Beet Armyworm Resistance to Fenvalerate and Methomyl: Resistance Variation and Insecticide Synergism[1]

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ABSTRACT Beet armyworm, *Spodoptera exigua* (Hübner), strains, originating from field-collected populations resistant to fenvalerate and methomyl, were selected with these insecticides in the laboratory. Resistance increased to the insecticide used as the selecting agent. The fenvalerate resistance ratio increased from 7.7 to 47.6, and the methomyl resistance ratio increased from 4.2 to 21.8. Selection with one of these insecticides did not increase resistance to the other insecticide. Resistance did not decrease to levels of the corresponding control populations with the same original resistance backgrounds that were not selected with either insecticide in the laboratory. The fenvalerate resistance ratio of a methomyl-resistant population (selected with methomyl in the laboratory and previously exposed to fenvalerate and methomyl in the field) was not as great as the fenvalerate-resistant population, but did not decrease to the levels of the control population. The trend was similar when a fenvalerate-resistant population was tested for susceptibility to methomyl and compared with a control population. The fenvalerate-resistant, methomyl-resistant, and control populations were exposed to the synergists piperonyl butoxide and S,S,S-tributyl phosphorotrithioate. Esterases were implicated as one causal mechanism of resistance to fenvalerate, but a notable part of fenvalerate resistance was unexplained. There was no indication that these synergists blocked methomyl resistance. An overlap in resistance activity among multiple insecticides, as displayed in beet armyworm resistance to fenvalerate and methomyl, should be considered when switching insecticides for control of beet armyworm.

KEY WORDS *Spodoptera exigua*, Lepidoptera, Noctuidae, fenvalerate, methomyl, insecticide synergism

Beet armyworm, *Spodoptera exigua* (Hübner), feeds on many cultivated hosts including tomato, cotton, celery, lettuce, and alfalfa (Metcalf & Flint 1962). On these hosts, it is exposed to a broad array of insecticides (Trumble 1990) that vary in rates of use and toxicological activity. In addition, beet armyworm is highly

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mobile (Mitchell 1979) and may be found on weedy hosts in uncultivated areas where insecticides are not used. The dynamics of beet armyworm biology and the different insecticides used in its control result in a high degree of variation in the exposure and response of beet armyworm to insecticides. Beet armyworm resistance to the carbamate methomyl (Meinke & Ware 1978, Brewer & Trumble 1989) and the pyrethroid fenvalerate (Chaufaux & Ferron 1986, Brewer & Trumble 1989) is variable in time, space, and homogeneity of resistance among populations (Brewer et al. 1990). It is therefore difficult to assign a cause to beet armyworm resistance to fenvalerate and methomyl. When 33 beet armyworm populations from nine geographic regions were monitored, fenvalerate resistance was positively correlated with frequency of use of fenvalerate and methomyl, but methomyl resistance was not correlated with use of either insecticide (Brewer et al. 1990). These biological and operational factors are complex and likely interacting (Georghiou & Taylor 1977a, 1977b) and may explain the resistance variation among populations.

Controlled resistance spectrum and insecticide synergism studies may assist in discerning the action of individual selecting agents. The resistance spectrum of two beet armyworm populations differing in susceptibility to fenvalerate and methomyl were compared with a susceptible population before and after further selection with the insecticides in the laboratory. The resistant and susceptible populations were then exposed to insecticide-synergist combinations to assist in explaining the pattern of resistance activity and to help devise better insecticide use strategies.

Materials and Methods

Insect Populations. A susceptible strain was maintained on artificial diet in the laboratory without exposure to insecticides since its establishment from collections made in Orange County, CA, in 1982. Rearing conditions were those described by Brewer & Trumble (1989). The Orange County strain was used as the susceptible population in all tests.

