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# Selectivity of pesticides used in peach orchards to eggs and pupae of the predators *Chrysoperla externa* and *Coleomegilla quadrifasciata*

# Seletividade de produtos fitossanitários utilizados na cultura do pessegueiro a ovos e pupas dos predadores *Chrysoperla externa* e *Coleomegilla quadrifasciata*

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## Abstract

This study aimed to assess, under laboratory conditions, the selectivity of nine pesticides used in peach to the egg and pupal stages of the predators *Chrysoperla externa* and *Coleomegilla quadrifasciata*. Tests consisted of the direct application of pesticides on eggs and pupae of predators and assessment of sublethal effects on fertility and fecundity of emerged adults. For eggs, the pesticides were classified according to the effects on the reduction of the percentage of larval hatching, while for pupae they were classified as a function of the total effect, according to the toxicity scale proposed by the International Organization for Biological and Integrated Control (IOBC). All the pesticides (used dose) were harmless (class 1) to eggs of *C. externa*, but abamectin (80) and copper + calcium (1%) were considered slightly harmful (class 2) to pupae. In bioassays with *C. quadrifasciata*, abamectin (80), deltamethrin (40), and malathion (150) were classified as slightly harmful (class 2), while fenitrothion (100) was moderately harmful (class 3) to predator eggs; in the pupal stage, abamectin (80), fenitrothion (100), and malathion (150) were considered harmful (class 4). Azadirachtin (1%), chlorantraniliprole (14), deltamethrin (40), copper + calcium (25% + 10%), and sulfur + calcium (3.5 Ba) were harmless (class 1) to eggs and pupae of *C. externa* and *C. quadrifasciata* and thus should be prioritized in sprayings for pest control in the IPM of peach.
**Key words:** Green lacewing. Ladybug. Fruticulture. Chemical control. Integrated pest management.

## Resumo

Objetivou-se com este trabalho avaliar em laboratório a seletividade de nove produtos fitossanitários utilizados na cultura do pessegueiro sobre as fases de ovo e pupa dos predadores *Chrysoperla externa* e *Coleomegilla quadrifasciata.* Os ensaios consistiram da aplicação direta dos agrotóxicos sobre ovos e pupas dos predadores e avaliação de efeitos subletais na fertilidade e fecundidade dos adultos emergidos. Para ovos, os produtos fitossanitários foram classificados em função dos efeitos na redução

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da porcentagem de eclosão de larvas, e para pupas em função do efeito total, conforme a escala de toxicidade proposta pela *International Organization for Biological and Integrated Control* (IOBC). Todos os produtos fitossanitários (dosagem utilizada) foram inócuos (classe 1) aos ovos de *C. externa*, entretanto, abamectina (80) e cobre + cálcio (1%) foram considerados levemente nocivos (classe 2) às pupas. Nos bioensaios com *C. quadrifasciata*, abamectina (80), deltametrina (40) e malationa (150) foram classificados como levemente nocivos (classe 2), enquanto fenitrotiona (100) apresentou-se como moderadamente nocivo (classe 3) aos ovos do predador; para a fase de pupa, abamectina (80), fenitrotiona (100) e malationa (150) foram nocivos (classe 4). Os produtos *Azadiractina* (1%), clorantraniliprole (14), deltametrina (40), cobre + cálcio (25% + 10%) e enxofre + cálcio (3,5 Ba) são inócuos (classe 1) aos ovos e pupas de *C. externa* e *C. quadrifasciata*, e assim devem ser priorizados em pulverizações visando ao controle de pragas no MIP pessegueiro.

**Palavras-chave:** Crisopídeo. Joaninha. Fruticultura. Controle químico. Manejo integrado de pragas.

## Introduction

Peach crop stands out in the temperate fruticulture. According to data from the Brazilian Institute of Geography and Statistics (IBGE, 2018), Brazil produced 222,613 tons of peach in the 2017 season. In this context, the state of Rio Grande do Sul is the main peach producer, standing out the municipalities of Pelotas and Canguçu (MADAIL, 2014), which account for more than 70% of the production destined to industry, a fact related to the number of agro-industries in the region (EHLERT, 2017).

The presence of insect pests in peach orchards may cause economic losses to producers. The fruit flies *Anastrepha fraterculus* (Wiedemann, 1930) and *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae), the oriental fruit moth *Grapholita molesta* (Busck, 1916) (Lepidoptera: Tortricidae), and the maize weevil *Sitophilus zeamais* (Motschulsky, 1885) (Coleoptera: Curculionidae) are the most important insect pests found in peach orchards (NAVA et al., 2014; NÖRNBERG et al., 2016). For satisfactory levels of production to be achieved, these pests are often controlled by applying chemicals (CHOUINARD et al., 2016). However, the indiscriminate use of these chemicals causes environmental imbalances mainly due to the mortality of biological control agents, such as natural enemies (DEGRANDE et al., 2002).

