SELECTION AND CROSS AND MULTIPLE RESISTANCE TO INSECTICIDES BY BEET ARMYWORM IN EASTERN UNITED STATES

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Abstract

Beet armyworm, *Spodoptera exigua* (Hubner), larvae, collected from flowering plants in Alva, FL, were selected for resistance for 13 generations. Selection was conducted with 10 insecticides which represented three classes of insecticides. First generation of larval treatment was filial one. A resistance threshold of 20 µg/larva for each insecticide was used to indicate resistance or susceptibility in each generation. None of the insecticides showed selection for resistance. Strain was susceptible to bifenthrin and profenofos in all generations. Cross resistance was shown in generation nine by chlorpyrifos and methyl parathion, but not by profenofos. Multiple resistance to non-cyclopropane pyrethroids, i.e. fenvalerate and esfenvalerate, and organophosphorus insecticides was evident in generations three, six and 10 through 12. Susceptibility (LD_'< 20 µg/larva) to the insecticides tested was present in 25%, 67%, 67%, 100%, 86%, 25%, 89%, 50%, 50%, 0%, 100% and 25% of this strain in generations 1 through 12, respectfully. A laboratory reference susceptible strain had lower LD_'s for each insecticide than shown by Alva strain in any generation. Only non-significant regressions were determined in generation 13. With this variation in response resistance factors could be polygenic.

Introduction

Three organophosphorus (chlorpyrifos, methyl parathion and profenofos), one carbamate (methomyl), five cyclopropane pyrethroids (bifenthrin, cypermethrin cyfluthrin, *lambda* cyhalothrin and permethrin) and two non-cyclopropane pyrethroids (esfenvalerate and fenvalerate) are used for control of beet armyworm on a wide range of vegetable crops in subtropical south Florida and cotton in northwestern Florida. The insect is sometimes difficult to control with one or more of these insecticides on these crops during certain years in different fields. Producers suggest that the insect is resistant to the above insecticides.

This insect was susceptible to fenvalerate, methomyl and permethrin in Perris, CA [Wolfenbarger and Brewer 1993). Other strains of beet armyworm from California were susceptible to fenvalerate and methomyl [Brewer and Trumble 1994). Strains of beet armyworm from Bascom and Walnut Hill in northwestern Florida and Alva in subtropical Florida were resistant and had LD_'s of methomyl, permethrin and fenvalerate which were eight, 65 and 22,258 fold greater, respectively, than shown in Perris, CA [Wolfenbarger and Brewer 1993). Selection for resistance was shown by cypermethrin to a strain from Tifton, GA; but not by a strain from Monroe, LA [Wolfenbarger. et al 1997).

Fenvalerate, a non-cyclopropane pyrethroid, was selected because it was a standard insecticide against this lepidopterous pest of cotton and vegetables (Brewer and Trumble 1994). Methomyl was selected because it is a standard insecticide against this insect in south Florida. Chlorpyrifos is a standard insecticide against this insect in cotton. Permethrin, methyl parathion, bifenthrin, cypermethrin, cyfluthrin, *lambda* cyhalothrin, esfenvalerate and profenofos were selected because they are used to control other cotton insect pests. Selection for resistance by this strain to insecticides of the three classes is unknown. Selection for resistance to fenvalerate, permethrin, methomyl and methyl parathion was emphasized. A resistance threshold of 20 µg/larva for each insecticide was used to separate insecticides which were resistant and those which were susceptible. LD50s were examined for amount of cross and multiple resistance each generation. Methods allowed us to examine the results with this focus. Toxicity of all insecticides was determined for a laboratory reference strain (DOW-Zeneca) and compared to the toxicity of all insecticides against the Alva, FL strain.

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Materials and Methods

Technical chlorpyrifos was obtained from DOW, Inc., Midland, MI. Technical of fenvalerate, esfenvalerate, methomyl, methyl parathion, bifenthrin, cypermethrin cyfluthrin, *lambda* cyhalothrin, and permethrin were obtained from sources shown in Wolfenbarger and Brewer (1993).

