THE USE OF SINGLE PAIRS OF TOBACCO BUDWORM (NOCTUIDAE:LEPIDOPTERA) TO EVALUATE RESISTANCE AND SUSCEPTIBILITY OF TWO PYRETHROIDS

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Abstract

Single pairs (81) of the tobacco budworm were established from different genotypes in 1983 and 1989-1990. Seventy of these single pairs were for two strains or crosses of the strains for two generations following a selection regime with permethrin. Progeny of 11 single pairs, collected as eggs or larvae from fields across the Lower Rio Grande Valley (LRGV), were treated with cypermethrin. LD_'s of 11, 15, 55 and all 81 single pairs consistently followed a continuum. With one exception there was no significant difference in LD_'s which were adjacent to another and ranked from the lowest to the greatest. The most resistant single pair was the reference strain. The most susceptible single pair was a reference x field cross. A difference of 8,105 was determined for both LD_'s. LD_'s of field collected strains were intermediate to LD_'s of the strains and crosses of strains. A resistance threshold of 0.2 µg pyrethroid/larva is offered.

Introduction

Toxicity of cypermethrin to larvae from single pairs of tobacco budworm, *Heliothis virescens* (F.), showed great variation [Wolfenbarger et al. 2000). Single pairs of moths are the minimum phenotypic size for this insect. LD_'s of insecticides to larvae reared from brother-sister mating of groups of moths have been the most widely used method of determining their response (Anonymous 1970). It is unknown what this LD_' means for progeny from each group of moths reared from eggs, larvae or pupae collected from host plants in a field or fields or the soil of a field or fields. Larvae, progeny of groups of moths, can be resistant, susceptible or have both responses present in some proportion. Genotype which control the response of each larva to each pyrethroid is unknown. Groups of moths collected from traps or by hand can also be paired for progeny but it is not known if they are brother-sisters. Moths may have been from a nearby field or from a location hundreds of miles away and their age is unknown.

The objective of the tests was to determine the magnitude of resistance and susceptibility to larvae from single pairs. Treatment with an insecticide to progeny of single pairs could and should be used to determine the proportion of resistance or susceptibility of populations in a field or fields in an area such as the LRGV. With a resistance threshold the proportion of resistance and susceptibility by each single pair can be determined.

Materials and Methods

Technical permethrin and cypermethrin (>93% purity) were obtained from FMC Corp., Princeton, NJ. Technical grade acetone was used to dilute doses of the insecticides.

Reference laboratory strain was continually reared at USDA-ARS laboratories at Brownsville and Weslaco, TX since 1968. Field strain was collected near Brownsville, TX, in 1983 and used in a selection experiment for 12 generations with brother-sister matings by groups of moths (Wolfenbarger 1986). LD_'s of Brownsville and reference strains and reciprocal crosses of the two strains were determined. In 1989 eggs and larvae of insect were collected once from a cotton field near Brownsville, Edinburg (north), Las Milpas, Los Fresnos and Weslaco. In 1990 eggs and larvae were collected twice from the same field near Weslaco [Wolfenbarger et al. 2000). In 1989 and 1990 progeny were treated in the first larval generation from the field.

In both tests larvae were reared to adults and paired singly for their lifetime in gauze covered 473 ml cylindrical cardboard cartons with 5% sugar water. Neonate larvae were placed singly in cups with artificial diet and treated when they weighed 22 ± 6 mg [Wolfenbarger 1986 and Wolfenbarger et al 2000). Topical application of doses were applied to dorsum of thorax in 1 microliter in acetone. Doses were 0.00095, 0.0019, 0.003875, 0.00775, 0.0155, 0.031, 0.0625, 0.125, 0.25, 0.5, 1.0 and 1.25 µg of both insecticides/larva. Ten to 30 larvae/dose in one to thirteen replicates were treated. All available larvae were treated. Each replicate was conducted on a different day.

Mortalities were determined after 72 h. LD_'s, slope ± standard error (SE) and 95% confidence intervals (CI) were determined by probit analysis of SAS (1988). LD_'s were ranked from lowest to greatest value. Females are listed first in all crosses. Log 10 was used to transform doses. A significant difference (at 95% probability) in LD_' values was determined by non-overlapping of confidence intervals.

