INHERITANCE OF CRY1AC RESISTANCE IN THE TRANSGENIC BT COTTON SELECTED STRAIN OF THE COTTON BOLLWORM, *HELICOVERPA ARMIGERA* (HÜBNER)

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Abstract

The Bt cotton selected strain of *Helicoverpa armigera* showed 82.9-fold resistance in its further selection to Cry1Ac over one generation vis-à-vis susceptible strain. Inheritance of Cry1Ac resistance was found partially dominant and autosomal on the basis of bioassay response to Cry1Ac in reciprocal cross between resistant male/female F_1. Cry1Ac was negatively correlated to Cry1Ac assay concentration. Consistent with earlier findings, resistance was recessive at high concentration of Cry1Ac. However, the dominance of resistance increased as the concentration of Cry1Ac decreased. Analysis of survival and growth of progeny from backcrosses (F_1 X resistant strain) suggested that resistance was controlled by single or a few loci. Overall the patterns observed can be explained by either a single resistance gene with three or more alleles or by more than one resistance gene.

INTRODUCTION

Lepidopteran insect protective transgenic crops with *Bacillus thuringiensis* (Bt) *Cry1Ac* gene proved highly effective against larval insect pests (Shelton *et al.,* 2002). Bt cotton is now cultivated in as many as 18 different countries over an area of 14 million acres. Bt cotton is effective against bollworm complex (Ferré and Van Rie 2002), which *Helicoverpa armigera* a major pest of cotton in India and elsewhere. *H. armigera* has shown ability to develop resistance to Cry1Ac to selection pressure under laboratory condition (Kranti, 2001 and Akhurst *et al.,* 2003).

Knowledge of inheritance of Cry1Ac resistance is important for its management. In particular, the refuge strategy for Bt cotton mandated by the United States Environmental Protection Agency is expected to work best if inheritance of resistance is functionally recessive, which means that progeny from mating between homozygous susceptible and homozygous resistant adults are killed by Bt cotton (Gould and Tabashnik 1998, Liu *et al.,* 2001a and Daly and Olsen 2004).

The present study describes the response of the Bt strain from cotton field at the peak of the infestation to additional selection with Cry1Ac, including exposed the individuals to sub lethal dose, resulting in a resistance ratio 82.9-fold resistance. Our primary objective in this study reported here was to determine the mode of inheritance of this high level of resistance to Cry1Ac, including evaluation of maternal effects, sex linkage, dominance, and the number of loci influencing resistance. To achieve this objective, we used bioassays with Cry1Ac in artificial diet to test progeny from various crosses involving the resistant strain, a susceptible strain, and their hybrid F_1 progeny.

MATERIALS AND METHODS

Insects. Larvae were reared on chickpea-based semi synthetic diet. We collected a susceptible strain from non-Bt cotton during peak of infestation and bioassay conducted to determine the LC_{50} for this population. The resistant strain larvae collected from Bt cotton field at the end of the season and then exposed to selection pressure for one generation, bioassay conducted to determine LC_{50}.

Preparation of Bt Cry1Ac Toxin. For selection and bioassays we used recombinant *E. coli* strain for *Cry1Ac* gene from Bacillus Genetic Stock Center, Ohio State University, Columbus, USA. The toxin of Cry1Ac was prepared by using procedure described by Lee *et al.,* (1995). Cells were growing in nutrient broth containing 50-µg/ml ampicillin for 72 h. Cells were harvested by centrifugation at 4500 *g* for 10 min at 4 ^{0}C and the pellet was suspended in lysis buffer (50 mM Tris, pH 8.0, 50 mM EDTA, 15 percent sucrose, lysozyme @ 2mg/ml) and incubated for 4 h. After incubation lysis buffer was replaced with Cry wash-1 solution (0.5 MNacl and 2% Triton x-100) and sonicatated for 3 min on ice. The pellet was collected by centrifugation at 4500 *g* and washed three times with Cry wash-1, three times with Cry wash-2 solution (0.5 M sodium chloride) and three times

with sterile doubled distilled water. Finally the pellet was solubilized in solubilizing buffer (50 mM sodium carbonate, 10 mM dithiothreitol, pH 10.5) at 37 ^{0}C for 6 h. Supernatant containing toxin was collected following centrifugation at 4500 *g* for 10 min and stored at -20 ^{0}C till further use. The quantification of toxin done using SDS PAGE technique (Laemmli, 1970) with 8% resolving gel, using Genei Mini Dual electrophoresis model (Bangalore Genei Pvt. Ltd. Bangalore, India). The toxin bands were identified by comparing with protein molecular weight markers. The toxin concentration was determined by comparing absorbance with BSA standard curve obtained by similar procedure.

