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BIOLOGICAL CONTROL

Side-Effects of Glyphosate to the Parasitoid *Telenomus remus* Nixon (Hymenoptera: Platygastridae)

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Abstract

The aim of this study was to compare the side-effects of glyphosate to the parasitoid *Telenomus remus* Nixon (Hymenoptera: Platygastridae) when parasitoids were exposed to this chemical at the pupal (inside host eggs) and adult stages. Bioassays were conducted under laboratory conditions according to the International Organization for Biological Control (IOBC) standard methods for testing side-effects of pesticides to egg parasitoids. Different glyphosate-based pesticides (Roundup Original®, Roundup Ready®, Roundup Transorb®, Roundup WG®, and Zapp Qi®) were tested at the same acid equivalent concentration. Treatments were classified following the IOBC toxicity categories as (1) harmless, (2) slightly harmful, (3) moderately harmful, and (4) harmful. When tested against *T. remus* adults, Roundup Original®, Roundup Ready®, Roundup Transorb®, and Roundup WG® reduced parasitism 2 days after parasitoid emergence, being classified as slightly harmful. Differently, when tested against *T. remus* pupae, all tested glyphosate-based products did not differ in their lethal effect and therefore did not reduce *T. remus* adult emergence or parasitism capacity, being classified as harmless. However, differences on sublethal toxicity were found. Parasitism of individuals emerging from parasitized eggs sprayed at the pupal stage of *T. remus* with Zapp Qi® was lower compared to control, but parasitism was still higher than 66%, and therefore, Zapp Qi® was still classified as harmless. In conclusion, all tested glyphosate-based products can be used in agriculture without negative impact to *T. remus* as none was classified as harmful or moderately harmful to this parasitoid when exposure occurred at the pupal or adult stages.

Introduction

Glyphosate inhibits the EPSP synthase enzyme (5-enolpyruvylshikimate 3-phosphate synthase) (Coutinho & Mazo 2005) and is today one of the most widely used herbicides throughout the world, mainly due to its broad-spectrum weed control (Travlos & Chachalis 2013, Shrestha *et al* 2014).

Spraying with this herbicide has become the main strategy of weed control in genetically engineered crops like soybean, maize, and cotton that carry resistant traits against this molecule. Glyphosate helps to prevent weed competition (Shaner 2000) that can reduce agricultural production by 30 to 40% (Lorenzi 2000). The classification of glyphosate as an herbicide does not account for its effects on beneficial organisms. Even

though it is applied exclusively for weed control, it can impact non-target organisms, such as insects (Rizzardi *et al* 2003).

Pesticide risk assessment has usually been based on short-term acute toxicity effects (lethal effects). Longer-term effects on population dynamics (sublethal effects), critical for biological control, and consequently, the success of integrated pest management (IPM) are often overlooked (Biondi *et al* 2013). However, sublethal effects of a given compound are also related to its toxicological properties acting on arthropod physiology and behavior (Biondi *et al* 2012). It has been pointed out that acute toxicity assessments alone cannot fully predict the actual impact of pesticides on non-target organisms (Ali *et al* 2012, Tan *et al* 2012, Guo *et al* 2013, Kim *et al* 2013, Planes *et al* 2013, Saber & Abedi 2013, Sohrabi *et al* 2013).

Sublethal effects related to species-specific life history traits must be carefully considered in order to assess pesticide risks to IPM programs (Biondi *et al* 2013). These effects are defined as physiological and/or behavioral effects on individuals that survive exposure to a toxic compound at low concentrations (Desneux *et al* 2007) and may be expressed as changes in the life history traits of a beneficial insect species, such as parasitoid-regarded parasitism rate, longevity, egg viability, consumption rate, and behavioral patterns (Croft 1990, Desneux *et al* 2007).

