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Status of Malathion Resistance in Five Genera of Beetles Infesting Farm-Stored Corn, Wheat, and Oats in the United States[1]

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ABSTRACT More than 100 strains of *Tribolium castaneum* (Herbst), *Rhyzopertha dominica* (F.), *Sitophilus* spp., *Cryptolestes* spp., and *Oryzaephilus* spp., were collected from grain bins on farms in 14 grain-producing states. These were screened for resistance to malathion by the discriminating dose technique, using impregnated filter papers. Measurable tolerance occurred in 31 of 36 strains of *Tribolium*, 11 of 13 strains of *Rhyzopertha*, 7 of 22 strains of *Sitophilus*, 5 of 42 strains of *Cryptolestes*, and none of 13 strains of *Oryzaephilus*. Resistance was widespread and severe only in *Tribolium castaneum*. Fifteen of the *T. castaneum* strains were tested further, and all achieved resistance levels of >20-fold after a single selection. Resistance in this species was largely or completely suppressed by triphenylphosphate in all 13 strains tested.

The weevils, *Sitophilus* spp., lesser grain borer, *Rhyzopertha dominica* (F.), sawtoothed and merchant grain beetles, *Oryzaephilus* spp., red flour beetle, *Tribolium castaneum* (Herbst), and flat and rusty grain beetles, *Cryptolestes* spp., are among the most serious pests of stored grain and cereal products in this country and worldwide (Cotton and Wilbur 1974). Malathion was registered for postharvest insect control in the United States in 1958 (Anonymous 1958) and has been the primary residual insecticide for direct treatment of stored grain since that time. Minimum effective residues of malathion for beetle control on wheat at 13% moisture are typically 0.3 to 0.5 ppm for *Oryzaephilus* spp., 0.5 to 2 ppm for *Cryptolestes* spp., 1 to 3 ppm for *Sitophilus* spp. and *T. castaneum*, and 5 to 20 ppm for *R. dominica* (Strong et al. 1967). The maximum legal tolerance for malathion residues on marketable grain is 8 ppm (Anonymous 1958). In practice, much higher residues often occur, particularly with surface treatments (Quinlan 1972).

Malathion resistance in beetles infesting stored commodities has become widespread on a global scale over the past 2 decades (Parkin 1965, Pasalu et al. 1974). In a broad survey of resistance covering eight species of beetles in 85 countries, Champ and Dyte (1977) found malathion resistance to be most frequently encountered in *Tribolium* spp., both worldwide and in North America. We know of no other published reports of malathion resistance in North American populations of these species, with the exception of *T. castaneum*.

With respect to *T. castaneum* in the United States, there has been a discernible association between resistance and malathion use, as well as a trend toward increasing resistance in more recent years. Malathion resistance in *T. castaneum* in the United States was first reported independently by Speirs et al. (1967) and by Vincent and Lindgren (1967). Both groups found relatively low resistance factors (~2- to 11-fold at LD_{50}).

A subsequent survey (Zettler 1974) showed that malathion resistance in *T. castaneum* had increased significantly between 1967 and 1974 in peanut warehouses in the southeastern United States. Zettler (1974) found high levels of resistance (resistance factors ~10 to 100 at LD_{50}) in all six strains collected in Georgia and Alabama. Bansode and Campbell (1979) found a high level of malathion-specific resistance in a composite strain of *T. castaneum* collected from grain bins on farms in several areas of North Carolina.

Strong et al. (1969) conducted an intensive statewide survey of malathion resistance in *Tribolium* spp. in California. Facilities for commodity storage were avoided in an attempt to more accurately sample "natural" populations with no history of exposure to malathion. No resistance was detected in 39 strains of *T. castaneum* from throughout the state.

All of these surveys are either long outdated, narrow in scope, or do not include farm-stored grain. We have recently reported on the status of malathion resistance in populations of the Indianmeal moth, *Plodia interpunctella* (Hübner), throughout the U.S. grain belt (Beeman et al. 1982). The present survey updates and expands our knowledge of the situation with regard to coleopterans.

