

# Impacts of seven insecticides on Cotesia flavipes (Cameron) (Hymenoptera: Braconidae)

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#### Abstract

The endoparasitoid wasp Cotesia flavipes (Cameron) (Hymenoptera: Braconidae) is inundatively released in Brazilian sugarcane plantations to control the sugarcane borers Diatraea saccharalis (Fabricius) and Diatraea flavipennella (Box) (Lepidoptera: Crambidae). In conjunction with these releases, several synthetic insecticides are used to control the neonate larvae of these pests. We assessed the lethal and transgenerational sublethal effects of seven of these insecticides on C. flavipes. Leaf discs were sprayed at the highest field concentrations of chlorantraniliprole, lambda-cyhalothrin + chlorantraniliprole, chlorfluazuron, triflumuron, lambda-cyhalothrin + thiamethoxam, tebufenozide, and novaluron. Distilled water was used as a negative control. Newly emerged females (24 h old) were placed in Petri dishes containing the treated leaves, and the lethal and transgenerational sublethal effects were assessed for the next two generations. Lambda-cyhalothrin  $+$ chlorantraniliprole and lambda-cyhalothrin + thiamethoxam caused  $100\%$  mortality of the parasitoid and were highly persistent, causing more than 30% mortality at 30 days after spraying. Chlorantraniliprole, chlorfluazuron, novaluron, and triflumuron did not cause significant mortality compared to the negative control, but did have transgenerational sublethal effects. The length of the tibia of the right posterior leg, used as a growth measurement, was reduced in the progeny  $(F<sub>1</sub>$  generation) of exposed female parasitoids. In addition, chlorantraniliprole increased and chlorfluazuron reduced the proportion of females in the  $F_1$  generation, whereas novaluron reduced the proportion of females in the  $F_2$  generation. Overall, only tebufenozide was considered harmless to C. flavipes. The results of this study suggest that lambda-cyhalothrin + chlorantraniliprole and lambda-cyhalothrin + thiamethoxam are harmful to  $C$ . *flavipes*, although field studies are needed to obtain results for actual sugarcane crops.

Keywords Diatraea saccharalis · Larval parasitoid · Toxicity · Transgenerational sublethal effects · Integrated pest management

## Introduction

Brazil is the world's largest sugarcane producer, with a harvest of 633 million tons on approximately 9 million hectares in 2017/2018 (Conab [2018\)](#page-7-0). In spite of this high production, the incidence of pests is one of the major factors limiting further increases in crop yield (Oliveira et al. [2014](#page-8-0)).

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The sugarcane borers Diatraea saccharalis (Fabricius) and Diatraea flavipennella (Box) (Lepidoptera: Crambidae) are key pests of sugarcane, due to their direct and indirect damage, biotic potential, adaptability to different environmental conditions, and the abundance of host plants (Joyce et al. [2014;](#page-8-0) Pavinato et al. [2018](#page-8-0); Pinto et al. [2006\)](#page-8-0). Neonate larvae initially feed on the leaf parenchyma, and the secondinstar larvae migrate to the base of the plant where they penetrate the stem, opening longitudinal and transverse galleries that block the sap flow, reducing sucrose accumulation and crop yield. On young plants, these borers can dry the shoots, which prevents further growth and eventually kills the plant (Dinardo-Miranda [2008](#page-7-0)); and they weaken older plant stalks, which may topple in the wind (Trevisan et al. [2016](#page-9-0)).

Because the larvae develop almost completely inside the stem, D. saccharalis has been controlled preferably by

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means of biological-control agents. The koinobiont parasitoid wasp Cotesia flavipes (Cameron) (Hymenoptera: Braconidae) is the most effective and widely used control agent to reduce the population levels and damage caused by sugarcane borers in production systems, where the levels of parasitism can vary from 13 to 43% (Dinardo-Miranda et al. [2014\)](#page-7-0). Following the introduction of C. flavipes in Brazil in the 1970s, several companies and biofactories have begun to mass-produce this natural enemy, which is released on approximately 3.5 million hectares of sugarcane crops (Parra [2014;](#page-8-0) Vacari et al. [2012](#page-9-0)). The parasitoid females efficiently locate the larvae inside the stem through olfactory stimuli (Molnár et al. [2016;](#page-8-0) Sétamou et al. [2002](#page-9-0)). Parasitized sugarcane-borer larvae show reductions in food consumption and cellular immune capacity (Mahmoud et al. [2011\)](#page-8-0). After the parasitoid exits, the larvae lose weight and die (Passos et al. [2014](#page-8-0); Rossi et al. [2014](#page-8-0)). In the 1990s, inundative releases of C. flavipes reduced the sugarcaneborer populations from 10 to 2%, preventing approximately U\$80 million in losses to growers (Vilela et al. [1998](#page-9-0)). Currently, the control of sugarcane borers with C. flavipes constitutes one of the largest applied biological-control programs in the world (Parra et al. [2014](#page-8-0); Santos et al. [2015](#page-8-0)).

