# Field Monitoring for Insecticide Resistance in Beet Armyworm (Lepidoptera: Noctuidae)

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ABSTRACT A technique for monitoring insecticide resistance in field populations of beet armyworm, Spodoptera exigua (Hübner), was developed and compared with conventional topical bioassay. Insecticides were incorporated into the adhesive of a pheromone trap, thereby combining insect collection with insecticide application. This attractant trap technique (ATT) provided stable LC50's with low control mortality when traps were incubated at 21°C for 30-36 h after insects were captured. L.C.so's of a laboratory colony tested with fenvalerate, permethrin, and methomyl were 95.6, 142, and 11.5 µg/g of adhesive, respectively. Slopes of probit regression for males were similar for topical and ATT bioassay, indicating a parallel response of the insect to the two methods. When larval and adult susceptibility were compared with topical application bioassay, toxicity differed less than 3-fold (adult LD<sub>50</sub> > larval LD<sub>50</sub>) based upon body weight, and slopes of the probit lines were nearly equal for fenvalerate and permethrin (pyrethroids). Larvae exposed to methomyl (a carbamate) were more tolerant (20 times at LD<sub>50</sub>) than adult males, and the slope of their probit regression was lower. Use of the ATT was further evaluated by monitoring a fresh market tomato field in southern California. Resistance ratios at LCso ranged from 3.0 to 7.3 for fenvalerate, 1.5 to 2.5 for permethrin, and 7.1 to 33.0 for methomyl.

KEY WORDS Insecta, insecticide resistance, resistance monitoring, Spodoptera exigua

BIOCHEMICAL AND BIOASSAY METHODS are the primary means of testing for insecticide resistance. Recent interest has focused on adapting these techniques to provide rapid, field-based monitoring of insecticide resistance. Biochemical techniques are advantageous because they test for activity that is directly linked to a resistance mechanism (Sawicki et al. 1978, Pasteur & Georghiou 1981, Miyata 1986). Unfortunately, such techniques are currently restricted to the testing of esterase activity and insensitive acetylcholinesterase, which limits their use to the organophosphate and carbamate pesticides. Conventional bioassays by topical application have been standardized for many species by the Food and Agriculture Organization of the United Nations Panel of Experts on Insecticide Resistance (Busvine 1980), but historically these tests have not been suitable for simple and routine monitoring. Topical bioassays with a discriminating dose require fewer insects for testing, but also are cumbersome for routine resistance monitoring. Because of the technical difficulties of topical application, World Health Organization (WHO) test kits for detection of resistance use insecticide-treated papers and solutions for mosquitoes (WHO 1976). For phytophagous arthropods, the recent development of a residual bioassay and diagnostic dose for Tetranychus spp. spider mites has been effective (Dennehy et al. 1987). The rapid increase in numbers of resistant arthropod species since the introduction of the synthetic organic insecticides (Georghiou 1986) underscores the need for inexpensive field

techniques that can detect resistance. The continued development of rapid monitoring techniques that are applicable to a range of species in the field is desirable.

Recently, several techniques have been developed that combine insect collection with insecticide application. For Lepidoptera, pheromone traps have been used with basic bioassay procedures (Riedl et al. 1985, Haynes et al. 1987). The technique of Riedl et al. (1985) requires topical application in the laboratory after insect capture in the pheromone trap. Haynes et al. (1987) eliminated the need for topical application by incorporating the insecticide into the insect trapping adhesive, thereby combining insect collection with insecticide application. Given knowledge of the concentration-mortality response of resistant and susceptible populations, selection of a discriminating dose further increases the usefulness of the technique in the field.

In this study, the attractant trap technique (ATT) developed by Haynes et al. (1987) was used to monitor insecticide resistance of the beet armyworm, Spodoptera exigua (Hübner). Beet armyworm feeds on various agricultural crops, including cotton and many vegetables, that are heavily treated with insecticides. Considerable variation in susceptibility to insecticides has been documented. Yoshida & Parrella (1987) found reduced levels of susceptibility to methomyl for a Florida strain compared with a California strain. Meinke & Ware (1978) did not find resistance in three Arizona strains

tested with methomyl. Chaufaux & Ferron (1986) reported resistance to the pyrethroid deltamethrin in Central America. In selection experiments with methyl parathion, Alava & Lagunes Tejeda (1976) found that beet armyworm developed resistance to malathion, endrin, and methomyl. Therefore, this insect is a good candidate to study resistance patterns in a polyphagous lepidopteran over time.

