

# Physiological selectivity of insecticides to the egg parasitoids *Telenomus podisi* and *Trissolcus teretis* (Hymenoptera: Scelionidae)

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## Research Article

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# Abstract

*Telenomus podisi* and *Trissolcus teretis* are important parasitoids of stink bug eggs and their preservation contributes to the ecological management of these pests. This work aimed to evaluate the physiological selectivity of the newest insecticides ethiprole and sulfoxaflor + lambda-cyhalothrin in comparison with thiamethoxam + lambda-cyhalothrin and chlorpyrifos, used to control stink bugs, for *T. podisi* and *T. teretis*. Bioassays were carried out in the laboratory following standardized protocols. Six independent bioassays were conducted to evaluate the effect of insecticides on the pupal and adult stages of the parasitoids and the effects of insecticide sprays over the host eggs before parasitism. The insecticides were applied using a Potter tower. Ethiprole at rates of 150 and 200 g/150L H<sub>2</sub>O was classified as harmless (class 1), according to the International Organization of Biological Control to both pupae and adults of *T. podisi*. When tested against *T. teretis*, ethiprole was classified as harmless (class 1) and slightly harmful (class 2), but it still was the most selectivity pesticide among studied chemicals. When adult parasitoids of both species were exposed to sprayed host eggs, wasps had similar parasitism. The other treatments triggered more severe negative side effects to the parasitoids, especially to adults. Overall, ethiprole was the least toxic compound and should be preferred in integrated pest management aimed at preserving these biocontrol agents, while the other tested insecticides should be evaluated under semi-field and field conditions to confirm their higher toxicity and be replaced for more selectivity pesticide whenever possible.

## Introduction

Stink bugs are among the most important pests of soybean (*Glycine max*) and maize (*Zea mays*), especially in South America, where they are responsible for significant yield loss when not properly managed (Gomes et al. 2020; Bueno et al. 2021). Among the different species that feeds on soybean and maize, *Euschistus heros* (Fabricius, 1794) (Hemiptera: Pentatomidae), is the most abundant in South America, mainly at latitudes between 0° and 23° (Panizzi and Corrêa-Ferreira 1997). Not only are these pests noteworthy for feeding directly from soybean pods, seriously affecting yields besides physiological and sanitary quality of the seeds but also injuring maize development when feeding on plant seedlings (Corrêa-Ferreira and Azevedo 2002; Gomes et al. 2020). In order to mitigate losses caused by those pests and consequently increase profits, growers control these phytophagous arthropods (Zalucki et al. 2009). Currently, the primary method of stink bug control adopted by growers is the use of chemical insecticides, often applied erroneously and excessively (Song and Swinton 2009; Panizzi 2013; Bueno et al. 2021).

The overuse of insecticides, especially the non-selective ones, has triggered several important adverse side-effects (Torres and Bueno 2018). Not only can the abusive use of synthetic chemicals lead to a reduced activity of biological control agents (Torres and Bueno 2018) but also to pest resurgence and occurrence of secondary pests (Bueno et al. 2021) besides selection for pest resistance (Sosa-Gomez et al. 2001; Sosa-Gómez and Silva 2010). Therefore, a more sustainable stink bug management is of major interest. Among the most eco-friendly and sustainable pest management tools available, augmentative

biological control stands out (Cock et al. 2010), being historically applied on more than 30 million ha worldwide (van Lenteren et al. 2018).

Among the different possibilities of biological control agents, egg parasitoids have been widely used in augmentative biological control and can be considered the most important stink bug biocontrol agents (Koppel et al. 2009; Laumann et al. 2010). Among the different species of egg parasitoids that can be used in augmentative biological control of stink bugs, *Telenomus podisi* Ashmead, 1893 (Hymenoptera: Scelionidae) is noteworthy due to its high parasitism and control efficacy against its hosts (Peres and Corrêa-Ferreira 2004; Queiroz et al. 2018; Silva et al. 2018). Not only *T. podisi* but also *Trissolcus teretis* (Johnson) (Hymenoptera: Scelionidae) are solitary egg parasitoids that limit the numerical increase of stink bugs in the Neotropical region (Medeiros et al. 1998). *Telenomus podisi* is the predominant egg parasitoid of species of the genus in different cropping systems (Tillman 2011) while there still is a huge lack of studies on *T. teretis*. Nevertheless, *T. teretis* is usually found in eggs of stink bugs in Central Brazil (Medeiros et al. 1998; Laumann et al. 2010).

Despite such importance of those biocontrol agents for stink bug control, neither chemical nor biological control acting alone can adequately address pest problems in multi-pest crop ecosystems or against some highly damaging pest species, in highly profitable crops such as the example of stink bug in soybean and maize crops (Torres and Bueno 2018). Thus, selective pesticides are of great value for crop management. A significant advantage of selective products is their effectiveness against target pests with minimal side-effects on natural enemies (Broadbent and Pree 1984; Bacci et al. 2007; Torres and Bueno 2018). Consequently, knowledge of the effects of chemicals, commonly used on soybean and maize crops, on egg parasitoids is extremely important. In this context, in the present study we evaluated possible side-effects of different insecticides frequently sprayed on soybean and corn to control stink bugs on the egg parasitoids *T. podisi* and *T. teretis*, aiming to determine the most selective chemicals to be preferably used in integrated pest management (IPM) programs whenever necessary.

## Material And Methods

Six bioassays were conducted to assess the side-effects of different insecticides (Table 1) on pupae and adults of *T. podisi* and *T. teretis*, as well on their parasitism capacity on treated host eggs. Trials were carried at  $25 \pm 2^{\circ}\text{C}$ ;  $70 \pm 10\%$  RH, and photoperiod of 14:10 h (L:D), with five replicates in a completely randomized design, in accordance with the protocols proposed by the “International Organization for Biological Control” (IOBC) (Hassan 1992; Hassan et al. 1995; Manzoni et al. 2007).

Table 1

Description of treatments (commercial products and doses) evaluated for selectivity to the egg parasitoids *Telenomus podisi* and *Trissolcus teretis* under controlled laboratory conditions.