Although C. flavipes is an important biological-control agent of sugarcane borers in production systems, synthetic insecticides are sprayed on leaves to reduce the population of newly hatched larvae present in the crop, since the firstand second-instar larvae of D. saccharalis remain outside the stems (Van Leerdam et al. [1985;](#page-9-0) Wiedenmann et al. [1992\)](#page-9-0). Diamide, benzoylurea, diacylhydrazine, pyrethroid, and neonicotinoid insecticides have been the synthetic insecticides most often used to control sugarcane borers and other pests that cause significant damage to the plants (MAPA [2019](#page-8-0)). However, the use of these compounds may also reduce the effectiveness of C. flavipes and affect D. saccharalis and D. flavipennella biological-control programs in sugarcane. In addition to mortality, these insecticides can also affect the biological (development of immature stages, fecundity, fertility, longevity, and sex ratio) and behavioral (parasitism, mobility, and orientation) parameters of natural enemies (Desneux et al. [2007](#page-7-0); Guedes et al. [2016\)](#page-7-0), thereby reducing the biocontrol services that they provide (Biondi et al. [2015\)](#page-7-0). Several studies have determined the toxicity levels of insecticides to parasitoids belonging to the genus *Cotesia* Cameron (Hymenoptera: Braconidae) (Haseeb et al. [2004](#page-7-0); Lin et al. [2007](#page-8-0); Mardani et al. [2016;](#page-8-0) Ohta and Takeda [2015\)](#page-8-0), but few have used the lethal and transgenerational sublethal effects to assess the impact of these compounds on C. flavipes (Fonseca et al. [2015\)](#page-7-0).

In view of the importance of C. flavipes as a biologicalcontrol agent of D. saccharalis and D. flavipennella, and the need for studies to assess the compatibility of this natural enemy with chemical control, in this study we assessed the lethal and transgenerational sublethal effects of seven commonly used insecticides on C. flavipes. The results will contribute not only to understanding the impact of these insecticides on C. flavipes, but also to evaluating the practical implications of releasing this parasitoid in combination with insecticide sprayings, and will help to develop management strategies to reduce the impacts of these compounds in sugarcane production systems.

#### Material and methods

#### Insects

A colony of D. saccharalis was established from a population maintained under laboratory conditions, reared on an artificial diet based on soybean meal, wheat germ, sugar, vitamins, Wesson salts, ascorbic acid, and water as described by Hensley and Hammond ([1968\)](#page-8-0) and modified by Araújo et al. ([1985\)](#page-7-0). When the larvae reached the fifth instar, they were subjected to parasitism by females of C. flavipes obtained from a colony kept in the laboratory for several generations at the Iracema Farm headquarters of the São Martinho Group, municipality of Pradópolis, São Paulo, Brazil. About 60 newly emerged adult parasitoids (≤24 h old) were placed in plastic cages (6.0 cm in diameter  $\times$  6.0 cm high) with honey drops for 24 h, to allow them to mate. D. saccharalis fifth-instar larvae were exposed to the parasitoids through a hole in the cover of each plastic cage. The parasitized larvae were placed in Petri dishes (10 cm in diameter  $\times$  2 cm high) with the diet. Fifteen days after parasitism, parasitoid larvae started to leave the host and pupate. The pupae were removed from the Petri dishes and transferred to new plastic cages, where they were kept until the adult parasitoids emerged. The rearing was performed in a climate-controlled room with a temperature of  $25 \pm 1$  °C, relative humidity (RH)  $70 \pm 10\%$ , and 14 L: 10D h photoperiod. For all assays, newly emerged females ( $\leq$ 24 h old) of *C. flavipes* were used.

## Chemicals

Seven commercial insecticides widely used for controlling D. saccharalis in sugarcane production systems were tested on C. flavipes, using the maximum field concentration recommended to control this pest by the manufacturers of the insecticides (MAPA [2019](#page-8-0)). The insecticides and concentrations assessed were as follows: chlorantraniliprole 84 mg L<sup>-1</sup> (Altacor 35% w/w, diamide, wettable granules, Du Pont do Brasil Ltd., Barueri, SP, Brazil); lambda-cyhalothrin + chlorantraniliprole  $33.5 + 67$  mg L<sup>-1</sup> (Ampligo  $5 + 10\%$  w/v, pyrethroid + diamide, concentrated suspension, Syngenta

