Lethal, Sublethal And Transgenerational Effects of Insecticides Labeled For Cotton On Immature *Trichogramma Pretiosum*

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Abstract

The egg parasitoid *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) regulates lepidopteran pest populations in cotton crops. However, cotton harbors dozens of pests, and growers rely on multiple insecticide applications to manage these damaging organisms. A harmonious integration of control tactics is required for proper pest management, and the use of selective insecticides (i.e., those promoting effective pest control while causing little impact on natural enemies) fits within this scope. This study aimed to assess the lethal, sublethal and transgenerational effects of insecticides from varying chemical groups on *T. pretiosum*. The insecticides were sprayed on parasitized host [*Ephestia kuehniella* (Zeller)] eggs with developing *T. pretiosum* stages (egg-larva, prepupa and pupa), and biological traits were assessed following adult emergence. Overall, pupae were more susceptible to the insecticides. We found thiodicarb and chlorfenapyr to reduce F0 adult emergence in rates comparable to the positive control (methomyl). Adult F0 deformation was the highest on flupyradifurone-treated organisms, and both the F0 parasitism rate and female survival were reduced by the insecticides (except for teflubenzuron). The sex ratio was affected by thiodicarb and flupyradifurone. Transgenerational effects occurred on adult emergence, which was reduced on the offspring (F1) of thiodicarb-, chlorfenapyr-, and flupyradifurone-treated *T. pretiosum*. In addition, thiodicarb lessened the F1 sex ratio. Combined, these results indicate that teflubenzuron is the safest insecticide; the other insecticides (especially thiodicarb and chlorfenapyr) are non-selective to *T. pretiosum*. Field and semifield studies are required to confirm the harmfulness of thiodicarb and chlorfenapyr towards *T. pretiosum*.

Introduction

Cotton is a crop widely cultivated in Brazil, one of the top five cotton-producing countries (Campos et al. 2019). Cotton cultivated area surpassed 1.6 million hectares in 2019, resulting in a production of 6.9 million tons of raw cotton (FAO 2021). The cotton wool is a commodity with multiple uses, and cotton cultivation provides substantial income and employment in Brazilian producing areas. The crop harbors dozens of yield-limiting arthropod species (Machado et al. 2019). These organisms are mainly managed through the application of broad-spectrum insecticides, sprayed in a calendar-based frequency, which can disrupt biological control exerted by beneficial arthropods (Moscardini et al. 2008).

The parasitoid wasp *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) is one of the main species used in biological control, being released in arable (e.g., cotton, corn, soybean, and vegetables) and wood crops for the control of lepidopteran pests (Smith 1996; Parra and Zucchi 2004; Laurentis et al. 2019). Under natural conditions, endemic populations of *T. pretiosum* parasitizes up to 98% of eggs of the cotton leafworm *Alabama argillacea* (Hübner) (Lepidoptera: Noctuidae), and parasitism rates increase over the crop cycle (Fernandes et al. 1999). Inundative releases of this parasitoid have been reported to yield 76% egg parasitism of *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae) in cotton (Saavedra et al. 1997), but establishment of augmented parasitoid populations is halted by spraying of non-selective insecticide (Stinner et al. 1974).

Trichogramma pretiosum controls pests in the egg stage, which poses a major advantage since it can potentially prevent pests from causing economic damage (Figueiredo et al. 2015); however, the diurnal behavior of this parasitoid renders it exposed to the direct application of insecticides (Pompanon et al. 1999; Reznik et al. 2009). Therefore, the use of selective insecticides is crucial for preserving this parasitoid populations and achieving effective rates of parasitism in the field (Parra and Zucchi 2004; Costa et al. 2014). Conceptually, selective insecticides are those that effectively control pests while concomitantly causing minimal toxicity on natural enemies and other beneficial organisms (Castle and Naranjo 2009; Bueno et al. 2017; Torres and Bueno 2018; Carvalho et al. 2019).

Thus, studies of the insecticides’ impacts on beneficial insects are central to achieving the compatibility of biological and chemical control methods; the assessment of lethal and sublethal effects of insecticides is required to thoroughly examine their risk upon non-target organisms (Desneux et al. 2007). Considering that the use of pesticides to control lepidopteran pests in cotton is a limiting factor for the success of the augmentative release of *Trichogramma* spp., this study aimed to evaluate the lethal and transgenerational effects of insecticides used in cotton on immature *T. pretiosum*.

Material And Methods

2.1 Insects

A colony of *T. pretiosum* was established in the Laboratory of Ecotoxicology at the Department of Entomology (Universidade Federal de Lavras). The colony was kept at 25 ± 2 C, 70 ± 10 % RH and a 12:12 h L:D photoperiod, and reared on UV-sterilized eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) (Insecta Produtos Biológicos, Lavras, Minas Gerais, Brazil). Host eggs (≤ 24 hours old), glued with arabic gum (50%) onto blue paper cards (8 × 1 cm²), were exposed to a 24-hours *T. pretiosum* kept in 1-L plastic containers sealed with plastic film. Then, the cards were transferred to new plastic containers and kept until adult *T. pretiosum* emergence, when the adults were supplied new host eggs. Adult wasps were fed honey droplets carefully placed on the container wall.