Among different species, the egg parasitoid *Telenomus remus* Nixon (Hymenoptera: Platygastridae) is an effective biological control agent for various pest species of the genus *Spodoptera* (Cave 2000, Morales *et al* 2000, Pomari *et al* 2012), mainly due to its high reproductive capacity (Bueno *et al* 2008). Its hosts include *Spodoptera frugiperda* (J.E. Smith), *Spodoptera eridania* (Cramer), and *Spodoptera cosmioides* (Walker) (Lepidoptera: Noctuidae), responsible for heavy production losses in soybean, maize, and cotton crops (Cruz *et al* 1999, Carmo *et al* 2009, Santos *et al* 2010). The parasitoid *T. remus* has been used extensively in pest management programs in Venezuela involving inundative release in maize-growing areas (Hernández *et al* 1989, González & Zocco 1996, Ferrer 2001). Inundative biological control aims at directly increasing mortality of the pest population, whereby the released natural enemy is used as a biological insecticide (Stinner 1977). The practice of inundative release therefore centers on its cost/benefit ratio as compared to other alternatives, especially pesticides.

Therefore, releasing but also preserving this egg parasitoid in agroecosystems where it naturally occurs is a very important step toward suppressing populations of *Spodoptera* spp. below levels that cause economic losses (Carmo *et al* 2010a). However, the use of herbicides for weed control might also be necessary and, in some situations, weed and pest control need to be applied simultaneously. Then, knowledge on the parasitization ability of this parasitoid after glyphosate exposure is of crucial importance to classify the side-effects of this herbicide on the egg parasitoid *T. remus* and select the most selective ones.

The glyphosate toxicity can be due to the chemical action of its active ingredient, which may be toxic to small insects such as *Trichogramma* (Bueno *et al* 2008). However, glyphosate sublethal toxicity on egg parasitoids may differ among products (Giolo *et al* 2005, Nörnberg *et al* 2008). Each of these products has a slightly different chemistry and surfactant mixture, and in comparison with the original Roundup formulation (Roundup Original®), some of these products might be less acutely toxic to non-target organisms (Howe *et al* 2004). It is therefore important to understand the chemical properties of glyphosate and its different glyphosate-based products as well as its lethal and sublethal toxicity to beneficial insects in order to point the most harmless glyphosate to beneficial insects to be used in IPM crops. Although there is information in the literature on the physical, chemical, and toxicological properties of glyphosate (Amarante Júnior *et al* 2002), little is known about the toxicity of different commercial glyphosate-based products to beneficial insects (Giolo *et al* 2005, Bueno *et al* 2008, Nörnberg *et al* 2008, Carmo *et al* 2009) and a diversity of glyphosate brands is available. These products are based on a variety of salts, such as potassium salt, isopropylamine salt, and ammonium salt, which, despite having the same mechanism of action, may have different effects on beneficial organisms (Giolo *et al* 2005). Therefore, the aim with this study was to comparatively assess lethal and sublethal effects of different glyphosate-based products applied to *T. remus* pupae (within host eggs) and on parasitoid adults emerged from these treated pupae. We also evaluated the parasitism activity of adults (emerged from non-treated pupae) directly exposed to dry residue on inert substrate as well as the transgenerational effect on the adult emergence of the F_1 generation (i.e., progeny of the treated generation).

Material and Methods

The bioassays were conducted in the laboratory under controlled conditions (25 ± 2°C, 70 ± 10% RH, 14L:10D h photoperiod) in a fully randomized arrangement, with seven treatments and five replications, in accordance with the protocols proposed by Pesticides and Beneficial Organisms from the International Organisation for Biological Control (IOBC) (Hassan *et al* 2000, Manzoni *et al* 2007) and modified by Carmo *et al* (2010a) for studies with *T. remus*.

