TOBACCO BUDWORM: VARIATION IN RESPONSE AND REPRODUCTIVE "FITNESS" OF STRAINS, CROSSES AND BACKCROSSES OF THE STRAINS TO METHYL PARATHION AND EPN[1/]

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Abstract

To test for possible resistance to methyl parathion and EPN, larvae of the tobacco budworm, Heliothis virescens (F.), were collected from cotton at two sites in Tamaulipas, Mexico, in 1968-1970 and reared to adulthood in the laboratory at Brownsville, TX. Adults were allowed to mate with members of their group or were crossed with a laboratory strain of the same insect. The progeny of these crosses were topically treated with methyl parathion or EPN to determine the LD_{50} values for these insecticides. Surviving insects were crossed and back-crossed in succeeding generations. Numbers of successful matings, eggs for female and the percentage of egg hatch did not change significantly from one generation to the next. In 1968 LD_{50} values of methyl parathion to the field-collected strain were 32 times higher than they were to the laboratory strain; in 1970, they were 136 and 75 times higher in the first and second generations tested. In 1968, crosses of the two strains indicated that resistance was sex-linked to the male; 1970 studies showed co-dominance of male and female. The strain collected in 1969 was treated with EPN and found to be 8 times less susceptible than was the laboratory strain to this insecticide. Progeny of reciprocal crosses of field-collected and laboratory insects exhibited co-dominance for resistance.

Response To Methyl Parathion by various strains of the tobacco budworm, collected in northeastern Mexico and the Lower Rio Grande Valley (LRGV) of Texas was reported by Wolfenbarger (1973), Wolfenbarger et al. (1981), and Wolfenbarger et al. (1982). One of these strains was highly resistant (R) to methyl parathion, whereas others expressed varying degrees of resistance. However, no reports of resistance by the tobacco budworm to EPN have been found. In fact, EPN was more toxic than methyl parathion to tobacco budworms collected from Platon Sanchez Altamira and Estacion Cuauhtemoc, in Tamaulipas, Mexico, in 1969 and in Brownsville, Texas, U.S.A., in 1970 (Wolfenbarger 1973).

We needed to know if field-collected strains of tobacco budworms were at a reproductive disadvantage as a result of exposure to these organophosphorus insecticides. Roush & Plapp (1982) suggested that information on the reproductive capacity of insecticide-resistant strains is necessary for the modeling of resistance patterns in (R) populations as compared to the productivity of susceptible (S) strains.

There is little information on the magnitude of variation in the response of filial crosses and back-crosses or R and S strains of the tobacco budworm to methyl parathion and EPN. Whitten (1978) crossed strains of this species and found that resistance to methyl parathion appeared to be influenced by a single major autosomal gene of incomplete dominance but that other genes may also have been involved.

We made similar crosses to those of Whitten (1978) with other strains of this insect and tested them with methyl parathion and EPN to determine response of three field collected strains (one strain per year) of tobacco budworm when crossed with our laboratory S strain. Back-crosses were also made. Reproductive "fitness" of members of treated populations and crosses was then determined for two or three consecutive generations of 1969 and 1970 collections. Two or three generations of this insect occur during the typical 90-to 100-day cotton fruiting season in the LRGV.

Methods and Materials

Technical methyl parathion was obtained from Monsanto Co., Inc., St. Louis, MO. Technical EPN was obtained from DuPont Inc., Wilmington, DE. An S strain of tobacco budworm was originally obtained from the USDA, ARS, Biological Control Laboratory, Tucson, AZ, in 1966; thereafter, this strain was reared under laboratory conditions at Brownsville for four years or 48 generations before the 1968 test.

Strains (R) were initiated with from 25, 30 and 71 larvae collected from cotton near Estacion Cuauhtemoc, in August 1968 and September 1969, and Mante in 1970, respectively. These towns are located in southern Tamaulipas, Mexico, and they are about 60 km apart. The R strain larvae were reared to the adult stage in the laboratory at Brownsville.

This report begins with the progeny of the adult survivors of the second generation called generation one here from the field (about 30D between generations) in November, 1968, 1969 and 1970. The second generation (third generation from the field) was treated in early 1969, 1970, 1971 and the third generation (fourth generation from the field) was treated from February to March in 1970 and 1971. In 1968 there was a fourth generation (fifth generation from the field).

