PESTICIDE EFFECTS ON INSECT NATURAL ENEMIES OF COTTON PESTS

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Abstract

We tested the effect of selected insecticides on the parasitic wasp *Cotesia marginiventris*. This wasp is an important parasite of nearly all caterpillars that attack cotto. It is particularly effective against armyworms, but also is a common parasite of bollworms, budworms, and loopers. As such, it is important that we do what we can to conserve this wasp in the field, and that we understand the potential impacts of pesticide applications on its ability to function.

For this study, we sprayed insecticides on wasps developing within their cocoons, as cocoons are fully exposed in the field during insecticide treatments, and are unable to escape. Further, cocoons in the field provide an important source of wasps for caterpillar biological control. We examined the effects of 6 insecticides: ?-cyhalothrin (Karate Z), dicrotophos (Bidrin), emamectin benzoate (Denim), novaluron (Diamond), and acetamiprid (Assail or Intruder). All were tested at field rates, although this was a laboratory study. Freshly-formed cocoons (< 12 h old) of *C. marginiventris* were collected and separated into groups. Each group of cocoons was sprayed with the designated insecticide, allowed to dry, and each cocoon was individually placed in a tissue culture tube. Treated cocoons were checked twice daily for wasp emergence. Developmental time (from treatment to adult emergence from the cocoons) and survival were recorded, and gender of emerging parasitoids was determined.

After adult emergence, parasitoids were paired within the insecticide treatments to allow detailed examination of their life histories in relation to the insecticide treatments. Each pair was held in a plastic petri dish provisioned with a streak of honey. Each day, 50 beet armyworm caterpillars (24-48 h old) were added to each dish to allow the female parasitoids to sting them, and caterpillars from the previous day were removed and placed on diet, where they were held until paraisoitd emergence, or the caterpillar pupated (indicating that it was not parasitized). In this manner we were able to assess insecticide effects on wasp fecundity and, by extension, the ability of the parasitoids to locate and attack hosts. A small piece of insect diet was placed in each dish for the host caterpillars to feed on during exposure to the wasps.

Developmental times of male and female wasps were affected by insecticide treatments. Development of both males and females was delayed by Karate, Bidrin, and Denim, although the delay was generally less than a single day. Survival was marginally affected by insecticides, with the lowest survival occurring in the Denim and Karate treatments. In all cases of mortality, the parasitoids died as adults within the cocoons, indicating that the insecticides did not impair pupal development.

Longevity and lifetime fecundity of adult females were unaffected by insecticide treatment. Daily egg production differed somewhat among treatments (although not significantly), but differences in daily production appear to have been compensated for by increased longevity to equalize the lifetime fecundity.

The insecticides tested in this study did not seriously affect the development, survival, longevity, or reproduction of *C. marginiventris* exposed to the pesticides through the cocoon, or through incidental ingestion while emerging from the cocoon. These results suggest that wasps within cocoons at the time of insecticide application will be protected from most adverse effects. This means that many insecticides used in a cotton field will not reduce survival or biological control potential of *Cotesia marginiventris* wasps that are in the pupal stage in the field. Adult wasps exposed in the field will likely not always be as fortunate.

Introduction

The reduced-insecticide environment of the cotton system that has resulted from boll weevil eradication and adoption of insecticide-transgenic plants creates an ideal opportunity for integrating biological control in integrated pest management (IPM). To take full advantage of existing biological control agents, it is important to understand the impact of insecticides on valuable natural enemy species. With new compounds in the pipeline and making their way to the market, it is important that we maintain a dynamic database of the effects of insecticides on natural enemies so that growers can make informed decisions about the products available to them.

