COMPARISONS OF THE BIOLOGY OF PYRETHROID RESISTANT AND SUSCEPTIBLE HELIOTHIS ARMIGERA.

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Introduction

With the appearance of pyrethroid resistance in Heliothis armigera during the 1982/83 cropping season a strategy aimed at limiting the development of widespread resistance was rapidly implemented. H. armigera had previously shown a propensity to acquire high levels of resistance to other insecticides (eg. DDT). As part of a number of studies on the genetics and ecology of pyrethroid resistance the work described here was commenced to evaluate the biological fitness of resistant individuals relative to their susceptible counterparts.

The evolution of insecticide resistance is a complex process resulting from the interaction of many genetic, ecological, physiological and operational factors. By using computer simulations a number of factors which may influence the rate at which resistance will develop have been identified. These factors include:

- the initial frequency and dominance of genes for resistance
- the selection pressure applied by spraying (frequency, timing and persistance of chemicals)
- immigration of susceptible individuals from unselected populations
- the fitness of individuals carrying genes for resistance relative to susceptibles

Apart from those selective factors under human control (spray frequency, persistence and timing etc), the simulations showed two factors to be most important. Firstly an influx of susceptible migrants may significantly reduce the rate at resistance may develop and in combination with appropriate insecticide rates and application schedules could even reverse the trend toward resistance, (at present our knowledge of local or long distance

movements by Heliothis is poor but programs are underway to examine this aspect of its ecology).

The second important factor was the influence of resistance on other aspects of the fitness of individuals. An insect's 'fitness' includes many factors: longevity of adults, their fecundity and fertility, the viability and rate of development of larvae, all of which influence its ability to survive and multiply. Resistant individuals obviously have an advantage in heavily sprayed cropping situations. However, if an increase in resistance is linked with say a reduction in the number of eggs laid or a reduction in the survival of larvae then the rate of increase of resistance genes may be retarded in the absence of insecticides. In one simulation study the combination of severe reproductive disadvantage in the resistant insects with the immigration of susceptibles into the population led to a dramatic reduction in the rate of resistance development. However in the absence of immigration there was little effect. Similarly, another study showed that a moderate reproductive disadvantage doubled to time taken for simulated resistant populations to regain their original size.

In the present case of pyrethriod resistant Heliothis in Australian cotton growing areas we need some understanding of the fitness of resistant and susceptible moths in order to interpret changes in the frequency of resistant individuals in the field and to evaluate the effectiveness of the measures introduced to control resistance.

Methods

Two aspects of fitness were examined:

1. The longevity and fecundity of adult female moths.
2. The survival and rate of development of larvae.

Since the genetic basis of pyrethroid resistance is not yet known most comparisons have been made between susceptible individuals and those which have survived a discriminating dose of insecticide, here designated as "resistant". Extensive preliminary studies by N. Forrester (N.S.W. Dept. of Agriculture) established the discriminating dose for fenvalerate as 0.2 µg/30-40 mg. larva. This dose will kill 99.9% of susceptible individuals. Individuals which survive this dose may be heterozygous or homozygous for the resistance gene(s) and bioassays (by N. Forrester) showed great differences in the level of resistance among these survivors in each stage of the season.

In other insects it has been possible to follow changes in the frequency of resistant genes through several generations by using large populations. However, the difficulties associated with Heliothis rearing and mating in the laboratory have restricted our studies to material collected directly from the field or reared for only 1-2 generations in the laboratory.

1. Fecundity of adults : field material

Larvae were collected from the field during stages 1, 2 and 3 of the pyrethroid management strategy and screened for resistance using the discriminating dose. All material was collected as eggs from commercial cotton fields and reared to testing size (30-40 mg) on an artificial diet. The performance of these selected individuals was compared with that of moths from susceptible strains or with moths from the field which were unselected (85%-90% of these were susceptibles). When adults emerged, groups of 4-5 pairs were allowed to mate for 4 nights and were then separated into single pairs in individual cages. Relatively few eggs were laid before moths were isolated. Food was provided as a 10% honey solution. The number of eggs laid on paper towelling was recorded each day for each female.