Commercial product	Formulation	Active ingredient (a.i.)	(g) a.i./150 L H <sub>2</sub> O
Water (control)	-	distilled water	-
Curbix®	200 SC	ethiprole	150
Curbix®	200 SC	ethiprole	200
Expedition®	100/150 SE	sulphoxaflor + lambda-cyhalothrin	20 + 30
Expedition®	100/150 SE	sulphoxaflor + lambda-cyhalothrin	30 + 45
Engeo Pleno S®	141/106 SC	thiamethoxam + lambda-cyhalothrin	28.2 + 21.2
Engeo Pleno S®	141/106 SC	thiamethoxam + lambda-cyhalothrin	35.3 + 26.5
Lorsban®	480 EC	chlorpyrifos	960

## Parasitoids and host colonies

*Telenomus podisi* were first collected from soybean fields (*E. heros* parasitized eggs) at the Embrapa Soybean Field Station (23° 11' 11.7" S and 51° 10' 46.1" W) during summer of 2015, sent to taxonomist and identified as *T. podisi*. *Trissolcus teretis* was originally collected in Brasilia, DF, Brazil, and grown at the parasitoid rearing facilities of Embrapa Cenargen from where some specimens were transferred to Embrapa Soybean, Londrina, PR, Brazil during 2017 when it was also sent to taxonomist and identified as *T. teretis*. Voucher specimens of *T. podisi* from IBCBE 003272 to IBCBE 003333 and voucher specimens of *T. teretis* from IBCBE 003334 to IBCBE 003425 were deposited at the "Coleção de Insetos Entomófagos Oscar Monte", Instituto Biológico de Campinas, Campinas, São Paulo, Brazil. After field collection, both parasitoids were kept in climatic chambers (ELETROLab®, model EL 212, São Paulo, SP, Brazil) regulated at 25 ± 2°C, RH 70 ± 10% and photoperiod of 14:10 h (L:D). Parasitoids were reared according to methodologies previously described by Peres and Corrêa-Ferreira (2004) and briefly summarized in the following.

Both *T. podisi* and *T. teretis* have been reared on *E. heros* eggs. Frozen eggs after being taken from nitrogen - 196°C were glued to pieces of card (5 cm × 8 cm). Those host eggs were introduced into plastic cages (8.5 cm high and 7 cm in diameter) (Plasvale Ltda., Gaspar, State of Santa Catarina, Brazil) together with eggs already parasitized and with imminent parasitoid emergence. Small drops of pure *Apis mellifera*-produced honey were placed inside these cages to provide food for the emerging adults. The cages were then closed, and parasitism allowed for 24 h. After that, the emerged adults were used for trials as well as for colonies maintenance.

Sting bugs were collected from soybean fields at the Embrapa Soybean Field Station (23° 11' 11.7" S and 51° 10' 46.1" W) and kept in the laboratory for approximately four years accordingly to methodology

previously described by Panizzi et al. (2000). New field insects have been introduced each year to maintain insect colony quality. The insects were kept in plastic screen cages (20 cm × 20 cm sides × 24 cm tall) (Plasvale Ltda., Gaspar, State of Santa Catarina, Brazil) lined with filter paper and fed *ad libitum* with a mixture of beans (*Phaseolus vulgaris* L.; Fabaceae), soybeans (*Glycine max* L. Merr.; Fabaceae), peanuts (*Arachis hypogaea* L.; Fabaceae), sunflower seeds (*Helianthus annuus* L.; Asteraceae) and privet fruits (*Ligustrum lucidum* Aiton; Oleaceae). A Petri dish (diameter 9 cm) with a cotton wad soaked in distilled water was added to each cage. Cages were cleaned, food replaced, and egg masses collected on a daily basis. After collection, egg masses were transferred to acrylic boxes (11 cm × 11 cm × 3.5 cm) lined with filter paper moistened with water. After eclosion, second instar nymphs were transferred to new cages identical to those previously described. The eggs were collected daily and used for colony maintenance or stored in liquid nitrogen (-196°C) (Silva et al 2008) for up to six months, during which time they maintain their quality for parasitism (Favetti et al 2014) prior to their use in the experiments.

Impact of *T. podisi* (bioassay 1) and *T. teretis* (bioassay 2) pupae exposure to the spray of different insecticides

The selectivity of different insecticides (Table 1) for *T. podisi* and *T. teretis* pupae was tested separately for each parasitoid species using the same methodology according to the standard protocols established by the “International Organization for Biological Control” - IOBC (Hassan 1992, Hassan et al 1995, Manzoni et al. 2007) modified by Carmo et al (2010). Cards measuring 3 cm<sup>2</sup> (1 card per replicate) containing approximately 100 host eggs from 0–24 h were exposed to newly emerged parasitoid females (24-48-h old). Parasitism was allowed for 24 h. Then, the cards were transferred to plastic cages (8.5 cm high and 7 cm in diameter) (Plasvale Ltda., Gaspar, State of Santa Catarina, Brazil) and kept until pupation, which is complete approximately 216 to 240 h after parasitism (Foerster et al 2004). Then, parasitoid pupae were submitted to insecticides (Table 1) sprays according to the methodology used by Carmo et al (2010) with five replications (Cards measuring 3 cm<sup>2</sup> (1 card per replicate) containing approximately 100 eggs with the pupal stage of the parasitoid) for each treatment. Spraying was performed using a Potter Spray Tower calibrated to deposit a volume of 1.25 ± 0.25 mg.cm<sup>-2</sup> according to established IOBC protocols (Hassan 1992, Hassan et al 1995). The cards with the treated host eggs, which was containing the parasitoid pupae, were left to completely dry in the room for about two hours to remove excess moisture. Then, they were placed in cages described by Hassan (1992) until the emergence of the adults, when they were fed with honey.

After adult emergence, new cards containing approximately 100 *E. heros* eggs were introduced into the cages [one card on the first day (1 DAE) and a second one on the third day after parasitoid emergence (3 DAE)]. A drop of honey was provided to the parasitoids at 1 DAE and 3 DAE. The cards remained in the cages until the fifth day after parasitoid emergence, when they were removed and stored in plastic bags distributed in a climatic chamber (ELETROLab®, model EL 212, São Paulo, SP, Brazil) at 25°C ± 2°C, 70% ± 10% RH and photoperiod of 14:10 h (L:D) to evaluate parasitism and parasitoid emergence. The emergence of parasitoid adults from sprayed eggs was calculated by dividing the number of *E. heros* eggs with emergence hole by the total number of parasitized eggs multiplied by 100.

