

# Acute toxicity of lethal and sublethal concentrations of neonicotinoid, insect growth regulator and diamide insecticides on natural enemies of the woolly apple aphid and the obscure mealybug

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Received: 1 February 2021; Accepted: 3 May 2021; doi:10.4067/S0718-58392021000300398

## ABSTRACT

Based on the importance of using low-risk compounds to protect beneficial agents, the present study evaluated the acute toxicity of lethal and sublethal concentrations of the insecticides thiacloprid, pyriproxyfen, and chlorantraniliprole on adults of *Aphelinus mali* (Haldeman), the main parasitoid of the woolly apple aphid, *Eriosoma lanigerum* (Hausmann). Similarly, acetamiprid, buprofezin, pyriproxyfen, chlorantraniliprole and cyantraniliprole were evaluated on adults of the parasitoid *Acerophagus flavidulus* (Brethés) and also on larvae and adults of the predator *Cryptolaemus montrouzieri* Mulsant, both species important natural enemies of the obscure mealybug, *Pseudococcus viburni* (Signoret). Natural enemies were exposed to insecticide residues of minimum recommended rate (1x) and between one to four concentrations (0.5x, 0.25x, 0.1x and 0.05x) of the minimum recommended rate on apple leaves. Our results indicate that thiacloprid was moderately harmful to *A. mali* at 1x, and acetamiprid was harmful to *A. flavidulus* causing 100% mortality from 0.1x to 1x concentrations. Buprofezin, pyriproxyfen and chlorantraniliprole were harmless or slightly harmful for both parasitoids. Cyantraniliprole was slightly harmful from 0.25x to 1x for *A. flavidulus*. For *C. montrouzieri*, acetamiprid was harmless or slightly harmful for larvae and adults on the concentrations evaluated. Buprofezin, pyriproxyfen and chlorantraniliprole were harmless for larvae and adults of *C. montrouzieri*. Cyantraniliprole was slightly harmful on larvae and moderately harmful on adults of *C. montrouzieri* at 1x. Our data suggest that buprofezin, pyriproxyfen and chlorantraniliprole could be compatible with the natural enemies evaluated, while thiacloprid and acetamiprid were less compatible with parasitoids than with the predator. Finally, cyantraniliprole seems to be less compatible than chlorantraniliprole at 1x with the natural enemies evaluated.

**Key words:** Aphelinidae, Coccinellidae, ecotoxicology, Encyrtidae, integrated pest management, mortality.

## INTRODUCTION

Apple (*Malus domestica* (Suckow) Borkh.) is an important fruit crop in many regions around the world, including central Chile and the West Coast of USA. Both regions share pest problems among which phloem-feeding pests, such as the woolly apple aphid (WAA), *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae), and mealybugs, *Pseudococcus* spp. (Hemiptera: Pseudococcidae), are important secondary pests. In apple orchards frequent applications of broad-spectrum insecticides can be the key factor explaining the limited success of some natural enemies, and the use of more selective insecticides is an important strategy to improve the integration of chemical and conservation biological control (Jones et al., 2009; Weddle et al., 2009).

Insecticides used to control codling moth (CM), *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), and other pests in apple orchards can disrupt biological control (Beers et al., 2016). Some of these are wide-spectrum neurotoxic insecticides, such as the neonicotinoids thiacloprid and acetamiprid (Casida, 2018) while other are more selective insect growth regulators (IGRs), such as buprofezin and pyriproxyfen (Tunaz and Uygun, 2004) or newer muscular contraction disruptors such as the anthranilinic diamides chlorantraniliprole and cyantraniliprole (Lahm et al., 2005; 2007; Selby et al., 2013; Ahumada and Chorbadjian, 2019).

Among the natural enemies of phloem-feeding pests, *Aphelinus mali* (Haldeman) (Hymenoptera: Aphelinidae) is the most important parasitoid of the WAA around the world. *Aphelinus mali* is a specialist, solitary, koinobiont endoparasitoid successfully introduced to many countries for the control of WAA (Howard, 1929; Asante, 1997; Cross et al., 1999). Regarding the natural enemies of the obscure mealybug (OMB), *Pseudococcus viburni* (Signoret) (Hemiptera: Pseudococcidae), in Chile the parasitoid *Acerophagus flavidulus* (Brethés) (Hymenoptera: Encyrtidae) is the most relevant (González, 2010; Charles, 2011). *Acerophagus flavidulus* is a specialist, gregarious, koinobiont endoparasitoid native to Argentina and Chile (Karamaouna and Copland, 2000; Noyes, 2003). On the other hand, the predator *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) is native to Australia and has been used in augmentative releases against mealybugs in many crops and countries (Kairo et al., 2013; Mani, 2018).

