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Monitoring for Resistance to Chlorpyrifos-Methyl, Pirimiphos-Methyl and Malathion in Kansas Populations of Stored-Product Insects[1]

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ABSTRACT: Strains of stored-product insect pests collected on farms and at country elevators throughout Kansas in 1987 were screened for resistance to chlorpyrifos-methyl (CM), pirimiphos-methyl (PM) and malathion. *Tribolium castaneum* and *Cryptolestes* spp. were uniformly susceptible to both CM and PM, but all *T. castaneum* strains were strongly resistant to malathion. Of 22 strains of *Oryzaephilus surinamensis* tested, all but three were susceptible to CM, but only about one-third were susceptible to malathion. One of the CM-resistant strains was cross-resistant to PM. Of 18 strains of *Rhyzopertha dominica* tested, all but three were susceptible to CM. These resistant strains were not cross-resistant to PM. The implications for resistance development are discussed.

Since its registration in 1958, malathion has been the only widely used residual insecticide for direct application to stored grain in the United States. Few protectants have been available for postharvest application because of 1) the relatively small size of the stored-product market compared to markets for broad-spectrum field insecticides, 2) strict regulation of pesticides on food and feed grains, and 3) lack of incentive for farmers to protect stored grains from insect damage (Reed et al., 1989). Two new grain protectants became available in 1985 and 1986 when Reldan® (chlorpyrifos-methyl, CM) was registered for direct application on wheat, rice, oats, barley, and sorghum (but not on corn), and Actellic® (pirimiphos-methyl, PM) was registered for stored corn and sorghum.

When malathion was introduced into the stored-product insecticide market, the probability of insect resistance to protectants was not well-appreciated. As a result, wild populations of target insects were not monitored adequately, and the malathion resistance that developed caught the market unprepared. Today malathion resistance is widely acknowledged, and resistant strains have largely supplanted the susceptible wild-types in many areas (Arthur et al., 1988; Haliscak and Beeman, 1983; Zettler, 1982).

We undertook a state-wide survey in Kansas to avoid repeating past mistakes with respect to lack of monitoring for resistance to stored-grain protectants. Because PM and CM have been on the market for only a short time, wild populations have had little opportunity to develop resistance. The present survey probably reflects for the most part the natural level of susceptibility present in wild populations of four species of stored product Coleoptera, including *Rhyzopertha dominica* (F.), *Tribolium castaneum* (Herbst), *Oryzaephilus surinamensis* (L.), and *Cryptolestes* (mixed species). We also surveyed resistance to malathion in the same populations.

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Table 1. Probit analysis and discriminating doses for chlorpyrifos-methyl and malathion against lab-S strains of four species of Coleoptera.[a]

Species	LD_{50}	LD_{99}	Slope	Discriminating dose[b]
	Chlorpyrifos-methyl			
Oryzaephilus surinamensis	0.41 (0.38–0.46)	1.19 (0.99–1.54)	5.06	1.25
Tribolium castaneum	0.07 (0.07–0.08)	0.24 (0.20–0.28)	4.57	0.35
Cryptolestes ferrugineus	0.18 (0.10–0.30)	0.47 (0.29–32.1)	5.53	0.50
Rhyzopertha dominica	0.37	2.9	3.25	1.25
	Pirimiphos-methyl			
Oryzaephilus surinamensis	0.36	0.84	6.20	1.25
Tribolium castaneum	0.04	0.12	5.56	0.20
Cryptolestes ferrugineus	0.33 (0.28–0.40)	3.93 (2.18–10.67)	2.16	1.0
Rhyzopertha dominica	0.63 (0.59–0.68)	2.13 (1.79–2.67)	4.42	1.75
	Malathion			
Oryzaephilus surinamensis	0.02 (0.02–0.03)	0.08 (0.07–0.11)	3.94	0.15
Tribolium castaneum	0.03 (0.02–0.04)	0.15 (0.10–0.44)	5.33	0.35

[a] Each species was tested at 4–6 dosages, using 25 beetles per dose. Each experiment was replicated four times; 95% confidence limits are given in parentheses. All values are expressed as mg per disc.
[b] Values for discriminating doses were determined empirically.