Impact of *T. podisi* (bioassay 3) and *T. teretis* (bioassay 4) adult exposure to the dry residue of different insecticides

Approximately 100 eggs of *E. heros* were glued on cardboard cards. These cards were then offered to freshly emerged *T. podisi* or *T. teretis* for oviposition for 24 h. After that, the parasitized *E. heros* eggs were placed into Duran® tubes (emergence vials, 0.6 cm diameter × 6 cm height) containing a droplet of honey. The Duran® tubes were then sealed with a plastic film and stored in a climatic chamber (ELETROLab®, model EL 212, São Paulo, SP, Brazil) at 25°C ± 2°C, 70% ± 10% RH, and photoperiod of 14:10 h (L:D) until parasitoid emergence. Potter Spray Tower was used to spray a volume of insecticide solution of 1.25 ± 0.25 mg.cm<sup>-2</sup> on the glass plates (13 x 13 cm) used to mount the cages and expose the parasitoids (Hassan 1992, Hassan et al 1995). After spraying, the plates were kept in ambient conditions for 2 h for drying, after which they were fixed in aluminum frames to form the exposure cage, where a circulating air flow allowed the elimination of possible toxic gases, according to the methodology described by Hassan (1992). Then, the tubes containing adults of the parasitoids were covered with aluminum foil and connected to holes in the cages for the introduction of insects, according to the methodology used by Carmo et al (2010). One and three days after exposure of the parasitoids to the dried residues of the products on the glass plates, cards (1 x 2 cm) containing about 100 *E. heros* eggs and honey droplets were introduced into the cages. The cards containing supposedly parasitized host eggs were removed on the fifth day of exposure, placed in transparent plastic bags and stored in an air-conditioned chamber at 25°C ± 2°C, 70% ± 10% RH and a photoperiod of 14:10 h (L:D). The design was completely randomized with 5 replications (cages). The number of parasitized eggs and the number of insects emerged in each treatment were evaluated.

Impact of host egg exposure to insecticides on parasitism by *T. podisi* (bioassay 5) and *T. teretis* (bioassay 6) (no-choice test)

The design was completely randomized with five replicates containing five females of *T. podisi* or *T. teretis* (24-48h old) each, individualized in glass tubes (75 mm high x 12 mm in diameter), totaling 25 females per treatment). These females were offered cards containing approximately 50 viable eggs (24 hours) of *E. heros*, sprayed with insecticides (Table 1) with a Potter tower. A droplet of honey was inserted into the wall of the glass tube, to serve as food for the females. After that, the glass tubes were stored in climatic chambers. Parasitism was allowed for 24 hours. Then, the number of dead females was counted and alive females were discarded. Cards containing the parasitized eggs were transferred to new tubes, placed in climatic chambers until the emergence of the parasitoids to evaluate the parasitism and parasitoid emergence (progeny viability %).

## Statistical analysis

Data obtained from all the six different bioassays were subjected to exploratory analysis to evaluate normality assumptions for the residuals (Shapiro & Wilk 1965), homogeneity of variance between treatments (Burr and Foster 1972) and additivity of the model in order to be subjected to analysis of variance (ANOVA). Data not following normality assumptions or homogeneity of variance were

transformed. Means were compared using Tukey`s HSD test (5% error probability) implemented in SAS (SAS Institute 2001). Furthermore, the insecticides effects on *T. podisi* and *T. teretis* as related to distilled water (used as control treatment) was computed by the equations: EP (Effects on pupae %) =  $(1 - \text{adult emergence observed for the tested treatment} / \text{adult emergence observed for the control treatment}) \times 100$ ; and E(Effects on adults %) =  $(1 - \text{parasitism observed for the tested treatment} / \text{parasitism observed for the control treatment}) \times 100$ . Then, the chemicals were classified according to the IOBC standards as follows: class 1, harmless (EP or E < 30%); class 2, slightly harmful ( $30\% \leq \text{EP or E} \leq 79\%$ ); class 3, moderately harmful ( $80\% \leq \text{EP or E} \leq 99\%$ ); and class 4, harmful (EP or E > 99%) (Hassan 1992).

## Results

Impact of *T. podisi* (bioassay 1) and *T. teretis* (bioassay 2) pupae exposure to the spray of different insecticides

Among the different tested insecticides (Table 1), the mildest side-effects on both *T. podisi* and *T. teretis* pupae were exerted by ethiprole, especially in the lowest studied rate of 150 g/150 L H<sub>2</sub>O, but also in the highest rate of 200 g/150 L H<sub>2</sub>O when compared to the other tested treatments. When eggs of *E. heros* containing *T. podisi* pupae inside, close to parasitoid emergence (13 days after egg parasitism), were sprayed with different insecticides, ethiprole 150 and 200 g/150 L H<sub>2</sub>O allowed the highest adult parasitoid emergence, statistically similar to the control (water), which varied from 33.7 to 48.4%. *Telenomus podisi* pupae sprayed with water (control) had 40.0% adult emergence. Moreover, parasitism capacity of *T. podisi* emerged from those treated pupae with ethiprole was equal to those parasitoids emerged from the control (water) considering both 1 and 3 days after parasitoid emergence (DAE) (Table 2). Consequently, ethiprole 150 and 200 g/150 L H<sub>2</sub>O was classified as harmless (class 1) when applied over *T. podisi* pupae in *E. heros* eggs (Table 3) not only due to the lack of impact above adult parasitoid emergence compared to pupae spray with water (control), but also due to the lack of any impact above parasitism capacity of adults which emerged from treated pupae. No sublethal effect was observed on parasitism or parasitoid emergence of this second parasitoid generation (progeny viability) (Table 2).

Table 2

Effects of exposure of parasitized host eggs at parasitoid pupal stage to insecticides on *Telenomus podisi* and *Trissolcus teretis* emergence (%) and on the parasitism (%) and progeny survival (%) of adults emerged from exposed eggs at one and three days after emergence (DAE).

