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RESISTANCE AND SELECTION FOR RESPONSE TO INSECTICIDES BY BEET ARMYWORM FROM LOWER RIO GRANDE VALLEY

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Abstract

A strain of beet armyworm larvae, collected in May, 1991, from peppers near Donna, TX, in the LRGV, was selected for resistance for nine generations to 11 insecticides in three classes. An LD_{50} of 20 µg/larva was used as the resistance threshold for each insecticide each generation. The strain was resistant to fenvalerate each generation. It was resistant to permethrin, methyl parathion and methomyl in certain generations, but was susceptible in other generations. Susceptibility of the strain was shown by 33%, 100%, 89%, 100%, 0%, 67%. 33%, 50% and 0 % of insecticides tested from generations one through nine, respectively. Multiple resistance was exhibited in generations one, five, six and eight.

Introduction

Beet armyworm, *Spodoptera exigua* (Hubner), larvae are sometimes difficult to control with registered insecticides in cotton and vegetable crops in the subtropical area of the LRGV. In 1989, in Brownsville, a strain of beet armyworm was susceptible to bifenthrin, cyfluthrin, cypermethrin, deltamethrin, *lambda* cyhalothrin, methomyl, profenofos and sulprofos; but the strain was resistant to methyl parathion, permethrin and tralomethrin (Wolfenbarger and Brewer 1993). At the same location in 1990 another strain was collected which was susceptible to methyl parathion (Wolfenbarger and Brewer 1993). In 1994 Wolfenbarger et al. (1997) showed a reversion to increased susceptibility by a strain from Weslaco to methomyl and chlorpyrifos after seven generations. In 1995 strains collected from cotton near Donna, Mercedes, Lyford and Weslaco were resistant to methomyl because mortalities were < 50% and the dose was greater than the resistance threshold; at 50 µg/larva mortalities ranged from 10% to 34% [Sparks et. al 1996). None of these field-collected strains were resistant to chlorpyrifos. Strains from Mercedes and Weslaco were resistant to methyl parathion, but strains from Lyford and Donna were susceptible (Sparks et al. 1996).

In the LRGV fenvalerate, methomyl, methyl parathion and permethrin are considered to be standard insecticides against the beet armyworm and other insect pests on cotton and vegetable crops. Bifenthrin, chlorpyrifos, cypermethrin, cyfluthrin, esfenvalerate, *lambda* cyhalothrin, and profenofos are used on cotton and vegetable crops for the control of other insect pests.

These registered insecticides were tested each generation against a strain from Donna. LD_{50}s of one to eight of these insecticides were determined each generation for nine generations. Insecticides were evaluated to determine if there was cross or multiple resistance, selection for resistance or reversion to susceptibility by this strain. A resistance threshold used an LD_{50} of >20 µg/larva to indicate which insecticides were resistant or susceptible. Toxicity of insecticides against this strain was compared to toxicity against a susceptible laboratory reared reference strain.

Materials and Methods

Technical of 11 insecticides was obtained from sources listed by Wolfenbarger and Brewer (1993) and Wolfenbarger and Wolfenbarger (2001). The classes of insecticides tested were organophosphorus (chlorpyrifos, methyl parathion and profenofos), carbamate (methomyl), cyclopropane pyrethroids (bifenthrin, cyfluthrin, cypermethrin, *lambda* cyhalothrin and permethrin) and non-cyclopropane pyrethroids (esfenvalerate and fenvalerate).

Thirty-eight larvae were collected from peppers in a field near Donna, TX (Wolfenbarger and Brewer 1993), placed on artificial diet (Shaver and Raulston 1974) and held in the laboratory until they pupated. All adults, as brothers-sisters, were placed in 3.78 L oviposition chambers with 5% sugar water for their six to 18 d lifetime. Moths mate within 12 to 48 h post-emergence. Plastic sheets were placed on the top and sides of the chamber as the site for egg masses. Sheets with egg masses were removed daily or every other day and held in a capped container for larval eclosion. Five to 100 egg masses were collected each generation. Eggs hatched over five to nine days. Each generation was about 30 d. Test was conducted for nine consecutive generations from February, 1991 to December, 1991.

