 1985, 1988; Hassan 1992; Sterk et al. 1999). However, no procedure has been tested to specifically study the selectivity of pesticides on *T. remus*. Therefore, we examined the selectivity of insecticides, herbicides, and fungicides—commonly used on soybean crops—on the egg parasitoid *T. remus*, using analytical methods adapted from those proposed by the IOBC for use on *Trichogramma cacoeciae* (Marchal) (Hymenoptera: Trichogrammatidae) (Hassan 1992). This methodology is most appropriate for ingredients that are active via exposure by contact and neglects ingestion exposure (Desneux et al. 2007; Stark and Banks 2003). On the other hand, the most important mode of exposure of natural enemies to pesticides is through contact; ingestion may occur, but certainly to a lesser extent. Moreover, an important benefit of using a standardized methodology to study pesticide selectivity to parasitoids is the possibility of comparing results from all over the world, albeit with the precaution of taking into consideration biological differences among parasitoid species (Hassan 1992).

Materials and methods

The experiments were carried out in a completely randomized experimental design with four replications according to standardized protocols described by Hassan (1992), with the following adaptations.

Small glass vials, each containing a droplet of honey on its inner wall, received egg masses (150 eggs) of *S. frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) parasitized by *T. remus*. Then the glass vials were sealed with plastic film and maintained at 25 ± 1°C, 70 ± 10% RH, with a 14/10 h (L/D) photoperiod, until emergence of insects. At parasitoid emergence, glass plates (13 × 13 cm) were each sprayed with one of the pesticides shown in Table 1, or distilled water (control), ensuring a deposit of 1.25 ± 0.25 mg cm^{-2}. When dry, the plates were set in aluminum frames, using the cage arrangement described by Hassan (1992). The vials containing the parasitoids were then connected to the cages, exposing the parasitoids to the glass plates. Twenty four hours after parasitoid release inside the cages, numbered cards, containing *S. frugiperda* egg masses (400 eggs) and honey droplets were inserted. A second card was placed inside each cage 24 h after the first card. Since the eggs were viable in order to avoid any change in *T. remus* behavior, the trials needed to be stopped on the third day to avoid *S. frugiperda* hatching.

Parasitism (%) and parasitoid emergence (%) were evaluated. We analyzed the assumption of normality (Shapiro and Wilk 1965) and homogeneity of variance of treatments in these parameters (Burr and Foster 1972). Whenever data did not follow the normality or homogeneity of variance, a transformation was performed. For the second day of contact with the insecticides and herbicides, the parasitism (%) data needed to be transformed into arcsin $\sqrt{X/100}$ to perform the analysis of variance (ANOVA). The means were compared by Tukey's test ($P \leq 0.05$) for statistical significance (SAS Institute 2001). Furthermore, the reduction in *T. remus* parasitism as related to

Table 1 Pesticides tested against the egg parasitoid *Telenomus remus* under controlled environmental conditions in the laboratory

