PESTICIDE EFFECTS ON INSECT NATURAL ENEMIES OF COTTON PESTS

John R. Ruberson, Melissa D. Thompson, and Phillip M. Roberts
Dept. of Entomology, University of Georgia, Tifton, GA

Introduction

Arthropod natural enemies are important components of integrated pest management (IPM) programs, and have become particularly important recently in cotton. The virtual elimination of the boll weevil has greatly reduced the need for broad-spectrum insecticide use in cotton, which is encouraging survival of arthropod natural enemies in a system once inimical to them. Further reductions in insecticide use due to the widespread adoption of Bt cotton also help to encourage the persistence and efficacy of natural enemies in cotton fields. In addition, novel insecticides are increasingly making their way to the market, many of which have very favorable environmental profiles that include 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. 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. The results of this report focus on studies with two species of natural enemies: (1) *Trichogramma pretiosum*, a parasitic wasp (Hymenoptera: Trichogrammatidae) that attacks the eggs of a number of caterpillar pests of cotton; and (2) *Orius insidiosus*, a predatory bug (common name “pirate bug”; Heteroptera: Anthocoridae) that preys on numerous small pests of cotton. Additional studies were conducted with the parasitic wasp *Cotesia marginiventris* (Hymenoptera: Braconidae), as originally proposed in the project proposal. However, in repeated studies (4 experiments), control mortalities ranged from 32 to 52% after 24 h of exposure, exceeding the established control mortality ($\leq$ 15%) by a substantial margin, and making interpretation of results from insecticidal treatments impossible. Thus, the results with *C. marginiventris* were discarded and are not presented here.

Methods

Sources of organisms. *Trichogramma pretiosum* wasps were obtained from a colony maintained in the laboratory since June 2001, when it was initiated with field-collected, parasitized heliothine eggs. The parasitoids were reared on eggs of the corn earworm, *Helicoverpa zea* (Lepidoptera: Noctuidae), and adults were provided with honey as a food resource (streaked on the walls of the vials in which wasps were held). The corn earworm eggs were provided by Ron Myers of the USDA-ARS (Tifton, GA).

Orius insidiosus was reared from field-collected bugs. Bugs were brought into the laboratory in July 2003, and were held in plastic containers with mesh-covered holes cut

in the lids to permit ventilation. Bugs were provided with pieces of green bean pods (replaced with fresh pieces three times weekly) and corn earworm eggs as food. The bean pod served as a source of moisture for the bugs, and also was the substrate into which they inserted their eggs (pirate bugs insert their eggs into plant tissue, much like tarnished plant bugs). Bean pods with eggs were collected three times weekly and the beans were placed in small, sealed cups in which the emergent nymphs were reared to adulthood. Nymphs were provided with fresh bean pod pieces and corn earworm eggs three times weekly.

Studies with *O. insidiosus*. The laboratory studies involved the use of an apparatus referred to as the "Tower". 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.

Treated cotton leaves were exposed to predators in small, circular chambers attached to the Tower through the ports described above. Cotton leaves were treated in a spray tower (at 20 psi) with 0.5 ml of the experimental treatment (insecticide mixed with water to achieve the desired rate for application of 8 gallons of water per acre; see Table 1). The leaf was then used as the bottom surface of the bioassay chamber, held in place by an underlying, untreated acrylic disc. The acrylic top of the chamber was untreated. Treated leaves were allowed to dry before five test insects (mated females, 3-5 days after adult emergence) were then introduced into each bioassay chamber with a small portion of food (4-5 corn earworm eggs per bug and a small piece of bean pod) and the chamber was affixed to the tower by inserting the vent tube in the chamber into the gasketed opening in the tower. Each chamber was examined 24, 72, and 120 hours after females were added to the chamber, and survival was recorded. In addition, the bean pod pieces were removed at the same intervals and the number of *O. insidiosus* eggs was recorded. The total number of eggs was divided by the average number of females in the dish (calculated as the sum of the number of females alive at 24 h and females alive at 120 h, divided by 2 $[(N_{24} + N_{120})/2]$) and the number of days (5) to produce the number of eggs laid per female per day. The experiment was repeated twice, with 4 replications (5 females per replicate) used for each treatment.

Survival data were transformed ($\arcsin\sqrt{\%}$) and analyzed using one-way ANOVA. When differences were significant, means were separated using the Waller-Duncan Bayesian *k* ratio (SAS Institute 1988).