The aim of the study was to provide the necessary data to build integrated pest management programs of pome fruit orchards. We assess the acute toxicity of lethal (minimum field recommended rate) and sublethal (dilutions from minimum field recommended rate) concentrations of six insecticides frequently used for the main apple pests in pome fruit orchards in Chile, on adults of the parasitoids *A. mali* and *A. flavidulus*, and also on larvae and adults of the predator *C. montrouzieri*. We exposed parasitoids and predator to insecticide residues on apple leaves under laboratory conditions, in order to evaluate mortality effect of insecticide concentrations on these natural enemies.

## MATERIALS AND METHODS

### Insect material

The parasitoids *Aphelinus mali* (Haldeman) were collected as mummies from organic apple (*Malus domestica* (Suckow) Borkh.) trees of the Sunrise Research Farm, Tree Fruit Research and Extension Center, Washington State University (WSU), Wenatchee, USA. The parasitoid mummies were carefully placed in 30 mL plastic cups covered with a plastic lid. These cups were maintained in a growth room with a controlled environment ( $25 \pm 1$  °C,  $40 \pm 5\%$  RH and 16:8 h photoperiod) until the emergence of the adult parasitoid. The parasitoid *Acerophagus flavidulus* (Brethés) and the predator *Cryptolaemus montrouzieri* Mulsant were reared at Xilema SpA, a biological control company located in Quillota, Chile. Parasitoid mummies, predator larvae and newly eclosed adults were sent weekly to laboratories of Sanidad Vegetal at Universidad de Talca (UTAL), Talca, Chile. The *A. flavidulus* mummies were kept in a growth chamber (Conviron, Winnipeg, Canada) at  $25 \pm 0.1$  °C,  $50 \pm 5\%$  RH and a photoperiod of 16:8 h until adult emergence (usually 2 to 5 d after laboratory arrival).

### Application of insecticides

*Aphelinus mali* was exposed to 96.0 and 9.6 mg L<sup>-1</sup> ai thiacloprid ([3-[(6-chloropyridin-3-yl)methyl]-1,3-thiazolidin-2-ylidene]cyanamide), 50 and 5 mg L<sup>-1</sup> ai pyriproxyfen (2-[1-(4-phenoxyphenoxy)propan-2-yloxy]pyridine) and 40 and 4 mg L<sup>-1</sup> ai chlorantraniliprole (5-bromo-N-[4-chloro-2-methyl-6-(methylcarbamoyl)phenyl]-2-(3-chloropyridin-2-yl)pyrazole-3-carboxamide), equivalent to the minimum recommended rate (1x) and one tenth of that concentration (0.1x) for codling moth (CM) (*Cydia pomonella* (L.); Lepidoptera: Tortricidae), woolly apple aphid (WAA) (*Eriosoma lanigerum* (Hausmann); Hemiptera: Aphididae) and obscure mealybug (OMB) (*Pseudococcus viburni* (Signoret); Hemiptera: Pseudococcidae) on apples (Table 1). *Acerophagus flavidulus* and *C. montrouzieri* were exposed to acetamiprid (*N*-[(6-chloropyridin-3-yl)methyl]-*N'*-cyano-*N*-methylethanimidamide *N*), buprofezin (2-*tert*-butylimino-5-phenyl-3-propan-2-yl-1,3,5-thiadiazinan-4-one), pyriproxyfen, chlorantraniliprole, and cyantraniliprole (5-bromo-2-(3-chloropyridin-2-yl)-*N*-[4-cyano-2-methyl-6-(methylcarbamoyl)phenyl]pyrazole-3-carboxamide) (Table 1).

