TOBACCO BUDWORM: FATE OF A BLACK-BODY DOMINANT-LETHAL MUTANT CROSSED WITH "WILD" FOLLOWING SELECTION WITH METHYL PARATHION

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Abstract

Response of a mutant strain and crosses of the mutant and laboratory-reared insecticide susceptible "wild" strain of the tobacco budworm, *Heliothis virescens* (F.), was determined. We wanted to determine if either one or both sexes of mutant could be eliminated by selection regime. The mutant was lethal in homozygous condition and the allele was dominant for black-bodied melanic character. Mutation was maintained in heterozygous status with the "wild" strain. LD_{20}, LD_{50}, and LD_{80} of methyl parathion to larvae of the mutant and crosses of mutant and the "wild" strain were determined. In the first generation the LD_{50} of methyl parathion for the heterozygous black-bodied strain was significantly and 22-fold greater than that of "wild" strain. LD_{50} of insecticide to larvae of reciprocal crosses indicated incomplete dominance or co-dominance of alleles, but no sex linkage. In subsequent three generations percentage black-bodied females and males of selected strain and crosses were compared to same percentages of non-selected strain and crosses. When larvae of both reciprocal crosses were treated with LD_{20} and LD_{50} in generation three percentage black-bodied females was reduced 60% and 80%, respectively, compared to the untreated population of the same crosses. When larvae of both reciprocal crosses were treated with LD_{80} in generation three all black-body males and females were killed. Both sexes of black-bodied strain treated with LD_{50} and LD_{80} were reduced 38% and 69%, respectively, in generation three compared to the untreated of the strain. Frequency of black-bodied males of black-bodied strain was reduced 0 to 38% with LD_{20}, LD_{50} and LD_{80} in that same generation.

Introduction

There is no information on the fate of a body color phenotype of the adult tobacco budworm, *Heliothis virescens* (F.), when challenged with methyl parathion. A male black-bodied individual was first observed in a population of moths which had been collected as larvae from cotton in 1976 near Brownsville, TX. It was then paired with females of the wild-type ("WW") laboratory strain. Black-body individuals were found in the next generation. Bartlett and Raulston (1982) designated the allele for black-body as "Bl" and determined it to be lethal in homozygous condition and dominant. They maintained this black-body strain in the heterozygous condition with the wild-type laboratory strain. Wild-type was not added each generation but only when black-bodied numbers fell in a generation were equal sexes of wild-type included the next generation. They were then maintained until numbers fell again. Wing colors of the black-bodied strain are dark green. There was no evidence of the natural coloration of the normal 'wild' strain.

Progeny of reciprocal crosses of this mutant and the "wild" laboratory colony phenotype were treated with methyl parathion in generation one to confirm mode of inheritance of the black-bodied phenotype. Methyl parathion is widely used in the Americas today for control of this pest. Selection regime of larvae of strain and crosses with methyl parathion was determined. Percentage of males and females of black-bodied strain and reciprocal crosses of the two strains was determined in each of four generations and compared to survival of the same strain and crosses in untreated populations. Objective of regime was to determine if populations of one of the sexes could be eliminated.

Materials and Methods

Technical methyl parathion (98%) was obtained from Monsanto Co., St. Louis, MO. Since 1976, the black body mutant has been maintained at the Brownsville laboratory. This test was conducted January through May, 1983. "Wild" laboratory strain has been maintained in the same laboratory for a decade prior to this test.

In the first generation progeny from 10 to 20 pairs of moths for each parent strain and each reciprocal cross were maintained separately in 3.78 liter containers. Females were listed first in each cross. In each of the subsequent generations 10 to 20 pairs were placed into containers for each LD_{50} of black-bodied and reciprocal cross until all moths were placed. Each and all larvae were reared separately on 10 ml diet in a 30 ml cup, Shaver & Raulston (1971).

LD_{50} of methyl parathion of "wild" phenotype was determined in generation one and not thereafter. In generation 2 one half of the total progeny of black-bodied strain and reciprocal crosses of the two strains was treated with the three LDs. Larvae weighed 25±5 mg (3rd instar) when topically treated with doses ranging from 0.024 to 50 µg methyl parathion/larva. In generations 3 and 4 the three LD values for the black-bodied strain and each reciprocal cross were applied to progeny of brother sister matings

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beginning in generation 1 and maintained in generations 2, 3 and 4. Basic methods for treatment were described by the Entomological Society of America (Anonymous 1970). Mortalities were determined 48 h after treatment when larvae did not move when probed. LD_{20}, LD_{50} and LD_{80} values, slope ± standard error (SE) and 95% confidence interval (CI) were calculated by probit analysis (SAS 1988). Significant differences in LD values were shown when 95% CI values did not overlap.

