INHERITANCE OF RESISTANCE TO PERMETHRIN BY THE TOBACCO BUDWORM, *HELIOTHIS VIRESCENS* (F)[1]: IMPLICATIONS FOR RESISTANCE MANAGEMENT

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ABSTRACT

A laboratory selected permethrin resistant strain of tobacco budworm, *Heliothis virescens* (F), was crossed with a susceptible strain to determine the nature of inheritance of the resistance. Crossing of these highly resistant and highly susceptible strains showed susceptibility to permethrin to be autosomal and incompletely dominant. Backcrosses of F_1 progeny with resistant males indicated either that more than one gene is responsible for the resistance in this strain, or that the strain was not homozygous for resistance. It is likely that more than one locus is influencing permethrin resistance. The crosses and backcrosses performed provided relevant information for resistance management in the field.

INTRODUCTION

The tobacco budworm, *Heliothis virescens* (F.), has long been a major pest of field crops in the Americas (Wolfenbarger *et al.* 1981) and in Arizona was first reported as a pest of cotton in 1972 (Watson 1974). Subsequently, it has been a sporadic pest in the major cotton-producing areas of the state with heavy, widespread populations occurring in 1976, 1977 and 1978.

During the initial tobacco budworm (TBW) outbreak in 1972 Lentz *et al.* (1974) obtained baseline data for susceptibility to several commonly-used insecticides, and found the population to be resistant to the organophosphates. In 1976, Crowder *et al.* (1979) established baselines for the synthetic pyrethroids, fenvalerate (benzeneacetic acid, 4-chloro-alpha-(1-methylethyl), cyano (3 phenoxyphenyl) methylester) (Pydrin®, Shell Chemical Co.), and permethrin (3-(Phenoxyphenyl) methyl (+) cis-trans-3-(2,2-dichloroethenyl),-2,2-dimethyl-cyclopropanecarboxylate) (Pounce®, F.M.C.; Ambush®, I.C.I. Americas, Inc.). Since then, field-collected populations have been monitored to determine susceptibility to methyl parathion and permethrin at the end of each growing season (Watson *et al.* 1986, Watson and Kelly 1991).

In 1980, a laboratory culture was established using TBW from Maricopa Co., AZ., to initiate studies on selection for permethrin resistance. Selection through the first 12 generations demonstrated the capacity of the TBW to develop resistance to pyrethroid insecticides (Jensen *et al.* 1984). Continued selection resulted in dramatic increases in the LD_{50}'s. Because of the capacity of TBW and other *Heliothis* spp. to develop resistance to pyrethroid insecticides in the laboratory, and because of control failures in the field (Gunning *et al.* 1984, Plapp and Campanhola 1986) interest has been generated in finding ways to manage resistance in the field (Anonymous 1984, Luttrell and Roush 1987, Plapp 1987).

The inheritance of resistance to pyrethroids in Lepidoptera has not been studied as extensively as in Diptera. A 700-fold resistance to fenvalerate in the diamondback moth, *Plutella xylostella* (L.) was autosomal, incompletely recessive, and due to more than one gene (Liu *et al.* 1981). Resistance to pyrethroids in *Heliothis armigera* in Australia was incompletely dominant and autosomal, but the number of genes responsible for the resistance was not conclusively demonstrated (Daly 1988, Gunning and Easton 1987). Roush and Luttrell (1987) crossed pyrethroid resistant and susceptible TBW and found the susceptible trait to be

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[2]Ariz. Coll. Agric., Agric. Exp. Sta. Jour. Ser. No. 7242

incompletely dominant, however no attempt was made to further elucidate the genetics in this case. Payne *et al.* (1988) conducted the most thorough study of the inheritance of pyrethroid resistance in TBW. They utilized several resistant strains, two of which were subjected to a crossing scheme to produce homozygosity for resistance. Reciprocal crosses demonstrated that susceptibility was autosomal and incompletely dominant, and backcrossing of the F_1 to a resistant parental strain indicated that a single gene is responsible for the resistance.

Knowledge of the inheritance of resistance is essential to the development of any sound resistance management strategy. The pyrethroid resistant strain developed in our laboratory attained a level of resistance much higher than any of the strains utilized by the above researchers, and was believed to be approaching homozygosity for resistance. Therefore, studies were initiated in 1985 to determine the inheritance of resistance to permethrin in the TBW. The information generated in these studies could be utilized in resistance management strategies.