Two laboratory strains originating from field populations were selected for enhanced levels of resistance. One strain was selected with fenvalerate and the other with methomyl. The strain selected with fenvalerate originated from 20 egg masses found on tomato collected in September, 1987, near Colonia Guerrero, Baja California Norte (Mexico). A fenvalerate resistance ratio of 12 measured at the LC_{50} was documented in the wild population using an adult susceptibility test. A methomyl resistance ratio of 5.6 was detected for the same population (Brewer et al. 1990). Fenvalerate resistance of this population was increased through selection of larvae with fenvalerate. Beginning with the first laboratory generation (F_1), the strain was exposed to selection pressure through 19 generations by topically applying fenvalerate (94%; E.I. du Pont de Nemours & Company, Wilmington, Del.) diluted in acetone to third instar larvae. An Arnold (Type LV 65) microapplicator (Burkard, Rickmansworth, Herts, England) was used to deliver 0.5-μl droplets to the thoracic dorsum of each of at least 660 larvae per generation. The application method was the same in all subsequent

tests. Selection pressure was increased in increments from 0.10 μg per F_1 larva to 3.20 μg per F_{19} larva. Mortality ranged from 45 to 85 percent; survivors were used to establish subsequent generations. Based on comparison with the susceptible population, the fenvalerate resistance ratio at the LD_{50} at the F_1 and F20 generation was 7.7 and 64.2, respectively, using a larval susceptibility test (Brewer et al. 1990). Selections were not conducted for generations 20 and 21. At generation 22, resistance was not different than the 20th generation. Before the use of any subsequent generation in resistance spectrum or synergism experiments, this fenvalerate-resistant population (Fen-R) was selected (3.20 μg per larva) in the generation preceding a test. A control population (Fen-C) derived from the same parent population was maintained without exposure to the selection regime.

The methomyl-resistant population was derived similarly. The laboratory strain originated from eight egg masses and approximated 50 larvae collected on lettuce located near Bakersfield, Kern County, CA, August, 1987. For the wild population, a methomyl resistance ratio of 19 and a fenvalerate resistance ratio of 6.0 at the LC_{50} was detected using an adult susceptibility test (Brewer et al. 1990). Methomyl resistance of this population was increased through selection of larvae using methomyl (98%; du Pont). The selection pressure was increased in increments from 4.0 μg per F_1 larva to 37.8 μg per F_{19} larva. Mortality ranged from 45 to 90 percent. Based on comparison with the susceptible population, the methomyl resistance ratio at the LD_{50} at the F_1 and F_{20} generation was 4.2 and 22.7, respectively, using a larval susceptibility test (Brewer et al. 1990). Before the use of any subsequent generation in resistance spectrum or synergism experiments, this methomyl-resistant population (Met-R) was selected (18.9 μg per larva) in the preceding generation. A control population (Met-C) derived from the same parent population was maintained without exposure to the selection regime.

Resistance Spectrum. The Fen-R, Met-R, Fen-C, Met-C and susceptible populations were tested for susceptibility to fenvalerate and methomyl. Six to 11 concentrations of each insecticide were applied separately to 20 - 30 third instars of each population. Larval weights ranged from 3.00 to 5.00 mg, and larvae were randomly assigned to each insecticide concentration. Bioassays using each insecticide were repeated three times on different days for each population. After exposure, larvae were held in 210-ml ice cream cups filled with 65 ml of artificial diet. The temperature and light conditions were 27 ± 1°C and 16:8 L:D. Mortality was recorded 24 h after application. A larva was considered dead if it remained immobile for 15 sec after being turned on its dorsum. Combined mortality (24 h) data for the three replicates were analyzed with probit regression (LeOra Software 1987). The mean larval weight was used to calculate the LD_{50} and its fiducial limits in μg insecticide per g body weight. Resistance ratios were calculated as (LD_{50} of the resistant population) ÷ (LD_{50} of the susceptible population).

