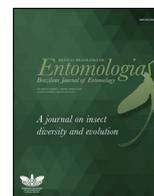




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Toxicity of insecticides to the egg parasitoids *Telenomus podisi* and *Trissolcus teretis* (Hymenoptera: Scelionidae)

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ABSTRACT

This work aims to evaluate the toxicity of insecticides used for stink bug control to the egg parasitoids *Telenomus podisi* and *Trissolcus teretis*. We tested ethiprole and sulfoxaflor + lambda-cyhalothrin in comparison with thiamethoxam + lambda-cyhalothrin and chlorpyrifos. Three independent bioassays were conducted in the laboratory and repeated for each parasitoid species, to evaluate the effect of insecticides on pupal and adult stages of the parasitoids and the effects of insecticide sprays on host eggs prior to parasitism. Ethiprole at concentrations of 100 and 133.3 g/100 L H₂O 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 selective pesticide among the studied chemicals. When adult parasitoids of both species were exposed to sprayed host eggs, parasitism rates were similar. The other treatments triggered more severe negative side effects to the parasitoids, especially to adults. Overall, ethiprole was the least toxic compound among the studied products 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 verify their higher toxicity.

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 species that feed 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; Bueno et al., 2021). Not only are stink bugs noteworthy for feeding directly on soybean pods but also for their impact on maize development when feeding directly on seedlings. The caused injuries can seriously affect yields as well as the physiological and sanitary quality of the produced grains (Corrêa-Ferreira and Azevedo, 2002; Gomes et al., 2020). In order to mitigate losses caused by stink bugs and consequently to increase profits, growers control these phytophagous arthropods (Bueno et al., 2015). Currently, the primary method for stink bug control adopted by growers is the use of chemical insecticides,

often applied incorrectly and excessively (Song and Swinton, 2009; Panizzi, 2013; Bueno et al., 2021).

The overuse of insecticides, especially non-selective ones, has triggered several important adverse side-effects (Bueno et al., 2022a). Not only can 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), in addition to selection for pest resistance (Sosa-Gómez et al., 2001, 2020; 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 (Bueno et al., 2020), being applied to more than 30 million ha worldwide (van Lenteren et al., 2018).

Among the possible 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; Bueno et al., 2022b). Among the appropriate species of egg parasitoids, *Telenomus podisi* Ashmead, 1893 (Hymenoptera: Scelionidae) is noteworthy due to its

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high parasitism and control efficacy against its hosts (Queiroz et al., 2018; Silva et al., 2018; Bueno et al., 2020). *Telenomus podisi* and *Trissolcus teretis* (Johnson, 1987) (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 its genus in different cropping systems (Tillman, 2011; Bueno et al., 2022b) while there still is a profound 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 the 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 such as stink bugs in soybean and maize crops (Torres and Bueno, 2018). Thus, selective pesticides are of great value for crop management, especially as a conservation biological control strategy for sustainable intensification of food production using integrated pest management (IPM) (Shields et al., 2019). A significant advantage of selective products is their effectiveness against target pests with minimal side-effects on natural enemies (Broadbent and Pree, 1984; Torres and Bueno, 2018; Bueno et al., 2022a). Consequently, knowledge on how chemicals that are commonly used on soybean and maize crops are affecting egg parasitoids is extremely important. In this context, we evaluated possible side-effects of different insecticides frequently sprayed on soybean and corn for the control of stink bugs (especially *E. heros*) on the egg parasitoids *T. podisi* and *T. teretis*, thereby aiming to determine the most selective chemicals to preferably be used in IPM programs. Although several studies have been published on this subject, this is the first to report the toxicity of ethiprole and sulfoxaflor + lambda-cyhalothrin, in addition to being the first study of insecticide selectivity on *T. teretis*.

Material and methods

Three bioassays were conducted to assess the side-effects of different insecticides on pupae and adults of *T. podisi* and *T. teretis*, as well as on their parasitism capacity on treated host eggs. Trials were carried out at 25±2°C; 70±10% RH, and a 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., 2000; Manzoni et al., 2007), and repeated for both parasitoid species (*T. podisi* and *T. teretis*). The newest insecticides (ethiprole and sulphoxaflor + lambda-cyhalothrin) as well as the insecticide (thiamethoxam + lambda-cyhalothrin) already tested on *T. podisi* (Stecca et al., 2018) (although in this study a different trademark, Engeo Pleno S® was used instead of Engeo Pleno®) were investigated in the higher and lower recommended concentrations against *E. heros*. In contrast, chlorpyrifos, which is known to be harmful to different egg parasitoid species (Bueno et al., 2017) was only tested in the highest concentration as a positive control (Table 1).

Parasitoids and host colonies

Telenomus podisi was 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, 630 m of altitude) during the summer of 2015, and identified by a taxonomist as *T. podisi*. *Trissolcus teretis* was originally collected in Brasília, DF, Brazil, and grown in 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 a 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 climate chambers (ELETROLab®, model EL 212, São Paulo, SP, Brazil) set to 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), briefly summarized in the following.

Both *T. podisi* and *T. teretis* were reared on *E. heros* eggs. After removal from liquid nitrogen (-196°C), frozen eggs were glued to pieces of cardboard (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 the 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 colony maintenance.