Arrangement of Crosses. In the first generation, the following crosses were made: RXR, SXS, RXS, and SXR. For the second generation the progeny of these four crosses

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were either produced by intra-strain mating (i. e., interbreeding of males and females from the same cross) or back-crossed with males or females from the R and S. In the third generation shown for methyl parathion-1968 and EPN-1969 progeny of certain crosses and the back-crosses were mated within strains or back-crossed a second time with males or females of either the R and S. A fourth generation of R strain-1968 was intra-strain mated. Male and female moths were paired in equal numbers. Two to 60 pairs per one to 14 four-liter containers were used. The female is listed first in all crosses.

All neonate larvae were placed singly in 21 g capacity plastic cups containing 10 g soybean-wheatgerm diet (Shaver & Raulston 1971). Fifty-percent serial dilutions of methyl parathion ranged from 100 to 0.00031 mg/ml of acetone; those for EPN ranged from 200 to 0.04875 mg/ml of acetone. Six to 11 doses per strain or cross were topically applied to the dorsum of the thorax of 15-40 larvae per replicate when, according to procedures suggested by the Entomological Society of America (1970). The larvae were 5-7 days and weighed 35$\pm$5 mg.

All tests were replicated 2 to 6 days (1 day = 1 replicate), depending on the numbers of larvae available. Live pupae that developed from each cross were separated according to sex for forthcoming R and S strains, crosses, or intra-strain matings. Then, the number of larvae treated was recorded.

Methyl parathion-1968. In generation one, S, R and reciprocal crosses were replicated 2 to 3 times with 14 to 40 pairs per replicate. All larvae were treated. In generation two, S, R, reciprocal crosses and seven back-crosses were replicated 1 to 3 times with 7-31 pairs per replicate. In generation three, 4-12 R and S pairs were replicated once. Generation four of R had one replicate of four females and five males.

EPN - 1969. In generation one, S, R and crosses were replicated 2-4 times with 10-20 moths per replicate. In each strain, cross or back-cross 5 to 15% of the larvae were not treated. In generation two, S, R, reciprocal crosses and back-crosses to S and R were replicated 4 to 12 times with 11 to 15 pairs per replicate. In generation three, R and S, filial crosses and reciprocal crosses were replicated twice with 10 or 11 pairs per replicate. Intr-strain mating of back-cross with resistant strain in generation two was replicated 6 times with 2 or 3 pairs per replicate; intr-strain mating of back-cross with susceptible strain in generation two was replicated 6 times, 4 to 10 pairs per replicate. Remaining back-crosses were replicated 7 times, 4 to 9 pairs per replicate.

Methyl parathion - 1970. All crosses and back-crosses of the strain were made once with 7 to 31 pairs in generation one and/or two. All larvae were treated as in 1968.

Data Analysis

Mortalities of larvae treated with each dose and 72 h. For 1968, 1969, and 1970, the difference in mortality after 24 h versus 72 h of 0.00625 to 0.1 mg/larva for methyl parathion and 1.56 to 25 for EPN of R and S was determined. Significant difference between these two times was compared by "t" at $P < 0.05$ at each dose. After 48 h all LD_{50} values, expressed as mg or insecticide per larva, were calculated from a line estimated by probit (SAS User's Guide 1979) as were number larvae treated. The standard error of the slope was determined by square root of the variance of slope of the co-variance matrix. When the "t" of slope $\pm$ SE was ≤ 1.96, the regression was judged to be not significantly different from zero. When a non-significant value was obtained, percentage mortality was shown for the highest dose tested. LD_{50} values whose 95% C.I.'s did not overlap, were considered to be significantly different.

Biotic Analysis-Reproductive fitness evaluations were determined for strains exposed to EPN in 1969 and methyl parathion in 1970. Tests were conducted separately from toxicity tests for methyl parathion-1970. An insect is considered to be "fit" if it reproduces normally despite exposure to these insecticides. Fitness, as related to models for resistance, is further discussed by Taylor (1983). Methods used to determine the numbers of insects that mated, the fecundity of females and fertility after exposure to one other insecticides were similar. When they died all females were dissected to determine the percentage of females whose bursa copulatrix contained one or more spermatophores, indicating that mating had occurred. Fecundity was determined by counts of eggs on all cheesecloth substrates throughout the ovipositional activity of all females of each cross or strain. Results were expressed as eggs per female. Fertility was determined by counting of all larvae that hatched from the counted eggs and results were expressed as percentage hatch. Equal number of female and male moths were in each container per strain, cross or back-cross each generation. For EPN-1969, confidence intervals of the measurements of "fitness" were determined for each mean of each strains, filial crosses or back-crosses between 1 versus 2 and 2 versus 3 generations. In generation one, two and three, we paired 4, 12 and 27 back-crosses, respectively.