Synergism. The synergists used were piperonyl butoxide (PB) (90-95%, ChemService, West Chester, PA) and S,S,S-tributyl phosphorotrithioate (TBPT) (86%, ChemService). PB is an inhibitor of monooxygenases

(Wilkinson 1976), and TBPT is a general esterase inhibitor (Jao and Casida 1974). Monooxygenases and esterases have been implicated in pyrethroid and carbamate resistance (Soderlund et al. 1983, and Kuhr and Dorough 1976). Concentrations of 0.5 and 2.0 μg/μl of PB and TBPT, respectively, were prepared by diluting each synergist in acetone and were applied to third instars in 0.5-μl droplets. These were maximal sublethal concentrations that were selected from a preliminary test using a series of five concentrations of each synergist (0.5, 1.0, 2.0, 5.0, and 10.0 μg/μl).

The effect of each synergist on fenvalerate resistance was tested by applying fenvalerate to larvae of the Fen-R and susceptible populations 2 h after exposure to PB or TBPT. Similarly, methomyl was applied to larvae of the Met-R and susceptible populations 2 h after exposure to PB or TBPT. Six or seven insecticide concentrations and a solvent-only control were used in combination with each synergist. Bioassays without synergists also were conducted. Bioassays were repeated two or three times. The number of larvae tested, incubation, mortality scoring, and analysis procedures were the same as in the resistance spectrum tests. Resistance ratios were calculated after probit analysis. Synergism ratios were calculated as (LD_{50} of the insecticide alone) ÷ (LD_{50} of the insecticide-synergist combination). Relative percent synergism of x (R%S[x]), where x is the Fen-R, Met-R, or susceptible population, was calculated (Brindley and Selim 1984) and expressed in the form

$$R\%S(x) = \frac{100 \log(\text{synergism ratio of } x)}{\log[(\text{synergism ratio, susceptible population})(\text{resistance ratio, unsynergized } x)]}$$

If R%S(resistant population) > R%S(susceptible population), then synergism in the resistant population would be attributable to blocking resistance.

Results and Discussion

Resistance Spectrum. Resistance to fenvalerate increased when fenvalerate was used as the selecting agent, and fenvalerate resistance declined when a population previously exposed to fenvalerate and methomyl was removed from selection pressure. The fenvalerate resistance ratio of the Fen-R population (selected with fenvalerate) and Fen-C population (not selected) was 47.6 and 1.5, respectively. The parent of both these populations, at least 22 generations prior to these tests, had a fenvalerate resistance ratio of 7.7 (Brewer et al. 1990). Selection with fenvalerate did not increase resistance to methomyl but appeared to prevent the Fen-R population from decreasing in methomyl resistance at the same rate as the Fen-C population, although the 95% fiducial limits of the LD_{50} overlapped (Table 1). Similarly, the fenvalerate resistance ratio of the Met-R population (selected with methomyl) was not as great as the Fen-R population (7.1 and 47.6, respectively), but did not decrease to levels of the Met-C population (95% fiducial limits of the LD_{50} did not overlap; Table 1).

Table 1. Toxicity of fenvalerate and methomyl to beet armyworm populations.

Population	n[a]	% C[b]	Slope ± SE[c]	LD_{50}[d]	R[e]
			fenvalerate exposure		
Susceptible	480	0.0	1.98 ± 0.17	4.8 (4.1 - 5.8)	-
Fen-R	363	0.0	1.62 ± 0.16	230 (98 - 412)	47.6
Met-R	419	0.0	2.33 ± 0.19	34.4 (27.4 - 43.8)	7.1
Fen-C	517	0.0	1.35 ± 0.12	7.3 (3.00 - 13.1)	1.5
Met-C	483	0.0	1.95 ± 0.16	6.3 (2.2 - 13.3)	1.3
			methomyl exposure		
Susceptible	536	0.0	0.81 ± 0.082	81.5 (45.6-129)	-
Fen-R	429	0.0	0.89 ± 0.087	141 (74.2-236)	1.7
Met-R	611	0.0	1.10 ± 0.083	1,773 (1,245-2,501)	21.8
Fen-C	470	0.0	0.91 ± 0.091	105 (68.8 - 215)	1.3
Met-C	469	0.0	1.14 ± 0.10	1,049 (703 - 1,618)	12.9