### Materials and Methods

Beet armyworm were obtained from two laboratory colonies that were maintained on artificial diet (Patana 1969) at 27 ± 1°C and a photoperiod of 16:8 (L:D) in our laboratory. One colony (CA colony) was established from collections made at Orange County, Calif., in 1982. The second colony (AZ colony) was obtained in 1988 from the Western Cotton Research Laboratory (USDA-ARS, Phoenix, Ariz.). The AZ colony has been in culture without insecticide pressure or introduction of field material for more than 20 yr at the USDA facility. At least 100 adults were allowed to mate and oviposit in a waxed cardboard ice cream carton (3.8 liters) provided with 10 ml of a 20% honey solution. Beet armyworm also were sampled directly with the ATT in a fresh market tomato field in Orange County from fall 1986 to spring 1987.

Two pyrethroids, permethrin (94.3%, FMC Corporation, Philadelphia) and fenvalerate (94%, E. I. du Pont de Nemours & Company, Wilmington, Del.), and a carbamate, methomyl (98%, Du Pont), were tested with the ATT. Technical grade pyrethroids were serially diluted in 9:1 hexane/ethanol to add to the insect trapping adhesive. Acetone was used to dilute methomyl because of its insolubility in 9:1 hexane/ethanol. Solutions were mixed into insect adhesive (Tanglefoot Company, Grand Rapids, Mich.) to obtain insecticide mixtures of 10, 40, 80, 160, 320, 500, 1,000, 2,000, and 4,000 µg (AI)/g adhesive. Incubation temperature and amount of adhesive in the range found satisfactory by Haynes et al. (1987) were used.

Approximately 5.5 g treated adhesive was spread evenly on wax-coated, pressed cardboard inserts (325 cm<sup>2</sup>) cut to fit into a Pherocon 1C pheromone trap (Trece, Salinas, Calif.). This procedure resulted in surfaces treated with insecticide of 0.17, 0.68, 1.35, 2.71, 5.42, 8.46, 16.92, 33.85, and 67.69  $\mu g/cm^2$ . These inserts were secured with paper clips to the bottom of the traps for use in the field or were used directly in laboratory studies. For ATT laboratory bioassays, moths were placed ventral side down onto the adhesive. For ATT field bioassays, a commercially available beet armyworm pheromone lure (Trece) was attached to the top of the trap. After one night's collection, the inserts were taken to the laboratory. For laboratory and field studies, the inserts were incubated at room temperature (21 ± 2°C) and about 95% RH. Humidity was stabilized by placing the inserts in a Plexiglas container (60 by 49 by 34 cm) with distilled water at the bottom; paper toweling was draped over the sides to serve as a wick. The inserts were held in place in wooden racks (33 by 38 by 24 cm) and spaced 2.4 cm apart. The Plexiglas container also was used to transport the inserts from the field to the laboratory.

For topical application bioassays, all insecticides were serially diluted in acetone. An Arnold (Type LV 65) microapplicator (Burkard, Rickmansworth, Herts, England) was used to deliver 0.5 and 1.0  $\mu$ l to the thoracic dorsum of larvae and adults, respectively. Each larva was held in a 30-ml plastic cup filled with diet; adults were incubated in groups of  $\leq$ 20 moths in oviposition containers. Incubation temperatures and light regimes were the same as those used for the insect colonies.