Pesticides exert a variety of effects on natural enemies, of which the most frequently reported is acute toxicity (Ruberson et al. 1998). Although understanding the acute toxicity of insecticides to natural enemies is important and relevant to IPM, pesticides can exert other effects which can be equally or more important for IPM. Studies of the effects of pesticides on natural enemies rarely consider how pesticides influence biological attributes of the natural enemies. Some pesticides have been reported to exert sublethal effects on natural enemies. In other words, the pesticides fail to kill the natural enemy outright, but adversely affect the biology of the natural enemy and reduce or eliminate its effectiveness. These effects cannot be detected in standard field studies of pesticides where natural enemies are evaluated only by counts. Presence of a natural enemy after pesticide application does not necessarily mean that the natural enemy is fully functional. Thus, lack of serious acute toxicity does not mean that biological control is working, and adverse sublethal effects can lead to secondary pest outbreaks, or the need for more frequent repeat applications of insecticides. The current emphasis on selective pesticides is generating materials that are less prone to kill natural enemies outright, which is a valuable property, but they may still exert important and relevant effects on the natural enemies.

In these studies we examined the effects of several insecticides, including older standards and newer selective compounds, on the life history of a natural enemy: the parasitic wasp *Cotesia marginiventris*. *Cotesia marginiventris* is an important parasite of nearly all caterpillars that attack cotton (Krombein et al. 1979). It is particularly effective against armyworms (Ruberson et al. 1994), but also is a common parasite of bollworms, budworms, and loopers (Boling and Pitre 1970).

Methods

Insect Acquisition and Maintenance. The insects used in the studies were obtained from field collections. A culture of *C. marginiventris* was initiated from parasitized beet armyworms (*Spodoptera exigua*) collected in cotton in July 2004, in Tift County, Georgia. The culture was maintained in the laboratory at 25 $\pm$ 2°C, L:D 14:10. *Cotesia marginiventris* is maintained with beet armyworm larvae as hosts.

Experimental Procedures. The treatments used in the test are presented in Table 1. For each treatment, the insecticide was combined with water to simulate a concentration that would be applied at a rate of 8 gallons per acre and was applied to the test subjects using a Potter Spray Tower (Burkard Scientific, Ltd., UK), with pressure set to 25 psi and a spray volume of 0.5 ml per application.

We used cocoons (that contained parasitoid pre-pupae and pupae) for the tests for two reasons. First, the cocoons are exposed in the field and immobile, so that they cannot escape treatments. Second, after wasps complete development to the adult stage inside the cocoon, they chew their way out of the cocoon, ingesting pesticide residues on the cocoons silk. Freshly-formed cocoons (< 12 h old) of *C. marginiventris* were collected and separated into groups of 10, with 3 groups randomly assigned to each insecticide treatment (total n = 30 per treatment). Each group of cocoons was sprayed with the designated insecticide, allowed to dry, and each cocoon was individually placed in a tissue culture tube. Treated cocoons were held at 25 $\pm$ 1°C, L:D 14:10, in environmental chambers and checked twice daily for parasitoid emergence. Developmental time (from treatment to adult emergence from the cocoons) and survival were recorded, and gender of emerging parasitoids was determined.

After adult emergence, parasitoids were paired within the insecticide treatments to allow detailed examination of their life histories in relation to the insecticide treatments. Each pair was held in a plastic petri dish (100x15 mm) provisioned with a streak of honey. Each day, 50 beet armyworm caterpillars (24-48 h old) were added to each dish to allow the female parasitoids to sting them, and caterpillars from the previous day were removed and placed on diet, where they were held until parasitoid emergence, or the caterpillar pupated (indicating that it was not parasitized). In this manner we were able to assess insecticide effects on wasp fecundity and, by extension, the ability of the parasitoids to locate and attack hosts. A small piece of insect diet was placed in each dish for the host caterpillars to feed on during exposure to the wasps.

Table 1. Insecticides and rates used in experiments with *Cotesia marginiventris*

Insecticide	Product name	Rate (lbs AI/A)
Water (control)	N/A	N/A
Diamond	novaluron	0.03
Diamond	novaluron	0.059
Intrepid (Assail)	acetamiprid	0.05
Denim	emamectin benzoate	0.01
Bidrin	dicrotophos	0.5
Karate Z	?-cyhalothrin	0.0019
S-1812	pyridalyl	0.150

Developmental times (separated for males and females), longevity, and fecundity were analyzed using one-way analysis of variance (PROC GLM, SAS Institute 1994). Pre-imaginal survival was analyzed using the Kruskal-Wallis test (PROC NPAR1WAY, SAS Institute 1994).