For methyl parathion-1970 a strain tested with 6 (total 24 pairs) and 3 (total 12 pairs) pairs per replicate in generation one and two, respectively. LSD at $P < 0.05$ was used to determine significance of percentage mated or fertility and fecundity of strain cross or back-cross between each generation.

Results

Methyl parathion - 1968. The LD_{50} value for the R strain larvae in generation 1 was 6.9 mg per larva with CIs of 5.43

- 8.58 (Table 1). Wolfenbarger (1973) obtained an LD_{50} value of 8.86 mg/larvae with CIs of 5.31 - 11.45 mg/larva for a similar R strain, and because the CIs overlapped, the LD_{50} values were considered to be alike. The LD_{50} value for the S strain (Table 1) indicate that these insects were 33 times more susceptible to methyl parathion than were the R strain insects. Resistance of the R strain in generations 2 and 3 was shown by LD_{50} values of 23.45 and 30.61 mg per larva, respectively, but these values were statistically the same.

In generations 1 and 2, reciprocal crosses of S and R clearly show that the trait for resistance was carried by the male insect, i.e., crosses containing R males had LD_{50} values of 60.92 and 11.44 as compared to those in which the female was from the R strain, 1.45 and 3.43 ug per larva, in generations 1 and 2, respectively (Table 1). The back-cross (S X R) X R (LD_{50} 23.45), R X (S X R) (LD_{50} 2.09) and the CIs did not overlap (Table 1). These results again indicate that the male of the R carries most of the resistance factors in this strain.

In generation 3, the R X R cross was significantly more resistant than it was in generation 1. We were unable to compute the LD_{50} values for the crosses made in generation 4 but found that the dose of 3.125 mg per larva of methyl parathion killed 21% of the larvae of the ((R X (R X S) X R back-cross and 100 mg per larva killed 100% of the larvae of the R cross.

In generation 1 the slope values for the R and S strains were equal. In the first two generations, slope values ranged from 0.16 to 4.58. More than half were 1; we consider slopes of 1 to be flat, and hence, that our test insects were heterogenous for response to methyl parathion.

The LD_{50} values of the R strain, with continued selection, increased 4.44-fold in three generations (Table 1). Moths did not lay viable eggs in the fourth generation.

Larval mortalities in the R and S strains after 24 h were compared with kill observed after 72 h following treatment (Table 2). All doses killed a greater percentage of the larvae of the S strain than of the R strain in 24 h. Mortalities of S and R were significantly different at the highest and lowest doses.

EPN - 1969. LD_{50} values for R larvae of generations 1, 2, and 3 were equal at mg per larva of 6.87 (CIs 5.2 - 9.1), 6.88 (CIs 5.5 - 8.7) and 10.6 (CIs 6.87 - 14.59) as shown in Table 3. Similar tests reported by Wolfenbarger (1973) showed an LD_{50} value of 14.3 mg per larva (0.5 mg/g dose) with CIs 10.01 - 20.31 which was significantly different from the LD_{50} values reported here.

LD_{50} values for the R and S strains differed in 1 and generation 3, although they were 8-, 2-, and 3-fold higher for R than S in generations 1, 2, and 3, respectively.

In generation 1, LD_{50} values of the reciprocal crosses R X S and S X R were similar. Their slopes were flat and < 1. The LD_{50} values or reciprocal crosses and R and S strains differed by 3.2- and 2.6-fold. The LD_{50} values of the reciprocal crosses were intermediate to those of the R and S strains, indicating co-dominance (incomplete dominance) for resistance.

The LD_{50} values of the reciprocal crosses in all three generations were statistically similar, and they were statistically similar to those of both parents in generation 2 but only to the S in generation 3. When either the R or S were backcrossed with second generation reciprocal crosses, LD_{50} values were statistically similar to second generation reciprocal crosses and both parents.

Because no increased resistance by the R male was indicated for EPN, the reciprocal crosses, as well as all subsequent crosses with each sex, were pooled into 17 groupings of 3, 5, and 9 in generations 1, 2, and 3, respectively. Grouping were made by crosses with the R or S strain or by intra-strain mating of the R and S males and females.

LD_{50} values for the S strain increased 4- to 4-fold from generation 1 to generations 2 and 3, but differences were not significant. However, the fact that the LD_{50} values did increase in a strain considered to be susceptible indicates that variation occurs.