Larvae (47) were collected from flowering plants near Alva, FL (Wolfenbarger and Brewer 1993), placed on artificial diet (Shaver and Raulston 1974) and sent to Weslaco, TX. Pupae were removed and adults handled as described by Wolfenbarger and Brewer (1993). Plastic sheets on the top and sides of the oviposition chambers were removed daily or every other day and held in a capped container for larval eclosion. Each generation strain was held as multiple pairs of 20 as brothers-sisters for their 6 to 18 d lifetime. Each generation lasted about 30 d. Duration of test was 13 generations. Test was conducted from February, 1991 (Wolfenbarger and Brewer 1993) to January, 1992.

DOW-Zeneca is a laboratory reference strain. It was considered a susceptible strain to all of the insecticides tested here. LD_'s of this strain are shown for all insecticides [Wolfenbarger and Brewer 1993, Sparks et al 1996, Teran 1997 and Wolfenbarger et al 1997).

Prior to treating larvae were held individually on diet in cups as described by Wolfenbarger and Brewer (1993). First generation of treating was filial one. Larvae were treated while on the surface of the diet. All larvae of desired size, three to six days, were treated each generation.

All larvae were selected for resistance each generation. Depending on availability of larvae each insecticide was replicated one to four times on different days each generation. Each day of treatment was a replicate. There were three to eight days of egg hatches each generation. Each egg hatch consisted of two to 15 egg masses of strain. Doses of chlorpyrifos ranged from 0.03875 to 25.0 µg/larva. Doses for the other insecticides are listed by Wolfenbarger and Brewer (1993).

Low doses of each insecticide were tested each generation. The low doses killed no larvae. This allowed us to determine the number of larvae which were dead or dying of disease(s) or unexplained causes. Following treatment and mortality determinations by all insecticides, survivors were pooled and reared to pupation for the next generation.

Mortalities were determined after 72 h as described by Wolfenbarger and Brewer (1993). Probit analysis, [SAS 1989), was used to determine slope ± SE, LD_' and 95% confidence interval of the mortalities [Wolfenbarger and Brewer 1993). For each dose totals of larvae treated and larvae killed by each insecticide were analyzed. Ratio of slopes/SE of regression which are <1.96 of t for ∞∞ are not significantly different from zero. These results are important because they show a response. Each generation LD50s were ranked from greatest to lowest.

LD_'s of beet armyworm which indicate a threshold of resistance are unknown for any insecticide. An LD_' of >20 µg/larva was arbitrarily selected as a resistance threshold for this strain. It was used to separate resistance from susceptibility for each insecticide each generation.

Results and Discussion

One of the four insecticides, i.e. fenvalerate, methomyl, methyl parathion and permethrin, was tested in each generation except generation two. Six other cyclopropane and non-cyclopropane pyrethroids and anticholinesterase insecticides were also tested in one or more generations.

We determined 67 significant and non-significant regressions (Table 1). LD_'s determined in generation one,[Wolfenbarger and Brewer 1993) through 12 showed that permethrin, profenofos, cypermethrin, bifenthrin, profenofos, chlorpyrifos, bifenthrin, methyl parathion, profenofos, methomyl, methomyl, and methomyl were the most toxic each generation, respectively. Selection for resistance through 12 generations by any of the most toxic insecticides was not shown. Four non-significant regressions were determined in generation 13. Strain was susceptible to anti-cholinesterase insecticides in 75% of the generations. LD_'s of the most toxic insecticide for each generation ranged from 3 to 4.99 in one to seven generations, 5 to 9.99 in eight and 11-12 generations, 10 to 19.99 in generation nine and >20 µg/larva in generation 10.

In generations one through 12 fenvalerate, esfenvalerate, fenvalerate, cypermethrin, cypermethrin, methyl parathion, methyl parathion, methomyl, fenvalerate, fenvalerate and methyl parathion were the least toxic, respectively. Selection for resistance by any of the least toxic insecticides was not shown. Fenvalerate (a mixture of 4 isomers) was the least toxic in four of the generations. Esfenvalerate (contains about 85% of the most toxic isomer) was the least toxic in one generation. Both pyrethroids were the least toxic in 42% of the generations. If resistance by all the pyrethroids was included 54% would be the least toxic to this strain during the selection regime. LD_'s of the least toxic insecticides by generation range from 1 to 9.99 in generation four, 10 to 49.99 in generation 11, 50 to 99.99 in generations two, seven and eight, 100 to 499.99 µg/larva in generations five and six, 500 to 999.99 µg/larva in generations three and nine and-10, 1000 to 4999.99 µg/larva in generation 12, and >5000 µg/larva in generation one.

