The *Journal of Cotton Science* 2:117-120 (1998)
http://journal.cotton.org, © The Cotton Foundation 1998

ARTHROPOD MANAGEMENT

Methomyl Resistance in Strains and Crosses of Tobacco Budworm: Degree of Dominance and Patroclinous Effects

D.A. Wolfenbarger* and D.J. Wolfenbarger

INTERPRETIVE SUMMARY

The heritability of response to methomyl was determined for a strain of tobacco budworm field-collected near Brownsville, Texas. Matings (brother-sister) of the field-collected strain to a reference strain produced larvae that responded much like the field-collected parent. Data indicated that inheritance of response to methomyl was dominant. The response levels of both the crosses found in generation one were the same in the second generation.

Larvae produced by matings of males of the field-collected strain and females of the reference strain were less susceptible to methomyl than were larvae produced by mating of males of the reference strain to females of the field-collected strain, indicating a possible linkage to the male of the field-collected strain (i.e. patroclinous effect). A previous study using the same methodology on a different field-collected strain of the tobacco budworm did not suggest this linkage to the male of the field-collected strain. This indicates that there are different modes of inheritance for response of insects from the field to methomyl among different strains of the tobacco budworm.

These results suggest that methomyl should not be used on a population of tobacco budworms against which it had performed poorly the previous generation. Increased tobacco budworm susceptibility should not be expected, even if moths of the strain collected from the field have mated with moths that are susceptible to methomyl. A shift should be made to another class of insecticide.

55 Calle Cenizo, Brownsville, TX 78520. Received 24 Dec. 1997. *Corresponding author (D59d@aol.com).

Abbreviations: LD_{50}, lethal dose for 50% of test insects; F_1, first filial generation; F_2, second filial generation.

ABSTRACT

A field-collected (field) and a susceptible reference strain of tobacco budworm [*Heliothis virescens* (F.)] were crossed to determine mode of inheritance for their response to methomyl (Lannate). The field strain was not selected because we wanted to determine the mode of inheritance of a non-selected strain. Our results were different from previous results on the inheritance of response to this insecticide with other strains of this insect. Methomyl was applied topically to progeny of the field and susceptible strains and crosses of them. LD_{50} values (as µg methomyl/larva) were determined after 48 h. Probit analysis was used to calculate 95% confidence intervals and slopes of regression. The LD_{50} value of the field strain was significantly greater (17-fold) than that of the susceptible strain. The LD_{50} values of both reciprocal crosses of F_1 and F_2 were not significantly different from the LD_{50} of the field strain, indicating that response to methomyl by this field strain was dominant. The LD_{50} of susceptible x field (female listed first) in the F_1 reciprocal cross was significantly greater than the LD_{50} of field x susceptible, indicating a possible sex linkage. Results indicate a possible patroclinous effect.

Variation in response to methomyl by field-collected strains of the tobacco budworm in Mexico and the United States has been shown by Roush and Wolfenbarger (1985) and Wolfenbarger et al. (1987), respectively. In 1972, Wolfenbarger (1973) reported that field-collected strains from Mexico and reference susceptible strains of this insect were equally susceptible to methomyl. Roush and Wolfenbarger (1985) further showed that the inheritance of resistance to methomyl by crosses of a field-collected strain from Torreon, Coahuila, Mexico and another reference strain was due to a single, autosomal, incompletely dominant gene.

In this study, a field strain (field-collected near Brownsville, Texas) and the same reference strain used by Wolfenbarger (1973) were crossed. Roush and Wolfenbarger (1985) reported the inheritance pattern of the F_1 offspring was compared to that of similar crosses. The same methods were used in both experiments.

MATERIALS AND METHODS

Technical methomyl (>96% purity) was obtained from DuPont, Inc., Wilmington, Delaware. The susceptible reference strain has been reared at the USDA laboratory in Brownsville since 1968. The field strain was reared without selection from 43 larvae collected from cotton near Brownsville in July 1974. The test was initiated in September with adults reared for one generation to ensure adequate insects. Throughout the test, larvae were reared on soybean-based artificial diet to pupation. Pupae of each strain and cross were sexed and maintained separately. Emerging moths of the field and susceptible strains were crossed reciprocally for the F_1 generation (the female listed first in each cross). Each strain was intra-strain mated. Depending on the number of adults available, 10 to 20 randomly selected pairs were placed in 3.78 L containers with cheesecloth coverings for oviposition at 23±4°C. Covers were removed daily. Eggs were held for 2 to 3 days for eclosion. One or two containers were used for each cross or strain each generation.

Single neonate larvae were placed in 22 mL plastic cups containing about 10 mL diet and held at 27°C. Larvae of each strain or cross were treated in about 5 days when they weighed 20 to 30 mg. Larvae were treated in another location at 25±4 °C. Care was taken to ensure the larvae were not molting at the time of treatment.