Pesticides	Formulation	Active ingredient (a.i.)	Chemical group	(g)a.i./200 l H_2O
Insecticides				
Acefato	750 PS	Acephate	Organophosphates	525
Cascade	100 EC	Flufenoxuron	Benzoilureis	10
Connect	100/12.5 SC	Imidacloprid + beta-cyfluthrin	Neonicotinoid/Pyrethroid	100 + 12.5
Dimilin	80 WG	Diflubenzuron	Benzoilureis	20
Intrepid	24 SC	Methoxyfenozide	Diacylhydrazine	36
Lorsban	480 EC	Chlorpyrifos	Organophosphates	384
Stallion	150 CS	Gamma-cyhalothrin	Pyrethroid	3.75
Talstar	100 EC	Bifentrin	Pyrethroid	5
Tracer	480 SC	Spinosad	Spinosins	24
Herbicides				
Alteza	240/30 SL	Glyphosate + imazethapyr	Glycine substituted/imidazolinone	533.4[a] + 90
DMA	806 SL	2,4-D Amine	Acid ariloxyialcanoic	1209
Dual Gold	960 EC	S-metolachlor	Chloroacetanilide	1920
Flumyzin	50 WP	Flumyoxazin	Cyclohexenodicarboximide	60
Gamit	500 EC	Clomazone	Isoxazolidinone	1000
Gliz	480 SL	Glyphosate	Glycine substituted	2880[a]
Gramocil	100/200 SC	Diuron + paraquat	Urea/bypiridyls	300 + 600
Gramoxone	200 SL	Paraquat	Bypiridyls	600
Roundup original	480 SL	Glyphosate	Glycine substituted	1200
Roundup ready	480 SL	Glyphosate	Glycine substituted	960[a]
Roundup transorb	648 SL	Glyphosate	Glycine substituted	1920[a]
Fungicides				
Celeiro	100/500 SC	Flutriafol + tiophanate methyl	Triazole/benzimidazole	60 + 300
Cercobim	500 SC	Tiophanate methyl	Benzimidazole	400
Derosal	500 SC	Carbendazin	Benzimidazole	250
Folicur	250 EC	Tebuconazole	Triazole	150
Impact	125 SC	Flutriafole	Triazole	125
Nativo	100/200 SC	Trifloxystrobin + tebuconazole	Strobirulin/triazole	60 + 120
Opera	50/133 SE	Epoxyconazole + pyraclostrobin	Triazole/strobirulin	30 + 79.8
Opus	125 SC	Epoxyconazole	Triazole	12.5
Priori	250 SC	Azoxystrobin	Strobirulin	50
Priori Xtra	200/80 SC	Azoxystrobin + cyproconazol	Strobirulin/triazole	60+24

[a] Active ingredient expressed in equivalent acid

the control treatment was computed by the equation: $E(\%) = (1 - Vt/Vc) \times 100$, in which $E(\%)$ is the percent reduction in parasitism, Vt is the median parasitism for the treatment tested, and Vc is the average parasitism observed for the control treatment. The chemicals were classified according to the IOBC standards as follows: class 1, harmless ($E < 30\%$); class 2, slightly harmful ($30\% \leq E \leq 79\%$); class 3, moderately harmful ($80\% \leq E \leq 99\%$); and class 4, harmful ($E > 99\%$) (Hassan 1992).

Results

Among the insecticides, the mildest side-effects on *T. remus* adults were exerted by the products from the insect growth regulator (IGR) group. Parasitism (%) and parasitoid emergence (%) of methoxyfenozide 21.6 ($P < 0.0001$, $F = 148.93$, $df_{error} = 10$, $df_{model} = 3$) and 36 g a.i. ha^{-1} ($P < 0.0001$, $F = 58.54$, $df_{error} = 19$, $df_{model} = 7$) treatments were statistically similar to control at both first and second day of parasitism (Table 2). Thus, this active ingredient was classified as harmless (class 1) to adults of *T. remus* for both doses (Table 2). Diflubenzuron and flufenoxuron, also IGRs, were classified as harmless (class 1) at the first day of parasitism, but impaired parasitism on the second day ($P < 0.0001$, $F = 26.8$, $df_{error} = 28$, $df_{model} = 7$) when they were classified as slightly harmful (class 2) (Table 2). Methoxyfenozide was the only tested insecticide that did not reduce parasitism on the first or second day after parasitoid emergence (Table 2).

The pyrethroid insecticides, gamma-cyhalothrin, bifentrin, and imidacloprid + beta-cyfluthrin and the organophosphates, acephate and chlorpyrifos, killed

Table 2 Side-effects of insecticides on the egg parasitoid *Telenomus remus* and placement of the product into IOBC survival side-effect classes