All tested glyphosate-based commercial products were adjusted to a concentration recommended for post-emergence application on plants. Furthermore, all treatments were set at the same acid equivalent concentration (720 g ha^{-1}) considering 200 L of water ha^{-1} to obtain a better

understanding of possible lethal and sublethal effects of different commercial brands and their chemical differences on the studied parasitoid. The studied treatments were (1) negative control (distilled water); (2) Roundup Original®, isopropylamine salt, 960 g of active ingredient (a.i.) ha^{-1}; (3) Roundup Ready®, isopropylamine salt, 972 g a.i. ha^{-1}; (4) Roundup Transorb®, isopropylamine salt, 972 g a.i. ha^{-1}; (5) Roundup WG®, ammonium salt, 792.5 g a.i. ha^{-1}; (6) Zapp Qi®, potassium salt, 892.8 g a.i. ha^{-1}; and (7) positive control (Lorsban® 480 BR), chlorpyrifos 480 g a.i. ha^{-1}. The active ingredient and dosage of the positive control were defined according to Carmo *et al* (2010a, b) who showed this treatment to be harmful to both *T. remus* pupae and adults.

Parasitoid and host colonies

All *S. frugiperda* eggs and *T. remus* used in the experiments were obtained from insect colonies kept at Embrapa Soybean, located in Londrina, state of Paraná, Brazil. *Spodoptera frugiperda* was originally collected on corn plants (*Zea mays*) in Rio Verde, state of Goiás, in 2007. After field collection, it was reared under laboratory-controlled environmental conditions (25±2°C 70±10% RH, and 14L:10D h photoperiod) and fed on the artificial diet proposed by Greene *et al* (1976) and Parra (2001) for about 136 generations. *Telenomus remus* was originally collected in Ecuador and was multiplied at the parasitoid-rearing facilities at ESALQ/USP (Escola Superior de Agricultura "Luiz de Queiroz"/Universidade de São Paulo), from where some specimens were transferred to Embrapa Soybean 8 years ago. Since then, *T. remus* has been reared in the laboratory using *S. frugiperda* egg masses (approximately 150 eggs each) for about 250 generations. These egg masses were glued onto a cardboard sheet (2 cm × 8 cm). Three of these sheets with eggs previously parasitized by *T. remus* were placed in a glass tube (8 cm long and 2 cm in diameter) sealed with a PVC film. Small drops of honey were placed in these tubes to feed the adults when they emerged. These tubes, containing the parasitoids, were then closed, and *T. remus* parasitism was allowed for 24 h. Adults emerging from these eggs were used for experiments or for colony maintenance. Quality control of the parasitoid colony was evaluated in accordance with Prezotti *et al* (2002) who performed each 10 generations of the insect, and endogamy issues were never observed.

Adult parasitoid side-effect bioassay

Spodoptera frugiperda eggs parasitized by *T. remus* (±250 pupae of *T. remus*) were placed in Duran tubes containing a drop of honey and sealed with a plastic film. They were kept in a controlled environment (25±2°C, 70±10% RH, a photoperiod of 14:10 h L/D) until parasitoids emerged. Up

to 24 h after the emergence of *T. remus* adults inside the tubes, glass plates (13×13 cm) were sprayed with the described treatments using a Potter tower calibrated to deposit 1.75±0.25 mg solution cm^{-2} (Hassan *et al* 2000, Manzoni *et al* 2007). The volume of application was controlled using a precision electronic balance both before and after spraying (Carmo *et al* 2010a).

After spraying, plates were left to dry for 2 h at 25±2°C, 70±10% RH, under constant illumination. Next, the plates were fixed into aluminum frames with forced air circulation using an extractor (Hassan 1992). After setting up the plates in the air flow, the Duran tubes containing the parasitoids (around 200 adults with a sex ratio of 0.6, 80% of the 250 pupae selected) were wrapped in aluminum foil and linked up to the emergence holes (Carmo *et al* 2010a). One day after adult release, the Duran tube was removed while visually ensuring that all parasitoids entered the arena (more than 90% of the replicates). When only a few parasitoids were left in the Duran tube, they were manually forced into the arena.