MATERIALS AND METHODS

Strains. The susceptible strain (S) used in this experiment was obtained from the USDA Western Biological Control Laboratory in Tucson, AZ, and was maintained in our laboratory for the duration of this experiment. This strain has been reared without exposure to insecticides since 1965. The permethrin resistant strain (PsHi=R) has been selected every generation with permethrin in the laboratory since the fall of 1980, and had attained >1000-fold resistance (LD_{50} 4000-9000μg/g vs 3-5μg/g for susceptible field strain) by the time these tests were conducted. Portions of the experiment were conducted using the F_{36}, F_{41} and F_{43} generations of the R strain.

Bioassays. Dosage mortality tests were modified from the standard method recommended by the Entomological Society of America (Anon. 1970). Third instar larvae weighing 22±6 mg were topically treated in the dorsal thoracic region with 1 μl droplets of serial dilutions of technical permethrin (98.4% purity) in acetone. Three or more replicates of 25 larvae were used at each of five to eight doses. Mortality was assessed 72 h after treatment. Controls were treated with acetone alone. No mortality was observed among the controls.

Crosses. Following eclosion of adults, reciprocal crosses [R♂xS♀(RS); S♂xR♀(SR)] were made in 3.8 L (1 gal) glass jars, with approximately 20 pairs per jar. Untreated F_1 progeny from the two crosses involving the R F_{36} generation were either mated with others from the same cross to produce F_2 generations, or separately backcrossed to S males. Untreated F_2 progeny of both RS and SR crosses were used to produce F_3 generations.

In the R F_{41} generation, R♂ were crossed with S♀ using the above technique. Some of the untreated F_1 generation were used to produce the F_2, and the remainder of the females were backcrossed with R F_{42}♂. This procedure was repeated with the R F_{43} generation, except that the initial cross was S♂ x R♀, and the backcross utilized R F_{44}♂.

Analysis of Data. Dosage mortality lines were obtained for all crosses at each generation, but not for the resistant strain during the generations in which they were being used as a parental strain. Dosage mortality results were obtained for R F_{37}, F_{39}, and F_{44}. Dosage mortality regressions were computed by probit analysis using a Fortran program to perform the calculations described by Finney (1971). LD_{50} values and slopes were compared using means, confidence intervals and standard errors.

RESULTS AND DISCUSSION

Table 1 presents dosage mortality results for the resistant and susceptible strains of TBW and the various crosses resulting from these parentages. The R F_{37} generation (that following the crossing experiments with R F_{36} generation) was highly resistant to permethrin. Reciprocal crosses of this resistant strain with the susceptible (S) strain produced F_1's with LD_{50}'s much closer to that of the S parent, but nevertheless with a 14- to 18-fold level of resistance, indicating that susceptibility apparently was inherited as an incompletely dominant trait. The F_1 generations from crosses involving the R F_{41} and R F_{43} produced similar results. There was no difference between the R♂ x S♀ and the S♂ x R♀ crosses, indicating that the gene(s) involved are not sex linked. Results similar to these were reported by Payne *et al.* (1988). Little change was noted in the LD_{50}'s of the F_1, F_2, and F_3 generations. However, when F_1♀ were backcrossed to S and R♂, LD_{50}'s were further reduced in the former and considerably increased in the latter. These are depicted graphically (using μg/larva) in Fig.'s 1 and 2 for the S♂ backcrosses and

Fig.'s 3 and 4 for the R♂ backcrosses. It should be noted that the R♂ backcrosses, as detailed in the methods section, utilized generations F_{42} for the R♂ x (RS F_1♀) backcross, and F_{44} for the R♂ x (SR F_1♀) backcross.

TABLE 1. Dosage Mortality Results for Resistant and Susceptible Strains and all Crosses.