2.2. Insecticides

Adhering to IOBC guidelines, the insecticides were tested at their highest recommended dose rates for the control of cotton pests based on a spray solution of 200 L/ha (AGROFIT 2020). The active ingredients (a.i.), trade names, chemical groups, and concentrations (g or mL of a.i./L of spray solution) were as follows: teflubenzuron 150 g/L (Nomolt, Benzoylphenylurea, 0.125 mL), thiodicarb 800 g/kg (Larvin, Oxime methylcarbamate, 2.5 g), chlorfenapyr 240 g/L (Pirate, Pyrazol analogue, 3.0 mL), flupyradifurone 170.9 g/L (Sivanto, Butenolide, 1.875 mL), and the positive control methomyl 215 g/L (Lannate, Oxime methylcarbamate, 1.0 mL). The negative control consisted in the application of distilled water, the solvent of all insecticides.

2.3. Bioassays

Twenty-five *T. pretiosum* females were individualized in glass vials (8.5 × 2.5 cm) sealed with plastic films and offered, for a 24-hours period, UV-sterilized *E. kuehniella* eggs (n = 125) glued with arabic gum (50%) onto blue paper cards (8 × 1 cm²). Then, females were removed and the vials were kept at rearing conditions for three pre-determined periods (1, 4 and 8 days) corresponding to the egg-larval, prepupal and pupal parasitoid stages, respectively. Thus, cards with host eggs containing *T. pretiosum* at the varying life stages were sprayed under a Potter tower (Burkard, Uxbridge, UK) calibrated at 15 lb/pol^2 pressure to deposit 1.5 ± 0.5 µL/cm^2, as recommended by the IOBC (Sterk et al. 1999). The sprayed cards were air-dried at room temperature and subsequently individualized in glass vials kept at rearing conditions until wasp emergence. Evaluations were performed with the aid of a stereoscopic microscope (40× magnification) to determine egg parasitism (indicated by the blackening of the vitelline membrane of the host egg) and parasitoid emergence (presence of a wasp exit hole in the host egg) and, following adult emergence, the wasp sex (male or female) and deformation status (occurrence of antennaless and/or wingless adults). The bioassay was held in a completely randomized design (CRD) and a factorial layout (3 parasitoid stages × 6 insecticidal treatments), yielding 18 treatments with 5 replicates (a vial with 5 cards with parasitized host eggs). The assessed endpoints included the F0 emergence rate (emerged adults × 100 ÷ parasitized host eggs), F0 deformation rate (deformed wasp adults × 100 ÷ total wasp adults) and F0 sex ratio [proportion female = Σ▯ ÷ Σ(▯+▯)].

Transgenerational effects of the insecticides were also tested on the surviving F0 adults. Twenty females surviving from the insecticidal treatments were randomly collected and individualized in glass vials, as described previously. Each female was offered 125 UV-sterilized *E. kuehniella* eggs, free from insecticidal treatment, glued onto paper cards (5 × 0.5 cm²) for 24 hours. F0 female *T. pretiosum* were held in the vials, honey-fed, and assessed daily for survival until death, whereas the board cars were transferred to new vials for further assessments. Evaluations were also done to determine the F0 parasitism (parasitized host eggs × 100 ÷ total host eggs) and, following the F1 cycle completion, the F1 emergence rate, F1 adult deformation rate and F1 sex ratio. The bioassay was held in a CRD and a factorial layout across a range of parasitoid stages (egg-larva, prepupa and pupa) by insecticidal treatment (negative control and five a.i.) combinations. Due to the low survival of F0 adults emerging from methomyl-treated *T. pretiosum*, transgenerational studies were not performed for this insecticide.

2.4. Data analysis

All analyses were performed using R software (V. 3.5.3) and RStudio (V. 1.2.5001) (R Core Team, 2019). Data on adult emergence and deformation (F0 and F1), parasitism (F0) and sex ratio (F0 and F1) were checked for normality (Shapiro-Wilk test) and homoscedasticity of residuals (Bartlett test). A two-way ANOVA (*ExpDes* package; Ferreira et al., 2018) was performed to verify the effect of parasitoid stage, insecticidal treatment and their interaction on the assessed biological traits. In case of significance of the main factors, the Scott–Knott cluster analysis was used to separate their means. Significant interactions were probed with additional ANOVA and Scott–Knott cluster analyses.

Data on F0 female survival were analyzed using Kaplan–Meier estimators to obtain survival curves and estimates of median lethal times (LT_{50}). The overall similarity of the survival curves was tested through the Log-Rank test, and pairwise comparisons among the curves were performed with the Holm–Sidak's test. These analyses were implemented with functions from *survival* and *survminer* packages (Therneau 2020; Kassambara et al. 2021).

The insecticides were also categorized into toxicity classes based on their reduction in beneficial capacity (adult emergence of F0 and F1 and F0 parasitism rates) (Sterk et al. 1999). The reduction (R) was calculated thus: R = 100 - (insecticide treatment value × 100 ÷ control value). Toxicity categories were as follows: class 1 = harmless (R < 30%), class 2 = slightly harmful (30% ≤ R < 80%), class 3 = moderately harmful (80% ≤ R ≤ 99 %), and class 4 = harmful (R > 99%).

Afterwards, a hierarchical clustering analysis was performed to assist on interpretation of the bioassays' outcomes by applying functions (*dist* and *hclust*) from *stats* package. Dissimilarity (Euclidean distance) between all insecticidal treatments was quantified using mean values of the assessed endpoints (emergence, deformation and sex ratio – for F0 and F1, and LT_{50} and parasitism – for F0). The attributes (pooled mean values for each combination of insecticidal treatment with parasitoid stage) were standardized (mean = 0 and variance = 1) prior to the analysis (Wickham 2018). The dendrogram was constructed using the Ward's minimum variance method ("ward.D"), and the cut-off point was determined as per Mojena (Mojena 1977).