Results

Strains and crosses. In generation 13 LD_'s of 15 single pairs ranged from 0.015 to 0.21 µg/larva, a 14 fold difference (Table 1). Groupings of LD_'s for the strains or both reciprocal crosses were not evident although the two lowest and the two greatest LD_'s were for the reference and the Brownsville strain, respectfully.

Slopes were as variable as the LD_'s; 40% were <1, 40% ranged from 1-2 and 20% were >2. SE of slopes ranged from 11% to 39% of slope. Number of insects treated for each single pair was variable and ranged from 34 to 205.

In generation 14 LD_'s of 55 single pairs showed 8,105 fold difference. LD_'s ranged from 0.0019 to 15.4 µg/larva. This is far greater variation than shown in generation 13. The greatest LD_' was for reference strain and it was 110 fold greater than the next value. The two CI values overlapped which indicates equal response. All LD_' values in both generation showed a continuum because the CI overlapped the next greater or lower LD_'.

Slopes were steeper in generation 14 than shown in generation 13; 18% were <1, 60% ranged from 1-2 and 22% were >2. SE of slope ranged from 1% to 47% of slope. With the three fold increase in number of single pairs the range was also greater. Number of insects treated ranged from 34 to 242, which is similar to generation 13.

Crosses of LRGV field strains. In 1989 and 1990 LD_'s of progeny from 11 single pairs from nine fields ranged from 0.027 to 1.75 µg cypermethrin/larva when treated in the first generation (Table 2). These two values were 65 fold different and significantly different from each other . The lowest value was determined by Weslaco x Weslaco cross in 1990 while the greatest value was determined in 1989 by the Santa Rosa x Brownsville cross. Larvae of this cross were resistant.

For all 11 pairs of crosses shown for 1989 and 1990 (Table 2) slope values ranged from 0.6 to 2.16; 18% were <1, 76% ranged from 1-2 and 6% were >2. SE of slopes ranged from 11% to 47% of slope. No trend for location and slope was indicated.

The number of larvae produced by these field-collected strains ranged from 47 to 364 (Table 2). This range is similar to those determined by strains and their crosses in generation 13 and 14 (Table 1). We treated 49 larva of the single pair with the lowest LD_' and 364 larvae at the next LD_'.

There is a continuum of LD_'s for permethrin in 1983 just as there was for cypermethrin in1989 and 1990. This allowed us to propose a resistance threshold of LD_' of 0.2 µg/larva for larvae of this pest. Resistance to permethrin comprised 7% and <l% in generations 13 and 14 in 1983, respectively. Resistance to cypermethrin was shown by one single pair or 13% in 1989 and 1990.

Discussion

This methodology and these results yield insights into sampling for identification of single pair populations of tobacco budworm which might be resistant to an insecticide. Samples of eggs and larvae were taken from nine of 5,000 to 10,000 cotton fields grown each year in the LRGV of Texas. This is not an adequate sample size, but resistant pairs were found. Resistance and susceptibility has to be part of a continuum based on these results, but it is unknown how many resistant single pairs and total single pairs are present on any given day in the LRGV.

This single pair methodology allows the mating of one female and one male for their lifetime. Yet progeny of 1 female and 1 male can have many genetic differences since the pair can mate 1+ times in their lifetime. Larval males can have twice as many factors for resistance because they have XX chromosomes while larval females have XY chromosomes.

Resistance to these pyrethroids has to be polygenic because a number of genes have to be involved. Polygenic factors for resistance or susceptibility can only be a genetic-environment legacy. Genes are influenced by their environment, but this gene-environment interaction is not understood for this insect. Are gene(s)-environment associated with resistance to pyrethroids the at same for all other insecticide classes? One result is clear: genes for resistance or susceptibility are not always constant. Resistance can occur and then disappear in the same strain (Wolfenbarger 1986). Resistance factors can be different in different matings. Genes can show resistance or susceptibility with one mating but the next mating by the same male will result in a completely different set of genes for resistance or susceptibility. There may be a certain allele which is variant in a section of DNA for a certain protein which is a trait for resistance in a certain environment. This allele may not develop this protein in every environment.

