RESPONSE OF STRAINS AND CROSSES OF BEET ARMYWORM (SPODOPTERA EXIGUA (HUBNER)) TO INSECTICIDES

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Abstract

Resistance to fenvalerate (Pydrin) and methomyl (Lannate) was linked to crosses of males from a field collected Tifton (T) strain and females of a susceptible (S) reference strain of beet armyworm, Spodoptera exigua (Hubn.). In F_1, LD50's of both insecticides from crosses of male T x female S and brother-sister matings of T strain were significantly greater than those from crosses of female T x male S and brother-sister matings of S strain. Results suggest that genetic factors for resistance to these insecticides are present on the Y chromosome of male even though insecticides have completely different modes of action. Backcrosses of either insecticide to either sex of S and T with F_1 reciprocal crosses showed variable LD50 values. When T male and T female were paired with either T x S and S x T of F_1 generation, LD50's of fenvalerate were similar but brother-sister matings of T showed a non-significant regression. The presence of S strain in the backcrosses maintained a dose-response. For fenvalerate two introductions of S in the backcross generation had LD50's which ranged from 0.12 to 0.41. Where two introductions of T were made LD50's of fenvalerate ranged from 0.34 to 14.33. For methomyl two introductions of S in the backcross generation had an LD50 of 0.72. Where two introductions of T were made LD50's to methomyl ranged from 1.32 to 3.45. An increase in the factors from T strain resulted in generally greater LD50's.

Introduction

Strains of the beet armyworm, Spodoptera exigua (Hubner), from Florida and Georgia were considered to be resistant to fenvalerate, methomyl and methyl parathion (Wolfenbarger & Brewer 1993). The strain from California was not resistant to fenvalerate (Wolfenbarger & Brewer 1993). Inheritance of resistance to any insecticide by any strain from any eastern state has not been shown. Brewer & Trumble (1991) conducted an inheritance of resistance study with fenvalerate against a field collected strain from western Mexico and a susceptible reference strain from California. They showed that LD_{50} values of the parental and filial generations overlapped and that a polygenic model of inheritance seemed most plausible to explain resistance expression.

Here resistance expression by a field collected strain from Tifton, GA and a susceptible reference strain and appropriate reciprocal and backcrosses were determined for generations two and three from the field with fenvalerate, methomyl and methyl parathion. LD50's of emamectin benzoate and emamectin hydrochloride, esfenvalerate, fenvalerate, methomyl and methyl parathion were shown for T strain in first generation from the field by Wolfenbarger and Brewer (1993). Fenvalerate was tested to compare results on the mode of inheritance of our field collected strain with the field collected strain of armyworm from Mexico of Brewer and Trumble (1991). Esfenvalerate (Asana), which is composed of the most active isomer of fenvalerate to insects, was tested to compare its toxicity with fenvalerate. Chlorpyrifos (Lorsban) and methomyl were tested because they are standard insecticides against this pest in the United States and Mexico. Methyl parathion was tested because it is used for control of other insect pests of cotton. Emamectin benzoate (Proclaim) and emamectin hydrochloride were tested because they are extremely toxic to larvae of the beet armyworm, Wolfenbarger & Brewer (1993). In addition, LD_{50} values of our two strains and F_1 crosses were determined for four days to determine if time of toxicity might be a mechanism for resistance by this insect.

Materials And Methods

Technical esfenvalerate, fenvalerate and methomyl were obtained from Dupont Inc., Wilmington, DE; chloropyrifos was obtained from DOW Inc., Midland, MI; emamectin benzoate and emamectin hydrochloride were obtained from Merck Inc., Parsipinay, NJ; and methyl parathion was obtained from Cheminova Inc., Lemvig, Denmark.

The field strain originated from 40 larvae collected from cotton near Tifton (T), GA in 1990. The larvae and subsequent generations were reared on artificial diet (Shaver & Raulston 1974). The susceptible reference (S) strain was obtained from Zeneca Inc., Richmond, CA and reared in the laboratory on the same diet since 1967 (Wolfenbarger & Brewer 1993).

