

ACUTE TOXICITY OF NOVEL INSECTICIDES TO *ORIVS INSIDIOSUS* AND *COTESIA MARGINIVENTRIS*

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Abstract

The acute toxicity of several novel insecticides was tested against two important natural enemies of cotton pests: the parasitic wasp *Cotesia marginiventris*, and the predatory bug, *Orius insidiosus*. The wasp is an important natural enemy of many caterpillar pests, particularly armyworms. The predatory bug is an effective predator of small pests, feeding heavily on thrips, mites, aphids, and eggs and small caterpillars. The tests were conducted in the laboratory and greenhouse, utilizing several bioassay techniques. The pesticides studied were Karate (l-cyhalothrin), Novaluron, Tracer (spinosad), Steward (indoxacarb), Centric (thiamethoxam), Intruder (acetamiprid), Trimax (imidacloprid), Denim (emamectin benzoate), and the numbered compounds S-1812 (a Valent insecticide targeting caterpillars), F-0570 (an FMC pyrethroid also targeting caterpillars), and the novel compound flonicamid (FMC's F-1785, targeting sucking pests).

The novel insecticides generally were less toxic than the standard insecticides, represented here by the pyrethroid Karate. Centric, however, was highly toxic to *O. insidiosus*, not differing significantly from Karate in its toxicity. Intruder and Trimax were toxic to *O. insidiosus*, though less so than was the case with Centric or Karate. Intruder exhibited no toxicity to *C. marginiventris* in previous tests. Flonicamid exhibited little or no toxicity to *Orius insidiosus* at any of the rates tested, although there were indications from the Leaf Trial that some mortality may occur after prolonged exposure to treated foliage. Novaluron was essentially non-toxic to *O. insidiosus*. Tracer was highly toxic to *C. marginiventris*, but not to *O. insidiosus*. Steward exhibited some toxicity to *C. marginiventris* after prolonged exposure to treated foliage, unlike observations in previous tests. Toxicity of Steward may require additional attention. Denim and S-1812 were not toxic to *C. marginiventris*.

Introduction

Arthropod natural enemies are important components of integrated pest management (IPM) programs, and have become particularly important recently in cotton, where natural enemy diversity is rather high relative to many other crop systems (Whitcomb and Bell 1964). The virtual elimination of the boll weevil from the southeastern United States has greatly reduced the need for broad-spectrum insecticide use in cotton in this region, which is encouraging survival of arthropod natural enemies in a system once inimical to them. In addition, novel insecticides are increasingly making their way to the market, many of which have favorable environmental profiles including reduced toxicity to arthropod natural enemies. The availability of selective insecticides that conserve natural enemies is a boon to further development of IPM in cotton.

Pesticides can exert a range of effects on arthropod natural enemies, not limited to acute mortality (Croft 1990, Ruberson et al. 1997). Sublethal effects also can be important in the population dynamics and efficacy of natural enemies. These effects should not be neglected, but they require considerably more effort to examine, and their overall impact in field populations can be difficult to predict (Stark and Wennergren 1995). The present study focused on acute toxicity of selected insecticides to two species of arthropod natural enemies: (1) *Cotesia marginiventris*, a parasitic wasp (Hymenoptera: Braconidae) that attacks a number of caterpillar pests of cotton; and (2) *Orius insidiosus*, a predatory bug (Heteroptera: Anthracoridae) that preys on numerous small pests of cotton.

Materials and Methods

Sources of Organisms

Cotesia marginiventris wasps were obtained from a colony maintained in the laboratory since April 2002, when it was initiated with field-collected cocoons. The parasitoids were reared on larvae of the beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae), and adults were provided with a honey:water blend (10:90) as a food resource. Only adult wasps were used in the acute toxicity trials. The beet armyworms also are maintained in the laboratory, where they are reared on a pinto bean diet.

Orius insidiosus were purchased as adults from IPM Labs in Locke, NY. The bugs were used within one day of delivery.

Laboratory Studies

The laboratory studies involved the use of an apparatus referred to as the "Tower" (see Fig. 1). The tower consists of a large, metal frame that is hollow with a fan at the bottom to pull air through the ventilation holes perforating the sides of the tower, and a moveable vent at the top to modify airflow. Airflow through the bioassay chambers is critical for reducing the risk of volatile accumulation in the enclosed chambers.