Treatment (grams/150 L H <sub>2</sub> O)	Sprayed pupae	1 DAE		3 DAE	
	Adult emergence (%)	Parasitism (%)	Progeny viability (%)	Parasitism (%)	Progeny viability (%)
<b>Telenomus podisi (bioassay 1)</b>					
Water	40.0 ± 8.2 ab <sup>a</sup>	52.5 ± 5.8 a	76.8 ± 2.6 a	55.6 ± 6.3 a	74.7 ± 1.5 ab
Ethiprole 150	33.7 ± 8.4 abc	55.3 ± 4.6 a	72.9 ± 4.5 a	51.2 ± 6.3 a	81.1 ± 2.2 a
Ethiprole 200	48.4 ± 6.7 a	54.6 ± 5.2 a	80.3 ± 3.6 a	56.0 ± 4.7 a	75.9 ± 2.7 ab
Sulphoxaflor 20 + lambda-cyhalothrin 30	4.4 ± 2.1 d	39.2 ± 9.9 a	87.7 ± 4.4 a	34.9 ± 10.0 a	79.0 ± 4.4 a
Sulphoxaflor 30 + lambda-cyhalothrin 45	4.9 ± 1.6 d	34.4 ± 5.8 a	81.7 ± 5.9 a	38.4 ± 8.6 a	80.2 ± 4.1 a
Thiamethoxam 28.2 + lambda-cyhalothrin 21.2	14.6 ± 3.1 cd	56.4 ± 6.0 a	74.1 ± 4.6 a	61.5 ± 6.0 a	79.6 ± 2.4 a
Thiamethoxam 35.3 + lambda-cyhalothrin 26.5	15.3 ± 6.1 cd	42.2 ± 8.8 a	67.7 ± 4.1 a	52.9 ± 7.5 a	76.5 ± 0.7 ab
Chlorpyrifos 960	11.3 ± 2.1 cd	39.4 ± 3.9 a	76.7 ± 5.9 a	38.9 ± 11.2 a	56.8 ± 12.2 b
F	9.42	2.15	1.70	1.55	2.80
P	< 0.0001	0.0688	0.1466	0.1862	0.0228
<b>Trissolcus teretis (bioassay 2)</b>					
Water	23.0 ± 3.3 ab	47.6 ± 3.0 a <sup>a</sup>	59.7 ± 2.6 a	27.6 ± 4.3 a <sup>a</sup>	42.1 ± 7.7 a
Ethiprole 150	25.3 ± 1.4 ab	25.3 ± 5.3 b	48.9 ± 4.5 a	15.6 ± 1.8 a	49.7 ± 4.6 a
Ethiprole 200	13.0 ± 2.5 cd	10.5 ± 4.3 c	52.2 ± 3.6 a	3.5 ± 1.7 b	58.9 ± 14.5 a
Sulphoxaflor 20 + lambda-cyhalothrin 30	23.4 ± 2.0 abc	0.0 ± 0.0 d	No parasitism	0.0 ± 0.0 b	No parasitism
Sulphoxaflor 30 + lambda-cyhalothrin 45	1.5 ± 1.2 d	0.0 ± 0.0 d	No parasitism	0.0 ± 0.0 b	No parasitism

Treatment (grams/150 L H <sub>2</sub> O)	Sprayed pupae	1 DAE		3 DAE	
	Adult emergence (%)	Parasitism (%)	Progeny viability (%)	Parasitism (%)	Progeny viability (%)
Thiamethoxam 28.2 + lambda-cyhalothrin 21.2	32.6 ± 2.0 a	0.0 ± 0.0 d	No parasitism	0.0 ± 0.0 b	No parasitism
Thiamethoxam 35.3 + lambda-cyhalothrin 26.5	19.9 ± 3.0 bc	0.0 ± 0.0 d	No parasitism	0.0 ± 0.0 b	No parasitism
Chlorpyrifos 960	20.3 ± 3.2 bc	0.0 ± 0.0 d	No parasitism	0.0 ± 0.0 b	No parasitism
F	13.79	56.16	2.44	47.53	0.97
P	< 0.0001	< 0.0001	0.0771	< 0.0001	0.4440

Means ± Standard Error (SE) in each column for each parasitoid species followed by the same letter did not differ from each other according to the Tukey test (5% probability). <sup>a</sup>Original data followed by statistics done on data transformed into arcsin  $\sqrt{X / 100}$  as request to perform ANOVA according to Burr & Foster (1972).

Table 3

Classification of insecticide selectivity to *Telenomus podisi* and *Trissolcus teretis* according to the "International Organisation for Biological Control" (IOBC) in different bioassays and days after emergence (DAE) of adults or days after spraying (DAS).

Treatment (grams/150 L H <sub>2</sub> O)	Bioassays with pupae						Bioassays with adults			
	Sprayed pupae		1 DAE		3 DAE		1 DAS		3 DAS	
	EP <sup>a</sup>	C <sup>b</sup>	E <sup>c</sup>	C	E	C	E	C	E	C
	Telenomus podisi (bioassay 1)						Telenomus podisi (bioassay 3)			
Ethiprole 150	15.8	1	0.0	1	8.0	1	13.0	1	17.5	1
Ethiprole 200	0.0	1	0.0	1	0.0	1	6.2	1	16.0	1
Sulphoxaflor 20 + lambda-cyhalothrin 30	88.9	3	25.2	1	37.2	2	89.8	3	83.9	3
Sulphoxaflor 30 + lambda-cyhalothrin 45	87.7	3	34.3	2	30.9	2	86.2	3	100	4
Thiamethoxam 28.2 + lambda-cyhalothrin 21.2	63.6	2	0.0	1	0.0	1	93.9	3	100	4
Thiamethoxam 35.3 + lambda-cyhalothrin 26.5	61.6	2	19.6	1	4.9	1	100	4	100	4
Chlorpyrifos 960	71.6	2	24.9	1	30.1	2	100	4	100	4
	Trissolcus teretis (bioassay 2)						Trissolcus teretis (bioassay 4)			
Ethiprole 150	0.0	1	46.8	2	43.2	2	46.1	2	50.6	2
Ethiprole 200	43.1	2	77.8	2	87.1	3	69.1	2	80.3	3
Sulphoxaflor 20 + lambda-cyhalothrin 30	0.0	1	100	4	100	4	100	4	100	4
Sulphoxaflor 30 + lambda-cyhalothrin 45	93.2	3	100	4	100	4	99.1	4	98.3	3
Thiamethoxam 28.2 + lambda-cyhalothrin 21.2	0.0	1	100	4	100	4	100	4	100	4

<sup>a</sup>EP (Effects on pupae %) = (1 – adult emergence observed for the tested treatment/ adult emergence observed for the control treatment) × 100; <sup>b</sup>Classes: 1 = harmless (EP or E < 30%), 2 = slightly harmful (30 ≤ EP or E ≤ 79%), 3 = moderately harmful (80 ≤ EP or E ≤ 99%), 4 = harmful (EP or E > 99%); <sup>c</sup>E (Effects on adults %) = (1 – parasitism observed for the tested treatment/ parasitism observed for the control treatment) × 100.

Treatment (grams/150 L H <sub>2</sub> O)	Bioassays with pupae						Bioassays with adults			
	Sprayed pupae		1 DAE		3 DAE		1 DAS		3 DAS	
	EP <sup>a</sup>	C <sup>b</sup>	E <sup>c</sup>	C	E	C	E	C	E	C
Thiamethoxam 35.3 + lambda-cyhalothrin 26.5	13.3	1	100	4	100	4	98,3	3	86.0	3
Chlorpyrifos 960	11.7	1	100	4	100	4	100	4	100	4

<sup>a</sup>EP (Effects on pupae %) = (1 – adult emergence observed for the tested treatment/ adult emergence observed for the control treatment)×100; <sup>b</sup>Classes: 1 = harmless (EP or E < 30%), 2 = slightly harmful (30 ≤ EP or E ≤ 79%), 3 = moderately harmful (80 ≤ EP or E ≤ 99%), 4 = harmful (EP or E > 99%); <sup>c</sup>E(Effects on adults %) = (1 – parasitism observed for the tested treatment/parasitism observed for the control treatment)×100.