Non-significant regressions were determined for permethrin (0.065 ± 0.99 for 47 larvae), methyl parathion (1.0 ± 0.51 for 64 larvae), and esfenvalerate (0.46 ± 0.35 for 94 larvae) in generation four, esfenvalerate (0.43±0.34 for 86 larvae) in generation six, *lambda* cyhalothrin (0.6 ± 0.66 for 63 larvae) in generation seven, fenvalerate (0.29 ± 0.18 for 107 larvae) in generation eight, methomyl (0.33 ± 0.25 for 97 larvae) in generation 10, permethrin (0.28 ± 0.29 for 97 larvae) in generation 11 and fenvalerate (0.38 ± 0.44 for 41 larvae), methomyl (0.45 ± 0.34 for 75 larvae), methyl parathion (-0.053 ± 0.43 for 44 larvae) and permethrin (0.6 ± 0.42 for 69 larvae) in generation 13. Ratio of slope/SE was <1.96 and did not differ from 0. Eight (75%) of the non-significant regressions were determined for the pyrethroids. None of the 12 insecticides killed more than 58% of the larvae with the greatest dose tested. The same insecticides which were the least toxic also showed non-significant regressions. However, non-significant regressions were shown for methomyl, methyl parathion and permethrin; they were also the most toxic and the least toxic. Results show a complete range of responses. All three classes of insecticides show non-significant regression.

Slope values of all regressions ranged from -0.053 to 2.59. Fifty-six percent of the slopes were <1, indicating flat regressions; 42% and 2% had slopes from 1 to 2 and >2, respectively. All non-significant regressions showed flat slopes.

Cross resistance was shown only in generation nine; strains were resistant to chlorpyrifos and methyl parathion, but not to profenofos. Cross resistance could have been exhibited in generations two through seven to either organophosphorus insecticides [generations two or six] or cyclopropane pyrethroids [generations three and four] or to both classes [generations five and seven].

Multiple resistance was first shown in generation three by fenvalerate and methyl parathion. Strain was susceptible to esfenvalerate, the resolved isomer of fenvalerate, to insects in this generation. We can only suggest that the resolved isomer insecticide was not affected by factors responsible for resistance by non-resolved isomer insecticide. In generation six multiple resistance was shown by fenvalerate, methyl parathion and permethrin. Larvae were susceptible to chlorpyrifos in this generation. In generation seven strain showed multiple resistant to methyl parathion and fenvalerate. Resistance was not shown to four cyclopropane pyrethroids, the carbamate and the other organophosphorus insecticides in this generation. In generation eight multiple resistance was shown to methomyl, esfenvalerate and permethrin, but not to methyl parathion. In generation nine multiple resistance was shown by fenvalerate, chlorpyrifos, and methyl parathion, but the strain was susceptible to methomyl and profenofos. In generation 10 multiple resistance was shown by strain to all three classes of insecticides. In generation 12 strain was resistant to fenvalerate, methyl parathion and permethrin, but not to methomyl.