Laboratory Studies. For the ATT bioassay, we evaluated effects of incubation time and moth age on toxicity. To determine the optimal time of incubation of the inserts, bioassays were done on the CA colony and the field population collected in 1986 from Orange County. Six concentrations of fenvalerate and eight concentrations of methomyl were used for the CA colony bioassay with 25 3-dold insects per concentration. For bioassays of the field population, seven concentrations of fenvalerate and eight concentrations of methomyl were used with at least 31 insects per concentration. Mortality was observed after incubation times of 12, 30, 36, 54, and 78 h. Data at each incubation time were analyzed with Polo-PC with correction of control mortality (LeOra Software 1987). LCso's and control mortality were compared over incubation times. To determine the effect of moth age, four age groups of CA colony males were treated at 1 to 2, 3 to 4, and 5 to 6 d after emergence. Treatments were controls without insecticide and concentrations of 10 and 80 µg (AI)/g adhesive for methomyl and fenvalerate, respectively. Each treatment was replicated four times on separate inserts with 20 insects per insert. Mortality was recorded after 30 h. Age comparisons were made by an analysis of variance (ANOVA; SAS Institute 1985) and Duncan's (1955) multiple range test applied to proportional mortality after the arcsine square root transformation.

If deterioration of the toxicant in the traps placed in the field occurred at a greater rate than in the laboratory, field mortality data could not be compared with data for the laboratory colony to determine whether resistance was present in the field collection. Therefore, effects of chemical aging in the field were simulated by comparing inserts aged for 0 and 24 h in a greenhouse before placing CA colony moths on the adhesive. The inserts were treated with methomyl and fenvalerate at 10 and 80 µg (AI)/g of adhesive, respectively. Controls without insecticide were included. Each concentration for each aging period was replicated four times on separate inserts; 20 male moths per insert were tested. Mortality was recorded at 30 h. An ANOVA was used to detect changes in mortality

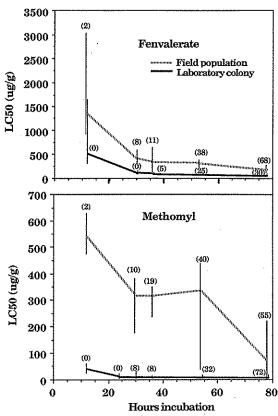


Fig. 1. Effect of incubation time on toxicity of ATT bioassay for fenvalerate and methomyl. Vertical lines are 95% fiducial limits; values in parentheses are percentage of control mortality.

due to aging after transformation of data by the arcsine square root.

Toxicity to CA and AZ larvae and adults was compared by topical application bioassays. For each colony, seven concentrations for each pyrethroid were applied to third instars (wt, 3–5 mg) and male and female 3-d-old adults (wt, 30–50 mg). Nine concentrations of methomyl were tested on third instars and adults. Twenty to 30 insects per concentration were tested. Each bioassay was replicated three times; data were pooled before probit analysis (LeOra Software 1987). Mortality was recorded 24 h after application. ATT bioassays with males and females were done with six concentrations per chemical. Mortality was recorded 30 h after application.

Field Studies. Field bioassays were done by placing the treated inserts in pheromone traps spaced ≥20 m apart in a tomato field. Seven to eight concentrations of each chemical were replicated with at least four inserts. The chemicals were tested separately in a randomized complete block design on three consecutive nights. Inserts were placed in the field in the late afternoon, retrieved the next morning before 0900 hours (temperature at retrieval was ≤21°C), and taken to the laboratory. Because

Table 1. Effect of age of CA colony male S. exigua on mortality in attractant trap toxicity tests

μg/g	MSE	Age, days <sup>a</sup>						
		1-2		3-4		5–6		
		n	Propor- tion	n	Propor- tion	n	Propor- tion	
			Fenva	lerate	;			
0	0.014	80	0.013a	80	0.038a	80	0.125b	
80	0.019	79	0.190a	78	0.215a	80	0.538b	
			Meth	omyl				
0	0.027	80	0.013a	80	0.038a	80	0.113a	
10	0.031	80	0.100a	80	0.163a	80	0.575b	

Means within a row followed by the same letter are not significantly different (P > 0.05; Duncan's [1955] multiple range test after arcsine square root transformation).

<sup>a</sup> MSE, Mean square error; n, total number insects tested; Proportion, proportion dead at 30 h incubation.

optimal male response to the pheromone has been estimated to occur about 8 to 10 h into scotophase (Shorey & Gaston 1965), mortality was recorded 38 to 40 h after scotophase began. This time corresponded with 30 h incubation from average time of capture. Data from the inserts of each concentration were pooled for probit analysis (LeOra Software 1987). Five bioassays of each chemical from August to October 1986 and one in April 1987 were used to assess the field performance of the technique and compare susceptibility levels over time. Resistance ratios at LC<sub>50</sub> or LC<sub>50</sub> were calculated as LC field population ÷ LC CA colony males.

