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Short communication

Effect of the ecdysone agonists, methoxyfenozide and tebufenozide, on the lady beetle, *Coleomegilla maculata*

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Introduction

The ecdysone agonists, methoxyfenozide (RH-2485) and tebufenozide (RH-5992), are insect growth regulators selective for lepidopteran insects (Dhadialla et al., 1998). These compounds have potential for controlling the European corn borer, *Ostrinia nubilalis* (Hübner), and the southwestern corn borer, *Diatraea grandiosella* Dyar, two of the most damaging insect pests of maize, *Zea mays* L. (Seymour et al., 1996; Trisyono & Chippendale, 1997, 1998). They were significantly more toxic to larvae than was carbaryl, a carbamate insecticide, and they were lethal to eggs of these species (Trisyono & Chippendale, 1997; 1998).

Thirteen different insect orders including beneficial, predatory, parasitic, and non-target insects have been reported to be unaffected by tebufenozide (Oakes, 1994). The lady beetle, *Coleomegilla maculata* (DeGeer), is an important predator for eggs of *O. nubilalis* (Conrad, 1959; Mason et al., 1996), and is abundant in maize ecosystems (Cottrell & Yeargan, 1998). Considering the potential of methoxyfenozide and tebufenozide for controlling *O. nubilalis* and *D. grandiosella*, and the importance of *C. maculata* as a predator, we examined the acute effect of methoxyfenozide and tebufenozide on eggs and larvae of *C. maculata*.

Materials and methods

Insects and chemicals. *C. maculata* eggs were provided by USDA, APHIS (Mission, TX). Technical tebufenozide (95% active ingredient [AI]), methoxyfenozide (≈94% [AI]) were obtained from Rohm and Haas (Spring House, PA), and technical carbaryl (99.8% [AI]) was provided by Rhone-Poulenc Ag Company (Research Triangle Park, NC).

Toxicity of the ecdysone agonists to C. maculata. Ovicidal tests were carried out by dipping the eggs of *C. maculata* (24–48 h post-oviposition) in a solution of 1400 mg (AI)/liter methoxyfenozide, tebufenozide, or carbaryl. This concentration was determined from a field experiment which applied 280 g AI/ha tebufenozide to maize with a spray volume of 21 gallons/acre (Seymour et al., 1996). The insecticide solutions were prepared by first dissolving the insecticides in acetone, and then adding an equal volume of distilled water. Solvent and untreated controls were included in the experiments. The eggs were dipped in the insecticide solution for 30 s, and the treated eggs were air dried and placed in 30 g clear plastic cups supplied with wetted cotton. The number of newly hatched larvae was recorded daily until the 7th day after treatment.

Larvicidal tests were conducted by feeding the newly hatched larvae of *C. maculata* with treated or untreated frozen egg masses of *O. nubilalis* incubated at 28 °C L16:D8. In the first bioassay, waxed paper containing frozen *O. nubilalis* egg masses was cut

and dipped in a solution of 100 or 200 mg (AI)/liter methoxyfenozide, tebufenozide, or carbaryl for 30 s, air dried, and transferred into 30 g plastic cups; whereas in the second bioassay, the concentration of these compounds was increased to 1400 mg (AI)/liter. In both bioassays, solvent and untreated controls were included. Each treatment was replicated five times for the first bioassay, except for the controls (three times), and four times for the second bioassay. Eight to fourteen newly hatched larvae of *C. maculata* were used in each replicate. Larvae from each replicate were released into two cups, and sufficient egg masses (>50 egg masses) were provided in each cup. Cotton wetted with 0.4 ml water was placed in the cups to provide moisture. Larval mortality was recorded daily, and the observations were terminated at the 4th day after treatment when all surviving larvae had ecdysed to the second instar.

Data analysis. Percentages of egg and larval mortality were transformed using arcsine $\sqrt{x}$ before being submitted to analysis of variance (ANOVA) (Gomez & Gomez, 1976). ANOVA was carried out using a completely randomized design. Least significant difference (LSD) with $\alpha = 0.05$ was used for means separation only when the F test ($\alpha = 0.05$) in the ANOVA was significant (Fisher protected LSD). Statistical analyses were carried out using MSTAT (Eisensmith & Russel, 1989).

Results and discussion

Methoxyfenozide and tebufenozide were significantly less toxic to eggs (Table 1) and larvae (Figures 1A, B) of *C. maculata* than was the conventional insecticide, carbaryl. Methoxyfenozide and tebufenozide applied at 1400 mg (AI)/liter did not cause significant mortality of *C. maculata* eggs, but these compounds caused >99% mortality to *O. nubilalis* eggs when they were applied at a much lower concentration (200 mg (AI)/liter) (Trisyono & Chippendale, 1997).