Selection. The larvae were collected from the Bt cotton (MECH 162 Bt and MECH 184 Bt) field and were fed on normal diet till pupal stage. The progeny was exposed to diet containing 0.26 µg Cry1Ac/g for 4 days. The LC_{50} of Cry1Ac was estimated against neonates of next generation. The selection pressure was continued for further generation to maintain resistance level.

Bioassay. Bioassays were carried out at 28 ± 1 ^{0}Cand 70 - 80% relative humidity by using Cry1Ac with 10 g of artificial diet. Ten-gram pre- cooled diet was mixing well with the Cry1Ac toxin-using pestle and mortar plate for each concentration. It was divided into three units each about of (3-4g) in 33-ml plastic cups. Ten neonates (12-18 h old) were transferred in each cup using fine brush. We used six different concentrations ranging from 0.035 to 3.0 µg/g as well as control; thirty neonates have been used to each concentration with three replicates. The observations were taken at 24 h interval and the LC_{50} calculated after 96 h.

Mass Crosses. To evaluate maternal effects, sex linkage, and dominance, we tested F_1 progeny of reciprocal mass crosses between resistant and susceptible strains. Pupa was kept individually in the 33-ml plastic cups till the emergence of the adult. In one experiment 10 resistant male adults were mated with 10 susceptible female adults and the adults were sexed on the basis of their wing characters (greenish in colour for male and brownish in colour for female). Adults were allowed to mate and eggs were collected to provide neonates for subsequent bioassays. The entire experiment with F_1 progeny was performed twice at the same week of December 2003. We obtained enough progeny to

test from the cross between susceptible males and resistant females as well as the cross between susceptible females and resistant males.

To estimate the number of loci influencing resistance. We tested progeny of reciprocal mass backcrosses between the F_1 progeny and the resistant strain. We chose these backcrosses (rather than F_1 x susceptible) because F_1 differed more from the resistant strain than from the susceptible strain (Roush and Daly, 1990). Thus, this choice increased the power of tests for distinguishing among modes of inheritance (Tabashnik, 1991). In one of the crosses 10 resistant males mated with 10 susceptible females to get F_1 and its LC_{50} calculated. Subsequently, 20 males of that F_1 mated with 20 females resistant and its reciprocal cross-made. Similarity, 10 resistant females mated with 10 susceptible males to get its F_1 and LC_{50} recorded. From this F_1, 20 males mated with 20 resistant females and 20 females of F_1 mated with 20 resistant males. Addition to this, 150-larvae exposed to 3.06 µg/g) as a discriminating dose of Cry1Ac toxin from resistant strain, F_1 strain, and backcross of F_1 x resistant strain as well as F_1 x susceptible strain.

Pair Limited Cross and growth Bioassay. To evaluate the number of loci influencing resistance, we compared growth of resistant larvae F_1 progeny and backcross progeny derived from single-pair crosses. In contrast to the survival bioassays, growth bioassays conducted November- December 2003 provided evaluation of responses to Cry1Ac on a continuous scale, which is especially useful for comparing observed distributions versus those expected under different hypotheses about inheritance (Gould *et al.,* 1995). In each single-pair crops, 3 virgin adult male and 3 virgin adults female were paired in a 60-ml cup .We obtained eggs and put neonates on diet with 3.06 µg toxin/g diet. Neonates were tested individually on Cry1Ac for 96 h., rather than determining their survival at 21 d, we weight them 11d after hatching (Tabashnik *et al.,* 2002). Cups were held at 27 ^{0}C with a photoperiod of 14:10 (L: D) h. We obtained weights of 50 larvae from two single-pair crosses, backcross (F_1 x resistant) and (F_1 x susceptible), susceptible and resistant strain.

