



## Plant Protection

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# Residual toxicity of four insecticides on larvae and adults of the predator *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae)

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**Abstract:** The objective was to evaluate the residual action of the insecticides acetamiprid + etofenprox, spinetoram, indoxacarb and methoxyfenozide on the predator *Chrysoperla externa* Hagen, 1861 (Neuroptera: Chrysopidae). The bioassays followed the methodologies proposed by the International Organization for Biological and Integrated Control (IOBC). The insecticides were sprayed on grapevine plants cv. Burgundy, using the maximum recommended field dosage for fruit trees. Larvae and adults of the predator were exposed to leaves containing the insecticide residues, at 3, 10, 17, 24 and 31 days after spraying, to determine the residual effect on the following biological parameters: mortality, fecundity and fertility. Based on the toxicity observed during the bioassays, the insecticides were classified according to the IOBC persistence scale. Spinetoram was classified as moderately persistent to larvae and slightly persistent to adults, indoxacarb also he was considered persistent for larvae and as a short-lived for the adult stage of the lacewing, thus showing the difference in susceptibility between the stages of development of *C. externa*. The insecticides acetamiprid + etofenprox and methoxyfenozide are the most suitable for ecologically safe application, in areas where the predator occurs because they are classified as short-lived insecticides for the larval and adult stages of *C. externa*.

**Index Terms:** Lacewings, Persistence, Chemical control, Biological control, Temperate climate fruits.

## Toxicidade residual de quatro inseticidas sobre larvas e adultos do predador *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae)

**Resumo:** O objetivo foi avaliar a ação residual dos inseticidas acetamipride + etofenproxi, espinetoram, indoxacarbe e metoxifenoizida sobre o predador *Chrysoperla externa* Hagen, 1861 (Neuroptera: Chrysopidae). Os bioensaios seguiram as metodologias propostas pela International Organization for Biological and Integrated Control (IOBC). Os inseticidas foram pulverizados em plantas de videira cv. Borgonha, usando a dosagem máxima de campo recomendada para frutíferas. Larvas e adultos do predador foram expostos às folhas contendo os resíduos do inseticida, aos 3, 10, 17, 24 e 31 dias após a pulverização, para determinar o efeito residual sobre os seguintes parâmetros biológicos: mortalidade, fecundidade e fertilidade. Com base na toxicidade observada durante os bioensaios, os inseticidas foram classificados de acordo com a classificação de persistência da IOBC. Espinetoram foi classificado como moderadamente persistente para larvas e levemente persistente para adultos, indoxacarbe também foi considerado moderadamente persistente para larvas e, como de vida curta para a fase adulta do crisopídeo, mostrando assim a diferença de suscetibilidade entre os estágios de desenvolvimento de *C. externa*. Os inseticidas acetamipride + etofenproxi e metoxifenoizida são os mais indicados para aplicação ecologicamente segura, em áreas onde o predador ocorre naturalmente, pois são classificados como inseticidas de vida curta (classe 1) para os estágios larval e adulto de *C. externa*.

**Termos de Indexação:** Crisopídeo, Persistência, Controle químico, Controle biológico, Frutíferas de clima temperado.

### Introduction

The eastern moth, *Grapholita (Cydia) molesta* (Busck, 1916) (Lepidoptera: Tortricidae) and the South American fruit fly *Anastrepha fraterculus* (Wiedemann, 1830) (Diptera: Tephritidae) are the main pest arthropods that occur in fruit trees in temperate climates in Brazil (MONTEIRO et al., 2020; STUPP et al., 2021). Chemical control, with the spraying of synthetic insecticides, is the method most used by producers to control these pest arthropods (LEIVAS et al., 2020; MONTEIRO et al., 2020). Despite its effectiveness, the use of this control method alone is associated with several negative effects, such as the selection of resistant individuals, outbreak of secondary pests and reduction or elimination of populations of natural enemies (DESNEUX et al., 2007; FERNANDES et al., 2010), and thus does not meet the precepts of Integrated Pest Management (IPM) (CARVALHO et al., 2019).

IPM recommends the use of selective insecticides in line with the presence of natural enemies, and the use of biological control should always be prioritized over the exclusive application of insecticides (SHAN et al., 2020). Among the natural enemies present in fruit crops, the generalist predator *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae) is of great importance due to its wide geographic distribution, high predatory capacity in the larval stage, wide range of prey, tolerance to some insecticides and high reproductive potential (PASINI et al., 2020; SUÁREZ-LÓPEZ et al., 2020). In addition, generalist predators such as *C. externa* have an advantage over specialists due to polyphagia and can exploit diverse food resources and survive in the agroecosystem without target pests, thus preventing their resurgence (DE ARMAS et al., 2020). Therefore, *C. externa* has great potential to act in the

suppression of secondary pests, such as the two-spotted spider mite *Tetranychus urticae* (Koch, 1836) (Acari: Tetranychidae), European red spider mite *Panonychus ulmi* (Koch, 1836) (Acari: Tetranychidae) and the aphid *Brachycaudus persicae* (Passerini, 1860) (Hemiptera: Aphididae), both in orchards in Brazil (CASTILHOS et al., 2019) and worldwide (RAMESHGAR et al., 2019).

IPM programs seek to increase the compatibility between control methods, including chemical and biological methods, so as to increase their effectiveness (SUÁREZ-LÓPEZ et al., 2020). One of the first steps in designing a biological control strategy is to study the toxicological profile of insecticides used to control pest arthropods against their natural enemies. This evaluation must be carried out not only in the laboratory, but also in greenhouse conditions, measuring not only the toxicity immediately after application, but also the permanence of the insecticide and the evolution of its toxicity over time (MORALES et al., 2019). Laboratory tests evaluate mortality in the absence of environmental factors/variables, and their results can be amplified or reduced in the greenhouse and field (ABDULLAHI et al., 2020). It is also necessary to understand bioecological aspects of the tested insects (QUESADA; SADO, 2020), and as immature and adult stages of *C. externa* may show differences in susceptibility, appropriate tests are required at both stages of development.

Several factors can interfere with the toxicity of a pesticide in semi-field and field conditions, requiring the use of tests with a validated methodology to measure the impacts of pesticides on non-target organisms (JANSEN, 2010). In this regard, the International Organization for Biological and Integrated Control (IOBC) proposes a globally recognized assessment, involving laboratory, semi-field and field tests (HASSAN, 1994). These evaluation steps make it possible to determine different toxicological

characteristics of pesticides and establish whether they are compatible for use in IPM. In Brazil, *C. externa* has been used to test the selectivity and persistence of several insecticides in peach crops (CASTILHOS et al., 2019) and in wheat (PASINI et al., 2020). Therefore, the aim of this study was to evaluate the residual action (duration of harmful activity) of four insecticides used to control *G. molesta* and *A. fraterculus*, in the larval and adult stages of the predator *C. externa*, using the methodology proposed by the IOBC.

## Material and Methods

### Insects.

Adults and larvae of *C. externa* used in the bioassays came from a population maintained in a controlled environment (temperature (T): 25±1 °C; relative humidity (RH): 70±10%; photophase (FT): 14 hours).

Predator eggs were deposited in plastic trays (43 cm × 27 cm × 13 cm) and covered with a voile fabric until hatching. Larvae of *C. externa* were separated and kept in individual test tubes (12 cm long × 5 cm diameter), closed with transparent PVC film and fed *ad libitum*, with eggs of the alternative host *Ephestia kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae) maintained according to the methodology proposed by Parra (1997) until pupation. Adults were kept in acrylic cages (15.5 cm high × 18.5 cm in diameter), closed with paper towels at both ends, which served as a substrate for oviposition. Distilled water was supplied by capillarity through an orifice in the cage, and an artificial diet as described by Vogt et al. (2000) was placed around the cage, at the height of the water inlet hole. The water and diet were replaced twice a week, and the paper towels with the eggs were removed and placed in the trays until the larvae hatched.

### Plants.

Due to its larger leaf area, grapevine plants (*Vitis labrusca* L.), variety “Bordô”, were

used as a plant substrate for the application of insecticides as recommended by the IOBC (Ternes et al. 2001). Non-flowering plants were kept in 10-L pots with plant substrate, fertilized according to technical recommendations (Melo et al. 2016), and irrigated daily. The plants were cultivated in a greenhouse, under the following climatic conditions: T: 30.0 ± 9.0 °C; RH: 60.0 ± 25%. Six plants 210 days old were used with at least 30 leaves measuring 12 cm in diameter, for each treatment and each stage of insect development evaluated (larva and adult).

### Tested insecticides.

The four insecticides selected for the bioassays (Table 1), from different chemical groups and with different modes of action (IRAC, 2022), are used in temperate climate fruit crops to control *G. molesta* and *A. fraterculus*. All insecticides evaluated are recommended for foliar spraying. Also, the products were previously diluted in distilled water at the maximum recommended field concentration (g or mL 100 L<sup>-1</sup>), with a recommended application volume of 1000 L ha<sup>-1</sup> (MAPA, 2021).

### Bioassays.

The bioassays were conducted following the IOBC methodologies proposed by Hassan and Abdelgader (2001), Castilhos et al. (2019) and Pasini et al. (2020). The plants were sprayed using a pressurized CO<sub>2</sub> sprayer with a flat jet nozzle (Teejet XR110015EVS). The working pressure was 50 psi, up to the runoff point. After the spray had dried, the plants were placed in a greenhouse (T: 30.0 ± 9.0 °C; RH: 60.0 ± 25%, photophase:14 h). A control treatment (distilled water) was added to all bioassays.