Proteção de Cultivos Ltd., São Paulo, SP, Brazil); chlorfluazuron 85 mg L<sup>-1</sup> (Atabron 5%, w/v, benzoylurea, emulsifiable concentrate, ISK Biosciences do Brasil Defensivos Agrícolas Ltd., Indaiatuba, SP, Brazil); triflumuron 129.6 mg  $L^{-1}$  (Certero 48% w/v, benzoylurea, concentrated suspension, Bayer S.A., São Paulo, SP, Brazil); lambda-cyhalothrin + thiamethoxam  $100 + 141$  mg L<sup>-1</sup> (Engeo Pleno  $10 + 14.1\%$ ) pyrethroid + neonicotinoid,  $w/v$  concentrated suspension, Syngenta); tebufenozide 480 mg L−<sup>1</sup> (Mimic 24% w/v, diacylhydrazine, concentrated suspension, Iharabras S.A. Indústrias Químicas, Sorocaba, SP, Brazil); and novaluron  $50 \text{ mg } L^{-1}$  (Rimon 10% w/v, benzoylurea, emulsifiable concentrate, Adama Brasil S.A., Londrina, PR, Brazil) (IRAC [2019](#page-8-0)). Distilled water was used as a negative control treatment.

## Toxicity of insecticides to Cotesia flavipes females

To assess the insecticide effects on C. flavipes females, discs (3.3 cm in diameter) of sugarcane leaves (cv. RB84-7515) were cut and placed in acrylic plates (3.5 cm in diameter) with a layer of an agar: water solution (2.5%), and used as experimental units. Then, the discs were sprayed with 2 mL of one of the solutions in a Potter tower (Burkard Scientific Co., Uxbridge, UK) adjusted to a 68.6 kPa pressure, resulting in  $1.8 \pm 0.1$  mg cm<sup>-2</sup> deposition of fresh residues, which is consistent with the criteria established by the Pesticides and Beneficial Organisms Working Group of the International Organization for Biological Control of Noxious Animals and Plants/West Palearctic Regional Section (IOBC/WPRS) for studies of pesticide toxicity to natural enemies (Hassan [1994\)](#page-7-0). After spraying, the discs were kept in a climate-controlled room for 2 h to allow the residues to dry. Twelve C. flavipes females (24 h old) were anesthetized with  $CO<sub>2</sub>$  for 10 s and released in each experimental unit, with one larva per female for parasitizing. The experimental units were the acrylic plates covered with plastic caps with centrally drilled holes (2.5 cm in diameter) screened with voile fabric to allow gas exchange and to prevent excess moisture accumulation. A honey droplet  $(-1 \text{ mm}^3)$  was used to feed the parasitoids during the assessment period. For each treatment, five replicates were used. The experimental units were kept in a climate-controlled room with a temperature of  $25 \pm 1$  °C,  $70 \pm 10\%$  RH, and 14 L: 10D h photoperiod. The number of live/dead parasitoids was recorded 24 h after exposure to the residues. Parasitoids that did not react to the touch of a thin brush were considered dead.

## Transgenerational sublethal effects of insecticides on Cotesia flavipes

The transgenerational sublethal effects of insecticides were assessed for products that caused lower mortalities in the

previous bioassay. For this purpose, 20 C. flavipes females surviving from each treatment were placed in individual flat-bottom glass tubes  $(2.5 \text{ cm in diameter} \times 8.5 \text{ cm high})$ . Then, a *D. saccharalis* fifth-instar larva was placed in each tube, to allow parasitism for 30 s. The parasitized larvae were transferred to acrylic plates (6 cm in diameter) containing a refeeding diet (Hensley and Hammond [1968\)](#page-8-0) with addition of acetic acid and no Wesson salts, and monitored until pupation.

The egg-pupa development time of the parasitoid was determined based on the duration (in days) between the parasitism and pupa formation  $(F_1 \text{ generation})$ . Parasitoid pupae emerging from each parasitized borer larva were counted and weighed, using an analytical balance with precision 0.0001 g (Ohaus Explorer E02140, Stacey Cochiara, Gaithersburg, MD), 24 h after formation. The pupae were then transferred to flat-bottom glass tubes and maintained until the adults emerged. The pupal development time was determined based on the duration (in days) between the formation of the pupa and the parasitoid emergence. Adults were separeted by gender to determine the sex ratio and released inside a small cage  $(20 \times 20 \times$ 20 cm) for mating for 24 h. Next, 20 mated females from each treatment were randomly collected and transferred to flat-bottom glass tubes in order to assess the parasitism capacity of the  $F_2$  generation, following the same procedure and criteria described above.