Data analysis. We estimated LC_{50} and slopes of concentration-mortality lines with probit analysis (Ross 1997, Tabashnik *et al.,* 1987). Resistance ratios were calculated as the LC_{50} for the susceptible strain. Dominance estimated as described previously by (Liu and Tabashnik 1997b) using two methods; Stone's (1968) estimation of *D* based on LC_{50}s as

follows: D = (2X2- X1- X3)/(X1-X3), where X1, X2, and X3 are the logarithms of the LC_{50s} for the resistant homozygotes, heterozygotes, and susceptible homozygotes, respectively. In such methods, which require LC_{50}s, D could be calculated only from mass crosses. In that case, D value ranged from −1 (completely recessive resistance) to 1 (completely dominant resistance). But based on the mortality at each of four concentrations: 0.28, 1.0, 3.0, and 6 µg/g Cry1Ac/g diet, the single-concentration method Stone's, (1968) estimated dominance (h) as follows: h = (W12-W22)/(W11-W22), where W11, W12, and W22 are the fitnesses at a particular toxin concentration for resistant homozygotes, heterozygotes, and susceptible homozygotes, respectively. While h ranges from zero (completely recessive) to one (completely dominant). We converted D to the same scale as h as follows: h = (D +1)/2 for comparison the two methods (Liu and Tabashnik (1997b). Here the fitness of treated resistant homozygotes always will be defined as 1. The fitness of hetrozygotes treated with Cry1Ac was estimated as (the survival rate of treated of F_1 progeny) / (the survival rate of treated resistant larvae). Similarly, the fitness of susceptible homozygotes treated with Cry1Ac estimated as (the survival rate of treated susceptible larvae) / (the survival rate of treated resistant larvae). In each case, initially we estimated mortality caused by Cry1Ac by adjusting total mortality for control mortality using Abbott's correction (1). The survival rate determined as 100% - mortality (expressed as a percentage). Since h ranged from zero (completely recessive resistance) to one (completely dominant resistance). When h is 0.5, in that case, resistance is called co-dominant or additive. Terms partially recessive used ($0 < h < 0.5$) and partially dominant ($0.5 < h < 1$).

The number of loci influencing resistance estimated by using three approaches to analyze response of progeny backcrosses to Cry1Ac: (1) tests of fit to models comparison of slopes and variance, and calculation of effective number of factors (Lande 1981, Tabashnik 1991, Tabshnik *et al.,* 1992); and distribution of weights (Gould 1995).

For the one-locus model and polygenic models in which the effects of locus were additive and equal, chi-square tests used (1) to determine significant deviation between observed and expected mortality at each concentration. We noted previously, these tests provided only approximate probability values (Tabashnik *et al.,* 1992).

(1) $X^2 = (O - E) / E$

Where O is the observed number dead in the backcross generation at dose x, E is the expected proportion.

We used the approach of Tabashnik *et al.,* (1992) to apply Lande's (Lande 1981) method to estimate the minimum number of effective factors. To determine if growth patterns were consistent with expectation from a single locus model, we examined the distribution of larval weights of the resistant strain, F_1 progeny and backcross progeny 11 days after feeding on diet with 1 µg Cry1Ac/g diet. With a single locus and two alleles, half of the backcross progeny are expected to be RS heterozygotes, with a weight distribution similar to that of F_1 progeny, the other half are expected to be RR homozygotes, with a weight distribution similar to that of the resistant strain (Gould *et al.,* 1995). Based on larvae body weight we used the lower end of the distribution of the resistant larvae (25 mg) as the threshold for separating larvae into two categories; small (i.e., < 25mg, similar to F_1) and large (i.e., similar to resistant). A chi-square test applied to check for fitting 1:1 ratio of small to large larvae predicted by the single-locus model.

RESULTS

Response to selection. Selection with 0.26 µg Cry1Ac/g diet after getting the population from Bt-cotton plant continuously while conducting all the experiments. The resistant strain had experienced one round of selection, with concentrations ranged from 0.035 to 3.0 µg Cry1Ac/g diet. The LC_{50} for resistant strain was 3.06 µg Cry1Ac/g diet. Based on data from resistant strain as well as susceptible strain, the ratio was 82.9 (Table 1).

Maternal Effects, Sex Linkage, and Dominance. LC_{50} did not differ between the F_1 hybrid progeny of the reciprocal crosses between the susceptible and resistant strains (Table 1). Likewise, the mean slope of concentration-mortality line did not differ between the reciprocal crosses (Table 1). Thus, inheritance was autosomal; neither maternal effects nor sex linkage were evident.

The LC_{50} of the hybrid F_1 progeny from mass crosses was higher than LC_{50} of limited pair families (Table 1). Although the resistance ratio was 82.9 for resistant strain, it was only 39.7 for the F_1 hybrid progeny. The LC_{50} values for the resistant strain, susceptible strain, and their F_1 hybrid progeny yielded a value of 0.62 for *D*, which is equivalent to a value of 0.81 for *h* and indicates partially dominant inheritance.