Stink bugs were collected from soybean fields at the Embrapa Soybean Field Station (23° 11' 11.7" S and 51° 10' 46.1" W, 630 m of altitude) and kept in the laboratory for approximately four years according to the methodology previously described by Panizzi et al. (2000). New field insects were 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) prior to their use in the experiments for up to six months, a period during which their quality for parasitism is maintained (Favetti et al., 2014).

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.) | (grams) a.i./100 L H ₂ O | Commercial product (mL/ha) |
|--------------------|-------------|-----------------------------------|-------------------------------------|----------------------------|
| Curbix® | 200 SC | ethiprole | 100 | 750 |
| Curbix® | 200 SC | ethiprole | 133.3 | 1000 |
| Expedition® | 100/150 SE | sulphoxaflor + lambda-cyhalothrin | 13.3 + 20 | 200 |
| Expedition® | 100/150 SE | sulphoxaflor + lambda-cyhalothrin | 20 + 30 | 300 |
| Engeo Pleno S® | 141/106 SC | thiamethoxam + lambda-cyhalothrin | 18.8 + 14.1 | 200 |
| Engeo Pleno S® | 141/106 SC | thiamethoxam + lambda-cyhalothrin | 23.5 + 17.7 | 250 |
| Lorsban® | 480 EC | chlorpyrifos | 640 | 2000 |

Impact of the parasitoid pupae exposure to the spray of different insecticides (bioassay 1)

The selectivity of different insecticides (Table 1) to *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., 2000; Manzoni et al., 2007) modified by Carmo et al. (2010). Cards measuring 3 cm² (1 card per replicate) containing approximately 100 24-h-old host eggs 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 insecticide sprays (Table 1) according to the methodology used by Carmo et al. (2010) with five replicates for each treatment in a completely randomized design. Each replicate consisted of a card, which measured 3 cm² and contained approximately 100 eggs with parasitoids in the pupal stage. Spraying was performed using a Potter Spray Tower (Burkard Manufacturing Co Ltda, Hertfordshire County, England) (Fig. 1A) regulated to a pressure of 1.5 kgf/cm² in order to deposit a volume of 1.25 ± 0.25 mg.cm⁻² according to established IOBC protocols (Hassan, 1992; Hassan et al., 2000). The cards with the treated host eggs containing the parasitoid pupae, were left to dry completely in the room for about 2 h to remove excess moisture. Next, they were placed in cages (Fig. 1B) described by Hassan (1992) until the emergence of the adults, which then 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 inside a climate 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 with the aid of a stereoscopic microscope (Leica-Wild M10, Wetzlar, Germany). The emergence of parasitoid adults from sprayed eggs was calculated by dividing the number of *E. heros* eggs with an emergence hole by the total number of parasitized eggs multiplied by 100.

Impact of parasitoid adult exposure to the dry residue of different insecticides (bioassay 2)

Approximately 100 eggs of *E. heros* were glued on cardboard cards. These cards were then offered to freshly emerged *T. podisi* and *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 (Fig. 1C). The Duran® tubes were then sealed with plastic film and stored in a climate 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. A Potter Spray Tower (Burkard Manufacturing Co Ltda, Hertfordshire County, England) (Fig. 1A) regulated to a pressure of 1.5 kgf/cm² was used to spray a volume of insecticide solution of 1.25 ± 0.25 mg.cm⁻² on the glass plates (13 × 13 cm) (Fig. 1D) used to mount the cages (Fig. 1E) and expose the parasitoids (Hassan, 1992; Hassan et al., 2000). The design was completely randomized with five replications