Among the natural enemies of peach pests is the green lacewing *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae). This predatory species presents a high predation capacity during its larval stage, host diversity, high reproductive potential, ease of rearing in the laboratory, and tolerance to some pesticides, showing its potential for use in biological control programs of insect pests (CASTILHOS et al., 2014; PASINI et al., 2018). Other important predators in an orchard are from the Coccinellidae family, which stand out as being generalists from larval to adult stage, preying on insect pests such as aphids, mealybugs, psyllids, whiteflies, mites, eggs and immature beetles, and lepidopteran (GIORGI et al., 2009; LIXA et al., 2010). One of the species of this family that occurs in peach is the ladybug *Coleomegilla quadrifasciata* (Schöenherr, 1808) (Coleoptera: Coccinellidae), being considered an important biological control agent (ZAZYCKI, 2010).

The presence of predators in orchards is vital to the success of integrated pest management (IPM) in the peach crop and to preserve them in the agroecosystem, it is essential to use selective pesticides to populations of natural enemies (REDDY, 2016). It should also be taken into account that the egg and pupal stages are immobile and exposed to direct sprayings in orchards, which can cause lethal and sublethal effects on these developmental stages (RUGNO et al., 2015). Thus, studying pesticide selectivity on the egg and pupal stages is necessary since any impact in one of them can inhibit the development cycle and prevent the establishment of populations of *C. externa* and *C.*

*quadrifasciata* in peach orchards (DEGRANDE et al., 2002).

In Brazil, there are few studies on pesticide selectivity in the peach crop using the egg and pupal stages of *C. externa* (CASTILHOS et al., 2014), and no study was found with *C. quadrifasciata*. Thus, due to the lack of data on pesticide selectivity, mainly using manipulated formulations, studies that address their lethal and sublethal effects on *C. externa* and *C. quadrifasciata* are required. Therefore, this study aimed to assess the selectivity of nine pesticides with commercial and manipulated formulations used in the peach crop on eggs and pupae of *C. externa* and *C. quadrifasciata* using methodology proposed by the International Organization for Biological and Integrated Control (IOBC).

## Material and Methods

*Rearing and maintenance of predators and alternative host*

Eggs and pupae of *C. externa* and *C. quadrifasciata* used in the bioassays were obtained from a rearing maintained at the Laboratory of Integrated Pest Management (LabMIP) of the Federal University of Pelotas (UFPel) under controlled conditions (temperature of 25±1 °C, relative humidity of 70±10%, and photoperiod of 14 hours), according to methodology adapted from Silva et al. (2009) and Castilhos et al. (2014). The population of the alternative host *Ephestia kuekniella* (Zeller, 1879) was reared according to the methodology proposed by Parra (1997) and used to feed the larval stage of *C. externa* and the larval and adult stages of *C. quadrifasciata*. Adult individuals of *C. externa* fed the diet proposed by Vogt et al. (2000).

*Pesticides*

Nine pesticides (Table 1) recommended in the integrated peach production were used (BRASIL, 2003). Most of them are also in accordance with the doses proposed for the conventional cultivation (MAPA, 2018). The products were chosen because they are usually recommended not only for the peach cultivation but also in other temperate climate crops. Six commercial products were used and the used dose corresponded to the maximum recommended dose peach crop (MAPA, 2018). The three pesticides with manipulated formulation are listed in the Standards for the Organic Production of Plants and Animals (BRASIL, 1999), being used as recommended for the crop (Table 1).

**Table 1.** Pesticides used in the maximum recommendation for the peach crop in bioassays of eggs and pupae of *Chrysoperla externa* and *Coleomegilla quadrifasciata*.

| Technical name | Active ingredient (a.i.) | Chemical group | Maximum dose[1] | C[2] |
|---|---|---|---|---|
| | | Commercial formulation | | |
| Altacor | Chlorantraniliprole | Anthranilamide | 14 | 4.90 |
| Decis 25 EC | Deltamethrin | Pyrethroid | 40 | 1.00 |
| Malathion 100 EC | Malathion | Organophosphorus | 150 | 10.00 |
| Neemax | Azadirachtin | Tetranortriterpenoid | 1% | 0.15 |
| Sumithion 500 EC | Fenitrothion | Organophosphorus | 100 | 5.00 |
| Vertimec 18 EC | Abamectin | Avermectin | 80 | 0.18 |
| | | Manipulated formulation | | |
| Concentrated Bordatec | Copper + calcium | Inorganic | - | 25% + 10% |
| Bordeaux mixture | Copper + calcium | Inorganic | - | 1% |
| Lime-sulfur solution | Sulfur + calcium | Inorganic | - | 3.5 Ba* |

[1]mL or g 100 $L^{-1}$ of water; [2]Concentration of a.i. in the solution, g 100 $L^{-1}$ or %; *Degree Baume (Ba).