In generation 3, LD_{50} values of reciprocal crosses and S and R were statistically similar. One grouping was of particular interest. The LD_{50} value of the second generation of R backcross intrastrain mating was significantly greater than all the other groupings of the crosses in all three generations, including the R strain or its backcross a second time to R. Alleles of genes for resistance to EPN may have combined in a manner to cause the larvae to exhibit this significant level of response. Also, it was interesting to note that the second generation of S backcross intrastrain mating had an LD_{50} value significantly greater than that of the second backcross to S (in generations 2 and 3). The backcross to S for the second time was the most susceptible of the groupings in generation 3.

About 70% of the slopes for the R and S and 17 groupings of crosses ranged from 1 - 1.3 (Table 3). The slopes for R were within the above range; two of the three for S were < 1, suggesting that there may have been more heterogeneity in S and in R for response to EPN. The slope (0.79) of R shown by Wolfenbarger (1973) was flatter than those shown for this strain for the three generations (Table 3).

The number of larvae available for treatment in each cross ranged from 2,801 (R strain of generation 1) to 0 (7 crosses in generation 3. The numbers of larvae treated in each grouping were adequate to determine an LD_{50} value when they ranged from 203 to 3,414 and 53% of the LD_{50} values

were calculated from fewer than 1000 larvae. R and S strain larvae that reached the size and age at which they could be treated decreased in numbers from generation 1 to generation 3. Heavy loss of larvae occurred in their first and second stages. In generation 1, 2, and 3, we expected 9,931, 8,797, and 2,334 third stage R larvae as predicted from numbers of females X fecundity X hatch, but only 22, 9, and 8% of the expected populations, respectively, became available for treatment. We expected 16,744, 8,516, and 4,004 third stage S larvae but obtained 8, 13, and 9% in the three respective generations. The other crosses showed the same trend. The senior author used the same rearing methods prior to and after this test without any serious loss of larvae and concludes that the rearing procedure is adequate and suggests that the larval mortality could be caused by conditions other than the contact with independent of response to the insecticide.

The untreated larvae included in this test were used to simulate "refugia" described by Taylor (1983). They were returned to the strain cross or backcross. Thus, alleles of genes of these nonselected (unexposed to insecticide) insects were part of the next generation and could have been present in backcross pairings of R and S or intrastrain populations. The presence of these untreated insects did not reduce the LD_{50} values of either R or S.

The means ± SD were determined for percentage mated, fecundity, and fertility of all backcrosses and crosses for each of the three generations (Table 4). As the LD_{50} values of EPN increased, the effect on percentage mated, fecundity, and fertility did not change significantly regardless of the generation. The LD_{50} values of all strains, crosses and backcrosses were averaged. They increased 28% from the first to the second generation and 50% from the first to the third generation. No significant differences were determined for 5 doses between 24 h and 72 h mortality (Table 2).

Methyl Parathion - 1970. Wolfenbarger (1973) reported an LD_{50} value of 57.44 mg per larva (2.01 mg/g), CI 31.42 - 81.39, for progeny of a first generation from the field in 1970. This value was significantly higher than that shown for generation 1 of our present test (17.6 mg per larva), but it was significantly lower than that for generation 2 (132.52 mg per larva), (Table 5).

The LD_{50} values for the reciprocal crosses of an R strain from Mante and our S strain in generation 1 were statistically similar and did not suggest sex linkage for resistance (Table 5) as did the crosses containing R insects from Cuauhtemoc in 1968 (Table 1). The LD_{50} of the R X S cross was 3.9X less than that of the R intrastrain cross and 39.5X more than that of the S intrastrain in generation 1. The S X R was 4.5X more susceptible than the R strain and 39.5X less susceptible than the S.

Incomplete dominance for resistance to methyl parathion was shown by Whitten (1978) whose evaluations of the response of resistant strains of the tobacco budworm were similar to ours.

The LD_{50} values of the second generation for S and R, like those of the Pl's (first parent generation, differed 135.4X. While LD_{50} values of reciprocal crosses were not significantly different, those of the F2's differed 8X, and backcrosses to the R females had the highest LD_{50} values. LD_{50} values of the S strain increased 12 fold from generation 1 to generation 2, indicating the magnitude of variation which is typical for this insect.