At 3, 10, 17, 24 and 31 days after application (DAA) of the insecticides, leaves with residues from each treatment were removed from the plants, with the aid of scissors, and transferred to the laboratory (T: 25± 1 °C; RH: 70±10%; FT: 14 hours) to be used in bioassays to evaluate harmful activity (persistence) against the larval and adult stages of *C. externa*.

### Larval exposure.

The cages for the larval stage were made using 5-L plastic trays with a methacrylate

**Table 1** Insecticides used in bioassays to access harmful activity on *Chrysoperla externa*.

Active ingredient	<sup>1</sup> Primary action site	<sup>2</sup> C.D.=	<sup>3</sup> A.I.	Target pest	Culture
<b>Acetamiprid + Etofenprox</b> Technical name: Eleitto <sup>1</sup> Chemical group: Neonicotinoid (4) + Diphenyl Ether (3) Manufacturer: Iharabras S.A.	Competitive modulators of nicotinic acetylcholine receptors + Sodium channel modulators	70	167 +300	<i>Anastrepha fraterculus</i> ; <i>Grapholita molesta</i>	Peach
<b>Spinetoram</b> Technical name: Delegate <sup>1</sup> Chemical group: Spinosyns (5) Manufacturer: Corteva Agriscience	Allosteric modulators of nicotinic acetylcholine receptor – site I	30	250	<i>Anastrepha fraterculus</i> ; <i>Grapholita molesta</i>	Apple and Peach
<b>Indoxacarb</b> Technical name: Avatar <sup>1</sup> Chemical group: Oxadiazine (22 A) Manufacturer: FMC Química do Brasil Ltda	Voltage-dependent sodium channel blockers	750	150	<i>Grapholita molesta</i>	Apple and Peach
<b>Methoxyfenozide</b> Technical name: Intrepid 240 SC <sup>1</sup> Chemical group: Diacylhydrazine (18) Manufacturer: Corteva Agriscience	Ecdysone receptor agonists	80	240	<i>Grapholita molesta</i>	Apple

<sup>1</sup>According to IRAC (2022);

<sup>2</sup>C.D. = Commercial dosage (g or mL.100 L<sup>-1</sup>);

<sup>3</sup>A.I.= Active ingredient concentration (g kg<sup>-1</sup> or g L<sup>-1</sup>).

base (34 cm long × 20 cm wide), which were covered with a cloth composed of 50% polyester and 50% viscose to maintain moisture. Grapevine leaves containing dried insecticide residues were detached from the plants and taken to the laboratory, where they were placed on the base which was covered with cloth. Two methacrylate plates (32 cm long × 8 cm wide), which had five holes each (5 cm in diameter) were placed on top. An open bottomed plastic cup (50 mL), previously sprinkled with talc powder, was attached to each orifice to prevent the escape of larvae, forming the exposure arenas.

First instar larvae (1–2 days old) were added to these arenas, with the aid of a fine brush, and remained in contact with the vine leaves until adult emergence, while being fed daily *ad libitum* with eggs from *E. kuehniella*. The design used was completely randomized. Each treatment consisted of four methacrylate plates with five arenas each, and each plate consisted of five insects, totaling 20 per treatment. Larval mortality was evaluated daily and the duration of development from larva to adult in days. The reproductive parameters were evaluated in those treatments that showed accumulated mortality of ≤ 50% (See subheading Assessment of sublethal effects on surviving insects).

### **Adult exposure.**

For adult exposure of *C. externa* to grapevine leaves containing insecticide residues, cages composed of two glass plates (14 cm × 14 cm) were made, where these plates served as the bottom and cover of the cages. The plate at the bottom of the cage was covered with fabric composed of 50% polyester and 50% viscose, onto which the previously detached grapevine leaf was placed and later also a methacrylate ring (diameter of 10 cm; height of 3 cm) with five holes (1.3 cm diameter), closed with voile-like fabric to allow ventilation. One hole was connected to a suction pump to eliminate toxic fumes

and another hole (0.8 diameter) was used to supply water to the insects. Artificial diet was placed on the side of the cage. One-week-old adults were placed in these cages and kept in an acclimatized room. The design used was completely randomized. Each treatment consisted of four cages, containing eight insects per replicate (See subheading Assessment of sublethal effects on surviving insects). Accumulated mortality was assessed after 120 h of exposure to insecticide residues (SCHMUCK et al., 2000). Sublethal tests were performed in treatments where the cumulative mortality was ≤ 50%.

### **Assessment of sublethal effects on surviving insects.**

In addition to mortality, the sublethal effects on reproductive parameters (fecundity and fertility) were evaluated in adults who survived in bioassays to evaluate the duration of harmful insecticide activity in the larval and adult stages of *C. externa*. Evaluations were only carried out in treatments with a mortality rate ≤ 50%. To analyze reproductive parameters, adults were separated in sex, thus, five to seven pairs of *C. externa* that survived the previous bioassays were sedated with CO<sub>2</sub> and transferred to acrylic cages and kept under the same climatic conditions as the source population. Three days after the first laying, eggs deposited on paper towels were collected for four consecutive days. The total number of eggs collected was divided by the number of females in the cage in order to determine the average fecundity (number of eggs per female per day). In addition, at each collection, the eggs were removed from the paper towel, with scissors and a brush, and incubated in 96-well cell culture plates (Kasvi Ltda., Pinhais, PR, Brazil) coated with transparent PVC film, to avoid cannibalism and escape, and the number of hatched eggs was evaluated daily in order to calculate the fertility of the eggs in each treatment.

### Selectivity rating.

The insecticides selectivity for larvae and adults, taking into account the mortality percentages, was determined for each treatment and corrected for the control using the formula of Schneider-Orelli (PÜNTENER, 1981). The total treatment effect was calculated using the formula proposed by Vogt et al. (1998):

$$E = 100\% - (100\% - M\%) \times R1 \times R2$$

where: E = total effect (%); M% = mortality in the treatment corrected for the control; R1 = ratio between the average daily eggs laid per treated and untreated female and R2 = ratio between the average viability of eggs laid per treated and untreated female.

The selectivity of the insecticides was classified as a function of the total effect, according to the toxicity classes proposed by the IOBC: 1) innocuous (<30%); 2) slightly harmful (30–79%); 3) moderately harmful (80–99%); and 4) harmful (>99%) (STERK et al., 1999). When the insecticides prove to be innocuous in two consecutive bioassays, or at the end of the bioassays, they will be classified according to the IOBC persistence scale as: 1) short-lived (<5 days); 2) slightly persistent (5–15 days); 3) moderately persistent (16–30 days), and 4) persistent (>30 days) (HASSAN, 1994).

### Statistical analysis.

Larval and adult mortality were analyzed separately for each exposure interval using a generalized linear model (GLM) with binomial error distribution through the probit linkage function. The number of dead insects was considered as a response variable (dependent), while time and treatments (acetamiprid + etofenprox, spinetoram, indoxacarb, methoxyfenozide and control) were included as explanatory (independent) variables. The treatment \* time interaction was also analyzed. Pearson's chi-square test was used to scale the parameters that explain overdispersion.

Data regarding larval and adult mortality, and the duration of the development period

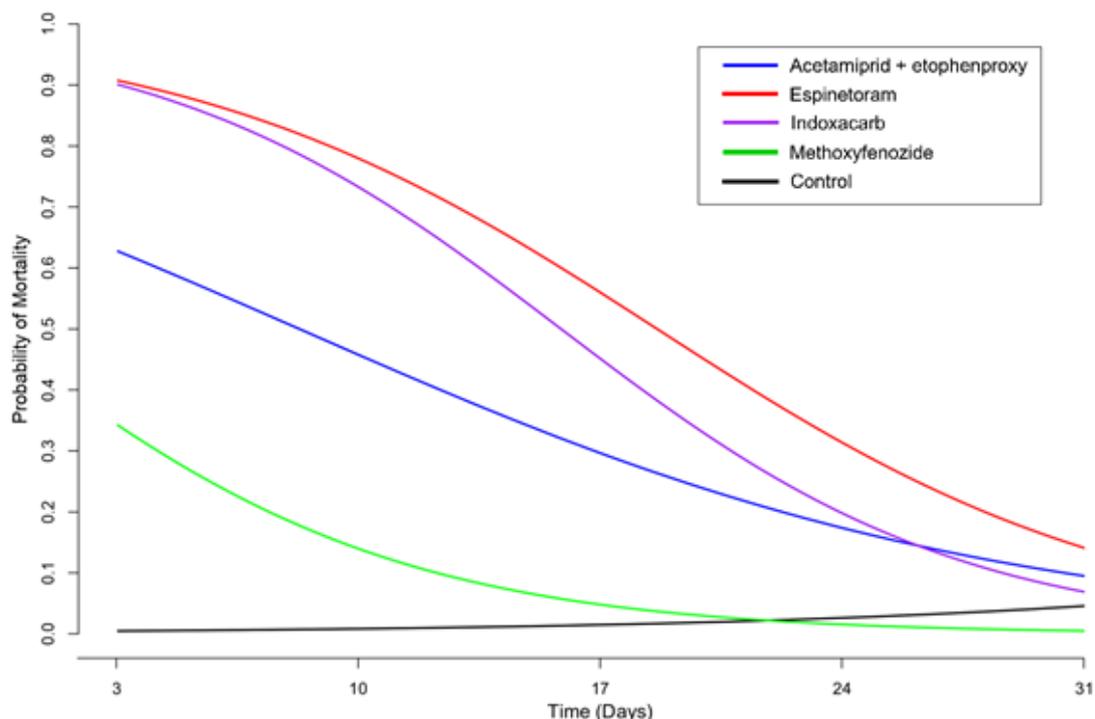
were also subjected to Kruskal-Wallis analysis to determine significance ( $p \leq 0.05$ ) and later to Dunn's mean test, with Bonferroni correction to 5% ( $p \leq 0.05$ ). The values obtained in the fecundity and fertility analyses were submitted to an exploratory analysis of residual normality using the Shapiro Wilk test and homoscedasticity was tested using Barlett's test. The independence of residues was graphically verified. Subsequently, these data were submitted to analysis of variance ( $p \leq 0.05$ ), and if significant, were submitted to the Tukey test ( $p \leq 0.05$ ). All statistical analyses were performed using R version 4.0.0. (R DEVELOPMENT CORE TEAM, 2021).