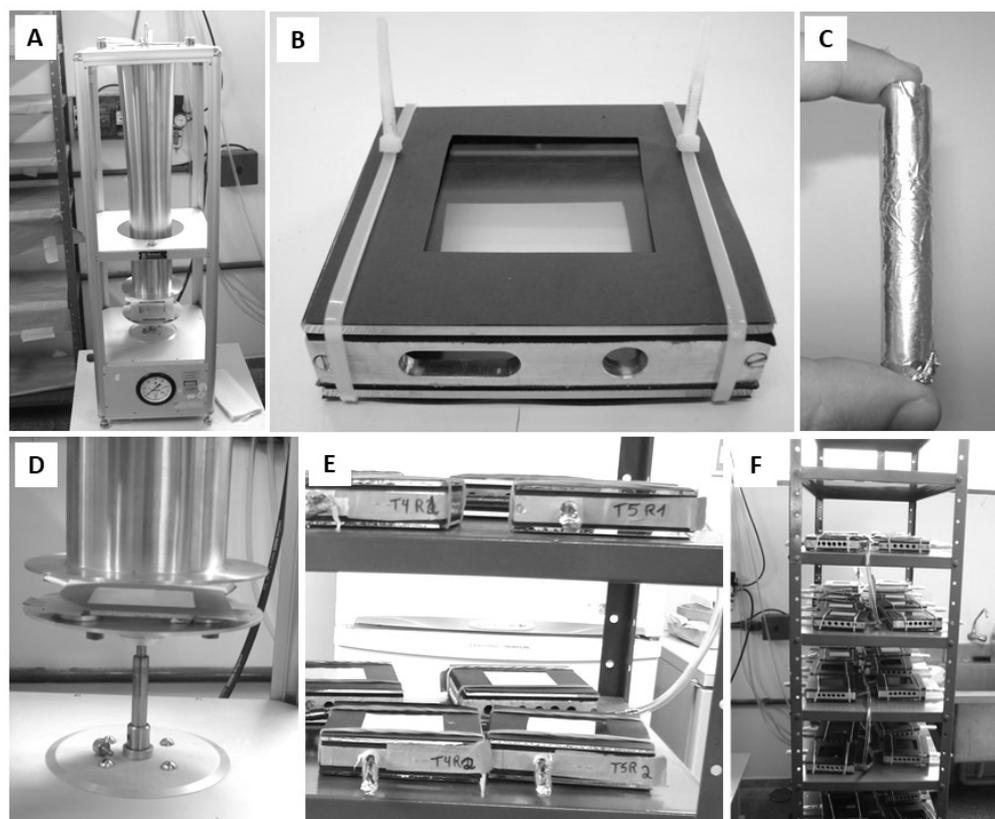


Figure 1 Bioassay setup. Potter Spray Tower (A), experimental cage (B), Duran tube with adult parasitoids inside covered with aluminum foil used to introduce the wasps into the experimental cage (C), glass plate being sprayed with the Potter Spray Tower before the setup of the experimental cage (D), adult cages mounted with the Duran tube connected (E), experimental cages setup with circulating air flow allowing the elimination of possible toxic gases (F).

(cages). 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 (Fig. 1F) 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 (Fig. 1C) and connected to holes in the cages for the introduction of insects (Fig. 1E), 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 a climate-controlled chamber at 25°C ± 2°C, 70% ± 10% RH and a photoperiod of 14:10 h (L:D). The number of parasitized eggs and the number of insects that emerged in each treatment were evaluated with the aid of a stereoscopic microscope (Leica-Wild M10, Wetzlar, Germany).

Impact of host egg exposure to insecticides on parasitism (no-choice test) (bioassay 3)

Telenomus podisi and *T. teretis* females were offered cards containing approximately 50 viable eggs (24 h) of *E. heros*, sprayed (volume of 1.25 ± 0.25 mg.cm⁻²) with insecticides (Table 1) using a Potter Spray Tower (Burkard Manufacturing Co Ltda, Hertfordshire County, England) (Fig. 1A) regulated to a pressure of 1.5 kgf/cm² in a completely randomized design with five replicates. Each replicate had five females of *T. podisi* or *T. teretis* (24–48h old), individualized in glass tubes (75 mm high x 12 mm in diameter), totaling 25 females per treatment. A droplet of honey was placed on the wall of the glass tube, to serve as food for the females. After that, the glass tubes were stored in climate chambers. Parasitism was allowed for 24 h in order to evaluate the immediate impact of the insecticides on parasitism and adult parasitoid mortality. Then, the number of dead females was determined and live females were discarded. Cards containing the parasitized eggs were transferred to new tubes, placed in climate chambers until the emergence of the parasitoids to evaluate parasitism and parasitoid emergence (progeny viability %) with the aid of a stereoscopic microscope (Leica-Wild M10, Wetzlar, Germany).

Statistical analysis

Data obtained from all three bioassays, and repeated for each parasitoid species were subjected to exploratory analysis to evaluate normality assumptions for the residuals (Shapiro and 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, insecticide effects on *T. podisi* and *T. teretis* in comparison to distilled water (used as control treatment) was computed by the following equations:

$$EP \text{ (Effects on pupae \%)} = \left(\frac{1 - \text{adult emergence observed for the tested treatment}}{\text{adult emergence observed for the control treatment}} \right) \times 100 \quad (1)$$

$$E \text{ (Effects on adults \%)} = \left(\frac{1 - \text{parasitism observed for the tested treatment}}{\text{parasitism observed for the control treatment}} \right) \times 100 \quad (2)$$

Using these data, the chemicals were classified according to the IOBC standards as follows: class 1, harmless (EP or E <30%); class 2, slightly harmful (30% ≤ EP or E ≤ 79%); class 3, moderately harmful (80% ≤ EP or E ≤ 99%); and class 4, harmful (EP or E > 99%) (Hassan, 1992).

Results

Impact of the parasitoid pupae exposure to the spray of different insecticides (bioassay 1)

Among the tested insecticides, the mildest side-effects on both *T. podisi* and *T. teretis* pupae were exerted by ethiprole, especially in the lowest studied concentration of 100 g/100 L H₂O, but also in the highest concentration of 200 g/100 L H₂O compared with the other tested treatments. When eggs of *E. heros* containing *T. podisi* pupae close to parasitoid emergence (13 days after egg parasitism) were sprayed with different insecticides, ethiprole 100 and 133.3 g/100 L H₂O 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* that emerged from pupae treated with ethiprole was equal to that of parasitoids that emerged from pupae treated with control (water) on both 1 and 3 DAE (Table 2). Consequently, ethiprole 100 and 133.3 g/100 L H₂O was classified as harmless (class 1) when applied on *T. podisi* pupae in *E. heros* eggs (Table 3) not only due to the lack of impact on adult parasitoid emergence compared with the control (sprayed with water), but also due to the lack of any impact on parasitism capacity of adults that emerged from treated pupae. No sublethal effect was observed on parasitism or parasitoid emergence of this second parasitoid generation (progeny viability) (Table 2).