Results

The insecticides significantly affected the emergence ($F_{5,72}$= 54.60, P < 0.001), adult deformation ($F_{5,72}$= 87.42, P < 0.001) and parasitism rate ($F_{4,45}$ = 132.23, P < 0.001) of the parental wasps (F0). Conversely, diferences in responses of the exposed *T. pretiosum* stage occurred only for F0 emergence ($F_{2,72}$ = 14.58, P < 0.001) and deformation ($F_{2,72}$ = 14.30, P < 0.001). Thiodicarb, chlorfenapyr and methomyl reduced the F0 emergence in all developmental periods (egg-larvae, prepupae and pupae) (Table 1). The greatest reductions in F0 emergence occurred in the pupal stage, especially for thiodicarb, chlorfenapyr and flupyradifurone. Unlike the other insecticides, flupyradifurone increased *T. pretiosum* adult deformation. F0 parasitism of teflubenzuron exposed insects did not differ from that of control; the same did not occur for thiodicarb, chlorfenapyr and flupyradifurone (Table 1).

Survival curves of F0 females emerging from insecticide-treated immatures differed among themselves [log-rank test: χ^2 = 400.68, *d.f.* = 4, P < 0.001 (egg-larva); χ^2 = 419.44, *d.f.* = 4, P < 0.001 (prepupa); χ^2 = 455.70, *d.f.* = 4, P < 0.001 (pupa)]. Irrespective of the immature stage, all insecticides reduced the life span of females, with the exception of teflubenzuron. Notably, tiodicarbe e flupiradifurone caused the fastest mortalities, with median lethal times (LT_{50}) ranging from 1 to 4 days (Fig. 1).

Insecticides significantly impacted emergence ($F_{4,45}$ = 28.12, P < 0.001), but not adult deformation of F1 generation ($F_{4,45}$ = 1.59, P = 0.19). The exposed *T. pretiosum* stage did not affect both variables (emergence: $F_{2,45}$ = 0.97, P = 0.39; deformation: $F_{2,45}$ = 0.11, P = 0.90). Treatment with thiodicarb, chlorfenapyr and flupyradifurone led to reduced F1 adult emergence (Table 2).

According to the IOBC classification, teflubenzuron was harmless (class 1) to *T. pretiosum* for all variables assessed (F0 emergence, F0 parasitism and F1 emergence), irrespective of the exposed parasitoid stage (Fig. 2). Thiodicarb was slightly harmful in most situations, whereas flupyradifurone and chlorfenapyr had mixed classes, ranging from harmless (class 1) to moderately harmful (class 3).

Insecticides significantly affected the sex ratio of F0 ($F_{5,72}$ = 35.22, P< 0.001) and F1 generation ($F_{4,45}$ = 26.58, P< 0.001), whereas the exposed *T. pretiosum* stages did not (F0: $F_{2,72}$ = 2.69, P= 0.07; F1: $F_{2,45}$ = 2.28, P= 0.11). Treatment with teflubenzuron and chlorfenapyr did not reduce F0 sex ratio (Fig. 3a). Thiodicarb and methomyl, in turn, yielded lower F0 sex ratio for all *T. pretiosum* exposed stages, and flupyradifurone reduced this variable when prepupa and pupa were treated. Differently from the other compounds, thiodicarb reduced the F1 sex ratio (Fig. 3b).

Based on the Ward.D' cluster dendrogram, the insecticidal treatments were categorized into two groups. Teflubenzuron was the safest insecticide (i.e., the one grouped with the negative control) to *T. pretiosum*, followed by flupyradifurone, thiodicarb and chlorfenapyr (Fig. 4).

Discussion

Insecticides from varying chemical groups and modes of action were tested for lethal, sublethal and transgenerational effects on *T. pretiosum*. Overall, the compounds affected at least one of the assessed biological traits, and the degree of parasitoid response was mediated by the treated developmental stage.

Insecticides exhibited varying degrees of toxicity to *T. pretiosum*, which is likely resulted from the characteristics of the host chorion (e.g., thickness and chemical composition) and to the physicochemical properties (e.g., lipophilicity and molecular weight) of the tested products (Bacci et al. 2007). The reduced emergence of F0 individuals treated with chlorfenapyr can be attributed to its high log Kow = 5.28, which makes this compound highly lipophilic and easily absorbed through the host chorion and translocated up to its action site (Hoffmann et al. 2008). Although they have allowed the development of *T. pretiosum* immatures, which was evidenced by the accumulation of urate granules and blackening of the host egg, methomyl and thiodicarb reduced adult emergence; in fact, we observed unemerged adults inside the host eggs. Such an effect results from the adult intoxication when opening the exit hole due to contact and/or ingestion of insecticide residues retained on the host egg surface (Cônsoli et al. 2001).

The application of flupyradifurone, a butenolide closely related to the neonicotinoids, did not reduce the emergence of *T. pretiosum* (F0) for the egg-larva and prepupae stages. We hypothesize that this insecticide had limited penetration through the host egg chorion, resulted from its low log K_{ow} (1.2) (Nauen et al. 2015). Results obtained with flupyradifurone in the present study are similar to those of Moura et al. (2005), who verified that the neonicotinoid thiamethoxam is innocuous to *T. pretiosum*. Teflubenzuron, an IGR insecticide, also did not compromise *T. pretiosum* emergence, possibly due to its high molecular weight (381.10 g/mol), which hinders the penetration of the compound through the chorion (Stock and Holloway 1993). Furthermore, this compound acts primarily by ingestion, which reduces its toxicity towards natural enemies (Merzendorfer 2013). Our findings are supported by earlier reports of low toxicity of IGR compounds towards *Trichogramma galloi* Zucchi, *Trichogramma atopovirilia* Oatman & Platner and *T. nubilale* Ertle & Davis (Bueno et al. 2008; Maia et al. 2010; Wang et al. 2012; Souza et al. 2013; Costa et al. 2014).