Pupae from both strains (20 to 50 of each sex) were placed in 3.78 liter plastic-lined cardboard containers for moth emergence and oviposition. A 5% sucrose solution was included as food for moths. Moths were mated to obtain parental, filial and backcross generations of larvae and exposed to insecticides. For consistency female was listed first in all crosses and backcrosses.

Individual neonate larvae were placed in 30 ml. plastic cups containing 12-15 ml. artificial diet. Larvae weighing 15 ± 6 mg were treated with insecticides in 1 μl acetone. All

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insecticides were serially diluted (50%) from the greatest to lowest dose.

Doses, as μg/larva, were applied in the following ranges; chlorpyrifos (25 to 0.195), emamectin benzoate and emamectin hydrochloride (1 to 0.0003875), esfenvalerate (50 to 0.195), fenvalerate (200 to 0.39), methomyl (100 to 0.195) and methyl parathion (100 to 0.01). Not all doses were tested against strains, crosses or backcrosses.

Speed of kill of fenvalerate, methomyl and methyl parathion to both parent strains and both F_1 reciprocal crosses in generation two were expressed as percentage reduction in LD50's 48 and 96 h after the 24 h LD_{50}.

Mortalities of the treated insects were determined after 24, 48, 72 and 96 h. Only larvae with no movement following gentle probing were counted as dead. LD_{50} values, slope ± standard error and 95% confidence interval (C.I.) were determined by Probit Analysis (SAS 1985). Significant differences between LD_{50} values were indicated by non-overlapping confidence intervals. Where slope/SE ratios were t= infinity <1.96 at P=0.05 the slope was not significantly different from zero. In addition to the use of non-overlapping of 95% C.I.'s, differences in susceptibility were determined among generations with likelihood ratio tests and parallelism tests which follow a chi^2 distribution (LeOra 1987).

Priorities of insecticide for each strain, cross or backcross were established; fenvalerate was the first to be treated, followed by methomyl, and methyl parathion was last. Chlorpyrifos, emamectin benzoate, emamectin hydrochloride and esfenvalerate were used to treat a strain or cross in the second generation from field.

Results And Discussion

LD_{50} values of the field collected strain to brother-sister matings of T to six insecticides are shown in second generation from the field (Table 1). Emamectin benzoate was the most toxic insecticide. LD50's were 0.001 to 0.0013 μg/larva for T and S x T cross, respectively. Chlorpyrifos and methomyl were equally toxic to T strain; their LD50's had overlapping 95% C.I. values. Esfenvalerate and fenvalerate were the least toxic; in generations two (Table 1) and three (Table 2) only non-significant regressions for both insecticides were determined for T strain. Mortalities were less than 50% for either insecticide with greatest dose tested. LD50's were found for esfenvalerate and fenvalerate in brother-sister matings of S crosses as well as several backcrosses. In fact they were less than 0.01 μg/larva in generation one and two. Of interest was the lack of toxicity by esfenvalerate; this insecticide contains about 86% of the most toxic isomer(SD 43775) of fenvalerate while fenvalerate contains 25% of this isomer (Wolfenbarger et al 1989). About 90% of the toxicity to insects is caused by this isomer. Results suggest that mechanism(s) which prevent the toxicity of this isomer are equal regardless of its concentration in the insecticide.

In generation two (Table 1) LD50 values for methyl parathion and methomyl were 48% and 87% less, respectively, than those shown in generation one (Wolfenbarger & Brewer 1993). LD50's of methyl parathion in both generations were equal because they had overlapping 95% C.I. The 95% C.I. values for methomyl in the two generations did not overlap and were significantly different.

In generation one, LD_{50} values of the reference S strain to emamectin benzoate or emamectin hydrochloride, fenvalerate, methyl parathion and methomyl were 111, 10,900, 220 and 16,-fold less than those of the T strain, respectively (Wolfenbarger & Brewer 1993).

In generation two (Table 1) LD50 for methyl parathion was 70 fold less, respectively, for the S strain than the T strain (Table 1). LD50's for insecticide were significantly different. In generations one and two LD_{50} values for methyl parathion to S were also statistically similar.