Twenty-four hours after parasitoid release into the arenas, numbered cards, containing *S. frugiperda* egg masses (300±100 eggs) and honey droplets, were inserted. A second card was placed inside each cage 24 h after the first card. Since superparasitism is rare for this species (Cave 2000), the first card was not removed from the cages because parasitoids tend to stay on the top and middle egg masses so that, inevitably, some test organisms would have been removed with this first card. Since eggs were not sterile, as used in *Trichogramma* selectivity tests, in order to avoid any change in the behavior of *T. remus*, the experiments had to be stopped on the third day to prevent *S. frugiperda* from hatching (Carmo *et al* 2010a). The cards were removed from the enclosures and placed in transparent plastic bags which were then stored at 25±2°C, 70±10% RH, and a photoperiod of 14:10 h L/D until emergence of the parasitoids so that parasitism could be assessed.

Side-effects to pupal parasitoid bioassay

Cards with 300±100 *S. frugiperda* eggs with *T. remus* pupae close to emergence (11 days after parasitism) were kept at 25°C under a 14:10 h L/D photoperiod (Pomari *et al* 2012) and were sprayed with the treatments previously described. Since there is no superparasitism in this parasitoid species (Pomari *et al* 2012), the number of parasitoid pupae was estimated by counting the number of parasitized *S. frugiperda* eggs under a stereoscopic microscope.

As in the adult bioassay, spraying was carried out using a Potter tower calibrated to deposit 1.75±0.25 mg cm^{-2} solution, in line with IOBC standards (Hassan 1992). Next, sprayed eggs containing *T. remus* pupae were kept at 25±2°C, 70±10% RH, for around 2 h under constant illumination to

eliminate excess moisture. Treated cards were then inserted into enclosures as described for the adult bioassay (Hassan 1992) until adults emerged (generation F_O). Honey droplets on small cards were inserted into the cage to feed the adults after emergence. Cards with *S. frugiperda* eggs (300±100 eggs up to 24 h old) were offered 1 and 2 days after adults emerged. On the third day, the cards were stored in air-filled plastic bags until evaluation. Viability of the sprayed pupae (number of parasitized eggs with a parasitoid emergence hole), parasitism capacity of the F_O generation (adults originating from treated pupae), and transgenerational effects on the percentage of emergence of the F_1 generation (progeny of the treated generation) were quantified.

Statistical analysis

The results were subjected to exploratory analysis to evaluate normality assumptions for the residues (Shapiro & Wilk 1965), homogeneity of the variance between treatments (Burr & Foster 1972), and additivity of the model in order to apply an analysis of variance (ANOVA). Data not following the normality assumptions or homogeneity of variance were transformed to arcsin $\sqrt{X/100}$. This transformation was performed for viability (%) at both the second day of herbicide contact [2 days after parasitoid emergence (DAE)] for both adult and pupal trials. Means were compared using Tukey's honest significant difference (HSD) test (5% error probability) implemented in the SAS statistical analysis program (SAS Institute 2001).

In addition, the effect (E) of each pesticide on *T. remus* was determined by comparison with a negative control (distilled water) and calculated using the formula by Hassan *et al* (1985, 2000) and Carmo *et al* (2010a): $E_1\% = (1 - \text{parasitism in the treatment} / \text{parasitism in the control}) \times 100$ for adult assays and $E_2\% = (1 - \text{viability of sprayed pupae} / \text{viability of pupae treated with the control}) \times 100$ for pupal assays. Treatments were classified as follows: class 1 = harmless ($E < 30\%$), class 2 = slightly harmful ($30\% \leq E < 80$), class 3 = moderately harmful ($80\% \leq E < 99$), and class 4 = harmful ($E \geq 99\%$).

Results

Adult parasitoid side-effect bioassay

When glyphosate was tested for *T. remus* adults, parasitism (%) was not affected by the tested glyphosate-based products on the first day after adult emergence (1 DAE) and, therefore, none of the glyphosate-based products differed from the negative control (Table 1) being classified as harmless (class 1) (Table 3). Differently, 2 DAE, Roundup Original®,

Roundup Ready®, Roundup Transorb®, and Roundup WG® reduced parasitism compared to negative control (Table 1), being classified as slightly harmful (class 2) (Table 3). At 2 DAE, Zapp Qi® was the only tested treatment to be classified as harmless (class 1) (Table 3).

