EFFECTS OF NOVEL PESTICIDES ON *TRICHOGRAMMA PRETIOSUM*

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Abstract

The effects of several pesticides on the foraging behavior and life history of *Trichogramma pretiosum* were evaluated. The pesticides tested were: (1) Tracer® (spinosad; Dow AgrosSciences); (2) Steward® (indoxacarb; DuPont); (3) Dimilin® (diflubenzuron; Uniroyal); and (4) Karate Z® (l-cyhalothrin; Syngenta). For the foraging study, eggs were treated with a single rate of each compound, the eggs were allowed to dry, and female parasitoids were each allowed to forage on two eggs. Four behaviors (antennating, stinging, host feeding, and cleaning) were timed and compared among treatments. For the life history study, eggs parasitized 3 days prior were treated with 2 rates of each of the compounds, and the developmental times, emergence rates, parasitoid size (right hind tibia length), female longevity, and fecundity were assessed. Female parasitoids spent significantly less time foraging on both eggs treated with Karate than on eggs treated with Steward and Tracer, where females spent the greatest amount of time. Most of this difference was due to shortened stinging and prolonged cleaning times for females on the Karate-treated eggs, and prolonged stinging times and cleaning times for females on the Steward- and Tracer-treated eggs, respectively. Pesticide treatment significantly affected preimaginal developmental times, but these differences were small and likely of limited biological significance. Parasitoid emergence was adversely affected by Tracer and Karate at both rates, and by Dimilin at the high rate (0.125 lbs AI/A). Females emerging from eggs treated with a low rate of Karate (0.015 lbs AI/A) were significantly larger than females emerging from eggs treated with Dimilin at the low rate (0.03 lbs AI/A) but no other differences were observed in size. Female longevity and offspring production were unaffected by the treatments the females had received while developing in the host egg.

Introduction

The advent of novel selective insecticides has created an ideal opportunity for integrating arthropod natural enemies with pesticide use in pest management programs. However, survival is not the sole criterion for detemining the suitability of an insecticide for integration with biological control agents. Insecticides can have sublethal effects on natural enemies that can severely disrupt their efficacy and their population dynamics without causing extensive mortality (see Croft 1990). These effects can be very subtle, but still can have substantive impacts. For example, recent work by Delpuech et al. (1998, 1999, 2001) has demonstrated that even at very low doses some pyrethroids and organophosphates can interfere with sex pheromone production by females and with responses to the pheromone by males of the parasitoid *Trichogramma brassicae* Bezdenko, potentially affecting mating success of the parasitoids. Sublethal effects are not considerations with most broad-spectrum insecticides, as survival of natural enemies is minimized or entirely eliminated. However, the newer selective materials often permit moderate to high levels of natural enemy survival, and under these conditions sublethal effects become quite important.

In screening pesticides against natural enemies it is important to select species that will function as good indicators of some component of the natural enemy complex, and which are of some importance to the target systems in which the pesticides will be used. *Trichogramma* species are widespread and can be important parasitoids of pests in numerous crop systems (Hassan et al. 2000). In addition, they tend to be relatively sensitive to pesticides, making them a conservative choice as an indicator species in pesticide bioassays. In this paper we present results of studies examing the effects of selected pesticides on the egg parasitoid *Trichogramma pretiosum*. The study focused on the effects of these pesticides on oviposition behavior of females and the success of offspring emerged from treated eggs. Our objective was to characterize the effects of these pesticides on female foraging behavior and on the development, emergence, and life-history attributes of females emerged from treated eggs.

Materials and Methods

Two areas were examined in this project. First, we examined the behavior of foraging females on eggs treated with the insecticides (placed on an untreated substrate). Second, we treated parasitized eggs and evaluated survival and relevant life history traits of females that had emerged.

Behavior of Foraging Females
Eggs of the corn earworm, *Helicoverpa zea* (Boddie), were treated with five different pesticide treatments: (1) water (control treatment); (2) Tracer® (spinosad; Dow AgrosSciences; 0.06 lbs AI/A); (3) Steward® (indoxacarb; DuPont; 0.11 lbs AI/A); (4) Dimilin® (diflubenzuron; Uniroyal; 0.125 lbs AI/A); and (5) Karate Z® (l-cyhalothrin; Syngenta; 0.03 lbs AI/A). A total of 50 eggs were treated with each treatment, and treatments were applied at a rate of 0.5 ml per unit area (the unit was a 100-mm diameter petri dish in which the eggs were held) using a Potter spray tower (in water at 20 psi). Pairs of eggs of the same treatment were placed in petri dishes and a single female *T. pretiosum* (48-72 h old) was introduced. Her behavior was observed on each of the eggs and specific behaviors (antennation, stinging, host feeding, and cleaning) were timed. Each of the females had had previous exposure to host eggs and was mated prior to the observations. We observed 6-12 females for each treatment. Duration of each behavior was analyzed using analysis of variance (ANOVA), and significant means were separated using the Waller-Duncan Bayesian *k* ratio, with *k*=100.

