CAN RESPONSE LEVELS TO ANY INSECTICIDE BE MAINTAINED BY A POPULATION OF BEET ARMYWORM? D. A. Wolfenbarger Brownsville, TX D. G. Riley University of Georgia Tifton, GA Bob Cartwright Merck Research Laboratories Stillwater, OK

Abstract

LD50's of emamectin benzoate, chlorpyrifos and methomyl to a field collected sample of a population of the beet armyworm, Spodoptera exigua (Hubner), were significantly lower after six or seven generations of selection than the first two generations. This is inbreeding depression. This beet armyworm population was highly resistant to methomyl (LD50 was >100µg/larva), intermediate in response to chlorpyrifos (LD50 >6µg/larva) but susceptible to emamectin benzoate (LD50 was <0.04 µg/larva) in generation 1-2. In generations 3 and 4 LD50's of seven and four groups of pairs showed a 4191 difference for emamectin benzoate. LD50's showed 3 and 8 fold differences for chlorpyrifos and methomyl, respectively. LD50's for DOW-Zeneca strain were 0.48, 0.064 and 5.18 µg/larva for chlorpyrifos, emamectin benzoate and methomyl, respectively.

Introduction

Concentrations which cause 50% mortality decreased >50% within 2 generations following selection with chlorpyrifos for seven strains of the beet armyworm collected in the southeastern United States (Chandler & Ruberson 1996). This is the first such report for this Lepidoptera species.

What does this mean? First, it does not mean that resistance by this insect will not and cannot occur. But results (Chandler & Ruberson 1996) clearly show that doses required for 50% mortality are lower following selection. This phenomena is called inbreeding depression by Lincoln et al [1982]. If resistance is lost, will this loss be maintained and if so for how long even though selection pressure is continually applied or will response levels increase again. We show variation in response to progeny from groups of moths to emamectin benzoate, chlorpyrifos and methomyl. Also, we show an example of in-breeding depression for a sample of a strain of beet armyworm for methomyl, a carbamate insecticide and chlorpyrifos, an organophosphorus insecticide which are widely used for control of this insect, and emamectin benzoate, an experimental insecticide with a different mode of action.

Materials and Methods

Technical chlorpyrifos, emamectin benzoate and methomyl were tested at 0.095-50, 0.000475-1, and 0.78-50 μ g/larva in one microliter of acetone, respectively.

This strain was collected as all stages of larvae (about 75) from cotton near Weslaco, TX, June 10, 1995, and placed on larval diet [Sparks et al 1996]. It was maintained on larval diet in all subsequent generations. The susceptible strain was obtained as eggs from Zeneca, Inc., who previously obtained the strain from DOW Inc.

Topical applications of insecticides were made to the dorsum of the thorax of 3^{rd} stage larvae 18 ± 7 mg with methodology described by Wolfenbarger & Brewer [1993]. Mortalities were determined 24, 48, 72 and 96 h for all insecticides tested.

LD50, slope \pm standard error (SE) and 95% confidence interval were determined by SAS (1988). Differences between LD50's were indicated by non-overlapping of confidence intervals. Non-significant regression was indicated when ratio of slope/SE was t0.05 = <1.96. Shown are percentage mortality at indicated µg/larva.

Each larva was treated with an insecticide each generation from 1-2 to 4, but populations were grouped into two populations in generations 5, 6 and 7. In these generations one of the populations was treated with emamectin benzoate while the other was treated with chlorpyrifos and methomyl. Selection with emamectin benzoate was discontinued after generation 6 because the population died. Selection with chlorpyrifos and methomyl was discontinued in generation 7 because the populations were considered to be susceptible. In generation 3 adults were grouped into 7 different oviposition containers (5 to 10 pairs/group). In generation 4 only three groups of the seven survived and they were paired again [5 to 10 pairs/group].

Results and Discussion

Emamectin benzoate showed LD50's of 0.037 [Sparks et al. 1996], 0.088, 0.062, 0.000021, and 0.00071 to the Weslaco strain of the beet armyworm after 72 h in generations 1-2, 3, 4, 5 and 6, respectively (Table 1) For comparison, the Zeneca-DOW strain showed an LD50 of 0.064 after 72 h; 308 insects were tested and slope \pm SE was 1.06 ± 0.29 with confidence interval of 0.015-1.12. LD50's of generations 1-4 and generations 5 and 6 were significantly different even though selection pressure had been applied each generation. This strain was lost after generation 6.