Similar results for ethiprole were observed when the insecticide was sprayed over *T. teretis* pupae, but only with the lowest rate of 150 g/150 L H<sub>2</sub>O (Tables 2 and 3). *Trissolcus teretis* pupae sprayed with ethiprole 150 g/150 L H<sub>2</sub>O had 25.3% of adult emergence, statistically similar to the emergence of 23% recorded for *T. teretis* pupae sprayed with water (control). Despite similar adult emergence, *T. teretis* which emerged from sprayed pupae with ethiprole had lower parasitism (25.3%) 1 DAE compared to the control (47.6%). Nevertheless, parasitism at 3 DAE was similar between adults emerged from treated pupae with ethiprole (15.6%) and control (27.6%) (Table 2). Therefore, ethiprole 150 g/150 L H<sub>2</sub>O was classified as harmless (class 1) and slightly harmful (class 2) when sprayed over *E. heros* containing *T. teretis* pupae inside, close to parasitoid emergence (13 days after egg parasitism), when taking parasitoid emergence and parasitism capacity of emerged adults into consideration, respectively (Table 3). Differently, ethiprole 200 g/150 L H<sub>2</sub>O reduced adult emergence from sprayed pupae compared to both water (control) and even ethiprole at lower rate (150 g/150 L H<sub>2</sub>O). Moreover, ethiprole 200 g/150 L H<sub>2</sub>O also impact parasitism of emerged adults from treated pupae, which was statistically inferior to water and ethiprole at lower rate (150 g/150 L H<sub>2</sub>O) both 1 and 2 DAE (Table 2). Thus, ethiprole 200 g/150 L H<sub>2</sub>O was classified as slightly harmful (class 2) and moderately harmful (class 3) when sprayed over pupae of *T. teretis* in eggs of *E. heros* considering adult emergence of sprayed pupae and parasitism capacity of adults which emerged from those sprayed pupae both 1 and 3 DAE (Table 3).

Both tested rates of thiamethoxam + lambda-cyhalothrin (28.2 + 21.2 and 35.3 + 26.5 g/150 L H<sub>2</sub>O) exhibit a stronger side-effect on both *T. podisi* and *T. teretis* when sprayed over the parasitoid pupae close to emergence (13 days after egg parasitism) compared to ethiprole (Tables 2 and 3). Side-effects included a significant reduction of adult emergence of *T. podisi* from sprayed pupae compared to the control, which did not trigger the expected reduction in parasitism of those parasitoid 1 and 3 DAE. Those

results were slightly different when the experiments were carried out with *T. teretis*. Thiamethoxam + lambda-cyhalothrin did not trigger the same reduction in *T. teretis* emergence from sprayed pupae compared to control, however, those parasitoids which emerged from treated pupae did not have parasitism (Table 2). Therefore, thiamethoxam + lambda-cyhalothrin (28.2 + 21.2 and 35.3 + 26.5 g/150 L H<sub>2</sub>O) was classified as slightly harmful (class 2) and harmless (class 1) for *T. podisi* and classified as harmless (class 1) and harmful (class 4) for *T. teretis* when sprayed over pupae (13 days after egg parasitism) taking adult emergence and parasitism capacity of emerged adults into consideration, respectively (Table 3).

Chlorpyrifos 960 g/150 L H<sub>2</sub>O had very similar results to thiamethoxam + lambda-cyhalothrin when it was sprayed over *T. podisi* and *T. teretis* pupae (Tables 2 and 3). Chlorpyrifos triggered a significant reduction of *T. podisi* emergence from sprayed pupae but not enough to reduce parasitism capacity of those emerged adults 1 or 3 DAE. Regarding *T. teretis*, adult emergence was not reduced but no parasitism of emerged adults was recorded (Table 2). Thus, chlorpyrifos 960 g/150 L H<sub>2</sub>O received the same classification of thiamethoxam + lambda-cyhalothrin (28.2 + 21.2 and 35.3 + 26.5 g/150 L H<sub>2</sub>O). It was also classified as slightly harmful (class 2) and harmless (class 1) for *T. podisi* and classified as harmless (class 1) and harmful (class 4) for *T. teretis* when sprayed over pupae (13 days after egg parasitism) taking adult emergence and parasitism capacity of emerged adults into consideration, respectively (Table 3).

Sulphoxaflor + lambda-cyhalothrin (20 + 30 and 30 + 45 g/150 L H<sub>2</sub>O) triggered a strongest reduction in *T. podisi* and *T. teretis* emergence from sprayed pupae compared to the other tested treatments. However, for *T. podisi* it was not enough to reduce parasitism capacity of those emerged adults while for no parasitism was recorded for *T. teretis* emerged from sprayed pupae (Table 2). Therefore, despite some results variation, on general sulphoxaflor + lambda-cyhalothrin was classified as moderately harmful (class 3) and slightly harmful (class 2) for *T. podisi* and classified as moderately harmful (class 3) and harmful (class 4) for *T. teretis* when sprayed over pupae (13 days after egg parasitism) taking adult emergence and parasitism capacity of emerged adults into consideration, respectively (Table 3).

Impact of *T. podisi* (bioassay 3) and *T. teretis* (bioassay 4) adult exposure to the dry residue of different insecticides

Parasitism and progeny viability of *T. podisi* and *T. teretis* on *E. heros* eggs were evaluated on the first and third days after the emergence of adults (DAE) exposed to different treatments (Table 1) by walking on dry residue of the sprayed surface. Similar to previous bioassays carried out with pupae, ethiprole at both tested rates (150 and 200 g/150 L H<sub>2</sub>O) exhibited lower impact above *T. podisi* and *T. teretis* adults when compared to the other tested insecticides (Tables 3 and 4). *Telenomus podisi* adults which had contact with ethiprole had similar parasitism capacity to the control (parasitoids which had contact with water). Not only have the parasitoids treated with this insecticide the same parasitism capacity to the control but also the same progeny viability, indicating no sublethal effect from this chemical (Table 4). Therefore, ethiprole (150 and 200 g/150 L H<sub>2</sub>O) was classified as harmless (class 1) to adults of *T. podisi*

accordingly to IOBC protocols (Hassan 1992) (Table 3). For adults of *T. teretis*, ethiprole reduced paratism, especially at the higher rate of 200 g/150 L H<sub>2</sub>O (Table 3). Therefore, this insecticide was classified as slightly harmful (class 2) for adults at the lower tested rate (150 g/150 L H<sub>2</sub>O), which varied from slightly harmful (class 2) to moderately harmful (class 3) for the insecticide at the higher rate (200 g/150 L H<sub>2</sub>O) at 1 and 3 DAE, respectively (Table 4).