Materials and Methods

Sampling

Corn, wheat, and oats from randomly selected bins on farms in 26 states were sampled in the summer of 1980 under a project coordinated by the Agricultural Stabilization and Conservation Service, designed to assess the quality of farm-stored grain reserves (Storey et al. 1982). All samples containing live adult coleoptera were saved, until a total of 127 strains representing five genera were accumulated from 14 states. Within each state, samples containing few insects were pooled until an average of ~50 beetles of a genus (minimum = 5) were accumulated for each strain. Most of the resulting strains contained insects from more than one bin, but no two strains contained insects from the same bin. *Si-*

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tophilus, Tribolium, and *Cryptolestes* spp. were found most frequently on corn, *R. dominica* on wheat, and *Oryzaephilus* spp. on oats. Standard laboratory (susceptible) strains of *S. oryzae* (L.) (rice weevil), *R. dominica, T. castaneum, Oryzaephilus surinamensis* (L.) (sawtoothed grain beetle), and *Cryptolestes ferrugineus* (Stephens) (rusty grain beetle) were used for comparison to field strains. All laboratory strains have been maintained for many years at the U.S. Grain Marketing Research Laboratory or at the Stored Products Laboratory, Department of Entomology, Kansas State University.

Rearing and Bioassy

Sitophilus spp. were reared on whole grain wheat, lesser grain borers on whole grain wheat containing a small amount of flour, *Cryptolestes* and *Oryzaephilus* spp. on a mixture of rolled oats, flour and corn meal (2:1:1, by volume), and red flour beetles on whole wheat flour containing 5% (wt/wt) brewer's yeast. All strains were maintained for at least two generations before testing. Adults 2 to 8 weeks old were used for all experiments.

Insects were treated by exposing them to filter papers impregnated with insecticide in an oil carrier. Technical-grade (95%) malathion, alone or in combination with triphenylphosphate, was dissolved in a mixture of hexanes (boiling point = 68–70°C), acetone, and sunflower oil (3:1:1, by volume). Whatman no. 1 7-cm filter paper discs were treated with 0.5 ml of the appropriate solution, and the solvents were allowed to evaporate overnight. The treated discs were laid on a glass plate, and the beetles were confined on the discs in glass rings 5 cm in diameter and 2.5 cm high. Rings were covered with fine-mesh screen to prevent immigration or escape by flight. In experiments with *Sitophilus* and *Oryzaephilus,* the rings were also coated with mineral oil or Teflon, respectively, to prevent escape by climbing. Mortality was assessed after 48 h. Insects were counted as dead if they were ataxic and unable to right themselves. Mortality in the controls was ≤2% in all cases.

All strains were tested by using discriminating doses of malathion, i.e., doses known to produce 100% mortality in the standard laboratory (lab-S) strains. Values for LD_{100} were determined empirically, and were set as low as possible to permit detection of marginal or low-level resistance. Probit analysis of data for the lab-S strains was conducted according to Finney (1971), using 100 insects at each of three to six concentrations for each species. FAO method no. 15 (Anonymous 1974) includes a discussion of the discriminating dose principle.

To determine whether levels of resistance could be increased by selection, survivors of discriminating doses were cultured, and their progeny were retested. A few strains were selected for several consecutive generations to determine their maximum potential for achieving high levels of resistance.

Field strains of *Sitophilus, Oryzaephilus,* and *Cryptolestes* collected in this survey were not identified to species. To gain valid information about resistance at the genus level, laboratory strains representing the most tolerant species within each genus were chosen as standards for comparison. In this way it was assured that no "false positives" would be recorded. In the genus *Sitophilus,* the rice weevil, *S. oryzae* is the most tolerant of the three major species (Strong et al. 1967), although species differences are only slight. Futhermore, interspecific hybridization can occur between *S. oryzae* and *S. zeamais* (Kiritani 1965) and species identity may therefore not exist in mixed populations. Species determination in the genus *Cryptolestes* is notoriously difficult (Howe and Lefkovitch 1957). We suspect that at least some of the strains were mixed species. *C. ferrugineus* and *C. pusillus* (Schönherr) (formerly *C. minutus*) are believed to be the two most common stored-grain pest species representing this genus in the United States (Howe and Lefkovitch 1957). Preliminary tests using standard laboratory strains revealed that *C. ferrugineus* was more tolerant to malathion than *C. pusillus* (the flat grain beetle). We therefore used *C. ferrugineus* as the standard for comparison. In the genus *Oryzaephilus,* the two pest species occurring in this country, *O. surinamensis* and *O. mercator* (Fauvel), are identical in malathion tolerance (Strong et al. 1967). Furthermore, of the two species only *O. surinamensis* is considered to be a significant pest on stored grain (Howe 1956). We therefore used *O. surinamensis* as the standard for comparison.