Estimation of *h* separately for each of four concentrations shows that dominant increased as Cry1Ac concentrations decreased, (Table 2; Fig.2). Resistance was completely recessive at 6 µg Cry1Ac/g diet (0.0); and was partially recessive at 1 and 3 µg Cry1Ac/ g diet (0.32 and 0.20), while it was partially dominant at 0.28 µg Cry1Ac/ g diet (0.61). Estimation of *D* based on LC_{50}s, Stone's (1968) similar results were observed, and ranged from completely recessive to partially dominance at (6, 3, 1, and 0.28 µg Cry1Ac/g diet) respectively (Table 3).

Number of locus affecting the trait. Results from analysis backcross data (Fig.1.D; Table.1) suggested that one or few loci conferred resistance to Cry1Ac in resistant strain. The observed mortality was corresponded more closely with the expected mortality of the monogenic model, since the mortality of the backcross larvae fitted 1:1 ratio after 96 h from the exposure to the discriminating dose.

Weight Distribution. The weight distributions of 50 surviving larvae fed for 11 days on diet contains 0.28 and 1.0, 3.0, 6.0 µg Cry1Ac/g diet were consistent with expectations from a model with a major resistance locus (Fig.1). The weight distributions were almost completely distinct form resistant and susceptible larvae. Larval body weight distribution of susceptible parent ranged from 1-35 mg (fig 1.A). The larval body weight distribution of F_1 progeny was similar to the larval weight distribution of susceptible parent. The larval body weight distribution of resistant ranged from 40-290 mg with the peak around 110-220 mg (Fig.1.B). The weight of backcross progeny between resistant and F_1 parent showed the weight distribution similar to resistant parent. However, the peak distribution of larvae was between 80-130 mg/each. The sharpness of the peak of backcross progeny weight suggested presence of resistant homozygous. The data analysis based on chi-square test survival ratio using discriminating dose for backcross progeny was show to be fitting into 1:1 ratio suggesting presence of one major gene in controlling the trait (Table 5).

Similarly the weight distribution curve for backcross progeny was in accordance with chi-square test, which again suggest that inheritance of Cry1Ac in *H. armigera* is controlled by one gene action (Fig. 1.D).

The concentration mortality curves for different populations namely, resistant and susceptible parent were wide a part. The mortality curve of F_1 progeny was close to susceptible parent while that of progeny of backcross was close to the resistant parent (Fig. 2).

DISSCUSION

The results suggest that inheritance of resistance to Bt toxin Cry1Ac in the field-selected strain of *H. armigera* was controlled primarily by one or a few major loci. However, a model with only one-locus and tow alleles cannot explain both the response of this strain to initial selection reported previously (Tabashnik *et al.,* 2000b) and increased resistance produced by additional selection reported here. After getting the population from Bt-cotton, in which the individuals were exposed to 0.26 µg Cry1Ac/ g diet sub lethal dose, the resistant strain had 82.9-fold resistance relative to the susceptible strain (Tabashnik *et al.,* 2000b). The observed increase in resistance after 80% survival at 6 µg Cry1Ac/ g diet was achieved indicates that the resistant strain harbored more than one allele contributing to resistance. Thus, either three or more alleles affecting resistance occurred at one locus or more than one locus influenced resistance.

Inheritance of 82.9-fold resistance here was autosomal and recessive at 6 µg Cry1Ac/ g diet. Tabashnik *et al.,* (2000b) reported that the increased resistance to Cry1Acin diet did not greatly boost survival generations of F_8 and F_9 AZR-R larvae of pink bollworm on Bt cotton plants relative to their survival on non-Bt cotton plants. The AZR-R strain showed 40% relative survival on Bt cotton (3.1% on Bt cotton and 7.8% on non-Bt cotton), when he tested F_{17} generation of the AZR-R strain showed relative survival on Bt cotton of 46% (5.3% on Bt cotton divided by 11.5% on non-Bt cotton) (Liu *et al.,* 2001b). Thus, any increase in resistance to Cry1Ac that occurred within one-year greenhouse tests did not greatly increase survival of resistant strain on Bt cotton relative to non-Bt cotton, because larval mortality was greater for Cry1Ac in bolls of Bt cotton than on diet contending 10 µg Cry1Ac/ g diet (Liu *et al.,* 1999, 2001).