Similar results for ethiprole were observed when sprayed over *T. teretis* pupae, but only with the lowest concentration of 100 g/100 L H₂O (Tables 2 and 3). Adult emergence of *Trissolcus teretis* pupae sprayed with ethiprole 100 g/100 L H₂O was 25.3%, statistically similar to the emergence of 23% recorded for *T. teretis* pupae sprayed with water (control). Despite similar adult emergence, parasitism of *T. teretis* 1 DAE that had emerged from ethiprole treated pupae was lower (25.3%) compared with the control (47.6%). Nevertheless, parasitism 3 DAE was similar for adults that emerged from pupae treated with ethiprole (15.6%) and the control (27.6%) (Table 2). Therefore, ethiprole 100 g/100 L H₂O was classified as harmless (class 1) to *T. teretis*. However, when considering parasitoid emergence and parasitism capacity of emerged adults, ethiprole 100 g/100 L H₂O was classified as slightly harmful (class 2) when sprayed over *E. heros* containing *T. teretis* pupae close to parasitoid emergence (13 days after egg parasitism) (Table 3). In contrast, ethiprole 133.3 g/100 L H₂O reduced adult emergence from sprayed pupae compared with water (control), as well as compared with ethiprole at the lower concentration (100 g/100 L H₂O). Moreover, ethiprole 133.3 g/100 L H₂O also impacted parasitism of emerged adults from treated pupae, with a stronger statistical effect than water and ethiprole at the lowest concentration (100 g/100 L H₂O) both 1 and 2 DAE (Table 2). Thus, ethiprole 133.3 g/100 L H₂O was classified as slightly harmful (class 2), but moderately harmful (class 3) when sprayed over *T. teretis* pupae in eggs of *E. heros* when 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 concentrations of thiamethoxam + lambda-cyhalothrin (18.8+14.1 and 23.5+17.7 g/100 L H₂O) exhibited a stronger side-effect on both *T. podisi* and *T. teretis* when sprayed over the parasitoid pupae compared with ethiprole (Tables 2 and 3). Side-effects included a significant reduction of adult emergence of *T. podisi* from sprayed pupae compared with the control, which

Table 2

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

| Treatment (grams/100 L H ₂ O) | Sprayed pupae | | 1 DAE | | 3 DAE | |
|---|---------------------|----------------|-----------------------|----------------|-----------------------|--|
| | Adult emergence (%) | Parasitism (%) | Progeny viability (%) | Parasitism (%) | Progeny viability (%) | |
| <i>Telenomus podisi</i> | | | | | | |
| Water | 40.0 ± 8.2 ab* | 52.5 ± 5.8 a | 76.8 ± 2.6 a | 55.6 ± 6.3 a | 74.7 ± 1.5 ab | |
| Ethiprole 100 | 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 133.3 | 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 13.3 + lambda-cyhalothrin 20 | 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 20 + lambda-cyhalothrin 30 | 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 18.8 + lambda-cyhalothrin 14.1 | 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 23.5 + lambda-cyhalothrin 17.7 | 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 640 | 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 | |
| DF _{residue} | 36 | 37 | 37 | 38 | 37 | |
| <i>Trissolcus teretis</i> | | | | | | |
| Water | 23.0 ± 3.3 ab | 47.6 ± 3.0 a* | 59.7 ± 2.6 a | 27.6 ± 4.3 a* | 42.1 ± 7.7 a | |
| Ethiprole 100 | 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 133.3 | 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 13.3 + lambda-cyhalothrin 20 | 23.4 ± 2.0 abc | 0.0 ± 0.0 d | No parasitism | 0.0 ± 0.0 b | No parasitism | |
| Sulphoxaflor 20 + lambda-cyhalothrin 30 | 1.5 ± 1.2 d | 0.0 ± 0.0 d | No parasitism | 0.0 ± 0.0 b | No parasitism | |
| Thiamethoxam 18.8 + lambda-cyhalothrin 14.1 | 32.6 ± 2.0 a | 0.0 ± 0.0 d | No parasitism | 0.0 ± 0.0 b | No parasitism | |
| Thiamethoxam 23.5 + lambda-cyhalothrin 17.7 | 19.9 ± 3.0 bc | 0.0 ± 0.0 d | No parasitism | 0.0 ± 0.0 b | No parasitism | |
| Chlorpyrifos 640 | 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 | |
| DF _{residue} | 37 | 39 | 14 | 37 | 13 | |

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% significance). *Original data shown, but statistics were performed on arcsin transformed data into $\sqrt{X/100}$ prior to ANOVA according to Burr and 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 1 and 3 days after emergence (DAE) of adults, and 1 and 3 days after spraying (DAS).