2. Viability and rate of development of larvae

The rate of development of larvae from the resistant and susceptible colonies was measured at 25° on an artificial diet. In stages 1 and 2 several batches of larvae were taken from eggs pooled over all the available females in each category, while in stage 3 the development of larvae from individual females was examined.

Larvae were transferred individually on the day of hatching to 20 ml plastic vials containing sufficient food for complete development. We recorded the following parameters of development:

1. % survival to 7 days of age
2. mean weight at 7 days of age (mg)
3. time taken to pupate (in days)
4. % survival to pupation
5. pupal weight (mg)
6. time taken to emerge as adults (in days)
7. % survival to adult emergence

Studies of laboratory strains selected for increased resistance

In November 1983 data was collected on the development of larvae from two strains which had been selected with the discriminating dose of either fenvalerate or deltamethrin for three consecutive generations. These strains originated from resistant moths collected in the Namoi Valley at the end of the 1982/83 season. In the generation when observations were made, the LD_{50} (i.e. the dose required to kill 50% of individuals) of the fenvalerate and deltamethrin strains was 3.2x higher and 7.8x higher than for susceptibles.

Further selections were made using survivors of the discriminating dose collected during stage 3. Progeny from these females were selected with doses of fenvalerate of 0.2 or 0.4 µg/larva, equivalent to 1 x and 2 x the discriminating dose. The survivors from each dose were mated in groups of 5 pairs upon emergence, then the fecundity of each female and the performance of her larvae were determined as outlined above.

Results

Fecundity and Longevity of Females

Figure 1 shows the mean number of eggs produced per female for selected and unselected females from the Namoi Valley in each stage of the season. Under the experimental conditions most females laid 800-1000 eggs and lived for 14-15 days. However there was much variation between females, with a range from <200 up to a maximum of 2462 eggs laid by one female. From the figure it appears that females which had survived the discriminating dose as larvae produced fewer eggs than did susceptible or unselected individuals (23% less in stage 1, 37% less in stage 2 and 29% less in stage 3). This difference of about 300 eggs, was evident in all stages of the season but was statistically significant only during stage 2 ($p<0.05$). There was no evidence of significantly greater variability among resistant females than among the unselected group.

However, egg laying in Heliothis is known to be stimulated by mating. If comparisons are restricted to mated females only, the differences between strains are reduced and none is significant. Table 1 gives details of individual fecundity and longevity for the mated females. The weighted mean fecundity for all Namoi females in the selected and unselected groups over all three stages was:

resistant females (n = 32) - 1041.0 eggs (±505)
unselected females (n = 41) - 1344.0 eggs (±582)

This difference of 23% is statistically significant ($p<0.05$). In stages 2 and 3 there was an indication that fewer selected females had mated successfully but given the difficulties of achieving higher mating success with Heliothis in the laboratory little can be made of this at present. There were no differences in the average length of life between groups.

During stage 3 the fecundity of female H. armigera from Emerald, Darling Downs and the Lockyer Valley was also measured. Note that, in contrast to the Namoi collections for stage 3, the moths from these regions were not screened with insecticide as larvae. Instead the resistance status of each female was assessed by screening a group of her progeny. A female was classified as resistant if >5% of her progeny survived the discriminating dose. Data was collected for a total of 108 females but only 53 of these had mated successfully. Comparisons of fecundity of the susceptible and resistant females showed no reduction in egg production in the resistant group (Table 2). The levels of resistance in the resistant females (as measured by the proportion of larvae which survived the discriminating dose) ranged from 5.5% to 64.0%, but there was no relationship between fecundity and this measure of resistance (Figure 2). That is, there was no decline in fecundity in the most highly resistant females. For all regions combined mean fecundity (±S.D) of susceptible and resistant females was not significantly different:

Resistant (n = 18) - 1233.4 eggs (±493)
Susceptible (n = 35) - 1025.7 eggs (±503)