## Results

### Residual effect on larvae.

The insecticides had a significant effect over time on predator mortality (Figure 1). The insecticides spinetoram, indoxacarb and acetamiprid + etofenprox had a higher probability of mortality than that observed for methoxyfenozide in all time periods evaluated (3, 10, 17, 24 and 31 DAA) (Figure 1).

In the evaluation of larval mortality at 3 DAA, the products acetamiprid + etofenprox, spinetoram and indoxacarb caused the significantly highest mortality levels, and being classified as harmful (class 4) (Table 2) to larvae of *C. externa*, due to the total effect of 100% (Table 3). The total effect of methoxyfenozide, despite not having caused high mortality, was 44% (Table 3), and thus it was considered slightly harmful (class 2) to the larvae (Table 2). At 10 DAA, significant differences between the treatments were observed (Table 2). The insecticides spinetoram and indoxacarb caused high larval mortality, differing significantly from the control, and were classified as harmful (class 4). On the other hand, at 17 DAA, these two insecticides were classified as slightly harmful (class 2) (Table 2). At 24 and 31 DAA, no treatment caused significant mortality in relation to the control and the total effects were lower than 30% (Table 3), thus being classified as harmless (class 1) to predator larvae (Table 2).



**Figure 1.** Probability of mortality of first instar larvae of *Chrysoperla externa* exposed to residues of insecticides at different time periods (3, 10, 17, 24 and 31) (mixed logistic model with random interception).

Statistics:  $\chi^2= 228.42$ ;  $df= 4$ ;  $p < 0.001$ .

**Table 2** Number of dead first instar larvae ( $\pm$ SE), selectivity classification and persistence (duration of harmful activity) of insecticides to *Chrysoperla externa*.

Treatment / ( <sup>1</sup> A.I.)	Days after application (DAA)					Persistence Days / <sup>4</sup> C
	3 <sup>2</sup> N° / <sup>3</sup> C	10 <sup>2</sup> N° / <sup>3</sup> C	17 <sup>2</sup> N° / <sup>3</sup> C	24 <sup>2</sup> N° / <sup>3</sup> C	31 <sup>2</sup> N° / <sup>3</sup> C	
control (---)	0.00±0.00 c	0.00±0.00 c (--)	0.25±0.22 c (--)	0.00±0.00 ns (--)	0.50±0.25ns (--)	-- (--)
acetamiprid + etofenprox (167+300)	4.00±0.61 ab (4)	1.50±0.75 bc (1)	1.00±0.35 abc (1)	0.75±0.22 (1)	0.75±0.41 (1)	<5 (1)
spinetoram (250)	4.50±0.43 a (4)	4.50±0.43 a (4)	2.00±0.35 ab (2)	1.50±0.26 (1)	1.00±0.35 (1)	16–30 (3)
indoxacarb (150)	4.50±0.25 a (4)	3.50±0.43 ab (4)	2.25±0.41 a (2)	1.50±0.56 (1)	0.00±0.00 (1)	16–30 (3)
methoxyfenozide (240)	2.25±0.24 b (2)	0.50±0.25 c (1)	0.50±0.25 bc (1)	0.00±0.00 (1)	0.00±0.00 (1)	<5 (1)
H	11.99	14.96	11.49	2.79	7.40	
df	4	4	4	3	4	
P	0.03	<0.001	0.02	0.42	0.11	

<sup>1</sup>A.I.= Active ingredient concentration (g kg<sup>-1</sup> or g L<sup>-1</sup>);

<sup>2</sup>N° = Number of dead insects. Means followed by the same letter in the column do not differ significantly from each other by Dunn test with Bonferroni correction (P> 0.05); ns= not significant.

<sup>3</sup>C= Initial IOBC toxicity classes: 1 = innocuous (<30%), 2 = slightly harmful (30-79%), 3 = moderately harmful (80-99%), 4 = harmful (>99%);

<sup>4</sup>C= IOBC persistence classes: 1 = short-lived (<5 days); 2=slightly persistent (5-15 days); 3 = moderately persistent (16-30 days); 4 = persistent (>30 days).

**Table 3** Cumulative larval mortality (%), ratio of female fecundity, egg fertility and total effect of insecticides applied on the larval stage of *Chrysoperla externa*.

	Treatment	<sup>1</sup> C.D.	<sup>2</sup> M	<sup>3</sup> R1	<sup>4</sup> R2	<sup>5</sup> E.T.
3 days after application (DAA)	acetamiprid + etofenprox	70	80	--	--	100
	spinetoram	30	90	--	--	100
	indoxacarb	75	90	--	--	100
	methoxyfenozide	80	45	0.97	1.05	44
10 days after application (DAA)	acetamiprid + etofenprox	70	30	1.06	1.08	20.05
	spinetoram	30	90	--	--	100
	indoxacarb	75	70	--	--	100
	methoxyfenozide	80	10	0.94	1.11	6.77
17 days after application (DAA)	acetamiprid + etofenprox	70	15	1.11	0.81	23.48
	spinetoram	30	35	1.05	0.96	34.34
	indoxacarb	75	40.20	0.92	1.00	45.26
	methoxyfenozide	80	5	1.05	0.94	5.80
24 days after application (DAA)	acetamiprid + etofenprox	70	15	1.42	1.00	0.00
	spinetoram	30	30	0.91	1.11	0.00
	indoxacarb	75	30	1.11	1.16	10.36
	methoxyfenozide	80	0	1.43	0.81	0.00
31 days after application (DAA)	acetamiprid + etofenprox	70	5	1.03	1.00	2.17
	spinetoram	30	10	0.81	1.05	23.81
	indoxacarb	75	0	0.90	0.96	13.45
	methoxyfenozide	80	0	1.00	1.10	0.00

<sup>1</sup>C.D. = Commercial product dosage (g or mL.100 L<sup>-1</sup>);

<sup>2</sup>M= Mortality of treatment based on the formula of Schneider-Orelli (Püntener 1981) (%);

<sup>3</sup>R1= Ratio between the daily average of eggs laid per treated and untreated female;

<sup>4</sup>R2= Ratio between the average viability of eggs laid per treated and untreated female;

<sup>5</sup>E.T.= Total effect estimated following the formula proposed by Vogt et al. (1998). E.T.=100-(100%-M) x R1 x R2.

Regarding the classification of persistence of harmful activity against the larval stage, the insecticides spinetoram and indoxacarb, which were harmful (class 4) to larvae up to 10 DAA, were classified as moderately persistent (class 3) between 16 and 30 days. On the other hand, acetamiprid + etofenprox and methoxyfenozide were considered short-lived insecticides (class 1), as they presented harmful activity for less than 5 days (Table 2).

It was not possible to assess the effect on the duration of the larval to adult development period at 3 DAA for acetamiprid + etofenprox, spinetoram and indoxacarb due to high larval mortality ( $\geq 50\%$ ) (Table 4). The insecticide methoxyfenozide showed a significantly higher value than the control (Table 4). At 10 DAA, the insecticide methoxyfenozide showed the highest value at 21.21 days, dif-

fering significantly from the control at 20.85 days (Table 4). At 17 DAA, the insecticides acetamiprid + etofenprox and spinetoram showed the highest values in this evaluation, differing significantly from the control (Table 4). There were no significant differences between treatments in the evaluations at 24 and 31 DAA (Table 4).

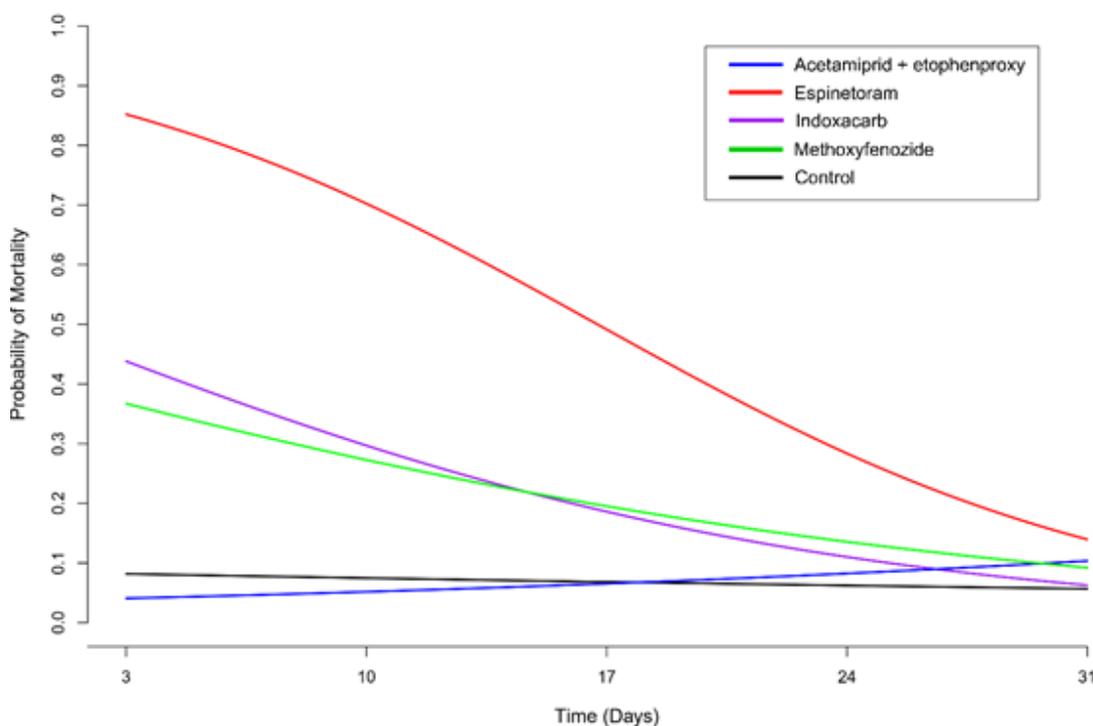
### Residual effect in adults.