LD_{50} values for the backcrosses of the R X S or S X R to R males and females were compared with those for the backcrosses of S males and females. Differences ranged from 6.8X to 125X. When R X S or S X R were backcrossed with the S strain, progeny were susceptible, but backcrosses with the R strain resulted in resistant progeny (Table 5).

In generation 2, slopes of R and S and 12 groupings ranged from 0.52 to 1.49. Of the 12 crosses slopes were less than 1 which equals ca. 58%. Fifty-eight percent of the values were 1.0, but all regressions were significant. Slopes of R and the reciprocal crosses were < 1.0 in generations 1 and 2, and 75% of the backcrosses to R were < 1.0 in generation 2. However, 75% of the slopes for backcrosses to S were (greater than sign) > 1.0.

Larvae of R and S treated with the same doses of methyl parathion in 1968 and 1970 (Table 2) killed the strains similarly when treated with the same dose.

A "t" test (P 0.05) showed that the percentage of females that mated and their fecundity did not differ between strains or among crosses (Table 6). Fertility of the R strain was significantly greater $t = 52.79$; df 5; $P = < 0.001$, than that of the S strain and the S X R cross in generation 1, but fertility of R and R X S did not differ significantly. The fertility of R, S, and crosses did not differ in generation 2.

Discussion

Our data suggest that the R strains collected from two sites in Mexico were heterogeneous for response to both methyl parathion and EPN (Tables 1, 3, and 5). Slopes for methyl parathion-treated generation 1 R strains were 2.11 and 0.92 in 1968 and 1970, respectively, and 1.15 for EPN in 1969. We suggest that the variation in these slopes was normal, and that it is not possible to predict with great accuracy the response levels of field-collected R strains methyl parathion or EPN.

Co-dominance of R and S alleles for the expression of resistance or susceptibility was evident in two of the three F1 populations with EPN-1969 or methyl parathion (1970).

The male moth is homogametic (XX) chromosomes) and the female is heterogametic (XO chromosomes). In 1968, resistance mechanisms to methyl parathion were associated with the male; thus, we conclude that there were gene(s) for resistance on the X chromosome for this strain. This is the first time resistance to methyl parathion in a strain of the tobacco budworm has been reported to be sex-linked.

In generation 1, the LD_{50} values of S strain were 0.21, 0.83 and 0.12 mg per larva for those larvae treated with methyl parathion in 1968, EPN in 1969, and methyl parathion in 1970, respectively. In separate tests conducted with this S strain, Wolfenbarger (1973) reported LD_{50} values for methyl parathion and EPN to be 1.52 and 1.89 mg per larva, respectively, in 1970. Thus we see > 10-fold differences in LD_{50} values to these insecticides to an inbred S strain which we consider to be as homozygous as any other S strain.

Wolfenbarger et al. (1984) reported an LD_{50} value of 128.6 mg per larva for methyl parathion to tobacco budworms from the LRGV during a growing season in which field control was only 12 percent. However, that high value was exceeded by resistant insects from Mante when intra-strain crossed, crossed with a susceptible strain, or back-crossed (R X (S X R)).

No field strain of tobacco budworm has been proven to be homozygous for its response to methyl parathion and EPN. Thus, these results are not a genetic analysis of response of methyl parathion and EPN of the tobacco budworm but they do indicate a quantitative response exhibited when different field collected strains are crossed with a laboratory strain. LD_{50} values of all 4 strains tested increased with selection pressure from either insecticide each generation. Back-crosses did not always respond as expected. In the future, we need to determine proportions of the different response levels within populations by single pairs. These results need to be determined within a defined area for three months, a normal cotton growing season. If the proportion that is resistant is 0.8 field control may not be obtained; if the proportion is 0.2 field control may be obtained. Also, the use of a particular dose with organophosphorus insecticides is not possible because our susceptible strain did not respond consistently and this occurred here with methyl parathion and EPN. Even if one insecticide such as methyl parathion indicate differences in response of sexes from one strain to another then strains negate the use of particular doses.

There are certain problems with mass pairings of moths of the tobacco budworms which were performed. For example, the inclusion of singly- and multiply-mated females is complicated by the differences in male contribution, but the results, despite the variation, suggest a continuum for each biotic factor as well as response of this insect to an insecticide. We suggest that this biotic variation is consistent with this species and this was previously shown by Robinson & Wolfenbarger (1978) for many field populations of this species.