Parasitoid viability by comparison with the negative control (water) was not altered by the application of any of the glyphosate treatments in the daily evaluations at both 1 and 2 DAE (Table 1). In contrast, for the chlorpyrifos insecticide (positive control), there was no parasitism on the days assessed (Table 1).

Side-effects to pupal parasitoid bioassay

Similar to the results reported for adults, the assessed glyphosate-based products did not differ in their effect on parasitoid emergence from sprayed pupae (Table 2). Furthermore, parasitism of generation F_O and viability of generation F_1 glyphosate-based products did not differ among each other or from the control (distilled water), with the exception of Zapp Qi®. In the latter treatment, we recorded the lowest 1 DAE parasitism, although this was not observed on 2 DAE (Table 2).

All glyphosate treatments were classified as harmless (class 1) (Hassan *et al* 1985) at the pupal development stage of the parasitoid (Table 3). However, the positive control (chlorpyrifos) differed from the negative control (distilled water) and the glyphosate treatments. It adversely affected parasitism and emergence of adults (Table 2) and was classified as harmful (class 4) at this stage of development of *T. remus* (Hassan *et al* 1985) (Table 3).

Discussion

In general, glyphosate did not severely impact the egg parasitoid *T. remus*. These results might be due to its mode of action. Glyphosate is a non-selective herbicide that inhibits plant growth through interference with the production of essential aromatic amino acids by inhibition of the enzyme enolpyruvylshikimate phosphate synthase. This enzyme is responsible for the biosynthesis of chorismate, an intermediate in phenylalanine, tyrosine, and tryptophan biosynthesis (Williams *et al* 2000). This pathway for aromatic amino acid biosynthesis is exclusive to plants; therefore, its blockage does not affect members of the animal kingdom, including beneficial arthropods (Williams *et al* 2000).

Despite this exclusive glyphosate action above plants, it is important to point out that commercial glyphosate products contain various other chemicals that may not be specified on the label. One of the reasons is that information regarding the surfactant components of herbicide formulations is often protected as proprietary information of the manufacturer

Table 1 Impact of glyphosate and chlorpyrifos commercial products on parasitism activity and viability of *Telenomus remus* adults (emerged from non-treated pupae) directly exposed to dry residue on inert substrate 1 and 2 days after parasitoid emergence (DAE) ($25 \pm 2°C$, $70 \pm 10\%$ RH, photoperiod of 14:10 h L/D).

Treatment	1 DAE		2 DAE	
	Parasitism (%)[a]	Viability (%)[a]	Parasitism (%)[a]	Viability (%)[a]
Control	74.3 ± 5.3 a	60.3 ± 8.8 ns	81.3 ± 6.4 a	61.4 ± 13.3 ns[b]
Roundup Original®	70.0 ± 5.6 a	79.7 ± 5.1	44.6 ± 1.6 b	80.2 ± 2.7
Roundup Ready®	68.0 ± 8.2 a	60.9 ± 5.4	25.3 ± 4.5 c	52.3 ± 14.2
Roundup Transorb®	74.7 ± 6.0 a	70.4 ± 10.2	42.2 ± 1.8 b	64.6 ± 16.3
Roundup WG®	69.9 ± 6.8 a	65.6 ± 3.3	24.1 ± 1.7 c	57.0 ± 2.0
Zapp Qi®	53.0 ± 9.4 a	61.4 ± 13.1	79.7 ± 3.9 a	58.1 ± 16.5
Lorsban® 480 BR	0.0 ± 0.0 b	–	0.0 ± 0.0 d	–
CV (%)	24.87	28.59	17.56	33.70
F	17.05	0.70	79.52	0.34
df_{total}	33	28	31	21
p	<0.0001	0.6286	<0.0001	0.8816

ns = ANOVA is not significant; – = non-existent parameter.

[a] Means followed by the same letter in columns do not differ significantly (Tukey's HSD test, 5% probability).

[b] Original results followed by statistical analysis of the data transformed to arcsin $\sqrt{X/100}$.