| Treatment (grams/100 L H ₂ O) | Bioassays with pupae | | | | | | Bioassays with adults | | | |
|---|----------------------|----------------|----------------|---|-------|---|-----------------------|---|-------|---|
| | Sprayed pupae | | 1 DAE | | 3 DAE | | 1 DAS | | 3 DAS | |
| | EP ^a | C ^b | E ^c | C | E | C | E | C | E | C |
| <i>Telenomus podisi</i> | | | | | | | | | | |
| Ethiprole 100 | 15.8 | 1 | 0.0 | 1 | 8.0 | 1 | 13.0 | 1 | 17.5 | 1 |
| Ethiprole 133.3 | 0.0 | 1 | 0.0 | 1 | 0.0 | 1 | 6.2 | 1 | 16.0 | 1 |
| Sulphoxaflor 13.3 + lambda-cyhalothrin 20 | 88.9 | 3 | 25.2 | 1 | 37.2 | 2 | 89.8 | 3 | 83.9 | 3 |
| Sulphoxaflor 20 + lambda-cyhalothrin 30 | 87.7 | 3 | 34.3 | 2 | 30.9 | 2 | 86.2 | 3 | 100 | 4 |
| Thiamethoxam 18.8 + lambda-cyhalothrin 14.1 | 63.6 | 2 | 0.0 | 1 | 0.0 | 1 | 93.9 | 3 | 100 | 4 |
| Thiamethoxam 23.5 + lambda-cyhalothrin 17.7 | 61.6 | 2 | 19.6 | 1 | 4.9 | 1 | 100 | 4 | 100 | 4 |
| Chlorpyrifos 640 | 71.6 | 2 | 24.9 | 1 | 30.1 | 2 | 100 | 4 | 100 | 4 |
| <i>Trissolcus teretis</i> | | | | | | | | | | |
| Ethiprole 100 | 0.0 | 1 | 46.8 | 2 | 43.2 | 2 | 46.1 | 2 | 50.6 | 2 |
| Ethiprole 133.3 | 43.1 | 2 | 77.8 | 2 | 87.1 | 3 | 69.1 | 2 | 80.3 | 3 |
| Sulphoxaflor 13.3 + lambda-cyhalothrin 20 | 0.0 | 1 | 100 | 4 | 100 | 4 | 100 | 4 | 100 | 4 |
| Sulphoxaflor 20 + lambda-cyhalothrin 30 | 93.2 | 3 | 100 | 4 | 100 | 4 | 99.1 | 4 | 98.3 | 3 |
| Thiamethoxam 18.8 + lambda-cyhalothrin 14.1 | 0.0 | 1 | 100 | 4 | 100 | 4 | 100 | 4 | 100 | 4 |
| Thiamethoxam 23.5 + lambda-cyhalothrin 17.7 | 13.3 | 1 | 100 | 4 | 100 | 4 | 98.3 | 3 | 86.0 | 3 |
| Chlorpyrifos 640 | 11.7 | 1 | 100 | 4 | 100 | 4 | 100 | 4 | 100 | 4 |

^aEP (Effects on pupae %) = (1 - adult emergence observed for the tested treatment/ adult emergence observed for the control treatment) × 100; ^bClasses: 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%); ^cE (Effects on adults %) = (1 - parasitism observed for the tested treatment/ parasitism observed for the control treatment) × 100.

did not trigger the expected reduction in parasitism 1 and 3 DAE. Results were slightly different when experiments were carried out with *T. teretis*. Thiamethoxam + lambda-cyhalothrin did not trigger the same reduction in *T. teretis* emergence from sprayed pupae compared with the control. However, the parasitoids that emerged from treated pupae did not parasitize any eggs (Table 2).

Therefore, thiamethoxam + lambda-cyhalothrin (18.8+14.1 and 23.5+17.7 g/100 L H₂O) 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) when considering adult emergence and parasitism capacity of emerged adults, respectively (Table 3).

Results for exposure to chlorpyrifos 640 g/100 L H₂O was very similar to those for thiamethoxam + lambda-cyhalothrin when sprayed over *T. podisi* and *T. teretis* pupae (Tables 2 and 3). It triggered a significant reduction of *T. podisi* emergence from sprayed pupae but not enough to reduce parasitism capacity of 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 640 g/100 L H₂O received the same classification as thiamethoxam + lambda-cyhalothrin (18.8+14.1 and 23.5+17.7 g/100 L H₂O). 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 (13.3+20 and 20+30 g/100 L H₂O) triggered the strongest reduction in *T. podisi* and *T. teretis* emergence from sprayed pupae compared with the other tested treatments. However, for *T. podisi* it was not enough to reduce parasitism capacity of emerged adults while no parasitism was recorded for *T. teretis* that had emerged from sprayed pupae (Table 2). Therefore, despite some variation in the results, in 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 parasitoid adult exposure to the dry residue of different insecticides (bioassay 2)

Similar to the previous bioassay carried out with pupae, ethiprole exhibited lower impact on *T. podisi* and *T. teretis* adults at both tested

concentrations (100 and 133.3 g/100 L H₂O) than the other tested insecticides when parasitism and progeny viability of the parasitoids were evaluated after the exposure of adults to the dry residue of the different studied insecticides (Tables 3 and 4). *Telenomus podisi* adults that had contact with ethiprole had similar parasitism capacity as the control (parasitoids that had contact with water). They also showed the same progeny viability, indicating no sublethal effect of this chemical (Table 4). Therefore, ethiprole (100 and 133.3 g/100 L H₂O) was classified as harmless (class 1) to adults of *T. podisi* according to IOBC protocols (Hassan, 1992) (Table 3). For adults of *T. teretis*, ethiprole reduced parasitism, especially at the higher concentration of 133.3 g/100 L H₂O (Table 3). Therefore, this insecticide was classified as slightly harmful (class 2) for adults at the lower tested concentration (100 g/100 L H₂O), and varied from slightly harmful (class 2) to moderately harmful (class 3) at the higher concentration (133.3 g/100 L H₂O) on 1 and 3 DAE, respectively (Table 4).