None of the LD_'s tested against the Alva strain had a lower LD50 than the Dow-Zeneca laboratory reference strain. LD_'s of all the insecticides determined here for strain from Alva, FL, are shown for the DOW-Zeneca laboratory reference strain [Wolfenbarger and Brewer 1993, Sparks et al 1996, Teran 1997 and Wolfenbarger et al 1997). A non-significant regression was shown for the non-cyclopropane pyrethroid esfenvalerate for this strain (Wolfenbarger et al 1997). The strain was susceptible. At 0.195, 0.39, 0.78, 1.56 and 3.125 µg esfenvalerate/larva mortalities ranged from 83% to 96% for 178 larvae. Mortalities were variable for the five doses. With the exception of LD_'s for methyl parathion and methomyl [Sparks et al 1996), reference strain showed that LD_'s were < 0.05 µg/larva for cyclopropane and non-cyclopropane pyrethroids and < 1.0 µg/larva for anticholinesterase insecticides. In 1995 LD_'s of methomyl and methyl parathion, tested against the same reference strain, were 5.48 and 1.02 µg/larva [Sparks et al 1996). They were significantly greater than the LD_'s of 0.8 and 0.16 µg/larva (Wolfenbarger et al 1997) for the same insecticides, respectively. The same strain showed LD_'s of 0.00097 and 0.12 µg/larva for methomyl and methyl parathion, respectively, [Teran 1997). Population was susceptible based on the threshold, but the results showed variation in susceptibility in LD_'s to the reference strain by the same insecticide.

Response of this field-collected strain to the five cyclopropane and both non-cyclopropane pyrethroids in the different generations was extremely variable. Susceptibility was shown to bifenthrin, cyfluthrin and *lambda* cyhalothrin each generation. Cypermethrin and permethrin were susceptible in 75% and 43% of the generations, respectfully. Cypermethrin has a cyano moiety while permethrin does not. Resistance factors for permethrin by this strain were not always evident for cypermethrin. Cross resistance by both pyrethroids was not shown in the same generation.

Toxicity of both or one of the non-cyclopropane pyrethroids was tested in generations three and five through nine. It was expected that the fully resolved isomer pyrethroid (esfenvalerate) was more toxic than fenvalerate, but this was not always shown. In generations three and seven esfenvalerate was significantly more toxic than fenvalerate. In generations five and nine esfenvalerate and fenvalerate were equally toxic. In generations six and eight the regression of fenvalerate was not significant and the strain exhibited resistance to esfenvalerate. Different factors for response in different generations were evident.

Great differences in LD_'s by the four anticholinesterase insecticides were determined. In generations three, seven, 11 and 12 strain was susceptible to methomyl. Strain was resistant in generation eight. Susceptibility to methomyl was evident in 80 % of the generations. In generations one, three, six, seven, nine, 10 and 12 strain was resistant to methyl parathion. In generations one, eight and 11 the strain was susceptible to methyl parathion. In generations two, five and six strain was susceptible to chlorpyrifos while in generation nine it was resistant. Susceptibility to chloropyrifos was shown in 75% of the generations. Susceptibility by strain to profenofos was shown in all five generations.

Susceptibility (LD_' <20 μg/larva) was present in 25%, 67%, 67%, 100%, 86%, 25% 89%, 50%, 50%, 0%, 100% and 25% in generations 1 through 12, respectively. These results indicate that resistance by this insect can be mostly polygenic. Results suggest that more than one factor for resistance was present in the larvae in the different generations. The same genes would probably not be responsible for resistant factors in all three classes of insecticides. The dramatic changes in LD_'s for one insecticide from one generation to the next showed resistance fluctuated and was not stable. Resistance is not an all or nothing situation. It cannot be stated that resistance will be present in all individuals of a population of this insect in the Americas if one population at one location on one day is shown to be resistant.

Number of larvae treated by all insecticides was variable each generation. All available larvae were placed on diet each generation. In generation one 855 were treated while in generation thirteen 229 larvae were treated. The brother-sister matings show a 83% reduction in number larvae treated from the first to last generation. Perhaps this reduction is the cost of resistance to all the insecticides tested against this strain of beet armyworm.

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Table 1. Toxicity of insecticides against beet armyworm for 12 generations. Alva, Florida.