Pesticides with manipulated formulation consisted of concentrated Bordatec, Bordeaux mixture, and lime-sulfur solution. Concentrated Bordatec was prepared by dissolving the product in 500 mL of water and using a portable pH meter to check whether the pH was in the range of 7 to 9. Bordeaux mixture was prepared according to the recommendations of Fortes (2002) for temperate fruit trees by homogenizing 30 g of copper sulfate and 30 g of quicklime in 5 L of water and measuring the pH with a portable pH meter until it reached the range of 8 to 9. Lime-sulfur solution was prepared according to the methodology described by Venzon et al. (2016), in which the solution is composed of 100 g of sulfur, 50 g of quicklime and 5 L of water. These components were homogenized and heated to a reddish-gray color. Then, the density of this solution was measured with a Baume-type densimeter and the concentration was adjusted to 3.5 degrees Baume (Ba).

*Bioassays*

The bioassays were conducted following the IOBC methodologies, as proposed by Medina et al. (2003). Product sprayings were carried out directly on eggs and pupae, both with approximately 24 hours of age. A manual sprayer with a 500 mL capacity (Guarany® Ultrajet, Itu, SP, Brazil) with a spray solution tank of 2±0.2 mg $cm^{-2}$, measured through a precision scale, was used.

*Bioassay with eggs*

The experimental design was a completely randomized design with four replications of 24 eggs each, totaling 96 eggs per experimental unit. After spraying and drying of the applied pesticides on the eggs of *C. externa* and *C. quadrifasciata*, they were individualized in 96-well cell culture plates and maintained in an acclimatized room. At five days after individualization, egg viability and a consequent reduction in larval hatching (RLH) were assessed for each treatment.

*Bioassay with pupae*

The experimental design of the bioassay with pupae of *C. externa* and *C. quadrifasciata* was a completely randomized design with four replications of six pupae each, totaling 24 pupae per experimental unit. The pupae of predators were individualized in 24-well cell culture plates after spraying and subsequent drying of pesticides and maintained in a room under the same conditions of rearing. At seven days after application, viability and reduction of adult emergence (RAE) were assessed for each pesticide.

*Sublethal effects of sprayed adults in the pupal stage*

In addition to the lethal effects, the sublethal effects on fecundity and fertility of surviving adults from the applications in the pupal stage were also assessed. For this, five to seven couples of *C. externa* were separated into acrylic cages (15.5 cm high × 18.5 cm in diameter) and five couples of *C. quadrifasciata* were separated into polyethylene containers (9 cm high × 12 cm in diameter). Both predators were maintained in a room with climatic conditions and feeding equal to that of rearing. At seven days after the observation of the first oviposition, eggs from both species were collected daily for 10 consecutive days. The average number of eggs/female/day was determined from the daily count of eggs of *C. externa* and *C. quadrifasciata*. These eggs were then incubated in 96-well cell culture plates to determine the average percentage of larval hatching in each treatment.

*Selectivity classes*

The variables RLH and RAE were corrected as a function of the control treatment by the Schneider-Orelli equation (PÜNTENER, 1981). The total effect of each pesticide for pupae was calculated using the equation proposed by Vogt et al. (1992): $E = 100\% - (100\% - RAE\%) \times R1 \times R2$, where E is the total effect (%), RAE% is the reduction of adult emergence, R1 is the ratio between the average daily number of eggs laid per treated and untreated female, and R2 is the ratio between the average viability of eggs laid per treated and untreated female. The pesticides were classified according to their selectivity to eggs as a function of the reduction in the percentage of hatching and to the pupae as a function of the total effect, according to the toxicity classes proposed by IOBC (STERK et al., 1999): 1) harmless (<30%); 2) slightly harmful (30-79%); 3) moderately harmful (80-99%); and 4) harmful (>99%).