Table 4

Effects of different insecticides on adults of *Telenomus podisi* and *Trissolcus teretis* one and three days after the emergence (DAE) from treated eggs of the host *Euschistus heros*.

Treatment (grams/150 L H <sub>2</sub> O)	1 DAE		3 DAE	
	Parasitism (%)	Progeny viability (%)	Parasitism (%)	Progeny viability (%)
<b>Telenomus podisi (bioassay 3)</b>				
Water	59.5 ± 2.9 a	64.2 ± 7.0 a	58.6 ± 3.5 a	80.6 ± 5.0 a
Ethiprole 150	51.8 ± 5.2 a	67.8 ± 3.5 a	48.3 ± 6.5 a	69.3 ± 7.6 a
Ethiprole 200	55.8 ± 3.0 a	68.0 ± 3.1 a	49.2 ± 10.8 a	68.4 ± 5.3 a
Sulphoxaflor 20 + lambda-cyhalothrin 30	6.0 ± 2.5 b	62.0 ± 9.9 a	9.5 ± 3.2 b	56.8 ± 6.8 a
Sulphoxaflor 30 + lambda-cyhalothrin 45	8.2 ± 4.2 b	54.1 ± 4.2 a	0.0 ± 0.0 b	No parasitism
Thiamethoxam 28.2 + lambda-cyhalothrin 21.2	3.6 ± 2.3 b	56.2 ± 6.2 a	0.0 ± 0.0 b	No parasitism
Thiamethoxam 35.3 + lambda-cyhalothrin 26.5	0.0 ± 0.0 b	No parasitism	0.0 ± 0.0 b	No parasitism
Chlorpyrifos 960	0.0 ± 0.0 b	No parasitism	0.0 ± 0.0 b	No parasitism
F	90.85	1.88	30.73	
P	< 0.0001	0.1475	< 0.0001	
<b>Trissolcus teretis (bioassay 4)</b>				
Water	24.2 ± 6.2 a <sup>a</sup>	40.8 ± 11.6 a <sup>a</sup>	21.3 ± 2.6 a	38.9 ± 4.8 ab
Ethiprole 150	13.0 ± 4.2 ab	40.2 ± 11.6 a	10.5 ± 1.3 b	38.1 ± 5.6 ab
Ethiprole 200	7.5 ± 1.1 b	47.4 ± 7.2 a	4.1 ± 1.4 c	65.0 ± 3.8 a
Sulphoxaflor 20 + lambda-cyhalothrin 30	0.0 ± 0.0 c	No parasitism	0.0 ± 0.0 e	No parasitism
Sulphoxaflor 30 + lambda-cyhalothrin 45	0.2 ± 0.2 c	50.0 ± 0.0 a	0.4 ± 0.2 de	50.0 ± 0.0 a
Thiamethoxam 28.2 + lambda-cyhalothrin 21.2	0.0 ± 0.0 c	No parasitism	0.0 ± 0.0 e	No parasitism

Treatment (grams/150 L H <sub>2</sub> O)	1 DAE		3 DAE	
	Parasitism (%)	Progeny viability (%)	Parasitism (%)	Progeny viability (%)
Thiamethoxam 35.3 + lambda-cyhalothrin 26.5	0.4 ± 0.4 c	50.0 ± 0.0 a	3.0 ± 1.0 cd	14.3 ± 0.0 b
Chlorpyrifos 960	0.0 ± 0.0 c	No parasitism	0.0 ± 0.0 e	No parasitism
F	20.91	0.12	37.95	6.85
P	< 0.0001	0.9712	< 0.0001	0.0064

Means ± Standard Error (SE) in each column for each parasitoid species followed by the same letter did not differ from each other according to the Tukey test (5% probability). <sup>a</sup>Original data followed by statistics done on data transformed into arcsin  $\sqrt{X / 100}$  as request to perform ANOVA according to Burr & Foster (1972).

All the other tested insecticides exhibited a strongest impact above adults of both parasitoid species (*T. podisi* and *T. teretis*) (Tables 3 and 4). Both sulphoxaflor + lambda-cyhalothrin (20 + 30 and 30 + 45 g/150 L H<sub>2</sub>O), thiamethoxam + lambda-cyhalothrin (28.2 + 21.2 and 35.3 + 26.5 g/150 L H<sub>2</sub>O) and chlorpyrifos 960 g/150 L H<sub>2</sub>O reduced *T. podisi* and *T. teretis* parasitism compared to control (water) (Table 4) and, therefore all of them were classified as moderately harmful (class 3) and harmful (class 4) (Table 3).

Impact of host egg exposure to insecticides on parasitism by *T. podisi* (bioassay 5) and *T. teretis* (bioassay 6) (no-choice test)

All the studied insecticides (Table 1) after being sprayed on *Euchistus heros* (F.) (Heteroptera: Pentatomidae) eggs triggered parasitoid mortality of those female wasps after getting in contact with those eggs, except ethiprole 150 g/150 L H<sub>2</sub>O. Despite such mortality when the number of parasitized eggs and progeny viability was analyzed not only ethiprole 150 g/150 L H<sub>2</sub>O but also ethiprole 200 g/150 L H<sub>2</sub>O had similar results to the control (water). All the other studied treatments negatively impact *T. podisi* and *T. teretis* parasitism when adults tried to parasitize eggs with residues of those insecticides. Sulphoxaflor + lambda-cyhalothrin (20 + 30 and 30 + 45 g/150 L H<sub>2</sub>O), thiamethoxam + lambda-cyhalothrin (28.2 + 21.2 and 35.3 + 26.5 g/150 L H<sub>2</sub>O) and chlorpyrifos 960 g/150 L H<sub>2</sub>O significantly increase adult female mortality, reduced both the number of parasitized eggs and parasitoid emergence (progeny viability) when female wasps of both of *T. podisi* and *T. teretis* were put together with *E. heros* which had been previously sprayed with those insecticides (Table 5).

Table 5

Number of dead parasitoids (N = 5), parasitized eggs (parasitism%) and progeny viability (%) after 24 hours of exposure of adult females of *Telenomus podisi* and *Trissolcus teretis* to *Euschistus heros* eggs sprayed with different insecticides.