Larval Viability and Rate of Development

1. Progeny for Selected and Unselected Namoi Females

Table 3 shows some parameters of development for larvae from selected and unselected females. (The apparently slower development rate during stage 1 was caused by faulty temperature control on the rearing cabinet). There were no consistent differences between the two groups in % suryival to 7 days of age, % survival to pupation, mean weight at 7 days or the time taken to pupate. As shown by the high coefficients of variation (CV%) there was much variation between larvae in their weight at 7 days of age. However, progeny from selected females were no more variable than those of unselected females, except in stage 3. This was due to a significantly higher proportion of 'slow' developing larvae in the selected group (Table 4). Larvae were

classified as 'slow' if they weighed less than half the average weight of their group at 7 days of age. Most often the 'slow' larvae weighed <5 mg at 7 days of age and many of them died at this weight. Others survived but grew very slowly and often weighed only 100 mg at 15-20 days when other larvae normally pupate. It was noticeable that these individuals were often a pale green colour form of H. armigera. Despite the higher proportion of slow larvae in the selected group there was no difference in the time taken to reach pupation (Table 3), since only about about 30% of the 'slow' larvae pupated successfully whereas 73% of the remainder did so.

2. Performance of Larvae from Different Areas

There was also no difference in the performance of larvae from resistant and susceptible females collected from different regions in Stage 3 (Table 5). As was the case with fecundity of these females, there was no trend towards reduced survival or longer development times associated with increasing resistance levels among the resistant females (Figure 3).

3. Performance of Larvae from Laboratory Resistant Strains

In contrast to the field resistant material discussed above, data from some laboratory strains suggest there may be a reduction in larval viability and rate of development when strains are selected for increased levels of resistance. Such as effect was first indicated by observations of larvae from the strains selected with either fenvalerate (ARSF strain) or deltamethrin (ARSD strain). These larvae weighed only 4-8 mg at 7 days of age and contained a high proportion of slow developing larvae (60%), most of which (90%) did not survive to pupation. In addition, those larvae which did survive took about 14 days longer than susceptibles to pupate (Table 6).

The performance of larvae from strains selected with varying doses of fenvalerate (Stage 3) also suggested a reduction in growth rate in the resistant colonies, particularly at the highest selection pressure. These strains had undergone two selections: firstly when collected from the field they were selected with 0.2 µg fenvalerate (1 x discriminating dose) and in the next generation some were left unselected while the remainder were selected with 0.2 or 0.4 µg fenvalerate (1 x and 2 x the discriminating dose). The results (Table 7) indicate a reduction in growth rates and survival to pupation as the selection pressure applied to the parental generation was increased. There was also an increase in the proportion of

'slow' developing larvae from 15.0% in the unselected strain to 50.0% in the strain selected most heavily. This experiment has continued through another generation of selection but results are not yet available.

Discussion

Two observations are most often invoked in support of the suggestion that, in the absence of insecticides resistant insects are at a disadvantage relative to susceptibles. Firstly, most insect populations are not resistant before exposure to insecticide and the genes for insecticide resistance are present only at low frequency (0.01-0.001). It seems reasonable to assume therefore that, prior to the introduction of the insecticide, the gene (or genes) conferring resistance were disadvantageous (or neutral) in their effect on biological fitness. Secondly it is commonly observed, both in laboratory populations and in the field, that when selection pressure from insecticides is removed the frequency of resistant individuals declines, sometimes within 1-2 generations. This has occurred in laboratory strains of H. armigera (N. Forrester pers. comm.).

There are now a number of laboratory studies which demonstrate a small, though significant reduction in reproductive fitness of resistant insects. Examples are known for mosquitos, grain beetles and houseflies. Those studies which have identified the underlying causes have found that lower fitness may be due to both reduced fecundity or fertility of adults and reduced viability of larvae. However, there are many other examples where the differences in reproductive potential of resistant and susceptible strains are so small as to impose only mild pressures of natural selection against the resistant genotypes Some studies also show that if selection continues for many generations, the level of resistance becomes more stable and does not decline when selection by insecticides is removed. This occurs as a result of modifications in other areas of the genome which allow resistant genotypes to regain normal fitness.