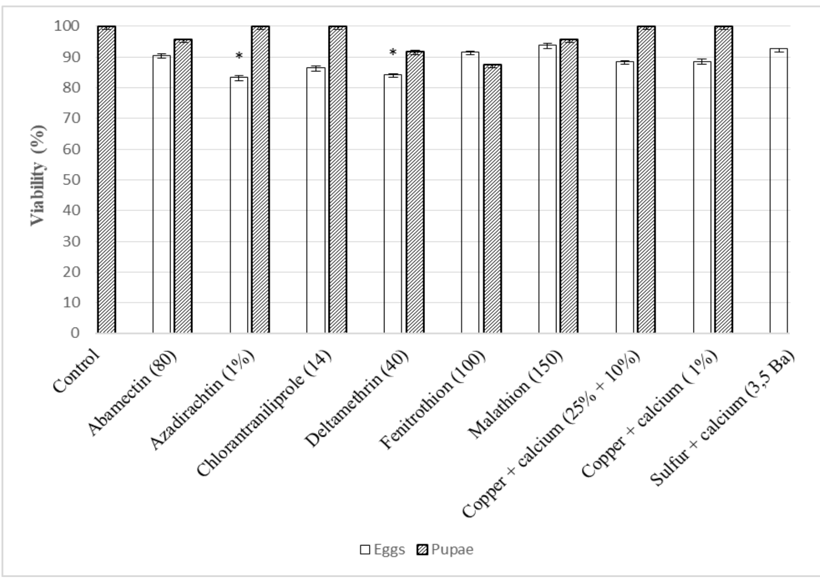
*Statistical analysis*

The data on egg and pupal viability, fecundity, and fertility were submitted to the exploratory analysis of normality of residuals by the Shapiro Wilk test and homoscedasticity by the Barlett test, with subsequent analysis of variance (ANOVA). The average viability of eggs and pupae of each treatment was compared with the control by the Dunnett's test ($\alpha = 0.05$), while the means of fecundity and fertility were compared by Tukey's test ($\alpha = 0.05$) when significant. The statistical analyses were performed using the statistical software Assistat version 7.7 (SILVA; AZEVEDO, 2016).

## Results and Discussion

Egg viability of *C. externa* after pesticide applications ranged from 81.25 to 95.83%. Products with the commercial formulation azadirachtin (1%) and deltamethrin (40) provided egg viability of 81.25 and 83.33%, respectively, differing significantly from the control (Figure 1). All the pesticides (commercial and manipulated formulations) were classified as harmless (class 1) to the egg stage of *C. externa* since RLH was lower than 30% (Table 2).

**Figure 1.** Viability of eggs and pupae of *Chrysoperla externa* sprayed with pesticides used for peach crop.




*Significant difference when compared to the control by the Dunnett's test at 5% probability.

**Table 2.** Total effect and toxicity classification of pesticides used in the peach crop and applied to eggs and pupae of *Chrysoperla externa*.

| Treatment | CP[1] | Eggs | | Pupae | | | | |
|---|---|---|---|---|---|---|---|---|
| | | RLH[2] | C[3] | RAE[4] | Fecundity | Fertility | E (%)[5] | C[3] |
| Commercial formulation | | | | | | | | |
| Control | - | - | - | 0.00 | 21.77 ± 0.93[ns] | 95.88 ± 0.22 a | - | - |
| Abamectin | 80 | 10.42 | 1 | 4.17 | 19.95 ± 1.98 | 73.75 ± 1.06b | 32.35 | 2 |
| Azadirachtin | 1% | 18.75 | 1 | 0.00 | 22.43 ± 0.89 | 94.79 ± 0.2a | 0.00 | 1 |
| Chlorantraniliprole | 14 | 12.50 | 1 | 0.00 | 22.04 ± 2.12 | 85.41 ± 0.25ab | 9.81 | 1 |
| Deltamethrin | 40 | 16.67 | 1 | 8.33 | 20.20 ±0.29 | 92.70 ± 0.00a | 17.76 | 1 |
| Fenitrothion | 100 | 8.33 | 1 | 12.50 | 19.55 ± 0.45 | 87.50 ±1.54ab | 28.29 | 1 |
| Malathion | 150 | 4.17 | 1 | 12.50 | 21.04 ±2.17 | 86.46 ± 0.96ab | 23.74 | 1 |
| Manipulated formulation | | | | | | | | |
| Copper + calcium | 25% + 10% | 12.50 | 1 | 0.00 | 19.29 ± 1.48 | 93.75 ± 0.25a | 13.36 | 1 |
| Copper + calcium | 1% | 10.42 | 1 | 0.00 | 17.20 ± 1.24 | 82.30 ± 0.65ab | 32.27 | 2 |
| Sulfur + calcium | 3.5 Ba* | 8.33 | 1 | 4.17 | 19.47 ± 1.25 | 92.67 ± 0.61ab | 16.01 | 1 |