(Chen *et al* 2004). These other components can carry their own toxicity and are reported to trigger harmful side-effects in some egg parasitoids (Nörnberg *et al* 2008). In larval amphibians, glyphosate-based products containing a common surfactant, polyethoxylated tallowamine (POEA), have been classified as moderately to highly toxic in laboratory, mesocosm, and pond enclosure experiments (Chen *et al* 2004). However, chemical side-effects above natural enemies might vary accordingly to the studied biological control species, indicating the importance of studying different parasitoid species, including *T. remus*. Previous results had shown noxious effects of some glyphosate-based herbicides on the egg parasitoid species *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) (Nörnberg *et al* 2008) what was not observed at the same degree of intensity for *T. remus* in our study.

Differences among glyphosate-based products for *T. remus* were observed for adult parasitoids 2 DAE (Table 1) and on parasitism 1 DAE of adults from sprayed pupae (Table 3). Differences for adult parasitoid (Table 1)

Table 2 Impact of glyphosate and chlorpyrifos commercial products on *Telenomus remus* pupae (inside host eggs) and on parasitoid adults emerged from treated pupae 1 and 2 days after emergence (DAE) ($25 \pm 2°C$, $70 \pm 10\%$ RH, photoperiod of 14:10 h L/D).

Treatment	Sprayed pupae Viability (%)[a]	Generation F_0 (1 DAE) Parasitism(%)[a]	Generation F_1 (1 DAE) Viability (%)[a]	Generation F_0 (2 DAE) Parasitism(%)[a]	Generation F_1 (2 DAE) Viability (%)[a]
Control	98.4 ± 0.8 a	88.6 ± 6.0 a	84.8 ± 5.5 ns	72.5 ± 5.3 a	85.8 ± 4.5 ab[b]
Roundup Original®	96.3 ± 1.3 a	86.9 ± 4.6 ab	85.4 ± 4.2	68.0 ± 9.1 a	80.8 ± 4.4 b
Roundup Ready®	98.1 ± 0.7 a	84.5 ± 2.8 ab	82.3 ± 6.0	66.2 ± 6.9 a	97.0 ± 2.2 a
Roundup Transorb®	96.1 ± 0.7 a	87.4 ± 6.5 ab	92.3 ± 2.7	81.7 ± 5.8 a	92.7 ± 2.4 ab
Roundup WG®	96.3 ± 0.9 a	84.6 ± 4.0 ab	95.8 ± 2.7	68.1 ± 5.9 a	95.6 ± 1.4 ab
Zapp Qi®	98.3 ± 0.5 a	66.5 ± 5.1 b	91.3 ± 3.9	80.9 ± 5.2 a	88.0 ± 4.5 ab
Lorsban® 480 BR	0.3 ± 0.2 b	0.0 ± 0.0 c	–	0.0 ± 0.0 b	–
CV (%)	2.06	14.22	10.94	21.54	9.89
F	2274.09	51.65	1.47	22.02	3.64
df_{total}	34	31	29	34	27
p	<0.0001	<0.0001	0.2377	<0.0001	0.0150

Generation F_0 is parasitoids originated from sprayed pupae. Generation F_1 is parasitoids from Generation F_0. – = non-existent parameter.

[a] Means followed by the same letter in columns do not differ significantly (Tukey's HSD test, 5% probability).

[b] Original results followed by statistical analysis of the data transformed to arcsin $\sqrt{X/100}$.

Table 3   Effect (*E*) of each of glyphosate and chlorpyrifos commercial product to Telenomus remus, followed by the classification of the product toxicology (C) according to the standards of the International Organization for Biological Control (IOBC) (25 ± 2°C, 70 ± 10% RH, photoperiod of 14:10 h L/D).