	Parasitism (%)[1]	Parasitoid emergence (%)[1]	E[2]	C[3]	Parasitism (%)[1]	Parasitoid emergence (%)[1]	E[2]	C[3]
Assay 1								
Acephate 525	0.0 ± 0.0 c	–	100	4	0.0 ± 0.0 c[4]	–	100	4
Chlorpyrifos 384	0.0 ± 0.0 c	–	100	4	0.0 ± 0.0 c	–	100	4
Diflubenzuron 20	84.0 ± 3.4 a	83.6 ± 2.6[ns]	7.1	1	19.7 ± 11.4 bc	64.5 ± 1.2 b[4]	76.9	2
Spinosad 24	26.5 ± 3.8 b	82.3 ± 2.9	70.6	2	0.0 ± 0.0 c	–	100	4
Flufenoxuron 10	65.2 ± 14.2 a	62.3 ± 20.7	27.8	1	26.1 ± 16.0 b	67.7 ± 7.7 b	69.3	2
Gamma-cyalothrin 3.75	0.0 ± 0.0 c	–	100	4	0.0 ± 0.0 c	–	100	4
Methoxyfenozide 36	81.4 ± 8.1 a	72.6 ± 6.5	9.9	1	86.1 ± 8.0 a	91.5 ± 2.8 a	0	1
Control (H_2O)	90.3 ± 4.2 a	84.5 ± 3.4	–	–	85.2 ± 4.1 a	81.9 ± 5.9 ab	–	–
CV (%)	25.6	21.5	–	–	49.4	9.7	–	–
F	58.54	1.04	–	–	26.80	6.31	–	–
P	<0.0001	0.4262	–	–	<0.0001	0.0211	–	–
df model	7	4	–	–	7	3	–	–
df error	19	12	–	–	28	10	–	–
Assay 2								
Methoxyfenozide 21.6	90.1 ± 6.4 a	81.3 ± 5.2 [ns]	0	1	63.9 ± 2,5 a	69.9 ± 0.4 [ns]	0	1
Imidacloprid 100 + beta-cyfluthrin 12.5	1.4 ± 1.4 b	–	98.2	3	0.00 ± 0.00 b	–	100	4
Bifentrin 5	0.0 ± 0.0 b	–	100	4	0.00 ± 0.00 b	–	100	4
Control (H_2O)	76.2 ± 6.6 a	77.8 ± 5.6	–	–	60.95 ± 2.67 a	78.8 ± 5.7	–	–
CV (%)	20.2	13.2	–	–	11.6	10.8	–	–
F	148.93	1.69	–	–	395.47	2.47	–	–
P	<0.0001	0.2615	–	–	<0.0001	0.1674	–	–
df model	3	1			3	1	–	–
df error	10	6	–	–	12	6	–	–

[1] Means ± SE followed by the same letter in each assay column are not statistically different according to Tukey's test ($P > 0.05$)

[2] Reduction in parasitism (%)

[3] Classes: 1, harmless ($E < 30\%$); 2, slightly harmful ($30 \leq E \leq 79\%$); 3, moderately harmful ($80 \leq E \leq 99\%$); 4, harmful ($E > 99\%$)

[4] Original data followed by statistics done on data transformed into arcsin $\sqrt{X/100}$ as request to perform ANOVA according to Burr and Foster (1972)

[ns] ANOVA non-significant

the insects that had contacted the dry residue (Table 2). They all reduced parasitism (%) on both the first and second days (Table 2). Thus, these products were classified as harmful (class 4), or as moderately harmful (class 3) for the imidacloprid + beta-cyfluthrin treatment on the first day of parasitism (Table 2).

Spinosad reduced parasitism and was classified as slightly harmful (class 2) on the first day of parasitism ($P < 0.0001$, $F = 58.54$, $df_{error} = 19$, $df_{model} = 7$) and harmful (class 4) on the second day ($P < 0.0001$, $F = 26.80$, $df_{error} = 28$, $df_{model} = 7$), when no parasitism was recorded. No effects on parasitoid emergence were registered for the survivors on the first day ($P = 0.4262$, $F = 1.04$, $df_{error} = 12$, $df_{model} = 4$) (Table 2).

Among the herbicides, only paraquat at 600 g a.i. ha^{-1} reduced parasitism (%) compared to the control on the first day of parasitism ($P < 0.0001$, $F = 50.1$, $df_{error} = 19$, $df_{model} = 7$) and was classified as moderately harmful (class 3) (Table 3). Overall, paraquat was the most harmful herbicide, causing parasitism reductions from 93.6% on the first day to 100% on the second day (Table 3). On the second day of parasitism, glyphosate + imazethapyr and 2,4-D amine treatments showed similar parasitism to the control and were classified as harmless (class 1) to *T. remus* adults ($P < 0.0001$, $F = 9.41$, $df_{error} = 26$, $df_{model} = 7$) (Table 3).