Thiodicarb, chlorfenapyr and flupyradifurone were more toxic to *T. pretiosum* pupae than to the other stages. Costa et al. (2014) and Tabebordbar et al. (2020) found a similar pattern (higher pupa susceptibility) when studying the insecticide effect on *T. galloi* and *Trichogramma evanescens* Westwood. Because of the shorter interval between the insecticides spray on pupa and parasitoid emergence, the newly-emerged adults faced higher insecticide residues on the host egg surface than those from the other insecticide-treated stages (egg-larva and prepupa), leading to higher mortality of adults originated from treated pupae.

We found that all compounds, with the exception of teflubenzuron, reduced *T. pretiosum* F0 parasitism rates. Teflubenzuron was the only insecticide that did not diminish F0 female survival, and it is reasonable to assume that, as the teflubenzuron-treated females lasted longer, they could parasitize more eggs. Besides, the other compound might have impaired reproductive traits (e.g., sex discrimination, mate choice, and locomotor activity), leading to reduced parasitism by *T. pretiosum* (Dupont et al. 2010; Delpuech et al. 2012; Wang et al. 2018). The deleterious effects of thiodicarb and chlorfenapyr on *T. pretiosum* parasitism success has been previously reported, and they were both classified as slightly harmful (Moura et al. 2005; Bueno et al. 2008).

In flupyradifurone-treated immatures, F0 adult deformation might also have contributed to the lower parasitism rate. Insects were weakened following emergence and did not fully distend the wings; some adults even lacked these appendages. Another study reports higher deformation of adult *T. pretiosum* from neonicotinoid-treated host eggs (Moura et al. 2005), which corroborates our findings.

Some of the insecticides caused transgenerational effects on *T. pretiosum* emergence; thiodicarb, chlorfenapyr and flupyradifurone promoted lower F1 emergence, as opposed to the IGR teflubenzuron. In previous studies, triflumurom (another IGR) was found to not interfere with this biological trait of *T. pretiosum* (Carvalho et al. 2003; Vianna et al. 2008; Souza et al. 2013). The non-selectivity of flupyradifurone contrasts with a previous report of the harmlessness of neonicotinoids (acetamiprid and thiamethoxam) to the F1 emergence of *T. pretiosum* (Moura et al. 2005).

Methomyl, thiodicarb and flupyradifurone reduced the F0 sex ratio, whereas only methomyl reduced the sex ratio of the offspring (F1). Like many egg parasitoids, *T. pretiosum* reproduces by arrhenotokous parthenogenesis. Thus, males originate from unfertilized eggs and females from fecundated ones. Insecticides impair sex ratios (i.e., the proportion of females) by causing selective male mortality (Matioli et al. 2019), male sterility (Umoru and Powell 2002), altered mating behavior (Wang et al. 2018; Kremer and King 2019) and reduced viability of stored sperm in females (Rosenheim and Hoy 1988). These effects, isolated or combined, might have reduced the number of fertilized eggs, ultimately leading to a male-biased sex ratio. Both teflubenzuron and chlorfenapyr did not affect the sex ratio of the F0 and F1 generations; these results are in line with earlier reports of innocuity of IGR (triflumuron) and chlorfenapyr to the sex ratio of *T. pretiosum* (Moura et al. 2005; Souza et al. 2013).

In summary, all tested insecticides, except for teflubenzuron, presented deleterious effects on *T. pretiosum*, and thiodicarb and chlorfenapyr caused the most striking negative responses. Therefore, teflubenzuron should be prioritized in IPM programs of lepidopteran pests in cotton. Since environmental conditions (e.g., temperature, radiation and rainfall) mediate insecticide degradation and potentially reduce their toxicity towards insects (Maia et al. 2016), further studies in semifield and field conditions are required to confirm the side effects of thiodicarb and chlorfenapyr on *T. pretiosum*.