Comparison of LD50's of fenvalerate to S and reciprocal crosses indicated that resistance was a sex-linked patroclinous effect (Table 1). In F_1 LD50 of S x T was significantly greater than LD50 of T x S. Both were significantly greater than those of S strain. Comparison of reciprocal F_1's indicate that sex-linkage of the resistance traits to males was consistent with the data. Hypotheses that the probit curves were the same (df=2, chi^2=68.3, P<0.001) or parallel (df=1, chi^2=30.2, P<0.001) were rejected. Such comparisons were not done for methyl parathion because not enough insects were available for treating in the T x S cross. LD50's of insecticide for the S x T cross were significantly greater than shown for the S strain, but equal to that shown by T strain.

Comparison of reciprocal backcrosses in generation three (Table 2) to T by likelihood ratio tests indicate that sex-linkage was a plausible explanation of the results because hypothesis that probit curves were the same (df = 6, chi^2 = 24.9, P < 0.001) or parallel (df = 3, chi^2 = 8.13, P < 0.043) was rejected. When male of T was crossed to female of T x S LD50's of fenvalerate was >14 μg/larva. Perhaps this is an example of dominant epistatis for resistance which is exhibited by genotype of that group of insects of that cross. When male of S was crossed to T x S no progeny were produced.

For comparison the LD_{50} value of fenvalerate of our S strain was 4-fold less than the LD_{50} of a California strain, while our T strain was 10-fold less than the LD50 of a Mexico strain used by Brewer and Trumble (1991). With this information, and given the possibility of sex-linkage by males, the resistance mechanisms of our strains and the strains of Brewer and Trumble (1991) are different.

LD_{50} values of methomyl in the T and S strains and F_1 of reciprocal crosses were also consistent with resistance being linked to the male (Table 1). LD50's for T and S differed 5-fold and were significantly different. LD50's for S x T and T x S differed 46-fold and were significantly different. LD50's of the T strain and the S x T cross showed overlapping C.I.'s, while LD50's of the T strain and S x T cross were significantly different from the T x S cross and S strain. LD50 of our S strain was twice as susceptible as the California strain used by Brewer and Trumble (1991). Our T strain was 8-fold more susceptible than the Mexico strain of the same authors.

For methomyl, comparison of probit curves of reciprocal crosses by likelihood ratio tests indicated that sex-linked effects of the resistance trait were consistent with the data. The hypotheses that curves were the same (df=2, chi^2=100.7, P<0.001) or parallel (df=1, chi^2=16.8, P<0.001) were rejected. Differences in parallelism of the regression of probit curves for second (Table 1) and third generations (Table 2) from the field of the S x T cross were not detected (df=1, chi^2=0.39, P=0.53). Differences in parallelism of the same generations of the T x S cross were also not detected (df=1, chi^2=1.82, P=0.18). Comparison of backcrosses (Table 2) to the resistant parent (T) indicates sex-linkage because hypothesis that the probit lines were the same (df = 4, chi^2 = 8.16, P < 0.09) was accepted. For methomyl, the large standard errors of the regression caused the model estimates to be highly variable. Difficulties with methomyl are probably related to the small differences in susceptibility of the parents in the second generation from the field.

Backcrosses of S and T females to the S x T males in generation 3 showed LD50's equal for both fenvalerate and methomyl (Table 2). Backcrosses of S x T females to S males showed non-significant regression for fenvalerate and methomyl. Backcrosses of male T to S x T females showed 60% reduction in LD50 compared with tests of the F_2 S x T using methomyl, but LD50's were equal when treated with fenvalerate. Esfenvalerate and fenvalerate were equally toxic to the S x T cross in generation 3.

Slopes were variable for insecticide, strain, cross or backcross during the two generations (Tables 1 and 2). Slopes of curves of fenvalerate ranged from 0.33 to 1.59 in the first generation. Slopes of curves by esfenvalerate in the second generation from the field ranged from 0.19 to 3.14, which were similar to fenvalerate in generation one (Wolfenbarger & Brewer 1993). Factors appeared to be similar in strains and crosses which affect toxicity of both insecticides.

Slopes of curves for methomyl were equal; they ranged from 0.52 to 1.77 in the first generation (Table 1) and 0.37 to 1.46 in the second (Table 2). Factors for resistance to methomyl appeared to be the same both generations. Slopes for methyl parathion ranged from 0.47 to 0.83 in generations one and two indicating that factors for resistance were similar for both generations.