Insecticides had significant effects over time on adult mortality (Figure 2). The insecticide spinetoram presented the highest probability of mortality in relation to the other insecticides at all time intervals analyzed (Figure 2). The insecticide acetamiprid + etofenprox was the insecticide with the lowest probability of mortality in the five time periods evaluated (Figure 2).

**Table 4** Duration of the developmental stages (larva to adult) in days ( $\pm$ SE) of *Chrysoperla externa* larvae exposed to insecticide residues.

Treatment	<sup>1</sup> A.I.	Days after application (DAA)				
		3	10	17	24	31
control	---	18.50 $\pm$ 0.20 b	20.85 $\pm$ 0.11 b	17.21 $\pm$ 0.09 b	20.35 $\pm$ 0.20 <sup>ns</sup>	21.16 $\pm$ 0.11 <sup>ns</sup>
acetamiprid + etofenprox	167+300	-----	21.14 $\pm$ 0.17 ab	18.75 $\pm$ 0.29 a	19.82 $\pm$ 0.49	21.23 $\pm$ 0.10
spinetoram	250	-----	-----	18.67 $\pm$ 0.41 a	20.07 $\pm$ 0.29	21.13 $\pm$ 0.16
indoxacarb	150	-----	-----	18.45 $\pm$ 0.49 ab	20.07 $\pm$ 0.21	21.10 $\pm$ 0.14
methoxyfenozide	240	19.27 $\pm$ 0.29 a	21.21 $\pm$ 0.16 a	18.05 $\pm$ 0.30 ab	20.05 $\pm$ 0.23	20.95 $\pm$ 0.15
H		2.50	6.24	14.70	1.88	2.12
df		1	2	4	4	4
P		<0.001	0.04	0.01	0.76	0.71

A.I.= Active ingredient concentration ( $\text{g kg}^{-1}$  or  $\text{g L}^{-1}$ ); Means followed by the same letter in the column do not differ significantly from each other by the Dunn test with Bonferroni correction ( $P > 0.05$ ); ns= not significant.



**Figure 2.** Probability of mortality when the adult stage of *Chrysoperla externa* was exposed to residues of insecticides at different time periods (3, 10, 17, 24 and 31) (mixed logistic model with random interception).

Statistics:  $\chi^2 = 187.67$ ;  $df = 4$ ;  $p < 0.001$ .

As for adult mortality, significant differences were observed at 3, 10 and 17 DAA (Table 5). At 3 DAA, acetamiprid + etofenprox did not differ significantly from the control, being considered innocuous (class 1) to adults of *C. externa* (Table 5). The total effect of the other treatments was 100% (Table 6), and therefore they were classified as harmful (class 4)

(Table 5). At 3 and 10 DAA, the insecticide spinetoram caused the highest mortality among the treatments evaluated, differing significantly from the control in these evaluations (Table 5). On the other hand, at 17 DAA, spinetoram did not differ significantly from the control, despite presenting the highest mortality of all treatments, and therefore all

**Table 5** Number of dead adults ( $\pm$ SE), selectivity and persistence classification of insecticides to the adult stage of *Chrysoperla externa*.

Treatment / <sup>1</sup> A.I.	Days after application (DAA)					Persistence	
	3	10	17	24	31	Days	<sup>4</sup> C
	<sup>2</sup> N° / <sup>3</sup> C						
control (---)	1.25 $\pm$ 0.22 b (---)	0.50 $\pm$ 0.13 b (---)	0.50 $\pm$ 0.13 ab (---)	0.00 $\pm$ 0.00 a (---)	1.00 $\pm$ 0.18 <sup>ns</sup> (---)	--	--
acetamiprid + etofenprox (167+300)	0.75 $\pm$ 0.41 b (1)	0.00 $\pm$ 0.00 b (1)	0.25 $\pm$ 0.11 b (1)	0.75 $\pm$ 0.11 a (1)	1.00 $\pm$ 0.18 (1)	<5	1
spinetoram (250)	8.00 $\pm$ 0.00 a (4)	5.00 $\pm$ 0.43 a (4)	3.00 $\pm$ 0.56 a (1)	1.25 $\pm$ 0.27 a (1)	2.50 $\pm$ 0.80 (1)	5-15	2
indoxacarb (150)	4.00 $\pm$ 0.61 ab (4)	1.00 $\pm$ 0.18 b (1)	2.50 $\pm$ 0.13 ab (1)	0.00 $\pm$ 0.00 a (1)	1.00 $\pm$ 0.43 (1)	<5	1
methoxyfenozide (240)	4.00 $\pm$ 0.61 ab (4)	1.00 $\pm$ 0.18 b (1)	1.25 $\pm$ 0.11 ab (1)	0.00 $\pm$ 0.00 a (1)	2.00 $\pm$ 0.18 (1)	<5	1
<b>H</b>	<b>12.90</b>	<b>13.40</b>	<b>11.02</b>	<b>12.05</b>	<b>3.59</b>		
<b>df</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>		
<b>P</b>	<b>0.02</b>	<b>0.01</b>	<b>0.03</b>	<b>0.02</b>	<b>0.46</b>		

<sup>1</sup>A.I.= Active ingredient concentration (g kg<sup>-1</sup> or g L<sup>-1</sup>);

<sup>2</sup>N° = Number of dead insects. Means followed by the same letter in the column do not differ significantly from each other by Dunn test with Bonferroni correction (P > 0.05); ns= not significant.

<sup>3</sup>C= Initial IOBC toxicity classes: 1 = innocuous (<30%); 2 = slightly harmful (30-79%); 3 = moderately harmful (80-99%); 4 = harmful (>99%);

<sup>4</sup>C= IOBC persistence classes: 1 = short-lived (<5 days); 2=slightly persistent (5-15 days); 3 = moderately persistent (16-30 days); 4 = persistent (>30 days)

**Table 6** Cumulative larval mortality (%), ratio of female fecundity, egg fertility and total effect of insecticides applied on the adult stage of *Chrysoperla externa*.

Tratamentos	<sup>1</sup> C.D.	<sup>2</sup> M	<sup>3</sup> R1	<sup>4</sup> R2	<sup>5</sup> E.T.	
3 days after application (DAA)	acetamiprid + etofenprox	70	0.00	1.05	1.03	0.00
	spinetoram	30	100	--	--	100
	indoxacarb	75	40.75	--	--	100
	methoxyfenozide	80	40.75	--	--	100
10 days after application (DAA)	acetamiprid + etofenprox	70	0	1.16	1.08	0.00
	spinetoram	30	60	--	--	100
	indoxacarb	75	6.67	0.88	1.27	0.00
	methoxyfenozide	80	6.67	1.10	0.94	3.47
17 days after application (DAA)	acetamiprid + etofenprox	70	0	0.97	1.02	1.29
	spinetoram	30	33.33	1.32	1.07	5.73
	indoxacarb	75	26.67	0.98	1.05	24.97
	methoxyfenozide	80	10.01	0.84	1.04	21.03
24 days after application (DAA)	acetamiprid + etofenprox	70	9.38	0.89	0.96	21.86
	spinetoram	30	15.63	0.95	1.02	18.05
	indoxacarb	75	0.00	0.93	0.99	7.95
	methoxyfenozide	80	0.00	0.89	0.86	22.91
31 days after application (DAA)	acetamiprid + etofenprox	70	0.00	1.17	1.01	0.00
	spinetoram	30	21.43	1.05	1.12	7.72
	indoxacarb	75	0.00	1.06	0.88	6.65
	methoxyfenozide	80	14.29	1.05	1.12	7.72

<sup>1</sup>C.D. = Commercial product dosage (g or mL.100 L<sup>-1</sup>);

<sup>2</sup>M= Mortality of treatment based on the formula of Schneider-Orelli (Püntener 1981) (%);

<sup>3</sup>R1= Ratio between the daily average of eggs laid per treated and untreated female;

<sup>4</sup>R2= Ratio between the average viability of eggs laid per treated and untreated female;

<sup>5</sup>E.T.= Total effect estimated following the formula proposed by Vogt et al. (1998). E.T.=100-(100%-M) x R1 x R2.

tested insecticides were classified as innocuous (class 1) (Table 5), with total effects < 30% (Table 6). In the evaluations at 24 and 31 DAA, all products tested were classified as innocuous (class 1) to the adult stage of *C. externa* (Table 5). There was no significant difference between treatments in these two evaluations (Table 5).