As yet our data do not suggest a major reduction in fitness, in either reproduction or larval viability, in resistant H. armigera from field populations. The most likely explanation for this is that the great majority of the "resistant" moths are heterozygous resistant (RS) individuals. The discriminating dose (0.2 µg/30-40 mg larva) applied to field collected larvae removes the susceptibles (SS), but does not distinguish heterozygotes (RS) from homozygotes (RR), both of which are resistant at this dose. During the

early stages of selection for resistance, when the frequency of resistance genes is low (as in the case in the Namoi Valley), most of the resistance genes will occur in the heterozygotes (RS). Studies with both houseflies and mosquitoes have found that while resistance homozygotes suffered a reduction in fitness, there was no difference between susceptibles (SS) and heterozygotes (RS). Thus while the resistance gene(s) may be effectively dominant in conferring resistance to RS individuals they may be recessive in their effects on biological fitness. If, as seems possible, this is the case for Heliothis, then in the absence of insecticides, the rate of selection against resistance genes will slow as its frequency declines and will reach a lower limit at which R genes will be maintained in the population in heterozygotes.

The observed reduction in fecundity of resistant females (23%) was not consistent between stages and may be explained in part by sublethal effects of the insecticide dose experienced by the selected individuals rather than by their resistance per se. Although larvae may survive the dose of insecticide there may be a reduction in vigour of the adult which results in slightly lower rate of egglaying. This is supported by the data from stage 3 where resistant females not treated with insecticides (from Emerald, Lockyer and Downs) showed no reduction in fecundity at all (Table 2). Furthermore, despite the small sample size, there was no reduction in fecundity of the resistant Namoi Valley moths in stage 3 (which were exposed to insecticide), some of which showed the highest levels of resistance yet detected in the Namoi Valley (30 x resistance, N. Forrester pers. comm.).

By contrast the observations for laboratory strains suggested that selection for increased levels of resistance, (i.e. a higher proportion of homozygous resistant individuals), was associated with a reduction in fitness, particularly in larval viability and development. At this stage we cannot be certain that all the observed effects in laboratory strains are a consequence of resistance per se. In studies of other insects where marked reductions in fitness of resistant insects have been found, the effects of inbreeding depression of other factors not related to resistance have been implicated. Similarly for Heliothis, laboratory culturing may result in reduced genetic variance, particularly in the resistant colonies, due to inbreeding in what are often small populations of moths. This may well explain the low viability and rate of development of larvae from the ARSD and ARSF colonies, which although selected with the discriminating dose for 3 generations, were not highly resistant (7.8 x and 3.2 x resistance). However,

the changes in larval performance observed after further selections of stage 3 resistant moths, seem more likely to be due to resistance. In these strains there was an increase in the proportion of slow individuals (which may perhaps be the homozygous resistant ones), while the remainder developed as rapidly as susceptibles. Thus these experiments suggest there may be strong selection against homozygous resistance while the studies of field moths suggest that heterozygotes suffer no reduction in fitness.

TABLE 1. INDIVIDUAL FECUNDITY AND LONGEVITY OF SELECTED AND UNSELECTED FEMALE H. ARMIGERA. MATED FEMALES ONLY.

Stage of season	Type of female	n	$\bar{x}$ eggs/♀	CV%	$\bar{x}$ days/♀	CV%
1	Selected * (81.3%) **	13	1114.1	43.00	14.31	19.22
	Susceptible (76.0%)	22	1463.9	47.00	14.14	23.90
2	Selected (63.6%)	14	877.4	58.08	14.64	29.99
	Unselected (81.8%)	9	1116.2	30.65	15.22	30.75
3	Selected (26.3%)	5	1309.0	37.90	15.00	12.47
	Unselected (50%)	10	1285.3	36.17	14.50	26.07

* Individuals collected from the field which survived a dose of 0.2 µg as 30-40 mg larvae.