Results and Discussion

Table 1 shows the results of log-probit analysis of data for the lab (susceptible) strains, as well as the empirically determined values for LD_{100}, which were used as the discriminating doses. The relative susceptibilities of these laboratory strains to malathion (in decreasing

Table 1. Probit analysis and discriminating doses for malathion against lab-S strains of four species of coleoptera

Species	LD_{50}[a]	LD_{95}[a]	Slope	Discriminating dose[b]
Oryzaephilus surinamensis	0.015 (0.014–0.017)	0.06 (0.05–0.07)	2.90	0.10
Tribolium castaneum	0.06 (0.04–0.07)	0.12 (0.10–0.19)	5.08	0.15
Sitophilus oryzae	0.10 (0.06–0.14)	0.17 (0.13–1.58)	6.89	0.15
Cryptolestes ferrugineus	0.14 (0.10–0.18)	0.48 (0.31–1.19)	3.08	1.0
Rhyzopertha dominica	0.6 (0.53–0.66)	1.50 (1.30–1.85)	4.12	1.75

[a]95% Confidence limits given in parentheses.
[b]Values expressed as mg per disc. Values for discriminating doses were determined empirically.

Table 2. Mortality in strains of *T. castaneum* treated with discriminating doses of malathion[a]

Strain[b]	Mortality (%) at indicated malathion dose (mg/disc): Before selection[c]	After selection[c]			R factor[d]
	0.15	0.15	2.5	10	
GA-1	0 ± 0	1.3 ± 1.9	4.0 ± 3.3	77.3 ± 4.6	>83
KS-1	0 ± 0				
MN-1	2.7 ± 1.9	12.0 ± 5.6	48.0 ± 20.0	100 ± 0	>20
IA-1	12.0 ± 9.5	6.7 ± 1.9	84.0 ± 8.6	100 ± 0	>20
IA-2	13.5 ± 8.5	3.3 ± 2.7	66.7 ± 5.0	100 ± 0	>20
MN-2	15.3 ± 7.1	4.5 ± 2.8	68.0 ± 5.6	100 ± 0	>20
MN-3	16.7 ± 2.7	8.7 ± 1.9	74.7 ± 10.5	100 ± 0	>20
NE-1	20.0 ± 7.6	13.3 ± 6.8	74.7 ± 9.4	100 ± 0	>20
IA-3	22.0 ± 11.5	2.0 ± 2.0	82.7 ± 5.0	100 ± 0	>20
IA-4	27.3 ± 7.4	15.3 ± 4.3	84.0 ± 3.3	100 ± 0	>20
NE-2	29.3 ± 6.8	12.7 ± 5.8	76.0 ± 4.0	100 ± 0	>20
NE-3	32.0 ± 3.3	21.3 ± 6.4			
IA-5	34.0 ± 3.8	13.3 ± 7.2	64.0 ± 3.3	98.7 ± 2.3	>20
IA-6	35.3 ± 7.1	14.7 ± 5.0	92.0 ± 8.0	100 ± 0	~20
IA-7	35.3 ± 7.1	7.3 ± 6.3	90.7 ± 5.0	100 ± 0	~20
MN-4	36.0 ± 16.4	16.7 ± 5.8			
NE-4	38.0 ± 6.8	23.3 ± 9.1			
IA-8	51.3 ± 10.2	12.7 ± 4.3	77.3 ± 3.8	100 ± 0	>20
SD-1	51.3 ± 16.4	26.0 ± 11.2			
NE-5	55.3 ± 16.4	26.0 ± 11.2			
MN-5	57.3 ± 15.4	17.3 ± 1.9			
ND-1	62.7 ± 9.7	13.3 ± 5.5	82.7 ± 10.0	100 ± 0	>20
IA-9	65.3 ± 12.4	31.8 ± 10.8			
WI-1	70.6 ± 6.4	26.7 ± 4.4			
MN-6	76.0 ± 12.4	20.7 ± 7.4			
SD-2	83.3 ± 9.6	19.3 ± 8.1			
SD-3	86.0 ± 3.8	35.3 ± 7.8			
SD-4	87.3 ± 5.4	33.3 ± 11.0			
IA-10	94.0 ± 7.2	100 ± 0			
OH-1	95.3 ± 4.5	28.7 ± 7.1			
SD-5	99.3 ± 1.5				
MN-7	100 ± 0				<1.25
ND-2	100 ± 0				<1.25
ND-3	100 ± 0				<1.25
SD-6	100 ± 0				<1.25
MI-1	100 ± 0				<1.25
LAB-S	100 ± 0				≡1.0