Larvae of crosses that survived treatment each generation were reared to pupation for brother-sister matings to produce the F_2 generation. This was done to determine the amount of variation between F_1 and F_2 generations.

Three to eight doses of methomyl, which ranged from 0.155 to 20 µg/larva, were diluted in 1 µL of reagent grade acetone. Doses were applied with a microapplicator to the dorsum of the thorax of 35 to 300 larvae/dose (two to five replicates conducted on different days) and mortalities were determined after 48 h. Control mortality of the field strain was not determined, but a dose of 0.0095 µg/larva was tested on 75 larvae. Control mortality of the susceptible strain was not determined because no disease was present. Doses were selected to provide an LD_{50} (as µg/larva). Slope of the regression and 95% confidence interval were obtained by probit analysis (SAS Institute,1988). The degree of heterogeneity for the regression was determined by chi-square (X^2) by the same probit analysis.

Larvae that survived the bioassay from each reciprocal cross were reared to pupation on artificial diet for brother-sister matings of the F_2 generation. This was done to determine the amount of variation caused by segregation that occurred between F_1 and F_2 of each reciprocal cross.

A significant difference in LD_{50} values among strains, crosses, and generations was indicated when 95% confidence intervals did not overlap. Georghiou and Garber (1965) described calculations of the expected mortality for 0.155, 0.625, and 5 µg methomyl/larva in F_2 to show effects at low, medium, and high doses in the second and first generations. The differences between expected and observed mortalities were then determined by X^2 (tested for significance at $P_{0.05}$ for 1 degree of freedom). Degree of dominance (D) was determined by the methods of Stone (1968). When D = 0, the heterozygote had the same degree of susceptibility to the field strain as it did to the susceptible strain. D = 1 was indicative of complete dominance and 0 < D > 1 of incomplete dominance.

RESULTS AND DISCUSSION

The LD_{50} value for field strain was significantly greater (17-fold) than the LD_{50} of the susceptible strain (Table 1). The LD_{50} of the field strain was not significantly different from the LD_{50} values for the F_1 and F_2 reciprocal crosses (Table 1). Inheritance of methomyl resistance is considered to be dominant. In a previous study, Roush and Wolfenbarger (1985) showed an incomplete dominance or co-dominance with their strains. The strains used in this study produced different results. Using Stone's calculations(1968), we determined that the degree of dominance for the F_1 of susceptible x field was 0.81 and that for the field x susceptible was 0.4. Dominance was expressed more strongly in the F_1 of susceptible x field than shown by field x susceptible as evidenced by the non-overlapping 95% confidence interval (Table 1).

Table 1. Toxicity of methomyl to 20 to 30 mg larvae of a field- collected (F) and susceptible (S), reference strain and crosses of tobacco budworm. Measurements made 48 h after exposure. Brownsville, TX 1974.

Dose	Strain		F_1		F_2	
(µg/larva)	F	S	F x S	S x F	F x S	S x F
% Mortality						
20				90	83	
10		97	90	80	84	
5	65	96	85	75	63	77
2.5		93	82	69	55	64
1.25		88	64	57	51	52
0.625	53	89	63	54	48	43
0.31		70	40	38	26	31
0.155	23	69	35	27	39	31
Treated larvae/dose						
	35	90	81	300	200	200
LD_{50}						
	0.92	0.054	0.4	0.7	0.96	1.05
95% confidence interval						
	0.44-3.54	0.022-0.096	0.27 - 0.54	0.59 - 0.82	0.78 - 1.18	0.85 - 1.41
Slope						
	0.79	0.94	0.91	0.82	0.72	0.65
Heterogeneity of regression line						
	2.2	5.2	5.7	8.4	12.9†	34.3†

† Significant at 5% level of probability by X^2.

The LD_{50} value of susceptible x field in F_1 was significantly and < two-fold greater than that exhibited by the field x susceptible. A patroclinous effect (i.e., sex) is suggested for offspring of the susceptible x field cross in the F_1. Perhaps there are factors on the male chromosomes that contribute to this dominance. A patroclinous effect was not evident in the crosses of the strains that Roush and Wolfenbarger (1985) used. No larvae of the field strain treated with 0.0095 µg/larva died in 48 h or at pupation. Thus, a disease, which would alter the results, was not evident.

The F_1 progeny of this non-selected strain are heterozygotes. It is not known what would have happened to genes that affect biotic or other factors if we had used a selection regime. We suggest that no field-collected strain of tobacco budworm is homozygous for resistance to any insecticide.

Firko and Wolfenbarger (1991) determined expected mortalities for one, two, four, and eight genes. Little difference in mortalities was shown for this number of genes so no attempt was made to indicate the number of genes involved in our strains.