STRAIN[a]	LD_{50}(95% CI)[b]	LD_{95}(95% CI)[b]	SLOPE(SE)	RR[c]
S	0.21(0.20-0.23)	0.59(0.51-0.68)	3.69(0.23)	1
R F_{37}[d]	5530(4850-6300)	38100(27700-52600)	1.96(0.15)	26100
R♂xS♀[e]				
F_1	2.92(2.33-3.60)	22.0(14.9-39.5)	1.88(0.13)	13.8
F_2	2.34(1.98-2.76)	39.2(24.2-63.6)	1.34(0.11)	11.0
F_3	2.73(2.29-3.30)	17.5(11.1-27.9)	2.04(0.13)	12.9
S♂xF_1♀	0.71(0.61-0.82)	3.54(2.56-4.89)	2.36(0.18)	3.3
S♂xR♀[e]				
F_1	3.86(3.03-4.84)	18.4(12.6-34.4)	2.43(0.19)	18.2
F_2	2.57(2.23-2.96)	26.0(17.0-39.8)	1.64(0.14)	12.1
F_3	3.80(3.09-4.76)	46.5(25.9-85.3)	1.51(0.10)	17.9
S♂xF_1♀	0.63(0.56-0.71)	3.46(2.77-4.33)	2.22(0.15)	3.0
R F_{39}[d]	4130(3650-4680)	31000(21500-44700)	1.88(0.16)	19500
R♂xS♀[f]				
F_1	4.87(3.92-5.88)	16.8(12.5-27.9)	3.05(0.19)	23.0
F_2	2.81(2.46-3.20)	26.2(18.9-36.2)	1.70(0.12)	13.3
R♂xF_1♀	39.8(27.7-57.2)	3670(1300-10400)	0.84(0.06)	188
R F_{44}[d]	8930(7890-10100)	61700(45900-83100)	1.96(0.14)	42100
S♂xR♀[g]				
F_1	4.89(4.50-5.32)	16.9(13.6-21.0)	3.05(0.25)	14.4
F_2	5.43(4.82-6.11)	40.1(29.1-55.0)	1.90(0.14)	25.6
R♂xF_1♀	42.7(30.5-59.9)	11200(4060-30700)	0.68(0.07)	201

[a]Crosses are grouped under the original parental cross.
[b]LD_{50} and LD_{95} values are expressed in μg insecticide/g larval weight. The 95% confidence interval is shown in parentheses.
[c]RR = resistance ratio = (LD_{50} of strain)/(LD_{50} of S).
[d]The resistant generations listed represent the closest generations to those used in the crosses for which dosage mortality results were obtained.
[e]The resistant F_{36} generation was used in this cross.
[f]The resistant F_{41} generation was used in this cross, and the F_{42} generation was used in the subsequent backcross.
[g]The resistant F_{43} generation was used in this cross, and the F_{44} generation was used in the subsequent backcross.

The backcrosses to S♂ provide little additional information. The LD_{50} is intermediate to that of the S and F_1, and the slope is steep. If monogenic inheritance were assumed, this would be expected since the progeny should be 50% homozygous susceptible (SS) and 50% heterozygous (Ss), and these phenotypes do not differ too greatly. However, this does not rule out polygenic influences.

Slopes of the log probit curves depicting the R♂ x (RS) and R♂ x (SR) backcrosses were much lower than those of either parental strain or the F_1, indicating greater heterogeneity of phenotypes, as well as increased resistance. The plateau seen by Payne *et al.* (1988) in the dosage mortality lines from their backcrosses of permethrin R x S F_1 generations with R strains, is absent in this study. A plateau is expected at 50% mortality if there is only one gene responsible for the resistance, because the genotypes of the backcross progeny should be 50% heterozygous (Ss) and 50% homozygous resistant (ss). The lack of a plateau probably indicates that more than one gene is involved in the resistance, but could also be due to a lack of sufficient homozygosity