For the progeny  $(F_1)$  of exposed female parasitoids, the tibia length of the right hind leg was also measured and used as a criterion to estimate the transgenerational sublethal effects of these insecticides on C. flavipes development. For this purpose, 20 parasitoid females from each treatment were randomly collected and stored in individual Eppendorf tubes with a 70% ethanol solution for 7 days. Next, each female was cleared, following the same procedure used for Trichogramma spp. (Querino and Zucchi [2011\)](#page-8-0), and mounted on a slide. The tibia length of each specimen was measured at 40 × magnification, under a Zeiss light microscope (Carl Zeiss do Brasil Ltd., São Paulo, SP, Brazil) coupled to a digital camera (IS300, Eurekam Bel 3.0 MP), using the software Bel Capture version 3.0.

## Persistence of the residual effects of insecticides on Cotesia flavipes

The duration of the residual effects of insecticides on C. flavipes was assessed for products that caused high mortalities in the first bioassay. For this purpose, 'RB84- 7515' sugarcane plants at five months of age (70–90 cm high) grown in 20-L pots in a greenhouse were used as a substrate for spraying the treatments. For each treatment, five plants were sprayed with a volume corresponding to ~125 mL per plant, using a Jacto PJH manual sprayer (Jacto do Brasil S.A., Pompéia, SP, Brazil) equipped with a FL-5VS conical nozzle (Teejet Technologies Company, São Paulo, SP, Brazil). At 5, 15, and 30 days after spraying (DAS), two leaves were randomly collected from each plant, and in the laboratory, discs (3.3 cm in diameter) were cut, placed in acrylic plates (3.5 cm in diameter) with an agar: water solution (2.5%), and used as experimental units. Twenty newly emerged adults  $(\leq 24 h$  old) of C. flavipes were anesthetized with  $CO<sub>2</sub>$  for 10 s and released in each experimental unit. The experimental units were the acrylic plates covered with plastic caps with centrally drilled holes (2.5 cm in diameter) screened with voile fabric to allow gas exchange and to prevent excess moisture accumulation, and kept in a climate-controlled room as described above. For each treatment and assessment date, five replicates were used.

The number of live/dead parasitoids was recorded 24 h after the parasitoids were released in the experimental units. Parasitoids that did not react to the touch of a thin brush were considered dead. The effect of each insecticide was determined based on the number of dead parasitoids after 24 h of exposure.

## Experimental design and data analysis

For all assays, a fully randomized experimental design was used. Generalized linear models (GLM) (Nelder and Wedderburn [1972\)](#page-8-0) with quasi-binomial, quasi-Poisson, Gaussian, and binomial distributions were used for analysis of the proportion data  $(F_0)$  generation female mortality), counts (number of pupae and adults), duration (egg-pupa and pupaadult development times), and sex ratio, respectively. In order to determine the duration of the residual effects of lambda-cyhalothrin + chlorantraniliprole and lambda-cyhalothrin  $+$  thiamethoxam on C. flavipes adults, the mortalities observed at different times (days after spraying, DAS) were subjected to a repeated-measure analysis using generalized linear mixed models (GLMM) of the "lme4" package (Bates et al. [2015](#page-7-0)) with binomial distribution. The effect of the explanatory variables (plants treated with insecticides and negative control) and the time were considered as fixed factors, while the repeated measure of each experimental unit (sugarcane leaf disc) in time was considered as random, assuming an intercept that was different for each individual. The effect of treatment  $\times$  time was assessed by likelihoodratio tests  $(P < 0.05)$  between a complete model and a model without the effect of treatment or time. The same test was used to determine the significance of the treatment  $interaction \times time$ , comparing two models: one with interaction and another without interaction. For each time, a GLM with quasi-binomial was used to analyze the mortality data. For all assays, goodness-of-fit was assessed through half-normal plots with simulation envelopes using the

"*hnp*" package (Demétrio et al. [2014](#page-7-0)). In case of significant differences among treatments, multiple comparisons with the Tukey test  $(P < 0.05)$  were performed, using the "glht" function of the " $multcomp$ " package, with adjusted  $P$  values (Hothorn et al. [2008\)](#page-8-0). All analyses were performed with the statistical software "R", version 3.4.4 (R Development Core Team [2018\)](#page-8-0).