Two different approaches were used with the tower. The first approach (called the “disc” method) involved treatment of the clear acrylic covers of each bioassay chamber with the desired insecticide rate. Applications were made using a Potter spray tower applying 0.5 ml of pesticide mixture evenly to the total treatment area (28.3 in²). In the second method (the “leaf” method) cotton leaves were treated in the spray tower with the same amount of material as that used in the disc method above. The leaf was then used as the bottom surface of the bioassay chamber, held in place by an underlying, untreated acrylic disc. In the leaf method the acrylic top of the chamber was untreated. In both methods the treated surfaces were allowed to dry before five test insects (not distinguished by gender) were introduced into each bioassay chamber with a small portion of food (4-5 corn earworm eggs per bug). The chamber was affixed to the tower by inserting the vent tube in the chamber into the gasketed opening in the tower. Only *Orius insidiosus* was successfully studied with the tower. Two trials with *Cotesia marginiventris* had unacceptable levels of control mortality and the results were discarded.

In the laboratory trials on the tower, we examined the following insecticides (most were at recommended use rates, although flonicamid also was tested at a high rate that was ca. 2.5-fold higher than the projected field use rate):

Insecticide	Rate (lbs AI/A)	<i>Orius</i> trials	
		No. reps: leaf	No. reps: disc
Water (control)	NA	6	10
Centric (thiamethoxam)	0.025	6	10
	0.050	6	10
Tracer (spinosad)	0.09	6	10
Karate (l-cyhalothrin)	0.03	6	10
Flonicamid (F-1785)	0.044	6	10
	0.13	6	10
	0.22	6	10
Novaluron	0.039	6	10
	0.075	6	10

Greenhouse Study

Whole cotton plants were treated with insecticides mixed to the desired rate and applied with a backpack sprayer calibrated to apply 8 gallons per acre. Five plants were treated for each experimental treatment. The plants were allowed to dry for 2 hours, after which one leaf was caged in the middle of each plant. The cage consisted of a 1-pint paper can with a hole punched in a side through which the leaf petiole could be slipped. Both ends of the paper can were punched open and covered with organza to permit ventilation and prevent insect escape. This method was used only for *O. insidiosus*. Five bugs were introduced per cage, and five cages were used for each treatment. Survival of the bugs was evaluated 24 hr after introducing them into the cages. The treatments evaluated in the greenhouse study were:

Insecticide	Rate (lbs AI/A)	No. reps
Water (control)	NA	5
Intruder (acetamiprid)	0.075	5
Trimax (imidacloprid)	0.047	5
Flonicamid (F-1785)	0.044	5
	0.22	5
Centric (thiamethoxam)	0.05	5
Novaluron	0.059	5

Semi-Field Study

Only *C. marginiventris* was evaluated using this method. Field plots (16 rows wide by 50' long) were treated with each of the insecticides using a John Deere Hi-Cycle sprayer, calibrated to apply 6.7 gallons of liquid per acre. The insecticides used in this test targeted lepidopteran pests. Fully-expanded leaves were removed from the upper 3-4 nodes of 12 plants from the center two rows of each plot 2 hr and 3 d after insecticide application. Leaves were returned to the laboratory, where they were placed in ventilated petri plates. Into each plate we added a small streak of honey to the lid of each dish and added five *C. marginiventris*. Each treatment was replicated five times each for the 2-h and the 3-d leaf samplings. Survival was examined 24 h (and 48 h for the 2-h leaves) after parasite introduction into the plates. Treatments evaluated in the semi-field trials were:

Insecticide	Rate (lbs AI/A)	No. reps
Water (control)	NA	5
S-1812 (pyridalyl)	0.10	5
	0.15	5
Tracer (spinosad)	0.09	5
Tracer (spinosad) + Karate (l-cyhalothrin)	0.09 + 0.016	5
Steward (indoxacarb)	0.11	5
Denim (emamectin benzoate)	0.01	5
F-0570	0.014	5
	0.018	5

Data Analysis

All raw data were in the form of percentages, and were transformed (arcsine of the square root of each value (plus one)) to stabilize the heavy skewing in the data. Data were then subjected to a one-way ANOVA, and where significant, means were separated using the Waller Duncan Bayesian *k* ratio.