Survival and Life History Traits
Eggs of the cotton bollworm, *H. zea*, were parasitized by female *T. pretiosum*, and parasitism was verified by direct observation. Parasites were allowed to develop in the eggs for 3 days after parasitism. On the 3rd day after parasitism, 9 insecticide treatments were applied to the eggs, using a Potter spray tower, as described above. The 9 treatments were: (1) water (control treatment); (2) Tracer low rate (0.03 lbs AI/A); (3) Tracer high rate (0.06 lbs AI/A); (4) Steward low rate (0.055 lbs AI/A); (5) Steward high rate (0.11 lbs AI/A); (6) Dimilin low rate (0.03 lbs AI/A); (7) Dimilin high rate (0.125 lbs AI/A); (8) Karate low rate (0.015 lbs AI/A); and (9) Karate high rate (0.03 lbs AI/A). All treated eggs were observed for parasite emergence, and dates of emergence were recorded (40 eggs for each treatment; 4 reps of 10 eggs each) to determine developmental times.

Females emerging from eggs that produced only 2 parasitoids were collected and held individually with mates in test tubes provisioned with honey, and their longevity observed. Females were collected from eggs producing only two parasitoids in an effort to maintain reasonable consistency in parasitoid size. In addition, irradiated *H. zea* eggs were provided to individual females every other day, and rates of parasitism of these eggs were tracked throughout the lifetimes of the females (8 to 15 females per treatment). Data collected were: (1) longevity of females, (2) lifetime parasitism rates of host eggs by individual females, and (3) number of offspring produced. After all parasitoid offspring had emerged, eggs were dissected to count any offspring that had failed to emerge from the host eggs. Offspring dead in eggs were added to those that emerged for each female to obtain the number of offspring produced per female. The length of the right hind tibiae of females also was measured (using an ocular micrometer) as an indicator of parasitoid size for those surviving to emerge. Measurements were made by removing the tibiae from females and measuring length with an ocular micrometer in a compound microscope. All evaluated parameters were analyzed using analysis of variance (ANOVA), and significant means were separated using the Waller-Duncan Bayesian *k* ratio, with *k*=100.

Results and Discussion

Behavior of Foraging Females
The behavior of ovipositing females was altered somewhat by the presence of Karate on the chorion of the eggs (Table 1). Females foraging on eggs treated with Karate spent significantly less time antennating, stinging, and host feeding on the first egg encountered than was the case for eggs of most of the other treatments. Females also spent significantly more time cleaning themselves after encountering the second egg treated with Karate. Karate may have irritated the parasitoids, and may make treated hosts less attractive. Preference studies are needed to establish this more clearly. Although the differences were not significant, females foraging on eggs treated with Tracer spent considerable time cleaning themselves. More observations are needed to determine whether or not this is significant for long-term survival of the females. Further work is also needed to establish whether or not exposure to numerous treated eggs would have adverse long-term effects on foraging females.

The total time females spent on the two eggs did differ significantly among treatments, with females spending the greatest amount of time on eggs treated with Steward and Tracer, and the least on eggs treated with water or Karate Z (Table 1). Reduced time on the Karate-treated eggs may have been due to some degree of irritation to the parasitoid, but the basis for the increased time spent on eggs treated with Steward is unclear, as there were no other effects of this pesticide on the parasitoid (see below). Females on Steward-treated eggs spent considerable amounts of time stinging, as did females on the first Tracer-treated egg encountered (Table 1). Females on Tracer-treated eggs also tended to spend more time cleaning (particularly on the second egg) than was the case for females in the other treatments, except the Karate treatment on the second egg.

Survival and Life History Traits
Insecticide affected development and survival of the parasitoids (Table 2). Development was most rapid in the Steward treatments, which yielded significantly shorter developmental times than the control, Tracer 0.06, and Karate 0.03. The

mechanism or significance of this difference is unknown. However, overall, none of the pesticides tested exerted serious adverse effects on the preimaginal development of the parasitoids. Consoli et al (1998) reported that Karate significantly prolonged preimaginal development of *Trichogramma pretiosum*, but we did not observe an effect as great as that which they observed. The range of differences in preimaginal development among treatments amounted to only 1 day, which likely has very limited biological significance. It may, however, point to relevant sublethal effects.