Chlorpyrifos showed LD50's of 6.61[Sparks et al. 1996], 2.24, 0.056, 0.087, and 0.22 after 72 h in generations 1-2, 3,

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5, 6 and 7, respectively (Table 1). For comparison the Zeneca-DOW showed an LD50 of 0.48 after 72 h; 342 insects were tested and slope \pm SE was 1.53 ± 0.24 with confidence interval of 0.24-0.75. LD50's of chlorpyrifos in generations 1-2 and generation 3 were significantly different (Table 1). LD50's of chlorpyrifos for generation 5 were significantly lower than shown in generation 6 and 7. But the LD50 in generation 1-2 was significantly greater than LD50's shown in generations 5, 6 and 7.

Methomyl showed a non-significant regression of slope as 0.21 ± 0.2 [Sparks et al. 1996], and 33% mortality at 50 µg/larva, 413.98, 36.29, 4.49, 4.71 and 1.88 after generations 1-2, 3, 4, 5, 6 and 7, respectively. For comparison, the Zeneca-DOW showed an LD50 of 5.18 after 72 h; 262 insects were treated with slope \pm SE of 0.7 \pm 0.21 and 95% confidence interval of 0.71-22.62. LD50's of methomyl in generations 3 through 6 were statistically similar while the LD50 of generation 7 was significantly lower than any of the others.

Methomyl showed LD50's from 36.29 to 303.38 μ g/larva, an 8 fold difference among 7 groups of 5 to 10 pairs of beet armyworm adults in generations 3 and 4 (Table 2). Among the same groups for the same generations LD50's ranged from 1.88 to 6.12 and 0.04 to 167.65 μ g/larva for chlorpyrifos and emamectin benzoate, respectively.. Differences in LD50's for chlorpyrifos and emamectin benzoate were 3 and 4191, respectively. Great variation in response to emamectin benzoate was shown among the groups of moths. Differences among the groups were not great for chlorpyrifos and methomyl.

Slope values indicate flat curves for methomyl; they ranged from a high of 0.62 to a low of 0.14 (Tables 1 and 2). They were flat for emamectin benzoate with one exception; one of the groupings showed a slope value of 1.13. The other slope values ranged from a high of 0.78 to a low of -0.0055. Results for emamectin benzoate and methomyl indicate not only great variation, but that most of the curves were flat and that factors which contribute to these curves are multiple.

Of the eight regression curves for chlorpyrifos 63% were greater than 1 and 25% ranged from 0.93 to 0.99. The lowest slope value for chlorpyrifos was 0.69 which was greater than the greatest value shown for methomyl. Thus, factors which contribute to response by chlorpyrifos are considered to be fewer than shown for emamectin benzoate and methomyl.

LD50's of emamectin benzoate 24 h post-treatment were 1.09, 7.5, 3.54, 0.006 and 0.064 for generations 1-2, 3, 4, 5 and 6, respectively. For the same sequence of generations the LD50's of emamectin benzoate 96 h post-treatment were 0.0068, 0.033, 0.03, 0.000025 and 0.00041, respectively. Thus, LD50's were 160, 227, 118, 240 and 156 fold less, respectively after 96 h.

LD50's of all three insecticides indicated greater sensitivity in generations 6 and 7 compared to the first three generations. We suggest that the level of response by methomyl indicates resistance, but this resistance was lost to levels below that shown by the susceptible strain.

Different modes of action are shown by the insecticides tested here. The mode of action for emamectin benzoate (Anonymous 1995) is distinctly different from methomyl and chlorpyrifos. We suggest that there are differences in the interaction of methomyl and chlorpyrifos with cholinesterases of this strain of beet armyworm.

The phenomena we show here is inbreeding depression (Lincoln et al. 1982) of factors responsible for response to these insecticides by this sample of a strain of beet armyworm. Continued selection of this sample of a population of beet armyworm resulted in greater susceptibility to three insecticides. If this occurs to all populations in a given area then resistance to these insecticides will not be maintained. We suggest that it is doubtful that samples of populations will respond alike to selection pressure. Some samples of populations may not show an inbreeding depression due to certain response factors to a specific insecticide.