Results

Developmental times of male and female wasps were affected by insecticide treatments (Table 2). Development of both males and females was delayed by Karate, Bidrin, and Denim, although the delay was generally less than a single day. Survival was marginally affected by insecticides, with the lowest survival occurring in the Denim and Karate treatments (Table 2). In all cases of mortality, the parasitoids died as adults within the cocoons, indicating that the insecticides did not impair pupal development.

Longevity and lifetime fecundity of adult females were unaffected by insecticide treatment (Table 3). Daily egg production differed somewhat among treatments (although not significantly), but differences in daily production appear to have been compensated for by increased longevity to equalize the lifetime fecundity.

Discussion

The effects of the various insecticides on *C. marginiventris* were variable, but generally limited. Developmental delays of less than one day would create few problems in the life history of the parasitoid, and mortality was relatively low (the highest was ~23% in the Denim treatement). Longevity and fecundity were not significantly affected by any of the insecticides. These results indicate that the parasitoids developing in the cocoons during application of the tested insecticides would suffer limited or no harmful effects, even after chewing out of the cocoon and ingesting contaminants in the silk.

It is well known that ?-cyhalothrin (Karate) adversely affects parasitic wasps, including *C. marginivenris*, when they are exposed to residues as adult wasps (Ruberson and Tillman 1999, Tillman and Mulrooney 2000). When adult *C. marginiventris* are exposed to fresh (ca. 1 h after treatment) Karate residues on leaves, mortality after 48 h is approximately 45-65% (Ruberson and Tillman 1999, Tillman and Mulrooney 2000). The present results indicate that much of this toxicity is ameliorated if the parasitoids are expose in the pupal stage, within the cocoon. Sublethal effects of Karate on parasitoids have not been as well-documented. Prior to this study there was no information available on *C. marginiventris*. Desneux et al. (2004) noted that exposure of the parasitoid *Aphidius ervi* (a braconid parasitoid of pea aphids) to sublethal dosages of ?-cyhalothrin impaired the ability if the wasps to locate hosts through anemotaxis. However, these effects disappeared after 24 h. In our study, there were no sublethal effects of Karate on the longevity of the wasps, or on their ability to parasitize hosts within the arenas in which they were housed.

Dicrotophos (Bidrin) has a broad spectrum of activity, and has been shown to be highly toxic to numerous natural enemies, including adult *C. marginiventris* (see Croft 1990 for general review). This insecticide is an important tool for managing stink bug pests in the Southeastern US (although its use is being increasingly limited by regulatory constraints). It appears from our study here that applications of Bidrin applied while parasitoids are in the cocoon will have a very limited impact on parasitoid development, but no other adverse effects.

Pyridalyl (S-1812) has not been found to be highly toxic to adult *C. marginiventris* (Ruberson and Tillman 1999, Tillman and Mulrooney 2000), and it is now apparent that it has no adverse effect on pupae. Nor does S-1812 exert any adverse effects on the life-history traits of females that have ingested the insecticide incidentally while emerging from the cocoon.

Novaluron (Diamond) is an insect growth regulator that has been researched for some time. However, there is very little information available on potential effects on natural enemies. The present results suggest that it will have little impact on *C. marginiventris* developing within the cocoons, or on the life histories of wasps emerging from the cocoons.

The effect of emamectin benzoate (Denim) on parasitoids has not been elucidated. Boyd and Boethel (1998) found that this insecticide had exhibited limited toxicity to predatory bugs in cotton, supporting field trials with a suite of predators (Dunbar et al. 1998). In our study, Denim slowed development of the parasitoids, and reduced survival significantly. It did not adversely affect the life histories of surviving females, however. The impact on adult *C. marginiventris* of exposure to Denim residues is not known, but two other Hymenopterans, the honeybee *Apis mellifera* and the eulophid parasitoid *Diglyphus isaea*, were highly susceptible to fresh residues of emamectin benzoate (Chukwudebe et al. 1997). Mortality after the residues had aged for 24 h, however, were negligible for both species. Thus, the impact would be of short duration.