Studies with *T. pretiosum*. Corn earworm eggs were exposed to female *T. pretiosum* and parasitism was verified visually. Parasitized eggs were held at 25ºC for 7 days (to allow parasitoid pupation in the host egg), then placed in plastic petri plates (100 mm by 15 mm). The eggs in the plates were treated with 0.5 ml of insecticide treatments (formulated at the rates presented in Table 2; mixed to the equivalent of 8 gallons of water per acre) applied to each dish using a spray tower (at 20 psi). Thirty parasitized eggs were treated for each insecticide (3 replicates of 10 eggs each).

Treated eggs were examined daily for parasitoid emergence. Longevity and reproduction of the wasps that emerged from treated eggs was evaluated to determine whether the insecticides had sublethal effects on the wasps. After emergence from parasitized eggs, parasitoids were held as individual pairs in 100x10 mm glass tissue culture tubes plugged with cotton batting. Honey was streaked in the tube to provide food for the wasps. Each wasp pair was provided with an excess of corn earworm eggs every other day throughout the female's life, and removed eggs were held at 25ºC to assess parasitism and reproduction of females emerged from treated eggs. Eggs were placed in tubes attached to a small square cut from the sticky section of a Post-It® note. After each female wasp died, the length of her right hind tibia was measured (using a micrometer mounted in the ocular piece of a dissecting microscope) to evaluate parasitoid size in relation to insecticide treatment and to permit analysis of the relationship between wasp size and number of eggs laid per female.

Developmental times and wasp emergence were compared among insecticide treatments using one-way ANOVA. Adult longevity and parasite size also were compared among insecticide treatment using ANOVA. Significantly different means were separated using the Waller-Duncan Bayesian *k* ratio.

Results and Discussion

Studies of *O. insidiosus*. The results from the tests are presented in Table 1. Centric, Karate, and Assail all were highly toxic to *O. insidiosus* within the first 24 hours of exposure. These results are in agreement with previous studies. The toxicity of Denim became apparent by 72 hours. The delayed response may account, at least in part, for the conflicting results with Denim and other natural enemies. Most toxicity studies tend to focus on survival in the first 24-48 hours of exposure. By observing survival over a longer period, we are more likely to detect cumulative effects. Prolonged studies with other natural enemy species may yield comparable results to those observed here for *O. insidiosus.* Survival in the Diamond treatment did not differ significantly from the control treatment during the course of the experiments. This is not surprising, as growth regulators often exert little acute toxicity on adult insects.

None of the females in the Assail, Centric, or Karate treatments laid eggs (Table 1). Among the remaining treatments, there were no significant differences in numbers of eggs laid per female per day. Sickening and death in the Assail, Centric, and Karate treatments likely occurred too rapidly to permit any egg production by dying females. Females in the Denim treated did lay eggs, although at a numerically reduced rate relative to the control and Diamond treatments. Egg production was unaffected by Diamond over the 5-day experimental period. In other studies with growth regulators and predatory stink bugs (*Podisus* spp.), reproduction of the bugs was reduced by growth regulators. It is possible that the duration of the experiment was insufficient to detect changes in reproduction. However, it is encouraging that there was no loss in reproductive capacity for at least five days following exposure to Diamond.

Studies of *T. pretiosum*. Developmental times of developing wasps in treated eggs was unaffected by insecticide treatment (Table 2). This indicates that the insecticides either failed to permeate the chorion of the host's egg, or the wasp pupae were not susceptible to the insecticides. Emergence of wasps was significantly affected by Tracer (Table 2). At the low rate of Tracer, the reduction in emergence was marginally different from the control and Steward treatments. However, at the high rate of Tracer (approximating the standard field usage rate) emergence was significantly reduced. Many of the wasps that emerged successfully in the Tracer treatments failed to survive more than a just few hours post-emergence. These wasps were excluded from the longevity/reproduction portion of the experiment. It appears likely that the wasps consume some of the Tracer as they chew their way out of the eggs, and that the ingested amounts of Tracer, particularly at the high rate, are sufficient to kill many of the wasps that chew out of the eggs.