## Results and Discussion

Laboratory Studies. For ATT bioassays, low control mortality at 30 h and a stabilization of LC<sub>50</sub>'s between 30 and 54 h incubation for methomyl and fenvalerate were evident for laboratory and field strains (Fig. 1). Beyond 36 h, control mortality increased dramatically in the field collection. Therefore, a standard incubation period of 30 h was selected for further ATT bioassays.

The age of moths for the ATT bioassays affected mortality after a 30-h incubation period at 21°C (Table 1). The 5- to 6-d-old moths had greater mortality (P < 0.05, Table 1) than younger moths for the fenvalerate control and the 80 and 10  $\mu$ g/g concentration of fenvalerate and methomyl, respectively. Because Shorey et al. (1968) found that beet armyworm response to pheromone peaked between 2 and 5 d after pupal emergence, the effect (i.e., greater mortality) of older moths may be diluted by the greater numbers of younger moths caught in the trap. However, we recommend not counting moths that lack hairs on the thoracic dorsum; this condition was generally associated with moths older than 4 d (M.J.B., unpublished data). The use of this strategy avoids potential shifts in LCs due to age fluctuations of the field population that are not indicative of resistance.

Table 2. Comparisons of toxicity in topical and attractant trap bioassays on CA colony adults and larvae of S. exigua

		Topical	Attractant Trap		
	రేదే	<b>φ</b>	Larvae	<b>ී</b>	오오
Fenvalerate					
n	440	440	510	360	360
Mortality <sup>a</sup>	0.0	1.4	1.1	0.0	5.0
Weight <sup>b</sup>	35.19	52.15	4.20	40.16	63.64
Slope ± SE	$1.99 \pm 0.16$	$1.86 \pm 0.16$	$1.73 \pm 0.17$	$1.83 \pm 0.30$	$1.84 \pm 0.57$
LD or LC <sub>50</sub> c	6.5	6.2	3.1	95.6	187.6
95% FL <sup>d</sup>	4.8-8.6	4.3-8.7	2.2-4.3	45.1-173.2	68.0-325.4
LD or LC <sub>90</sub> c	28.7	30.0	16.8	478.7	934.6
95% FL <sup>d</sup>	19.7-51.3	18.8-66.8	10.4-40.1	242.4-2,990	467-27,932
Pe	0.15	0.074	0.21	0.24	0.30
Permethrin					
n	462	431	540	339	381
Mortality <sup>a</sup>	1.3	0.0	0.0	1.9	7.9
Weight <sup>b</sup>	35.59	55.42	3.86	40.97	61.24
Slope ± SE	$2.07 \pm 0.17$	$2.14' \pm 0.18$	$2.15 \pm 0.16$	$2.39 \pm 0.31$	$2.73 \pm 0.33$
LD or LC50c	5.2	2.9	1.7	142.1	120.4
95% FL <sup>d</sup>	4.4-6.1	2.2-3.6	0.8 - 2.7	113.3-172.2	96.4-144.3
LD or LC90c	21.7	11.4	6.9	490	355.4
95% FL <sup>d</sup>	17.2-29.5	8.2-18.8	3.8-31.1	375.6-733.6	286.8-480.5
$P^e$	0.47	0.24	0.85	0.60	0.75
Methomyl					
$\eta$	363	341	685	360	370
Mortality®	4.0	0.0	0.0	8.0	6.7
Weight <sup>b</sup>	40.61	61.90	4.18	40.61	61.90
Slope ± SE	$4.02 \pm 0.55$	$4.95 \pm 0.85$	$0.80 \pm 0.08$	$3.55 \pm 0.92$	$2.12 \pm 0.45$
LD or LC50c	9.1	7.2	186.2	11.5	13.7
95% FL <sup>d</sup>	7.5-110	6.0-8.6	139.2-259.9	6.9-14.8	4.5 - 22
LD or LC90°	18.9	13.0	7,288	26.5	55.1
95% FL <sup>d</sup>	14.9-27	10.5-18.7	3,522-20,766	20.5-46.5	35.5-135.5
$P^e$	0.96	0.93	0.75	0.82	0.36

<sup>&</sup>lt;sup>a</sup> Mortality, percentage of control mortality.