The contribution of genes for resistance in their environment may or may not be additive. They may be synergistic. Certain genes may control other genes. Genes for resistance may produce proteins in many different environments, in one

environment but not another. The environment may include other insecticides with the same or different modes of action, natural or secondary compounds in the plant which larvae ingest, different concentrations of an insecticide or the natural compounds, ambient temperatures and many other factors. The environment may be too harsh for the development of proteins which destroy insecticides. The interaction of the gene and its environment can only be dynamic in each larva. The larva can be resistant or susceptible in one generation and change to another condition the next generation because genes may have been triggered to change with a change of environment.

Conclusion

Resistance in 81 single pairs was not predominant based on the resistance threshold. Single pairs can be used to define resistance and susceptibility by any population of tobacco budworm. If eggs, larvae and pupae are collected from fields over any area every other day moths could be paired singly to determine the proportion of the population which is resistant or susceptible.

References

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Table 1. Toxicity of permethrin to progeny of single pairs of tobacco budworm by two strains and their crosses for two generations. 1983.

Number Treated Larvae	Slope ± SE	LD_' (µg/larva)	95% Confidence Interval
		Generation 13	
		Reference x Reference	
130	1.67±0.24	0.012	0.0000024-0.034
		Reference x Reference	
97	0.73±0.19	0.014	0.0089-0.02
		Brownsville x Brownsville	
34	2.4±0.39	0.015	0.014-0.026
		Reference x Reference	
121	1.42±0.27	0.019	0.011-0.28
		Reference x Reference	
150	1.79±0.42	0.023	0.014-0.032
		Reference x Brownsville	
170	2.1±0.72	0.029	0.017-0.43
		Brownsville x Reference	
155	1.62±0.63	0.03	∞-∞
		Reference x Reference	
101	0.79±0.19	0.035	0.01-0.07
		Reference x Reference	
45	1.93±0.35	0.047	0.027-0.07
		Brownsville x Reference	
93	0.96±0.21	0.055	0.038-0.08
		Brownsville x Reference	
87	0.93±0.17	0.058	0.04-0.08
		Reference x Brownsville	
171	0.71±0.17	0.085	0.06-0.12
		Reference x Brownsville	
205	1.76±0.12	0.12	0.09-0.17
		Brownsville x Brownsville	
83	0.83±0.091	0.15	0.1-0.24
		Generation 14	
		Reference x Brownsville	
75	2.14±1.01	0.0019	∞-∞
		Reference x Reference	
56	0.75±0.097	0.0023	0.000071-0.0076
		Reference x Reference	
63	1.41±0.53	0.0074	∞-0.031
		Reference x Reference	
170	1.01±0.15	0.0083	0.0011-0.019
		Reference x Reference	
132	1.75±0.17	0.0086	0-2.11
		Reference x Reference	
180	1.7±0.32	0.01	0.007-0.14
		Reference x Reference	
140	1.35±0.35	0.011	0.007-0.014
		Reference x Reference	
103	1.21±0.42	0.012	0026-0.03
		Reference x Reference	
125	1.42±0.32	0.013	0.0085-0.19
		Reference x Reference	
75	0.75±0.31	0.16	∞-∞
		Reference x Brownsville	
93	1.04±0.67	0.017	∞-0.046
		Reference x Reference	
103	2.1±0.45	0.018	0.012-0.23
		Reference x Reference	
142	0.64±0.29	0.019	∞-∞
		Brownsville x Reference	
124	1.75±0.15	0.019	0.012-0.026
		Reference x Reference	
64	2.1±0.021	0.021	0.0078-0.41
		Reference x Reference	
191	1.64±0.082	0.024	0.013-0.048
		Reference x Reference	
187	0.57±0.24	0.024	∞-∞
		Brownsville x Reference	
151	1.85±0.75	0.024	0.0068-0.042
		Brownsville x Reference	