[a] n, number of larvae exposed to insecticide.
[b] % C, percent control mortality.
[c] Slope ± SE, slope of the probit regression line ± the standard error.
[d] LD_{50}, point estimate of LD_{50} in µg insecticide per g body weight (95% fiducial limits).
[e] R, resistance ratio = (LD_{50} of resistant population) ÷ (LD_{50} of susceptible population).

The trend was similar when populations selected and not selected with methomyl were tested for susceptibility to methomyl and fenvalerate. The methomyl resistance ratios of the Met-R and Met-C populations were 21.8 and 12.6, respectively. The parent of both these populations had a methomyl resistance ratio of 4.2 (Brewer et al. 1990). The methomyl resistance ratio of the Fen-R population was not as great as the Met-R population (1.7 and 21.8, respectively). Selection with methomyl maintained fenvalerate resistance at higher levels in the Met-R population than when no selection pressure was applied to the Met-C population (Table 1). Multiple resistance, cross resistance, or both may be responsible for the initial evolution of resistance to these insecticides. But once resistance was evolved to these insecticides, resistance spectrum tests indicated that there was an overlap in fenvalerate and methomyl resistance activity.

Synergism. Fenvalerate was synergized with PB and TBPT in the susceptible and Fen-R populations (Table 2). Monooxygenases were not implicated as a cause of resistance because relative percent synergism of fenvalerate in the Fen-R population did not exceed that of the susceptible population (R%S[Fen-R] < R%S[susceptible]) when the two populations were exposed to PB and fenvalerate (Table 2). In contrast, relative percent

Table 2. Response of susceptible and fenvalerate-resistant populations of beet armyworm when exposed to the insecticide fenvalerate and the synergists piperonyl butoxide (PB) and S,S,S - tributyl phosphorotrithioate (TBPT).

	susceptible						fenvalerate-resistant						
Treatment	n[a]	% C[b]	Slope ± SE[c]	LD_{50}[d]	S[e]	R%S[g]	n	% C	Slope ± SE	LD_{50}	S	R[f]	R%S
Fenvalerate	335	0.0	1.57 ± 0.17	3.33 (1.90-6.43)			389	0.0	2.21 ± 0.19	388 (329-460)		116.3	
PB+Fenvalerate	240	8.0	1.90 ± 0.36	0.23 (0.041-0.42)	14.3	35.9	484	0.0	1.28 ± 0.11	42.9 (31.0-61.9)	9.1	184.1	29.8
TBPT+Fenvalerate	240	3.0	1.48 ± 0.21	2.86 (1.33-5.00)	1.2	3.7	317	7.0	1.88 ± 0.22	214 (78.6-443)	1.8	74.8	11.9

[a] n, number of larvae exposed to the treatment.

[b] % C, percent control mortality.

[c] Slope ± SE, slope of the probit regression line ± the standard error.

[d] LD_{50}, point estimate of LD_{50} in µg insecticide per g body weight (95% fiducial limits).

[e] S, synergist ratio = (LD_{50} of unsynergized treatment) ÷ (LD_{50} of synergized treatment).

[f] R, resistance ratio = (LD_{50} of resistant population) ÷ (LD_{50} of susceptible population).