Indeed all studies till date did not investigate in details the inheritance of Cry1Ac resistance in *H. armigera*; most of the work has been done to develop the resistance in this insect to Cry1Ac under laboratory conditions (Kranti 2001; Akhurst *et al.*, 2003; and Chandrashaker and Gujar. 2004). As per our experiences, the insect under the investigation is so difficult to be reared beyond 6th generation under laboratory condition due to unfertilized eggs, which will not be able to hatch. Like the other cases of resistance to *B. thuringiensis* analyzed so far (Ferré and Van Rie 2002), resistance to Cry1Ac was autosomally inherited. However, unlike resistance to Cry1C toxins, Cry1F toxin, and Cry1A toxins in the *P. xylostella* (Ferré *et al.*, 1995, Metz *et al.*, 1995, and Tabashnik *et al.*, 1997). Similar to Cry1Ac in the CP73-3 strain of *Heliothis virescens* (Gould *et al.*, 1992), the extent of dominance of resistance to Cry1Ac depended on the concentration of toxin. However, the resistance strain was more recessive at high concentration. In the present study, the mortality of treated larvae and estimated dominance at given concentration also varied between the different experiments.

The determinations of dominance were based on the assumption that the resistant strain and susceptible strain were completely homozygotes when F_1 progeny were produced. But the presence of heterozygotes in resistance strain leads to lower the survival rate of F_1 progeny and thus leads to underestimates of dominance. Heterozygotes in the susceptible strain would have the opposite effect (Tabashnik *et al.*, 1997). Despite many examples of partially or completely recessive resistance in moth (Tabashnik, 1994), the resistance to Cry1Ac in the *H. armigera* found partially dominant which can guarantee that the assumption of resistance to Bt toxin always recessive was not accurate. We assumed here, that the Bt resistance strain of *H. armigera* moth harbors at least one partially dominant mutation that confers resistance to Cry1Ac and this finding is in accordance with that (Tabashnik *et al.*, 1997; Liu and Tabashnik 1997). In related example, the dominance of resistance to Bt toxins in laboratory-selected strain of *H. virescens* depended on the toxin and the particular strain (Gould *et al.*, 1992; Gould *et al.*, 1995; Sims and Stone 1991).

Our study can conclude that single or a few genes are controlled the Cry1Ac resistance trait in *H. armigera* and is inherited as a partially dominant. When the Cry1Ac concentration increased the susceptibility of the resistant population will increased, but

when the lower dose used the susceptibility of resistant population decreased and as a result of that, we will have a lot of numbers from susceptible individuals. Keep this in view, variation in dominance of resistance as a function of the toxin concentration and other environmental factors complicates resistance management. Simulation models show that refuges are likely to work best when resistance is recessive, i.e., when heterozygotes are killed (Gould, 1988, McGaughey and Whalon 1992). In principle, one could render resistance recessive simply by spraying sufficiently high concentrations of formulations or ensuring sufficiently high expression of toxins in transgenic plants. However, achieving functional recessivity by increasing the toxin concentrations vary in space and time, with either foliar applications or transgenic plants. Addition to this increasing the concentration of Cry1Ac might increase the cost of foliar applications or reduce yields of transgenic plants. Perhaps more importantly, if the optimistic assumptions of some models are violated and mating between resistant and susceptible insects is limited, increasing the toxin concentration could quickly eliminate susceptible individuals and thus greatly accelerate evolution of resistance (Liu and Tabashnik 1997).

Thus, the best option to deal with the dominant resistance is to increase the sizes of refuges. Another option is to use lower dose from the toxin that are low enough to enable survival of significant numbers of susceptible insects (Tabashnik 1994). These approaches will almost certainly delay resistance but may require integration with other tactics, such as biological control, to achieve adequate suppression of pests.