**Table 1. Insecticides and concentrations applied on apple leaf discs in toxicity test for natural enemies of the woolly apple aphid and the obscure mealybug.**

Commercial products (cp)	Active ingredient (chemical group)	Manufacturer	Minimum recommended cp rate mL or g L <sup>-1</sup>
Calypso 480 SC	Thiacloprid (neonicotinoid)	Bayer, AG, Germany	0.2 mL
Hurricane 70 WP	Acetamiprid (neonicotinoid)	Anasac SpA, Chile	0.12 g
Applaud 25 WP	Buprofezin (thiadiazine)	Anasac SpA, Chile	0.8 g
Delico 100 EC	Pyriproxyfen (pyridine)	Anasac SpA, Chile	0.5 mL
Coragen 20 SC	Chlorantraniliprole (anthranilic diamide)	FMC, USA	0.2 mL
Exirel 10 SE	Cyantraniliprole (anthranilic diamide)	FMC, USA	0.75 mL

Both OMB natural enemies were exposed to 84.00, 42.00, 21.00, 8.40 and 0.42 mg L<sup>-1</sup> ai acetamiprid; 200, 100, 50, 20 and 10 mg L<sup>-1</sup> ai buprofezin; 50.0, 25.0, 12.5, 5.0 and 2.5 mg L<sup>-1</sup> ai pyriproxyfen; 40, 20, 10, 4 and 2 mg L<sup>-1</sup> ai chlorantraniliprole and 75.00, 37.50, 18.75, 7.50 and 3.75 mg L<sup>-1</sup> ai cyantraniliprole. Each active ingredient concentration mentioned was equivalent to 1x, 0.50x, 0.25x, 0.10x and 0.05x of the minimum rate recommended for CM, WAA or OMB on apples. These concentrations were used to represent the degradation of insecticides residues over time. A volume of 2 mL of each insecticide concentration was applied at 0.045 MPa with a Potter precision laboratory spray tower (Burkard Scientific, Uxbridge, UK) on an apple leaf disk with the lower surface facing up on a pad of moist cotton inside a 30 mL plastic cup (Beers et al., 2009). Distilled water was used as control.

### Bioassays

The same methodology of exposure to dry insecticide residues on apple leaves was used for the three natural enemies as described by Gontijo (2011). The bioassay arena consisted in a 30 mL plastic cup with which had a lid with a small orifice ( $\approx$  5 mm in diameter) on the top covered by Micropore tape (3M, St. Paul, Minnesota, USA). Inside the cup, an apple leaf disk of 40 mm diameter was sprayed with insecticides or distilled water. Once the treated surface dried, one adult or larva of each natural enemy was placed over the leaf. All plastic cups were maintained in a Conviron chamber at  $25 \pm 0.1$  °C,  $50 \pm 5\%$  RH and a photoperiod of 16:8 h. Parasitoid adults which were 2-3 d old were used in the experiments in the WSU and UTAL laboratories. *Cryptolaemus montrouzieri* second-third instar larvae and newly eclosed adults were used on the day of arrival to the UTAL laboratories. Natural enemies were not fed during the exposure period. Evaluation of mortality was performed after 24 and 48 h exposure. Parasitoids and predators were considered dead if they did not move when probed with a fine brush (Mgocheki and Addison, 2009; Rogers et al., 2011; Wang et al., 2019). The experimental unit was an individual natural enemy. For each treatment, between 12-30 experimental units were used simultaneously. These whole bioassays were replicated three times.

### Statistical analysis

Data were analyzed as categorical with binary response (dead or alive) using a general linear model (GLM) with binomial distribution (R software; R Foundation for Statistical Computing, Vienna, Austria.) in order to compare different concentrations within each tested insecticide. Results were reported and analyzed only when control mortality was  $\leq 30\%$ . Significant differences ( $P \leq 0.05$ ) between treatments were evaluated with Tukey test using the “multcomp” R package. Mortality data were corrected using Abbott’s formula and negative values are shown as zero when presenting data in the tables (Abbott, 1925).