All other tested insecticides exhibited a strong impact on adults of both parasitoid species (*T. podisi* and *T. teretis*) (Tables 3 and 4). Both sulphoxaflor + lambda-cyhalothrin (13.3+20 and 20+30 g/100 L H₂O), thiamethoxam + lambda-cyhalothrin (18.8+14.1 and 23.5+17.7 g/100 L H₂O) as well as chlorpyrifos 640 g/100 L H₂O reduced *T. podisi* and *T. teretis* parasitism compared with the 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 (no-choice test) (bioassay 3)

All studied insecticides (Table 1) sprayed on *E. heros* eggs triggered parasitoid mortality of female wasps after contact with those eggs, except for ethiprole 100 g/100 L H₂O. Despite the recorded mortality, when

Table 4
Effects of different insecticides on adults of *Telenomus podisi* and *Trissolcus teretis* one and three days after emergence (DAE) from treated eggs of the host *Euschistus heros*.

| Treatment (grams/100 L H ₂ O) | 1 DAE | | 3 DAE | |
|---|----------------|-----------------------|----------------|-----------------------|
| | Parasitism (%) | Progeny viability (%) | Parasitism (%) | Progeny viability (%) |
| <i>Telenomus podisi</i> | | | | |
| Water | 59.5 ± 2.9 a | 64.2 ± 7.0 a | 58.6 ± 3.5 a | 80.6 ± 5.0 a |
| Ethiprole 100 | 51.8 ± 5.2 a | 67.8 ± 3.5 a | 48.3 ± 6.5 a | 69.3 ± 7.6 a |
| Ethiprole 133.3 | 55.8 ± 3.0 a | 68.0 ± 3.1 a | 49.2 ± 10.8 a | 68.4 ± 5.3 a |
| Sulphoxaflor 13.3 + lambda-cyhalothrin 20 | 6.0 ± 2.5 b | 62.0 ± 9.9 a | 9.5 ± 3.2 b | 56.8 ± 6.8 a |
| Sulphoxaflor 20 + lambda-cyhalothrin 30 | 8.2 ± 4.2 b | 54.1 ± 4.2 a | 0.0 ± 0.0 b | No parasitism |
| Thiamethoxam 18.8 + lambda-cyhalothrin 14.1 | 3.6 ± 2.3 b | 56.2 ± 6.2 a | 0.0 ± 0.0 b | No parasitism |
| Thiamethoxam 23.5 + lambda-cyhalothrin 17.7 | 0.0 ± 0.0 b | No parasitism | 0.0 ± 0.0 b | No parasitism |
| Chlorpyrifos 640 | 0.0 ± 0.0 b | No parasitism | 0.0 ± 0.0 b | No parasitism |
| F | 90.85 | 1.88 | 30.73 | 1.72 |
| P | <0.0001 | 0.1475 | <0.0001 | 0.2121 |
| DF _{residue} | 35 | 23 | 39 | 18 |
| <i>Trissolcus teretis</i> | | | | |
| Water | 24.2 ± 6.2 a* | 40.8 ± 11.6 a | 21.3 ± 2.6 a* | 38.9 ± 4.8 ab |
| Ethiprole 100 | 13.0 ± 4.2 ab | 40.2 ± 11.6 a | 10.5 ± 1.3 b | 38.1 ± 5.6 ab |
| Ethiprole 133.3 | 7.5 ± 1.1 b | 47.4 ± 7.2 a | 4.1 ± 1.4 c | 65.0 ± 3.8 a |
| Sulphoxaflor 13.3 + lambda-cyhalothrin 20 | 0.0 ± 0.0 c | No parasitism | 0.0 ± 0.0 e | No parasitism |
| Sulphoxaflor 20 + lambda-cyhalothrin 30 | 0.2 ± 0.2 c | 50.0 ± 0.0 a | 0.4 ± 0.2 de | 50.0 ± 0.0 a |
| Thiamethoxam 18.8 + lambda-cyhalothrin 14.1 | 0.0 ± 0.0 c | No parasitism | 0.0 ± 0.0 e | No parasitism |
| Thiamethoxam 23.5 + lambda-cyhalothrin 17.7 | 0.4 ± 0.4 c | 50.0 ± 0.0 a | 3.0 ± 1.0 cd | 14.3 ± 0.0 b |
| Chlorpyrifos 640 | 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 |
| DF _{residue} | 39 | 22 | 38 | 19 |

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% significance).

*Original data shown, but statistics were performed on arcsin transformed data into $\sqrt{X/100}$ prior to ANOVA according to Burr and Foster (1972).

the number of parasitized eggs and progeny viability were analyzed not only ethiprole 100 g/100 L H₂O but also ethiprole 133.3 g/100 L H₂O had results similar to control (water). All other studied treatments negatively impacted *T. podisi* and *T. teretis* parasitism when adults attempted to parasitize eggs with residues of those insecticides. Sulphoxaflor + lambda-cyhalothrin (13.3+20 and 20+30 g/100 L H₂O), thiamethoxam + lambda-cyhalothrin (18.8+14.1 and 23.5+17.7 g/100 L H₂O) and chlorpyrifos 640 g/100 L H₂O significantly increased adult female mortality, and reduced both the number of parasitized eggs and parasitoid emergence (progeny viability) when female wasps of both of *T. podisi* and *T. teretis* were brought into contact with *E. heros* that had been previously sprayed with those insecticides (Table 5).

