Pesticides and Beneficial Organisms
IOBC-WPRS Bulletin Vol. 103, 2014
pp. 41-45

Lethal effects of two reduced risk insecticides on *Harmonia axyridis* and *Coleomegilla maculata* (Col., Coccinellidae) following two routes of exposure

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Abstract: Reduced-risk pesticides are new compounds noted for their lower impact on human health and environment, and lower toxicity onto non-target organisms than traditional products. Novaluron (Rimon® EC 10) and chlorantraniliprole (Altacor® 35 WG), two reduced-risk insecticides, have been registered to control the codling moth, *Cydia pomonella* (L.) (Lep., Tortricidae), in Canada in 2008. Novaluron inhibits the chitin synthesis and belongs to the benzoylphenyl urea chemical class. Chlorantraniliprole, part of a new class of selective insecticides, the anthranilic diamides, causes paralysis of the muscle cells by interfering with the ryanodine insect receptors. Both compounds act by ingestion and contact, exhibiting ovicidal and larvicidal activities. Due to the high toxicity to codling moth and their low negative impact on the environment, novaluron and chlorantraniliprole are recommended for use in IPM programs. However, several studies have shown negative effects on beneficial arthropods. The aim of this study was to evaluate the effects of novaluron and chlorantraniliprole on two aphidophagous predators commonly found in apple orchards in south eastern Canada, *Harmonia axyridis* (Pallas) and *Coleomegilla maculata* (De Geer) (Col., Coccinellidae). We studied specifically the lethal effects of novaluron and chlorantraniliprole on larvae of *H. axyridis* and *C. maculata* following ingestion of treated prey and exposure to residues. Insecticide field rates (100 g a.i./ha novaluron and 50.75 g a.i./ha chlorantraniliprole) were tested against the predators in laboratory conditions. To evaluate the lethal effect of exposure to insecticides via ingestion, 1st instar larvae were fed with treated aphids for 24 hours. In a second bioassay, larvae were placed individually in dry Petri dishes previously dipped in insecticide solutions. Mortality was assessed at 24 72 h and 6 d after start of exposure. Following ingestion of treated prey, novaluron was highly toxic for *H. axyridis* causing 96.7% mortality after 6 days. *C. maculata* was not sensitive to novaluron and none of the species were sensitive to chlorantraniliprole. Both insecticides were toxic to *H. axyridis* and *C. maculata* via contact with residues. At 6 d, Chlorantraniliprole caused 100% mortality of both species, and exposure to novaluron resulted in 100% mortality for *H. axyridis* and 98.9% for *C. maculata*.

Key words: Reduced risk insecticides, chlorantraniliprole, novaluron, selectivity, *H. axyridis*, *C. maculata*, coccinellids, apple orchards

Introduction

Reduced risk insecticides have been recently adopted to control major pests in apple orchards in south-eastern Canada. However their effect on aphidophagous predators, important in preventing significant aphid infestations (Frèchette *et al.*, 2008), has not been fully assessed. Chlorantraniliprole (Altacor® 35 WG) and Novaluron (Rimon® EC 10) have been registered against the codling moth, *Cydia pomonella* (L.) (Lep., Tortricidae), in Canada in 2008. Chlorantraniliprole belongs to a new class of selective insecticides: the anthranilic diamides.

This insecticide interferes with the insect ryanodine receptors (RyRs) causing paralysis of muscle cells and ultimately death of sensitive species (Cordova *et al.*, 2006). Novaluron is a benzoylphenyl urea insecticide that inhibits the chitin synthesis. Insects die from abnormal endocuticular deposition and abortive molting (Cutler & Scott-Dupree, 2007). Both compounds act by ingestion and contact, exhibiting ovicidal and larvicidal activities (Bassi *et al.*, 2009; Cutler & Scott-Dupree, 2007). Due to the high toxicity to codling moth and their low negative impact on the environment, novaluron and chlorantraniliprole are recommended for use in IPM programs. However, several studies have shown negative effects on beneficial arthropods (Cutler *et al.*, 2006; Lefebvre *et al.*, 2012; Lefebvre *et al.*, 2011).