** Percentage of group which had mated.

TABLE 2. FECUNDITY OF FEMALE *H. ARMIGERA* FROM DIFFERENT AREAS (FERTILE FEMALES ONLY)

REGION	Type of female	n	$\bar{x}$ survival of larvae at 0.2 µg fenvalerate	$\bar{x}$ eggs/♀	CV%
MAREEBA *	Susceptible	12	0.0%	1338.8	18.66
NAMOI	Resistant	5	44.7%	1309.0	37.90
	Susceptible	10	0.1%	1285.3	36.17
EMERALD	Resistant	5	9.8%	1283.5	45.0
	Susceptible	8	0.9%	1185.6	43.0
DOWNS	Resistant	5	21.1%	938.2	50.0
	Susceptible	15	0.6%	759.8	62.0
LOCKYER	Resistant	3	24.3%	1488.3	29.0
	Susceptible	2	0.0%	1083.0	8.0
All Regions Combined (except Mareeba)	Resistant	18	25.1%	1233.35	40.0
	Susceptible	35	0.5%	1025.74	49.0

* Measured during Stage 1.

TABLE 3. PERFORMANCE OF LARVAE FROM SELECTED AND UNSELECTED FEMALES COLLECTED IN THE FIELD.

Stage of season	Type of female	% S to 7 days	$\bar{x}$ wt at 7 days (mg)	CV%	$\bar{x}$ days to pupate	% S to pupate
Stage 1	Selected	95.0	28.9	61.0	24.8	48.4
	Susceptible	99.5	46.9	50.0	22.6	64.5
Stage 2	Selected	86.3	83.2	52.0	18.9	70.0
	Unselected	88.2	76.3	67.0	20.9	42.9
Stage 3	Selected	96.7	90.8	65.0	17.3	64.4
	Unselected	96.7	101.1	43.0*	17.6	85.0

% S = percentage survival

TABLE 4. THE PROPORTION OF 'SLOW' DEVELOPERS AMONG GROUPS OF PROGENY FROM SELECTED AND UNSELECTED FEMALES.

Stage	Strain	% slow larvae	% of these which pupated	% of others which pupated
1	Selected	13.3	25.0	52.7
	Susceptible	13.7	49.8	67.7
2	Selected	18.4	14.1	81.8
	Unselected	18.3	22.1	70.0
3	Selected	20.0	45.8	95.2
	Unselected	5.0	26.3	73.3

TABLE 5. PERFORMANCE OF PROGENY OF SUSCEPTIBLE AND RESISTANT FEMALES FROM DIFFERENT REGIONS

Region	Type of female	$\bar{x}$ wt. at 7 days (mg)	% S to 7 days	Time to pupate (days)	% S to pupate	Pupal wt. (mg)	Sex Ratio ♀/♂
Namoi	Resistant	90.8	96.7	17.3	64.4	418.0	0.90
	Susceptible	101.1	96.7	17.6	85.0	439.6	1.53
Emerald	Resistant	99.9	100.0	18.6	85.0	432.6	3.25 *
	Susceptible	102.5	100.0	17.3	78.9	444.2	0.87
Downs	Resistant	40.7	100.0	17.0	75.7	437.8	0.96
	Susceptible	79.0	97.1	16.3	80.6	434.3	0.96
Lockyer	Resistant	39.9	100.0	14.9	91.5	428.7	1.28
	Susceptible	39.1	100.0	16.5	93.7	428.0	1.33

% S = percentage survival

TABLE 6. PERFORMANCE OF LARVAE FROM TWO HIGHLY SELECTED STRAINS OF H. ARMIGERA COMPARED WITH SUSCEPTIBLES (RESISTANT STRAINS DERIVED FROM MOTHS COLLECTED IN THE NAMOI VALLEY, APRIL/MAY 1983).