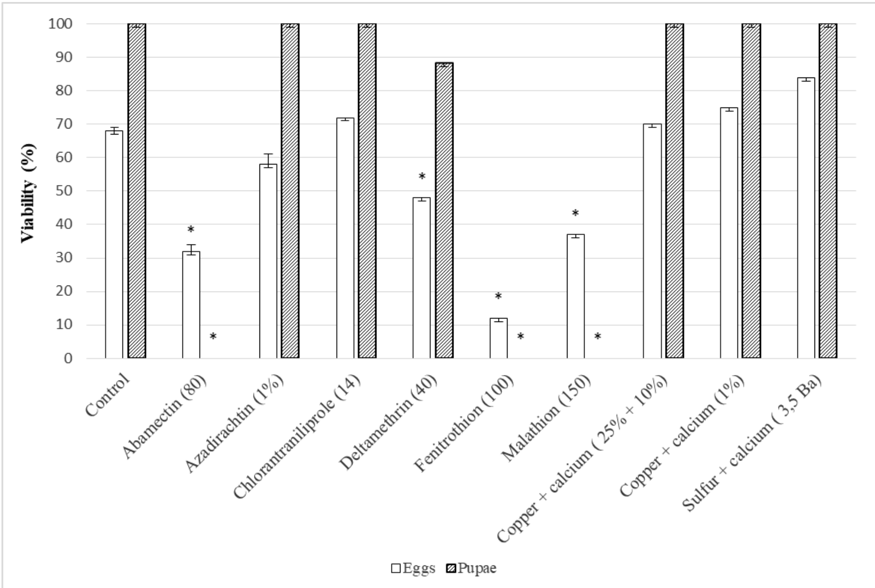
[1]CP = dose of commercial product (g or mL 100 $L^{-1}$) or percentage of concentration of the active ingredient in the solution; [2]RLH = reduction in larval hatching corrected by Schneider-Orelli (%); [3]C = IOBC classes: 1 = harmless (<30%), 2 = slightly harmful (30-79%), 3 = moderately harmful (80-99%), and 4 = harmful (>99%); [4]RAE = reduction of adult emergence corrected by Schneider-Orelli (%); [5]E = total effect (%); *Degree Baume; [ns] = not significant at 5% probability (ANOVA). Means followed by the same letter in the column do not differ significantly from each other by the Tukey's test at 5% probability.

For the ladybug *C. quadrifasciata*, egg viability ranged from 11.45 to 84.37% so that the commercial formulation fenitrothion (100) caused the lowest index and the manipulated product sulfur + calcium (3.5 Ba) led to the highest index. The commercial products abamectin (80), deltamethrin (40), fenitrothion (100), and malathion (150) provided significantly lower egg viability when compared to the control (Figure 2). Regarding selectivity classification of products tested on the ladybug eggs, azadirachtin (1%), chlorantraniliprole (14), copper + calcium (25% + 10%), all products with manipulated formulation were considered harmless (class 1); abamectin (80), deltamethrin (14), and malathion (150) were slightly harmful (class 2); and fenitrothion (100) was classified as moderately harmful (class 3) since it caused an RLH of 83.33% (Table 3).

Most of the tested pesticides caused a low RAE of *C. externa* and pupal viability submitted to application ranged from 87.5 to 100% (Figure 1). Regarding the pupal viability of *C. quadrifasciata*, the commercial products abamectin (80), fenitrothion (100), and malathion (150) caused an RAE of 100%, significantly reducing predator viability (Figure 2). The products did not significantly reduce the fecundity of *C. externa*. However, when the fertility of these eggs was assessed, abamectin (80) provided significantly lower viability when compared to the control (Table 2).

Azadirachtin (1%), chlorantraniliprole (14), deltamethrin (40), malathion (150), fenitrothion (100), copper + calcium (25% + 10%), and sulfur + calcium (3.5 Ba) showed a total effect lower than 30% for *C. externa*, being considered harmless (class 1) (Table 2). Studies conducted by Zanuncio et al. (2016) showed that larvae of the predator *Podisus nigrispinus* (Dallas, 1851) (Heteroptera: Pentatomidae) exposed to concentrations of azadirachtin above 50% caused adult malformation and reproductive problems, but when the concentrations were higher than 25%, they did not cause mortality or sublethal effects, thus evidencing the influence concentration on compound selectivity. These data are in accordance with the results obtained in our study, in which the used concentration was 1% and was also selective to *C. externa*.

**Figure 2.** Viability of eggs and pupae of *Coleomegilla quadrifasciata* sprayed with pesticides used for peach crop.




*Significant difference when compared to the control by the Dunnett's test at 5% probability.

