

Inheritance and Linkage of Malathion Resistance in the Red Flour Beetle¹

RICHARD W. BEEMAN

U.S. Grain Marketing Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Manhattan, Kansas 66502

J. Econ. Entomol. 76: 737-740 (1983)

ABSTRACT A strain of *Tribolium castaneum* (Herbst) from Georgia was 73-fold resistant to malathion and was highly cross-resistant to phenthoate ($\times 53$), but not to structurally dissimilar carboxylate esters; cross-resistance to any of a variety of other organophosphates, carbamates, chlorinated hydrocarbons or pyrethroids ($\leq \times 2.7$) was not detected. Malathion resistance (*Rmal*) was inherited as a simple autosomal semidominant trait. Linkage analysis showed *Rmal* to be located on group VI, 24.6 ± 1.0 map units from Microphthalmic.

Malathion resistance in the red flour beetle, *Tribolium castaneum* (Herbst), has become a worldwide problem since its first recorded occurrence in groundnut stores in Nigeria in 1961 (Parkin 1965, Champ and Dyte 1977). The situation is much the same in the United States, where such resistance is widespread and seems to have intensified over the past 15 years (Zettler 1982, Haliscak and Beeman 1983). High levels of resistance to DDT (Dyte and Blackman 1967, Bhatia and Bansode 1971) and to the cyclodiene insecticides (Bhatia and Pradhan 1972) have also been achieved by laboratory selection in strains of *T. castaneum*. A strain with nonspecific resistance was collected in Australia (Champ and Campbell-Brown 1970) and was subsequently shown to possess elevated levels of microsomal cytochrome P-450 (Wool et al. 1982).

High-level malathion resistance is commonly encountered in many pest species and is usually of the malathion-specific, triphenyl-phosphate-suppressible type (Plapp et al. 1963, Plapp and Tong 1966, Dyte and Rowlands 1968, Townsend and Busvine 1969). Such resistance is usually under the control of single, major genes; these have been located and mapped in several species of diptera (Tadano 1969, Sawicki 1973).

In this paper I describe the cross-resistance spectrum, mode of inheritance, linkage relationships, and map position of triphenyl-phosphate-suppressible malathion resistance in the red flour beetle.

Materials and Methods

Strains

The malathion-resistant (R) strain was collected in a farmer's corn bin in Georgia in the summer of 1980, and is identical with strain GA-1 described by Haliscak and Beeman (1983). The strain was already homozygous for resistance when collected (resistance factor >83 at LD_{50}) and did not respond to intense selection pressure applied in the laboratory for several generations. The laboratory susceptible (lab-S) strain is of Kansas origin

and has been under continuous culture at the U.S. Grain Marketing Research Laboratory for many years.

Mutant stocks bearing the following autosomal markers were obtained from the *Tribolium* Stock Center, Department of Biological Sciences, California State College, San Bernardino (abbreviation and linkage group in parentheses): pearl (*p*, II), missing abdominal sternites (*mas*, II), black (*b*, III), sooty (*s*, IV), jet (*j*, V), Microphthalmic (*Mo*, VI), chestnut (*c*, VII), antennapedia (*ap*, VIII), and alate prothorax (*apt*, IX). Descriptions of these mutants can be found in Sokoloff (1966). All marker strains proved to be homozygous for susceptibility to malathion.

Rearing and Bioassay

All strains were reared in continuous darkness at 28°C and 70% relative humidity (RH) on wheat flour fortified with 5% (wt/wt) brewer's yeast. For bioassay, the impregnated filter paper method of Stringer (1949) was used, as modified by Haliscak and Beeman (1983). To determine the cross-resistance spectrum of the R strain with respect to the lab-S strain, LT_{50} values were determined graphically for a single dose of each insecticide, using 5 replicates of 30 beetles each. In the case of lindane, dibutylphthalate was added to the carrier in a 2:1 ratio with sunflower oil. Glutaryl-methamidophos was applied topically with 0.5 μ l of acetone. All insecticides were technical or analytical grade unless otherwise indicated. Adults 1 to 3 weeks old were used for all bioassays. Beetles within this range of ages were obtained by harvesting pupae and holding them for 16 to 21 days at 28°C and 70% RH before bioassay.