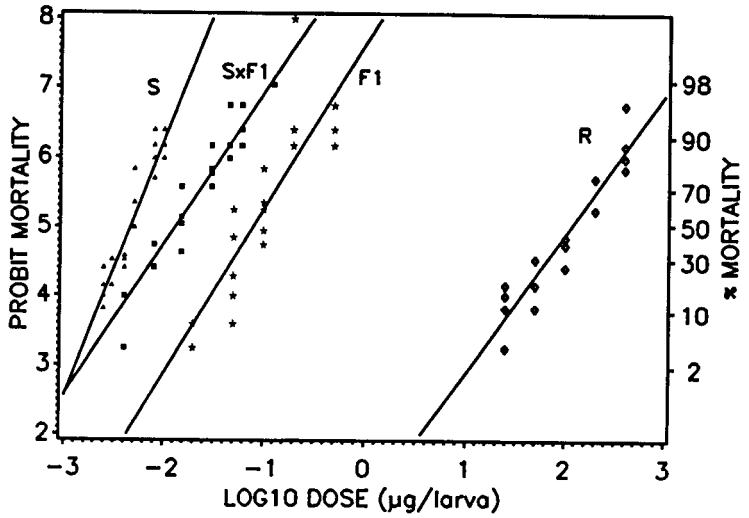

FIG. 1. Dosage mortality regression lines using permethrin against susceptible (S), resistant F_{37} (R), R F_{36}♂ x S♀ (F1) and S♂ x F_1♀ (SxF1) backcross.

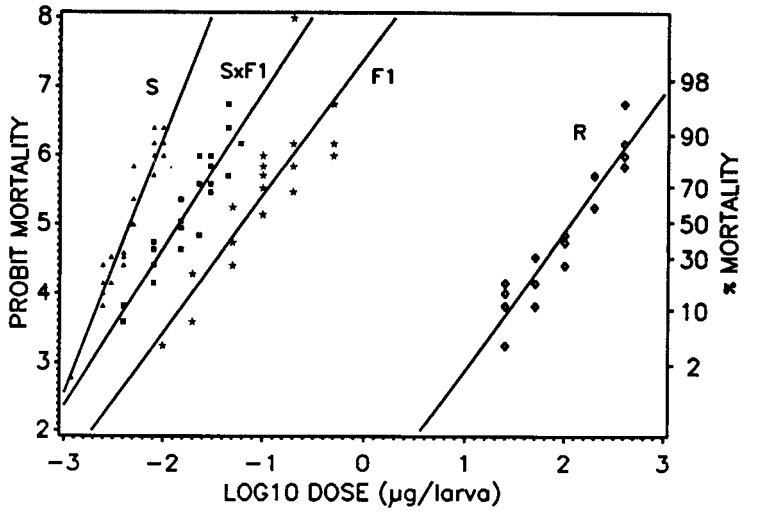

FIG. 2. Dosage mortality regression lines using permethrin against susceptible (S), resistant F_{37} (R), S♂ x R F_{36}♀ (F1) and S♂ x F_1♀ (SxF1) backcross.

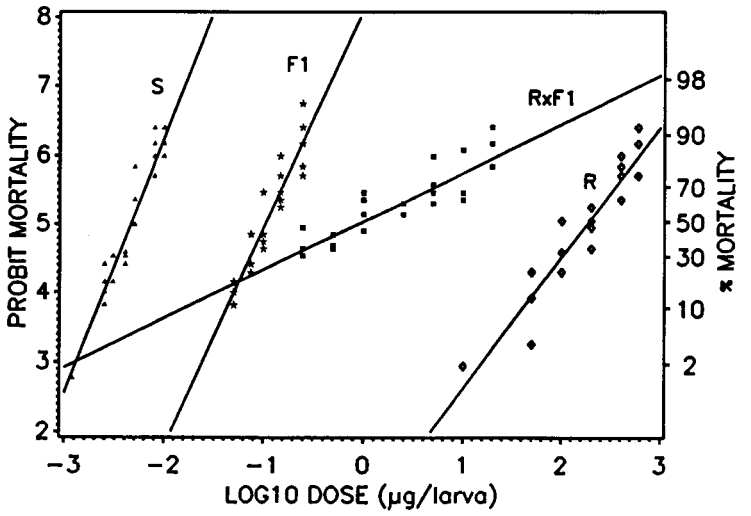

FIG. 3. Dosage mortality regression lines using permethrin against susceptible (S), resistant F_{39} (R), R F_{41}♂ x S♀ (F1) and R F_{42}♂ x F_1♀ (RxF1) backcross.