[g] R%S(x), relative percent synergism ratio of x = 100[log(S of x)]/[log(S of susceptible) (R of unsynergized treatment)], where x is the susceptible or resistant population.

synergism on the Fen-R population did exceed that of the susceptible population when the two populations were exposed to TBPT and fenvalerate (Table 2). Therefore, esterases were implicated as one mechanism of resistance to fenvalerate. Delorme et al. (1988) reported that esterases were involved in the metabolism of deltamethrin (also an α-cyano 3-phenoxybenzyl ester) in a resistant Guatemalan population of beet armyworm. Similarly, our data suggest the involvement of esterases in pyrethroid resistance. Alternative mechanisms may also be involved. A notable part of the fenvalerate resistance was unexplained using these synergists; the resistance ratio remained high for the PB+fenvalerate and TBPT+fenvalerate treatments (Table 2). Campanhola & Plapp (1989) concluded that target site insensitivity was a mechanism of pyrethroid resistance in adults and larvae of another noctuid, *Heliothis virescens* (F.) and such non-metabolic mechanisms may be expressed in adults and larvae. Such studies are appropriate for beet armyworm because similar expression of fenvalerate resistance has been found in adults and larvae (Brewer et al. 1990).

Methomyl was synergized with PB and TBPT in the susceptible and Met-R populations (Table 3). Even though both PB and TBPT displayed synergistic activity, monooxygenases and esterases were not implicated as a cause of resistance because R%S(Met-R) < R%S(susceptible) when the two populations were exposed to PB+methomyl or TBPT+methomyl (Table 3). Resistance to methomyl appears to be related to other factors not considered here.

Controlled resistance spectrum and insecticide synergism studies were helpful in analyzing the resistance spectrum of this pest. An overlap in fenvalerate and methomyl resistance activity was detected and should be considered when switching insecticides for control of beet armyworm populations that have been previously exposed to these insecticides. In part justified by these data and those documenting resistance in tomato pinworm, *Keiferia lycopersicella* (Walsingham) (Lepidoptera: Gelechiidae) (Brewer et al. 1993), growers in Sinaloa, Mexico, have adopted use of non-insecticidal control measures (e.g., biological control and pheromone confusion) and insecticides with modes of action differing from fenvalerate and methomyl (e.g., *Bacillus thuringiensis* var. *kurstaki* Berliner) (Trumble & Alvarado-Rodriguez 1993).

Table 3. Response of susceptible and methomyl-resistant populations of beet armyworm when exposed to the insecticide methomyl and the synergists piperonyl butoxide (PB) and S,S,S - tributyl phosphorotrithioate (TBPT).

	susceptible						methomyl-resistant						
Treatment	n[a]	% C[b]	Slope ± SE[c]	LD_{50}[d]	S[e]	R%S[g]	n	% C	Slope ± SE	LD_{50}	S	R[f]	R%S
Methomyl	620	2.0	0.85 ± 0.081	49.4 (31.1-71.6)			330	0.0	1.07 ± 0.073	1,878 (4,938-8,195)		38.0	
PB+Methomyl	681	1.8	0.64 ± 0.073	1.63 (0.45-5.34)	30.3	48.4	423	1.7	0.93 ± 0.12	255 (57.5-596)	7.4	156	28.4
TBPT+Methomyl	633	0.0	0.53 ± 0.079	2.68 (0.13-7.56)	18.4	44.5	469	1.9	0.88 ± 0.12	126 (5.29-326)	14.9	47.0	41.2

[a] n, number of larvae exposed to the treatment.
[b] % C, percent control mortality.
[c] Slope ± SE, slope of the probit regression line ± the standard error.
[d] LD_{50}, point estimate of LD_{50} in µg insecticide per g body weight (95% fiducial limits).
[e] S, synergist ratio = (LD_{50} of unsynergized treatment) ÷ (LD_{50} of synergized treatment).
[f] R, resistance ratio = (LD_{50} of resistant population) ÷ (LD_{50} of susceptible population).
[g] R%S(x), relative percent synergism ratio of x = 100[log(S of x)]/[log(S of susceptible) (R of unsynergized treatment)], where x is the susceptible or resistant population.

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Handbook of Sampling Methods for Arthropods in Agriculture

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