Mode of Inheritance

To determine the mode of inheritance of malathion resistance, reciprocal crosses were made between the R strain and the susceptible mutant *s* strain. Beetles were sexed as pupae and were mass-mated (10 to 15 pairs per cross) in 1-pint (ca. 473-ml) jars of medium. Male F_1 progeny were backcrossed to *s* females. Backcross of heterozygous resistant male progeny to susceptible *s* females was repeated for five consecutive generations to detect segregation of potentially multifactorial resistance. Before each backcross, heterozygous resistant males were

¹Mention of a proprietary product does not constitute a recommendation or an endorsement by the USDA. Received for publication 6 January 1983; accepted 15 March 1983.

selected by exposure to malathion (10 mg per filter paper) for a discriminating time of 2 h.

Analysis of Linkage

All test crosses were of the type $AaBb\delta \times aabb\varphi$. To test for linkage of the gene for resistance, *Rmal*, to the recessive markers *p*, *mas*, *b*, *s*, *j*, *c*, *ap*, and *apt*, marker-free, *Rmal* males were mass mated (10 to 15 pairs) to susceptible, mutant females. F_1 males (doubly heterozygous for *Rmal* and the recessive marker, coupling phase) were then mass-backcrossed to females of the appropriate marker strain. These adults were transferred twice to fresh medium at ca. 1-week intervals to obtain three batches of backcross progeny from each test cross. The progeny were scored for *Rmal* and visible marker phenotype. *Rmal* phenotype was determined by selecting the beetles with malathion at the discriminating time as described previously. The data were subjected to chi-square analysis for significant deviations from the 1:1:1:1 ratio of phenotypes predicted by independent assortment.

To test for linkage of *Rmal* to the dominant marker *Mo*, *Rmal* males were mated to *Mo* females. Since *Mo* is also a recessive lethal, these females were heterozygous for *Mo*. F_1 males expressing the *Mo* phenotype were outcrossed to females from the malathion-susceptible, marker-free lab-S strain. Since *Mo* is dominant and *Rmal* proved to be functionally dominant, this was a test-cross to a double recessive of the type $AaBb \times aabb$ (repulsion phase).

The expression of *Mo* is variable and overlaps the wild type. It is therefore not possible to distinguish wild type from *Mo* absolutely. For this reason, the test-cross progeny were carefully examined (as pupae) under a binocular microscope, and all pupae which could be unambiguously classified as *Mo* were segregated. Only this class was used for analysis of linkage, since the remaining pupae were an unresolvable mixture of wild-type and slightly expressed *Mo*. However, all beetles from both classes were scored as to *Rmal* phenotype to test for segregation of *Rmal*.

Results and Discussion

Cross-Resistance Spectrum

Resistance was of the malathion-specific type (Table 1). The R strain was 73-fold resistant with respect to the lab-S strain at LT_{50} . Intense cross-resistance extended to phenthoate, but not to structurally dissimilar carboxylate esters, nor to any of a variety of other organophosphates, carbamates, chlorinated hydrocarbons, or pyrethroids. As expected, malathion resistance in this strain is triphenylphosphate suppressible (Haliscak and Beeman 1983) and is associated with high malathion carboxylesterase activity (J. P. Haliscak and R. W. Beeman, unpublished data). Cross-resistance to phenthoate has also been reported in a strain from Kano, Nigeria, with this type of malathion resistance (Dyte and Blackman 1972).

Table 1. Cross-resistance spectrum for the Georgia (R) strain of *T. castaneum*

Insecticide	Dose (mg) ^a	LT_{50} (h)		R factor
		S ^b	R	
Malathion	10.0	0.60	44.0	73.0
Phenthoate	10.0	0.94	50.0	53.0
Methacrifos	1.0	0.67	0.54	0.81
Bomyl	1.0	5.0	8.5	1.7
Propetamphos	2.5	0.72	0.91	1.3
Isofenphos	2.5	5.2	6.2	1.2
Mevinphos	0.25	10.0	10.0	1.0
Glutarylmetamidophos	0.1	4.7	4.9	1.0
Pirimiphos methyl EC	2.5	0.79	1.4	1.7
Chlorpyrifos methyl EC	0.5	1.1	3.0	2.7
Fenitrothion EC	2.5	1.2	2.0	1.7
Diazinon EC	10.0	1.6	2.5	1.6
Fenvalerate	1.0	3.0	2.8	0.93
Decamethrin	0.1	0.78	1.8	2.3
Methoxychlor	15.0	4.4	7.8	1.8
Lindane	15.0	1.6	1.6	1.0
Cis-Chlordane	10.0	8.3	17.0	2.0

^aDose (AI) per paper (dose per insect in the case of glutarylmetamidophos).