Results and Discussion

Laboratory Studies

The results from the laboratory tests are presented in Table 1. Generally, survival of *O. insidiosus* adults was greater in the Leaf Trials than in the Disc trials. This is not surprising, given the strong tendency of these bugs to feed on plant sap, which was not available in the Disc trials. In both laboratory tests on the tower, both Karate and Centric induced high levels of mortality, significantly greater than that caused by any of the other treatments. Novaluron exhibited no toxicity in either test, regardless of the rate used. Tracer at the recommended field rate appeared to have a slight effect in the Disc trial after 48 h of exposure, but no such effect was observed in the Leaf Trial. Toxicity of flonicamid was negligible in both tests at 24 h, although there were some indications of mortality in the Leaf Trial after 48 h of exposure. It is possible that the bugs were able to acquire some of the flonicamid through the leaf. As noted above, the rates of flonicamid used in the trials ranged from projected usage rates to approximately 2.5 times the expected use rate.

Greenhouse Study

The results from this test are presented in Table 2. Centric and Intruder were both highly toxic to *Orius insidiosus*. Trimax also exhibited considerable toxicity to the bugs. Novaluron and flonicamid were not toxic relative to the controls after 24 h even at very high rates (ca. 2.5 times the projected use rate for flonicamid). The experiment could not be continued beyond 24 h due to the entry of fire ants into a number of the cages between the 24 h and 48 h evaluations, wherein they created holes through which the bugs were able to escape. As a result, we were unable to further examine the effect of flonicamid on *O. insidiosus* after 48 h of exposure on treated, intact plants.

Semi-Field Study

The results from this test are presented in Table 3. The relatively fresh residues (dried for 2 h) of Tracer, the Tracer/Karate mixture, and the high rate of F-0570 were highly toxic to *Cotesia marginiventris* after 24 h of exposure. The low rate of F-0570 was moderately toxic, and all other treatments exhibited no significant toxicity to *C. marginiventris* after 24 h of exposure. The pattern was similar after 48 h of exposure to 2-h-old residues, except that mortality in the Steward treatment increased considerably over the initial 24 h of exposure. Toxicity of Steward after prolonged exposure to recent residues had not been observed in our previous studies (Ruberson and Tillman 1999). As in our previous studies (Ruberson and Tillman 1999), S-1812 exhibited no toxicity to *C. marginiventris*. None of the insecticide residues were toxic to *C. marginiventris* after weathering 3 d in the field on the cotton plants, indicating that the residues all aged rather rapidly to a nontoxic level in the field.

Conclusions

The novel insecticides generally were less toxic than the standard insecticides, represented here by the pyrethroid Karate. Centric, however, was highly toxic in all tests, not differing significantly from Karate in its toxicity to *O. insidiosus*. Intruder and Trimax were toxic to *O. insidiosus*, although not as severely as Karate and Centric. Flonicamid exhibited little or no toxicity to *Orius insidiosus* at any of the rates tested (which ranged up to 2.5 times the expected use rate), although there were indications from the Leaf Trial that some mortality may occur after prolonged exposure to treated foliage. F-0570 exhibited toxicity to *C. marginiventris* comparable to levels typically observed with other pyrethroids. Novaluron was essentially nontoxic to *O. insidiosus* in all tests. Tracer was highly toxic to *C. marginiventris*, but not to *O. insidiosus*. Steward exhibited some toxicity to *C. marginiventris* after prolonged exposure to treated foliage, which had not been observed in previous studies. Toxicity of Steward may require additional attention. Denim and S-1812 were not toxic to *C. marginiventris*.

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Table 1. Survival (%; mean \pm sd) of the predator *Orius insidiosus* 24 and 48 h after exposure to dried insecticide residues on acrylic tops and bottoms (Disc Trial) or on treated cotton leaves forming the bottoms of the bioassay chambers.¹

Insecticide	Rate (lbs AI/A)	Disc Trial		Leaf trial	
		24 h	48 h	24 h	48 h
Water (control)	NA	79.5 \pm 20.61 A	67.5 \pm 21.11 AB	96.7 \pm 8.16 A	83.3 \pm 8.16 AB
Centric (thiamethoxam)	0.025	20.0 \pm 25.49 B	4.5 \pm 9.56 C	10.0 \pm 16.73 B	3.3 \pm 8.16 D
	0.050	7.3 \pm 15.48 C	2.0 \pm 6.32 C	6.7 \pm 10.33 B	0.0 \pm 0.00 D
Tracer (spinosad)	0.09	67.1 \pm 27.7 A	48.5 \pm 29.54 B	86.7 \pm 16.33 A	83.3 \pm 15.06 AB
Karate (l-cyhalothrin)	0.03	0.0 \pm 0.00 C	0.0 \pm 0.00 C	0.0 \pm 0.00 C	0.0 \pm 0.00 D
Flonicamid (F-1785)	0.044	73.2 \pm 27.35 A	58.7 \pm 34.69 AB	80.0 \pm 24.49 A	63.3 \pm 19.66 C
	0.13	68.2 \pm 20.55 A	48.2 \pm 24.90 B	87.8 \pm 14.15 A	66.7 \pm 16.33 BC
	0.22	70.0 \pm 27.59 A	60.0 \pm 35.67 AB	78.3 \pm 27.14 A	64.2 \pm 27.64 C
Novaluron	0.039	73.7 \pm 27.73 A	60.3 \pm 36.26 AB	96.7 \pm 8.16 A	96.7 \pm 8.16 A
	0.075	86.2 \pm 15.67 A	74.0 \pm 22.53 A	90.0 \pm 10.95 A	86.7 \pm 16.33 A