**Table 2. Mortality of *Aphelinus mali* at 24 and 48 h after exposure to insecticide treatments on apple leaf discs.**

Insecticide	Active ingredient concentration	Commercial product rate	n	Corrected mortality		IOBC class
				24 h	48 h	
	mg L <sup>-1</sup>			%	%	
Thiacloprid	0	Control	30	15.0 ± 10.6a	26.7 ± 18.8a	
	9.6	0.1x	30	43.1 ± 15.0b	45.5 ± 13.3b	2
	96.0	1x	30	84.3 ± 6.7c	93.2 ± 1.7c	3
		χ <sup>2</sup> , P		68.49 ≤ 0.01	67.28 ≤ 0.01	
Pyriproxyfen	0	Control	30	10.0 ± 3.3a	10.0 ± 3.3a	
	5	0.1x	30	3.7 ± 0.0a	7.4 ± 3.3a	1
	50	1x	30	63.0 ± 6.7b	70.4 ± 0.0b	2
		χ <sup>2</sup> , P		28.69 ≤ 0.01	33.24 ≤ 0.01	
Chlorantraniliprole	0	Control	34	0	0	
	4	0.1x	27	0	0	
	40	1x	29	0	3.5 ± 0.2	1
		χ <sup>2</sup> , P		0 ≥ 0.05	2.29 ≥ 0.05	1

Values within columns followed by a different letter are significantly different according to Tukey's test ( $P \leq 0.05$ ). Values are mean ± SE; n: number of insects tested. Control non corrected by Abbott's formula.

IOBC class: 1: Harmless (mortality < 30%), 2: slightly harmful (30 ≤ mortality ≤ 79%), 3: moderately harmful (80 ≤ mortality ≤ 99%), and 4: harmful (mortality > 99%).

## RESULTS

The mortality of adult *A. mali* was significantly increased with the minimum recommended (96 mg L<sup>-1</sup>) and 9.6 mg L<sup>-1</sup> concentrations of ai thiacloprid in comparison with the control after 24 and 48 h (Table 2). Pyriproxyfen applied at the lowest concentration (5 mg L<sup>-1</sup>) showed no difference in mortality with the control, but at the minimum recommended rate (50 mg L<sup>-1</sup>) mortality increased significantly for 24 and 48 h. On the contrary, chlorantraniliprole did not increase adult parasitoid mortality of *A. mali* in both concentrations evaluated.

From the lowest concentration (0.42 mg L<sup>-1</sup>) to the minimum recommended rate (84 mg L<sup>-1</sup>), acetamiprid significantly increased adult mortality of *A. flavidulus* in relation with the control treatment (Table 3). For all concentrations of insect growth regulators (IGRs) buprofezin and pyriproxyfen tested, adult mortality of *A. flavidulus* was not significantly different from control treatment. For chlorantraniliprole, only 0.25x concentration (10 mg L<sup>-1</sup>) was significantly higher than control. For cyantraniliprole all treatments except for the two lowest ones (3.75 and 7.5 mg L<sup>-1</sup>) were significantly higher than control (Table 3).

Higher mortality than control treatment was found for larvae of *C. montrouzieri* exposed to 0.5 and 1x rates of acetamiprid at 24 h, equivalent to 42 and 84 mg L<sup>-1</sup>, respectively (Table 4). Larvae of *C. montrouzieri* exposed to all concentrations tested of buprofezin and pyriproxyfen, showed nonsignificant differences in mortality from the control treatment at 48 h. The same was observed for chlorantraniliprole at 24 h. Only the minimum recommended rate of cyantraniliprole at 48 h significantly increased larval mortality relative to the control (Table 4).

Similar results were obtained for adults of *C. montrouzieri*, with neither of the IGRs (all concentrations) causing a significant level of mortality relative to the control (Table 5). For chlorantraniliprole, only 10 and 40 mg L<sup>-1</sup> significantly increased adult mortality at 48 h. Acetamiprid significantly increased adult mortality at all concentrations tested at 48 h. Similarly, cyantraniliprole at all concentrations over 18.75 mg L<sup>-1</sup> significantly increased adult mortality relative to the control at 48 h (Table 5).

## DISCUSSION

The International Organization for Biological Control (IOBC) recommends a tiered approach to evaluate insecticide compatibility with natural enemies, whereby insecticide testing is performed in the laboratory and depending upon the results obtained, semi-field or field tests may be included. The IOBC classifies pesticides into four categories depending on the extent of mortality or reduction in life history performance that they cause to natural enemies: harmless (class 1), slightly harmful (class 2), moderately harmful (class 3) and harmful (class 4), which correspond to reductions below 30%, between 31% and 79%, between 80% and 99% and higher than 99%, respectively (Sterk et al., 1999).