Materials and Methods

INSECT POPULATIONS: During a 1987 state-wide survey of infestations in country elevators and farms in 22 counties throughout Kansas (Wright, unpubl. data), various species of stored-product insects were collected from food-baited traps. Wire mesh envelop traps containing equal parts of cracked corn, wheat, sorghum, and rolled oats plus kibbled carob were placed at various locations at storage sites for 2 weeks. A minimum of 50 adults of each species was removed from the trap contents by aspiration and placed in culture.

REARING: Wild-caught colonies were maintained for at least 2–3 generations prior to bioassay. *R. dominica* was reared on whole wheat supplemented with ca. 5% whole wheat flour, and *Sitophilus* spp. on wheat or corn. *T. castaneum* was reared on whole wheat flour, and *O. surinamensis* and *Cryptolestes* (mixed species) on rolled oats, each with 5% by weight brewer's yeast supplement. All insects were reared in ca. 0.5 or 1 liter glass jars at 30 ± 1°C and 60 ± 5% relative humidity.

BIOASSAY: For bioassay, we used the impregnated filter paper method of Stringer (1949) with modifications (Haliscak and Beeman, 1983; Beeman, 1983). Treated papers were air-dried for 30 min with the aid of a fan to remove the volatile solvents. Adults 2–4 wk old were used for bioassay. The exposure time was 24 hr, unless otherwise indicated. The carrier consisted of a mixture of hexane : acetone : sunflower oil (3:1:1) when malathion was the test chemical. With CM or PM as test chemical, we added 1 part of n-butyl phthalate to 5 parts (v/v) of the above carrier to improve solubility. Technical grade insecticides were used for all bioassays. All colonies were tested initially with discriminating doses of insecticides. A discriminating dose is one that kills susceptible but not resistant

Table 2. Mortality in Kansas strains of *Oryzaephilus surinamensis* treated with discriminating doses of chlorpyrifos-methyl or pirimiphos-methyl.[a]

	Mortality (%) at indicated dose (mg/disc):			
	Chlorpyrifos-methyl		Pirimiphos-methyl	
Strain	1.25	1.5	1.25	10.0
Lab-S	100	100	100	
DK01	100		94 ± 9	
DK03	100		99 ± 2	
KW59	100			
MC62	100			
SA74	100			
MN61	100			
GO57	100			
DK02	99 ± 2			
CD54	99 ± 2		100	
BA50	99 ± 2			
CY53	99 ± 2		100	
BT51	99 ± 2			
PL65	99 ± 2		100	
SG68	99 ± 2		97 ± 6	
OB53	97 ± 4			
RN73	97 ± 4		91 ± 11	
DC55	96 ± 4			
LC60	95 ± 8	100		
TH69	85 ± 6	100		
HP71	91 ± 4[c]	87 ± 6[c]		
HP70	90 ± 8[c]	90 ± 8[c]		
PN64	71 ± 11[c]	66 ± 15[b,c]	18 ± 10[c]	92 ± 7[b,c]

[a] Values for percent mortality represent means ± SD of four independent determinations of 25 beetles each.

[b] These tests were conducted on progeny of the survivors of the first discriminating dose selections, i.e., the "once-selected" stocks.

[c] Significantly different from Lab-S ($P < 0.05$, *t*-test for comparison of means).

insects. We attempted to set discriminating doses as low as possible (LD_{95}-LD_{99} for susceptible strains) in order to permit detection of low-level resistance. Suspect resistant strains were re-tested at higher doses to determine both the intensity of resistance and the frequency of resistant individuals.