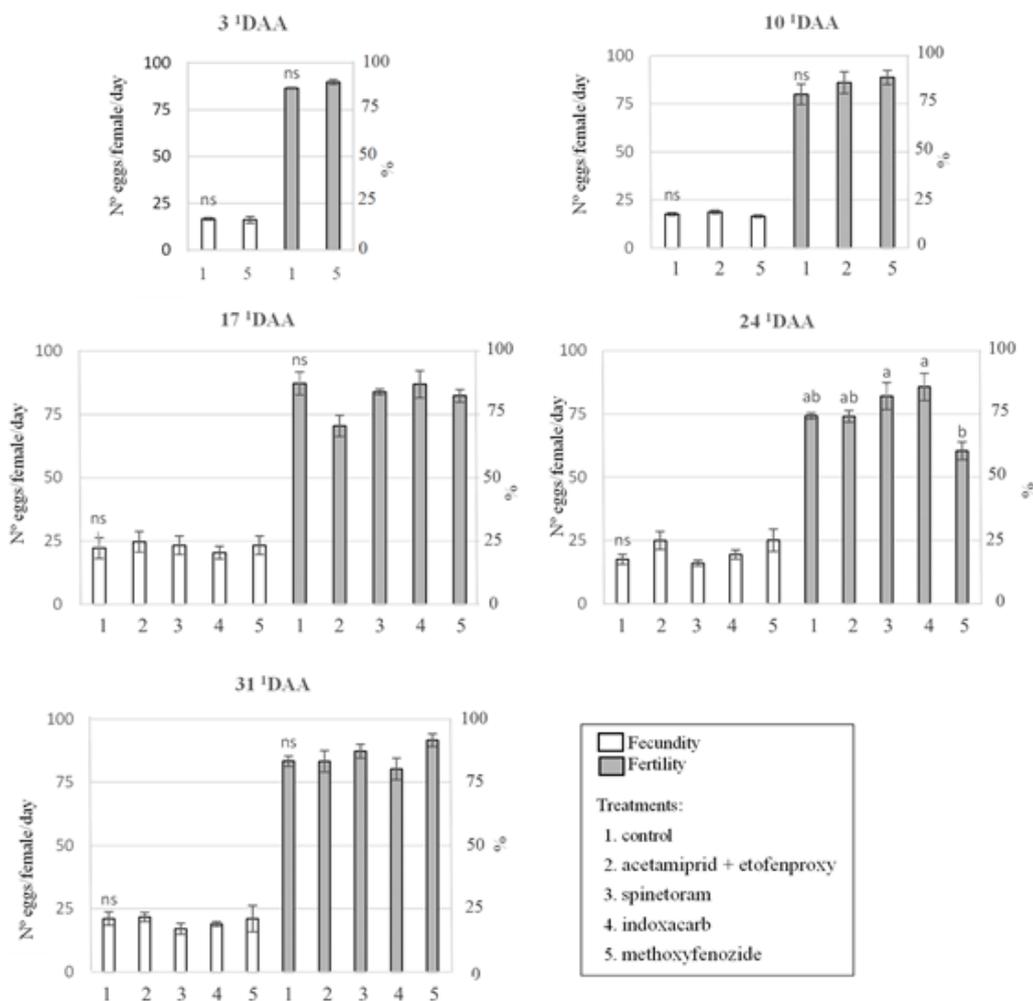
Regarding the classification of persistence of harmful activity against the predator’s adult phase, the insecticides acetamiprid + etofenprox, indoxacarb and methoxyfenozide were classified as short-lived (class 1) due to their harmful activity lasting less than 5 days. The insecticide spinetoram was classified as

slightly persistent (class 2), as residual activity lasted between 5 and 15 days (Table 5).

**Sublethal effects on surviving insects.**

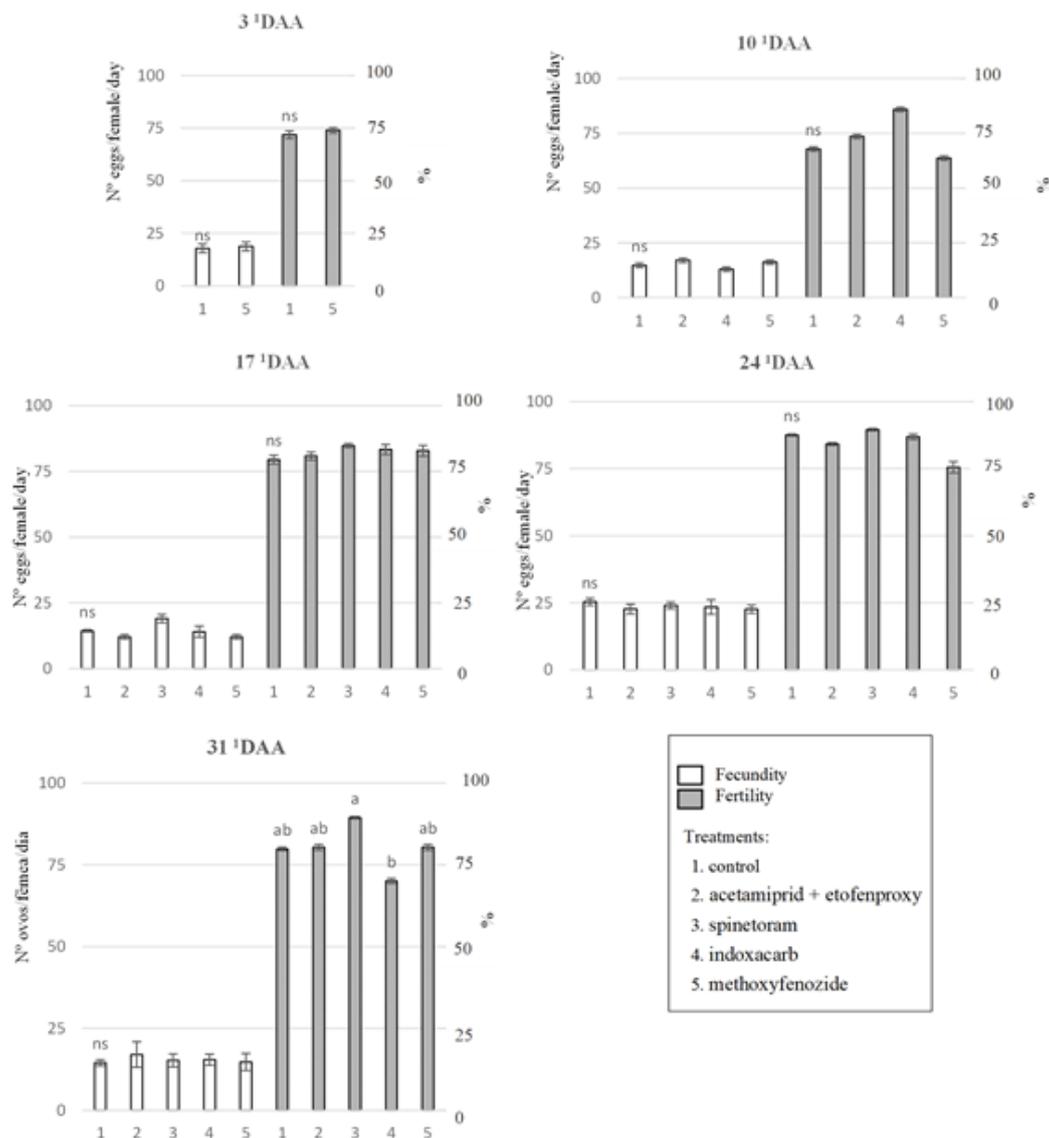
There was no significant difference in the reproductive parameters between the treatments evaluated at 3, 10 and 17 DAA, either in the evaluation of fecundity or fertility in larvae (Figure 3), or in adults (Figure 4).

At 24 DAA, there was no statistical difference in the fecundity of females exposed at first larval instar between treatments (Figure 3). In the fertility assessment, methoxyfenozide resulted in the lowest value (60.37%), differing significantly from spinetoram and indoxacarb (Figure 3).



**Figure 3.** Sublethal effects four insecticides on fecundity and fertility of females of *Chrysoperla externa* that survived larval exposure to insecticide residues. Means followed by the same letter in the column do not differ significantly from each other by Tukey test ( $p \leq 0.05$ ); ns= Not significant.

<sup>1</sup>DAA= days after application. Statistics: 3 DAA: Fecundity (FEC) df= 1; F= 0.05; p= 0.82; and Fertility (FER): df= 1; F= 0.42; p= 0.53; 10 DAA: FEC: df= 2; F= 1.50; p= 0.27 and FER: df= 2; F= 0.60; p= 0.57; 17 DAA: FEC: df= 4; F= 1.38; p= 0.85 and FER: df= 4; F= 2.37; p= 0.09.



**Figure 4.** Fecundity and fertility of females of *Chrysoperla externa* that survived adult exposure to insecticide residues. Means followed by the same letter in the column do not differ significantly from each other by Tukey test (ANOVA) ( $P > 0.05$ ); ns= Not significant.

<sup>1</sup>DAA= days after application. Statistics: 3 DAA: Fecundity (FEC):  $df = 1$ ;  $F = 0.09$ ;  $p = 0.77$  and Fertility (FER):  $df = 1$ ;  $F = 0.03$ ;  $p = 0.86$ ; 10 DAA: FEC:  $df = 3$ ;  $H = 3.03$ ;  $p = 0.38$  and FER:  $df = 3$ ;  $H = 5.37$ ;  $p = 0.14$ ; 17 DAA: FEC:  $df = 4$ ;  $F = 8.24$ ;  $p = 0.08$  and FER:  $df = 4$ ;  $F = 1.27$ ;  $p = 0.86$

At 31 DAA, evaluation of the fecundity of females and egg fertility when exposure occurred at first larval instar showed no significant differences between the evaluated treatments (Figure 3). Evaluation of fecundity at 31 DAA when exposure occurred at the adult stage showed no statistical difference between the treatments evaluated (Figure 4). On the other hand, in the fertility evaluation, the indoxacarb group had the lowest value (70.07%), differing significantly from spinetoram (89.44%), where as the acetamiprid

+ etofenprox and methoxyfenozide groups both showed 80.36% of egg viability, which was not significantly different from the control (Figure 4).