DOW-Zeneca is a laboratory reference strain considered to be susceptible to all the insecticides tested here. This information is offered to show that there is a strain of insect which is consistently susceptible to the 11 insecticides tested. LD_{50}s of all insecticides were previously tested against this strain [Wolfenbarger and Brewer 1993, Sparks et al. 1996, Wolfenbarger et al. 1977 and Teran-Vargas 1997).

Prior to treating larvae were held individually on diet in cups [Wolfenbarger and Brewer 1993 and Wolfenbarger and Wolfenbarger 2001). First generation of larva treated was filial generation one.

Doses, from low to high as µg /larva, of all insecticides tested are listed here; the method of treating was described by Wolfenbarger and Brewer (1993) and Wolfenbarger and Wolfenbarger (2001). All larvae were treated while on the surface of the diet when they weighed 15 ± 6 mg, which was between three and six days. The number of larvae available for testing each generation determined the number of insecticides to test. Untreated larvae were not used because a low dose of each insecticide which killed none to few larvae was used to determine natural mortalities. The low dose allows us to determine larvae which were dead or dying of disease(s) or unexplained causes. It also allowed us to incorporate these larvae into the pool of survivors of all doses of insecticides so the strain could be reared to the next generation.

Mortalities were determined after 72 h. Probit analysis of dead larvae and total larva treated, for each dose, as described by SAS (1988), was used to determine regressions of the mortalities (Wolfenbarger and Brewer 1993 and Wolfenbarger and Wolfenbarger 2001). Statistics of non-significant regressions are shown because they represent a response by that insecticide. LD_{50}s were ranked from greatest to lowest each generation.

LD_{50} values which indicate a threshold of resistance for beet armyworm are unknown for any insecticide. An LD_{50} value >20 µg/larva for all insecticides was arbitrarily selected as a resistance threshold. The threshold LD LD_{50} value was used to separate resistance from susceptibility for each insecticide each generation. This value was selected because larvae of this strain would be difficult to control with insecticides if the threshold LD_{50} value was exceeded.

Results

One of four insecticides, i.e. fenvalerate, methomyl, methyl parathion or permethrin was tested each generation, except generation two (Table 1). During the nine generations the strain showed 42 significant and non-significant regressions. LD_{50} values determined in generations one through eight showed that permethrin, chlorpyrifos, bifenthrin, permethrin, permethrin, methomyl, permethrin, and methomyl were the most toxic insecticides, respectively. In generation nine the strain was resistant to methyl parathion, the only insecticide tested. LD_{50} values of the most toxic insecticides ranged from <1 µg/larva for generation four, >1 to 9.99 µg/larva for generations two, three, and seven, 10 to 19.99 µg/larva for generations one and eight and >20 µg/larva for generation five. Selection for resistance to any of the most toxic insecticides was not shown.

In generation two the strain was susceptible to all five insecticides tested: esfenvalerate, cypermethrin, bifenthrin, chlorpyrifos and profenofos. Susceptibility to all insecticides tested was not indicated in any other generation.

In generations one through eight methyl parathion, profenofos, fenvalerate, fenvalerate, methyl parathion, methyl parathion, fenvalerate and fenvalerate were the least toxic, respectively. LD_{50} values in generation one are shown by Wolfenbarger and Brewer (1993). LD_{50} values of the least toxic insecticides ranged from 1 to 9.99 in generation two, 10 to 49.99 µg/larva in generations five and seven, 50 to 99.99 µg/larva in generations three and six, 100 to 499.99 in generations one and four and >500 in generation eight. Selection for resistance was not shown for any of the least toxic insecticides.

The strain was resistant to fenvalerate each generation. LD_{50} values were determined in generations three, seven and eight, but mortalities were so poor in generation one and four through six that a significant regression could not be determined.

Resistance by this strain to the most toxic insecticides was indicated in 13% of generations one through eight. Resistance by the least toxic insecticides was indicated in 75% in the same generations.