**Table 3. Mortality of adult *Acerophagus flavidulus* at 24 h after exposure to insecticide treatments on apple leaf discs.**

Insecticide	Active ingredient concentration	Commercial product rate	n	Corrected mortality, 24 h	IOBC class
	mg L <sup>-1</sup>			%	
Acetamiprid	0	Control	12	27.8 ± 6.2a	
	0.42	0.05x	10	65.4 ± 6.2b	2
	8.40	0.10x	12	100.0 ± 0.0b	4
	21.00	0.25x	12	100.0 ± 0.0b	4
	42.00	0.50x	12	100.0 ± 0.0b	4
	84.00	1x	12	100.0 ± 0.0b	4
		χ <sup>2</sup> , P		93.48 ≤ 0.01	
Buprofezin	0	Control	27	14.8 ± 6.4	
	10	0.05x	-	-	
	20	0.10x	13	0.6 ± 7.3	1
	50	0.25x	13	0.4 ± 8.7	1
	100	0.50x	13	1.7 ± 6.4	1
	200	1x	13	0.3 ± 8.8	1
		χ <sup>2</sup> , P		0.26 ≥ 0.05	
Pyriproxyfen	0	Control	18	23.7 ± 8.8	
	2.5	0.05x	-	-	
	5.0	0.10x	13	6.4 ± 10.2	1
	12.5	0.25x	13	1.4 ± 16.2	1
	25.0	0.50x	13	16.3 ± 10.8	1
	50.0	1x	13	18.4 ± 12.2	1
		χ <sup>2</sup> , P		3.46 ≥ 0.05	
Chlorantraniliprole	0	Control	12	3.3 ± 3.3a	
	2	0.05x	12	6.9 ± 1.4a	1
	4	0.10x	12	8.3 ± 2.9a	1
	10	0.25x	13	41.2 ± 8.4b	2
	20	0.50x	12	28.4 ± 4.6a	1
	40	1x	13	23.5 ± 5.9a	1
		χ <sup>2</sup> , P		33.06 ≤ 0.01	
Cyantraniliprole	0	Control	12	11.7 ± 7.3a	
	3.75	0.05x	12	20.8 ± 6.9a	1
	7.50	0.10x	12	20.8 ± 6.9a	1
	18.75	0.25x	12	39.0 ± 2.4b	2
	37.50	0.50x	12	47.8 ± 2.4b	2
	75.00	1 x	12	54.0 ± 0.6b	2
		χ <sup>2</sup> , P		28.5 ≤ 0.01	

Values within columns followed by a different letter are significantly different according to Tukey's test ( $P \leq 0.05$ ). Values are mean ± SE; n: number of insects tested. Control not corrected by Abbott's formula. IOBC class: 1: Harmless (mortality < 30%), 2: slightly harmful ( $30 \leq$  mortality  $\leq 79\%$ ), 3: moderately harmful ( $80 \leq$  mortality  $\leq 99\%$ ), and 4: harmful (mortality > 99%).

Our results indicate that neurotoxic neonicotinoid thiacloprid was moderately harmful to *A. mali* at the minimum recommended rate, and acetamiprid was harmful to *A. flavidulus* on almost all concentrations evaluated.

IGRs were harmless at low concentrations on *A. flavidulus* and slightly harmful at minimum rate only on *A. mali*, which is associated with the mode of action on this group of insecticides on immature stages. The diamide chlorantraniliprole was harmless to both parasitoids with the exception of 10 mg L<sup>-1</sup> ai, which was slightly harmful for *A. flavidulus*. On the other hand, the newer diamide cyantraniliprole was slightly harmful from 18.75 to 75 mg L<sup>-1</sup> ai for *A. flavidulus*. For *C. montrouzieri* acetamiprid was harmless on larvae and slightly harmful for adults, suggesting a less detrimental effect on the predator than on the parasitoid of the OMB. Both IGRs and chlorantraniliprole were harmless for larvae and adults. Cyantraniliprole was slightly harmful on larvae and moderately harmful on adults of *C. montrouzieri* at the minimum recommended rate.