The LD_{50} of the field strain was 1576-fold less than that shown for larvae of the field strain that Roush and Wolfenbarger (1985) used. Also, 80% of the 46 field strains of tobacco budworm that Wolfenbarger et al. (1987) tested had greater LD_{50} values than shown in this study. It is unknown what this difference means in the interpretation of these mode of inheritance results for methomyl. Only further crossings of other strains and testing will elucidate what levels of response are needed to conduct mode of inheritance experiments with this insect. There was no significant difference in LD_{50} values of our reference strain and those from St. Croix-Virgin Islands that Roush and Wolfenbarger (1985) used.

Firko (1991) stated that inheritance mechanisms that make an F population resistant may be polygenic. This may be shown here as F_1 reciprocal crosses show dominance for resistance and a possible sex linkage for the male. We suggest that each is controlled by alleles of different genes. Reduced sensitivity at the site of action, activity of various enzymes, reduced penetration of the cuticle, and increased excretion rate of the insecticide by the field strains are a few mechanisms that could be involved in the tolerance or elevated LD_{50} values to methomyl -- even if a small proportion of the population contributed a dominant allele for each of these mechanisms. These mechanisms could be present in reference strains but at a lower incidence .

The regression lines for the field and susceptible strains and both reciprocal crosses in F_1 were homogeneous as indicated by a non-significant X^2 (Table 1). Observed mortalities did not differ from expected mortalities. Curves for both F_2 crosses were heterogeneous. These results suggest there was variation for response to this insecticide that was greater in the F_2 than the F_1.

To determine differences between expected (data not shown in table) and observed mortalities for three doses in the F_2 reciprocal crosses, we used calculations of Georghiou and Garber (1965) for expected mortalities compared to mortalities in the parents and the F_1 (Table 1). This was done to further validate the amount of variation by another method of heterogeneous crosses. In the field x susceptible cross, expected and observed mortalities for 5 and 0.625 µg/larva were significantly different; expected mortalities were 83 and 67%; and X^2 was 4.8 and 5, respectively. Doses were selected for the calculations at the top, middle, and bottom of those tested because we wanted to determine the consistency of similarity of response. At 0.155 µg/larva, expected and observed mortalities were not significantly different; expected mortality was 41%; and X^2 was 0.1. In the susceptible x field cross, expected and observed mortalities for 5 and 0.155 µg/larva were not significantly different; expected mortalities were 78 and 36%; and X^2 was 0.01 and 0.5, respectively. At 0.625 µg/larva, expected and observed mortalities were significantly different; expected mortality was 63%; and X^2 was 5. This method of calculation shows that at the different doses there is variation in response.

CONCLUSIONS

Results indicate that methomyl resistance was dominant. The LD_{50} value for the field strain was significantly greater (by 17-fold) than that for a susceptible strain. LD_{50} values of both reciprocal crosses in the F_1 and F_2 were not significantly different from the LD_{50} for the field strain, but were significantly greater than the LD_{50} for the susceptible strain. Methomyl resistance in this study was dominant and indications of sex linkage and polygenic resistance factors were present.

REFERENCES

Firko, M.J. 1991. Insecticide resistance: The myth of single gene inheritance. p. 651–653. *In* D.J. Herber (ed.) Proc. Beltwide Cotton Insect and Control Conf., San Antonio, TX. 8–13 Jan1991. Natl. Cotton Council Am., Memphis, TN.

Firko, M.J., and D.A. Wolfenbarger. 1991. Tolerance to cypermethrin in Texas and Mississippi tobacco budworm: Genetic analysis and relationships between LD_{50} estimates and observation time. p. 657–659. *In* D. J. Herber (ed.) Proc. Beltwide Cotton Insect and Control Conf., San Antonio, TX. 8–13 Jan. 1991. Natl. Cotton Council Am., Memphis, TN.

Georghiou, G.P., and M.J. Garber. 1965. Studies on the inheritance of carbamate-resistance in the housefly (*Musca domestica* L.). Bull. World Health Organ. 32:181–196.

Roush, R.T., and D.A. Wolfenbarger. 1985. Inheritance of methomyl resistance in the tobacco budworm (Lepidoptera:Noctuiidae). J. Econ. Entomol. 78:1020–1022.

SAS Institute. 1988. Probit analysis. Additional SAS/SAT Procedures. P-179. Release 6.03. SAS Institute, Inc., Cary, NC.

Stone, B.F. 1968. A formula determining degree of dominance in cases of monfactorial inheritance of resistance to chemicals. Bull. World Health Organ. 38:325–326.

Wolfenbarger, D.A. 1973. Tobacco budworm: Cross resistance to insecticides in resistant strains of the tobacco budworm and in a susceptible strain. J. Econ. Entomol 61:37–40.

Wolfenbarger, D.A., S.M. Mayeux, R.J. Palazzo, J.B. Graves, J.R. Raulston, and L. Guerra-Sobrevilla. 1987. Bollworm and tobacco budworm: Susceptibility to methomyl after a decade of use. J. Agric. Entomol. 4:141–152.