The strain showed that 33%, 100%, 89%, 100%, 0%, 67%, 33%, 50% and 0% of LD_{50} values indicated susceptibility (LD_{50} values were <20 µg/larva) in generations one through nine, respectively. Results indicate that resistance has to be polygenic. Resistance is not an all or none situation. LD_{50}s of one insecticide in one generation can change significantly in the next generation.

One non significant regression was determined in each of the first seven generations of this strain. For fenvalerate (0.3 ± 0.26 for 216 larvae) in generation one, *lambda* cyhalothrin (1.22 ± 0.68 for 72 larvae) in generation two, esfenvalerate (0.091 ± 0.32 for 90 larvae) in generation three, fenvalerate (0.5 ± 0.31 for 75 larvae) in generation four, fenvalerate (0.47 ± 0.51 for 82 larvae) in generation five, fenvalerate (0.46 ± 0.28 for 81 larvae) in generation six and methyl parathion (0.73 ± 0.48 for 85 larvae) in generation seven (data not shown in table). Greatest doses tested were 200 µg/larva for fenvalerate and methyl parathion, 10 µg/larva for *lambda* cyhalothrin, and 100 µg/larva for esfenvalerate. For the greatest dose tested mortalities were 48%, 27%, 63%, 16%, 37%, 52% and 45%, respectively, for the insecticides tested during the seven generations. Fenvalerate and methyl parathion were resistant in generations one, four, five and seven because two mortalities were >48% and doses were >20 µg/larva. The remaining regressions had maximum doses of <20 µg/larva or mortalities >50%. Six (86%) of the non-significant regressions were determined for the pyrethroids. Many of the same insecticides which showed non-significant regressions for the Alva, FL strain (Wolfenbarger and Wolfenbarger 2001) showed the same for this strain. Response of strains from the LRGV in 1989, 1990, 1991 and 1995 was similar to those from FL (Wolfenbarger and Brewer 1993, Sparks et al. 1996 and Wolfenbarger and Wolfenbarger 2001).

Slope values for all regressions ranged from 0.091 to 6.23. Most (62%) showed slopes <1 and 33%, 2% and 3% showed slopes from 1-2, 2.1-4 and >4, respectively. Slopes < 1 are considered to be flat. Slopes of all non-significant regressions were flat except one. Its slope was between 1-2.

In generation one the strain showed multiple resistance to methomyl and methyl parathion. In generations five and six the strain showed multiple resistance to permethrin and methyl parathion. In generation eight the strain showed multiple resistance to permethrin, fenvalerate and methyl parathion.

All available larvae were treated each generation (Table 1). In generation one 819 larvae were treated with all insecticides (Wolfenbarger and Brewer 1993) while in generation nine 173 were treated. This is a 79% reduction in larvae from the first to the last generation. Perhaps this reduction is the cost of resistance to insecticides by this strain of the beet armyworm. There was no attempt to regulate the number of moths available for mating from generation to generation.

Discussion

It is difficult to determine a resistance threshold for any strain of beet armyworm that will define resistance or susceptibility in populations. More research is needed on LD_{50} values versus field control in the LRGV. Comparison was shown for the strain from Lyford, LRGV with chlorpyrifos [Sparks et al. 1996); field control was 51% and 30% 2 and 4 d following application and the LD_{50} was 3.96 µg/larva. The LD_{50} value was less than the threshold value, yet control was not satisfactory. Resistance was shown in both the laboratory and the field to methomyl; field control was 10% and 17% 2 and 4 d following application and toxicity with a dose of 50µg/larva was 28% [Sparks, et al. 1996)

Resistance by this strain (LD_{50} value >20 µg/larva) to an insecticide for one or more generations might result in susceptibility the next generation. Resistance might also be shown the next generation. For example, methomyl was resistant in generations one and susceptible in generations three through eight. This is a reversion to susceptibility. Methyl parathion was also resistant in generation one, susceptible in generations three and four and resistant in all subsequent generations. These results show a reversion to susceptibility and then a selection for resistance. Permethrin was susceptible in generations one, three and four, resistant in generations five and six, susceptible in generation seven and resistant in generation eight. The scenario of sequence of resistance and susceptibility was different for these three insecticides against this strain.