Number treated	Slope ±SE	LD_' μg/larva]	95% Confidence Interval
Generation 2 - March, 1991			
esfenvalerate			
168	0.46±0.14	66.31	6.46-3.5x10^{6}
chlorpyrifos			
67	1.99±0.57	8.94	5.38-14.88
profenofos			
83	1.49±0.28	2.96	1.64-5.49
Genearation 3 - April, 1991			
fenvalerate			
102	0.35±0.11	198.16	32.6-21,860.0
methyl parathion			
75	0.6±0.24	98.26	20.69-5x10^{6}
permethrin			
79	1.02±0.25	9.84	3.2-19.75
esfenvalerate			
81	0.92±0.18	9.61	4.03-30.29
methomyl			
74	0.67±0.25	5.52	0.3-15.68
cypermethrin			
102	1.6±0.23	0.95	0.54-1.77
Generation 4 - May, 1991			
cypermethrin			
97	1.11±0.29	4.61	1.33-17.17
profenonos			
84	1.05±0.3	0.5	0.076-1.06
bifenthrin			
55	1.25±0.37	0.21	0.073-0.42
Generation 5 - June, 1991			
cypermethrin			
102	0.69±0.24	329.54	94.2-68,721.0
fenvalerate			
126	1.2±0.2	9.07	5.45-17.16
chlorpyrifos			
108	1.14±0.23	7.44	4.14-12.99
esfenvalerate			
103	0.73±0.22	4.15	1.3-9.9
lambda cyhalothrin			
120	0.77±0.23	1.37	0.31-16.94
bifenthrin			
117	0.44±0.2	0.71	∞-∞
profenofos			
78	1.33±0.31	0.42	0.17-0.69
Generation 6 - July, 1991			
methyl parathion			
108	0.55±0.22	261.13	46.78-1.2x10^{9}
fenvalerate			
85	1.13±0.48	216.56	∞-∞
permethrin			
76	1.26±0.33	29.27	14.94-86.47
chlorpyrifos			
100	1.76±0.35	0.93	0.6-1.36

Table 1, continued

Number treated	Slope ±SE	LD_' µg/larva]	95% Confidence Interval
Generation 7 - August, 1991			
methyl parathion			
95	0.5±0.21	65.34	15.74-2.4x10^{6}
fenvalerate			
98	1.2±0.25	12.96	7.2-22.64
methomyl			
99	1.39±0.34	8.24	2.86-22.92
cypermethrin			
124	0.58±0.14	6.68	2.62-28.86
permethrin			
102	1.07±0.23	6.06	2.78-10.76
profenofos			
108	0.72±0.21	4.82	2.15-16.85
cyfluthrin			
89	1.54±0.58	2.79	0.48-56625.0
esfenvalerate			
95	0.6±0.24	0.46	0.0015-1.37
bifentrhin			
58	1.51±0.37	0.31	0.11-0.56
Genration 8 - August, 1991			
methomyl			
102	0.9±0.21	73.05	31.48-399.13
esfenvalerate			
134	0.48±0.12	20.46	5.9-322.87
permethrin			
116	1.4±0.24	12.65	7.57-22.8
methyl parathion			
123	1.12±0.19	7.77	4.54-13.5
Generation 9 - September, 1991			
fenvalerate			
83	0.39±0.29	977.41	∞-∞
chlorpyrifos			
106	0.46±0.24	297.71	∞-∞
methyl parathion			
93	0.65±0.32	236.79	57.05-9.37x10^{21}
esfenvalerate			
123	0.77±0.19	17.28	7.31-119.28
methomyl			
71	0.91±0.53	11.95	∞-∞
profenofos			
59	1.09±0.22	3.78	2-7.59
Generation 10 - October, 1991			
fenvalerate			
69	1.4±0.49	723.66	191.79-2.4x10^{13}
permethrin			
41	1.89±0.65	62.88	33.99-385.26
methyl parathion			
61	2.59±0.67	47.98	32.63-76.66
Generation 11 - November, 1991			
methyl parathion			
84	1.09±0.25	18.82	9.98-43.79
methomyl			
86	0.8±0.23	9.11	3.15-22.4

Table 1, continued

Number treated	Slope ±SE	LD_' µg/larva]	95% Confidence Interval
Generation 12 - December, 1991			
fenvalerate			
87	0.49±0.22	1,823.0	203.1-2.1x10^{16}
methyl parathion			
135	0.71±0.32	277.44	∞-∞
permethrin			
152	0.59±0.25	32.19	∞-∞
methomyl			
113	0.73±0.17	9.72	4.21-23.8