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Toxicity of two ecdysone agonists, halofenozide and methoxyfenozide, against the multicoloured Asian lady beetle *Harmonia axyridis* (Col., Coccinellidae)

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Abstract: The insecticidal activity of two ecdysone agonists, methoxyfenozide (RH-2485) and halofenozide (RH-0345), was tested against last-instar larvae of the natural predator *Harmonia axyridis* (Col., Coccinellidae). In addition, the relative weight gain of the larvae after application was followed. Both products proved to be equally toxic at concentrations of 25, 50 and 100 mg/l. The ecdysteroidal activity of the compounds caused premature induction of larval moulting, cessation of feeding and incomplete pupation in affected larvae. Although, compared with previous results with methoxyfenozide and halofenozide in target pests such as the Colorado potato beetle, *Leptinotarsa decemlineata*, these compounds caused mortality only after application at relatively high concentrations.

Key words: *Harmonia axyridis*, ecdysone agonist, halofenozide, methoxyfenozide, toxicity, larval weight

1 Introduction

Halofenozide and methoxyfenozide are two ecdysone agonists, belonging to a novel group of insect growth regulators (IGRs). They have a dibenzoylhydrazine-based structure of which RH-5849 is the precursor. They induce a precocious and lethal larval moulting in holometabolous insects shortly after oral uptake or topical application. Although they are structurally very different from the steroidal moulting hormone 20-hydroxyecdysone, their mode of action is through direct binding and stimulation of the ecdysteroid receptors (Wing, 1988; Wing et al., 1988; Smagghe et al., 1996a,b; Dhadialla et al., 1998). Interestingly, methoxyfenozide (RH-2485) and tebufenozide (RH-5992) (another structural analogue) exert a high potency and strong selectivity towards different Lepidoptera whereas halofenozide (RH-0345) is more potent and selective against Coleoptera (Dhadialla et al., 1998; Carton et al., 2000). This selectiveness and the differences in potency between species and orders are thought to be caused by differences in affinity for the ecdysteroid receptors and differences in pharmacokinetic properties.

Insects from different orders, including beneficials and non-target insects have been reported to be unaffected by these compounds (Le et al., 1996). The multicoloured Asian lady beetle *Harmonia axyridis* (Pallas) has been introduced in the USA and Europe from Japan for the control of mainly tree-inhabiting aphids but their predatory diet also includes scales and psyllids (Chapin and Brou, 1991; Onagna et al., 1993). The ecdysone agonists have a considerable potential for controlling Lepidoptera in orchards, such as the codling moth *Cydia pomonella* (L.) in fruit trees and the tree defoliating spruce budworm *Choristoneura fumiferana* (Clemens) (both Lep., Tortricidae). In this study, we have tested the effects of methoxyfenozide and halofenozide on last-instar larvae of the orchard coccinellid *H. axyridis* in a laboratory test. As such, we provide information on the possibilities for a combined use of these compounds together with the natural enemy *Harmonia* in integrated pest management programmes.

2 Materials and methods

2.1 Chemicals

Technical methoxyfenozide and halofenozide were obtained from the Rohm and Haas Co. (Spring House, PA, USA). All other products and solvents were of analytical grade.

2.2 Insects

All larval stages of *H. axyridis* were fed eggs of the flour moth *Ephestia kuehniella* (Zeller) (Lep., Pyralidae). The eggs were kept frozen until feeding time. All stages were reared under standard laboratory conditions of 24 ± 2°C, 70 ± 5% relative humidity and a 16 : 8 h (L : D) photoperiod.