Moderately harmful results of thiacloprid on *A. mali* agree with results from Gontijo (2011) but differ from Rogers et al. (2011; 2015) which found thiacloprid to be harmless on this parasitoid. In addition, Ebadollahi and Sadeghi (2020) found thiacloprid slightly harmful at 48 h, which only occurred in our study with 0.1x rate of the commercial product (9.6 mg L<sup>-1</sup> ai). For other encyrtid parasitoids of mealybugs, acetamiprid was harmless at the recommended rate on

*Leptomastix dactylopii* Howard, but when 4x the recommended rate was applied acetamiprid produced 100% mortality on this parasitoid (Cloyd and Dickinson, 2006). We found acetamiprid harmful to *A. flavidulus* at even low concentrations, which might be related with differences in body size between adult parasitoid species. In fact, the head width of *A. flavidulus* is between 0.26-0.29 mm (Karamaouna and Copland, 2000) while the head width of *L. dactylopii* is twice around 0.53-0.58 mm (Jong and van Alphen, 1989). Cloyd and Dickinson (2006) found acetamiprid harmful on adult *C. montrouzieri*, although less toxic than organophosphate insecticides in other study (Dumaniya et al., 2015). However, we found that acetamiprid was slightly harmful to adults. This could be related with methodological differences in bioassays, because Cloyd and Dickinson (2006) used direct spray on adults of *C. montrouzieri*, a different formulation (30 water soluble granules vs. 70 wettable powder) and water volume (4 vs. 2 mL) to spray acetamiprid that resulted in a lower amount of active ingredient used in our study (24 vs. 16 mg per experimental unit). Furthermore, in semi-field bioassays, acetamiprid residues were harmless for adults of *C. montrouzieri* 35 d after treatment (DAT) (Alvear et al., 2016). Foliar surface residues of acetamiprid on apple decreased to lower than 50% 7 DAT (Jamil et al., 2019), which might result in foliar surface residues lower than 0.42 mg L<sup>-1</sup> ai at 35 DAT.

**Table 4. Mortality of second-third instar larvae of *Cryptolaemus montrouzieri* at 24 and 48 h after exposure to insecticide treatments on apple leaf discs.**

Insecticide	Active ingredient concentration	Commercial product rate	n	Corrected mortality		IOBC class
				24 h	48 h	
	mg L <sup>-1</sup>			%	%	
Acetamiprid	0	Control	18	16.8 ± 5.7a	-	
	0.42	0.05x	-	-	-	
	8.40	0.10x	18	0.0 ± 3.1a	-	1
	21.00	0.25x	18	7.4 ± 2.7ab	-	1
	42.00	0.50x	19	33.9 ± 2.9b	-	2
	84.00	1x	19	15.4 ± 5.4b	-	1
		χ <sup>2</sup> , P		22.95 ≤ 0.01	-	
Buprofezin	0	Control	18	6.7 ± 3.9	12.9 ± 3.9	
	10	0.05x	18	0.0 ± 3.9	3.3 ± 4.2	1
	20	0.10x	15	11.9 ± 8.0	15.8 ± 7.7	1
	50	0.25x	18	0.0 ± 3.9	8.7 ± 0.5	1
	100	0.50x	15	4.8 ± 5.9	10.7 ± 5.9	1
	200	1x	15	0.0 ± 6.7	0.0 ± 0.0	1
		χ <sup>2</sup> , P		5.31 ≥ 0.05	6.23 ≥ 0.05	
Pyriproxyfen	0	Control	13	17.8 ± 15	22.2 ± 13.5	
	2.5	0.05x	13	0.0 ± 7.8	0.0 ± 2.2	1
	5.0	0.10x	13	0.0 ± 8.0	0.0 ± 0.0	1
	12.5	0.25x	13	0.0 ± 10.2	0.0 ± 0.0	1
	25.0	0.50x	13	0.0 ± 3.9	0.0 ± 0.0	1
	50.0	1x	13	0.0 ± 12.4	2.85 ± 13.5	1
		χ <sup>2</sup> , P		3.25 ≥ 0.05	2.62 ≥ 0.05	
Chlorantraniliprole	0	Control	17	22.1 ± 7.7	-	
	2	0.05x	17	0.8 ± 6.2	-	1
	4	0.10x	17	6.8 ± 1.4	-	1
	10	0.25x	17	2.1 ± 6.5	-	1
	20	0.50x	17	0.0 ± 0.0	-	1
	40	1x	17	6.5 ± 11.4	-	1
		χ <sup>2</sup> , P		2.69 ≥ 0.05	-	
Cyantraniliprole	0	Control	20	20.0 ± 15.0ab	25.0 ± 15.3a	
	3.75	0.05x	20	0.0 ± 0.0ab	0.0 ± 0.0a	1
	7.50	0.10x	20	0.0 ± 0.0a	0.0 ± 0.0a	1
	18.75	0.25x	20	12.5 ± 8.7ab	15.6 ± 8.8ab	1
	37.50	0.50x	20	4.1 ± 6.0ab	13.3 ± 15.3ab	1
	75.00	1x	20	18.8 ± 7.6b	35.6 ± 6.0b	2
		χ <sup>2</sup> , P		11.11 ≤ 0.05	15.78 ≤ 0.05	