Treatment	Number of dead parasitoids	Number of parasitized eggs (parasitism%)	Progeny viability (%)
<b>Telenomus podisi (bioassay 5)</b>			
Water	0.0 ± 0.0 c <sup>a</sup>	32.0 ± 2.1 a (64.0%)	81.5 ± 3.5 a
Ethiprole 150	0.0 ± 0.0 c	34.0 ± 2.4 a (68.0%)	86.7 ± 3.9 a
Ethiprole 200	2.0 ± 0.3 b	30.4 ± 1.6 a (60.8%)	81.9 ± 3.6 a
Sulphoxaflor 20 + lambda-cyhalothrin 30	2.0 ± 0.7 b	8.8 ± 1.6 b (17.6%)	27.7 ± 3.7 cd
Sulphoxaflor 30 + lambda-cyhalothrin 45	1.5 ± 0.3 b	5.8 ± 0.8 b (11.6%)	14.3 ± 6.4 d
Thiamethoxam 28.2 + lambda-cyhalothrin 21.2	2.8 ± 0.4 b	10.4 ± 1.9 b (20.8%)	37.5 ± 6.3 bc
Thiamethoxam 35.3 + lambda-cyhalothrin 26.5	1.8 ± 0.3 b	9.4 ± 0.9 b (18.8%)	12.0 ± 4.6 d
Chlorpyrifos 960	5.0 ± 0.0 a	13.2 ± 1.6 b (26.4%)	52.0 ± 2.3 b
F	22.84	50.27	45.97
P	< 0.0001	< 0.0001	< 0.0001
<b>Trissolcus teretis (bioassay 6)</b>			
Water	0.0 ± 0.0 d <sup>a</sup>	33.4 ± 2.5 a <sup>a</sup> (66.8%)	90.1 ± 3.9 a
Ethiprole 150	0.0 ± 0.0 d	32.8 ± 2.0 a (65.6%)	80.1 ± 3.4 ab
Ethiprole 200	2.8 ± 0.4 b	38.2 ± 1.2 a (76.4%)	73.9 ± 5.3 b
Sulphoxaflor 20 + lambda-cyhalothrin 30	2.2 ± 0.5 bc	1.4 ± 0.5 b (2.8%)	0.0 ± 0.0 c
Sulphoxaflor 30 + lambda-cyhalothrin 45	3.6 ± 0.7 ab	0.4 ± 0.4 b (0.8%)	0.0 ± 0.0 c
Thiamethoxam 28.2 + lambda-cyhalothrin 21.2	0.8 ± 0.4 cd	1.2 ± 0.4 b (2.4%)	0.0 ± 0.0 c
Thiamethoxam 35.3 + lambda-cyhalothrin 26.5	1.0 ± 0.3 cd	0.4 ± 0.4 b (0.8%)	0.0 ± 0.0 c
Chlorpyrifos 960	5.0 ± 0.0 a	2.4 ± 0.9 b (4.8%)	0.0 ± 0.0 c
F	26.61	181.42	18.22

Treatment	Number of dead parasitoids	Number of parasitized eggs (parasitism%)	Progeny viability (%)
<i>P</i>	< 0.0001	< 0.0001	< 0.0001

Means  $\pm$  Standard Error (SE) in each column for each parasitoid species followed by the same letter did not differ from each other according to the Tukey test (5% probability). <sup>a</sup>Original data followed by statistics done on data transformed into  $\sqrt{x+1}$  as request to perform ANOVA according to Burr & Foster (1972).

## Discussion

It is crucial to consider a variety of aspects using well-established methodologies when classified the selectivity of insecticides to natural enemies (Bueno et al. 2017). Overall, taking the impact of different studied chemicals when sprayed over the pupae and adults of the parasitoids (*T. podisi* and *T. teretis*) and also over the *E. heros* eggs before parasitism, ethiprole was the most selective insecticide in this study, which can be classified as harmless (class 1) inside the pre-established IOBC categories (Hassan 1992). This higher selectivity is especially true for the lowest tested rate of 150 g/150 L H<sub>2</sub>O, which was slightly more selective than the chemical at the rate of 200 g/150 L H<sub>2</sub>O for some of the evaluated parameters. This dose-dependent side-effects of ethiprole had previously been reported for honeybee (Liu et al. 2021) but it is the first report for egg parasitoids.

Ethiprole is a new phenylpyrazole insecticide with structure analogue to fipronil. It is effective against a broad spectrum of sucking insects with pronounced plant systemic activity (Carboni et al. 2003), the reason why it has been widely used against stink bugs in soybeans. However, not only are stink bugs hard to be controlled but also harmful to soybean and maize plants severely reducing yield when not well managed (Gomes et al. 2020; Bueno et al. 2021) which has increased the use of insecticides and consequently pest resistance reports (Sosa-Gómez et al. 2001; Sosa-Gómez and Silva 2010). The insecticides used against stink bugs are restricted to a few different modes of action, worsening resistance issues with *E. heros*, which is the most frequent stink bug species occurring in the soybean field, especially in South America. Ethiprole is described as having some positive characteristics such as high level of selective toxicity (Simon-Delso et al. 2015) and thus cross resistance would be not likely. In particular, ethiprole binds to the gamma-aminobutyric acid receptor (GABAR) on the membrane of nervous system cells of the target organism, inhibiting the central nervous system of the insect (Cole et al. 1993; Garrod et al. 2015). This differs from the other modes of action available for stink bug control and then it is of crucial importance for insecticide resistance management. Being selective to the most important egg parasitoids of the pest is also another important positive feature that makes etriprole and important tool to stink bug manage in soybean and maize fields.

Despite its selectivity to the egg parasitoids *T. podisi* and *T. teretis*, it is important to emphasize the need of using ethiprole only, when necessary, which is when the economic threshold of 2 sting bugs/meter is

reached or surpassed (Bueno et al. 2015). *Telenomus podisi* and *T. teretis* are only some of many species of beneficial organisms that should be preserved into the agroecosystem. Ethiprole has been observed to cause developmental deficiencies, disordered immune action, and abnormal reproduction and neurobehavior in some other nontarget organisms (Tanaka and Inomata 2017; Tanaka et al. 2018). Sublethal doses of ethiprole were reported to have physiologically toxic effects in honeybee larvae and adult honeybees inhibiting the pupation and eclosion rate of honeybee larvae (Liu et al. 2021).

Despite the taxonomic proximity of *T. podisi* and *T. teretis*, which belong to the same family (Scelionidae), the different results recorded between *T. podisi* and *T. teretis* when sprayed with the same insecticide and rate can have different reasons that should be studied in more details in future researches. Those variations in results are probably due to differences among the hosts species related to their size, chemical composition and thickness of cuticles among other reasons, for example. The greater the body volume of the beneficial organism, the smaller the specific area and, consequently, the lesser the exposure to insecticides (Picanço et al. 1997). Different insecticide penetration rates, related to physiological differences, chemical composition and thickness of *T. podisi* and *T. teretis* cuticles might also help to explain the varying responses of those species to the studied insecticides (Fernandes et al. 2010). More hydrophobic insect cuticles result in higher affinity to insecticides, consequently, higher insecticide penetration and possibly higher insect mortality (Leite et al. 1998). Furthermore, insecticide selectivity might also be associated to ethion metabolism by cytochrome P450-dependent monooxygenase enzymes of beneficial organisms. These enzymes usually detoxify lipophilic compounds, converting them into metabolites and allowing natural enemies to eliminate toxic compound through their feces (Brattsten et al. 1986), a process which might differ between *T. podisi* and *T. teretis*.