Differences in parasitoid size were limited to a significant difference between females emerging from eggs treated with the high rate of Dimilin and those emerging from eggs treated with the low rate of Karate (Table 2). No other significant differences were observed.

The number of eggs from which parasitoids successfully emerged also was affected by the insecticide treatment (Table 2). The Tracer and Karate treatments both reduced the rates of emergence of parasitoids from eggs. The high rates of Dimilin and Steward, however, also reduced the emergence rate. However, once parasitoids were able to open the egg, nearly all emerged from the eggs. Many of the parasitoids that emerged successfully from eggs treated with Tracer and Karate died during emergence or within a short time (a few minutes to hours) after emergence, unlike those emerging in the other treatments. Similar observations were made by Suh et al. (2000) with the parasitoid *Trichogramma exiguum* Pinto and Platner for Karate and Tracer. Suh et al. (2000) also observed higher levels of brachyptery among parasitoids emerging from eggs treated with Tracer than among parasitoids emerging from eggs treated with Karate, Ammo, Curacron, Larvin, Intrepid, or Confirm. These results suggest that the pesticides were for the most part unable to penetrate the chorion of the eggs after they were parasitized (with the exception of Tracer), but that accumulation of pesticides in the chorion could be ingested by the parasitoids as they emerge, killing them if the pesticide is toxic. This is in contrast with results of Karate and Tracer application to unparasitized eggs. When these two insecticides are applied to unparasitized eggs, many (if not all) of the embryos in the treated eggs fail to complete development (JRR personal observation), indicating penetration of the chorion by the pesticides. It appears that the chorion becomes a more effective barrier when parasitoids are in the egg.

Pesticide treatments had no effect on the reproduction or longevity of female parasitoids surviving to adulthood and successfully emerging (Table 3). Suh et al. (2000) obtained similar results for *T. exiguum* emerging from eggs treated 3 days after parasitization. This result indicates that parasitoid females surviving to emerge from eggs treated with any of the insecticides tested will be able to perform comparably and will experience comparable longevity.

These studies indicate that one can expect a possible decline in foraging efficiency for females attacking eggs treated with Steward and Tracer, and possible improvement on eggs treated with Karate (assuming long-term intoxication does not occur), but that little impact should be expected from the other insecticides. It is important to point out, however, that the substrate on which the eggs were placed in these tests was untreated. In the field where both the egg and the substrate are treated, the results may differ substantially. For example, in previous studies we and others have found Tracer to be quite toxic to *Trichogramma* spp. (Suh et al. 2000). Thus, the mortality of foraging females may be increased when Tracer has been used, even though there may be little impact on females if they survive to forage on individual, treated eggs. However, our results indicate that there is reasonable to excellent compatibility of these insecticides with a sensitive natural enemy exposed only through the host egg.

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Table 1. Duration of foraging behaviors of female *Trichogramma pretiosum* on two eggs of *Helicoverpa zea* treated with selected insecticides. All times are presented in seconds.

Treatment	Rate (lbs AI/A)	n	Antennating	Stinging	Feeding	Cleaning
First egg						
Control	—	14	101.4 ± 60.96 BC	307.4 ± 306.81 AB	50.4 ± 68.18 AB (7)	0 A
Dimilin	0.125	18	123.9 ± 68.95 AB	359.2 ± 190.73 AB	66.2 ± 75.96 AB (10)	17.6 ± 48.08 A (2)
Karate	0.03	12	53.8 ± 36.57 C	175.2 ± 51.1 B	0 B	7.5 ± 14.19 A (6)
Steward	0.06	12	165.8 ± 81.62 A	416.4 ± 258.72 A	71.0 ± 110.61 AB (6)	18.1 ± 62.64 A
Tracer	0.11	12	162.2 ± 122.13 AB	440.7 ± 250.58 A	112.0 ± 156.96 A (9)	19.8 ± 51.17 A (2)
Second egg						
Control	—	14	92.4 ± 71.24 A	275.2 ± 245.20 AB	25.9 ± 52.70 A (5)	0.7 ± 2.67 B (2)
Dimilin	0.125	18	82.4 ± 54.86 A	205.0 ± 96.03 AB	23.4 ± 81.14 A (2)	0
Karate	0.03	12	60.1 ± 34.92 A	155.8 ± 69.86 B	2.2 ± 7.51 A (2)	96.7 ± 135.55 A (7)
Steward	0.06	12	105.3 ± 47.99 A	323.5 ± 156.14 A	13.8 ± 34.97 A (3)	4.8 ± 16.45 B (3)
Tracer	0.11	12	111.8 ± 105.82 A	282.3 ± 155.31 AB	0 A	32.5 ± 81.88 B (2)

Parenthetical numbers under means represent the number of females observed engaging in the described activity. All female antennated and stung eggs, but not all fed on the host eggs or cleaned themselves.
Values in columns followed by the same letter were not significantly different (ANOVA; Waller-Duncan Bayesian k, k=100).