After 30 h incubation at 21°C, no differences in mortality were detected for insects on inserts aged for 0 or 24 h at all concentrations and the control (F < 0.19; df = 1, 6; P > 0.20 for each ANOVA).For 80 µg of fenvalerate per gram of adhesive, the proportion dead was 0.41 at 0 h and 0.44 at 24 h. For 10  $\mu$ g of methomyl per gram of adhesive, the proportion dead was 0.19 at 0 and 24 h. The proportion dead in all controls was <0.06. To further reduce variation in mortality caused by changing field conditions, inserts were left in the field for <24 h. They were placed in the field after 1500 hours to minimize exposure to sunlight and retrieved before 0900 hours the next morning. This protocol further reduced the possibility that deterioration of the toxicant differed in the field and laboratory and supported comparison of mortality data for the laboratory colonies with data for field populations.

Slopes of the probit regressions for the ATT and conventional topical bioassay were compared to determine if the two methods gave similar ratings across a range of concentrations. Slopes obtained by the two procedures were similar for each chemical, indicating that responses of the CA colony to the test procedures were parallel (Table 2). In addition,  $P(\chi^2)$  values indicated that data for both methods fit the probit model equally well (Table 2).

When responses of AZ colony (>20 yr old) and CA colony (6 yr old) were compared at LD<sub>50</sub>, toxicity of each insecticide differed less than 2-fold for adults and larvae. Slopes of the AZ colony were generally higher than those for the CA colony for topical application and ATT, but never exceeded 4.5. The higher slopes resulted in up to five times lower LD<sub>90</sub>'s (Table 2 for CA colony; AZ colony data not presented). The higher slopes were the only indication that the older AZ colony may be more genetically homogenous in susceptibility to these insecticides. The difference may reflect greater inbreeding in the older AZ colony (assuming susceptibility is linked to physiological efficiency) or strain differences between California and Arizona beet armyworm populations. Because of these similarities in susceptibility despite a 14-yr difference in duration of laboratory culture, our use of the CA insects as a susceptible reference appears to be justified. In addition, the CA colony was chosen as the reference for the field monitoring study

<sup>&</sup>lt;sup>b</sup> Weight, mg pretest body weight.

<sup>&</sup>lt;sup>c</sup> Based on μg/g body weight for topicals (LD) and μg/g of adhesive for attractant trap (LC).

 $<sup>^</sup>d$  FL, Fiducial limits for the preceding lethality estimate.  $^e$  Probability of  $\chi^2$  testing the hypothesis that the data does not differ from probit model.

Table 3. Attractant trap bioassay of S. exigua caught in a tomatoe field in Orange County, Calif.