## Results

Exposure of C. flavipes females to the insecticide residues indicated that lambda-cyhalothrin  $+$  chlorantraniliprole and lambda-cyhalothrin + thiamethoxam were highly harmful to this parasitoid (100% mortality). In contrast, chlorantraniliprole, chlorfluazuron, novaluron, tebufenozide, and triflumuron did not cause significant mortality, compared to the negative control (Table [1](#page-4-0)). Furthermore, chlorantraniliprole, chlorfluazuron, novaluron, tebufenozide, and triflumuron did not affect the egg-pupa and pupa-adult development periods, number of pupae, pupal weight, and progeny emergence  $(F_1 \text{ generation})$  (Table [1\)](#page-4-0). However, these treatments did cause transgenerational sublethal effects. In the  $F_1$  generation, chlorantraniliprole increased the proportion of females, whereas chlorfluazuron reduced the proportion of females; the effects differed from each other, but both did not differ statistically from the negative control (Table [1\)](#page-4-0). In comparison to the negative control, significant reductions in tibia length due to triflumuron, novaluron, chlorfluazuron and chlorantraniliprole treatments were observed, whereas the tebufenozide treatment had no effect on C. flavipes  $(F_1$  generation) (Table [1\)](#page-4-0). The largest reductions in tibial length were observed in the offspring of females exposed to chlorfluazuron and tri-flumuron (Table [1\)](#page-4-0).

For the  $F_1$  progeny ( $F_2$  generation), chlorantraniliprole, chlorfluazuron, novaluron, tebufenozide, and triflumuron did not affect the egg-pupa and pupa-adult development times, number of pupae, or pupal weight (Table [2](#page-5-0)). Chlorantraniliprole, chlorfluazuron, novaluron, tebufenozide, and triflumuron did not affect the sex ratio of emerging adults; however, novaluron significantly reduced the proportion of females of the  $F_2$  $F_2$  generation (Table 2).

The duration of the residual effects indicated a significant interaction between treatment and time [days after spraying (DAS)]  $(\chi^2 = 13.00; d.f. = 4; P = 0.0012)$ . The mortality of C. flavipes was significantly higher in lambda-cyhalothrin  $+$ thiamethoxam and lambda-cyhalothrin + chlorantraniliprole than in the negative control treatment at 5 DAS ( $F =$ 178.28;  $d.f. = 2$ , 27;  $P < 0.0001$ ), 15 DAS ( $F = 190.18$ ,  $d.f. = 2, 27; P < 0.0001$ , and 30 DAS ( $F = 73.34, d.f. = 2$ , 27;  $P < 0.0001$  $P < 0.0001$ ) (Fig. 1). Among treatments, the longest duration of the residual effects was observed for lambda-

<span id="page-4-0"></span>

cData (mean ± SE) followed by the same letter in a column do not differ significantly (GLM with a quasi-Poisson distribution, followed by post-hoc Tukey test; P < 0.05) Data (mean  $\pm$  SE) followed by the same letter in a column do not differ significantly (GLM with a binomial distribution, followed by post-hoc Tukey test;  $P < 0.05$ )

Data (mean ± SE) followed by the same letter in a column do

not differ significantly (GLM with a binomial distribution, followed by post-hoc Tukey test; P < 0.05)

cyhalothrin + thiamethoxam (100% mortality, 30 DAS), while lambda-cyhalothrin  $+$  chlorantraniliprole caused 45% mortality at the same assessment time (Fig. [1](#page-5-0)).

## **Discussion**

This study evaluated the toxicity levels and transgenerational sublethal effects of seven insecticides on the parasitoid wasp C. flavipes. The results showed that lambda $c$ yhalothrin + chlorantraniliprole and lambda-cyhalothrin + thiamethoxam were highly toxic to adults of this parasitoid. Similar toxicity has also been observed for other parasitoid species, including *Telenomus podisi* Ashmead (Hymenoptera: Platygastridae) (Pazini et al. [2017](#page-8-0)), Tamarixia radiata (Waterston) (Hymenoptera: Eulophidae) (Beloti et al. [2018](#page-7-0)), and Trichogramma pretiosum Riley (Hymenoptera: Trichogrammatidae) (Paiva et al. [2018\)](#page-8-0) treated with lambda-cyhalothrin + thiamethoxam. Lambda-cyhalothrin + chlorantraniliprole caused lower mortality than lambda-cyhalothrin + thiamethoxam only after 15 and 30 DAS. These differences might be associated with the components of the commercial product, and mainly with the percentage of lambda-cyhalothrin in the formulation. Lambda-cyhalothrin acts on voltage-gated sodium channels and is a non-systemic insecticide that acts rapidly through contact and ingestion (IRAC [2019;](#page-8-0) Soderlund and Bloomquist [1989](#page-9-0)), causing high mortality to natural enemies (Momanyi et al. [2012\)](#page-8-0). Thiamethoxam, which is a systemic insecticide and can act by contact, is a neonicotinoid that acts on the nicotinic acetylcholine receptors of the postsynaptic membranes of nerve-cell junctions (IRAC [2019;](#page-8-0) Wiesner and Kayser [2000](#page-9-0)), producing lethal and sublethal neurological effects over a long period of time (Prabhaker et al. [2011](#page-8-0); Yao et al. [2015\)](#page-9-0). There is a need to evaluate the potential sublethal effects of thiamethoxam on the behavior of C. flavipes, as recently found for another neonicotinoid, imidacloprid, on the braconid parasitoid Lysiphlebus fabarum (Marshall) (Hymenoptera: Braconidae) (Jam and Saber [2018](#page-8-0)). Chlorantraniliprole is also a systemic insecticide but is a member of the diamide group, acting on arthropod ryanodine receptors (Ebbinghaus-Kintscher et al. [2007](#page-7-0); IRAC [2019](#page-8-0)). Upon activation, stored calcium is released from the sarcoplasmic reticulum, impairing the regulation of muscle contraction and causing paralysis and death of sensitive species (Cordova et al. [2006\)](#page-7-0). This insecticide is highly selective to parasitoids since it acts mainly by ingestion, with little contact activity (Stecca et al. [2014](#page-9-0)). Even so, the results of this study suggest that lambda-cyhalothrin + chlorantraniliprole and lambda-cyhalothrin + thiamethoxam may reduce the effectiveness of C. flavipes as a biocontrol agent of D. saccharalis and D. flavipenella in sugarcane production systems.