One third of the larvae of the treated half in generations two, three and four were treated with the LD_{20}, another third with LD_{50} and the last third with $LD_{80,}$ calculated from the first generation.

The other half of the progeny of the black-bodied strain and each reciprocal cross was reared separately beginning in generation 1 and not treated.

Each third of the treated and untreated black-bodied strain and reciprocal crosses was reared separately. Six colonies were maintained and no “wild” strain insects were added to any of the populations during the four generations. The number of black-body males and females from the black-bodied strain and each cross was determined in each treated and untreated population. Differences were compared by contingency analysis of Sokal and Rohlf (1969). Expected number of black-bodied males and females was 67% for the black-bodied strain and 50% for reciprocal crosses of black-bodied and “wild” moths. Number in each category was combined for generations two, three and four. Differences between numbers of expected and observed were determined by x^2 at $P_{0.05}$.

Differences in female populations in treated vs untreated generations two, three and four were determined by “t” test as described by Steele and Torrie (1960) when treated with LD_{20}, LD_{50} and LD_{80}. Test was to determine if there were significant differences between female populations in the treated and untreated black-bodied strain and both reciprocal crosses.

In each generation, progeny of the treated and untreated halves were reared to the adult stage for determination of black- body and “wild” phenotype and their sex. Chi-square ($P_{0.05}$) of number of black-bodied moths versus "wild" moths was determined in generation one (Steele and Torrie 1960) to confirm the ratios of black-bodied and “wild” insects of Bartlett and Raulston (1982).

Results and Discussion

In the first generation LD_{50}'s for the black-body strain (Table 1) were significantly different (C.I. values did not overlap) and 22-fold greater than that of the "wild" strain which was 0.11 with 95% C.I. of 0.05-0.17 µg/larva and a slope 1.33±0.17 for 378 larvae (data not shown in table). Results suggest that the black-bodied strain was resistant and that this accounted for its increase in LD_{50}.

There were no significant differences in LD_{50} values to larvae produced by the reciprocal crosses (confidence intervals overlapped). LD_{50} values for the reciprocal crosses were 5-fold and significantly lower (confidence intervals did not overlap) than the black-body melanic mutant (BlW X BlW). LD_{50}s of reciprocal crosses were 4-fold and significantly greater than the (WW X WW) wild strain. These results indicate incomplete dominance (co-dominant) autosomal inheritance to methyl parathion. These results do not suggest any number of genes for the resistance factors. Firko (1991) suggests that incomplete dominance is most likely polygenic. If the genotypes were dominant LD_{50} values of reciprocal crosses would have been equal to the black-bodied strain.

LD_{50}'s of both reciprocal crosses in generations one, two and three were significantly lower than those of the black-body strain (Table 1). In generation 4, all three LD_{80} values were greater than the three LD_{80}'s shown in generation one for BlBl (as BlW x BlW genotype), BlW (as BlW x WW genotypes) and WBl (as WW x BlW genotype) phenotypes. This was especially true for the BlW male reciprocal cross; its LD_{50} value was 207-fold greater in generation four than generation one. Continued selection to the 4th generation caused expression of sex-linkage to the male of the black-bodied strain and resistance was not indicated by the black-bodied strain.

In the first generation, slopes ± standard error of the wild strain and both reciprocal crosses were steeper than those shown by black-bodied strain (Table 1). The flat slope for the black-bodied was undoubtedly influenced by the resistance and lethality factors which it possessed compared to the “wild” strain. Slope values and standard error were variable and showed no trend in generations two and three. Slope values were greater for all strains and crosses in generation four than for generations one through three, suggesting decreased genetic variability through selection.

In generations one 309, 425 and 292 were treated for the black-bodied strain and reciprocal crosses BlWW x WW and WW x BlWW, respectively (data not shown in table). This was an adequate number of larvae to be treated for probit analysis. This is also an adequate number to determine percentages of black-bodied and “wild” insects from each strain in the following three generations.