Values within columns followed by a different letter are significantly different according to Tukey's test ( $P \leq 0.05$ ). Values are mean ± SE; n: number of insects tested. Control not corrected by Abbott's formula.

IOBC class: 1: Harmless (mortality < 30%), 2: slightly harmful (30 ≤ mortality ≤ 79%), 3: moderately harmful (80 ≤ mortality ≤ 99%), and 4: harmful (mortality > 99%).

**Table 5. Mortality of adults of *Cryptolaemus montrouzieri* at 24 and 48 h after exposure to insecticide treatments on apple leaf discs.**

Insecticide	Active ingredient concentration	Commercial product rate	n	Corrected mortality		IOBC class
				24 h	48 h	
	mg L <sup>-1</sup>			%	%	
Acetamiprid	0	Control	16	11.8 ± 0.9a	22.0 ± 7.6a	
	0.42	0.05x	16	15.7 ± 7.8a	48.3 ± 9.9b	2
	8.40	0.10x	16	16.9 ± 6.7ab	41.4 ± 17.4b	2
	21.00	0.25x	16	48.2 ± 11.1bc	68.8 ± 4.3b	2
	42.00	0.50x	16	39.5 ± 6.7b	60.3 ± 17.4b	2
	84.00	1x	16	55.4 ± 6.4c	70.1 ± 14.5b	2
		$\chi^2$ , P		45.5 ≤ 0.01	55.16 ≤ 0.05	
Buprofezin	0	Control	15	6.7 ± 3.8	15.3 ± 2.3	
	10	0.05x	15	0.0 ± 0.0	0.0 ± 0.0	1
	20	0.10x	15	2.5 ± 2.0	0.0 ± 0.0	1
	50	0.25x	15	2.5 ± 2.0	8.3 ± 9.6	1
	100	0.50x	15	0.0 ± 0.0	8.3 ± 4.7	1
	200	1x	15	7.1 ± 3.8	10.6 ± 8.1	1
		$\chi^2$ , P		5.28 ≥ 0.05	6.19 ≥ 0.05	
Pyriproxyfen	0	Control	15	11.3 ± 8.0	26.7 ± 7.8	
	2.5	0.05x	15	2.3 ± 6.7	0.0 ± 0.0	1
	5.0	0.10x	15	0.0 ± 0.0	0.0 ± 0.0	1
	12.5	0.25x	15	0.0 ± 0.0	0.0 ± 0.0	1
	25.0	0.50x	15	2.3 ± 3.8	0.0 ± 0.0	1
	50.0	1x	15	0.0 ± 0.0	0.0 ± 0.0	1
		$\chi^2$ , P		5.29 ≥ 0.05	3.08 ≥ 0.05	
Chlorantraniliprole	0	Control	20	3.3 ± 1.7	10.0 ± 2.9a	
	2	0.05x	20	12.1 ± 10.0	16.7 ± 15.3ab	1
	4	0.10x	20	5.2 ± 4.4	20.4 ± 7.3ab	1
	10	0.25x	20	15.5 ± 8.3	24.1 ± 11.7b	1
	20	0.50x	20	10.3 ± 8.3	18.5 ± 9.3a	1
	40	1x	20	15.5 ± 7.3	29.6 ± 14.8b	1
		$\chi^2$ , P		11.63 ≤ 0.05	15.37 ≤ 0.01	
Cyantraniliprole	0	Control	20	1.7 ± 1.7a	5.0 ± 2.9a	
	3.75	0.05x	20	13.6 ± 2.9a	21.1 ± 5.8a	1
	7.50	0.10x	20	22.0 ± 6.0abc	42.1 ± 11.6ab	2
	18.75	0.25x	20	35.6 ± 17.6c	64.9 ± 14.5bc	2
	37.50	0.50x	20	47.5 ± 9.3ab	73.7 ± 12.6cd	2
	75.00	1x	20	40.7 ± 10.9c	80.7 ± 7.3d	3
		$\chi^2$ , P		58.18 ≤ 0.01	131.56 ≤ 0.01	

