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Inheritance of resistance to malathion in *Tribolium castaneum* (Herbst)

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Abstract. Studies on the inheritance of resistance to malathion in an originally field collected malathion-resistant strain of *T. castaneum* by making genetic crosses between the resistant and a susceptible strain revealed that resistance in this strain is controlled by an autosomally inherited single major gene which is incompletely dominant.

Keywords. Resistance; malathion; *T. castaneum*.

1. Introduction

The development of resistance to insecticides in field populations of stored product pests is being increasingly reported from various parts of the world. The FAO global survey report (Champ and Dyte 1976) showed that the resistance in rust red flour beetle, *Tribolium castaneum* was of world-wide occurrence. Malathion resistance in *T. castaneum* was reported earlier from India by Bhatia *et al* (1971). Inspite of its wide prevalence, there is little information regarding the inheritance of malathion resistance in this species. The present investigations were, therefore, undertaken with a view to finding the nature of inheritance of resistance to malathion in *T. castaneum*.

2. Material and methods

The malathion resistant (*R*) strain of *T. castaneum* employed in the present studies was initially collected from the field (Bhatia *et al* 1971) and reared in the laboratory for six generations under malathion pressure. A portion of the emerging adults was subjected to treatment with malathion 10% (impregnated filter-papers) and survivors picked up to start 40 single pair colonies. Pure malathion-resistant colonies were isolated by subjecting the single pair colonies in the second generation to a discriminating dose of 1% malathion (a dose which gave 100% kill in susceptible and no kill in *R* individuals) and those showing no mortality were pooled to rear the resistant strain used in the present studies. A portion of this strain was reared for two generations without insecticidal pressure and the tests carried out showed that it maintained the same level of resistance to malathion. Similarly, a susceptible strain (*S*) was isolated from single pair colonies obtained from a laboratory culture reared simultaneously without any insecticidal pressure.

The general rearing of the test insect in wheat flour consisted of the method given by Bhatia and Pradhan (1968).

2.1 *Bioassay technique*

The toxicity to malathion was measured by employing the impregnated filter-paper method recommended by FAO (Anonymous 1970) which consisted of exposing the adults in 5 cm diameter glass rings to Whatman No.1, 7 cm filter-papers impregnated with scalar concentrations of malathion in a 3:1:1:2 mixture of petroleum ether (60-80°C b.p.), acetone, Risella-17 oil and dibutyl phthalate. A complete test comprised three replicates of six to ten concentrations and a control. Ten to 40 adults of two weeks age were used after 1 hr starvation in each treatment, the number being constant in any one experiment. The exposure time was 24 hrs at 28±1°C at the end of which mortality was recorded.

2.2 *Genetical techniques*

Initially, base line toxicity data were obtained for malathion to both the *S* and *R* strains. Reciprocal mass crosses between *S* and *R* were performed to provide sufficient off-spring for testing and for further crosses. Twelve randomly selected freshly emerged adults of each sex (sexed in the pupal stage) were utilized per cross. The adults of each cross were released in jars containing wheat flour and after 4-6 days of oviposition were sieved out and transferred to fresh jars. The newly emerged adults were used either for bioassay tests or for crossing. The F_1, F_2 and black-cross progenies were thus reared in untreated flour and their susceptibility to malathion was determined.

2.3 *Statistical analysis*

The log concentration-probit mortality (*Ld-p*) lines were determined for parental, F_1, F_2 and back-cross populations and LC_{50} values calculated, only for parental and F_1 population, by probit analysis (Finney 1952). The expected F_2 segregation on the basis of single factor Mendelian inheritance was calculated by for formula given by Georghiou (1969) $X(F_2) = a_1(S)\,0.25 + a_2(SR)\,0.50 + a_3(R)\,0.25$, where X is the expected response of F_2 to a given dose, and a_1, a_2 and a_3 are the observed responses of *S*, *SR* and *R* populations to that dose, read from their respective regression lines. Similarly, the expected segregations of the back-crosses were calculated as: back cross to *S* parent, $X(BC) = a_1(SR)\,0.50 + a_2(S)\,0.50$; back-cross to *R* parent, $X(BC) = a_1(SR)\,0.50 + a_2(R)\,0.50$. The agreement of the observed response to the expected was tested by the x^2 method given by Finney (1952) as $x^2 = (r-np)^2/np(1-P)$ where, r=actual number affected, n=total number treated, p=expected proportion affected.

Significant deviations of the observed from the expected are indicated by $x^2 > 3.8$ for 1 degree of freedom at 0.05 *P* level.

2.4 *Presentation of data*

In all instances reciprocal crosses were made. Since the results were found statistically similar only results from one set of the crosses have been presented.