None of the fungicides reduced parasitism or parasitoid emergence, as compared to the control. They were classified as harmless (class 1) to *T. remus* adults on both the first and second days of parasitism, with the exception of azoxystrobin + cyproconazol and tiophanate methyl, which were classified as slightly harmful (class 2) on the second day of parasitism (Table 4). The other fungicides were harmless to *T. remus* adults (Table 4).

Discussion

The IGR insecticides (diflubenzuron, flufenoxuron and methoxyfenozide) were selective for *T. remus* adults. These chemicals inhibit chitin synthesis and kill target insects slowly by disturbing exoskeleton formation after molting (Reynolds 1987). Acting at specific points in the course of molting, these products may show some selectivity since the hormones that trigger this physiological process differ across insect taxonomic orders. Since pests and their natural enemies commonly differ in terms of taxonomic order, these active ingredients (IGRs) are generally able to specifically kill the target insect while preserving the beneficial counterpart. Examples of IGR selectivity have been reported in the literature. Diflubenzuron was described as harmless to *T. cacoeciae* (Hassan et al. 1987). Clorfluazuron and lufenuron, also examples of benzoylphenylurea compounds—the same chemical group of diflubenzuron and flufenoxuron—had only mild side-effects on *Trichogramma dendrolimi* (Takada et al. 2001) and *T. cacoeciae* (Hassan et al. 1998), which are also egg parasitoids.

Methoxyfenozide, a substitute for dibenzoylhydrazine, works slightly differently from the benzoylphenylureas. It accelerates the molting process by acting as an ecdysone agonist or ecdysonoid, substituting for the natural 20-hydroxyecdysone insect-molting hormone (Dhadialla et al. 1998). This hormone is specific to Lepidoptera which explains its selectiveness to the egg parasitoid *T. remus*. Similar results were reported by Bueno et al. (2008a) who evaluated the effects of methoxyfenozide on *Trichogramma pretiosum*, confirming its compatibility with biological control strategies.

In the literature, the IGRs are usually regarded as less harmful to beneficial insects when compared to other chemical groups, even though negative side-effects have been reported (Santos et al. 2006). For example, the IGR triflumuron was found to be moderately harmful (class 3) or harmful (class 4) to eggs and larvae of *T. pretiosum* (Bueno et al. 2008a). Moreover, lufenuron, another IGR, has been proven to have trans-ovarian activity in some insects, as demonstrated by Pratissoli et al. (2004) and Ávila and Nakano (1999) for *S. frugiperda* and *Diabrotica speciosa* (Coleoptera: Chrysomelidae), respectively. Trans-ovarian activity has also been observed for biological control agents. Although not killing adults, lufenuron significantly reduced egg viability in *Chrysoperla externa* (Neuroptera: Chrysopidae) after ingestion of the insecticide, thus classified as harmful (class 4) at that stage of development (Bueno and Freitas 2004). The IGR trans-ovarian activity was not observed here for *T. remus*. However, it is important to bear in mind that our bioassays evaluated dry-residue effects from contact exposure. Ingestion of IGR by adults of *T. remus* might occur in field

Table 3 Side-effects of herbicides on the egg the parasitoid *Telenomus remus* and placement of the product into IOBC survival side-effects classes