ARSF - selected with fenvalerate
ARSD - selected with deltamethrin

Strain	% S to 7 days (mg)	$\bar{x}$ wt at 7 days	% 'slow' developers	% S to pupation	$\bar{x}$ days to pupate
ARSF	76.0	7.8	61.0	40.0	30.6
ARSD	89.3	4.2	61.0	22.0	36.4
Susceptibles	99.5	46.9	13.7	64.5	22.6

TABLE 7. PERFORMANCE OF LARVAE FROM STRAINS SELECTED IN ONE GENERATION WITH THE DISCRIMINATING DOSE OF FENVALERATE (0.2µg) AND IN THE NEXT GENERATION WITH 0.2 OR 0.4µg COMPARED WITH THAT OF SUSCEPTIBLE OR UNSELECTED STRAINS.

		Selection applied in 2nd generation.		
Parameter of development	Susceptible Strain	Unselected	0.2µg	0.4µg
% S to 7 days	90.0	99.0	94.2	86.3
$\bar{x}$ wt at 7 days (mg)	129.1	130.7	97.6	70.1
CV %	55.9%	47.2%	75.9%	100.5%
% of 'slow' developers	8.4%	15.0%	34.6%	50.0%
% S to pupation	66.4%	72.0%	68.8%	33.9%
$\bar{x}$ days to pupate	16.4	16.2	16.9	18.5
Pupal wt (mg)	385.7	495.2	410.6	396.3

FIG. 1.

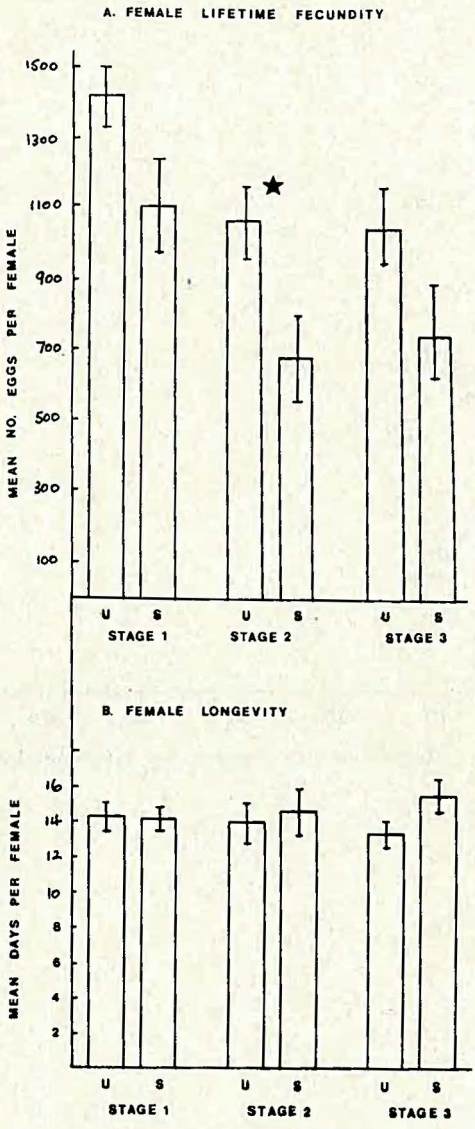
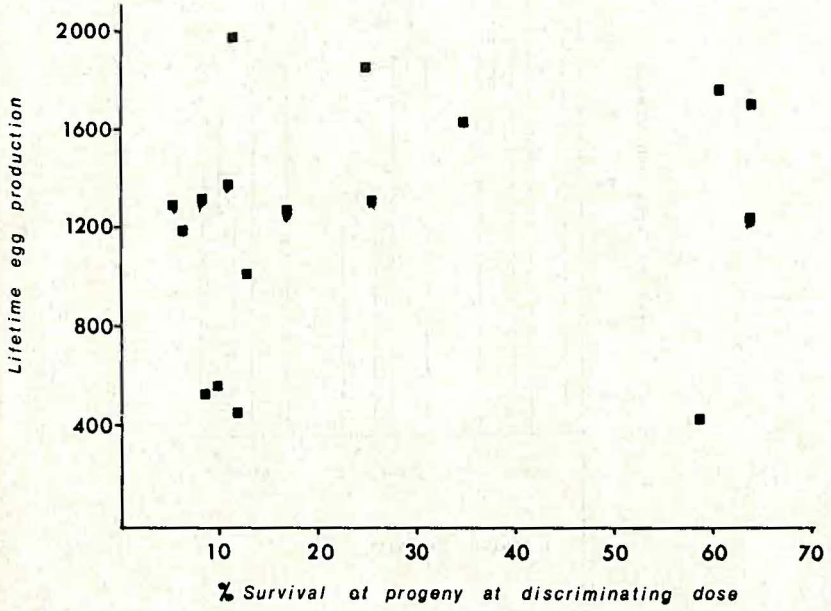