3. Results and discussion

The toxicity data of malathion to parents and the respective crosses are summarised in table 1. A comparison of the LC_{50} values of the parents shows that the *R*-parent is 30.73 times resistant to malathion than the *S*-parent. Based on the LC_{50} values, the resistance ratios of the reciprocal F_1's over the *S*-parent are 11.05 and 10.26 times. The LC_{50} values of F_1 from the reciprocal crosses are statistically similar, implying thereby that the resistance ratios of the two F_1's do not differ significantly.

The statistical agreement between the LC_{50} values of the reciprocal F_1's shows that there is no maternal effect and evidence of sex-linkage. Therefore, the character of resistance is autosomal, being transmitted to the off-spring by either the male or the female parent and no significant extrachromosomal (cytoplasmic) inheritance of resistance is involved. Further, susceptibility of the *S*, *R* and F_1 populations shows that the LC_{50} for F_1 is different from either of the parents. The resistance ratios for the *R* and the F_1's were 30.73, 11.05 and 10.26, respectively and according to Georghiou (1969) this indicates incomplete dominance of the character. It is also revealed from figure 1 wherein the regression lines for F_1 fall in between those for the two parents.

The various log concentration-probit mortality points for the F_2 population when plotted and connected, the resultant line showed a tendency for deflection in the range of 20 to 50% mortality giving rise to the formation of distinct plateaus in the line (figure 1). The appearance of such deflections and plateaus in the Ld-*p* line indicates that the F_2 population is segregating into three classes of phenotypes and consequently resistance may be due to a major factor. Assuming that a major factor is involved in this malathion-resistant strain and that the factor segregates in the usual Mendelian 1:2:1 ratio in the F_2 population, the observed mortalities were

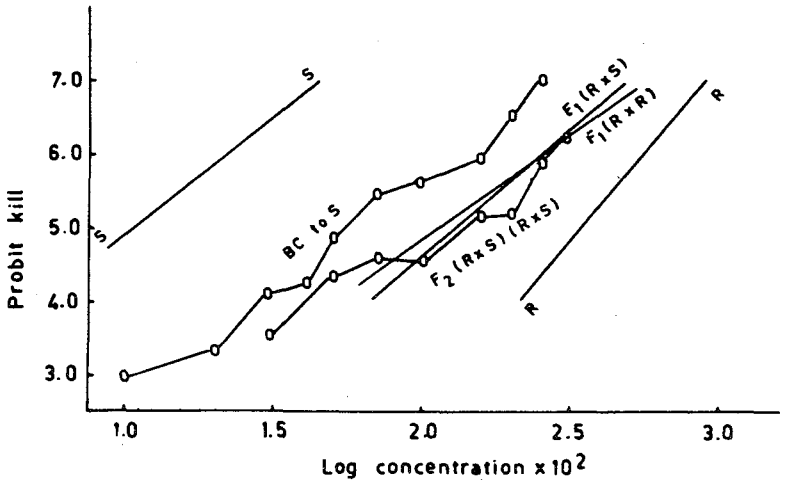

Figure 1. Ld-*p* lines for malathion applied to the adults of susceptible *(S)* and malathion-resistant *(R)* parents and their various crosses of *T. castaneum* (straight lines have been drawn for parents and F_1 populations whereas for F_2 and back cross (*BC*) to S-parent points have been connected to show the deflection in the lines).

Table 1. Susceptibility to malathion of parents and the population of various crosses of malathion resistant (*R*) and susceptible (*S*) *T. castaneum*.

Malathion Conc. %	*S*	*R*	F_1		F_2			Back crosses					
					R×*S*			(S×*R*) × *S*			(*R*×*S*) × *R*		
			R×*S*	*S*×*R*	*O*	*E*	x^2	*O*	*E*	x^2	*O*	*E*	x^2
0.05	11												
0.10	51							03	23	6.65*			
0.15	65							—	—	—			
0.20	79							17	42	7.62*			
0.25	88							—	—	—			
0.30	99				7	25	7.73*	27	48	5.62*			
0.40					—	—	—	37	53	3.32			
0.50			12	16	24	30	0.63	60	57	0.10			
0.70			—	—	33	36	0.17	67	64	0.08			
1.00			40	47	31	45	3.65	70	72	0.06	26	21	1.41
1.50			52	58	56	61	0.48	77	82	0.55	41	35	1.68
2.00		14	76	73	58	67	1.89	87	88	0.04	47	46	0.01
2.50		25	90	87	80	75	0.58	100	92	2.57	62	57	1.00
2.75		36	—	—	—	—	—				—	—	—
3.00		48	92	91	89	80	2.14				66	66	0.00
3.50		—			96	87	3.17				—	—	—
4.00		65									80	79	0.06
5.00		73											
6.00		95											
LC_{50}	0.109	3.380	1.205	1.119									
Fudicial	0.084	3.210	1.096	1.009									
Limits	0.140	3.540	1.324	1.242									
Slope (*b*)	3.2640±0.2615	4.5640±0.3220	3.3220±0.2860	2.8542±0.2433									
x^2	3.02	4.71	6.88	3.72									
DF	3	4	4	4									
R ratio	—	30.73	11.05	10.26									