Bartlett and Raulston (1982) showed that 67% of the moths had a black-body after the BlW x BlW were paired and that 50% of the moths were black-bodied after the BlW x WW were paired. We used the same “wild” strain they used. These percentages were the same regardless of the sex. This

percentage exhibited monogenic inheritance for the black-body mutant. After the larvae were treated in the first generation the number of moths, the percentage of black-bodied moths as observed, (expected) and x^2 for BlW x BlW, BlW x WW and WW x BlW crosses were 267, 63%(67%), and 0.67, 270 51%(50%) and 0.07 and 213, 45%(50%) and 1.13, respectively (data not shown in table). There were no significant differences (by $x^2 = 0.67$ and 0.65; 1 and 1 df; P> 0.3 and P> 0.3) from the expected 67%. For untreated of black-bodied strain, BlW x WW and WW x BlW the number of moths, percentage black-bodied moths and observed and (expected) and x 2 were 56, 59%(67%) and 0.65, 100, 37%(50%) and 3.38 and 30, 47%(50%) and 0.07, respectively (data not shown in table). For BlW X WW x^2 was 0.07 and 3.38 for 1 and 1 df P> 0.5 and P> 0.1, and for WW X BlW x^2 was 1.13 and 0.07 for 1 and 1 df; P> 0.2 and P=3.93. There was no significant difference in observed and expected black-bodied insects in the treated and untreated in generation one. The same expected and observed mortalities were determined by us and by Bartlett and Raulston (1982) for the same strains. Selection was initiated with these results in the first generation.

Percentage black-bodied males treated with LD_{20}, LD_{50} and LD_{80} ranged from 18 to 100% during the 4 generations with no consistent trend (Table 2). Percentage black-bodied females treated with LD_{20}, LD_{50} and LD_{80} ranged from 0 to 100%. Ten percent or less females of this mutant were determined from all LD's of methyl parathion in all three crosses in generations two, three and four.

In generations two 358, 374 and 748, three 384, 801 and 614 and four 592, 795 and 1000 moths (total of all three doses/generation) were obtained from black-bodied strain, BlWW x WW and WW x BlW, respectively. In generations two 349, 240 and 248, three 268, 329 and 352 and four 432, 198 and 294 larvae were treated for the above sequence of strain and crosses (data not shown in table). Ratio of larvae/moth of black-bodied strain, and the BlW x WW and WW x BlW crosses for each of the three LDs from the lowest to greatest were 0.97, 0.64 and 0.33 in generation 2, 0.7, 0.41 and 0.57 for generation 3 and 0.73, 0.24 and 0.29, respectively, for each of the three LDs from the lowest to the greatest. The greatest ratio was shown for the black-bodied but this was expected since “wild” moths were not added in the first generation. Ratios of crosses were about equal.

Replication in this test was not possible because the number of larvae from each adult was low. We expect 100 larvae/moth from the “wild” strain.

There was 100% reduction in percentage black-body males and females in both reciprocal crosses treated with LD_{80} in generation three (Table 2). In generation four, LD_{20} and LD_{50} dosages caused reductions of about 99% of females and no reduction of black-bodied males in both reciprocal crosses. Percentage black-body females of untreated BlW x WW reciprocal cross increased each generation from two through four; percentage black-body males of untreated BlW x WW reciprocal cross were variable. Despite the complete kill of black-bodied females, there were no significant differences for all three strains in generations two, three and four for any LD versus the untreated colonies; t = 0.82 for df = 53; P> 0.3.

Contingency table analysis compared differences in number of male and female of black-bodied strain and both reciprocal crosses in treated and untreated populations of generations 2, 3 and 4, which were grouped (data not shown in table). There were no significant differences between percentage male versus female of black-bodied strain for treated and untreated; x^2 was 3.4 (P>0.1) for treated and 0.81 (P>0.5) for the untreated. In the treated there were significantly more males of the black-bodied strain than females in both reciprocal crosses; x^2 was 8.0 (P<0.005) for BlWW and 14.8 (P<0.005) for WW x BlWW. In the untreated of both reciprocal crosses there were no significant differences in males and females of black-bodied insects; for B1W x WW x^2 was 1.68 (P>0.25) and for WW x BlWW it was 2.4 (P>0.25). These results confirm that females of the black-bodied strain were reduced compared to males when larvae were treated with methyl parathion.

Conclusion

Following a selection regime for four generations methyl parathion killed all females of a heterozygote black-bodied phenotypic mutant of tobacco budworm after four generations when crossed with wild moths. Reciprocal crosses of both parents were intermediate in response to each parent to insecticide in the first generation indicating incomplete dominance by phenotypic mutant. Black-bodied strain was resistant compared to wild strain in generation one. Black-bodied males were more resistant than black-bodied females in generations three and four. If black-bodies females were released into field populations of tobacco budworm they would be eliminated following the selection regime.

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Table 1. Toxicity of methyl parathion as µg/larva after 48 h for four generations of black-body strain and crosses of black-body strain and wild strain of tobacco budworm.

