RESPONSE OF TOBACCO BUDWORM[1] ADULTS AND LARVAE EXPOSED TO CYPERMETHRIN USING DIFFERENT BIOASSAY TECHNIQUES

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ABSTRACT

Studies were established to compare the effects of cypermethrin on adults of the tobacco budworm, *Heliothis virescens* (F.), using the adult vial test (AVT) and a modified adult vial test (MAVT). Insects were treated with cypermethrin at rates approximating those used in the field. The susceptibility of adults and larvae from the same population was also compared using petri dishes treated in the field with different concentrations of cypermethrin. Larvae were also tested by topical exposure to cypermethrin. Results indicated that rates of 163-224 ug/vial of cypermethrin in the AVT were equivalent to MAVT rates of 0.067-0.077 lb ai/A in causing adult mortality. A very high correlation (r=.99) was obtained for moth and neonate larval (0-10 hours old) mortality. LC_{50} and LC_{90} values for adults and neonate larvae were less than those obtained for older first instar larvae (24-48h post-eclosion) and third instar topically treated larvae. These results also suggest that optimum efficacy is obtained when pyrethroid sprays are targeted toward first instar larvae less than 24 hours old.

INTRODUCTION

Pyrethroid insecticides, introduced into the U.S. cotton market for control of cotton pests in 1979, have been used extensively to control the tobacco budworm, *Heliothis virescens* (F.) and the cotton bollworm, *Helicoverpa zea* (Boddie). Programs have been conducted annually across the cotton belt to monitor the susceptibility of third instar tobacco budworms to different pyrethroids using topical application techniques (Staetz 1985). The topical test method has been a standard in monitoring insecticide susceptibility levels in insect populations since the 1950's (Anonymous 1970). The greatest advantage of this method is the precision and accuracy with which desired doses can be applied to different life stages of insects. More recently, an adult vial test (AVT) was developed and used extensively to measure changes in the susceptibility of the tobacco budworm to pyrethroid insecticides (Plapp et al. 1987). This method is

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a rapid test conducted in the field with male moths that are easily obtained from pheromone traps. Both bioassay methods allow comparisons of the susceptibility of one population with another and of changes in susceptibility from one year to the next.

It has been suggested that the susceptibility of larval offspring can be predicted from the results of the AVT. Roush and Luttrell (1989) demonstrated a very high correlation between the activity of cypermethrin on pyrethroid resistant third instar tobacco budworms with bioassays using treated cotton terminals and the AVT for adults. However, further work is needed to define the relationship between AVT results and those which might be expected with field application rates required for acceptable control of larvae in the field. This knowledge is critical if the AVT or other monitoring techniques are to be used to predict the impact of resistance on commercial applications.

Herein we report the results of studies conducted in 1988 and 1989 to determine if there were relationships between results obtained with the AVT and other bioassay techniques using application rates equivalent to those recommended for field applications of cypermethrin.

MATERIALS AND METHODS

Male tobacco budworm moths were collected from wire cone traps baited with sex pheromone during August, 1988 and 1989 near Hearne, TX. Moths were removed from the traps before sunrise and bioassayed with the AVT using cypermethrin (technical) concentrations of 1, 5, 10, 30 and 100 ug ai/vial. One moth was tested per vial and 100 moths were tested per concentration. Equal numbers of moths placed in untreated vials served as controls. Vials used for controls were previously cleaned with acetone and allowed to dry. Vials were held 24h at room temperature (23° C.) and then moths were removed from the vials and tossed into the air. Those unable to fly were recorded as dead.

A modified vial test (MAVT) was used for bioassays of adults from the same populations used for the AVT. In the MAVT, vials were treated with concentrations of a commercial formulation of cypermethrin (Ammo 2.5 EC) equivalent to rates (lb ai/A) used in practical field applications. Concentrations equivalent to field rates were calculated for vial treatments as described in the following example. First, the amount (ml) of formulated insecticide required for an application rate of 0.01 lb ai/A (based on one gallon of cypermethrin) was determined by calculating the lb. ai/ml equivalents in 2.5 lb. ai/gallon; a volume of 17.74 ml of Ammo 2.5 EC contains 0.01 lb ai. The amount required for an application rate of 0.04 lb ai is 17.74 ml X 4 or 70.96 ml. To calculate the amount needed to treat a small plot (6 feet wide and 50 feet long) with four replications at a rate of 0.04 lb ai/A, 70.96 ml X 0.027 acres = 1.91 ml/acre. The weight of 1.91 ml of Ammo 2.5 EC is 1.75 grams. The amount of active ingredient in the formulated product, is 1.75 grams X 30.6% (% ai in formulation) or 0.54 grams ai. Then 0.54 grams X 1000 = 540 milligrams in 2415 ml water used to spray