Most cases of malathion resistance in *T. castaneum* in the United States are caused by either of two allelic mutations at an esterase locus on the sixth linkage group (Beeman, 1983; Beeman and Nanis, 1986). These are designated Rmal-1 and Rmal-2, conferring high-level and low-level resistance, respectively. To determine which allele is predominant in our Kansas populations, we tested five randomly chosen strains of *T. castaneum* at a dose that would discriminate between these two alleles. All tests were replicated four times. Data were analyzed by the *t*-test for comparison of means or by the probit procedure of Finney (1971).

Results and Discussion

Log-probit parameters for CM, PM and malathion against susceptible laboratory strains of *Oryzaephilus, Tribolium, Cryptolestes* and *Rhyzopertha* are shown

Table 3. Mortality in Kansas strains of *Oryzaephilus surinamensis* treated with discriminating doses of malathion.[a]

Strain	Mortality (%) at indicated dose (mg/disc)		
	0.15	0.5	1.5
Lab-S	100	100	
GO57	100		
LC60	100		
TH69	100		
DK03	100		
PL65	100		
RN73	98 ± 2	100	
OB63	96 ± 6	99 ± 2	
DK02	96 ± 0[b]		
SG68	96 ± 0[b]		
CD54	95 ± 4[b]	100	
MC62	91 ± 6[b]	97 ± 2[b]	
DC55	89 ± 10[b]		
CY53	87 ± 9[b]	99 ± 2	
KW59	81 ± 8[b]	88 ± 2[b]	
DK01	62 ± 17[b]	90 ± 5[b]	
RN72	60 ± 3[b]	97 ± 2[b]	
HP71	54 ± 9[b]	87 ± 9[b]	
BT51	54 ± 4[b]	92 ± 3[b]	
BA50	53 ± 11[b]	88 ± 6[b]	
HP70	35 ± 8[b]	86 ± 10[b]	
SA74	21 ± 8[b]	57 ± 6[b]	98 ± 2
PN64	14 ± 10[b]	56 ± 6[b]	99 ± 2

[a] Values for percent mortality represent means ± SD of four independent determinations of 25 beetles each.

[b] Significantly different from Lab-S ($P < 0.05$, *t*-test for comparison of means).

in Table 1. Discriminating doses (derived empirically from the data in Table 1 and from other data for doses in the LD_{95}-LD_{100} range) are also shown.

Of 22 strains of *O. surinamensis* tested, only three (HP70, HP71 and PN64) were resistant to CM (Table 2), whereas about two-thirds (15 of 22) were resistant to malathion (Table 3). Of the three CM-resistant strains, PN64 was the most resistant. PN64 was strongly cross-resistant (ca. ten-fold) both to PM (Table 2) and to malathion (Table 3) and, in fact, was the most resistant *O. surinamensis* strain tested for all three insecticides. The other two CM-resistant strains (HP70 and HP71) were also strongly cross-resistant to malathion (Table 3), but neither of these was tested for cross-resistant to PM.

Of 18 strains of *R. dominica* tested, three (SG68, MN61 and RN72) were resistant to CM (Table 4). The two most resistant strains (MN61 and RN72) were retested at a higher discriminating dose and their resistance was confirmed. Fifteen of the 18 strains (including the two with highest CM-resistance) were subsequently tested against PM, and all were susceptible (Table 4). None were tested for malathion resistance. Our finding of limited CM resistance in Kansas populations of *R. dominica* contrasts to that of R. Cogburn (pers. comm.), who found CM resistance in all five Texas populations of *R. dominica* surveyed. In addition, J.