Date <sup>a</sup>	n	% Control mortality	Slope ± SE	LC <sub>50</sub> (95% FL) <sup>b</sup>	RR <sub>50</sub> c	LC <sub>90</sub> (95% FL) <sup>b</sup>		$RR_{90}^c$	$P^d$
Fenvalerate									
8/28	195	15.0	$1.84 \pm 0.42$	469 (286-754)	4.9	2,833 (	1,272-9,148)	4.9	0.68
9/4	390	8.0	$2.46 \pm 0.36$	418 (290-592)	4.4	1,388	(880-4,053)	2.9	0.20
9/25	576	11.1	$1.85 \pm 0.24$	362 (177-640)	3.8	1,780	(908-12,744)	. 3.7	0.01
10/15	138	6.7	$1.71 \pm 0.43$	289 (144-466)	3.0	1,629	(880-7,627)	3.4	0.40
11/6	644	10.0	$1.72 \pm 0.20$	395 (230-580)	4.1	2,198 (	1,323-6,204)	4.6	0.05
4/26	390	2.6	$1.63 \pm 0.17$	700 (402-1,239)	7.3	4,301 (	2,120-20,408)	9.0	0.01
Permethrin									
8/19	135	15.0	$6.61 \pm 2.22$	338 (260-450)	2.4	529	(412-1,381)	1.1	0.75
9/3	459	4.0	$3.14 \pm 0.41$	263 (157-346)	1.9	673	(511-1,110)	1.4	0.15
9/26	278	17.9	$2.31 \pm 0.36$	283 (149-431)	2.0	1,016	(635-2,793)	2.1	0.10
10/16	119	7.1	$1.98 \pm 0.73$	218 (49-359)	1.5	965	(606-3,197)	2.0	0.55
11/5	293	6.7	$3.63 \pm 0.73$	264 (142-349)	1.9	595	(447-1,194)	1.2	0.25
4/25	329	9.5	$4.05 \pm 0.63$	351 (287-410)	2.5	727	(604-970)	1.5	0.75
Methomyl									
8/29	182	15.8	$7.86 \pm 2.28$	356 (265-402)	31.0	518	(458-706)	19.5	0.98
9/5	438	10.3	$7.33 \pm 1.69$	335 (288-368)	29.1	500	(442-659)	18.9	0.90
9/24	450	10.3	$5.10 \pm 1.07$	224 (159-313)	19.5	400	(294-1,310)	15.1	0.90
10/17	71	14.3	$5.50 \pm 2.20$	110 (76–162)	9.6	188	(134-600)	7.0	0.99
11/4	538	9.3	$6.18 \pm 0.91$	379 (325-432)	33.0	611	(519-834)	23.1	0.30
4/24	271	0.0	$2.52 \pm 0.26$	82 (66-100)	7.1	265	(207-368)	10.0	0.55

<sup>a</sup> August 1986 through April 1987.

b Lethal concentration in µg/g of adhesive at 50 and 90% mortality, respectively; followed by 95% fiducial limits.

d Probability of  $\chi^2$  testing the hypothesis that the data does not differ from probit model.

because it probably represented a less inbred line and was established from the location of the field monitoring study.

Comparisons of topical toxicities for the pyrethroids, taking body weight into account, indicated that larvae were more susceptible than adults by  $\leq$  3-fold at LD<sub>50</sub> (Table 2). A change in position of the probit lines was largely responsible for this difference. Nearly equal slopes of the adults and larvae indicated a parallel response to both pyrethroids

In contrast, larvae were 20 and 26 times more tolerant of methomyl at LD<sub>50</sub> than male and female beet armyworm, respectively (Table 2). The nearly flat slope for the larvae reflected the relatively low change in response per unit concentration for larvae compared with adults. Although this difference may be a result of higher mixed-function oxidase activity in larval Lepidoptera compared with nectar feeding adults (Krieger & Wilkinson 1969, Wilkinson 1983), the same relationship of mixed-function oxidase activity between larva and adult for resistant strains is not well understood. Dittrich et al. (1980) found that resistance to monocrotophos (organophosphate) was primarily due to mixedfunction oxidase activity in a closely related species, Spodoptera littoralis (Boisduval), in which larvae and adults displayed resistance. Unfortunately, direct comparison of degree of adult and larval susceptibility was difficult in their study because modes of application differed between stages. Additional investigation on the larval and adult susceptibility in resistant strains of beet armyworm is warranted because larvae are usually the main target of control with insecticides. If the same rate of decrease of toxicity for adults and larvae for resistant and susceptible strains is evident, the use of males as an indicator of resistance development in the larvae would still produce reliable estimates. The need to compare larval and adult susceptibility in strains resistant to fenvalerate and permethrin is not as critical because of the approximately equal slopes of the probit lines for adults and larvae (Table 2).

Field Studies. The ATT performed well as a bioassay for monitoring field population susceptibility to the chemicals. Control mortalities were generally higher than in the laboratory studies (Tables 2 and 3), but did not substantially reduce the effectiveness of the test. Retrieving the adhesive trap inserts before temperatures increased above 21°C and humidity decreased and placing them in a constant 21°C and high-humidity environment were essential to maintain a range of 0 to 15% control mortality (Table 3). Haynes et al. (1987), monitoring in a desert region, retrieved traps at 0600 hours to obtain similar control mortality. ATT effectiveness was indicated by the good fit to the probit lines for permethrin, for which the populations can be assumed to be near homogenous because of the susceptibility levels similar to those of the CA colony (range of resistance ratios = 1.1 to 2.5, Table 3).