In generation two, slopes (Table 1) of the S x T cross for all insecticides ranged from 0.47 to 3.14 (80% of slopes were >1). Slopes of the more susceptible reciprocal T x S cross ranged from 0.33 to 0.79. Slopes of brother-sister matings for both the S and T strains range from 0.47 to 1.77 and 0.19 to 1.14, respectively. Slopes of both F_2 reciprocal crosses ranged from 0.42 to 1.12, while those of backcrosses ranged from 0.18 to 1.46. Backcrosses to the male or female of the S strain show slopes from 0.16 to 0.69, while backcrosses to the male or female of the T strain show slopes which ranged from 0.18 to 1.46. While slopes of backcrosses to T are generally steeper than those with S, variation of values was too great to suggest any special meaning .

LD_{50}'s of the T strain in the second generation for fenvalerate, methomyl, and methyl parathion 24 h after treatment were 171, 673, 11,515-fold greater, respectively, than S strain (Table 3). LD50's for fenvalerate of the S strain and S x T were 60% less after 48 h, while T and T x S were greater than 90%. After 96 h LD50 of fenvalerate for S and S x T were 78% less, while T and T x S were greater than 95%. After 48 h LD50 of methomyl for S, T and S x T were 33 to 35% less, while the T x S was 71% less. The more susceptible reciprocal cross (T x S) was killed faster with methomyl than the S x T. After 96 h LD50 of methomyl to S, T and S x T was 64% less, while LD50 for T x S was 93% less. LD50 of methyl parathion to S and T were 58% or less after 48 h, while that of T x S was 92%. After 96 h LD50 for methyl parathion to S was 55% less, while that for T and S x T was 82% or greater. Speed of kill by methyl parathion was faster for T x S than either S or T after 96 h. In general the larger LD50's with all three insecticides after 24 h had the greatest reduction in LD50's after 96 h. There was no special relationship of time and toxicity for any strain or cross of the three insecticides.

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Table 1. Toxicity of insecticides to Tifton (T) and reference (S) strains of beet armyworm larvae (15 ± 6 mg.) in second generation from field, after 72 h. 1990.

Insecticide	Number Treated	Slope ± SE
T STRAIN		
Emamectin benzoate	105	1.14 ± 0.2
Methyl parathion	153	0.83 ± 0.2
Fenvalerate	112	0.41 ± 0.2
Methomyl	194	0.52± 0.1
Chlorpyrifos	105	1.14 ± 0.2
Esfenvalerate	71	0.19 ± 0.2
S STRAIN		
Fenvalerate	239	0.82 ± 0.2
Methomyl	191	1.77 ± 0.4
Methyl parathion	267	0.47 ± 0.1
T x S		
Methomyl	262	0.79 ± 0.2
Fenvalerate	276	0.33 ± 0.1
S x T		
Methomyl	270	1.33 ± 0.2
Fenvalerate	141	1.59 ± 0.3
Esfenvalerate	67	3.14 ± 0.7
Methyl parathion	241	0.47 ± 0.09
Emamectin hydrochloride	72	1.05 ± 0.3
T STRAIN		
Emamectin benzoate	0.001	($3.8X10^{-10}$-0.0064)
Methyl parathion	18.25	(9.18-67.40)
Fenvalerate		
Methomyl	2.23	(0.61-5.02)
Chlorpyrifos	2.1	(0.92- 3.77)
Esfenvalerate		
S STRAIN		
Fenvalerate	0.0031	(0.00065-0.0071)
Methomyl	0.42	(0.24 - 0.57)
Methyl parathion	0.26	(0.12- 1.26)
T x S		
Methomyl	0.14	(0.016 - 0.34)
Fenvalerate	0.058	(0.0037 - 0.2)
S x T		
Methomyl	6.48	(4.74 - 8.73)
Fenvalerate	7.78	(5.40 - 11.22)
Esfenvalerate	3.36	(2.20 - 5.27)
Methyl parathion	11.20	(4.68 - 45.23)
Emamectin hydrochloride	0.0013	(0.00035 - 0.0031)

Table 2. Toxicity of insecticides to Tifton [T] and reference strain of beet armyworm larvae (15 ± 6 mg) in third generation from field after 72h. 1990.