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2.3 Toxicity of methoxyfenozide and halofenozide after oral treatment

Toxicity via ingestion on newly ecdysed last-instar larvae (L_4d_0) of *H. axyridis* was assessed by treating both food and drinking water with different concentrations of both compounds. Frozen *E. kuehniella* eggs were dipped for 30 s in an aqueous solution of ecdysone agonist after which they were air dried and fed to the larvae *ad libitum* in small Petri dishes (Falcon, Becton Dickinson Benelux, Erembodegem, Belgium), with one Petri dish containing five larvae. Drinking water was supplied via a cotton plug, soaked in an equal concentration of ecdysone agonist. All solutions were prepared by dissolving the agonists in acetone and then adding water (final concentration of acetone is 5%). For each compound, we tested five different concentrations (25, 50, 100, 500 and 1000 mg/l) and for each concentration, at least 25 larvae were tested. Control treatment consisted of treatment with water containing 5% acetone. Mortality was recorded 5 days after treatment which was the time when the control larvae underwent pupation. The mortality data were subjected to probit analysis by means of POLO-PC (LeOra Software, 1987) to calculate LC_{50} values and 90% feducial limits (90% FL). These data were compared with mortality data recorded after treatment of last-instar larvae of the Colorado potato beetle *Leptinotarsa decemlineata* (Say) (Col., Chrysomeloidae) (Carton et al., 2000).

2.4 Effect of ecdysone agonists on development and weight gain

After treatment, average weight gain of the *Harmonia* larvae was determined on a daily basis. Dead larvae were excluded from the experiment. This and other visible effects caused by the agonists are discussed.

3 Results and discussion

3.1 Toxicity of methoxyfenozide and halofenozide after oral treatment

Figure 1 shows that methoxyfenozide was clearly less toxic than halofenozide at concentrations of 25 and 50 mg/l, but at higher concentrations of 100 and 500 mg/l they were equally toxic to last-instar larvae. At an extremely high concentration of 1000 mg/l, both compounds caused 100% mortality. After probit analysis, we estimated an LC_{50} value of 67.1 (38.4–115) mg/l for halofenozide and 71.3 (50.1–105) mg/l for methoxyfenozide. When tested against Lepidoptera and Coleoptera, a clear difference is noted between the toxicity of methoxyfenozide and halofenozide (Le et al., 1996; Farinòs et al., 1999; Carton et al., 2000). In contrast, our results show that these differences in selectivity of both compounds are not apparent in this non-target insect.

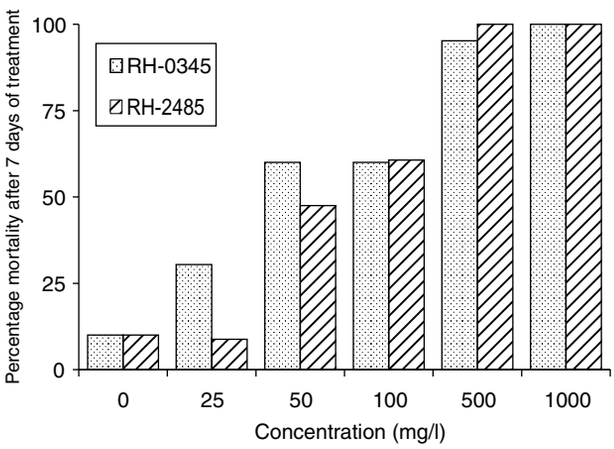

Fig. 1. *Toxicity by different concentrations of halofenozide (RH-0345) and methoxyfenozide (RH-2485) after 7 days of oral treatment of last-instar* Harmonia axyridis *larvae*

When we compare the LC_{50} values of halofenozide in last-instar larvae of *H. axyridis* (67.1 mg/l) and *L. decemlineata* (1.41 mg/l) (table 1), the coccinellid proved to be 27 times less susceptible to the agonist.