Acetamiprid (Assail, Intruder) is a recently-developed insecticide and there is relatively little known about its nontarget profile. Acetamiprid has been shown to be relatively non-toxic to several predators (Fitzgerald 2004, Tillman and Mullinix 2004), but highly toxic to others (Ruberson et al. 2004). This insecticide had no adverse effects on *C. marginiventris* through the cocoon in the present study. The effects of acetamiprid residues on adult wasp survival are not known at present, but will be investigated in the coming year.

In conclusion, the insecticides tested in the present study did not have serious adverse effects on the development, survival, longevity, or reproduction of *C. marginiventris* exposed to the pesticides through the cocoon, or through incidental ingestion while emerging from the cocoon. These results suggest that parasitoids within cocoons at the time of application of a variety of insecticidal compounds will be protected from most adverse effects. This is significant, as the pupal stage within the cocoon is in an exposed position, and is unable to move and escape insecticides as they are applied. Further, the wasps emerging from the cocoons in the treated field are already in the field, and can provide some level of biological control for hosts in the cotton field without having to rely on colonization from outside of the field.

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Table 2. Developmental times (in days) and percent survival of *C. marginiventris* in the pre-pupal and pupal stages, in relation to insecticide treatment.

Insecticide	Rate (lbs AI/A)	Developmental time (d)		% survival
		Males	Females	
Water	N/A	5.4 ± 0.60	5.7 ± 0.50	96.7
n		20	9	
Diamond	0.03	5.3 ± 0.45	5.8 ± 0.44	96.7
n		12	17	
Diamond	0.059	5.2 ± 0.43	5.9 ± 0.29	86.7
n		14	12	
Intrepid (Assail)	0.05	5.5 ± 0.51	5.8 ± 0.45	90.0
n		22	5	
Denim	0.01	5.8 ± 0.38	6.2 ± 0.63	76.7
n		13	10	
Bidrin	0.5	5.9 ± 0.86	6.4 ± 0.50	90.0
n		13	14	
Karate Z	0.0019	5.9 ± 0.27	6.1 ± 0.83	80.0
n		14	11	
S-1812	0.150	5.2 ± 0.42	5.4 ± 0.52	96.7
n		19	10	
df		7,119	7,80	7
F		5.49	3.77	Chi-Sq = 12.89
P		<0.0001	0.0014	0.0749

Table 3. Longevity (in days) and fecundity of, and eggs laid per day by adult female *Cotesia marginiventris* emerged from cocoons treated with various insecticides.

Insecticide	Rate (lbs AI/A)	n	Longevity (d)	Fecundity	Eggs/d
Water	N/A	9	10.8 ± 3.28	132.7 ± 50.83	14.0 ± 3.01
Diamond	0.03	10	12.6 ± 4.33	128.4 ± 49.17	10.5 ± 2.72
Diamond	0.059	10	11.7 ± 2.87	138.0 ± 26.85	12.1 ± 1.86
Intrepid (Assail)	0.05	5	10.5 ± 3.87	129.8 ± 66.92	15.2 ± 1.85
Denim	0.01	9	11.6 ± 3.24	150.2 ± 40.10	13.4 ± 3.16
Bidrin	0.5	10	10.9 ± 3.11	141.9 ± 46.54	13.0 ± 2.15
Karate Z	0.0019	10	10.4 ± 4.79	122.4 ± 58.66	11.8 ± 1.60
S-1812	0.150	9	10.6 ± 3.53	134.7 ± 69.3	11.7 ± 4.21
df			7,59	7,64	7,61
F			1.75	0.26	2.09
P			0.1139	0.9657	0.0584