[a]Values for percent mortality represent means ± SD of four independent determinations of 25 beetles each.
[b]Strain identifications indicate the state of origin.
[c]Survivors of the first test (dose = 0.15 mg) were used as seeding stock for "selected" cultures. Progeny were used for subsequent tests without further selection.
[d]The resistance factor refers to once-selected strains. It is the ratio of the estimated LD_{95} value for the field strain to that for the lab-S strain.

order of susceptibility) were as follows: sawtoothed grain beetle > rice weevil ~ red flour beetle > rusty grain beetle > lesser grain borer. This ranking is similar to that observed by Strong et. al. (1967).

T. castaneum

Of the species examined in this survey, the red flour beetle alone showed widespread and severe resistance to malathion. Of 36 strains from 10 states, only 5 suffered 100% mortality and almost half suffered < 50% mortality at the discriminating dose (Table 2) (Fig. 1). The resistant strains responded sharply to a single selection in almost every case (Table 2). The only exceptions were strains already highly resistant (GA-1 and MN-1) and strain IA-10, which was only slightly tolerant. The latter strain probably had vigor tolerance rather than specific resistance. Vigor tolerance would be expected to decline from inbreeding after intense selection pressure, whereas specific resistance would increase (cf. strains IA-10 and OH-1 in Table 2). Of the 15 strains tested at higher doses, all showed resistance factors of ≥20 after only one generation of selection (Table 2). In all 13 resistant strains tested, the resistance was largely or completely suppressed by the carboxylesterase inhibitor triphenylphosphate (TPP) (Table 3). However, in a few strains (IA-3, IA-4, and IA-7) a significant portion of the resistance was TPP nonsuppressible at the doses used (Table 3). This suggests that secondary mechanisms in addition to carboxylesterase may contribute to resistance in these strains.

On a national scale, there appears to be a tendency toward more severe resistance in more southern areas. Zettler (1974, 1982) found generally higher levels of resistance in Georgia, Alabama, and Florida than those reported here. Among the 15 strains tested at several

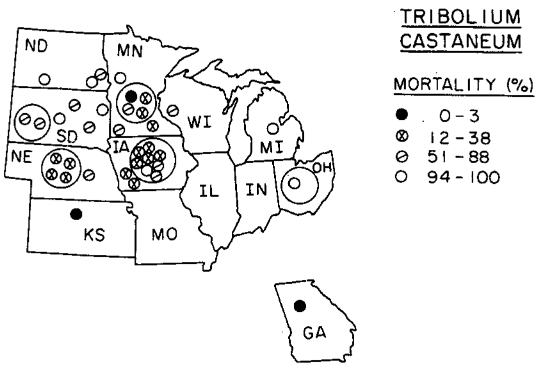

FIG. 1. Distribution of malathion resistance in populations of *T. castaneum*. Data for percent mortality are from Table 2. Data points enclosed by large circles indicate pooled strains from many counties, or from scattered localities within the state. Data points not enclosed by large circles indicate the county of origin, or the county contributing the largest number of samples in the case of composite strains from a few adjacent counties.

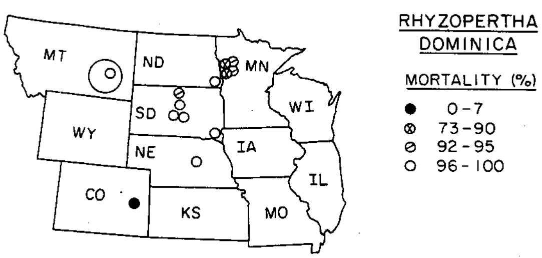

FIG. 2. Distribution of malathion resistance in populations of *Rhyzopertha dominica*. Data for percent mortality are from Table 4. See also legend to Fig. 1.