Discussion

It is crucial to consider a variety of aspects using well-established methodologies when studying the selectivity of insecticides to natural enemies (Bueno et al., 2017; Carvalho et al., 2021; Bueno et al., 2022a). Overall, concerning the impact of the different studied chemicals when sprayed over pupae and adults of parasitoids (*T. podisi* and *T. teretis*) as well as over *E. heros* eggs prior to parasitism, ethiprole was the most selective insecticide in this study, and can be considered selective inside the pre-established IOBC categories (Hassan, 1992; Bueno et al., 2022a). This higher selectivity is especially true for the lowest tested concentration of 100 g/100 L H₂O, which was slightly more selective than the concentration of 133.3 g/100 L H₂O for some of the evaluated parameters. This dose-dependent side-effect of ethiprole has previously been reported for honeybees (Liu et al., 2021) but this is the first report for egg parasitoids.

Ethiprole is a new phenylpyrazole insecticide with a structure analogue to fipronil. It is effective against a broad spectrum of sucking

insects with pronounced plant systemic activity (Caboni et al., 2003), which is why it has been widely used against stink bugs in soybeans. Because of its greater selectivity compared with the other tested insecticides used to control stink bugs, ethiprole strongly aligns with the IPM principle of prioritizing the most selective insecticides whenever possible (Bueno et al., 2021). Stink bugs are hard-to-kill pests which are prejudicial to both soybean and maize plants, severely reducing yields when not well managed (Gomes et al., 2020; Bueno et al., 2021). Their outbreaks have triggered the increase of insecticide sprays and, consequently, reports of pest resistance (Sosa-Gómez et al., 2001; Sosa-Gómez and Silva, 2010; Sosa-Gómez et al., 2020). 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 soybean fields, especially in South America (Panizzi and Corrêa-Ferreira, 1997; Bueno et al., 2021). Therefore, ethiprole has been described as having some positive characteristics such as a high level of selective toxicity (Simon-Delso et al., 2015), making cross-resistance to other used insecticides against stink bugs unlikely to happen. In particular, ethiprole binds to the gamma-aminobutyric acid (GABA) receptor on the membranes of nervous system cells of the target organism, inhibiting the central nervous system (Cole et al., 1993; Garrood et al., 2015). This differs from other modes of action available for stink bug control and, therefore, 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 ethiprole an important tool for stink bug management in soybean and maize fields.

Despite its selectivity to *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 stink bugs/meter is reached or surpassed (Bueno et al., 2015). *Telenomus podisi* and *T. teretis* are only

Table 5

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

| Treatment (grams/100 L H ₂ O) | Number of dead parasitoids (mortality%) | Number of parasitized eggs (parasitism%) | Progeny viability (%) |
|---|---|--|-----------------------|
| <i>Telenomus podisi</i> | | | |
| Water | 0.0 ± 0.0 c* (0%) | 32.0 ± 2.1 a (64.0%) | 81.5 ± 3.5 a |
| Ethiprole 100 | 0.0 ± 0.0 c (0%) | 34.0 ± 2.4 a (68.0%) | 86.7 ± 3.9 a |
| Ethiprole 133.3 | 2.0 ± 0.3 b (40%) | 30.4 ± 1.6 a (60.8%) | 81.9 ± 3.6 a |
| Sulphoxaflor 13.3 + lambda-cyhalothrin 20 | 2.0 ± 0.7 b (40%) | 8.8 ± 1.6 b (17.6%) | 27.7 ± 3.7 cd |
| Sulphoxaflor 20 + lambda-cyhalothrin 30 | 1.5 ± 0.3 b (30%) | 5.8 ± 0.8 b (11.6%) | 14.3 ± 6.4 d |
| Thiamethoxam 18.8 + lambda-cyhalothrin 14.1 | 2.8 ± 0.4 b (56%) | 10.4 ± 1.9 b (20.8%) | 37.5 ± 6.3 bc |
| Thiamethoxam 23.5 + lambda-cyhalothrin 17.7 | 1.8 ± 0.3 b (36%) | 9.4 ± 0.9 b (18.8%) | 12.0 ± 4.6 d |
| Chlorpyrifos 640 | 5.0 ± 0.0 a (100%) | 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 |
| DF _{residue} | 37 | 39 | 37 |
| <i>Trissolcus teretis</i> | | | |
| Water | 0.0 ± 0.0 d* (0%) | 33.4 ± 2.5 a* (66.8%) | 90.1 ± 3.9 a |
| Ethiprole 100 | 0.0 ± 0.0 d (0%) | 32.8 ± 2.0 a (65.6%) | 80.1 ± 3.4 ab |
| Ethiprole 133.3 | 2.8 ± 0.4 b (56%) | 38.2 ± 1.2 a (76.4%) | 73.9 ± 5.3 b |
| Sulphoxaflor 13.3 + lambda-cyhalothrin 20 | 2.2 ± 0.5 bc (44%) | 1.4 ± 0.5 b (2.8%) | 0.0 ± 0.0 c |
| Sulphoxaflor 20 + lambda-cyhalothrin 30 | 3.6 ± 0.7 ab (72%) | 0.4 ± 0.4 b (0.8%) | 0.0 ± 0.0 c |
| Thiamethoxam 18.8 + lambda-cyhalothrin 14.1 | 0.8 ± 0.4 cd (16%) | 1.2 ± 0.4 b (2.4%) | 0.0 ± 0.0 c |
| Thiamethoxam 23.5 + lambda-cyhalothrin 17.7 | 1.0 ± 0.3 cd (20%) | 0.4 ± 0.4 b (0.8%) | 0.0 ± 0.0 c |
| Chlorpyrifos 640 | 5.0 ± 0.0 a (100%) | 2.4 ± 0.9 b (4.8%) | 0.0 ± 0.0 c |
| F | 26.61 | 181.42 | 18.22 |
| P | <0.0001 | <0.0001 | <0.0001 |
| DF _{residue} | 39 | 39 | 39 |

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% significance).