*Significant at P=0.05 for 1 DF, O=observed mortality, E=expected mortality.

analysed for goodness of fit to monofactorial inheritance. The results show a good agreement and the observed response fits well with the expected in 8 out of 9 concentrations. Hence, it is concluded that malathion resistance is controlled by a major single factor.

The Ld-*p* line for the test-cross population shows a tendency for deflection around 50% mortality (figure 1) which suggests a segregation of groups of phenotypes in the population. The x^2- analysis of the observed and expected mortalities on the basis of 1:1 segregation at each concentration for the test cross population reveals that there is a close agreement between the two (table 1). However, some deviations in the range of mortality lower than 50% were observed. Similar agreement at each concentration has been found for the population of back cross to *R*-parent. This agreement in test-cross and back cross to *R*-parent further supports the conclusion that a major single factor is involved in the inheritance of resistance to malathion in this strain.

Further, the absence of overlapping in the regression lines for *SS* and *SR* genotypes suggests that a discriminating concentration of 0.5% malathion separates the susceptible phenotypes (*SS*) from that of the heterozygotes (*SR*). Based on this, at 0.5% concentration (table 2) the two F_2 populations showed 24 and 27% mortalities whereas the back crosses to *S*-parent showed 44, 60, 52 and 49% mortalities having a good fit to monofactorial inheritance by x^2 method which also reveals that a major single factor is involved in the inheritance of malathion resistance in this strain.

In F_2 and back crosses to *S*-parent significant deviations were observed in certain lower concentrations. 0.3% concentration expected to give 25% mortality in F_2 and 0.4% and below expected to give mortalities ranging from 53 to 22% in the back cross to *S*-parent, significantly lower mortalities distorted the segregations wherever the homozygous susceptible genotypes were expected to appear. These deviations may be attributed to the elimination of susceptible genotypes due to a reduction in egg hatch (3.11%) and larval survival (11.93%) in the susceptible strain as compared to the resistant as observed by authors from the biology studies (unpublished data).

Table 2. Use of discriminatory concentration of 0.5% malathion to the adults of different populations of crosses between malathion-resistant (*R*) and susceptible (*S*) *T. castaneum*.

Cross	Total no. of insects treated	% of insects		Agreement of observed and expected frequency
		Survived	Dead	$x^2=(O-E)^2/E$
$F_2(R\times S)\times(R\times S)$	45	76	24	0.63
$F_2(S\times R)\times(S\times R)$	90	73	27	1.17
$F_1(R\times S)\times S$	45	56	44	0.59
$S\times F_1(R\times S)$	60	48	52	0.21
$F_1(S\times R)\times S$	30	40	60	0.10
$S\times F_1(S\times R)$	60	51	49	1.88

All values of x^2 are not significant at 1 degree of freedom and 0.05 level of probability as $x^2<3.84$.

Thus, the results of the present studies on the inheritance of malathion resistance in *T. castaneum* show that it is inherited as an autosomal, incompletely dominant major factor. The evidence is mainly based on (i) the absence of continuous distribution of the character of resistance in the F_2 and back cross progenies and (ii) the statistical agreement of the observed responses to those which may be expected in the case of monofactorial inheritance.

References

Anonymous 1970 Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides; *FAO Plant Prot. Bull.* **18** 107-113

Bhatia S K and Pradhan S 1968 Studies on resistance to insecticides in *T. castaneum* (Herbst) — I. Selection of a strain resistant to p,p'DDT and its biological characteristics; *Indian J. Entomol.* **30** 13-32

Bhatia S K, Yadav T D and Mookherjee P B 1971 Malathion resistance in *T. castaneum* (Herbst) in India; *J. Stored Prod. Res.* **7** 227-230

Champ B R and Dyte C E 1976 Report of the FAO global survey of pesticide susceptibility of stored grain pests; *FAO Plant Production and Protection Series No.5* FAO of the United Nations Rome pp.297

Finney D J 1952 *Probit Analysis* 2nd edn (London: Cambridge University Press)

Georghiou G P 1969 Genetics of resistance to insecticides in house flies and mosquitoes; *Exp. Parasitol.* **26** 224-255