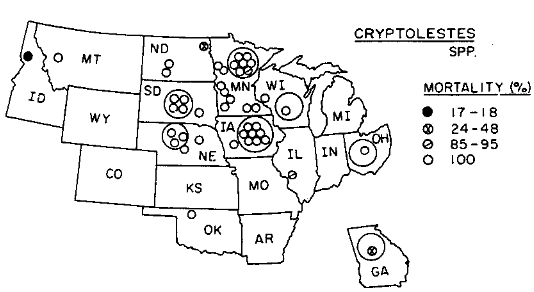

FIG. 3. Distribution of malathion resistance in populations of *Cryptolestes*. See also legend to Fig. 1.

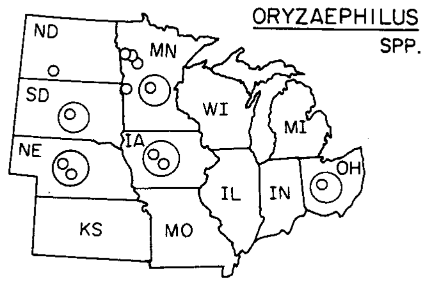

FIG. 4. Distribution of *Oryzaephilus* populations screened for resistance to malathion. See also legend to Fig. 1.

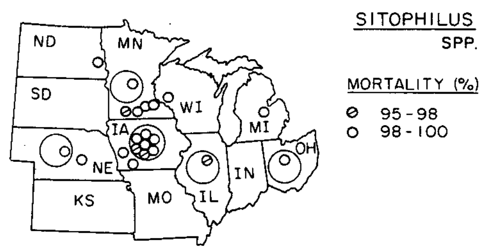

FIG. 5. Distribution of malathion resistance in populations of *Sitophilus*. See also legend to Fig. 1.

Table 3. Suppression of malathion resistance by triphenylphosphate in strains of *T. castaneum*[a]

Strain	Mortality (%) after exposure to [b]: Malathion (0.15 mg)	Malathion + TPP (0.15 mg + 0.75 mg)
GA-1	1.3 ± 1.9	99.0 ± 1.7
MN-1	12.0 ± 5.6	96.0 ± 4.9
IA-1	6.7 ± 1.9	100 ± 0
IA-2	3.3 ± 2.7	100 ± 0
MN-2	4.5 ± 2.8	100 ± 0
MN-3	8.7 ± 1.9	100 ± 0
NE-1	13.3 ± 6.8	96.2 ± 2.5
IA-3	2.0 ± 2.0	77.8 ± 7.3
IA-4	15.3 ± 4.3	80.0 ± 7.2
IA-5	13.3 ± 7.2	99.0 ± 1.7
IA-6	14.7 ± 5.0	100 ± 0
IA-7	7.3 ± 6.3	65.0 ± 9.9
IA-8	12.7 ± 4.3	92.0 ± 4.9
LAB-S	100 ± 0	

[a]Values for percent mortality represent means ± SD of four independent determinations of 25 beetles each.
[b]Mortality after exposure to TPP (0.75 mg) in all strains listed was 0 ± 0%.

doses in the present survey, the single strain from the deep south (GA-1) was distinctly more resistant than any of the 14 from the north-central region.

The confused flour beetle, *Tribolium confusum* Jacquelin du Val, is relatively insignificant as a pest of farm-stored grain. Of 38 strains of *Tribolium* collected in this survey, only 2 proved to be *T. confusum*, the remainder being *T. castaneum*.

R. dominica

Thirteen strains from six states were screened. Of these, 10 suffered ≥ 92% mortality at the discriminating dose, but only 2 suffered 100% mortality (Fig. 2 Table 4). This suggested that most of the strains were at least slightly tolerant. For six of the most tolerant strains, survivors of the initial screening were subcultured, and the F-1 progeny were retested at several doses. In four of the six strains (MN-1, MN-2, MN-4, and MT-1) the

Table 4. Mortality in strains of *R. dominica* treated with discriminating doses of malathion[a]