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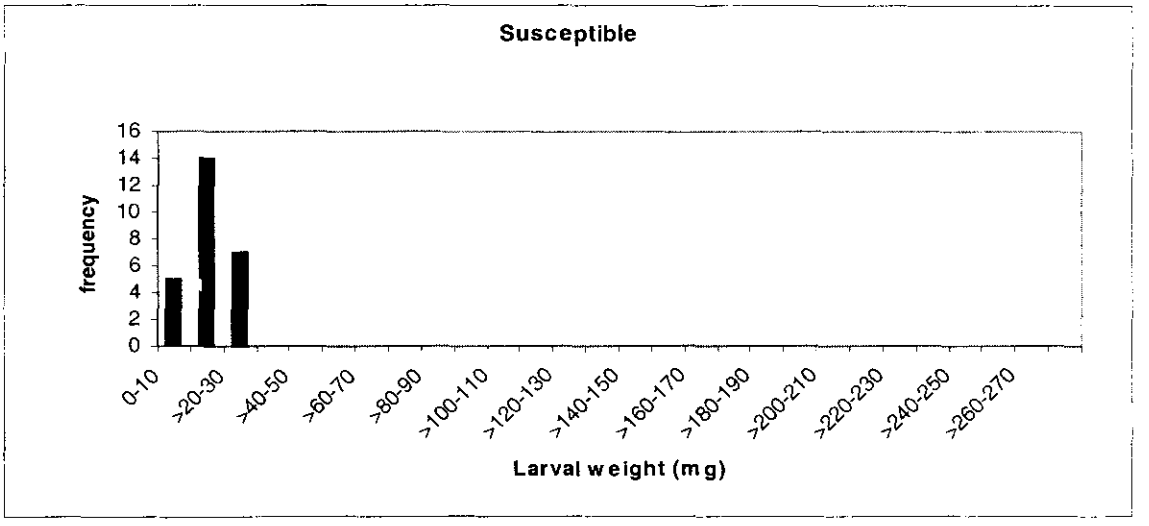
Susceptible
frequency
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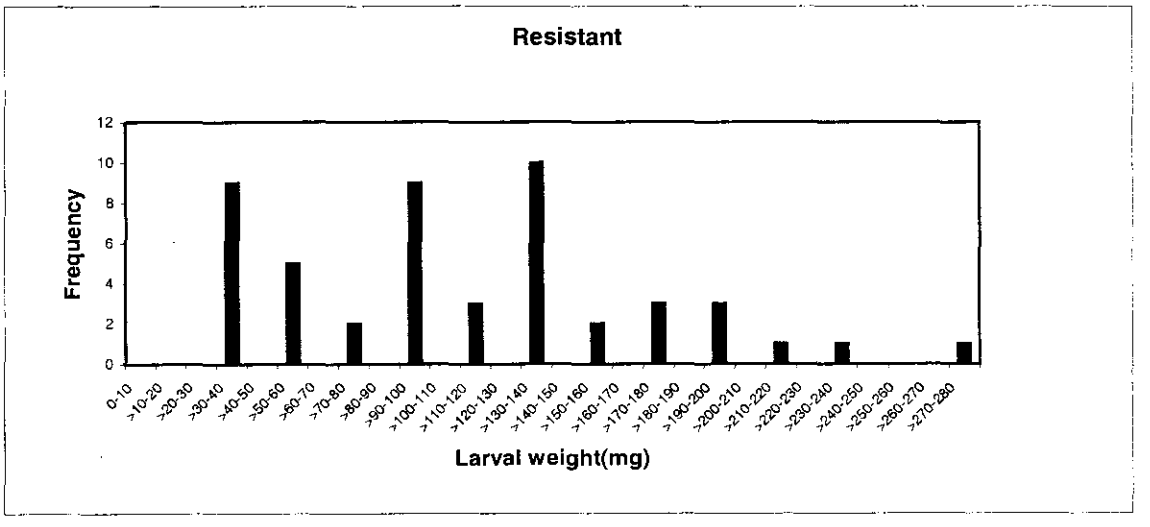
14
12
10
8
6
4
2
0
0-10
>20-30
>40-50
>60-70
>80-90
>100-110
>120-130
>140-150
>160-170
>180-190
>200-210
>220-230
>240-250
>260-270
Larval weight (mg)

Fig. 1. A


Resistant
Frequency
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10
8
6
4
2
0
0-10
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>60-70
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Larval weight(mg)