Table 4. Mortality in Kansas strains of *Rhyzopertha dominica* treated with discriminating doses of chlorpyrifos-methyl or pirimiphos-methyl.[a]

Strain	Mortality (%) at indicated dose (mg/disc): Chlorpyrifos-methyl 1.25	Chlorpyrifos-methyl 1.5	Pirimiphos-methyl 1.75
Lab-S	92 ± 9	99 ± 2	100
KW59	100		99 ± 2
DK03	100	100	
LC60	100		100
BA50	100		100
DK05	100		100
MC62	98 ± 2		99 ± 2
DK02	97 ± 4		
DK01	96 ± 6		100
CN52	95 ± 6		100
CD54	95 ± 4		100
RO67	93 ± 5		100
TH69	91 ± 2		99 ± 2
RN73	91 ± 6		99 ± 2
BT51	84 ± 11		
SA74	83 ± 5		100
SG68	65 ± 9[b]		
MN61	43 ± 8[b]	80 ± 13[b]	95 ± 5
RN72	26 ± 15[b]	54 ± 8[b]	100

[a] Values for percent mortality represent means ± SD of four independent determinations of 25 beetles each.

[b] Significantly different from Lab-S ($P < 0.05$, *t*-test for comparison of means).

L. Zettler (pers. comm.) found that Oklahoma populations of *R. dominica* are uniformly resistant to CM. The relative scarcity of CM resistance in this species in Kansas compared to Texas and Oklahoma may reflect more intense insecticide use, and therefore more intense selection pressure, in the more southern states.

T. castaneum and *Cryptolestes* spp. were uniformly susceptible to both CM and PM. Of 15 *Cryptolestes* strains tested against CM (12 of these further tested against PM) and 20 strains of *T. castaneum* tested against both CM and PM, all were susceptible to both insecticides (Table 5). Twenty-one strains of *T. castaneum* were subsequently tested for malathion resistance. All 21 were highly resistant, in most cases ca. 100-fold (Table 6). Of five strains of *T. castaneum* tested for the presence of Rmal-2, four suffered <50% mortality at a dose that kills all homozygous Rmal-2 beetles, indicating that the high-resistance form (Rmal-1) predominates in these strains (Table 6, last column).

The extent of malathion resistance among Kansas populations of *O. surinamensis* was unexpected in view of earlier data. In a survey of insects collected from farm-stored grain in 1980 we found no trace of malathion resistance among 12 strains of *Oryzaephilus* representing six northcentral states not including Kansas (Haliscak and Beeman, 1983). It seems likely either that malathion resistance in *Oryzaephilus* has increased substantially in the United States within the last decade, or that such resistance is much more prevalent in Kansas than in more

Table 5. Mortality in Kansas strains of *Cryptolestes* spp. and *Tribolium castaneum* treated with discriminating doses of chlorpyrifos-methyl (CM) or pirimiphos-methyl (PM).[a]

	Mortality (%)			
	Cryptolestes		*T. castaneum*	
Strain	CM	PM	CM	PM
Lab-S	100	100	100	100
RO66	100	100	100	97 ± 5
CY53	100	100	100	100
DC55	100	100	100	100
RO67	100			
DK04	100	100	100	100
HP70	100	100		
SA74	100	100	100	100
KW59	100	100	100	100
GO57	100	100	10	94 ± 8
DK01	100		100	100
BA50	100			
EL56	99 ± 2	100		
PL65	99 ± 2	100		
TH69	97 ± 6	100	100	99 ± 2
RN73	94 ± 8	100	100	100
SG68			100	100
MC62			100	96 ± 5
BT51			100	100
CD54			100	100
DK02			100	100
DK03			100	100
LC60			100	100
RN72			100	100
CN52			100	100
HP71			100	99 ± 2

[a] Values for percent mortality represent means ± SD of four independent determinations of 25 beetles each. Discriminating doses of CM for *Cryptolestes* and *Tribolium* were 0.5 and 0.35 mg/disc, respectively; for PM doses were 1.0 and 0.2 mg/disc, respectively.

northern states. Similarly, the intensity of malathion resistance among Kansas populations of *T. castaneum* as revealed in the present survey (Table 6) was far greater than that observed in our 1980 survey in more northern states (Haliscak and Beeman, 1983).