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Treatment	Development period (days) <sup>a</sup>		Number of pupae <sup>b</sup>	Pupal weight <sup>a</sup> $(mg)$	Emergence rate <sup>a</sup> $(\%)$	Sex ratio <sup>c</sup> $(9/3 + 9)$
	Egg-pupa	Pupa-adult				
Control	$14.7 \pm 0.21$	$6.5 \pm 0.15$	$61.0 \pm 5.81$	$0.07 \pm 0.005$	$95.2 \pm 1.04$	$0.75 \pm 0.04^{\circ}$
Chlorantraniliprole	$14.7 \pm 0.28$	$6.6 \pm 0.15$	$63.4 \pm 5.35$	$0.07 \pm 0.004$	$91.2 \pm 2.15$	$0.76 \pm 0.05^{\text{a}}$
Chlorfluazuron	$14.9 \pm 0.24$	$6.2 \pm 0.13$	$52.8 \pm 3.69$	$0.06 \pm 0.006$	$94.3 \pm 0.99$	$0.74 \pm 0.05^{\circ}$
Novaluron	$14.8 \pm 0.25$	$6.3 \pm 0.19$	$69.1 \pm 5.71$	$0.08 \pm 0.005$	$94.7 \pm 1.44$	$0.53 \pm 0.09^b$
Tebufenozide	$14.6 \pm 0.24$	$6.0 \pm 0.26$	$67.9 \pm 4.16$	$0.08 \pm 0.004$	$92.4 \pm 2.57$	$0.71 \pm 0.06^{\circ}$
Triflumuron	$14.6 \pm 0.15$	$6.4 \pm 0.19$	$71.0 \pm 4.92$	$0.07 \pm 0.004$	$94.7 \pm 1.81$	$0.65 \pm 0.07^{\text{a}}$
	$F = 0.310$	$F = 1.196$	$F = 1.588$	$F = 1.636$	$F = 0.788$	$\chi^2 = 7.348$
	$d.f. = 5, 103$	$d.f. = 5, 103$	$d.f. = 5, 102$	$d.f. = 5, 103$	$d.f. = 5, 102$	$d.f. = 5$
	$P = 0.9060$	$P = 0.3160$	$P = 0.1700$	$P = 0.2770$	$P = 0.5680$	P < 0.0001

<span id="page-5-0"></span>Table 2 Transgenerational sublethal effects on the  $F_2$  generation (egg-pupa and pupa-adult stage development time, number of pupae, pupal weight, emergence rate and sex ratio) of the parasitoid *Cotesia flavipes* when females  $(F_0)$  were exposed to treated leaves

a Data (mean ± SE) followed by the same letter in a column do not differ significantly (GLM with a Gaussian distribution, followed by post-hoc Tukey test;  $P < 0.05$ )

 $b$ Data (mean  $\pm$  SE) followed by the same letter in a column do not differ significantly (GLM with a quasi-Poisson distribution, followed by posthoc Tukey test;  $P < 0.05$ )

c Data (mean ± SE) followed by the same letter in a column do not differ significantly (GLM with a binomial distribution, followed by post-hoc Tukey test;  $P < 0.05$ )



Fig. 1 Mortality of *Cotesia flavipes* adults after exposition to insecticide residues in sugarcane leaves at 5, 15 and 30 days after spraying (DAS) of insecticides. Data (mean ± SE) followed by the same letter at each time (DAS) did not differ significantly (GLM with a quasibinomial distribution, followed by post-hoc Tukey test;  $P < 0.05$ )