^bMarker-free, Manhattan laboratory strain.

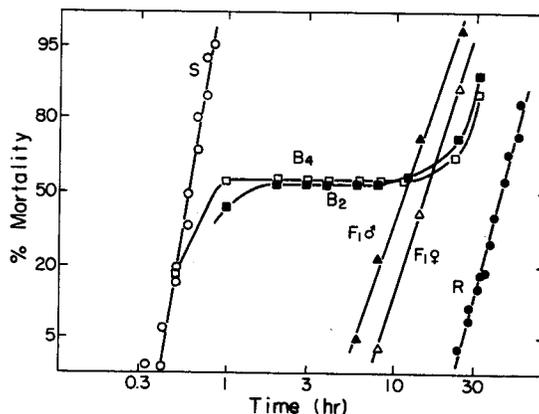


FIG. 1. Time-mortality response of susceptible, resistant, F_1 , and backcross populations of *T. castaneum* to malathion (10 mg per filter paper). Symbols: \circ , sooty; \bullet , Georgia; \blacktriangle and \triangle , F_1 (sooty $\varphi \times$ Georgia δ); \blacksquare and \square , second and fourth consecutive backcrosses, respectively, of selected hybrid males to sooty females (mixed sex).

Mode of Inheritance

Steep time-response curves indicated that both the *s* and *R* strains were uniform in their sensitivities to malathion (Fig. 1). Data points for the *s* and *R* strains in Fig. 1 include separate points for males and females; since no sex difference occurred however, the points are not differentiated and a single line is drawn for each strain. Resistance was semidominant. The f_1 data shown in Fig. 1 represent the cross $s\varphi \times R\delta$. The reciprocal cross (data not shown) gave almost identical results, confirming an autosomal mode of inheritance. A slight sex difference occurred in the F_1 population depicted in

Fig. 1, but not in the reciprocal cross. Repeated backcrossing of resistant hybrids to the *s* strain consistently yielded two classes of offspring (resistant and susceptible) of about equal size. There was no segregation of the resistant class into partially resistant subclasses even after five consecutive generations of backcross. Results for the second and fourth backcross populations are shown in Fig. 1. These observations constitute good evidence that resistance is primarily controlled by a single, semidominant allele or closely linked set of alleles (Crow 1957). Figure 1 shows that *Rmal* is best treated as dominant for the purpose of linkage analysis, since a wide range of discriminating times (2 to 6 h) can be used to distinguish *Rmal*/+ from +/+ genotypes, but no discriminating time completely segregates *Rmal*/+ from *Rmal*/*Rmal*.

Wool et al. (1982) inferred the presence of a Y-linked factor, in addition to a major autosomal factor, enhancing malathion resistance in the Kano strain based on the greater resistance level of Kano males compared with females, and the observation that F_1 males derived from Kano males were more resistant than their female siblings, whereas no sex difference was found in the reciprocal cross. Such a Y-linked factor was not present in the Georgia R strain described herein, since there was no sex difference in the resistance level of the R strain, and since F_1 males were not more resistant than their female siblings. The overdominance of resistance in the Kano strain further differentiates it from the Georgia strain.

Analysis of Linkage

Linkage analysis using group I markers (the X chromosome) was not performed, since *Rmal* was not sex linked. The results of linkage tests using markers on autosomal groups II to IX are given in Table 2. Significant deviation from the predicted ratio of 1:1:1:1 for independent assortment occurred in test crosses involving the recessive markers *p*, *s*, *j*, *ap*, and *apt*, and the dominant marker *Mo*. However, it is clear from the data in Table 2 that only *Mo* shows true linkage with *Rmal*. For *p*, *j*, *ap*, and *apt*, the deviations from prediction

reflect a deficiency in the mutant class and do not reflect linkage. For example, in the test-cross involving *p*, if the *Rmal* phenotype is ignored, equal numbers of pearl and wild type should be present. The value of χ^2 ($= 13.9$, $df = 1$, $P < 0.0002$) indicates that the pearl class is obviously deficient. In the case of *s*, the deviation from prediction reflects the combined effect of slight deficiencies in both the sooty and resistant classes, and again does not reflect linkage.