Treatment	Adults				Sprayed pupae		Generation F_0			
	1 DAE		2 DAE				1 DAE		2 DAE	
	E_1%[a]	C[b]	E_1%[a]	C[b]	E_2%[c]	C[b]	E_1%[a]	C[b]	E_1%[a]	C[b]
Roundup Original®	5.8	1	45.2	2	2.1	1	1.9	1	6.2	1
Roundup Ready®	8.5	1	68.9	2	0.3	1	4.6	1	8.8	1
Roundup Transorb®	0	1	48.1	2	2.3	1	1.4	1	0	1
Roundup WG®	6.0	1	70.4	2	2.1	1	4.5	1	6.1	1
Zapp Qi®	28.6	1	2.1	1	0.0	1	25.0	1	0	1
Lorsban® 480 BR	100	4	100	4	99.7	4	100	4	100	4

[a] Effect (*E*) of each pesticide on adults: E_1% = (1 – parasitism of the treatment / parasitism of the control) × 100 (Hassan et al 1985).

[b] Classification: class 1 = harmless ($E < 30$%), class 2 = slightly harmful ($30\% \leq E < 80$), class 3 = moderately harmful ($80\% \leq E < 99$), and class 4 = harmful ($E \geq 99$%).

[c] Effect (*E*) of each pesticide on pupae: E_2% = (1 – viability of pupae sprayed with the treatment / viability of pupae for the control) × 100 (Carmo *et al* 2010a).

are probably due to the fact that, at this stage of development, the insect is more susceptible to pesticides when exposed to contamination by contact when moving on treated surfaces (Hassan 1992, Bacci *et al* 2001). Differently, the pupa is protected inside the chorion of the host egg and is usually reported as more tolerant to chemicals (Hassan 1992) if compared to the adult stage. However, low parasitism 1 DAE of adults emerged from host eggs containing the parasitoid pupae sprayed with Zapp Qi® (generation F_0) (Table 2) might indicate an effect of this commercial product on parasitoid emergence, leading to a delay in the beginning of parasitism by females if compared to other treatments. A delay on parasitism might also be explained by a period of detoxification, when parasitoids used its energy to circumvent any possible chemical damage instead of using energy to search for new eggs. However, the explanation for such effects and validation of these hypotheses still need investigation. It is important to point out that protection offered by the host egg to the parasitoid pupa strongly depends on chorion permeability, which differs among host species (Biondi *et al* 2012) and might be different for each chemical.

Host egg chorion must provide protection against external changes and also have the necessary texture and hardness to be accepted by *T. remus* females. Shape, size, and chorionic structure of host eggs can differ widely among species and are key factors in the host selection process for various parasitoid species (Salt 1938, Schmidt & Smith 1987, Pak *et al* 1990, Schmidt 1994). *Spodoptera frugiperda* eggs have a moderate number of aeropyles located at the intersection between bridges and ridges. Their chorion is 2.50 to 11.95 μm thick, forming a discontinuous mucous layer (Cônsoli *et al* 1999). Differences in thickness of the chorion, particularly the exochorion (the most protein-rich layer), can affect

parasitism (Pak *et al* 1990) as well as oxygen balance with the environment (Hinton 1981), influencing, therefore, the permeability of the host egg after pesticide application. Thus, our data may be different from other host species. These differences might be related to sensitivity of host eggs to external changes which appears to heighten with an increasing number of aeropyle openings and to decrease with an increasing thickness of the mucous layer (Cônsoli *et al* 1999) that can be presented by different host species. Such effects of the relationship between chorion structure and host egg sensitivity on the provision of shelter to parasitoid development have been reported for many species of Lepidoptera (Barbier & Chauvin 1974, Chauvin & Chauvin 1980) and might play an important role in lethal and sublethal effects of pesticide application on immature parasitoid stages. These might explain differences among parasitoid species. However, a difference among chemicals in the same species is probably due to different toxicological properties of each pesticide (van der Werf 1996, Wijnands 1997). Differences in glyphosate-based products include different salts and surfactants used in the formulation of commercial brands. Salt formulations include isopropylamine, diammonium, monoammonium, or potassium as counterions. Also, glyphosate-based herbicides can be formulated with glyphosate either alone or with added cationic surfactants. The most common surfactant is polyethoxylated tallow referred to as polyoxyethyleneamine (POEA). Much of the available information reveals that endued (formulated) glyphosate products are more toxic to aquatic organisms than the glyphosate acid active ingredient alone and the results from the toxicity of the surfactant used in the formulation (Giesy *et al* 2000). It has also been shown that the toxicity of the surfactant varies with temperature, pH,

species, and stage of the animal exposed (Folmar *et al* 1979). In addition to differences in acute toxicity (Mann & Bidwell 1999), several non-ionic surfactants (i.e., nonyl- and octylphenols) may act as endocrine-disrupting compounds (EDCs) and have shown estrogenicity in EDC assays (Routledge & Sumpter 1996).