**Table 3.** Total effect and toxicity classification of pesticides used in the peach crop and applied to eggs and pupae of *Coleomegilla quadrifasciata*.

| Treatment | CP[1] | Eggs | | Pupae | | | | |
|---|---|---|---|---|---|---|---|---|
| | | RLH[2] | C[3] | RAE[4] | Fecundity | Fertility | E (%)[5] | C[3] |
| Commercial formulation | | | | | | | | |
| Control | – | – | – | 0.00 | 24.41±3.22[ns] | 88.33 ± .82[ns] | – | – |
| Abamectin | 80 | 54.55 | 2 | 100.00 | – | – | 100.00 | 4 |
| Azadirachtin | 1% | 15.55 | 1 | 0.00 | 26.16 ± 2.12 | 79.17 ± 1.58 | 1.81 | 1 |
| Chlorantraniliprole | 14 | 0.00 | 1 | 0.00 | 28.24 ±2.94 | 71.87 ± 1.49 | 5.86 | 1 |
| Deltamethrin | 40 | 43.94 | 2 | 8.33 | 27.27 ± 2.69 | 70.00 ± 1.43 | 26.97 | 1 |
| Fenitrothion | 100 | 83.33 | 3 | 100.00 | – | – | 100.00 | 4 |
| Malathion | 150 | 45.45 | 2 | 100.00 | – | – | 100.00 | 4 |
| Manipulated formulation | | | | | | | | |
| Copper + calcium | 25% + 10% | 0.00 | 1 | 0.00 | 28.60 ± 3.18 | 69.79 ±3.18 | 0.64 | 1 |
| Copper + calcium | 1% | 0.00 | 1 | 0.00 | 28.96 ± 1.19 | 75.00 ± 0.71 | 0.73 | 1 |
| Sulfur + calcium | 3.5 Ba* | 0.00 | 1 | 0.00 | 26.17 ± 2.32 | 81.00 ± 2.32 | 1.69 | 1 |

[1]CP = dose of commercial product (g or mL 100 $L^{-1}$) or percentage of concentration of the active ingredient in the solution; [2]RLH = reduction in larval hatching corrected by Schneider-Orelli (%); [3]C = IOBC classes: 1 = harmless (<30%), 2 = slightly harmful (30-79%), 3 = moderately harmful (80-99%), and 4 = harmful (>99%); [4]RAE = reduction of adult emergence corrected by Schneider-Orelli (%); [5]E = total effect (%); *Degree Baume; [ns] = not significant at 5% probability (ANOVA). Means followed by the same letter in the column do not differ significantly from each other by the Tukey's test at 5% probability.

The insecticide abamectin (80) presented a total effect of 32.35%, being considered slightly harmful (class 2) to *C. externa*, mainly due to a reduction in the fertility of adults emerged from treated pupae (Table 2). Similar results were found in the coffee crop, in which the same active ingredient was considered slightly harmful to eggs of *C. externa*, with egg viability of 65 and 57% for concentrations of 0.0067 and 0.0225 g a.i. $L^{-1}$, respectively (VILELA et al., 2010). Abamectin acts as a GABA (gamma-aminobutyric acid) agonist, with the ability to mimic the molecules of this neurotransmitter in the nervous system of insects. Thus, it binds to receptors and stimulates the flow of chlorine ions into nerve cells and neuromuscular junctions, leading to the blockade of nerve stimulus transmission and hence the death of these arthropods (AZOD et al., 2016). Studies with predatory mites of the Phytoseiidae family (HAN et al., 2010) and the ladybird *Menochilus sexmaculatus* (Fabricius, 1781) (Coleoptera: Coccinellidae) (AZOD et al., 2016) reported that despite the immediate mortality of natural enemies when exposed to high doses of this product, it does not cause sublethal damage to surviving populations or exposed to a lower dose, thus being a viable alternative to IPM programs.

Both fecundity and fertility of adults of *C. quadrifasciata* that survived the applications on pupae showed no significant reduction. Azadirachtin (1%), chlorantraniliprole (14), deltamethrin (40), copper + calcium (25% + 10%), copper + calcium (1%), and sulfur + calcium (3.5 Ba) were classified as harmless (class 1) to pupae of *C. quadrifasciata*, with a total effect lower than 30%. In contrast, abamectin (80), fenitrothion (100), and malathion (150) were considered harmful (class 4), as they caused an RAE of 100% (Table 3).

The fact that most of the products did not affect the fecundity and fertility of *C. externa* (Table 2) and *C. quadrifasciata* (Table 3) is very important for the viability of the biological control exerted by these predators in the peach crop since populations of natural enemies may succumb not only to mortality caused by pesticides but also to sublethal effects that affect the physiology or behavior of the insects (DESNEUX et al., 2007).

The active ingredient chlorantraniliprole is recognized by its selectivity to mammals and natural enemies, acting on the muscular system of insects by means of the activation of ryanodine receptors and release of calcium in the sarcoplasmic reticulum of muscular cells, thus regulating the intramuscular calcium balance (SATTELLE et al., 2008). This commercial product, even causing a reduction of 12.50% in RLH of *C. externa*, was considered harmless (class 1) (Table 2). In *C. quadrifasciata*, chlorantraniliprole did not alter the percentage of larval hatching (Table 3). This result was also observed in the pupal stage, in which a total effect of 9.81 and 5.86% was observed in *C. externa* and *C. quadrifasciata*, respectively, being considered selective to both (Tables 2 and 3).