Lambda-cyhalothrin is a pyrethroid that was tested mixed with a neonicotinoid (thiamethoxam) or a sulfoximine (sulphoxaflor) at different rates. Chemicals from the pyrethroid group act at sodium channels in the axon, causing hyperexcitation in the insects and killing them very quickly (Bueno et al. 2008). Treatment containing lambda-cyhalothrin triggered more severe negative side-effects to both *T. podisi* and *T. teretis* pupae and adults because this chemical is a neurotoxin that act similarly on the different species of insects, beneficials or pests, which share very similar nervous system. Thus, pyrethroids have a broad spectrum and are generally classified as non-selective for most beneficial arthropod species (Carmo et al. 2010). Various insecticides in this chemical group have been previously reported as harmful to different beneficial arthropods (Croft 1990; Croft and Whalon 1982; Sterk et al. 1999, Carvalho et al. 1999, Stecca et al. 2018). However, those negative side-effects can vary accordingly to the used chemical rate. Studied treatments containing lambda-cyhalothrin at higher rates (30 and 45 g/150 L H<sub>2</sub>O) were more noxious than treatments with lower rates of the pyrethroid (21.2 and 26.5 g/150 L H<sub>2</sub>O) to both parasitoid species. Furthermore it is important to note that both parasitoid species as pupae were more tolerant to the negative side-effects of insecticides when compared to adults. The higher tolerance of parasitoid pupae to chemicals had already reported in literature as a consequence of the protection offered by the chorion of the host egg to the parasitoid that develops inside its interior and is not reached by the sprayed chemicals (Stecca et al. 2016). This protection offer by the chorion of the host egg can

vary accordingly to the insecticide because the ability of a chemical to penetrate the chorion of an insect egg may be related to their physicochemical properties. For example, chemicals with higher molecular weight have greater difficulty in crossing the chorion (Stock and Holloway 1993), which may explain the higher tolerance of *T. podisi* and *T. teretis* pupae inside host eggs to chemicals that are harmful to adults of the same species. However, this protection depends on how close the spraying occurs to adult parasitoid emergence. Pesticide residue that remains on the chorion of the eggs can be enough to kill wasps during emergence since those wasps use their mouthparts to cut the chorion during emergence, the moment they can get contaminated and die due to the insecticide. Then, despite not having the ability to penetrate the chorion, some chemicals with longer residual can still be able to kill natural enemies at the moment of adult emergence because of pesticide spraying that occurred on the pupae stage.

Thiamethoxam is a neonicotinoid which acts as a neurotoxin and interferes with the transmission of nerve impulses by binding to specific acetylcholine receptors (Talebi-Jahromi 2007). Sulfoxaflor is the first insecticide of the sulfoximine group (Zhu et al. 2011) acting on insect nicotinic acetylcholine receptors (nAChRs), but with a distinct combination of attributes from the neonicotinoids (Sparks et al. 2013). Both thiamethoxam and sulfoxaflor were only tested in mixture with the pyrethroid as recommended in the field to manage stink bugs. Therefore, it does not allow to make further inferences about their selectivity on this study. However, both neonicotinoids, sulfoximines and as already mentioned also pyrethroids are reported as harmful to most natural enemies (Tomizawa and Casida 2005; Jiang et al. 2019). The main difference among those chemical groups is that pyrethroids have a contact action, which facilitates the exposure and action of this insecticide to parasitoids and other beneficial organisms in the field. On the other hand, neonicotinoids and sulfoximines are systemic products with little contact action. These insecticides need to be ingested by insects and, therefore, are more specific against phytophagous sucking insects that, due to their feeding habits, come into contact with the product when they feed on the sap and/or nectar of plants, which is the case with parasitoids of *T. podisi* and *T. teretis* (Santos et al. 2006). Therefore, sulfoxaflor possess similar adverse effects on parasitoid wasps like neonicotinoids as reported for the egg parasitoids *Trichogramma dendrolimi* (Matsumura, 1926), *Trichogramma ostrinae* (Pang and Chen, 1974) and *Trichogramma confusum* (Viggiani, 1976) (Hymenoptera: Trichogrammatidae) (Jiang et al. 2019).

Chlorpyrifos is an organophosphate that kill insects primarily by phosphorylation of the acetylcholinesterase enzyme (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, sulfoximine and neonicotinoids tested in this study, 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. 2008) and *T. cacoeciae* (Hassan et al. 1988).

Among the tested insecticides used to manage stink bug outbreaks, the mixture of neonicotinoids + pyrethroids and the organophosphates are among the cheapest insect-control products available to farmers, what can lead to a overuse of those chemicals. However, their application is not compatible with

the preservation of the most important stink bug biological control agents, the egg parasitoids from the Scelionidae family, as shown in this work. Therefore, those chemicals should be used with caution, always adopting the stink bug economic thresholds and whenever possible replaced, by less harmful products in IPM programs. Good alternatives to those products, when feasible, is the ethiprole, since their effects on *T. podisi* and *T. teretis* are less injurious as shown in this work.

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 some of the tested pesticides may be reduced, since *T. podisi* and *T. teretis* can benefit from refuge areas or may avoid chemical-treated areas (Hassan 1992, Carmo et al. 2010).

## Conclusion

Among all tested treatments available to manage stink bugs in soybeans, ethiprole was the least toxic compound to *T. podisi* and *T. teretis* and should be preferred in integrated management programs aimed at preserving those egg parasitoids whenever possible, while the other tested insecticides should be evaluated under semi-field and field conditions to confirm their higher toxicity and be replaced for more selectivity pesticide whenever possible.

## Declarations

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### Competing interest statement

The authors declare that there is no conflict of interest.

### Authors' contributions

Conceptualization: Ribeiro, W., Bueno, A.F. Bioassays development: Ribeiro, W., Bueno, A.F. Data analysis: Ribeiro, W., Bueno, A.F., Silva, D.M. Writing and editing: Bueno, A.F., Silva, D.M., Carvalho, G.A., Biondi, A. Reference analysis: Bueno, A.F., Silva, D.M., Carvalho, G.A., Biondi, A. Final draft correction: Bueno, A.F., Silva, D.M., Carvalho, G.A., Biondi, A.

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