Herbicide (g of a.i./200 l H_2O)	1 day after parasitoid emergence				2 days after parasitoid emergence			
	Parasitism (%)[1]	Parasitoid emergence (%)[1]	E^2	C^3	Parasitism (%)[1]	Parasitoid emergence (%)[1]	E^2	C^3
Assay 3								
Paraquat 600	4.8 ± 2.8b	89.0 ± 4.9[ns]	93.6	3	0.0 ± 0.0d[4]	–	100	4
Diuron 300 + paraquat 600	74.7 ± 1.2a	79.7 ± 6.5	1.1	1	4.8 ± 4.8d	88.5 ± 4.4[ns]	92.3	3
2,4-D Amine 1209	83.9 ± 3.2a	90.4 ± 3.4	0	1	68.0 ± 21.0ab	78.8 ± 6.2	0	1
Clomazone 1000	86.1 ± 2.9a	91.6 ± 1.3	0	1	8.4 ± 5.3cd	96.3 ± 2.1	86.3	3
Glyphosate 1200 (Roundup Original)	86.9 ± 4.0a	93.4 ± 0.8	0	1	5.4 ± 5.1cd	92.0 ± 6.7	91.2	3
Glyphosate 533.4* + imazethapyr 90	68.2 ± 7.2a	88.3 ± 5.5	9.8	1	83.7 ± 5.4a	91.4 ± 2.9	0	1
S-metolachlor 1920	81.5 ± 5.1a	88.0 ± 5.2	0	1	21.4 ± 21.4bcd	88.5 ± 1.5	65.3	2
Control (H_2O)	75.6 ± 5.2a	86.6 ± 3.7	–	–	61.6 ± 13.0ab	86.2 ± 6.8	–	–
CV (%)	11.0	8.7	–	–	55.4	10.6	–	–
F	50.1	0.89	–	–	9.41	1.05	–	–
P	<0.0001	0.5354	–	–	<0.0001	0.4377	–	–
df model	7	7	–	–	7	6	–	–
df error	19	19	–	–	26	14	–	–
Assay 4								
Flumyoxazin 60	76.2 ± 13.4[ns]	70.0 ± 7.9[ns]	0	1	24.6 ± 2.7b	76.4 ± 10.1[ns]	56.6	2
Glyphosate (Gliz) 2880*	74.6 ± 12.5	80.3 ± 9.3	2.1	1	0.0 ± 0.0c	–	100	4
Glyphosate (roundup transorb) 1920*	91.9 ± 3.8	91.9 ± 1.3	0	1	0.0 ± 0.0c	–	100	4
Control (H_2O)	76.2 ± 6.6	77.8 ± 5.6	–	–	61.0 ± 2.7a	78.8 ± 5.7	–	–
CV (%)	20.5	15.3	–	–	14.8	18.2	–	–
F	0.9	2.18	–	–	284.80	0.05	–	–
P	0.4781	0.1485	–	–	<0.0001	0.8311	–	–
df model	3	3	–	–	3	1	–	–
df error	9	11	–	–	9	5	–	–

[1] Means ± SE followed by the same letter in each assay column are not statistically different according to Tukey's test ($P > 0.05$)

[2] Reduction in parasitism (%)

[3] Classes: 1, harmless ($E < 30\%$); 2, slightly harmful ($30 \le E \le 79\%$); 3, moderately harmful ($80 \le E \le 99\%$); 4, harmful ($E > 99\%$)

[4] Original data followed by statistics done on data transformed into arcsin $\sqrt{X/100}$ as request to perform ANOVA according to Burr and Foster (1972)

[ns] ANOVA non-significant

*Active ingredient expressed in equivalent acid

conditions if honeydew or other sources of food are contaminated by the IGR spray. This possibility needs to be tested and its frequency established.

The pyrethroids (gamma-cyhalothrin, bifentrin, and imidacloprid + beta-cyfluthrin) were harmful to *T. remus* adults probably because they are neurotoxins that act similarly on all kinds of insects, beneficial and pest species. Various insecticides in this group have been reported as harmful to beneficial arthropods (Croft 1990; Croft and Whalon 1982; Sterk et al. 1999).

Organophosphates kill insects primarily by phosphorylation of the acetylcholinesterase enzyme

Table 4 Side-effects of fungicides on the egg parasitoid *Telenomus remus* and placement of the product into IOBC survival side-effects classes