In the case of *Mo*, there was no overall deficiency in the resistant class, since, of a total of 6,307 testcross progeny, 3,153 (50.0%) were resistant. However, in the sample of 1799 *Mo* beetles segregated as pupae for linkage analysis, only 443 (24.6%) proved to be resistant. Thus *Rmal* is linked to *Mo* at a map distance of 24.6 ± 1.0 . The frequency of recombination between *Rmal* and *Mo* was calculated separately for early, middle and late test-cross progeny. The values were 24.2, 22.9, and 26.5%, respectively.

Major genes for insecticide resistance in pest insects have been previously mapped only in species of diptera and orthoptera (e.g., Klassen 1966, Sawicki 1973, Ross and Cochran 1975, Arnold and Whitten 1976). The availability of good markers for 9 of the 10 linkage groups of *T. castaneum* makes this species, at present, the only coleopteran pest for which there is a reasonable prospect of obtaining extensive genetic information about insecticide resistance. Several of the linkage groups have been extensively mapped (Sokoloff 1977). Such genetic information should make possible the detection and recovery of radiation-induced chromosomal aberrations which might, in turn, be tailored for use as transporting mechanisms to introduce insecticide susceptibility or other desirable genes into natural populations (Curtis 1968, Foster et al. 1972).

T. castaneum has several attributes which recommend it over the dipterans as a model for evaluating genetic methods of population manipulation. It is very easy to rear and handle, and seldom flies under laboratory conditions. Extensive knowledge of the population dynamics of *T. castaneum* and related species is available because the *Tribolium* group has been a favorite of ecologists for years (e.g., Sokoloff 1974). This, coupled with its lon-

Table 2. Tests of linkage of malathion resistance (*Rmal*) with autosomal markers in *T. castaneum*

Marker ^a	No. of progeny of given phenotype ^b				χ^2	P	exp
	R,m	+ ,m	R, +	+ , +			
<i>p</i> (II)	606	597	716	677	15.1	0.002	649
<i>mas</i> (II)	552	527	554	582	2.74	0.43	554
<i>b</i> (III)	1,079	1,119	1,124	1,104	1.11	0.78	1,106
<i>s</i> (IV)	347	375	371	423	8.02	0.045	379
<i>j</i> (V)	882	941	1,553	1,609	362	<0.0001	1,246
<i>Mo</i> (VI)	443	1,346	—	—	463 ^c	<0.0001	900
<i>c</i> (VII)	964	937	1,019	949	4.07	0.25	967
<i>ap</i> (VIII)	374	378	467	434	14.8	0.002	413
<i>apt</i> (IX)	1,062	1,034	1,141	1,189	13.8	0.003	1,106

^aSee text for definitions of abbreviations.

^b"m" Refers to the marker indicated in the left-hand column.

^cBased on *Mo* phenotypic class only (see text for explanation).

gevity and, in particular, its long reproductive life, makes *T. castaneum* a logical candidate for use in the study of subtle or long-term effects of genetic manipulation on population equilibria in the laboratory as well as in a field or warehouse.

Acknowledgment

I thank A. Sokoloff for providing the mutant stocks.