Declarations

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References

1. AGROFIT (2020) Sistema de agrotóxicos fitossanitários. http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons. Accessed 19 Aug 2020
2. Bacci L, Crespo AL, Galvan TL et al (2007) Toxicity of insecticides to the sweetpotato whitefly (Hemiptera: Aleyrodidae) and its natural enemies. Pest Manag Sci 63:699–706
3. Bueno AF, Bueno RCOF, Parra JRP, Vieira SS (2008) Effects of pesticides used in soybean crops to the egg parasitoid *Trichogramma pretiosum*. Cienc Rural 38:1495–1503
4. Bueno AF, Carvalho GA, Santos AC et al (2017) Pesticide selectivity to natural enemies: Challenges and constraints for research and field recommendation. Ciência Rural 47:6
5. Campos KL, Araújo TA, Showler AT et al (2019) Integration of cotton plant resistance with selected organic boll weevil, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae). J Agric Sci 11:1–15
6. Carvalho GA, Grützmacher AD, Passos LC, Oliveira RL (2019) Physiological and ecological selectivity of pesticides for natural enemies of insects. In: Souza B, Vázquez L, Marucci R (eds) Natural enemies of insect pests in neotropical agroecosystems: Biological control and functional biodiversity. Springer, New York, pp 469–478
7. Carvalho GA, Roberto J, Parra P, Casadei Baptista G (2003) Bioactivity of pesticides used in tomato (*Lycopersicon esculentum* Mill.) crop to *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) in F1 and F2 generations. Ciência e Agrotecnologia 27:261–270
8. Castle S, Naranjo SE (2009) Sampling plans, selective insecticides and sustainability: The case for IPM as 'informed pest management'. Pest Manag Sci 65:1321–1328
9. Cônsoli FL, Botelho PSM, Parra JRP (2001) Selectivity of insecticides to the egg parasitoid *Trichogramma galloi* Zucchi, 1988, (Hym., Trichogrammatidae). J Appl Entomol 125:37–43
10. Costa MA, Moscardini VF, Gontijo PC et al (2014) Sublethal and transgenerational effects of insecticides in developing *Trichogramma galloi* (Hymenoptera: Trichogrammatidae). Ecotoxicology 23:1399–1408
11. Delpuech JM, Dupont C, Allemand R (2012) Effects of deltamethrin on the specific discrimination of sex pheromones in two sympatric *Trichogramma* species. Ecotoxicol Environ Saf 84:32–38
12. Desneux N, Decourtye A, Delpuech J-M (2007) The sublethal effects of pesticides on beneficial arthropods. Annu Rev Entomol 52:81–106
13. Dupont C, Allemand R, Delpuech JM (2010) Induction by chlorpyrifos, of the confusion of males in discriminating female sexual pheromones used for mate finding by two sympatric *Trichogramma* species (Hymenoptera: Trichogrammatidae). Environ Entomol 39:535–544
14. FAO (2021) FAOSTAT: Agriculture data. http://www.fao.org/faostat/en/#data/QC. Accessed 8 Aug 2021
15. Fernandes MG, Busoli AC, Paulo E, Degrande E (1999) Natural parasitism of *Alabama argillacea* Hüb. and *Heliothis virescens* Fab. (Lep.: Noctuidae) by *Trichogramma pretiosum* Riley (Hym.: Trichogrammatidae) on cotton in the state of Mato Grosso do Sul. An da Soc Entomológica do Bras 28:701
16. Ferreira EB, Cavalcanti PP, Nogueira DA (2018) ExpDes.pt: Pacote experimental designs (Portuguese). https://cran.r-project.org/package=ExpDes.pt. Accessed 7 Jul 2021
17. Figueiredo MLC, Cruz I, Silva RB, Foster JE (2015) Biological control with *Trichogramma pretiosum* increases organic maize productivity by 19.4%. Agron Sustain Dev 35:1175–1183