We used the 1:1 ratio of males to females because Guerra et al. (1972) determined that four pairs per 3.78 liter (do you mean 3.78 liter capacity container?) were more fecund and percentage mated was significantly greater than when 1, 2, and 8 pairs (1:1 ratio males to females) were held in the same 3.78 liters of space. By ratio calculation, 2.5 pairs would be the optimum number for 3.78 liter containers, but few of our crosses were made with 2 or 3 pair. What role the densities used played is unknown.

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Table 1. Toxicity after 48 h by methyl parathion topically applied to crosses and backcrosses of a resistant (R) strain of tobacco budworms from Estacion Cuauhtemoc, Tamaulipas, Mexico and a susceptible (S) strain from Brownsville, Texas, U.S.A., 1968.

Strains crosses and backcrosses	Number pairs moths	Number larvae tested	Slope ± SE
	Generation 1		
R	40	790	2.11 ± 0.18
S	20	520	2.14 ± 0.31
S X R	15	231	0.29 ± 0.081
R X	14	19	0.69 ± 0.21
	Generation 2		
R	31	175	0.92 ± 0.12
S X R	15	176	1.00 ± 0.22
R X S	25	240	0.16 ± 0.095
S X (R X S)	16	75	4.58 ± 0.32
(R X S) X S	18	209	2.20 ± 0.15
S X (S X R)	20	175	1.80 ± 0.23
(S X R) X R	10	143	1.23 ± 0.1
R X (S X R)	9	13	0.54 ± 0.15
R X (R X S)	7	156	0.69 ± 0.17
(R X S) X R	11	91	1.03 ± 0.12
	Generation 3		
R	12	510	0.69 ± 0.12
(S X (S X R)) X S	14	175	1.77 ± 0.12
((S X R) X S)	7	93	0.15 ± 0.12
Intrastrain Mated ((R X S) X R)	5	173	0.46 ± 0.092
Intrastrain Mated ((S X R) X R)	8	93	0.20 ± 0.17
Intrastrain Mated (R X (R X S) X R	5	112	0.21 ± 0.15
	Generation 4		
R	4	73	0.32 ± 0.20

Table 1 (cont"d

Strains crosses and backcrosses	LD_{50} (µg/larva)	(95%Confidence Interval)
	Generation 1	
R	6.90	(5.43-8.58)
S	0.21	(0.00066-0.63)
S X R	60.92	(18.59-3766.0)
R X S	1.45	(0.92-2.23)
	Generation 2	
R	23.45	(17.16-32.32)
S X R	3.43	(1.69-6.0)
R X S	11.44	(7.15-34.89)
S X (R X S0	0.49	(0.000046-1.54)
(R X S) X S	0.27	(0.0097-0.66)
S X (S X R)	2.09	(1.49-2.80)
(S X R) X R	23.45	(16.0-43.19)
R X (S X R)	18.02	(9.44-47.48)
R X (R X S)	2.38	(1.43-3.43)
(R X S) X R	9.72	(6.58-14.3)
	Generation 3	
R	30.61	(20.89-44.90)
(S X (S X R)) X S	0.51	(0.29-0.8)
((S X R) X S)	12.5 µg/larva killed 74%	
Intrastrain Mated ((R X S) X R)	0.77	(21.74-365.22)
Intrastrain Mated ((S X R) X R)	6.25 µg/larva killed 86%	
Intrastrain Mated (R X (R X S) X R	3.125 µg/larva killed 21%	
	Generation 4	
R	100.0 µg/larva killed	

Table 2. Difference in percentages kill by methyl parathion and EPN in 24 h versus 72 h of a field collected (R-Estacion Cuauhtemoc and Mante) and a susceptible (S) Brownsville laboratory strain of the tobacco budworm in generation 1[a/].

Methyl parathion R S (Collected in 1968) µg/larva			EPN R S (Collected in 1969) µg/larva			Methyl parathion R S (Collected in 1970) µg/larva		
0.1	78*	98	25.0	80	69	0.1	72*	91
0.05	80	97	12.5	93	75	0.05	86	89
0.025	80	96	6.25	71	71	0.025	87	94
0.0125	40*	84	3.125	80	73	0.0125	54*	95
0.00625	0*	89	1.56	63	61	0.00625	0*	92

a *Asterisk indicates significance by LSD (t; 2 df; P = 0.05) for R versus S

Table 3. Toxicity after 48 h of EPN by topical application to crosses (intrastrain mating) and backcrosses to a resistant (R) strain from Estacion Cuauhtemoc, Tamaulipas, Mexico, and a susceptible (S) strain from Brownsville, TX of the tobacco budworm. 1969-70.