*Original data shown, but statistics were performed on arcsin transformed data into $\sqrt{X/100}$ prior to ANOVA according to Burr and Foster (1972).

two of many species of beneficial organisms that should be preserved in the agroecosystem. Pesticide selectivity can deeply differ when tested on different beneficial organisms (Bueno et al., 2017). Ethiprole has been observed to cause developmental deficiencies, disordered immune action, and abnormal reproduction as well as 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 on 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 differences recorded between *T. podisi* and *T. teretis* when sprayed with the same insecticide and concentration can have various causes that should be studied in more detail in future research. Nevertheless, the recorded differences are probably due to species-specific characteristics such as body size, chemical composition and cuticle thickness among other reasons (Fernandes et al., 2010; Bueno et al., 2017). The greater the body volume of a beneficial organism, the smaller the specific area and, consequently, the lesser the exposure to insecticides (Picanço et al., 1997; Bueno et al., 2017). Different insecticide penetration rates, related to physiological differences, chemical composition and cuticle thickness of *T. podisi* and *T. teretis* might also help to explain the specific responses of both species to the studied insecticides (Fernandes et al., 2010; Bueno et al., 2017). More hydrophobic insect cuticles result in higher affinity to some insecticides, and consequently, in higher insecticide penetration and possibly higher insect mortality (Leite et al., 1998; Bueno et al., 2017). Insecticide selectivity might also be associated with metabolism by cytochrome P450-dependent monooxygenase, esterase, glutathione S-transferase and other enzymes of beneficial organisms. These enzymes usually detoxify lipophilic compounds, converting them into metabolites and allowing natural enemies to eliminate toxic compounds through their feces (Brattsten et al., 1986; Sturm and Hansen, 1999), 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 concentrations. Treatments 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 acts similarly on different insect species, beneficials or pests, with very similar nervous systems. Thus, pyrethroids have a broad spectrum and are generally classified as non-selective for most beneficial arthropod species (Carmo et al., 2010; Carvalho et al., 2021; Bueno et al., 2022a). Various insecticides in this chemical group have been previously reported as harmful to different beneficial arthropods (Croft and Whalon, 1982; Carvalho et al., 1999; Sterk et al., 1999; Stecca et al., 2018). Their use should be avoided and a replacement with more selective insecticides should be considered whenever possible. However, it is worth mentioning that the negative side-effects can vary according to the used concentration. Studied treatments containing lambda-cyhalothrin at higher concentrations (20 and 30 g/100 L H₂O) were more noxious than treatments with lower concentrations of the pyrethroid (14.1 and 17.7 g/100 L H₂O) to both parasitoid species. Furthermore, it is important to note that both parasitoid species pupae were more tolerant to the negative side-effects of insecticides than adults. The higher tolerance of parasitoid pupae to chemicals had already been reported in the 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 offered by the chorion of the host egg can vary according to the insecticide because the ability of a chemical to penetrate the chorion of an insect egg is related to its physicochemical properties. For example, chemicals with higher molecular weight have greater difficulty in crossing the

chorion (Stock and Holloway, 1993; Bueno et al., 2017), 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 and therefore can get contaminated at that point and die. Because of this, despite not having the ability to penetrate the chorion, some pesticides with longer residual times may still be able to kill natural enemies at the moment of adult emergence as a result of spraying that occurred at the pupae stage (Bueno et al., 2022a).

Both thiamethoxam and sulphoxaflor were only tested in mixtures with the pyrethroid as recommended to manage stink bugs in the field. Therefore, further assumptions about their selectivity cannot be made in 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). Chlorpyrifos was also reported herein to be harmful to the tested parasitoids. Similar reports were made for other organophosphates to different biocontrol agents (Carvalho et al., 2021; Bueno et al., 2022a). Noxious results of organophosphates were reported for *Trichogramma pretiosum* Riley and *Trichogramma cacoeciae* Marchal (Hymenoptera: Trichogrammatidae) (Bueno et al., 2008; Carvalho et al., 2021; Bueno et al., 2022a), among other beneficial insects. All those non-selective insecticides should be avoided whenever possible or replaced by other more selective insecticides inside IPM.

Among the tested insecticides, the mixture of neonicotinoids+pyrethroids and the organophosphates are among the most inexpensive insect-control products available to farmers, which can lead to an overuse. However, their application is not compatible with the preservation of the most important biological control agents of stink bugs, the egg parasitoids from the Scelionidae family, as shown here. Therefore, these chemicals should be used with caution, always adopting stink bug economic thresholds, and whenever possible be replaced by less harmful products in IPM programs. Good alternatives to those products, when feasible, include ethiprole, since its 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; Carvalho et al., 2021).

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 verify their higher toxicity and, consequently, be replaced with more selective pesticides.

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Conflicts of interest

The authors declare no financial interest.

Author contribution statement

WRS and AFB conceptualization, bioassays development. AFB and DMS data analysis. AFB, DMS and GAC writing and editing. AFB, DMS and GAC reference analysis. AFB and GAC final draft correction.

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