Fig. 1. B

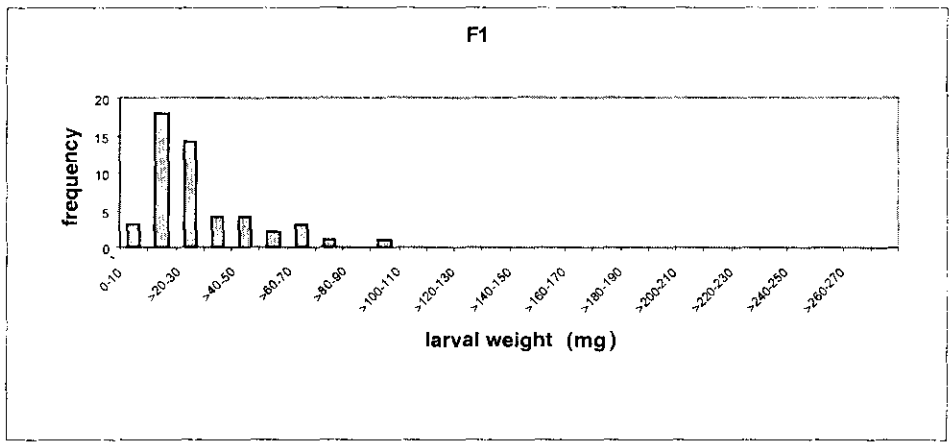

Fig. 1.C.

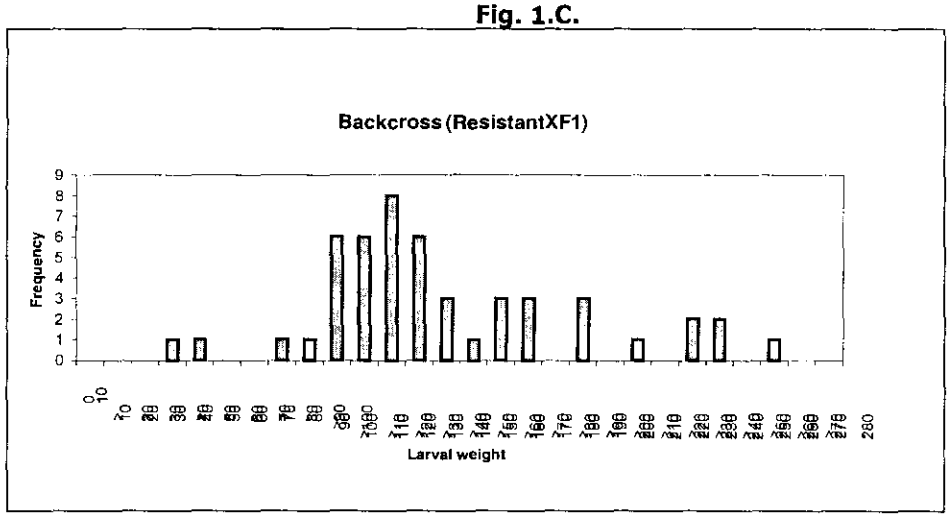

Fig. 1. D.

Fig. 1. Weight distribution of larvae 11days after hatching survived on diet treatments (µg/g). The surviving larvae, 50, were preferably selected from lower concentration for weighing. A) Susceptible, B) Resistant, C) F_1, and D) Backcross progeny (F_1 X resistant)

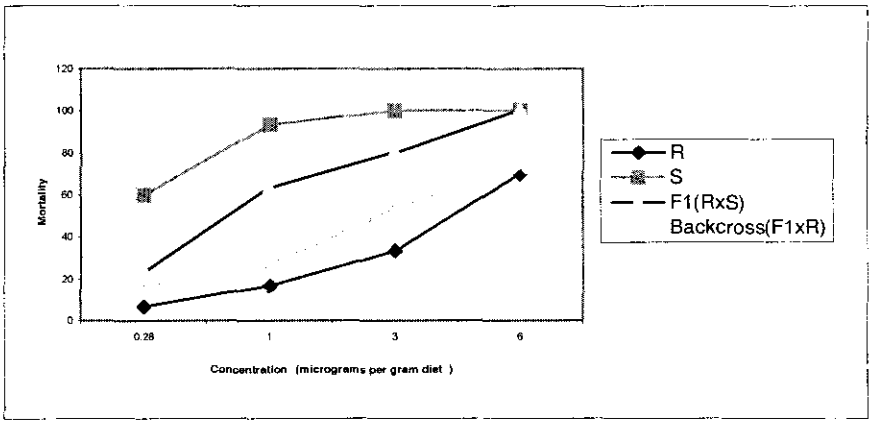

Fig. 2. Response to Cry1Ac of *H. armigera* larvae from susceptible strain, a resistant strain, F_1 progeny (resistant X Susceptible), and backcross progeny (resistant X F_1).

Table.1. Response of Susceptible, resistant and hybrid F_1 progeny of *H. armigera* to ry1Ac.