## Discussion

This study provides important new information on the toxicity patterns of four insecticides with five different modes of action (MoAs) for *C. externa*. Many factors are involved in the foliar persistence of chemi-

cal insecticides, such as climatic conditions, type of application, plant species, dosage, interval between applications and leaf age (JACOBSEN et al., 2015; PÉREZ-AGUILAR et al., 2018; MORALES et al., 2019).

The ready-mix insecticides acetamiprid + etofenprox belongs to Group 3A and Group 4A (IRAC, 2022), and the two components act as competitive modulators of nicotinic acetylcholine receptors at the post synapse (CASIDA; DURKIN, 2013; VANACLOCHA et al., 2019), and as sodium channel modulators (SOARES; CARVALHO, 2018), respectively. Acetamiprid + etofenprox, despite being a short-lived insecticide for larvae and adults of *C. externa*, showed differences in selectivity depending on the evaluation time, being harmful in the larval stage and innocuous in the adult stage of *C. externa*, in terms of mortality three days after application. The reason for greater adult survival and greater larval mortality may be the greater contact of larvae compared to the adult, due to their complex foraging activity, which increases the possibility of interaction with an insecticide-treated surface (GARZÓN et al., 2015). In addition, acetamiprid toxicity has been reported for *Chrysoperla rufilabris* (Burmeister, 1839) (Neuroptera: Chrysopidae) and *Hippodamia convergens* (Guérin-Ménéville, 1842) (Coleoptera: Coccinellidae) after exposure to blueberry leaves 14 DAA (ROUBOS et al., 2014). Other authors also found high levels of toxicity of imidacloprid, another insecticide from the neonicotinoid group, soon after application, followed by a decrease in mortality during subsequent days, when application occurred on leaves, in insects such as *Ceraeochrysa cubana* (Hagen, 1861) (Neuroptera: Chrysopidae) (RUGNO et al., 2015). *Orius insidiosus* (Say, 1832) (Hemiptera: Anthocoridae) (FERNANDES et al., 2016) and *Engytatus varians* (Distant, 1884) (Hemiptera: Miridae) (MORALES et al., 2019). It is important to emphasize that this insecticide acts by contact and the systemic route, has a broad spectrum of action and is easily translocated in plants through the xy-

lem and phloem (RORTAIS et al., 2005), despite being considered short-lived for *C. externa*. Other study using thiamethoxam. from the same chemical group, showed a greater residual effect on predators *Eriopis connexa* (Germar, 1824) (Coleoptera: Coccinellidae). *C. externa*, *O. insidiosus* and *Podisus nigrispinus* (Dallas, 1851) (Heteroptera: Pentatomidae), which was attributed to it being a systemic insecticide, and potentially remaining longer on the plant, thus potentially impairing biological control (MACHADO et al., 2019).

The insecticide spinetoram was classified as moderately persistent (class 3) to larvae, and slightly persistent (class 2) to adults of the predator. Spinetoram belongs to group 5 (IRAC, 2022) and acts by binding first to nicotinic acetylcholine receptors and, later, to gamma amino butyric acid receptors, causing the opening of ion channels and leading to the death of insects (SANTOS-JUNIOR et al., 2019). Despite the reported selectivity of spinosyns towards predators (CASTRO et al., 2018; SANTOS-JUNIOR et al., 2019), climatic conditions can directly influence the speed of chemical reactions, which can increase or decrease insecticide efficiency, being able to change the concentration of leaf residue in pesticide persistence experiments (MORALES et al., 2019). Temperature may be able to accelerate chemical reactions, often causing accelerated product degradation (ITOIZ et al. 2012; MORALES et al. 2019). However, Mansoor et al. (2015) reported that spinosyns have a negative coefficient and perform better at lower temperatures, such that the toxicity of this compound decreased by 1.27-fold at 28 °C and 1.47-fold at 40 °C in *C. carnea*. However, other factors must be taken into account, such as relative humidity and solar radiation (ITOIZ et al., 2012; MORALES et al., 2019). The low relative humidity throughout the experiment may partly explain the low rate of degradation of the chemical reactions that took place in the solution. In addition, the low incidence of ultraviolet (UV) radiation in experiments carried out in the greenhouse,

such as the one in this work, may also be a crucial factor, since in the field direct light causes photo degradation of the product, and despite not being measured in the bioassays, there may be a 75% decrease in UV intensity under these conditions due to the presence of plastic (MORALES et al., 2019). Our results corroborate those found by Jamil et al. (2019), with the predatory mite *Neoseiulus fallacis* (Garman, 1948) (Acari: Phytoseiidae), which also showed the residual effect of spinetoram up to 14 DAA. This decrease in toxicity was attributed to insecticide dilution on the leaves due to high enzymatic activity (BIONDI et al., 2012).

Indoxacarb belongs to group 22A (IRAC, 2022), is a voltage-gated sodium channel blocker (SILVA et al., 2017), and has been classified as moderately persistent (class 3) to larvae and as a short-lived insecticide (class 1) against adults of *C. externa*. Several studies have indicated that indoxacarb is selective against several natural enemies, such as parasitoids, lacewings and coccinellids (PEREIRA et al., 2014; ROUBOS et al., 2014; ARAUJO et al., 2017). One of the factors that may be related to this selectivity is the involvement of the cytochrome P450 monooxygenase enzyme, which may be related to a detoxification process in the predator, as reported for *Solenopsis saevissima* (Smith, 1855) (Hymenoptera: Formicidae) in residual toxicity tests with the adult stage of the ant in a greenhouse (ARAUJO et al., 2017).

In the current study the insecticide methoxyfenozide was classified as short-lived (class 1) for lacewing larvae and adults. This insecticide, belonging to group 18 (IRAC, 2022), is a diacylhydrazine that acts as an agonist of ecdysteroid receptors (RIMOLDI et al., 2008). The selectivity of this compound against natural enemies such as parasitoids and predators was recognized (ZOTTI et al., 2013; ONO et al., 2017). Insecticides that inhibit chitin synthesis, such as methoxyfenozide, act mainly by ingestion, but some compounds may have contact toxicity and adversely af-

fect reproductive parameters such as fecundity and especially fertility (ONO et al., 2017; SHAN et al., 2020). In addition, they act mainly on lepidopteran larvae (ZOTTI et al., 2013; PEREZ AGUILAR et al., 2018). The low toxicity of this insecticide on larvae and adults observed in the present study corroborates the literature, confirming the selectivity against *C. externa*, but altered the fecundity and fertility of females in some tests, as reported with *C. cubana* in laboratory tests (ONO et al., 2017).

It is important to emphasize that the vine plants used in the experiments were kept in a greenhouse for 31 days after the application of insecticides, protected from rain and also from ultraviolet radiation. In addition, relative humidity was low on most days, unlike in the field, which may have influenced the duration of harmful activity, as this can be favored or harmed by temperature, relative humidity, solar radiation, plant age, plant health and water conditions (MACHADO et al., 2019; MORALES et al., 2019; PASINI et al., 2020). Factors related to the insect, such as age, developmental stage, resistant strains, production of esterases and monooxygenases in cytochrome P450, can also have an influence (LUNA et al., 2018; CARVALHO et al., 2019; MORALES et al., 2019; QUESADA; SADO, 2020).

The insecticides spinetoram and indoxacarb, which were classified as moderately persistent (class 3), should be tested under field conditions, as they may have lower impacts on *C. externa* due to climatic effects on the product and also the ability of the insect to avoid treated areas (HASSAN, 1994). Differences in persistence for the two stages of development evaluated for each species were observed for spinetoram and indoxacarb, with spinetoram being moderately persistent for larvae and slightly persistent for adults, and indoxacarb being moderately persistent to larvae and considered to have short-lived effects on adult *C. externa*. Pasini et al. (2020) also found difference in

persistence of harmful activity against larvae and adults of *C. externa* and *E. connexa*, with the larval stage also being more sensitive than the adult stage. This difference in selectivity may be due to the foraging capacity of the two stages (GÁRZON et al., 2015). Quesada and Sadof (2020) also reported that the difference in selectivity between lacewings and coccinellids may be due to their feeding habits, whereby chewing coccinellids are more sensitive than immature lacewings that feed only on the cellular content of the prey.

The persistence of insecticides is not always evaluated in studies involving the selectivity of pesticides, but this information helps determine the impact of insecticides on non-target organisms and to estimate the survival of the population of natural enemies after exposure to chemical control (CASTILHOS et al., 2019; PASINI et al., 2020). Castilhos et al. (2019) also reported the importance of persistence tests in orchards, such as peach orchards, which are highly dependent on chemical control to control primary pests. Maintaining predators such as *C. externa* in orchards is thus important, and persistence tests would assist in decision making regarding whether to flood an area with this predator, as this should take place after the persistent interval. In this way it would be possible to achieve compatibility between chemical and biological control, favoring the presence of *C. externa* in orchards, and helping mainly in the suppression of pests that are considered secondary, thus reducing the indiscriminate use of insecticides.

Finally, we highlight those insecticides are still the main approach for maintaining pest arthropods below the threshold of economic damage, but the integration of chemical and biological controls is fundamental for the success of IPM. This is only possible with the use of selective insecticides that do not cause significant damage to natural enemies. In addition, preservation of natural en-

emies will likely reduce outbreaks of secondary pests, and will primarily act on surviving individuals of target pest species under selection pressure for resistance, thus delaying resistance selection (CARVALHO et al., 2019; MACHADO et al., 2019). Likewise, strategies aimed at reducing resistance are also essential in IPM, such as rotating insecticides with different mode of action or using insecticides containing two active ingredients. Therefore, we highlight the importance of this work evaluating the residual period of harmful activity of insecticides such as acetamiprid + etofenprox, spinetoram, indoxacarb and methoxyfenozide, which belong to five different groups (IRAC, 2022).