The results of exposure of *C. flavipes* adults to residues of chlorantraniliprole, chlorfluazuron, novaluron, or triflumuron indicated that these insecticides did not cause significant mortality but did cause transgenerational sublethal effects (Desneux et al. [2007](#page-7-0); Guedes et al. [2016](#page-7-0)). These effects have been observed when pests are exposed to sublethal doses or concentrations of pesticides, resulting in transgenerational hormesis (increasing the pre-oviposition period, fecundity, and larval and pupal survival) (Chen et al. [2016;](#page-7-0) Wang et al. [2017a;](#page-9-0) Margus et al. [2019](#page-8-0)) and reducing the longevity of the progeny and population increases (Hedayati et al. [2019](#page-7-0)). For natural enemies, the studies indicate that the insecticide residues can also alter the fitness of insects, due to contact or ingestion, and can cause negative effects on the next generations (Guedes et al. [2016](#page-7-0)). Reported negative transgenerational effects on parasitoids include reduction of emergence, alteration of the sex ratio (reducing the proportion of females) (Costa et al. [2014](#page-7-0)), reduction of fertility (Biondi et al. [2015\)](#page-7-0), and increased egg-to-adult period (Fonseca et al. [2015](#page-7-0)). The alteration in the tibia length is often used as a model to observe alterations of insect growth by the action of pesticides and other factors (Fonseca et al. [2015;](#page-7-0) Soler et al. [2007](#page-9-0)).

Chlorantraniliprole also shows little or no toxicity to other beneficial parasitoid wasps, including Cotesia chilonis (Matsumura) (Hymenoptera: Braconidae) (Huang et al. [2011](#page-8-0); Jia et al. [2011](#page-8-0)), Cotesia plutellae (Kurudjumov) (Hymenoptera: Braconidae) (Kishore et al. [2014\)](#page-8-0), Eretmocerus eremicus Rose & Zolnerowich (Hymenoptera: Aphelinidae) (Gradish et al. [2011](#page-7-0)), and Bracon hebetor Say (Hymenoptera: Braconidae) (Muslim et al. [2018](#page-8-0)). High selectivity was recorded for *C. chilonis* (Huang et al. [2011\)](#page-8-0), C. plutellae (Haseeb and Amano [2002](#page-7-0); Shi et al. [2004\)](#page-9-0), Trichogramma spp. (Hymenoptera: Trichogrammatidae) (Goulart et al. [2012\)](#page-7-0), Psyttalia (Opius) concolor Szèpligueti (Hymenoptera: Braconidae) (Bengochea et al. [2012\)](#page-7-0), and T. pallidus (Amarasekare et al. [2016\)](#page-7-0), as well as the predators Macrolophus basicornis (Stal) (Hemiptera: Miridae) (Passos et al. [2018;](#page-8-0) Soares et al. [2019](#page-9-0)), Orius laevigatus (Fieber) (Hemiptera: Anthocoridae) (Biondi et al. [2012\)](#page-7-0), Harmonia axyridis (Pallas) (Coleoptera: Coccinellidae), and

Coleomegilla maculata DeGeer (Coleoptera: Coccinellidae) (Cabrera et al. [2018](#page-7-0)) treated with chlorfluazuron, novaluron, or triflumuron. The low toxicity of these insecticides is attributed not only to the low contact activity of chlorantraniliprole, but also to the action of the chitin synthesisinhibitor insecticides on the endocrine system of immature stages of insects (Biddinger et al. [2014](#page-7-0); Staal [1975](#page-9-0); Wang et al. [2017b\)](#page-9-0), which may explain the low toxicity to adult parasitoids. In addition, these insecticides have a low capacity to penetrate the insect cuticle (Sun et al. [2015](#page-9-0); Wang et al. [2017b](#page-9-0)).

Despite this low penetration of the integument, these insecticides have high affinity to and retention capacity at target sites, which may lead to several sublethal effects (Schneider et al. [2003](#page-9-0)). In this study, chlorfluazuron, novaluron, and triflumuron reduced the tibia length of the  $F_1$  generation of C. *flavipes*. Due to the action mechanism (chitin synthesis inhibitors), these compounds disorganize the integument ultrastructure, reducing and/or delaying the formation of new integument (Castro et al. [2012](#page-7-0); Desneux et al. [2007;](#page-7-0) Perez-Farinos et al. [1998\)](#page-8-0). This process may also increase the energy cost and consequently reduce the growth rate and size of insects (Schneider et al. [2003](#page-9-0)). Therefore, in this study C. *flavipes* females ( $F_0$  generation) may have expended more energy to detoxify these compounds, and less on body growth of the  $F_1$  generation. Reduction in parasitoids' body size can negatively affect their parasitism capacity (King [2000;](#page-8-0) Silva-Torres et al. [2009\)](#page-9-0) and the effectiveness of natural enemies in pest control (Riddick [2006](#page-8-0); Sétamou et al. [2002\)](#page-9-0). However, no significant effect on the parasitism capacity was observed here, suggesting that these insecticides can be considered safe to use in sugarcane-borer control with minimal impact on C. flavipes.