¹Means in columns followed by the same letter are not significantly different (Waller Duncan Bayesian *k* ratio, *k* = 100).

Table 2. Survival (%; mean \pm sd) of the predator *Orius insidiosus* after 24 h of exposure to dried insecticide residues on treated cotton leaves in cages on intact plants.¹

Insecticide	Rate (lbs AI/A)	% survival
Water (control)	NA	96.0 \pm 5.48 A
Intruder (acetamiprid)	0.075	12.0 \pm 16.43 C
Trimax (imidacloprid)	0.047	27.5 \pm 9.57 B
Flonicamid (F-1785)	0.044	94.0 \pm 8.94 A
	0.22	94.0 \pm 5.48 A
Centric (thiamethoxam)	0.05	0.0 \pm 0.00 D
Novaluron	0.059	94.0 \pm 8.94 A

¹Means in columns followed by the same letter are not significantly different (Waller Duncan Bayesian *k* ratio, *k* = 100).

Table 3. Survival (%; mean \pm sd) of the predator *Cotesia marginiventris* after 24 h or 48 h of exposure to insecticide residues dried on field-treated cotton 2 hr and 3 d previous to exposure to parasites.¹

Insecticide	Rate (lbs AI/A)	2 d		3 d
		24 h	48 h	24 h
Water (control)	NA	92.0 \pm 10.95 A	88.0 \pm 10.95 A	100.0 \pm 0.00 A
S-1812 (pyridalyl)	0.10	92.0 \pm 10.95 A	88.0 \pm 10.95 A	100.0 \pm 0.00 A
	0.15	96.0 \pm 8.94 A	80.0 \pm 14.14 A	96.0 \pm 8.94 A
Tracer (spinosad)	0.09	8.0 \pm 10.95 CD	0.0 \pm 0.00 D	96.0 \pm 8.94 A
Tracer (spinosad) + Karate (l-cyhalothrin)	0.09 + 0.016	4.0 \pm 8.94 D	0.0 \pm 0.00 D	92.0 \pm 10.95 A
Steward (indoxacarb)	0.11	84.0 \pm 16.73 A	36.0 \pm 26.08 B	96.0 \pm 8.94 A
Denim (emamectin benzoate)	0.01	96.0 \pm 8.94 A	80.0 \pm 20.00 A	96.0 \pm 8.94 A
F-0570	0.014	60.0 \pm 42.42 B	44.0 \pm 44.36 B	100.0 \pm 0.00 A
	0.018	20.0 \pm 14.14 C	12.0 \pm 10.95 C	100.0 \pm 0.00 A

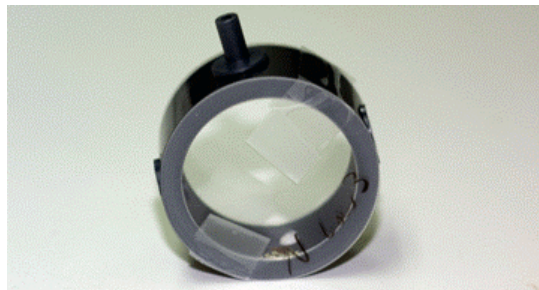
¹Means in columns followed by the same letter are not significantly different (Waller Duncan Bayesian *k* ratio, *k* = 100).



(A)



(B)



(C)

Figure 1. (A) The tower used in the trials, with the individual bioassay chambers arranged on its side. (B) A closeup of the bioassay chambers placed into the ventilation ports on the tower. (C) Closeup of a bioassay chamber with the acrylic covers taped in place, and showing the ventilation port that inserts into the tower.