18. Hoffmann EJ, Middleton SM, Wise JC (2008) Ovicidal activity of organophosphate, oxadiazine, neonicotinoid and insect growth regulator chemistries on the northern strain plum curculio, *Conotrachelus nenuphar*. J Insect Sci 8:29
19. Kassambara A, Kosinski M, Biecek P (2021) survminer: Drawing survival curves using "ggplot2." https://cran.r-project.org/package=survminer. Accessed 7 Jul 2021
20. Kremer AN, King BH (2019) A neonicotinoid affects the mating behavior of *Spalangia endius* (Hymenoptera: Pteromalidae), a biological control agent of filth flies. Environ Entomol 48:489–495
21. Laurentis VL, Ramalho DG, Santos NA et al (2019) Performance of *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) on eggs of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). Sci Rep 9:1156
22. Machado AVA, Potin DM, Torres JB et al (2019) Selective insecticides secure natural enemies action in cotton pest management. Ecotoxicol Environ Saf 184:109669
23. Maia JB, Carvalho GA, Leite MIS et al (2010) Selectivity of insecticides used in corn crops to adult *Trichogramma atopovirilia* (Hymenoptera: Trichogrammatidae). Rev Colomb Entomol 36:202–206
24. Maia JB, Carvalho GA, Medina P et al (2016) Lethal and sublethal effects of pesticides on *Chrysoperla carnea* larvae (Neuroptera: Chrysopidae) and the influence of rainfastness in their degradation pattern over time. Ecotoxicology 25:845–855
25. Matioli TF, Zanardi OZ, Yamamoto PT (2019) Impacts of seven insecticides on *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae). Ecotoxicology 28:1210–1219
26. Merzendorfer H (2013) Chitin synthesis inhibitors: Old molecules and new developments. Insect Sci 20:121–138
27. Mojena R (1977) Hierarchical grouping methods and stopping rules: An evaluation. Comput J 20:359–363
28. Moscardini VF, Moura AP, Carvalho GA, Lasmar O (2008) Residual effect of synthetic insecticides on *Trichogramma pretiosum* Riley (Hym., Trichogrammatidae) in different generations. Acta Sci Agron 30:177–182
29. Moura AP, Carvalho GA, Rigitano RLO (2005) Toxicity of insecticides used in tomato crop to *Trichogramma pretiosum*. Pesqui Agropecuária Bras 40:203–210
30. Nauen R, Jeschke P, Velten R et al (2015) Flupyradifurone: A brief profile of a new butenolide insecticide. Pest Manag Sci 71:850–862
31. Parra JRP, Zucchi RA (2004) *Trichogramma* in Brazil: Feasibility of use after twenty years of research. Neotrop Entomol 33:271–281
32. Pompanon F, Fouillet P, Bouletreau M (1999) Physiological and genetic factors as sources of variation in locomotion and activity rhythm in a parasitoid wasp (*Trichogramma brassicae*). Physiol Entomol 24:346–357
33. R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
34. Reznik SY, Voinovich ND, Karpova SG (2009) Daily rhythms in parasitization of the Angoumois grain moth *Sitotroga cerealella* Oliv. (Lepidoptera, Gelechiidae) eggs by the egg parasitoid *Trichogramma principium* Sug. et Sor. (Hymenoptera, Trichogrammatidae) females. Entomol Rev 89:5–15
35. Rosenheim JA, Hoy MA (1988) Sublethal effects of pesticides on the parasitoid *Aphytis melinus* (Hymenoptera: Aphelinidae). J Econ Entomol 81:476–483
36. Saavedra JLD, Torres JB, Ruiz MG (1997) Dispersal and parasitism of *Heliothis virescens* eggs by *Trichogramma pretiosum* (Riley) in cotton. Int J Pest Manag 43:169–171
37. Smith SM (1996) Biological control with *Trichogramma*: Advances, successes, and potential of their use. Annu Rev Entomol 41:375–406
38. Souza JR, Carvalho GA, Moura AP et al (2013) Impact of insecticides used to control *Spodoptera frugiperda* (J.E. Smith) in corn on survival, sex ratio, and reproduction of *Trichogramma pretiosum* Riley offspring. Chil J Agric Res 73:122–127
39. Sterk G, Hassan SA, Baillod M et al (1999) Results of the seventh joint pesticide testing programme carried out by the IOBC/WPRS-Working Group 'Pesticides and Beneficial Organisms. ' BioControl 44:99–117
40. Stinner RE, Ridgway RL, Coppedge JR et al (1974) Parasitism of *Heliothis* eggs after field releases of *Trichogramma pretiosum* in cotton. Environ Entomol 3:497–500
41. Stock D, Holloway PJ (1993) Possible mechanisms for surfactant-induced foliar uptake of agrochemicals. Pestic Sci 38:165–177
42. Tabebordbar F, Shishehbor P, Ziaee M, Sohrabi F (2020) Lethal and sublethal effects of two new insecticides spirotetramat and flupyradifurone in comparison to conventional insecticide deltamethrin on *Trichogramma evanescens* (Hymenoptera: Trichogrammatidae). J Asia Pac Entomol 23:1114–1119
43. Therneau TM (2020) A package for survival analysis in R. https://cran.r-project.org/package=survival. Accessed 7 Jul 2021
44. Torres JB, Bueno AF (2018) Conservation biological control using selective insecticides – A valuable tool for IPM. Biol Control 126:53–64
45. Umoru PA, Powell W (2002) Sub-lethal effects of the insecticides pirimicarb and dimethoate on the aphid parasitoid *Diaeretiella rapae* (Hymenoptera: Braconidae) when attacking and developing in insecticide-resistant hosts. Biocontrol Sci Technol 12:605–614
46. Vianna UR, Pratissoli D, Zanuncio JC et al (2008) Insecticide toxicity to *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) females and effect on descendant generation. Ecotoxicology 18:180–186
47. Wang D, Lü L, He Y (2018) Effects of two conventional insecticides on male-specific sex pheromone discrimination and mate choice in Trichogramma chilonis (Hymenoptera: Trichogrammatidae). Environ Entomol 46:328–334
48. Wang Y, Yu R, Zhao X et al (2012) Susceptibility of adult *Trichogramma nubilale* (Hymenoptera: Trichogrammatidae) to selected insecticides with different modes of action. Crop Prot 34:76–82
49. Wickham H (2018) scales: Scale functions for visualization. https://cran.r-project.org/package=scales. Accessed 10 Oct 2020

Tables

Table 1. F0 emergence (%) and adult deformation (%), and parasitism (%) of *Trichogramma pretiosum* exposed to different insecticidal treatments at varying life stages (egg-larva, prepupa and pupa).

Treatment	F0 emergence ^{a} (%)			Adult F0 deformation ^{b} (%)			F0 parasitism ^{c} (%)	
	Egg-larva	Prepupa	Pupa	Egg-larva	Prepupa	Pupa	Egg-larva	Prepupa
Control	88.3±2.79aA	85.4±4.02aA	84.5±3.18aA	1.56±0.6cA	0.24±0.1bB	0.0±0.0bB	83±1.87aA	73.1±3.45aA
Teflubenzuron	85.6±5.97aA	83.8±3.10aA	84.7±8.07aA	1.64±0.5cA	0.8±0.32bB	0.84±0.4bB	72.6±5aA	58.1±5.92bA
Thiodicarb	52.6±1.95bA	40.5±8.93bA	14.5±1.51cB	2.24±0.6bA	0.44±0.1bB	0.68±0.2bB	21.8±5.19bA	20.7±4.49cA
Chlorfenapyr	60.8±10.86bA	42.0±12.91bB	15.6±1.48cB	0.8±0.23dA	0.76±0.2bA	0.16±0.0bA	18.5±1.97bA	28.1±11.3cA
Flupyradifurone	76.6±10.39bA	78.5±1.28aA	53.2±6.31bB	4.0±1.08aB	4.8±0.75aA	2.76±0.4aC	9±1.6cA	14.3±2.9dA
Methomyl	16.7±7.45cA	14.0±6.36cA	11.3±5.23cA	0.16±0.0dA	0.12±0.0bA	0.24±0.0bA	-	-

Means (± SEM) with different letters, uppercase in the row and lowercase in the column, differ by the Scott-Knott test ($P < 0.05$).