Although chlorantraniliprole, chlorfluazuron, novaluron, and triflumuron were considered harmless to C. flavipes adults, they caused transgenerational sublethal effects, modifying the sex ratio of the  $F_1$  and/or  $F_2$  generations. Insecticides that affect the endocrine system can induce deformations in the ovaries and testes (Desneux et al. [2007](#page-7-0)). In this study, the proportion of females was reduced in both treated and untreated groups. Idris and Grafius ([1993\)](#page-8-0) suggested some possible reasons why insecticides may alter the sex ratio, including disruption of ovule fertilization, especially in haplodiploid species, and differential susceptibility between the sexes when exposed prior to the adult stage. The reason for the reduction in the proportion of females is unknown, but it may be due to a selective male mortality, as indicated by the reduction in adult emergence. Insects are particularly sensitive to IGRs (Insect Growth Regulators) during the larval to pupal molt, whereas the larval instars are rather insensitive (Menn and Beroza [1972](#page-8-0); Tunaz and Uygun [2004](#page-9-0)). Additionally, chlorfluazuron,

flufenoxuron, and triflumuron (insecticides of the same group) reduced the parasitism capacity of C. plutellae (Haseeb and Amano [2002](#page-7-0)), while flufenoxuron and lufenuron reduced the emergence rates of Bracon brevicornis (Wesmael) (Hymenoptera: Braconidae) and Trichogramma evanescens Westwood (Hymenoptera: Trichogrammatidae) (Tabozada et al. [2014\)](#page-9-0) when females were treated with these insecticides.

In addition to toxicity, lambda-cyhalothrin  $+$  chlorantraniliprole and lambda-cyhalothrin  $+$  thiamethoxam maintained their harmful activity for more than 30 DAS. Similar persistence of these compounds was observed for other parasitoid species. In greenhouse conditions, Momanyi et al. [\(2012](#page-8-0)) found that lambda-cyhalothrin (Karate 1.75% EC) had a long residual persistence for the parasitoids Trichogrammatoidea sp. nr. lutea and Trichogramma sp. nr. mwanzai (Hymenoptera: Trichogrammatidae). High insecticide persistence can reduce the effectiveness of biocontrol agents in IPM programs. It also reduces the recolonization capacity of natural enemies and interferes with the establishment of ecological balance in agroecosystems (Parra [2014\)](#page-8-0). Among the pyrethroids tested, lambda-cyhalothrin + chlorantraniliprole caused harmful effects to C. flavipes for shorter periods. The low concentration of lambda-cyhalothrin in the formulation may have contributed to the lower mortality compared to the lambda $c$ yhalothrin + thiamethoxam formulation. In addition, lambda-cyhalothrin + thiamethoxam includes a neonicotinoid (thiamethoxam) with high systemic and contact properties. This neonicotinoid also has a low log Kow (−0.13), which facilitates cuticle penetration and translocation in the insect body (Tomizawa and Casida [2005\)](#page-9-0).

To establish an integrated management program for sugarcane borers involving C. flavipes, knowledge of the effects of commonly used insecticides on the parasitoid is essential. Few studies have assessed the lethal and transgenerational sublethal effects of insecticides on this parasitoid. Parasitoids were exposed to the insecticides via residual contact on leaves, because in fields the compounds are sprayed on the aerial parts of plants to control neonate larvae of sugarcane borers. Assessments of the height that the insecticides can reach on the sugarcane plant and the parasitoids' contact with the residues are needed to confirm the possible harmful effects in the field. The present results showed that lambda-cyhalothrin  $+$  chlorantraniliprole and  $lambda-cyhalothrin + thiamethoxam$  were highly harmful and persistent to C. flavipes. On the other hand, chlorantraniliprole, chlorfluazuron, novaluron, and triflumuron did not cause high mortality, but did induce transgenerational sublethal effects. Tebufenozide was the only safe active ingredient for C. flavipes, causing no lethal or sublethal effects. However, field studies where environmental conditions cannot be manipulated are needed to better

<span id="page-7-0"></span>understand the effects of these insecticides on this parasitoid. Therefore, these insecticides should be used at different times from the C. flavipes releases. In conclusion, residues of the novel, low-risk insecticide tebufenozide appeared to be relatively harmless to C. flavipes adults, and therefore this insecticide could be safely applied prior to release and/or establishment of this parasitoid in sugarcane production systems.

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#### Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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