Moths which emerge will disperse and randomly mate. Moths may be descendants of brother-sisters or more distant relatives. In this laboratory test only matings of brothers-sisters were used. The 10 females in each chamber mate 0 or 1+ times with one or more of the same or different males. The genotype of each treated larva in each generation will be unknown. In addition there will generally be fewer pupae than larvae and fewer adults than pupae each generation. Thus, there is a loss of stadia each generation. The females emerge, mate and lay egg masses from which neonate larvae emerge but percentages which accomplish these activities each generation are not always consistent. This means that the number involved in these happenings cannot be predicted in any generation.

Except for esfenvalerate LD_{50} values of all insecticides shown here were determined against the laboratory reference strain DOW-Zeneca. A non-significant regression was shown for esfenvalerate, but there was 79% mortality at 0.195 µg/larva. This result indicates susceptibility. LD_{50} values were <0.05 µg/larva for cyclopropane and non-cyclopropane pyrethroids; LD_{50} values were <0.5 for anticholinesterase insecticides against this strain [Wolfenbarger and Brewer 1993, Teran-Vargas 1997 and Wolfenbarger et al. 1997). In 1995, LD_{50} values of methyl parathion and methomyl ranged from 2 to 5 µg/larvae for this same strain, yet they were less than the threshold [Sparks et al. 1996). LD_{50} values of the same insecticides against this Donna strain in any generation were much greater than shown by the laboratory reference strain.

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Table 1. Toxicity of insecticides against beet armyworm for nine generations, Donna, TX.

Insecticide	Number treated	Slope ± SE	LD_{50} [μg/larva]	95% Confidence Interval
Generation 2 - July, 1991				
Profenofos	50	6.23±1.95	4.27	3.08-5.46
Esfenvalerate	89	0.82±0.26	3.1	0.38-6.94
Cypermethrin	93	0.98±0.22	1.64	0.44-3.42
Bifenthrin	80	1.88±0.38	1.49	1.0-2.44
Chlorpyrifos	58	1.96±0.71	1.18	0.095-2.12
Generation 3 - August, 1991				
Fenvalerate	100	0.85±0.25	69.32	30.9-612.17
Cyfluthrin	94	0.82±0.29	19.96	6.34-3259.0
Methomyl	81	1.32±0.3	13.67	7.8-31.81
Methyl parathion	87	1.33±0.3	7.58	4.08-12.68
Cypermethrin	128	0.92±0.22	6.84	2.21-36.91
Permethrin	69	1.9±0.66	5.49	∞-∞
Chlorpyrifos	147	1.38±0.42	4.27	1.77-333.45
Bifenthrin	129	0.3±0.55	4.01	∞-∞
Generation 4 - September, 1991				
Methyl parathion	69	0.67±0.34	15.26	∞-∞
Methomyl	89	0.95±0.25	9.55	4.02-19.49
Profenofos	81	1.36±0.58	1.64	0.81-15.66
Permethrin	90	1±0.16	0.79	0.4-1.14
Generation 5 - October, 1991				
Methyl parathion	87	1.48±0.36	44.39	27.73-100.58
Permethrin	98	0.94±0.26	24.15	11.55-52.2
Generation 6 - November, 1991				
Methyl parathion	133	0.86±0.21	94.54	50.94-286.2
Permethrin	183	1.32±0.2	37.55	26.38-57.42
Methomyl	161	0.92±0.36	12.92	∞-∞
Generation 7 -December, 1991				
Fenvalerate	84	0.55±0.2	134.67	12.22-1.64x10 10
Methomyl	91	1.28±0.25	11.38	6.26-19.65
permethrin	148	0.8±0.11	1.52	0.69-3.17
Generation 8 - January, 1992				
Fenvalerate	83	0.63±0.24	820.31-	164.76-10x10 6
Methyl parathion	106	0.53±0.22	344.78	50.86-8.9x10 9
Permethrin	71	0.85±0.21	31.85	12.74-130.03
Methomyl	108	1.16±0.2	11.22	6.36-20.21
Generation 9 - February, 1992				
Methyl parathion	41	4.4±1.17	48.75	35.6-67.86