Among aphidophagous predators, two ladybeetles are commonly found in apple orchards in Quebec: *Harmonia axyridis* (Pallas) and *Coleomegilla maculata* De Geer. *H. axyridis*, the multicolored Asian ladybeetle, is an invasive species in North America (LaMana & Miller, 1996) that was first found in apple orchards in Quebec in 1994 (Coderre *et al.*, 1995). This species outcompetes and preys on other coccinelids (Cottrell, 2004; Koch, 2003; Lucas *et al.*, 2007) and it is the most voracious ladybeetle in the orchards. The twelve-spotted ladybeetle, *C. maculata*, is a common indigenous ladybeetle in southeastern Canada, and it preys on several pests (Roger *et al.*, 1995). Based on the limited literature, the toxicity of chlorantraniliprole and novaluron on coccinelids are species dependent. (Chappell, 2007; Dinter *et al.*, 2008; Wankhede & Kale, 2010). The aim of this study was to evaluate in laboratory conditions the lethal effect of chlorantraniliprole and novaluron on the multicolored Asian ladybeetle and the twelve-spotted ladybeetle, following two routes of exposure, at field rates used in apple orchards in Quebec, Canada.

Material and methods

Insects

H. axyridis and *C. maculata* individuals were collected in Quebec at Sainte-Agathe and Saint-Edmond respectively, and were reared in a growth chamber at 24 °C, 16 L: 8 D and 70% RH. Preys consisted of the aphid, *Myzus persicae* (Sulz.), reared in potato plants. Pollen and sweetened solution were also part of the diet.

Insecticides

The insecticides were used at field rates: 50.75 g a.i./ha chlorantraniliprole (Altacor® 35 WG, DuPont Canada) and 100 g a.i./ha novaluron (Rimon® EC 10, Makhteshim Agan of North America, Inc). Concentration of insecticides considered a spray volume of 1000 l/ha, most frequently applied by apple producers in Quebec.

Bioessais

The ingestion bioassay consisted of 30 first instar coccinelid larvae placed individually in 5 cm diameter Petri dishes and tested in three replicates. A mass of 1 mg of *M. persicae* aphids, previously treated with a Potter tower (1 ml solution and 2 mg/cm^2 aqueous insecticide deposit), were offered to the predators for 24 h. After this period, larvae were fed with non-treated fresh aphids daily. To assess the residual effect of the insecticides on ladybeetles a second bioassay was conducted. Thirty first instar coccinelid larvae (three replicates) were placed individually in ventilated dry Petri dishes previously dipped in insecticide solutions. Larvae were fed with non-treated fresh aphids daily. Experimental units were transferred to a growth chamber at 24 °C, 16 L: 8 D, and 70% RH for the test period. Three treatments were considered for both bioessais: distilled water as a control, chlorantraniliprole and novaluron.

Larval mortality was assessed at 24, 72 h and 6 days after start of exposure. Mortality data for larvae were arcsine transformed (Sokal & Rohlf, 1981) before repeated measures analysis of variance. Mean separation was carried out with the Tukey test (α = 0.05), using the JMP 10 software. Back-transformed data are presented in the results.

Results and discussion

H. axyridis

Chlorantraniliprole did not have a significant effect on the mortality of *H. axyridis* larvae following ingestion of treated prey (Table 1). Other studies have also concluded that chlorantraniliprole is safe for many natural enemies of pests in several crops (Gradish *et al.*, 2011; Shaw & Wallis, 2010). However, our results show that after contact with fresh chlorantraniliprole residues *H. axyridis* larvae were already sensitive at 24 h, and at 6 days 100% of the population was killed (Table 1), which classifies this insecticide as harmful for the multicoloured Asian ladybeetle (IOBC class 4).

Toxicity of novaluron on larvae, after ingestion, started at 72 h. By this time this insecticide was harmful as it was at 6 days (Table 1). After contact with fresh residues, toxicity of novaluron was high at 24 h. At 6 days novaluron caused 100% mortality, classifying the compound as harmful for *H. axyridis* (IOBC class 4) (Table 1).