Fungicides (g of a.i./200 l H_2O)	1 day after parasitoid emergence				2 days after parasitoid emergence			
	Parasitism (%)[1]	Parasitoid emergence (%)[1]	E^2	C^3	Parasitism (%)[1]	Parasitoid emergence (%)[1]	E^2	C^3
Assay 5								
Azoxystrobin 50	46.0 ± 12.8[ns]	71.4 ± 11.9[ns]	14.8	1	42.1 ± 8.6[ns]	83.6 ± 4.3[ns]	23.4	1
Azoxystrobin 60 + cyproconazol 24	49.3 ± 11.6	83.4 ± 5.4	8.8	1	20.3 ± 14.3	68.7 ± 27.0	63.2	2
Carbendazin 250	46.8 ± 13.9	71.2 ± 10.2	13.3	1	49.1 ± 6.9	90.4 ± 4.2	10.7	1
Epoxyconazole 30 + pyraclostrobin 79.8	48.5 ± 1.2	97.9 ± 1.7	10.2	1	39.0 ± 12.2	91.3 ± 1.3	29.1	1
Tebuconazole 150	64.7 ± 2.2	87.1 ± 5.3	0	1	45.3 ± 5.3	87.0 ± 1.8	17.7	1
Tebuconazole 120 + trifloxystrobin 60	61.0 ± 7.1	85.8 ± 2.0	0	1	50.0 ± 6.0	87.5 ± 2.2	9.1	1
Tiophanate methyl 400	57.4 ± 7.4	73.5 ± 9.8	0	1	28.5 ± 7.3	89.4 ± 3.7	48.2	2
Control (H_2O)	54.0 ± 2.9	70.4 ± 6.4	–	–	55.0 ± 6.0	83.8 ± 3.7	–	–
CV (%)	30.6	17.3	–	–	37.8	12.8	–	–
F	0.56	1.61	–	–	1.44	1.03	–	–
P	0.7773	0.1947	–	–	0.2514	0.4424	–	–
df model	7	7	–	–	7	7	–	–
df error	18	18	–	–	18	18	–	–
Assay 6								
Flutriafol 125	66.7 ± 13.8[ns]	74.7 ± 10.2[ns]	6.5	1	81.3 ± 7.9[ns]	97.4 ± 2.1[ns]	8.5	1
Flutriafol 60 + Tiophanate methyl 300	75.8 ± 7.6	91.9 ± 2.6	0	1	83.9 ± 10.1	92.7 ± 2.1	5.7	1
Control (H_2O)	71.3 ± 6.2	72.0 ± 1.9	–	–	88.9 ± 10.0	83.1 ± 8.3	–	–
CV (%)	23.4	12.8	–	–	20.6	6.6	–	–
F	0.22	3.70	–	–	0.12	3.39	–	–
P	0.8065	0.08	–	–	0.8920	0.1036	–	–
df model	2	2	–	–	2	2	–	–
df error	7	7	–	–	6	6	–	–
Assay 7								
Epoxyconazole 12.5	84.5 ± 5.1[ns]	87.0 ± 3.5[ns]	0	1	79.9 ± 7.8[a]	88.9 ± 4.3[ns]	0	1
Control (H_2O)	76.2 ± 6.6	77.8 ± 5.6	–	–	61.0 ± 2.7b	78.8 ± 5.7	–	–
CV (%)	13.3	13.6	–	–	16.5	12.06	–	–
F	1.03	11.03	–	–	5.32	1.99	–	–
P	0.3574	1.97	–	–	0.0605	0.2079	–	–
df model	1	1	–	–	1	1	–	–
df error	5	0.2102	–	–	6	6	–	–

[1] Means ± SE followed by the same letter in each assay column are not statistically different according to Tukey's test ($P > 0.05$)

[2] Reduction in parasitism (%)

[3] Classes: 1, harmless ($E < 30\%$); 2, slightly harmful ($30 \leq E \leq 79\%$); 3, moderately harmful ($80 \leq E \leq 99\%$); 4, harmful ($E > 99\%$)

[ns] ANOVA non-significant

(AChE) at the nerve endings. This type of poisoning causes loss of the available AChE and over-stimulation of organs by excess acetylcholine at the nerve endings and affects beneficial and pest insects similarly. Therefore, like pyrethroids, organophosphates are generally harmful to all insect groups. Noxious results of organophosphates on beneficial arthropods have been reported in the literature for

T. pretiosum (Bueno et al. 2008a) and *T. cacoeciae* (Hassan et al. 1988), also examples of egg parasitoids.

Because pyrethroids and organophosphates are among the cheapest insect-control products available to growers, they are often overused. However, their application is not compatible with biological control methods, as shown in this work. Therefore, pyrethroids and organophosphates should be replaced, whenever possible, by less harmful products in IPM programs. Good alternatives to those products, when feasible, are the IGRs compounds, since their effects on beneficial arthropods are less injurious.