Longevity of those female wasps surviving beyond the first few hours following emergence did not differ significantly among treatments (Table 2). Females in all treatments lived 3-4 weeks. Similarly, wasp size (as indicated by tibia length) was unaffected by insecticide treatment. Given that the size of *Trichogramma pretiosum* correlates positively with reproductive capacity (Ruberson and Kring 1993), this would further support the idea that emerged female wasps would be comparably effective regardless of the insecticide treatment to which they were exposed while inside the host egg.

Conclusions

The novel insecticides were variable in their toxicity to *O. insidiosus* relative to the standard insecticides, represented here by the pyrethroid Karate. Centric and Assail exhibited high acute toxicity to the bugs, not differing significantly from Karate. Denim also was highly toxic to *O. insidiosus,* but its toxicity was delayed 1-2 days relative to Assail, Centric, and Karate. Females exposed to Denim successfully laid eggs prior to death, unlike the females in the Assail, Centric, and Karate treatments. Diamond had no apparent adverse effects on the survival or reproduction of female *O. insidiosus.* Tracer and Steward had no negative effects on the developmental times of *T. pretiosum* wasps developing within the host eggs. However, when wasps chewed their way out of the eggs, Tracer was quite toxic to the wasps. None of the treatments adversely affected longevity of female wasps. Thus, adult wasps exposed to Tracer in the field will suffer considerable toxicity, and many wasps will also be killed in the process of chewing their way out of the eggs. Those wasps that are not seriously intoxicated during the process of emergence, however, will not live for any shorter period, and will likely experience little if any reproductive loss. Wasps exposed to Steward will not be harmed, either as adults (based on previous studies) or as immatures in the eggs. Steward appears to be entirely compatible with *T. pretiosum.*

References

Ruberson, JR & TJ Kring. 1993. Parasitism of developing eggs by *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae): host age preference and suitability. Biological Control 3: 39-46.

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Table 1. Survival (%; mean ± sd) of adult females of the predator *Orius insidiosus* 24, 72, and 120 h after exposure to dried insecticide residues on treated cotton leaves forming the bottoms of the bioassay chambers. Also includes number of eggs laid per female per day in the various treatments.[1]

Insecticide	Rate (lbs AI/A)	% survival			Eggs/fem/d (± sd)
		24 h	72 h	120 h	
Water (control)	NA	95.0 ± 10.00 ab	85.0 ± 10.00 a	55.0 ± 18.03 a	5.2 ± 2.11 ab
Centric (thiamethoxam)	0.050	13.3 ± 11.55 c	0 b	0 b	0 b
Assail (acetamiprid)	0.03	6.67 ± 11.55 c	0 b	0 b	0 b
Karate (l-cyhalothrin)	0.03	0 c	0 b	0 b	0 b
Denim (emamectin benzoate)	0.01	85.0 ± 10.00 b	5.0 ± 10.00 b	5.0 ± 10.00 b	3.7 ± 3.16 ab
Diamond (novaluron)	0.039	95.0 ± 10.00 ab	85.0 ± 19.14 a	70.0 ± 25.82 a	6.4 ± 4.24 ab
	0.059	100.0 ± 0.00 a	65.0 ± 10.00 a	62.0 ± 21.12 a	10.6 ± 4.73 a
F value		79.79	51.57	13.71	3.36
P		<0.0001	<0.0001	<0.0001	0.0362

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

Table 2. Developmental time (days following treatment) to adult emergence, % wasp emergence from parasitized eggs treated with insecticides, female size (length of right hind tibia in mm), and longevity of wasp females emerging from treated eggs for the parasitic wasp *Trichogramma pretiosum*.[1] Wasps were exposed to insecticides only in parasitized eggs 7 d after parasitism occurred.

Insecticide	Rate (lbs AI/A)	Developmental time (d, ± sd)	% emergence	Female tibia length (mm)	Female longevity (d, ± sd)
Water (control)	NA	4.0 ± 1.46 ab	88.9 a	0.18 ± 0.021 a	22.8 ± 7.76 a
Tracer (spinosad)	0.06	3.2 ± 0.43 b	66.7 a	0.17 ± 0.016 a	26.8 ± 0.41 a
	0.09	4.7 ± 2.66 a	25.0 b	0.19 ± 0.005 a	27.0 ± 0.00 a
Steward (indoxacarb)	0.09	3.9 ± 1.38 ab	96.4 a	0.19 ± 0.024 a	22.8 ± 8.54 a
	0.11	3.9 ± 1.32 ab	86.7 a	0.19 ± 0.019 a	25.4 ± 1.33 a

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