REFERENCES CITED

- Arnold, J. T. A., and M. J. Whitten. 1976. The genetic basis for organophosphorus resistance in the Australian sheep blowfly, *Lucilia cuprina* (Wiedemann) (Diptera, Calliphoridae). *Bull. Entomol. Res.* 66: 561-568.
- Bhatia, S. K., and P. C. Bansode. 1971. Studies on resistance to insecticides in *Tribolium castaneum* (Herbst). IV. Susceptibility of p, p'-DDT-resistant strains to some fumigants. *Indian J. Entomol.* 33: 45-49.
- Bhatia, S. K., and S. Pradhan. 1972. Studies on resistance to insecticides in *Tribolium castaneum* (Herbst). V. Cross-resistance characteristics of a lindane-resistant strain. *J. Stored Prod. Res.* 8: 89-93.
- Champ, B. R., and M. J. Campbell-Brown. 1970. Insecticide resistance in Australian *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae). II. Malathion resistance in Eastern Australia. *Ibid.* 6: 111-131.
- Champ, B. R., and C. E. Dyte. 1977. FAO global survey of pesticide susceptibility of stored grain pests. *FAO Plant Prot. Bull.* 25: 49-67.
- Crow, J. F. 1957. Genetics of insect resistance to chemicals. *Annu. Rev. Entomol.* 2: 227-246.
- Curtis, C. F. 1968. Possible use of translocations to fix desirable genes in insect pest populations. *Nature (London)* 218: 368-369.
- Dyte, C. E., and D. G. Blackman. 1967. Selection of a DDT-resistant strain of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *J. Stored Prod. Res.* 2: 211-228.
1972. Laboratory evaluation of organophosphorus insecticides against susceptible and malathion-resistant strains of *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae). *Ibid.* 8: 103-109.
- Dyte, C. E., and D. G. Rowlands. 1968. The metabolism and synergism of malathion in resistant and susceptible strains of *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae). *Ibid.* 4: 157-173.
- Foster, G. G., M. J. Whitten, T. Prout, and R. Gill. 1972. Chromosome rearrangements for the control of insect pests. *Science* 176: 875-880.
- Haliscak, J. P., and R. W. Beeman. 1983. Status of malathion resistance in five genera of beetles infesting farm-stored corn, wheat and oats in the United States. *J. Econ. Entomol.* 76: 717-722.
- Klassen, W. 1966. Genetics of resistance in mosquitoes. *Mosq. News* 26: 309-318.
- Parkin, E. A. 1965. The onset of insecticide resistance among field populations of stored-product insects. *J. Stored Prod. Res.* 1: 3-8.
- Plapp, F. W., Jr., and H. H. C. Tong. 1966. Synergism of malathion and parathion against resistant insects: phosphorus esters with synergistic properties. *J. Econ. Entomol.* 59: 11-15.
- Plapp, F. W., Jr., W. S. Bigley, G. A. Chapman, and G. W. Eddy. 1963. Synergism of malathion against resistant house flies and mosquitoes. *Ibid.* 56: 643-649.
- Ross, M. H., and D. G. Cochran. 1975. The German cockroach, *Blattella germanica*, pp. 35-62. In R. C. King [ed.], *Handbook of genetics*. Vol. 3. Invertebrates of genetic interest. Plenum Press, New York. 874 pp.
- Sawicki, R. M. 1973. Recent advances in the study of the genetics of resistance in the housefly. *Musca domestica*. *Pestic. Sci.* 4: 501-512.
- Sokoloff, A. 1966. The genetics of *Tribolium* and related species. Academic Press, New York. 212 pp.
1974. The biology of *Tribolium*, with special emphasis on genetic aspects. Vol. 2. Clarendon Press, Oxford. 610 pp.
1977. The biology of *Tribolium* with special emphasis on genetic aspects. Vol. 3. Clarendon Press, Oxford. 612 pp.
- Stringer, A. 1949. A simple method for assaying contact toxicities of insecticides, with results of tests of some organic compounds against *Calandra granaria* L. *Ann. Appl. Biol.* 36: 213-224.
- Tadano, T. 1969. Genetical linkage of malathion-resistance in *Culex pipiens* L. *Jpn. J. Exp. Med.* 39: 13-16.
- Townsend, M. A., and J. R. Busvine. 1969. The mechanism of malathion-resistance in the blowfly *Chrysomya putoria*. *Entomol. Exp. Appl.* 12: 243-267.
- Wool, D., S. Noiman, O. Manheim, and E. Cohen. 1982. Malathion resistance in *Tribolium* strains and their hybrids: inheritance patterns and possible enzymatic mechanisms (Coleoptera, Tenebrionidae). *Biochem. Genet.* 20: 621-636.
- Zettler, J. L. 1982. Insecticide resistance in selected stored-product insects infesting peanuts in the southeastern United States. *J. Econ. Entomol.* 75: 359-362.