## Conclusion

In general, our results suggest that the insecticides acetamiprid + etofenprox and methoxyfenozide could be the most indicated in orchards that target IPM techniques, since these two insecticides are considered to have short-lived (class 1) harmful effects on first instar larvae and adults of *C. externa*. The insecticides spinetoram and indoxacarb should be avoided when *C. externa* first instar larvae are present, as they are classified as having moderately persistent (class 3) harmful effects on the larval stage of the predator. Although spinetoram is classified as a slightly persistent insecticide (class 2) and indoxacarb as a short-lived insecticide (class 1) against the adult stage of the predator, they should be used sparingly in orchards, especially when there are first instar larvae, as they are more sensitive than adults.

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## References

- ABDULLAHI. G.; OBENG-OFORI. D.; AFREH-NUAMAH. K.; BILLAH. M.K. Acute and residual concentration-dependent toxicities of some selected insecticides to adult *Bactrocera invadens* Drew. Tsuruta and White (Diptera: Tephritidae). **The Journal of Basic and Applied Zoology**. Giza. v.1. n.1. p.1-10. 2020.
- ARAÚJO. T.A.D.; PICANÇO. M.C.; FERREIRA. D.D.O.; CAMPOS. J.N.; ARCANJO. L.D.P.; SILVA. G.A. Toxicity and residual effects of insecticides on *Ascia monuste* and predator *Solenopsis saevissima*. **Pest Management Science**. Medford. v.73. n.11. p.2259-66. 2017.
- BIONDI. A.; DESNEUX. N.; SICARO. G.; ZAPPALÀ. L. Using organic-certified rather than synthetic pesticides may not be safer for biological control agents: selectivity and side effects of 14 pesticides on the predator *Orius laevigatus*. **Chemosphere**. Oxford. v.87. n.7. p.803-12. 2012.
- CARVALHO. G.A.; GRÜTZMACHER. A.D.; PASSOS. L.C.; OLIVEIRA. R.L. Physiological and ecological selectivity of pesticides for natural enemies of insects. In: SOUZA. B.; VÁZQUEZ. L.; MARUCCI. R. (ed.). **Natural enemies of insect pests in neotropical agroecosystems**. Cham: Springer. 2019. p.469-78.
- CASIDA. J.E.; DURKI. K.A. Neuroactive insecticides: targets. selectivity. resistance. and secondary effects. **Annual Review of Entomology**. Stanford. v.58. n.. p.99-117. 2013.
- CASTILHOS. R.V.; GRÜTZMACHER. A.D.; KRÜGER. L.R.; SIQUEIRA. P.R.B.; MORAES. I.L. Persistence of insecticides used in peach orchards to larvae and adults of the predator *Chrysoperla externa* (Neuroptera: Chrysopidae). *Arquivos do Instituto Biológico*. São Paulo. v.83. n.. p.1-8. 2019.
- CASTRO. A.A.; LEGASPI. J.C.; TAVARES. W.D.S; MEAGHER JUNIOR. R.L.; MILLER. N.; KANGA. L.; HASEEB. M.; SERRÃO. J.E.; WILCKEN. C.F.; ZANUNCIO. J.C. Lethal and behavioral effects of synthetic and organic insecticides on *Spodoptera exigua* and its predator *Podisus maculiventris*. **Plos One**. San Francisco. v.13. p.e0206789. 2018.
- DE ARMAS. F.S.; GRUTZMACHER. A.D.; NAVA. D.E.; PASINI. R.A.; RAKES. M.; PAZINI. J.B. Non-target toxicity of nine agrochemicals toward larvae and adults of two generalist predators active in peach orchards. **Ecotoxicology**. Berlin. v.29. n.. p.327-39. 2020.
- DESNEUX. N.; DECOURTYE. A.; DELPUECH. J.M. Sublethal effects of pesticides on beneficial arthropods. **Annual Review of Entomology**. Stanford. v.52. n.1. p.81-106. 2007.
- FERNANDES. F.L.; BACCI. L.; FERNANDES. M.S. Impact and selectivity of insecticides to predators and parasitoids. **EntomoBrasilis**. Vassouras. v.3. n.1. p.1-10. 2010.
- FERNANDES. M.E. ALVES. F.M.; PEREIRA. R.C.; AQUINO. L.A.; FERNANDES. F.L.; ZANUNCIO. J.C. Lethal and sublethal effects of seven insecticides on three beneficial insects in laboratory assays and field trials. **Chemosphere**. Oxford. v.156. n.1. p.45-55. 2016.
- GARZÓN. A.; MEDINA. P.; AMOR. F.; VINUELA. E.; BUDIA. F. Toxicity and sublethal effects of six insecticides to last instar larvae and adults of the biocontrol agents *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) and *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae). **Chemosphere**. Oxford. v.132. n.1. p.87-93. 2015.
- HASSAN. S.A. Activities of the IOBC/WPRS working group pesticides and beneficial organisms. **IOBC/WPRS Bulletin**. Montfavet. v.17. n.10. p.1-5. 1994.
- HASSAN. S.A.; ABDELGADER. H.A. Sequential testing program to assess the effects of pesticides on *Trichogramma cacoeciae* Marchal (Hym.. Trichogrammatidae). **IOBC/WPRS Bulletin**. Montfavet. v. 24. n. 4. p.24-81. 2001.
- IRAC - Insecticide Resistance Action Committee. **The Irac classification: an interactive mode of action (MoA) tool**. 2002. Disponível em: <http://www.irac-online.org/modes-of-action/>. Acesso em: 24 out. 2022.

- ITOIZ. E.S.; FANTKE. P.; JURASKE. R.; KOUNINA. A.; VALLEJO. A.A. Deposition and residues of azoxystrobin and imidacloprid on greenhouse lettuce with implications for human consumption. **Chemosphere**. Oxford. v.89. n.9. p.1034-41. 2012.
- JACOBSEN. R.E.; FANTKE. P.; TRAPP. S. Analysing half-lives for pesticide dissipation in plants. **SAR and QSAR in Environmental Research**. London. v.26. n.. p.325-42. 2015.
- JAMIL. R.Z.R.; VANDERVOORT. C.; WISE. J.C. Residual toxicity of insecticides to *Neoseiulus fallacis* (Acari: Phytoseiidae) in apples. **Journal of Economic Entomology**. Laham. v.112. n.5. p.2262-7. 2019.
- JANSEN. J.P. Beneficial arthropods and pesticides: building selectivity list for IPM. **IOBC/WPRS Bulletin**. Montfavet. v.55. n.1. p.23-47. 2010.
- LEIVAS. G.; SCHIAVON. A.V.; MARQUES L.O.D.; HELLWIG. C.G.; ALQUINO. E.L.; SILVA. G.F.; MARTINS. C.R. Caracterização fitotécnica dos sistemas de produção de pêssegos na Região de Pelotas-RS. **Brazilian Journal of Development**. Curitiba. v.6. n.5. p.32594-618. 2020.
- LUNA. R.F.; BESTETE. L.R.; TORRES. J.B.; SILVA-TORRES. C.S.A. Predation and behavioral changes in the neotropical lacewing *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) exposed to lambda-cyhalothrin. **Ecotoxicology**. Berlin. v.27. n.6. p.689-702. 2018.
- MACHADO. A.V.; POTIN. D.M.; TORRES. J.B.; TORRES. C.S.S. Selective insecticides secure natural enemies action in cotton pest management. **Ecotoxicology and Environmental Safety**. Orlando. v.184. n.1. p.109669. 2019.
- MANSOOR. M.M.; AFZAL. A.B.M.; RAZA. Z.; AKRAM. A.; WAQAR. M.B.S.; AFZAL. Post-exposure temperature influence on the toxicity of conventional and new chemistry insecticides to green lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). **Saudi Journal of Biological Sciences**. Riyadh. v.22. n.3. p.317-21. 2015.
- MAPA - Ministério Da Agricultura, Pecuária E Abastecimento. **Sistema de agrotóxicos fitossanitários**. Brasília. 2021. Disponível em: [http://agrofit.agricultura.gov.br/agrofit\\_cons/](http://agrofit.agricultura.gov.br/agrofit_cons/) principal\_agrofit\_cons. Acesso em: 07 jan. 2021.
- MELO. G.W.B.; ZALAMENA. J.; BRUNETTO. G.; CERETTA. C.A. **Calagem, adubação e contaminação em solos cultivados com videiras**. Bento Gonçalves: Embrapa Uva e Vinho. 2016. 138p. (Documentos. 100).
- MONTEIRO. L.B.; WITT. L.G.; GUILOSKI. I.C.; SANTOS. R.S.S.; ASSIS. H.C.S. Evaluation of resistance management for the oriental fruit moth (Lepidoptera: Tortricidae) to insecticides in Brazilian apple orchards. **Journal of Economic Entomology**. Laham. v.113. n.3. p.1411-8. 2020.
- MORALES. S.I.; MARTÍNEZ. A.M.; FIGUEROA. J.I.; CAMPOS-GARCÍA. J.; GÓMEZ-TAGLE. A.; LOBIT. P.; SMAGGHE. G.; PINEDA. S. Foliar persistence and residual activity of four insecticides of different mode of action on the predator *Engytatus varians* (Hemiptera: Miridae). **Chemosphere**. Oxford. v.235. n.1. p.76-83. 2019.
- ONO. É.K.; ZANARDI. O.Z.; SANTOS. K.F.A.; YAMAMOTO. P.T. Susceptibility of *Ceraeochrysa cubana* larvae and adults to six insect growth-regulator insecticides. **Chemosphere**. Oxford. v.168. n.1. p.49-57. 2017.
- PARRA. J.R.P. 1997. Técnicas de criação de *Anagasta kuehniella*. hospedeiro alternativo para produção de *Trichogramma*. In: PARRA. J.R.P.; ZUCCHI. R.A. (ed.). **Trichogramma e o controle biológico aplicado**. Piracicaba: FEALQ. 1997. p.121-50.
- PASINI. R.A.; RAKES. M.; CASTILHOS. R.V.; DE ARMAS. F.S.; PAZINI. J.B.; ZANTEDESCHI. R.; GRÜTZMACHER. A.D. Residual action of five insecticides on larvae and adults of the neotropical predators *Chrysoperla externa* (Neuroptera: Chrysopidae) and *Eriopis connexa* (Coleoptera: Coccinellidae). **Ecotoxicology**. Berlin. v.30. n.1. p.44-56. 2020.