Deltamethrin (40) did not present toxicity to *C. externa* in any of the stages in which it was applied (Table 2), being also classified as harmless (class 1) to the pupae of *C. quadrifasciata*, presenting 26.97% of total effect (Table 3). Similar results were obtained by Godoy et al. (2004), who classified this pyrethroid as selective to eggs of *C. externa* in citrus. A hypothesis for the selectivity of predators to pyrethroids is the metabolization of product molecules through the metabolic detoxification of microsomal oxidases and esterases, which is one of the factors favoring insect pest resistance to this insecticide group (LILLY et al., 2016), being also a route of metabolization in natural enemies.

However, in the egg stage of *C. quadrifasciata,* deltamethrin (40) was classified as slightly harmful (class 2) (Table 3). Fogel et al. (2016) used cypermethrin at a dose of 25 mg a.i. $L^{-1}$ in pupae of *E. connexa* pupae and observed a 41% reduction in pupal survival, which is similar to the result found in our study for deltamethrin (RLH of 43.94%) (Table 3). Products of the chemical group of pyrethroids act in the opening of sodium channels, influencing the ionic permeability of cellular membranes. The selectivity observed for deltamethrin may be related to the low penetration rate due to the lipophilicity of the compound and thickness and composition of eggs and pupae (DONG et al., 2014).

The organophosphorus fenitrothion (100) and malathion (150) were harmless (class 1) to both stages of development of *C. externa* (Table 2). Castilhos et al. (2014) found similar results when using fenthion (0.050) and phosmet (0.100), belonging to the same toxicological class, which were harmless to eggs and pupae of *C. externa.* These insecticides act by inhibiting the enzyme acetylcholinesterase in the nerve synapses, causing the continuous passage of impulses and causing insect death. However, a lower sensitivity of the enzyme acetylcholinesterase on predators or even changes in specific genes may explain the selectivity of these products (BACCI et al., 2007) as they are known to be harmful to natural enemies and pollinators (BOTTON et al., 2011).

Fenitrothion was moderately harmful (class 3) for *C. quadrifasciata* because it reduced larval hatching by 83.33%. Malathion reduced to 45.45% this parameter, being considered as moderately harmful (class 2) (Table 3). Torres et al. (2013) obtained similar results when using chlorpyrifos (0.60) on pupae of *C. externa* in the coffee crop, leading to an RAE of 62%, which is attributed to the residual period of organophosphorus. Adult death occurs during the emergence because of contamination through contact of the newly emerged insect or insect body parts during ecdysis with the integument of sprayed pupae.

The insecticide/acaricide abamectin (80) was selective to eggs of *C. externa,* which was also verified by Carvalho et al. (2011) in the apple tree. For the Coccinellidae *C. quadrifasciata,* abamectin caused a reduction of 54.55% in the egg stage, being classified as slightly harmful (class 2), in addition to causing mortality of 100% of pupae (Table 3). According to Galvan et al. (2002), abamectin was moderately harmful to *Brachygastra lecheguana* (Latreille, 1824) and *Protopolybia exigua* (Saussure, 1906) and toxic to *Protonectarina sylveirae* (Saussure, 1954) (Hymenoptera: Vespidae) at concentrations corresponding to 50% (underdose) and 100% (dose) in the citrus crop. These effects were due to the high molecular weight and complex structure of the insecticide/ acaricide molecule, which decreases the cuticle penetration rate and makes it more susceptible to the action of detoxifying enzymes, respectively (HORNSBY et al., 1996).

The commercial formulations azadirachtin (1%) and chlorantraniliprole (14) and the manipulated formulations copper + calcium (1%) and sulfur + calcium (3.5 Ba) were not toxic to the egg and pupal stages of *C. externa* and *C. quadrifasciata,* being considered harmless (class 1) (Tables 2 and 3). These results are similar to those obtained with pupae of the parasitoid *Telenomus remus* (Nixon, 1937) (Hymenoptera: Platygastridae) when using commercial mixtures such as Borda-Ferti pH 7 (7% copper + 3.3% calcium) and Lime-sulfur mixture (20% sulfur + 10% quicklime), which were classified as slightly harmful (class 2) (SILVA et al., 2016). Similarly, selective tests carried out by Silva and Bueno (2014), who used the commercial mixture Borda-Ferti pH 7 (7% copper + 3.3% calcium) in the pupal stage of *Telenomus podisi* (Ashmead, 1893) (Hymenoptera: Platygastridae), showed no RAE and was considered as harmless (class 1). Lime-sulfur mixture (20% sulfur + 10% quicklime) was tested in that experiment and showed to be harmless in all assessments, not causing a reduction in the adult emergence rate or changing the parasitism rate.