Strain or generation	No. of insect	Slope ± SE	LC_{50} (µg/g) (Fiducial limits)	RR□
S-strain	210	0.8 ± 0.22	0.037 (0.006-0.07)	
R strain	210	1.2 ± 0.29	3.067 (2.00-7.73)	82.9
F1 Mass cross				
$^{10}{}_{R}{}^{\square} \times {}^{10}{}_{S}{}^{\square}$	210	1.3 ± 0.24	1.47 (1.03-2.45)	39.7
$^{10}{}_{S}{}^{\square} \times {}^{10}{}_{R}{}^{\square}$	210	1.2 ± 0.23	1.22 (0.85-2.01)	32.9
Pooled	420	1.3 ±0.16	1.34 (1.04-1.86)	36.2
F1 Single pair				
$^{3}{}_{R}{}^{\square} \times {}^{3}{}_{S}{}^{\square}$	210	1.4 ± 0.23	0.51 (0.35-0.70)	13.7
$^{3}{}_{S}{}^{\square} \times {}^{3}{}_{R}{}^{\square}$	210	1.7 ± 0.26	0.71 (0.52-0.96)	19.1
Pooled	420	1.6 ±0.18	0.63(0.50-0.78)	17.0

□ RR resistance ratio (the LC_{50} for the resistance strain divided by the LC_{50} for the susceptible strain).

Table 2. Dominance of cotton bollworm resistance as a function of Cry1Ac concentration.

Concentration (μg/mg diet)	Survival (%)		Dominance (h)
	Susceptible	F_1	
0.28	40	76.6	0.61
1.0	6.6	36.6	0.32
3.2	0.0	20.0	0.20
6.0	0.0	0.0	0.00

Adjusted survival was calculated survival on treated diet divided by survival on untreated diet, based on survival of the resistant strain, we estimated that its survival was 100% at the four concentrations was calculated as: (survival of F_1- survival of susceptible strain) / (100%- survival of susceptible strain).

Table.3. Dominance (h) of resistance to Cry1Acin *H. armigera* as a function of concentration of Cry1Ac.

Concentration	Strain generation	No. of larvae treated	Mortality%	Fitness	h^D
0.28	S strain	30	60.0	0.43	
	R strain	30	6.6	1.0	0.70
	F_1	30	23.3	0.82	
	S strain	30	93.3	0.07	
1.0	R strain	30	16.6	1.0	
	F_1	30	63.3	0.44	0.39
	S strain	30	100	0.0	
	R strain	30	33.3	1.0	
3.0	F_1	30	80.0	0.30	
	S strain	30	100	0.0	0.30
	R strain	30	73.3	1.0	
6.0	F_1	30	100	0.0	0.00

F_1 are hybrid progeny pooled from the two reciprocal mass crosses between the two parental strains.

h^D varies from 0 for completely recessive to 1 for completely dominant resistance.

Table 4. The response of F_1 backcross populations to Cry1Ac toxin

Parents/cross	LC_{50} (µg/g)	Fiducial limits 95%	Slope ± SE
S-strain	0.037	(0.006-0.07)	0.8 ± 0.22
R strain	3.067	(2.00-7.73)	1.2 ± 0.29
10 $R^{\square}$ x10 $S^{\square}$ (F_1)	1.47	(1.03-2.45)	1.3 ± 0.24
20 $F_1^{\square}$ x 20 $R^{\square}$ (backcross)	0.57	(0.37-0.95)	1.2 ± 0.18
20 $F_1^{\square}$ x 20 $R^{\square}$ (backcross)	0.92	(0.52-2.56)	0.8 ± 0.18
10 $R^{\square}$ x 10 $S^{\square}$ (F_1)	1.22	(0.85-2.01)	1.2 ± 0.23
20 $F_1^{\square}$ x 20 $R^{\square}$ (backcross)	0.64	(0.44-1.01)	1.2 ± 0.21
20 $F_1^{\square}$ x 20 $R^{\square}$ (backcross)	1.32	(0.86-2.58)	1.03 ± 0.23

Table 5. The mortality and survivals of different strain using discriminating dose of 3.06µg/g diets.

Population	Dose (µg /g diet)	**Mortality**	**Survival**	$\chi^2_{(1)}$		
				Calculated	Tabulated 5%	Tabulated 1%
F_2 (intermating F_1)	3.06	77	73	52.802	5.991	9.210
Backcross X R	3.06	74	76	0.026	3.841	6.635
Backcross X S	3.06	104	46	81.810	3.841	6.635