the 0.027 acre plot area or 0.22 milligrams/ml. Because 1 milligram/1 ml = 1000 ppm, 0.22 X 1000 = 220 ppm (0.04 lb ai/acre). The volume of solution used to coat the inside of a 23 ml vial was 0.5 ml. Acetone was used in place of water to dilute both the EC formulation and the technical cypermethrin used to treat the vials. The amount (0.5 ml technical + acetone) is equivalent to 1 ug/vial or 2 ppm. Vials treated with application rates equivalent to 0.000625, 0.00125, 0.0025, 0.005, 0.01, 0.02, 0.04 and 0.06 lb ai/acre were used in this study. One moth was tested per vial and 50-138 moths were tested per concentration. One hundred moths placed individually in untreated vials served as controls.

Another method used to test field application rates of cypermethrin against adults involved the use of glass petri dishes (100 X 15 mm) treated with sprays in the field. A backpack sprayer was used to treat dishes placed at ground level with EC formulations of cypermethrin at concentrations equivalent to 0.1, 0.08, 0.04, 0.02, 0.01, 0.005, 0.0025 and 0.00125 lb ai/A. A Thrush S2R aircraft was used for aerial applications of 0.10, 0.08, 0.06 and 0.04 lb ai/A of EC formulations of cypermethrin to petri dishes placed on a stand in a cotton field at maximum canopy height. For each concentration, 40-60 moths were exposed to the cypermethrin residues using one moth per dish. Similar numbers of moths placed individually in untreated petri dishes served as controls. A separate set of petri dishes for each dose was sent to the FMC laboratory at Princeton, NJ for testing of F1 and F2 larval progeny of field collected female moths.

At the time adult males were collected from pheromone traps for use in vial tests, adult females were also collected at night from the same fields. Moths were spotted using a fluorescent lantern and caught with a hand held net. Eggs from these moths were shipped to Princeton, NJ and the ensuing F1 larvae were used to initiate contact toxicity studies using the petri dishes that had been treated in the field with cypermethrin as described above. Similar petri dish tests were done with F2 progeny. Additionally, 440 larvae were held until the third instar (22$\pm$2mg) and treated with a topical applicator to obtain dose-mortality data for older larvae from the same population. Another group of 440 larvae was allowed to pupate and used to propagate the colony. Larvae less than 10h from eclosion were designated as neonate first instars for this study, while those between 24h and 48h posteclosion were designated as older first instars. Mortality data were subjected to a computerized SAS probit analysis (SAS 1982).

RESULTS AND DISCUSSION

Table 1 lists field application rates (lb ai/A) equivalent to concentrations of cypermethrin used in the AVT. This information can serve as a guide for those using the AVT in a monitoring program to indicate the relationship between the dilutions and equivalent field rates.

TABLE 1. Calculated Equivalent Rates of Cypermethrin (2.5EC) Expressed in Terms of Field Application Rates (lb ai/A), Concentration (ppm) and ug/vial Used in AVT and MAVT.

Rate lb ai/A	PPM	Ug/Vial
0.0018	10	5.0
0.0036	20	10.0
0.01	55	27.5
0.02	110	55.0
0.04	220	110.0
0.06	330	165.0
0.08	440	220.0
0.10	550	275.0

A comparison of LC_{50} values (Table 2) from AVT bioassays with technical cypermehtrin (ug/vial) with those from MAVT bioassays using the EC formulation of cypermethrin at equivalent field rates (lb ai/A) were statistically similar for moths obtained from mid to late August. Although previous AVT bioassays indicated susceptibility changes can occur from one sampling period to another (Riley 1988, Staetz 1989), our data indicate that groups of insects tested during this two week period were equally susceptible to cypermethrin. These results also show that the mortality caused by a technical cypermethrin concentration of 22-26 ug/vial was similar to that caused by an EC formulation of cypermethrin applied at a field rate of .0053-.0057 lb ai/A. Additionally, an AVT dose of 163-224 ug/vial, required for 90% mortality, was comparable in activity to a field use rate equivalent to 0.067-0.077 lb ai/A. This result suggests that even though concentrations of cypermethrin used in the AVT may be appropriate to show susceptibility changes from one population to another, they are too low to be used as a potential predictive test for field rate performance. This is particularly true if population levels are not taken into account. Based on the comparative treatment rates (Table 1) and the results of bioassays (Table 2), the AVT rates required to cause mortality similar to equivalent field application rates would be approximately 165 ug/vial for 0.06 lb ai/A, 220 ug/vial for 0.08 lb ai/A, and 275 ug/vial for 0.10 lb ai/A.