Table 2. Total time spent by female *Trichogramma pretiosum* foraging on host eggs (corn earworm, *Helicoverpa zea*) in relation to pesticides applied to eggs approximately one hour prior to observation. All times are presented in seconds.

Treatment	Rate (lbs AI/A)	n	Total time on first egg	Total time on second egg	Total for both eggs
Control	—	14	500.1 ± 377.12 AB	391.8 ± 293.55 A	824.38 ± 470.69 AB
Dimilin	0.125	18	566.4 ± 280.40 A	311.8 ± 161.37 A	890.6 ± 403.71 AB
Karate	0.03	12	236.5 ± 84.56 B	314.7 ± 159.46 A	551.2 ± 198.32 B
Steward	0.06	12	671.3 ± 450.58 A	447.3 ± 200.50 A	1118.6 ± 600.05 A
Tracer	0.11	12	734.7 ± 483.01 A	426.7 ± 263.55 A	1161.3 ± 670.02 A

Values in columns followed by the same letter were not significantly different (ANOVA; Waller-Duncan Bayesian k, $k=100$).

Table 3. Effects of pesticides on development, emergence, and size of emerging adults of *Trichogramma pretiosum* parasites in host eggs (corn earworm, *Helicoverpa zea*) treated 3 days after parasitism ($25 \pm 2°C$). Sample size was 40 eggs for each treatment (except the Steward 0.055 treatment, which had 44).

Treatment	Rate[1]	Developmental time (d)[2]	% of eggs yielding parasites	% emergence from eggs	Tibia length[3] (mm)
Control	NA	11.8 ± 0.76 A	100.0	98.2 ± 9.22	0.134 ± 0.009
Dimilin	0.03	11.2 ± 0.40 ABC	92.5	97.6 ± 8.54	0.147 ± 0.013
	0.125	11.2 ± 0.49 ABC	72.5	99.1 ± 4.64	0.130 ± 0.042
Tracer	0.03	11.9 ± 0.91 A	67.5	96.6 ± 13.59	0.148 ± 0.003
	0.06	11.4 ± 0.81 BC	65.0	100.0 ± 0.00	0.153 ± 0.103
Steward	0.055	10.9 ± 0.40 A	100.0	100.0 ± 0.00	0.154 ± 0.018
	0.11	11.1 ± 0.49 BC	82.5	100.0 ± 0.00	0.142 ± 0.022
Karate	0.015	11.2 ± 0.51 BC	76.9	97.0 ± 9.36	0.170 ± 0.028
	0.03	11.5 ± 1.02 B	75.0	96.2 ± 12.15	0.145 ± 0.021

[1]Rates are presented in lbs per acre equivalents.
[2]Means in columns followed by the same letter are not significantly different ($p > 0.05$; Waller Duncan bayesian k; $k=100$).
[3]Tibiae of females emerging from eggs treated with Karate at the low rate were significantly larger than those of females from eggs treated with Dimilin at the high rate. No other differences were observed.

Table 4. Effects of pesticides on longevity and reproduction of female *Trichogramma pretiosum* reared from host eggs (corn earworm, *Helicoverpa zea*) treated with selected insecticides 3 days after parasitism ($25 \pm 2°C$). None of the means within a column differed significantly (Waller-Duncan bayesian k ratio, $k=100$).

Pesticide	Rate	Longevity (d)	Offspring	Hosts parasitized
Control	NA	16.7 ± 8.86	39.4 ± 20.56	30.8 ± 18.58
Dimilin	0.03	14.9 ± 5.78	45.0 ± 26.12	33.3 ± 18.79
	0.125	12.8 ± 7.59	44.5 ± 25.63	32.3 ± 21.00
Tracer	0.03	19.2 ± 5.81	52.6 ± 30.34	40.6 ± 28.34
	0.06	23.5 ± 6.75	66.2 ± 40.56	50.9 ± 32.73
Steward	0.055	22.3 ± 6.75	66.2 ± 44.53	54.4 ± 38.10
	0.11	19.6 ± 6.65	35.8 ± 22.58	23.8 ± 15.84
Karate	0.015	17.3 ± 9.50	51.7 ± 31.85	40.5 ± 27.58
	0.03	14.3 ± 6.13	43.0 ± 35.82	27.0 ± 22.85