In summary, our data indicate that Kansas populations of *O. surinamensis, T. castaneum, R. dominica,* and *Cryptolestes* spp. are generally susceptible to CM and PM as of 1987. The only exceptions to this generalization were incipient resistance to CM in three out of 22 strains of *O. surinamensis* and in three out of 18 strains of *R. dominica*. Most notable was a single strain of *O. surinamensis* that was ca. ten-fold resistant to all three organophosphates tested. These results indicate that the potential for growth and spread of CM and/or PM resistance exists in *Oryzaephilus* and *Rhyzopertha.* There is a need to perform similar tests on *Sitophilus* populations. Our tests on 1987 Kansas populations of *Sitophilus* spp. could not be completed because of unexplained high mortality in untreated populations.

Table 6. Mortality in Kansas strains of *Tribolium castaneum* treated with discriminating doses of malathion.[a]

Strain	24 hr mortality (%) at indicated dose (mg/disc)			40 hr mortality (%) at indicated dose (mg/disc)	
	0.35	1.0	10.0	1.0	10.0
Lab-S	98 ± 2	100	100	100	100
Rmal-1	0 ± 0	1 ± 2	10 ± 7	1 ± 2	19 ± 7
Rmal-2	0 ± 0	0 ± 0	81 ± 8	0 ± 0	100[NS]
RN73	0 ± 0	1 ± 2	18 ± 8		
MC62	0 ± 0	4 ± 3	79 ± 4		
SA74	0 ± 0	0 ± 0	3 ± 4		
RO66	0 ± 0	0 ± 0	29 ± 29		
GO57	0 ± 0	1 ± 2	14 ± 8		
CN52	0 ± 0	0 ± 0	1 ± 2		
DK04	0 ± 0	0 ± 0	6 ± 5		
DC55	5 ± 10	0 ± 0	15 ± 2		
HP70	16 ± 7				
KW59	37 ± 14	52 ± 11	100[NS]		
DK02		21 ± 2	31 ± 4		
CD54		3 ± 4	16 ± 3		
CY53		8 ± 6	35 ± 6		
BT51		1 ± 2	17 ± 6		
TH69		3 ± 2	43 ± 7		
HP71		0 ± 0	20 ± 11	0 ± 0	35 ± 5
LC60		1 ± 2	25 ± 16	1 ± 2	45 ± 5
DK03		2 ± 4	45 ± 11	2 ± 2	74 ± 8
SG68		1 ± 2	11 ± 8	0 ± 0	
DK01		0 ± 0	26 ± 15	1 ± 2	47 ± 12
RN72		0 ± 0	25 ± 15	0 ± 0	25 ± 15

[a] Values for percent mortality represent means ± SD of four independent determinations of 25 beetles each. Unless indicated by "NS" superscript, all values are significantly different from Lab-S ($P < 0.05$, t-test for comparison of means).

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Literature Cited

Arthur, F., J. L. Zettler, and W. R. Halliday. 1988. Insecticide resistance among populations of Almond moth and Indianmeal moth (Lepidoptera: Pyralidae) in stored peanuts. J. Econ. Entomol. 81:1283–1287.

Beeman, R. W. 1983. Inheritance and linkage of malathion resistance in the red flour beetle. J. Econ. Entomol. 76:737–740.

Beeman, R. W., and S. N. Nanis. 1986. Malathion resistance alleles and their fitness in the red flour beetle (Coleoptera: Tenebrionidae). J. Econ. Entomol. 79:580–587.

Finney, D. J. 1971. Statistical Method in Biological Assay. Griffin, London. 212 pp.

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.

Reed, C. R., V. F. Wright, J. R. Pedersen, and K. Anderson. 1989. Effects of insect infestation of farm-stored wheat on its sale price at country and terminal elevators. J. Econ. Entomol. 82: 1254–1261.

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.

Zettler, J. L. 1982. Insecticide resistance in selected stored product insects infesting peanuts in the southeastern United States. J. Econ. Entomol. 75:359–362.