Small differences in the effect of glyphosate-based products may have remained undetected, especially when applied on pupae, considering that *T. remus* pupae might be better protected than adults and less sensitive to lethal and sublethal effects of pesticides. All glyphosate-based products were therefore classified as harmless (class 1) to the pupal stage of *T. remus*. This higher tolerance of pupae in comparison to adults has already been documented in the literature for other species of egg parasitoids (Giolo *et al* 2005, Nörnberg *et al* 2008), but this is the first time this phenomenon has been documented for *T. remus*. Moreover, the higher tolerance of pupae compared to adult can be sometimes less perceptible when product is harmful as what happened for chlorpyrifos.

In agreement with our findings for *T. remus*, lethal and sublethal effects of different glyphosate-based products on adults of *T. pretiosum* (Hymenoptera: Trichogrammatidae) were dependent on the salt present in the herbicide (Giolo *et al* 2005). Glyphosate-based products based on potassium (Zapp® Qi) and ammonium salts (Roundup® WG) were slightly harmful to *T. pretiosum* adults, causing 30 to 80% reduction in parasitism. The other glyphosate-based products based on isopropylamine salt (Roundup®, Polaris®, Gliz® 480 CS, Glifosato Nortox®, Glifosato 480 Agripec®, and Roundup® Transorb) were less selective (considering the different degrees of selectivity) to this beneficial insect and were classified as moderately harmful to adults of *T. pretiosum*, with 80 to 99% reduction in parasitism (Giolo *et al* 2005).

Chlorpyrifos was the most toxic treatment (classified as harmful) in all assessments and bioassays, as expected for a positive control (Tables 1 and 2). These negative effects of chlorpyrifos on *T. remus* were previously reported by Carmo *et al* (2009). This insecticide killed all parasitoid adults after entering the experimental arenas. When applied on pupae, 100% of the adults died as they attempted to emerge. Our data corroborate previous reports describing this pesticide as incompatible with the use of *T. remus* in biological control, whether in the adult or pupal stage. In general, neurotoxic compounds are less selective to natural enemies than others, like insect growth regulators (IGRs), for example. However, it is important to point out that the side-effects of IGR to natural enemies have been also reported in characteristics of the biology of many species with higher sensitivity in early stages of development (Cônsoli *et al* 2001). Insecticides that do not affect the survivorship of pre-imaginal stages of the parasitoid when the host eggs are treated could still reduce the capacity of parasitism of the adult parasitoid emerged (Cônsoli *et al* 1998). That is one of the reasons why

insecticides must always be rationally used in agriculture according to the IPM guidance. In this context, it is helpful to explain that chlorpyrifos acts in the nervous system of the insects. Since the functioning of the nervous system and the enzymes involved in nerve pulse transmission processes is very similar to all arthropods, this insecticide is harmful to all insect species, whether pests or natural enemies. Therefore, the lack of selectivity of chlorpyrifos can be obviously explained by its mode of action on the nervous system of insects (Yu 1987).

It is important to take into consideration that when choosing an herbicide for crop treatment, glyphosate-based herbicides that are least harmful to natural enemies should be given priority. Therefore, bearing in mind the importance of using selective chemicals with low impact on beneficial arthropods, we can conclude that all tested glyphosate-based products can be used in agriculture without negative impact to *T. remus* since they were not classified as harmful or moderately harmful to when parasitoid was exposed at the pupal or adult stages even in the most extreme exposure assays under laboratory-controlled conditions.

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