Mortality of *C. maculata* larvae fed with *O. nubilalis* egg masses treated with 100 or 200 mg (AI)/liter methoxyfenozide or tebufenozide for 4 days was not significantly higher than those fed with untreated *O. nubilalis* egg masses (Figure 1A). However, these compounds caused a significant mortality of *C. maculata* larvae when they were applied at 1400 mg (AI)/liter (Figure 1B). All surviving larvae were second instar by the 4th day after treatment. All larvae

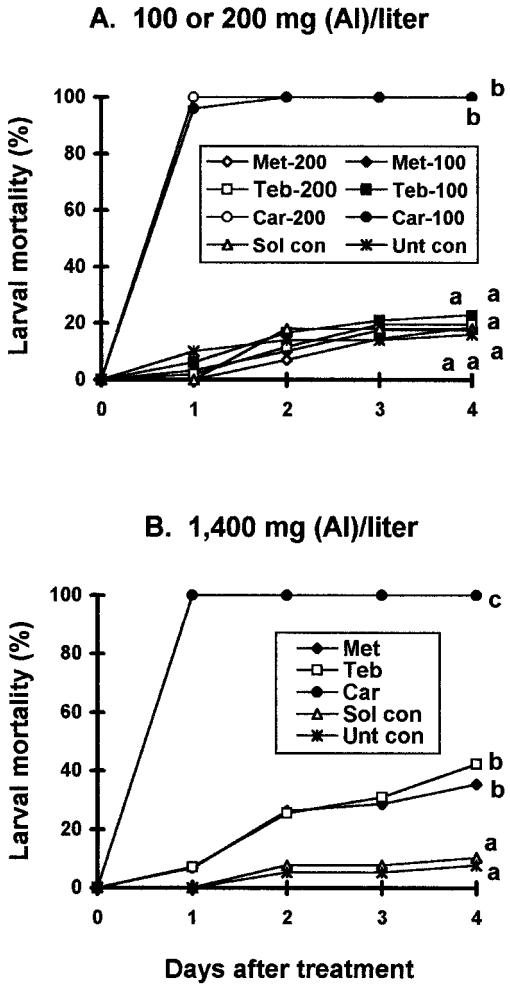

Figure 1. Larval mortality of *Coleomegilla maculata* fed with frozen egg masses of *Ostrinia nubilalis* treated with (A) 100 or 200 mg (AI)/liter and (B) 1400 mg (AI)/liter methoxyfenozide (Met), tebufenozide (Teb), or carbaryl (Car). Solvent (Sol con) and untreated control (Unt con) were included. $n = 27 - 50$ larvae/treatment for the first bioassay (A), and $n = 37 - 48$ larvae/treatment for the second bioassay (B). Larvae were maintained at 28 °C and L16:D8. Means at the 4th day after treatment followed by the same letter are not significantly different, Fisher protected LSD; P > 0.05.

fed with *O. nubilalis* egg masses treated with 200 or 1400 mg [AI]/liter carbaryl died by the first day after treatment, whereas 100% larval mortality occurred by the 2nd day for larvae fed with *O. nubilalis* egg masses treated with 100 mg (AI)/liter (Figures 1A, B).

These findings indicate that *C. maculata* is significantly less sensitive to the ecdysone agonists tested than is *O. nubilalis*. Using diet incorporation, the LC_{50} values of methoxyfenozide and tebufenozide at 7 days after treatment against newly hatched larvae of *O. nu-*

Table 1. Ovicidal activity of methoxyfenozide, tebufenozide, and carbaryl on eggs of *C. maculata*

Ecdysone agonist/ insecticide	Concentration (mg [AI]/liter)	Eggs[a] (No.)	Egg mortality[b],% (mean ± SD)
Methoxyfenozide	1400	99(6)	27.7 ± 26.7a
Tebufenozide	1400	132(7)	28.2 ± 28.8a
Carbaryl	1400	93(7)	100.0 ± 0b
Solvent control	–	94(6)	17.8 ± 25.7a
Untreated control	–	88(6)	12.5 ± 17.1a

[a]Numbers in parentheses represent the number of egg masses (each as a replicate) used in each treatment. Egg masses were incubated at 28 °C and L16:D8.
[b]Observation was terminated at the 7th day after treatment. Means followed by the same letter are not significantly different, Fisher protected LSD; $P > 0.05$.

bilalis were 0.031 and 0.101 μg (AI)/g, respectively (Trisyono & Chippendale, 1997). The difference in sensitivity of these species to the ecdysone agonists may be due to differences in the binding affinity of these compounds to the ecdysone receptors (Smagghe et al., 1996). The present study suggests that the effect of these compounds on *C. maculata* could be minimized by (1) selecting a discriminating concentration that will be lethal to *O. nubilalis*, but does not kill *C. maculata*, and (2) applying the selected concentration when most *O. nubilalis* eggs are close to eclosion. For example, application of 200 mg (AI)/liter of the ecdysone agonists to *O. nubilalis* eggs caused mortality of most eggs, but this treatment did not cause a significant mortality to eggs and larvae of *C. maculata*. However, field trials are necessary to determine the selectivity of methoxyfenozide and tebufenozide against target and non-target insects.

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