BlWW x BlWW	
Generation 1	
LD_{20} (95% CI)	0.27(0.18-0.35)
D50 (95% CI)	2.46(1.77-3.8)
LD_{80} (95% CI)	22.86(11.67-63.51)
Slope ± SE	0.87±0.13
Generation 2	
LD_{20} (95% CI)	0.13(0.033-0.31)
D50 (95% CI)	0.98(0.8-1.17)
LD_{80} (95% CI)	15.07(2.78-8.14)
Slope ± SE	0.94±0.17
Generation 3	
LD_{20} (95% CI)	0.26(0.12-0.39)
D50 (95% CI)	0.92(0.8-1.01)
LD_{80} (95% CI)	3.33(0.1-7.42)
Slope ± SE	0.87±0.21
Generation 4	
LD_{20} (95% CI)	0.41(0.32-0.59)
D50 (95% CI)	3.01(2.54-3.56)
LD_{80} (95% CI)	21.86(17.35-26.34)
Slope ± SE	1.95±0.32
BlWW x WW	
Generation 1	
LD_{20} (95% CI)	0.15(0.12-0.18)
D50 (95% CI)	0.56(0.49-0.64)
LD_{80} (95% CI)	2.14(1.77-2.26)
Slope ± SE	1.46±0.24
Generation 2	
LD_{20} (95% CI)	0.1(0.057-0.54)
D50 (95% CI)	0.33(0.23-0.43)
LD_{80} (95% CI)	1.11(0.84-1.64)
Slope ± SE	1.25±0.43
Generation 3	
LD_{20} (95% CI)	0.17(0.082-0.24)
D50 (95% CI)	0.48(0.36-0.61)
LD_{80} (95% CI)	1.43(.58-2.32)
Slope ± SE	1.42±0.51
Generation 4	
LD_{20} (95% CI)	0.16(0.82-0.24)
D50 (95% CI)	0.85(0.66-1.03)
LD_{80} (95% CI)	4.62(2.58-13.09)
Slope ± SE	1.73±0.42
WW X BIW	
Generation 1	
LD_{20} (95% CI)	0.19(0.16-0.24)
D50 (95% CI)	0.59(0.52-0.66)
LD_{80} (95% CI)	1.75(1.48-2.11)
Slope ± SE	1.78±0.32
Generation 2	
LD_{20} (95% CI)	0.091(0.045-0.14
D50 (95% CI)	0.37(0.28-0.41)
LD_{80} (95% CI)	1.49(1.10-2.36)
Slope ± SE	1.92±0.32
Generation 3	
LD_{20} (95% CI)	0.2(0.13-0.27)
D50 (95% CI)	0.6(0.47-0.73)
LD_{80} (95% CI)	1.8(1.32-3.88)
Slope ± SE	0.83±0.17
Generation 4	
LD_{20} (95% CI)	0.27(0.0013-0.48)
D50 (95% CI)	9.79-(7.72-11.63))
LD_{80} (95% CI)	361.54(15.82-4.0x10- 25)
Slope ± SE	3.42±0.72

Table 2. Number and percentage black body males and females from strain and reciprocal crosses of black body and wild-type strains exposed to LD_{20}, LD_{50} and LD_{80} by methyl parathion in three generations.

	Number moths treated		
Generation	**LD_{50}**	**LD_{50}**	**LD_{50}**
	BlW x BlW		
2	220	46	92
3	339	39	6
4	270	300	12
	BlW x WW		
2	265	83	26
3	313	432	56
4	421	374	0
	WW x BlW		
2	489	222	37
3	327	279	8
4	505	435	
	Percentage males treated		
Generation	**LD_{50}**	**LD_{50}**	**LD_{50}**
	BlW x BlW		
2	48	38	59
3	47	100	64
4	81	59	75
	BlW x WW		
2	43	18	50
3	36	70	0
4	33	66	
	WW x BlW		
2	46	38	75
3	50	46	0
4	100	79	
	Percentage females treated		
Generation	**LD_{50}**	**LD_{50}**	**LD_{50}**
	BlW x BlW		
2	31	28	18
3	100	10	5
4	8	15	3
	BlW x WW		
2	21	13	8
3	7	2	0
4	<1	<1	
	WW x BlW		
2	28	18	11
3	13	10	0
4	<1	3	
	Untreated		
		Percentage	
Generation	**Number moths**	**Male**	**Female**
	BlW x BlW		
2	473	43	38
3	107	68	16
4	46	25	10
	BlW x WW		
2	308	49	22
3	132	14	30
4	42	60	31
	WW x BlW		
2	368	44	23
3	135	45	23
4	74	50	9