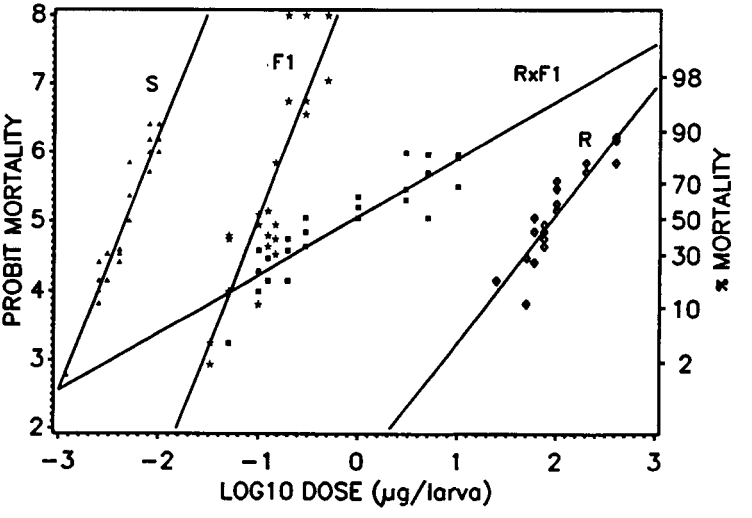

FIG. 4. Dosage mortality regression lines using permethrin against susceptible (S), resistant F_{44} (R), S♂ x R F_{43}♀ (F1) and R F_{44}♂ x F_1♀(RxF1) backcross.

in the R and S strains. In order to determine which of these factors is responsible for the lack of a plateau, a resistance purification scheme similar to that used by Payne *et al.* (1988) should be performed. This technique will produce homozygosity if there is a single gene responsible for the resistance. Furthermore, if there is one major mechanism of resistance and other mechanisms play a lesser role, the technique may produce homozygosity for the major mechanism, while eliminating some of the minor factors.

The results from the F_2 generations also do not conclusively indicate how many genes are involved in the resistance. Assuming a single gene and simple Mendelian inheritance, the F_2 would be expected to contain 25% homozygous R (ss), 25% homozygous S (SS), and 50% heterozygotes (Ss). There are no obvious inflections in the dosage mortality lines at either 25% or 75% mortality, and the mortality exceeds 80% at dosage levels far too low to produce any mortality in the resistant strain. Unfortunately, no doses yielding over 87% mortality were used, so it is unknown if any highly resistant individuals were present. It is possible that differential mortality of eggs or early instars of the resistant strain could have skewed the expected ratios. While the LD_{50} values of the F_2 generations do not differ from the corresponding F_1 values, the slopes of the F_2's are significantly lower in all cases. This indicates a greater heterogeneity of phenotypes, which is expected whether one or several genes are involved (F_2 progeny should include SS, Ss and ss genotypes for any single locus for resistance).

It is likely that there is more than one locus for resistance, possibly corresponding to more than one mechanism of resistance. Other studies we are conducting indicate that more than one mechanism of resistance may be present in this strain. These mechanisms have not yet been quantified, and it is possible that the results observed here are due to more than one mechanism playing a role in the resistance. There is evidence that target site insensitivity is a major resistance mechanism in this strain, and that metabolism may play a lesser role (Kelly 1988). This would account for the agreement of our results with those of Payne *et al.* (1988), who also found susceptibility to be incompletely dominant in strains whose major mechanism of resistance was target site insensitivity. In contrast, Daly (1988) found resistance to be incompletely dominant in strains of *H. armigera* in which increased metabolism by mixed function oxidases was responsible for the resistance.

These data show two important points that would be most relevant to insecticide resistance management, susceptibility is incompletely dominant and it is autosomal. Because the resistance is recessive, it would be easily selected, and therefore it would be important to avoid widespread treatments of successive generations with a pyrethroid. These precautions would ensure a source of susceptible individuals which could mate with pyrethroid-selected populations and thus maintain the dominant susceptible genes in local populations. While resistance developed in the field might differ from that of the laboratory selected strain examined here, this resistance management strategy could be applied at least until more specific information is obtained.

Populations usually do not develop in cotton in AZ until late summer and fall and are subjected to insecticidal pressure for a relatively short period of time, if at all. Moreover, TBW develop initially on a number of wild hosts which never receive insecticidal treatments (Rathman and Watson 1985), thereby providing a reservoir of susceptible individuals for mating with those subjected to insecticidal pressure. These phenomena probably help explain why populations of TBW in AZ are not yet resistant to the pyrethroid insecticides.

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