Strain[b]	Mortality (%) at indicated malathion dose (mg/disc)				R factor[d]
	Before selection[c]	After selection[c]			
	1.75	1.75	5	15	
CO-1	6.7 ± 6.0	21.3 ± 5.0	44.0 ± 8.6	93.3 ± 2.3	~10
MN-1	73.0 ± 10.6	25.3 ± 3.8	84.0 ± 6.5	100 ± 0	~4
MN-2	89.3 ± 6.0	70.7 ± 5.0	86.7 ± 1.9	100 ± 0	~4
MN-3	92.0 ± 6.5	86.7 ± 3.8	100 ± 0	100 ± 0	~2
MN-4	94.0 ± 5.5	40.0 ± 8.6	76.0 ± 14.2	100 ± 0	~4
SD-1	94.7 ± 7.9				
MT-1	96.0 ± 3.3	60.0 ± 9.8	100 ± 0	100 ± 0	~2
SD-2	97.3 ± 4.4				
NE-1	98.0 ± 3.0				
ND-1	98.0 ± 3.0				
SD-3	98.0 ± 3.0				
SD-4	100 ± 0				
SD-5	100 ± 0				
LAB-S	100 ± 0				≡1.0

[a]Values for percent mortality are means ± SD of six independent determinations of 25 beetles each.
[b]Strain identifications indicate the state of origin.
[c]Survivors of the first test (dose = 1.75 mg) were used as seeding stock for "selected" cultures. Progeny were used for subsequent tests without further selection.
[d]The resistance factor refers to once selected strains. It is the ratio of the estimated LD_{95} value for the field strain to that for the lab-S strain.

level of resistance increased sharply (Table 4). Although strain CO-1 did not seem to respond to selection, it remained the most resistant of the strains tested (resistance factor ~ 10 at LD_{95}) (Table 4). Strain CO-1 was further selected for two consecutive generations at ~LD_{80} (10 mg per disc) but remained nonresponsive to selection. Resistance in strain CO-1 was totally suppressed by TPP (data not shown), indicating that this resistance is probably the esterase-related, malathion-specific type.

The lesser grain borer appears to be a relatively recent introduction to this country, not having become established here until about the latter half of the nineteenth century (Schwardt 1933, Potter 1935). More recently, patterns of distribution of this species have continued to shift to the extent that it has become a major pest of wheat across the United States in the last 40 years (Barak, 1981, Storey et al. 1982). The establishment of malathion resistance in the lesser grain borer may be in its initial stages at the time of the present survey.

Cryptolestes *spp.*

Of the 44 strains of this genus representing 12 states, all but 5 suffered 100% mortality at the discriminating dose (Fig. 3). Survivors in the Georgia strain were subcultured, and the progeny were retested at several doses. The strain was nonresponsive to selection, and the resistance factor at LD_{95} was ~3.1 (data not shown).

Oryzaephilus *spp.*

Beetles in this genus were uniformly susceptible in the six states represented. All 12 strains suffered 100% mortality at the discriminating dose used, indicating resistance factors of <1.7 at LD_{95} in each case. The geographical origins of the strains are shown in Fig. 4.

Sitophilus *spp.*

A similar situation was found in the weevils (Fig. 5). Of the 21 strains tested, all suffered >95% mortality at the discriminating dose, indicating resistance factors of ≤1.0 at LD_{95} in each case. Seven of the strains had one or more survivors, suggesting the possibility of at least a low-level tolerance in some of these strains. A strain from Iowa had the lowest mortality (95.3%). This strain was selected at the discriminating dose for six consecutive generations, the survivors being subcultured after each selection. After this procedure, the strain had 84.6% mortality at the discriminating dose (0.15 μg per disc) and 99.6% mortality at the slightly higher dose of 0.175 μg per disc, indicating a resistance factor at LD_{95} of <1.03. Thus, malathion tolerance in this strain is only marginal and is unresponsive to selection pressure.

In summary, the present work confirms that TPP-suppressible malathion resistance is widespread in red flour beetle populations infesting farm-stored grain throughout the U.S. grain belt. Considerable resistance was also encountered in the lesser grain borer. Levels of resistance achieved in strains of the lesser grain borer were comparable to those in the red flour beetle, but resistance factors were considerably less in the former species. The reason for this is the natural tolerance to malathion of the lesser grain borer as a species, as can be seen in Table 1. Malathion resistance appears to be marginal, scarce, or absent in the other three genera of beetles surveyed.

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