The product sulfur + calcium (3.5 Ba) was harmless (class 1) to the egg and pupal stages of both predators (Tables 2 and 3). Studies using the acaricide Kumulus, which contains sulfur in its formulation, showed that the product was selective to eggs of *C. externa* in coffee (SILVA et al., 2006) and to the egg and pupal stages of this predator in peach orchards (CASTILHOS et al., 2014). For predators of the Arachnida class, sulfur + calcium (3.5 Ba) was less selective, causing a reduction in the populations of the predatory mite *Iphiseiodes zuluagai* and *Euseius* spp. in citrus (ANDRADE et al., 2010).

In this sense, *C. quadrifasciata* was more affected, mainly in the egg stage. The commercial formulations abamectin (80), deltamethrin (40), fenitrothion (100), and malathion (150) showed higher toxicity, which was repeated in the pupal stage with abamectin (80), fenitrothion (100), and malathion (150) (Table 3). It is important to emphasize that in addition to the formulation of insecticides, morphological issues related to each species may explain the permeability of some insecticides, which could serve as channels for the penetration of insecticides, which may have occurred with the products abamectin (80), deltamethrin (40), fenitrothion (100), and malathion (150). However, the data found in the literature on these peculiarities are still scarce (RIMOLDI et al., 2017). In our study, the assessment of insecticide toxicity on eggs was restricted to the verification of a reduction in the percentage of larval hatching. However, possible sublethal effects such as changes in embryo duration and harmful effects on larvae and adults from treated eggs may have influenced the impact of a certain insecticide on the egg stage of *C. externa* and *C. quadrifasciata* in the peach crop.

Egg chorion constitutes a chemical and physical barrier to insecticides, favoring the development of the embryo and preventing the entry of hydrophilic substances and the exit of water (NATION, 2008; RIMOLDI et al., 2017). In addition, according to Cosme et al. (2009), the silk cocoon that protects the pupae of *C. externa* has holes that allow breathing and where pesticides can possibly reach the insect. However, as can be observed, the insecticides probably were not able to penetrate through these holes, demonstrating that the pupa acts as a physical barrier, avoiding insect contamination and, consequently, not reducing the emergence of adults of *C. externa* (Table 2). Another issue related to the penetration of insecticides into the outer layer of eggs and pupae is related to the molecular weight of these products (MOSCARDINI et al., 2013). Thus, part of the insecticides, including the neurotoxic ones, were not toxic because their formulations were not able to penetrate the egg chorion.

Most of the tested pesticides were harmless to the egg and pupa stages of *C. externa.* However, many pesticides were toxic to the egg and pupal stages of the ladybug *C. quadrifasciata.* Therefore, caution is needed in the use of these products in the peach IPM since they may adversely affect the natural enemies present in orchards, especially the commercial formulations abamectin (80), fenitrothion (100), and malathion (150), which affected both stages of the predator development. Thus, these products should be avoided, preferring the adoption of selective products to *C. externa* and *C. quadrifasciata* in order to favor the maintenance of the natural biological control exerted by both predators in peach orchards.

This study assessed the selectivity, under laboratory conditions, of several pesticides using a standardized methodology of IOBC. For those products that presented classes 3 (moderately harmful) and 4 (harmful), new studies are needed under semi-field and field conditions (HASSAN, 1988) to determine its real impact on the predators *C. externa* and *C. quadrifasciata.* The results of this study have great importance for peach cultivation since selective pesticides should have their use prioritized in IPM, thus contributing to the preservation of the predators *C. externa* and *C. quadrifasciata* in orchards.

Therefore, abamectin (80), azadirachtin (1%), chlorantraniliprole (14), deltamethrin (40), fenitrothion (100), malathion (150), copper + calcium (25% + 10%), copper + calcium (1%), and sulfur + calcium (3.5 Ba) are harmless (class 1) to eggs of *C. externa.* Abamectin (80) and copper +

calcium (1%) are slightly harmful (class 2) to pupae of *C. externa.* The products azadirachtin (1%), chlorantraniliprole (14), copper + calcium (25% + 10%), copper + calcium (1%), and sulfur + calcium (3.5 Ba) are harmless (class 1) to the egg stage of *C. quadrifasciata.* Abamectin (80), deltamethrin (40), and malathion (150) are slightly harmful (class 2), while fenitrothion (100) is moderately harmful (class 3) to the egg stage. Abamectin (80), fenitrothion (100), and malathion (150) are harmful (class 4) to the pupae of this natural enemy.

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