- PEREIRA. R.R.; PICANÇO. M.C.; SANTANA JUNIOR. P.A.; MOREIRA. S.S.; GUEDES. R.N.; CORRÊA. A.S. Insecticide toxicity and walking response of three pirate bug predators of the tomato leaf miner *Tuta absoluta*. **Agricultural and Forest Entomology**. Oxford. v.16. n.3. p.293-301. 2014.
- PÉREZ-AGUILAR. D.A.; SOARES. M.A.; PASSOS. L.C.; MARTÍNEZ. A.M.; PINEDA. S.; CARVALHO. G.A. Lethal and sublethal effects of insecticides on *Engytatus varians* (Heteroptera: Miridae). a predator of *Tuta absoluta* (Lepidoptera: Gelechiidae). **Ecotoxicology**. Berlin. v.27. n.6. p.719-28 2018.
- PÜNTENER. W. **Manual for field trials in plant protection**. 2.ed. Basle: Ciba-Geigy. 1981. 205p.
- QUESADA. C.R.. C.S. SADO. Residual toxicity of insecticides to *Chrysoperla rufilabris* and *Rhyzobius lophanthae* predators as biocontrol agents of pine needle scale. **Crop Protection**. Guildford. v.130. n.1. p.105044. 2020.
- R DEVELOPMENT CORE TEAM. **R: A language and environment for statistical computing**. Versão 4.0.0. Viena: R Foundation for Statistical Computing. 2021. Disponível em: <http://r-project.org>. Acesso em: 10 fev. 2021.
- RAMESHGAR. F.; KHAJEHALI. J.; NAUEN. R.; DERMAUW. W.; VAN. L.T. Characterization of abamectin resistance in Iranian populations of European red mite. *Panonychus ulmi* Koch (Acari: Tetranychidae). **Crop Protection**. Guildford. v.125. n.1. p.104903. 2019.
- RIMOLDI. F.; SCHNEIDER. M.I.; RONCO. A. Susceptibility of *Chrysoperla externa* eggs (Neuroptera: Chrysopidae) to conventional and biorational insecticides. **Environmental Entomology**. Annapolis. v.37. n.1. p.1252-7. 2008.
- RORTAIS. A.S.; ARNOLD. G.R.; HALM. M.P.; TOUFFET-BRIENS. F. Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. **Apidologie**. Versailles. v.36. n.1. p.71–83. 2005.
- ROUBOS. C.R.; RODRIGUEZ-SAONA. C.; HOLDCRAFT. R.; MASON. K.S.; ISAACS. R. Relative toxicity and residual activity of insecticides used in blueberry pest management: Mortality of natural enemies. **Journal of Economic Entomology**. Laham. v.107. n.1. p.277-85. 2014.
- RUGNO. G.R.; ZANARDI. O.Z.; YAMAMOTO. P.T. Are the pupae and eggs of the lacewing *Ceraeochrysa cubana* (Neuroptera: Chrysopidae) tolerant to insecticides? **Journal of Economic Entomology**. Laham. v.108. n.6. 2630-9. 2015.
- SANTOS-JUNIOR. V.C.; MARTÍNEZ. L.C.; PLATA-RUEDA. A.; BOZDOĞAN. H.; ZANUNCIO. J.C.; SERRÃO. J.E. Exposure to spinosad induces histopathological and cytotoxic effects on the salivary complex of the non-target predator *Podisus nigrispinus*. **Chemosphere**. Oxford. v.225. n.1. p.688-95. 2019.
- SCHMUCK. R.. CANDOLFI. M.P.; KLEINER. R.; MEAD-BRIGGS. M.; MOLL. M.; KEMMETER. F.; JANS. D.; WALTERSDORFER. A.; WILHELMY. H.A. Laboratory test system for assessing effects of plant protection products on the plant dwelling insect *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). In: CANDOLFI. M.P.; BLUMEL. S.; FORSTER. R.; BAKKER. F.M.; GRIMM. C.; HASSAN. S.A.; HEIMBACH. U.; MEAD-BRIGGS. M.A.; REBER. B.; SCHMUCK. R.; VOGT. H. (ed.). **Guidelines to evaluate side-effects of plant protection products to non-target arthropods**. Reinheim: IOBC/WPRS. 2000. p.45-56.
- SHAN. Y.X.; ZHU. Y.; LI. J.J.; WANG. N.M.; YU. Q.T.; XUE. C.B. Acute lethal and sublethal effects of four insecticides on the lacewing (*Chrysoperla sinica* Tjeder). **Chemosphere**. Oxford. v.250. n.1. p.126321. 2020.
- SILVA. B.K.D.A.. M.S.D. GODOY. A.G.D. LIMA. A.K.S.D. OLIVEIRA. P.L. PASTORI. Toxicity of insecticides used in muskmelon on first-instar larvae of *Chrysoperla genanigra* Freitas (Neuroptera: Chrysopidae). **Revista Caatinga**. Mossoró. v.30. n.3. p.662-9. 2017.
- SOARES. A.D.F.T.; CARVALHO. G.A. Physiological selectivity of insecticides to eggs and larvae of predator *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae). **Coffee Science**. Lavras. v.13. n.3. p.292–303. 2018.

- STERK. G.; HASSAN. S.A.; BAILLOD. F.; BAKKER. F.; BIGLER. F.; BLÜMEL. S.; et al. Results of the seventh joint pesticide testing programme carried out by the IOBC/WPRS-working group 'Pesticides and beneficial Organisms'. **BioControl**. Netherlands. v.44. n.1. p.99-117. 1999.
- STUPP. P.; MACHOTA JUNIOR. R.; CARDOSO. T.D.N.; PADILHA. A.C.; HOFFER. A.; BERNARDI. D.; BOTTON. M. Mass trapping is a viable alternative to insecticides for management of *Anastrepha fraterculus* (Diptera: Tephritidae) in apple orchards in Brazil. **Crop Protection**. Guildford. v.139. n.1. p.105391. 2021.
- SUÁREZ-LÓPEZ. Y.A.; HATEM. A.E.; ALDEBIS. H.K.; VARGAS-OSUNA. E. Lethal and sublethal effects of lufenuron on the predator *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). **Crop Protection**. Guildford. v.134. n.. p.105217. 2020.
- TERNES. P.; CANDOLFI. M.P.; UFER. A.; VOGT. H. Influence of leaf substrates on the toxicity of selected plant protection products to *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) and *Aphidius rhopalosiphi* DeStefani Perez (Hymenoptera: Aphidiidae). **IOBC/WPRS Bulletin**. Montfavet. v.24. n.4. p.7–15. 2001.
- VANACLOCHA. P.. M.M JONES. J.A. TANSEY. C. MONZÓ. X. CHEN. P.A. STANSLY. Residual toxicity of insecticides used against the Asian citrus psyllid and resistance management strategies with thiamethoxam and abamectin. **Journal of Pest Science**. Berlin. v.92. n.2. p.871-83. 2019.
- VOGT. H.; BIGLER. F.; BROWN. K.; CANDOLFI. M.P.; KEMMETER. F.; KÜHNER. C.; MOLL. M.; TRAVIS. A.; UFER. A.; VIÑUELA. E.; WLADBURGER. M.; WALTERSDORFER. A. Laboratory method to test effects of plant protection products on larvae of *Chrysoperla carnea* (Neuroptera: Chrysopidae). In: CANDOLFI. M.P.; BLUMEL. S.; FORSTER. R.; BAKKER. F.M.; GRIMM. C.; HASSAN. S.A.; HEIMBACH. U.; MEAD-BRIGGS. M.A.; REBER. B.; SCHMUCK. R.; VOGT. H. (ed.). **Guidelines to evaluate side-effects of plant protection products to non-target arthropods**. Reinheim: IOBC/WPRS. 2000. p. 27–44.
- VOGT. H.; DEGRANDE. P.; JUST. J.; KLEPKA. S.; KUHNER. C.; NICKLESS. A.; UFER. A.; WALDBURGER. M.; WALTERSDORFER. A.; BIGLER. F. Side-effects of pesticides on larvae of *Chrysoperla carnea* (Neuroptera. Chrysopidae): actual state of the laboratory method. In: HASKELL. P.T.; MCEWEN. P. (ed.). *Ecotoxicology*. Boston: Springer. 1998. p. 123-36.
- ZOTTI. M.J.; GRUTZMACHER. A.D.; LOPES. I.H.; SMAGGHE. G. Comparative effects of insecticides with different mechanisms of action on *Chrysoperla externa* (Neuroptera: Chrysopidae): Lethal, sublethal and dose–response effects. **Insect Science**. Hoboken. v.20. n.6. p.743-52. 2013.