Higher resistance ratios at LC<sub>50</sub> and LC<sub>90</sub> (three to five times different from the CA colony) were evident for fenvalerate in 1986, with an increase in April 1987 to 7.3 at LC<sub>50</sub> and 9.0 at LC<sub>90</sub> (Table

c Resistance ratios for preceding LC value based upon comparison with attractant trap bioassay of CA colony males (Table 2).

3). These elevated resistance ratios, in contrast to those of permethrin, were probably related to the grower's pesticide program that included fenvalerate but not permethrin. Applications averaged one per month during the spring but increased to one every 14 to 21 d in response to increased insect pressure in fall 1986. Alternatively, the differences were relatively small and might be explained by the natural variation in the field or laboratory populations (95% FL are one indicator; Tables 2 and 3).

In contrast, tests with methomyl generated markedly higher resistance ratios than the pyrethroids in fall 1986 (Table 3). The small sample size on October 17 may explain why results differed from the other sampling dates before and after. In the following spring collection, resistance ratios at the LC<sub>50</sub> and LC<sub>90</sub> decreased to 7.1 and 10.0, respectively. Accompanying this decrease was a reduced slope (Table 3). The low slope on the spring date (2.52 ± 0.26) compared with that of the previous fall and that of the CA colony in ATT bioassay on males (Table 2), as well as intermediate LC<sub>50</sub>'s and LC90's, suggest a heterogenous population of susceptible and resistant individuals. The steeper slopes for the fall compared with the spring test, coupled with the high LC50's and LC90's during the fall, suggest an increase in the proportion of resistant individuals in the population (toward resistance homogeneity) through the season.

We suspect that the frequency of resistant individuals depends upon seasonal chemical selection pressure, overwintering mortality, spring migration, or a combination of these factors. Methomyl was applied on vegetable plantings adjacent to the monitoring site and is used frequently in Orange County from the late spring through the fall seasons. The high resistance ratios for methomyl in the fall observed in this study coincided with high methomyl use. From mid-December through March, populations of beet armyworm are generally low as a result of slow development in cool temperatures and of winter mortality (generally <1 moth per trap per week) (Trumble & Baker 1984). If reduced fitness of resistant individuals compared with those that are susceptible occurs during the winter, dilution of resistance in the spring would result by migration of susceptible insects from weed hosts on hillsides in the area.

The sensitivity of this bioassay technique to detect fluctuations in resistance to methomyl was encouraging, despite the differences in larval and adult susceptibility found in the laboratory colonies (Table 2). Eurther comparisons of larval and adult susceptibility are warranted for strains with different histories of selection with methomyl to determine if resistance development is expressed in larvae and adults by elevated mixed-function oxidase activity or other mechanisms. The field results indicated that resistance expression occurs to some degree in adults. Comparisons of larval and adult susceptibility for resistant populations would define

this relationship and increase the reliability of this resistance monitoring technique for estimating resistance in the larvae.

Field data from the ATT revealed a potential management problem of the beet armyworm with methomyl. Knowledge of the level of resistance present in a population before insecticide use can reduce the numbers of ineffective applications. The resulting benefits of reduced pest control costs, environmental contamination, and human exposure to pesticides are desirable. This technique allows monitoring of susceptibility levels over time, as well as in different locations that have differing histories of insecticide selection pressure. Such information is critical to developing effective resistance management programs. Estimation of a diagnostic or discriminating dose for the ATT would improve the practicality of assessing resistance of the beet armyworm in the field.

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### References Cited

Alava, D. & A. Lagunes Tejeda. 1976. Resistencia cruzada a varios tipos de insecticidas despues de producir resistencia a paration metilico en Spodoptera exigua (Hübner). Folia Entomol. Mex. 36: 77-78.

Busvine, J. R. 1980. Recommended methods for measurement of pest resistance to pesticides. FAO Plant Production and Protection Paper 21. United Nations Food and Agriculture Organization, Rome.

Chaufaux, J. & P. Ferron. 1986. Sensibilite differente de deux populations de Spodoptera exigua Hüb. (Lepid., Noctuidae) aux baculovirus et aux pyrethrinoides de synthese. Agronomie 6: 99-104.