Source	Treated	± SE
T STRAIN		
Methomyl	155	0.64 ± 0.1
Fenvalerate	98	0.16 ± 0.2
T x S		
Methomyl	55	0.43 ± 0.2
Methyl Parathion	211	0.71 ± 0.1
S x T		
Methomyl	44	1.12 ± 0.3
Fenvalerate	24	0.42 ± 0.1
S x (T x S)		
Fenvalerate	88	0.69 ± 0.1
Methomyl	75	0.37 ± 0.2
(T x S) x T		
Fenvalerate	44	0.71 ± 0.3
T x (T x S)		
Fenvalerate	75	0.50 ± 0.1
Methomyl	68	0.96 ± 0.2
T x (S x T)		
Fenvalerate	57	0.47 ± 0.2
Methomyl	102	0.60 ± 0.2
S x (S x T)		
Fenvalerate	37	0.53 ± 0.2
Methomyl	41	0.69 ± 0.3
(S x T) x T		
Fenvalerate	252	0.18 ± 0.07
Methomyl	103	1.46 ± 0.3
Methyl Parathion	154	0.65 ± 0.2
Source	LD_{50} (µg/larva)	(95% C.I.)
(S x T) x S		
Fenvalerate	51	0.33 ± 0.2
Methomyl	50	0.16 ± 0.3
T STRAIN		
Methomyl	11.76	(5.45- 29.58)
Fenvalerate		
T x S		
Methomyl	1.98	(0.00019-18.53)
Methyl Parathion	0.15	(0.075 - 0.28)
S x T		
Methomyl	7.78	(3.09 -28.80)
Fenvalerate	0.62	(0.0018-24.86)
S x (T x S)		
Fenvalerate	0.12	(0.020 - 0.35)
Methomyl		
(T x S) x T		
Fenvalerate	14.33	(3.47 -26.68)
T x (T x S)		
Fenvalerate	24.14	(5.76-399.04)
Methomyl	3.45	(1.25- 7.95)
T x (S x T)		
Fenvalerate	0.34	(0.01- 1.94)
Methomyl	1.32	(0.11- 3.65)
S x (S x T)		
Fenvalerate	0.41	(0.011- 3.94)
Methomyl	0.72	($1.16X10^{-6}$-2.89)
(S x T) x T		
Fenvalerate	1.67	(0.19-133.77)
Methomyl	2.56	(1.41- 4.00)
Methyl Parathion	3.15	(1.14- 7.36)

Table 3. Speed of kill of four insecticides tested against both parent strains and reciprocal crosses in generation two from field.

		LD_{50} after 24 h		Reduction (%) in LD_{50} after [h]	
Strain or Cross	Slope ± SE	LD?? [µg/larva]	[95% C.I.]	48	96
		Fenvalerate			
S	1.71 ± 0.23	0.059	[0.046 - 0.078	14	53
		Methomyl			
S	1.10 ± 0.22	1.36	[1.02 - 2.17]	35	52
		Methyl Parathion			
S	1.02 ± 0.12	0.33	[0.23 - 0.51]	30	55
		Fenvalerate			
T	0.39 ± 0.16	10,128.69	[450 - $5.32x10^{16}$]	99	96
		Methomyl			
T	0.96 ± 0.16	14.72	[9.6 - 24.4]	33	63
		Methyl Parathion			
T	0.48 ± 0.18	169.78	[39.85- $6.16x10^{5}$]	58	82
		Fenvalerate			
T x S	1.30 ± 0.23	23.30	[15.24 - 41.74]	58	78
		Methomyl			
T x S	1.40 ± 0.16	12.50	[9.41 - 16.92]	33	64
		Methyl Parathion			
T x S	0.49 ± 0.12	303.56	[60.64 - 17,394.0]	92	97
		Fenvalerate			
S x T	0.41 ± 0.18	2.54	[0.44 - ∞∞]	97	99
		Methomyl			
S x T	1.003 ± 0,15	1.87	[1.26 - 2.71]	71	93