Values within columns followed by a different letter are significantly different according to Tukey's test ( $P \leq 0.05$ ). Values are mean ± SE; n: number of insects tested. Control not corrected by Abbott's formula. IOBC class: 1: Harmless (mortality < 30%), 2: slightly harmful ( $30 \leq$  mortality  $\leq 79\%$ ), 3: moderately harmful ( $80 \leq$  mortality  $\leq 99\%$ ), and 4: harmful (mortality > 99%).

Both IGRs, buprofezin and pyriproxyfen, were harmless or slightly harmful to adults of several encyrtid parasitoid species of mealybugs (Wakgari and Giliomee, 2003; Rothwangl et al., 2004; Cloyd and Dickinson, 2006; Suma et al., 2009; Karmakar and Shera, 2018). However, when the field rate of buprofezin was applied on parasitized vine mealybug an increase in development time was found for *Anagyrus* sp. near *pseudococci* (Girault) and *Coccidoxenoides perminutus* (Timberlake) (Mgocheki and Addison, 2009). Our results agree with harmless or slightly harmful effects of both IGRs on adult *A. flavidulus*, which have been also described for *A. flavidulus* exposed to residues of 7 DAT in semi-field trials (Alvear et al., 2016).

For larvae and adults of *C. montrouzieri* pyriproxyfen and buprofezin were harmless (Cloyd and Dickinson, 2006; Alexander et al., 2013) which agree with our experimental results. However, other studies show that when pyriproxyfen-sprayed prey were used to fed larvae a slightly harmful effect was found (Planes et al., 2013; El Aalaoui et al., 2019).

Chlorantraniliprole has been found harmless for *A. mali* (Gontijo, 2011; Mills et al., 2016), which agree with our results. We found chlorantraniliprole harmless at almost all concentrations (except for 0.25x) and at the lower concentrations of cyantraniliprole on *A. flavidulus*. It seems that chlorantraniliprole could be safer than cyantraniliprole on *A. flavidulus*. For *C. montrouzieri* both diamides were mainly harmless or slightly harmful, with cyantraniliprole moderately harmful on adults at the minimum recommended rate.

It is important to emphasize that our results were from laboratory tests, which cannot be directly extrapolated to field conditions. Further bioassays should be performed to study the sublethal effects of those individuals who managed to survive this exposure and evaluate their effects on parasitism, predation, sex rate, clutch size, among others, in order to have a holistic view of the insecticide effects on natural enemies.

## CONCLUSIONS

This study has determined the acute toxicity of various concentrations of six insecticides used in apple orchards on adults of *Aphelinus mali* and *Acerophagus flavidulus* and also on larvae and adults of *Cryptolaemus montrouzieri*. Neonicotinoids such as thiacloprid and acetamiprid were moderately harmful to harmful especially on the two parasitoids, and harmless and slightly harmful on larvae and adults of *C. montrouzieri*. Growth regulators such as buprofezin and pyriproxyfen, and the diamide chlorantraniliprole seem to be safe for adult parasitoids and for larvae and adults of *C. montrouzieri*. Finally, the newer diamide cyantraniliprole was less compatible than chlorantraniliprole with the natural enemies evaluated.

## ACKNOWLEDGEMENTS

Thanks to XILEMA SpA for providing insects for bioassays. Also to CONICYT-ANID for Beca de Doctorado Nacional 2014 and CONICYT + PAI/Concurso Nacional de Tesis de Doctorado en el Sector Productivo 2015 + Folio 7815110001. Finally, thanks to Programa de Doctorado en Ciencias Agrarias, Universidad de Talca, for resources given to develop laboratory bioassays.

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