References

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Table 1. Contact toxicity of three insecticides applied topically to beet armyworms collected from cotton, Weslaco, TX. 1995.				
	ost-treatment [h]	Number larvae		
		treated		
Generat	ions 1 and 2 [June 25 -			
Emamectin	72	488		
benzoate				
Methomyl	96	120		
Chlorpyrifos	72	277		
Generation 3 [August 27 - 31]				
Emamectin	72	3727		
benzoate				
Methomyl	72	688		
Chlorpyrifos	72	1155		
1.4	Generation 4 [August 28 - September 9]			
Emamectin	72	3174		
benzoate				
Methomyl	72	344		
	ion 5 [September 29 -	October 6]		
Emamectin	72	2688		
benzoate				
Methomyl	72	1095		
Chlorpyrifos	72	1124		
Generation 6 [November 13 - 19]				
Emamectin	72	532		
benzoate				
Methomyl	72	790		
Chlorpyrifos	72	659		
Ger	neration 7 [December 1	5 18]		
Methomyl	72	540		
Chlorpyrifos	72	542		

Table 1 continued.

Insecticide	Slope \pm SE	
Generations 1 and 2 [June 25 - August 3]		
Emamectin benzoate	0.73 ± 0.12	
Methomyl	0.21 ± 0.2	
Chlorpyrifos	1.09 0.15	
Generation 3 [.	August 27 - 31]	
Emamectin benzoate	0.48 0.13	
Methomyl	0.47 ± 0.13	
Chlorpyrifos	1.05 ± 0.061	
Generation 4 [Augu	st 28 - September 9]	
Emamectin benzoate	0.51 ± 0.062	
Methomyl	0.25 ± 0.12	
Generation 5 [Septer	mber 29 - October 6]	
Emamectin benzoate	0.45 ± 0.13	
Methomyl	0.3 ± 0.077	
Chlorpyrifos	0.69 ± 0.01	
Generation 6 [N	ovember 13 - 19]	
Emamectin benzoate	0.66 ± 0.11	
Methomyl	0.33 ± 0.096	
Chlorpyrifos	0.93 ± 0.21	
Generation 7 [I	December 15 18]	
Methomyl	$0.5 \pm ^{\circ}.073$	
Chlorpyrifos	1.58 ± 0.14	

Insecticide	LD50[µg/larvae]or mortality [%] µg/larva	[95%Confidence Level]
Genera	tions 1 and 2 [June 25 - A	August 3]
Emamectin benzoate	0.037	[0.015 - 0.08]
Methomyl	33% at 50	
Chlorpyrifos	6.61	[4.68 - 9.41]
C	Generation 3 [August 27 -	31]
Emamectin benzoate	0.088	[0.0044 - 0.54]
Methomyl	413.98	[78.19 - 6.39x10 ⁸]
Chlorpyrifos	2.24	[1.87 - 2.68]
Genera	ation 4 [August 28 - Sept	ember 9]
Emamectin benzoate	0.062	[0.024 - 0.14]
Methomyl	36.29	[10.17 - 1.99x10 ¹⁶]
Genera	ation 5 [September 29 - C	ctober 6]
Emamectin benzoate	0.000021	[4.41x10 ⁻¹⁰ - 0.0003]
Methomyl	4.49	[1.11 - 14.52]
Chlorpyrifos	0.056	[0.0081 - 0.14]
Ge	neration 6 [November 13	- 19]
Emamectin benzoate	0.00071	[0.00011 - 0.0019]
Methomyl	4.71	[90.83 - 20.79]
Chlorpyrifos	0.087	[0.0031 - 0.25]
G	eneration 7 [December 15	5 18]
Methomyl	1.88	[1.12 - 3.24]
Chlorpyrifos	0.22	[0.17 - 0.28]

Table 2. Contact toxicity of three insecticides applied topically to 7 groups	
of 5 to 10 pairs of beet armyworm. Weslaco, TX. 1995.	

Table 2 continued.