75	1.93±0.17	0.026	0.015-0.04
121	0.85±0.39	Brownsville x Brownsville 0.026	∞-∞
94	1.34±0.17	Reference x Reference 0.027	0.015-0.4
101	1.24±0.17	Reference x Reference 0.028	0.21-0.37
211	1.67±0.27	Reference x Reference 0.032	0.021-0.064
75	0.75±0.34	Reference x Reference 0.032	∞-∞
34	1.6±0.21	Brownsville x Brownsville 0.033	0.019-0.05
64	2.1±0.64	Reference x Brownsville 0.037	0.0075-0.07
65	1.93±0.17	Reference x Brownsville 0.038	0.027-0.057
122	0.95±0.44	Reference x Brownsville 0.039	∞-∞
93	1.69±0.45	Reference x Reference 0.042	0.024-0.071
151	2.35±0.023	Reference x Reference 0.045	0.0038-0.096
154	1.83±0.45	Brownsville x Reference 0.046	0.016-0.18
145	1.23±0.32	Reference x Brownsville 0.047	0.012-0.11
123	1.75±0.14	Reference x Brownsville 0.052	0.046-0.087
110	2.11±0.52	Brownsville x Reference 0.052	0.033-0.087
95	1.21±0.32	Reference x Reference 0.052	0.041-0.74
76	1.45±0.41	Brownsville x Reference 0.057	0.039-0.99
109	1.81±0.54	Brownsville x Reference 0.064	0.052-0.079
142	2.01±0.33	Reference x Brownsville 0.064	0.045-0.11
67	0.47±0.22	Reference x Brownsville 0.067	∞-∞
131	0.75±0.27	Reference x Brownsville 0.069	0.05-0.08
242	2.03±0.072	Reference x Brownsville 0.076	0.055-0.11
52	1.84±0.55	Reference x Brownsville 0.077	0.018-2.0
83	2.75±0.082	Reference x Reference 0.08	0.055-0.11
42	2.01±0.35	Reference x Brownsville 0.08	0.03-0.23
92	0.75±0.29	Reference x Reference 0.081	∞-∞
45	1.75±0.14	Reference x Brownsville 0.088	0.024-2.16
52	1.75±0.12	Reference x Brownsville 0.1	0.08-0.13
124	1.87±0.14	Reference x Reference 0.11	0.09-0.14
121	1.94±0.72	Reference x Brownsville 0.12	0.067-0.29
145	2.4±0.49	Brownsville x Brownsville 0.12	0.091-0.17
63	1.84±0.34	Reference x Brownsville 0.12	0.086-0.17
175	2.03±0.13	Reference x Brownsville 0.14	0.077-0.29
131	1.94±0.086	Brownsville x Brownsville 0.14	0.11-0.21
164	1.01±0.31	Reference x Reference 15.4	0.064-∞

Table 2. Toxicity of cypermethrin to larvae from single pairs by topical application. Lower Rio Grande Valley. 1989-1990.

Number treated Larvae	Slope ± SE	LD_' (µg/larva)	95% Confidence Interval
		Weslaco x Weslaco	
79	1.13±0.3	0.027	0.011-0.05
		Brownsville x Los Indios	
133	0.9±0.1	0.53	0.0069-∞
		Weslaco x Weslaco	
59	0.83±0.3	0.71	0.025-0.71
		La Feria x Brownsville	
50	0.86±0.4	0.074	0.028-8.6x10 8
		Weslaco x Las Milpas	
71	1.4±0.3	0.077	0.042-0.14
		Weslaco x Weslaco	
191	1.7±0.2	0.079	0.059-0.11
		Weslaco x Weslaco	
182	1.48±0.3	0.089	0.046-0.18
		Las Milpas x Edinburg	
311	0.77±0.1	0.096	0.049-0.17
		Weslaco x Weslaco	
96	1.84±0.5	0.12	0.04-0.8
		Weslaco x Weslaco	
61	1.4±0.3	0.14	0.069-0.28
		Santa Rosa x Brownsville	
92	0.6±0.22	1.75	0.36-88.61