Because spinosad is a mixture of A and D spinosyns, which are tetracyclic macrolide compounds, produced by the actinomycete *Saccharopolyspora spinosa*, its use on organic crops is permitted in many countries. This chemical acts primarily as a stomach poison, with some contact activity (Bret et al. 1997). Although it is sometimes classified as environmentally and toxicologically of reduced risk and usually less harmful to predators, Hymenoptera parasitoids are significantly more susceptible to its effects (Williams et al. 2003). Although the negative effects of spinosad have been reported in the literature for *T. pretiosum* (Ruberson and Tillman 1999) and other parasitoids, its mode of action on parasitoid physiology remains unclear.

The issue of side-effects from herbicides on parasitoid wasps is very important, since their use with soybean crops increases each year with the planting of Roundup Ready® varieties. Although noxious effects of herbicides on egg parasitoids have been reported for *T. pretiosum* (Bueno et al. 2008a), the mode of action remains unclear. Also, male-fertility reduction was reported for *Anisopteromalus caladrae* (Hymenoptera: Pteromalidae) due to the effects of paraquat (Lacoume et al. 2009).

Similar to herbicides, fungicides have been applied more to soybean crops in recent years, mainly due to the occurrence of soybean rust. However, in contrast with the herbicides, the fungicides—except azoxystrobin + cyproconazol and tiophanate methyl—were harmless on both the first and second days of parasitism. Similar results with fungicides were reported for *T. pretiosum* (Bueno et al. 2008a) and *T. cacoeciae* (Hassan et al. 1994; Sterk et al. 1999). These results show that fungicides have little impact on the use of parasitoids as biological-control agents. However, this might be different for other biological control agents. Entomopathogenic fungi, for example, which are important in soybean, are more likely to be affected by fungicide use.

When highlighting the importance of selective products to be used in IPM programs, not only insecticides should be considered, but herbicides and fungicides as well. These latter chemicals are usually neglected in this type of evaluation, whereas they may exert negative impacts on natural enemies of insect pests—as shown within this work—thus contributing to ecosystem imbalance.

In conclusion, results reported herein show the effects of a range of pesticides on *T. remus* adults. These data will help in the choice of pesticides most appropriate for use in IPM programs. Furthermore, the analytical methods adapted from those proposed by the International Organization for Biological Control (IOBC) Pesticides and Beneficial Organisms Working Group for *T. cacoeciae* were successfully applied to the study of side-effects on *T. remus*. The most important effects on parasitism were similar to the IOBC side-effect classification from class 1 to class 4. Therefore, in future studies, this standardized methodology may be followed with added confidence.

It is important to emphasize that these experiments were carried out under controlled environmental conditions in the laboratory, where parasitoids were subjected to the highest possible pressure from the pesticides. Under field conditions, however, the negative impact of pesticides may be reduced, since the biological control agents can benefit from refuge areas or may avoid chemical-treated areas. Moreover, sunlight degradation plays an important role in the field, and may decrease strong pesticide impact seen on beneficial insects in the laboratory (Hassan 1992). Deltamethrin, which is recognized as harmful in the laboratory to various natural enemies has been shown to be rapidly degraded in semi-field conditions allowing aphid parasitoids to recolonize treated plants only a few days after deltamethrin treatment (Desneux et al. 2005, 2006). Therefore, neither isolated laboratory assays nor semi-field or field assays alone can fully explain pesticide effects on natural enemies. Furthermore, products that are non-selective in the laboratory need also to be tested in semi-field conditions to evaluate the residual persistence and also in the field to take into consideration sunlight degradation, shelters and other factors that might impact the pesticide effect. Therefore, further studies with products classified as harmful (class 4) under laboratory conditions should be carried out under field conditions to evaluate their persistence and to fully understand any negative impact on the biological control approach.

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