[a]Significant interaction between the factors (insecticide and parasitoid stage): $F_{10,72} = 3.17$, $P = 0.002$

[b]Significant interaction between the factors (insecticide and parasitoid stage): $F_{10,72} = 11.61$, $P < 0.001$

[c]Significant interaction between the factors (insecticide and parasitoid stage): $F_{8,45} = 3.52$, $P = 0.003$

Table 2. F1 emergence (%) and adult deformation (%) of *Trichogramma pretiosum* exposed to different insecticidal treatments at varying life stages (egg-larva, prepupa and pupa).

Treatment	F1 emergence ^{a} (%)			Adult F1 deformation ^{b} (%)		
	Egg-larva	Prepupa	Pupa	Egg-larva	Prepupa	Pupa
Control	88.7±0.4a	90.7±0.52a	86.8±5.79a	0.8±0.28a	0.0±0.0a	0.9±0.33a
Teflubenzuron	80.8±2.43a	88.9±1.55a	85.5±3.39a	0.8±0.29a	0.4±0.21a	1.0±0.29a
Thiodicarb	42.4±2.36c	51.4±3.25c	40.3±13.46b	1.0±0.67a	0.5±0.31a	1.2±0.67a
Chlorfenapyr	62.3±1.66b	70.5±0.32b	64.3±5.81b	0.15±0.1a	0.5±0.26a	0.35±0.15a
Flupyradifurone	41.4±11.31c	55.1±4.52c	59.7±4.68b	2.8±1.17a	1.2±0.62a	3.1±1.17a
Methomyl	-	-	-	-	-	-

Within each column, means (± SEM) with different letters differ by the Scott-Knott test ($P < 0.05$).

[a]Non-significant interaction between the factors (insecticide and parasitoid stage): $F_{8,45} = 0.82$, $P = 0.59$

[b]Non-significant interaction between the factors (insecticide and parasitoid stage): $F_{8,45} = 0.63$, $P = 0.75$

Figures

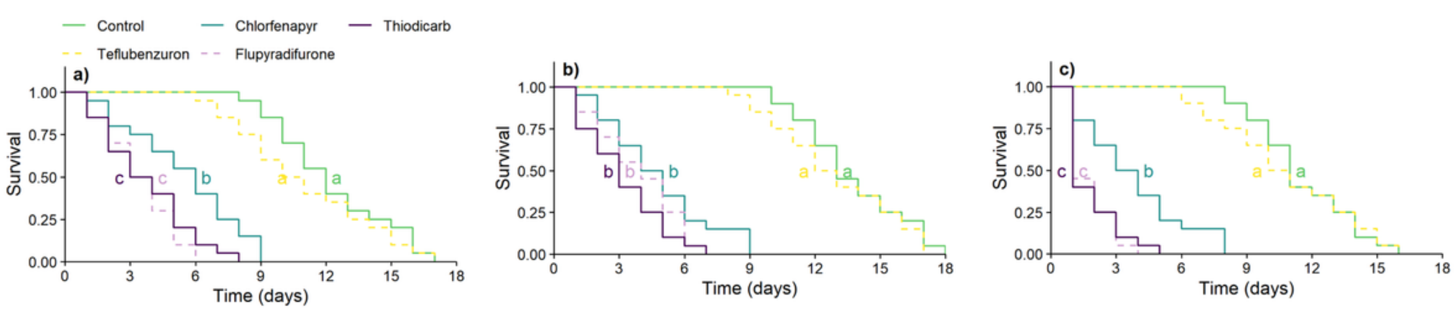

Figure 1

Survival curves of Trichogramma pretiosum females exposed to insecticidal treatments (control, teflubenzuron, chlorfenapyr, flupyradifurone, and thiodicarb) at varying life stages [a) egg-larva, b) prepupa, and c) pupa]. Curves with different lowercase letters differ significantly among themselves (P < 0.05, Holm–

Sidak test).

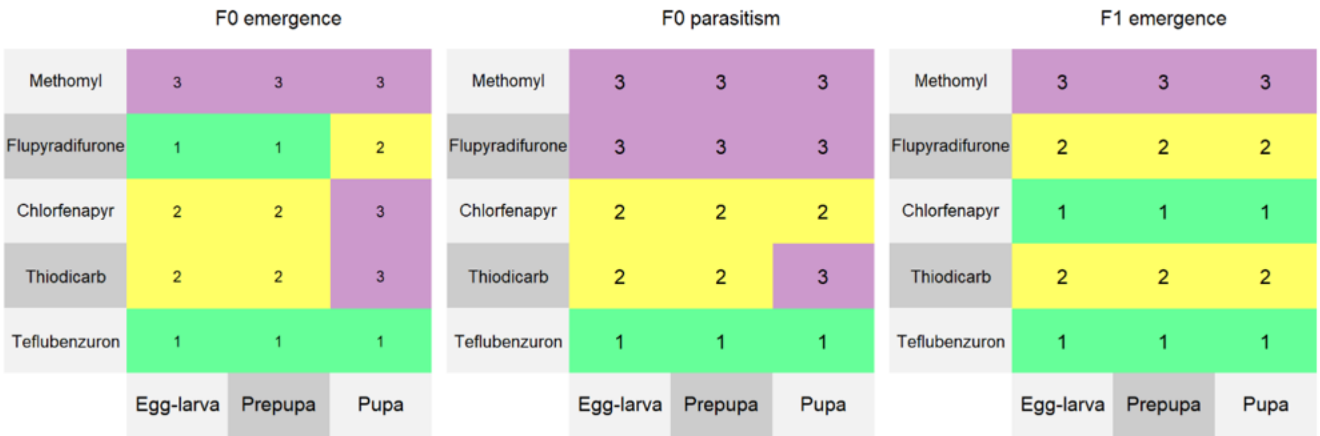

Figure 2

Heatmap diagram of the IOBC classification of insecticides (teflubenzuron, chlorfenapyr, flupyradifurone, thiodicarb, and methomyl) based on their effects on Trichogramma pretiosum biological traits (F0 emergence, F0 parasitism and F1 emergence) when the parasitoid was treated at varying life stages (egg-larva, prepupa and pupa).