Dennehy, T. J., E. E. Grafton-Cardwell, J. Granett & K. Barbour. 1987. Practitioner-assessable bioassay for detection of dicofol resistance in spider mites (Acari: Tetranychidae). J. Econ. Entomol. 80: 998– 1003.

Dittrich, V., N. Luetkemeier & G. Voss. 1980. OPresistance in Spodoptera littoralis: inheritance, larval and imaginal expression, and consequences for control. J. Econ. Entomol. 73: 356–362.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics 11: 1-42.

Georghiou, G. P. 1986. The magnitude of the resistance problem, pp. 14-43. In Committee on strategies for the management of pesticide resistant pest populations. Pesticide resistance, strategies and tactics for management. National Academy, Washington, D.C.

- Haynes, K. F., T. A. Miller, R. T. Staten, W.-G. Li & T. C. Baker. 1987. Pheromone trap for monitoring insecticide resistance in the pink bollworm moth (Lepidoptera: Gelechiidae): new tool for resistance management. Environ. Entomol. 16: 84-89.
- Krieger, R. I. & C. F. Wilkinson. 1969. Microsomal mixed-function oxidases in insects. I. Localization and properties of an enzyme system effecting aldrin epoxidation in larvae of the southern armyworm (*Pro*denia eridania). Biochem. Pharmacol. 18: 1403–1415.
- LeOra Software. 1987. Polo-PC: a user's guide to probit or logit analysis. LeOra Software, Berkeley, Calif.
- Meinke, L. J. & G. W. Ware. 1978. Tolerance of three beet armyworm strains in Arizona to methomyl. J. Econ. Entomol. 71: 645–646.
- Miyata, T. 1986. Detection and monitoring methods for resistance in arthropods based on biochemical characteristics, pp. 99-116. In G. P. Georghiou & T. Saito [eds.], Pest resistance to pesticides. Plenum, New York.
- Pasteur, N. & G. P. Georghiou. 1981. Filter paper test for rapid determination of phenotypes with high esterase activity in organophosphate resistant mosquitoes. Mosq. News 41: 181–183.
- Patana, R. 1969. Rearing cotton insects in the laboratory. USDA Production Research Report 108, Washington, D.C.
- Riedl, H., A. Seaman & F. Henrie. 1985. Monitoring susceptibility to azinphosmethyl in field populations of the codling moth (Lepidoptera: Tortricidae) with pheromone traps. J. Econ. Entomol. 78: 692-699.
- SAS Institute. 1985. SAS user's guide: statistics, version 5 ed. SAS Institute, Cary, N.C.
- Sawicki, R. M., A. L. Devonshire, A. D. Rice, G. D.

- Moores, S. M. Petzing & A. Cameron. 1978. The detection and distribution of organophosphorus and carbamate insecticide-resistant *Myzus persicae* (Sulz.) in Britain in 1976. Pestic. Sci. 9: 189–201.
- Shorey, H. H. & L. K. Gaston. 1965. Sex pheromones of noctuid moths. V. Circadian rhythm of pheromone-responsiveness in males of Autographa californica, Heliothis virescens, Spodoptera exigua, and Trichoplusia ni (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 58: 597-600.
- Shorey, H. H., K. L. Morin & L. K. Gaston. 1968. Sex pheromones of noctuid moths. XV. Timing of development of pheromone-responsiveness and other indicators of reproductive age in males of eight species. Ann. Entomol. Soc. Am. 61: 857-861.
- Trumble, J. T. & T. C. Baker. 1984. Flight phenology and pheromone trapping of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) in southern coastal California. Environ. Entomol. 13: 1278-1282.
- Wilkinson, C. F. 1983. Role of mixed-function oxidases in insecticide resistance, pp. 175-204. In G. P. Georghiou & T. Saito [eds.], Pest resistance to pesticides. Plenum, New York.
- World Health Organization. 1976. Resistance of vectors and reservoirs of disease to pesticides, 22nd report of the WHO expert committee on insecticides. WHO Technical Report Series 585, Geneva, Switzerland.
- Yoshida, H. A. & M. P. Parrella. 1987. The beet armyworm in floricultural crops. Calif. Agric. 41(3,4): 13-15.

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