Table 1. Lethal effect (mean % of first instar larvae mortality ± sd) of chlorantraniliprole and novaluron on *H. axyridis* following ingestion of treated prey and contact with fresh dried insecticide residues.

Treatments	Ingestion		
	24 h	72 h	6 days
Control	2.2 ± 1.9 a	8.0 ± 8.0 ab	13.6 ± 10.4 ab
Chlorantraniliprole	12.2 ± 1.9 ab	24.4 ± 8.4 b	28.9 ± 9.6 b
Novaluron	8.9 ± 8.4 ab	94.4 ± 1.9 c	96.7 ± 0.0 c
	Contact with residues		
	24 h	72 h	6 days
Control	3.7 ± 3.9 a	4.8 ± 2.5 a	7.2 ± 2.3 a
Chlorantraniliprole	57.8 ± 15.8 b	97.8 ± 1.9 c	100 ± 0.0 c
Novaluron	45.6 ± 24.8 b	97.7 ± 2.0 c	100 ± 0.0 c

ANOVA and Tukey test (p = 0.05). Results followed by the same letter are not statistically different.

C. maculata

None of the two insecticides had an impact on *C. maculata* larvae following ingestion of treated prey (Table 2). However, chlorantraniliprole acted faster than novaluron after the larvae were in contact with fresh residues. In this case, both insecticides were harmful to the twelve-spotted ladybeetle. At 72 h the two compounds were harmful and at 6 days chlor-antraniliprole could be classified in the OIBC class 4 and novaluron in the class 3 (Table 3).

Table 2. Lethal effect (% of first instar larvae mortality ± sd) of chlorantraniliprole and novaluron on *C. maculata* following ingestion of treated prey and contact with fresh dried insecticide residues.

Treatment	Ingestion		
	24 h	72 h	6 days
Control	2.3 ± 4.0 a	4.9 ± 8.6 a	10.9 ± 6.6 a
Chlorantraniliprole	0.0 ± 0.0 a	1.1 ± 1.9 a	4.4 ± 5.1 a
Novaluron	0.0 ± 0.0 a	18.9 ± 13.5 a	25.6 ± 10.7 a
	Contact with residues		
	24 h	72 h	6 days
Control	0.0 ± 0.0 a	3.4 ± 3.3 ac	10.0 ± 6.6 c
Chlorantraniliprole	46.0 ± 7.7 b	89.8 ± 3.6 de	100 ± 0.0 f
Novaluron	7.8 ± 10.7 c	86.5 ± 10.0 e	98.9 ± 1.9 ef

ANOVA and Tukey test (p = 0.05). Results followed by the same letter are not statistically different.

At field doses the two coccinelid species present a huge difference in their sensitivity to novaluron via ingestion, being *H. axyridis* the most sensitive species. This could be explained by metabolic differences (Cutler & Scott-Dupree, 2007) such as detoxifying enzyme activity. Both ladybeetle species were not sensitive to chlorantraniliprole via ingestion, but this insecticide was harmful by residual contact as it was the case of novaluron. However, even if our results show that under certain conditions residues of both insecticides can be highly toxic for coccinelids, toxicity of residues deposited on leaves should be validated in treated apple trees. Ingestion of contaminated prey must be assessed in the orchard as well, because in the present study coccinelids were offered treated aphids during only 24 h. Thereafter, a suitable insecticide treatment strategy, such as the choice of the insecticide according to the presence of beneficial arthropods in the orchard, could be adopted.

Acknowledgements

A part of the funding of this project was provided by the regional industry council of Quebec that executes the Canadian Agricultural Adaptation Program for Agriculture and Agri-Food Canada. This study was also funded by the Programme Prime-Vert, sous-volet 11.1 from the Ministry of Agriculture, Fisheries and Food from Quebec, the Natural Sciences and Engineering Research Council of Canada and Canada Foundation for Innovation.

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