TABLE 2. Mortality of Tobacco Budworm Adult Males in AVT and MAVT Bioassays Using Vials Treated with Different Concentrations of Technical or EC formulations Cypermethrin.[a]

Moth Collection Date	MAVT n	Rate (lb ai/A)	LC 95% C.I.	Slope ± SE	AVT n	(ug/vial)	LC 95% C.I.	Slope ± SE
LC50								
8/16	538	0.005	0.004-0.008	1.17 ± 0.93	500	26.2	15.35-44.24	1.38 ± 0.85
8/27	500	0.006	0.004-0.008	1.14 ± 0.94	500	22.5	10.43-45.17	1.49 ± 0.82
LC90								
8/16	538	0.067	0.046-0.117	1.17 ± 0.93	500	224	111-859	1.38 ± 0.85
8/27	500	0.077	0.038-0.238	1.14 ± 0.94	500	163	72-1283	1.49 ± 0.82

[a]Technical and EC formulations were both diluted with acetone prior to treatment of vials.

Results of tests with moths exposed to petri dishes treated with aerial or ground applications of cypermethrin (2.5 EC) showed that the field use rates provided 89-97% mortality. Application of cypermethrin at rates of 0.06 and 0.08 lb ai/A killed 94 and 95%, respectively, of moths when petri dishes were treated by ground application and 89 and 92%, respectively, when dishes were treated by aerial application in this population. Mortality in AVT bioassays of cypermethrin were similar to the LC_{90} values obtained (Table 2) for the MAVT. This provides evidence that the MAVT gave adult mortality results in this population which would be expected for a comparable field spray rate and that currently used commercial field rates can cause high mortality to adults that are contacted directly by sprays or come in contact with residual insecticide.

LC_{50} and LC_{90} values for field collected moths, older first instar larvae (0-10 and 24-48 hours old, respectively), and third instar larvae from the same population are shown in Table 3. First instar larvae were exposed to vials treated with field use rates using the same method as for adults, and third instars were treated topically. A very high correlation (r=.99) was obtained for results of tests with adult and neonate (0-10 hours of age) larvae indicating similar susceptibility to cypermethrin when exposed to cypermethrin 2.5 EC at field use rates. However, after larvae reached 24-48 hours of age, LC (50-90) values indicate it takes 2.5 times more active ingredient to produce the same mortality as that of neonates and adults. These results could be related to increases in metabolic activity which occurs as larvae mature or changes in the ability to penetrate larval integument (McCaffery et al. 1989). Thus, AVT results may be used to estimate expected mortality in newly hatched larvae but not for older first and third instars. Results of topical tests

TABLE 3. Mortality of Tobacco Budworm Adult Males Treated with Cypermethrin Using MAVT, AVT and Topical Test Procedures.

Moth Bioassay				Larval Bioassay								
				Field Treated Petri Dishes								
MAVT (lb ai/A) (n=500)[c]		AVT (ug/vial) (N=500)[c]		Neonate[a] F1 and F2 (lb ai/A) (n=393)[c]			First Instar[b] F1 and F2 (lb ai/A) (n=226)[c]			Topical Application Third Instar F1 (ug/gram) (n=440)[c]		
LC^{50}	95% C.I.	LC^{50}	95% C.I.	LC^{50}	95% C.I.	Slope±SE	LC^{50}	95% C.I.	Slope±SE	LC^{50}	95% C.I.	Slope±SE
0.006	0.004-0.008	22.5	10.4-54.2	0.006	0.005-0.007	1.55±0.80	0.015	0.011-0.002	1.60±0.79	14.4	2.7-26.4	1.44±0.03
LC^{90}	95% C.I.	LC^{90}	95% C.I.	LC^{90}	95% C.I.		LC^{90}	95% C.I.		LC^{90}	95% C.I.	
0.077	0.038-0.237	163	71.5-1287	0.039	0.027-0.064		0.095	0.057-0.21		112.4	57.2-1329.4	

[a] Unfed larvae (Neonate) 1-10 hours old
[b] Unfed larvae 24-48 hours old
[c] Number tested excluding controls

with third instar larvae indicate a slightly higher LC_{50} and a lower LC_{90} for the population tested in this study compared to those reported by Staetz et al. (1989) for another population in the same area in 1988.

Additionally, our results confirm a number of previous observations which indicate that applications of pyrethroids and other insecticides to newly hatched larvae will provide the most effective control. This reinforces the established management concept that pyrethroid sprays should be timed in such a manner to ensure maximum contact with newly-hatched tobacco budworm larvae.

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