A. FEMALE LIFETIME FECUNDITY
MEAN NO. EGGS PER FEMALE
1500
1300
1100
900
700
500
300
100
U S U S U S
STAGE 1 STAGE 2 STAGE 3
B. FEMALE LONGEVITY
MEAN DAYS PER FEMALE
16
14
12
10
8
6
4
2
U S U S U S
STAGE 1 STAGE 2 STAGE 3

FIG. 2.


2000
1600
1200
800
400
Lifetime egg production
10
20
30
40
50
60
70
% Survival of progeny at discriminating dose

LEGEND TO FIGURES

Figure 1.

(A) Lifetime fecundity and (B) length of life of female H. armigera collected in the Namoi Valley in each stage of the 1983/84 season. U = unselected susceptible females, S = selected resustant females (survivors of the discriminating dose of fenvalerate). Means SE are shown. The difference in fecundity between susceptible and resistant females was significant ($p<0.05$) only in stage 2.

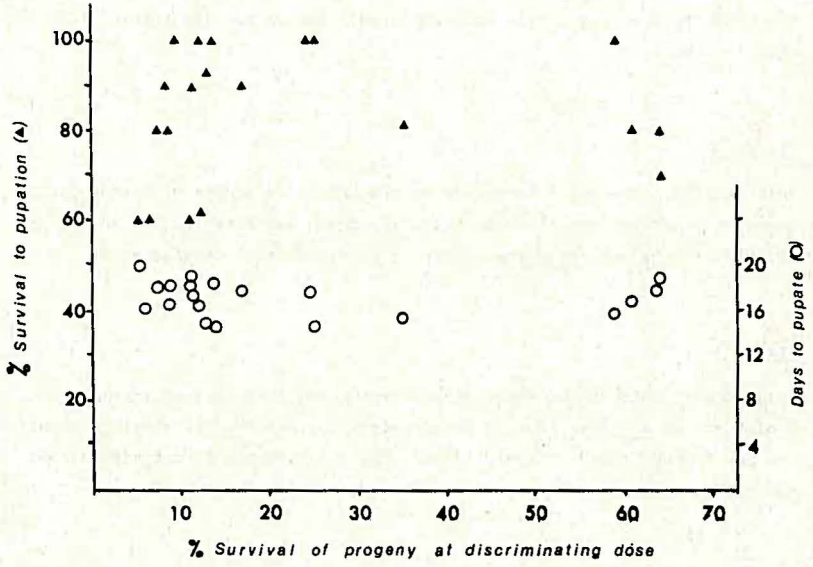
Figure 2.

Relationship between the frequency of resistance in groups of larvae (as shown by % survival at the discriminating dose) and lifetime fecundity for individual female H. armigera. There is no significant relationship.

Figure 3.

Relationship between the frequency of resistance in groups of larvae from individual H. armigera and two developmental parameters (survival to pupation and development time) of their larvae. The relationship is not significant for either parameter.

FIG.3.


100
80
60
40
20
% Survival to pupation (▲)
20
16
12
8
4
Days to pupate (○)
10
20
30
40
50
60
70
% Survival of progeny at discriminating dose