Strains crosses or backcross	Number crosses	Number pairs	Number insects tested
	Generation 1		
R		26	2801
S		50	1369
RXS and SXR	2	40	3414
	Generation 2		
R		56	870
S		60	1066
RXS and SXR	2	90	1704
RX(RXS and SXR)	4	177	3383
SX(RXS and SXR)	4	195	3012
	Generation 3		
R		21	203
S		20	359
RXS and SXR		22	272
RX(RX(RXS and SXR)	6	13	413
SX(SX(RXS and SX (RXS and SXR)XS	6	40	826
Intrastrain mate SX(RXS and SXR)XR	7	52	1188
Intrastrain mat ((RXS and SXR)XR)XR	7	42	871
((RXS and SXR)XS)XS	7	39	563
	7	67	1255

Strains crosses or backcrosses	% insects untreated	Slope ±SE
	Generation 1	
R	6	1.15 ± 0.082
S	11	0.87 ± 0.18
RXS and SXR	13	0.84 ± 0.19
	Generation 2	
R	10	1.05 ± 0.07
S	5	0.7 ± 0.17
RXS and SXR	15	0.84 ± 0.09
RX(RXS and SXR)	14	0.78 ± 0.13
SX(RXS and SXR)	16	1.02 ± 0.18
	Generation 3	
R	15	1.25 ± 0.21
S	9	1.16 ± 0.19
RXS and SXR	10	1.30 ± 0.088
RX(RX(RXS and SXR)	9	0.91 ± 0.15
SX(SX(RXS and SXR) (RXS and SXR)XS	9	1.09 ± 0.25
Intrastrain mated SX(RXS and SXR)XR	12	1.07 ± 0.17
Intrastrain mated ((RXS and SXR)XR)XR	11	1.05 ± 0.27
((RXS and SXR)XS)XS	10	1.08 ± 0.16
	11	1.01 ± 0.12

Table 3 (cont'd)

Strains crosses or backcross	LD_{50} (μ/larva)	(95% Confidence Interval)
	Generation 1	
R	6.87	(5.2 - 9.1)
S	0.83	(0.2 - 4.1)
RXS and SXR	2.15	(1.40 - 3.15)
	Generation 2	
R	6.88	(5.5 - 8.7)
S	3.39	(0.7 - 9.5)
RXS and SXR	3.21	(3.43 - 10.58)
RX(RXS and SXR)	3.43	(2.20 - 7.72)
SX(RXS and SXR)	4.29	(3.44 - 6.89)
	Generation 3	
R	10.6	(6.87 - 14.59)
S	3.72	(1.65 - 6.86)
RXS and SXR	3.70	(2.49 - 5.14)
RX(RX(RXS and SXR)	38.9	(14.87 - 276.85)
SX(SX(RXS and SXR)	6.0	(4.86 - 7.43)
(RXS and SXR)XS Intrastrain mated	8.87	(6.57 - 13.44)
SX(RXS and SXR)XR Intrastrain mated	5.9	(4.29 - 7.72)
((RXS and SXR)XR)X	2.5	(0.57 - 6.89)
((RXS and SXR)XS)XS	0.40	(3.43 - 0.46)

Table 4. Biotic response of three generations of resistant and susceptible strains, crosses and backcrosses of these strains of tobacco budworms topically treated with EPN. Cuauhtemoc, Tamaulipas, Mexico. 1969-1970.

Parameter	Response in Generation First (4 Crosses)	95% Confidence Interval Lower	Upper
Percentage mated	78.3 ± 19.5	70.3	85.9
Eggs/female	265.2 ± 189.2	189.2	340.9
Percentage hatch	62.8 ± 19.9	44.9	60.8
Number moths/cross	29.0 ± 14.0		

Table 4 Cont'd).

Parameter	Response In Generation Second (12 crosses)	95% Confidence Interval Lower	Upper
Percentage mated	89.0 ± 9.0		
Eggs/female	221.1 ± 81.91	170.9	237.3
Percentage hatch	62.8 ± 15.3	57.7	79.7
Number moths/cross	48.2 ± 6.46		

Table 4 (cont'd)

Parameter	Response in Generation Third (27 crosses)	95% Confidence Interval Lower	Upper
Percentage mated	79.0 ± 26.0		
Eggs/female	163.8 ± 101.9	128.6	199.1
Percentage hatch	60.1 ± 29.7	49.8	70.4
Number moths/cross	9.3 ± 4.6		

Table 5. Toxicity after 48 h of methyl parathion by topical application to stage larvae of crosses of a resistant (R) strain from Mante, Tamps., Mexico and a susceptible (S) strain from Brownsville, Texas, U.S.A. 1970-1971.