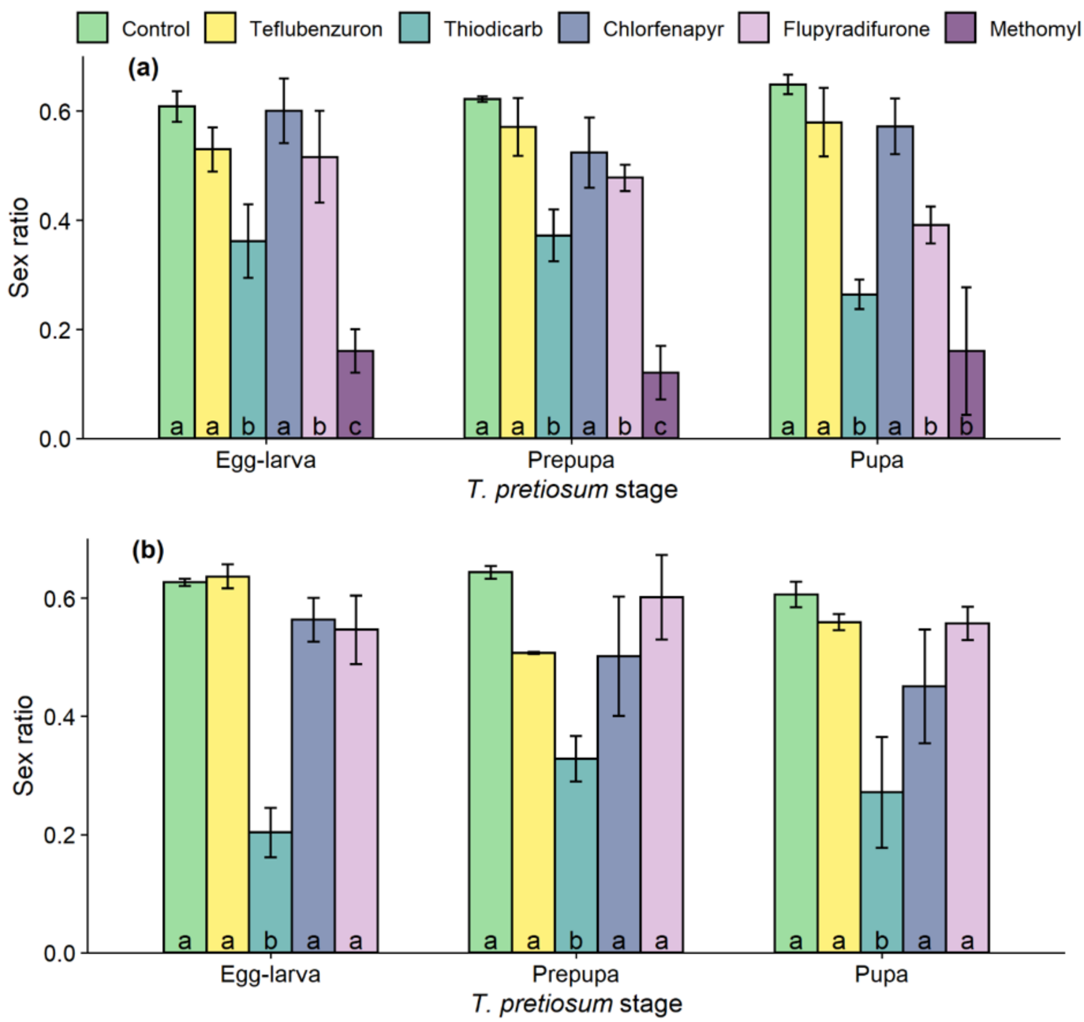

Figure 3

Sex ratio of the F0 and F1 generation of Trichogramma pretiosum exposed to different insecticidal treatments at varying life stages (egg-larva, prepupa and pupa). Within each T. pretiosum stage, bars (mean ± SEM) with different letters differ by the Scott-Knott test (P < 0.05).

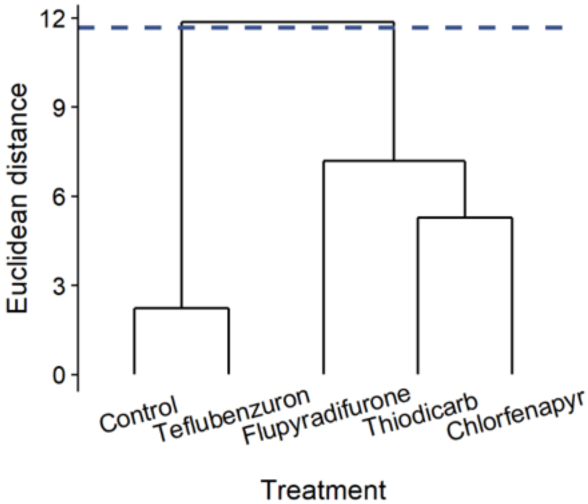

Figure 4

Cluster dendrogram (built with Ward's method) of six insecticidal treatments (negative control, teflubenzuron, flupyradifurone, thiodicarb, and chlorfenapyr) based on the dissimilarity (Euclidean distance) of their effects on biological traits of Trichogramma pretiosum. Two groups were formed, as indicated by the cut-off line.