 $Slope \pm SE$

 0.26 ± 0.084 0.52 ± 0.22

 0.11 ± 0.26 0.6 ± 0.29 1.11 ± 0.073

 0.26 ± 0.19 0.14 ± 0.22

 0.39 ± 0.18 0.46 ± 0.27 0.99 ± 0.17

 1.13 ± 0.19 0.85 ± 0.29 1.64 ± 0.29

 $\textbf{-0.39} \pm 0.19$

 0.22 ± 0.17 0.39 ± 1.69

 $\textbf{-0.0055} \pm 0.16$ 0.25 ± 0.12

 0.55 ± 0.073

 0.68 ± 0.29

of 5 to 10 pairs of beet arm Insecticide	Post-treatment		Insecticide	Slope :
Insecticide	,	nj Number larvae treated		Generation 3
~ .	Generation 3		Group 1	
Group 1			Emamectin benzoate	0.26 ±
Emamectin benzoate	96	1849	Methomyl	$0.52 \pm$
Methomyl	72	122	Group 2	
Group 2			Emamectin benzoate	$0.11 \pm$
Emamectin benzoate	72	288	Methomyl	0.6 ± 0.6
Methomyl	72	181	Chlorpyrifos	1.11 ±
Chlorpyrifos	72	732	Group 3	
Group 3			Emamectin benzoate	0.26 ±
Emamectin benzoate	96	310	Methomyl	$0.14 \pm$
Methomyl	96	100	Group 4	
Group 4			Emamectin benzoate	0.39 ±
Emamectin benzoate	72	239	Methomyl	$0.46 \pm$
Methomyl	72	156	Chlorpyrifos	$0.99 \pm$
Chlorpyrifos	72	211	Group 5	
Group 5			Emamectin benzoate	1.13 ±
Emamectin benzoate	72	145	Methomyl	$0.85 \pm$
Methomyl	72	122	Chlorpyrifos	1.64 ±
Chlorpyrifos	72	137	Group 6	
Group 6			Emamectin benzoate	-0.39 ±
Emamectin benzoate	48	143	Group 7	
Group 7			Emamectin benzoate	$0.22 \pm$
Emamectin benzoate	72	125	Methomyl	0.39 ±
Methomyl	72	143	j.	Generation 4
	Generation 4		Group 2	
Group 2			Emamectin benzoate	-0.005
Emamectin benzoate	72	97	Methomyl	0.25 ±
Methomyl	72	344	Group 3	0.20 _
Group 3			Emamectin benzoate	$0.55 \pm$
Emamectin benzoate	72	2320	Group 4	0.55 ±
Group 4			Emamectin benzoate	$0.68 \pm$
Emamectin benzoate	72	343	Emaneeun benzoate	0.00 ±

Table 2 continued;		
Insecticide	LD50 [µg/larva] or mortality [%] at µg/larva	[95% Confidence Level]
Generation 3		
Group 1		
Emamectin benzoate	5.99	[0.68 - 6.7x10 ¹⁰]
Methomyl	303.38	[42.38 - 2.5x10 ¹¹]
Group 2		
Emamectin benzoate	5% at 1.0	
Methomyl	174.69	[∞∞ -∞∞]
Chlorpyrifos	1.95	[1.57 - 2.42]
Group 3		
Emamectin benzoate	25% at 1.0	
Methomyl	44% at 50.0	
Group 4		
Emamectin benzoate	0.6	[∞∞ - ∞∞]
Methomyl	29% at 100	
Chlorpyrifos	6.12	[3.97 - 9.63]
Group 5		
Emamectin benzoate	0.043	[0.026 - 0.7]
Methomyl	187.87	[5±.77- 5.2x10 ⁴]
Chlorpyrifos	1.88	[1.07 - 2.77]
Group 6		
Emamectin benzoate	167.65	[4.23- 3.67x10 ⁷]
Group 7		
Emamectin benzoate	13% at 0.5	
Methomyl	33% at 25.0	
Generation 4		
Group 2		
Emamectin benzoate	61% at 10.0	
Methomyl	36.29	[10.17 - 1.99x10 ¹⁶]
Group 3		
Emamectin benzoate	0.063	[0.022- 0.15]
Group 4		
Emamectin benzoate	0.040	[∞∞-∞∞]