Strain cross or-back-crosses	No. of pairs per cross	No. of larvae treated	Slope ± SE
		Generation 1	
R	15	210	0.92 ± 0.17
S	12	196	0.53 ± 0.12
RXS	17	320	0.93 ± 0.072
SXR	18	879	0.76 ± 0.20
		Generation 2	
R	15	742	0.97 ± 0.18
S	12	370	1.01 ± 0.061
RXS	13	280	0.63 ± 0.11
SXR	7	421	0.57 ± 0.2
(RXS)	25	720	0.74 ± 0.21
SX(RXS)	20	321	1.06 ± 0.31
(RXS)XR	31	431	1.22 ± 0.10
(RXS)XS	17	275	0.52 ± 0.019
RX(SXR)	21	432	0.64 ± 0.17
SX(SXR)	10	312	1.49 ± 0.21
(SXR)XR	12	517	0.82 ± 0.17
(SXR)XS	11	436	1.34 ± 0.091

Table 5 (cont'd)

Strain cross or-back-crosses	LD50 (μg/larva)	(95% Confidence Intervals)
		Generation 1
R	17.60	(1.4 - 27.3)
S	0.13	(0.02 - 0.3)
RXS	4.48	(2.9 - 8.2)
SXR	3.90	(2.9 - 5.5)
		Generation 2
R	132.52	(86.8 - 242.6)
S	1.76	(1.3 - 2.4)
RXS	293.93	(43.0 - 2608.9)
SXR	36.58	(27.4 - 50.7)
RX(RXS)	94.44	(56.3 - 207.4)
SX(RXS)	10.20	(6.9 - 14.4)
(RXS)XR	85.87	(60.0 - 138.3)
(RXS)	0.62	(0.3 - 1.1)
RX(SXR)	152.28	(100.0 - 275.7)
SX(SXR)	1.90	(1.5 - 2.4)
(SXR)XR	67.20	(46.5 - 108.2)
(SXR)XS	1.75	(1.4 - 2.2)

Table 6. Biotic response of two generations resistant and susceptible strains, crosses, and backcrosses of strains of tobacco budworms topically treated larvae with methyl parathion. Mante, Tamaulipas, Mexico. 1970-1971.

Strain Cross or Backcross	No. of Crosses (4 pairs each)	Biotic Response: Percentage pairs mated	Biotic Response: No. of ± SD eggs per female
		Generation 1	
R	6	71.0 ± 18.8	251.1 ± 41.9
S	6	79.0 ± 24.6	224.5 ± 142.3
SXR	6	79.0 ± 24.6	434.8 ± 300.7
RXS	6	83.0 ± 12.9	150.2 ± 82.1
		Generation 2	
R	3	91.7 ± 14.4	297.0 ± 84.0
S	3	83.3 ± 14.4	281.9 ± 100.3
(RXS) XR	3	91.7 ± 14.4	287.0 ± 50.0
(RXR) XS	3	91.7 ± 14.4	281.6 ± 122.9
RX (RXS)	3	100.0 ± 0.0	228.7 ± 79.8
SX (RXS)	3	100.0 ± 0.0	266.7 ± 132.7
(SXR) XR	3	75.0 ± 43.3	511.7 ± 170.0
(SXR) XS	3	91.7 ± 14.4	207.8 ± 60.4
RX(SXR)	3	91.7 ± 14.4	301.5 ± 28.1

Table 6. (cont'd)

Strain Cross or Backcross	No. of Crosses (4 pairs each)	Biotic Response: Percentage Hatch
		Generation 1
R	6	70.0 ± 17.5
S	6	44.0 ± 16.6
SXR	6	45.0 ± 16.0
RXS	6	53.0 ± 23.3
		Generation 2
R	3	70.0 ± 5.6
S	3	66.0 ± 13.2
(RXS) XR	3	77.0 ± 7.6
(RXR) XS	3	78.0 ± 6.7
RX (RXS)	3	74.0 ± 11.5
SX (RXS)	3	80.0 ± 3.5
(SXR) XR	3	53.0 ± 20.4
(SXR) XS	3	77.0 ± 7.7
RX (SXR)	3	57.0 ± 30.0