For the codling moth *C. pomonella*, an important pest in apple orchards, an LC_{50} of 1.47 mg/l was calculated after oral treatment with tebufenozide, an agonist less toxic to caterpillars than methoxyfenozide (Pons et al., 1999). This indicates that there is a very substantial difference in susceptibility between these different insects for these new IGRs. This result confirms previous research (Trisyono et al., 2000) with ecdysone agonists on the lady beetle *Coleomegilla maculata* (DeGeer), an important predator of the eggs of the European corn borer *Ostrinia nubilalis* (L.). The caterpillar larvae were significantly more sensitive to the ecdysone agonists as compared with the larvae of its predator *C. maculata*. These results suggest that the toxicological effects of halofenozide and methoxyfenozide on *H. axyridis* could be minimized by selecting a discriminating concentration that is lethal to target insect pests and not against *H. axyridis*. Nevertheless, further research is required before final conclusions can be drawn. Little is known about the results and effects of these compounds in field trials. Yet, results indicate that application of these products have little or no adverse effects on beneficial insects and organisms such as pollinators and predatory insects (Le et al., 1996; Dhadialla et al., 1998). Also, other factors such as toxicity through penetration, residual toxicity and effects on fertility and reproduction, influence the toxic potential of these compounds.

3.2 Effect of ecdysone agonists on weight development

After oral treatment, development and weight gain was inhibited by methoxyfenozide in a dose-dependent way (fig. 2). At a concentration of 25 mg/ml, fresh weight (6 days after treatment) was reduced by 12.5% when compared with the control (28.3 and 32.3 mg, respectively). The morphologically unaffected larvae underwent normal pupation in spite of their lesser body weight. At higher concentrations the pupal weight was reduced even more, and a substantial percentage of the pupae were malformed. At all concentrations, all affected individuals showed clear signs of head capsule apolysis, indicating the induction of a premature larval moulting because of an ecdysteroidal activity. Also, affected larvae ceased feeding. Another visible symptom of the effects of treatment was the visualization of the old unshed cuticle through black colouration. In some cases

Table 1. *LC values (expressed as mg AI/l) for halofenozide and methoxyfenozide after 7 days of oral treatment of last-instar* Harmonia axyridis *larvae*

	Harmonia axyridis			*Leptinotarsa decemlineata*[1]
Compound	LC_{10} (90% FL)	LC_{50} (90% FL)	LC_{90} (90% FL)	LC_{50} (90% FL)
Halofenozide	16.3 (3.94–30.2)	67.1 (38.4–115)	277 (150–1100)	1.41 (1.20–1.65)
Methoxyfenozide	25.9 (7.88–39.7)	71.3 (50.1–105)	196 (125–710)	6.23 (5.15–7.22)

[1] Data from Carton et al. (2000).

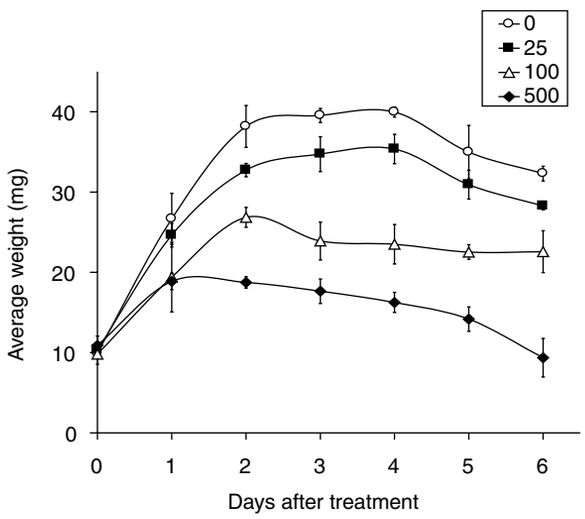

Fig. 2. *Fresh weight development of last-instar* Harmonia axyridis *larvae after oral treatment with methoxyfenozide (RH-2485) at different concentrations (0, 25, 100 and 500 mg/l). Data are expressed as means ± SE based on at least five replicates of five larvae each*

rectal prolapse was observed. We assume that the incapability to shed the old cuticula is caused by the inhibition of release of the eclosion hormone and as such prevents normal ecdysis. These symptoms are in agreement with previous results with these compounds in *L. decemlineata* where the induction of a premature moulting led to head capsule apolysis, cessation of feeding and unsuccessful pupation (Smagghe and Degheele, 1993; Carton et al., 2000).

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