

INSECTICIDE RESISTANCE AND RESISTANCE MANAGEMENT

Dose-Response Tests on Red Flour Beetle and Confused Flour Beetle (Coleoptera: Tenebrionidae) Collected from Flour Mills in the United States

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ABSTRACT Adults of 14 field strains of the red flour beetle, *Tribolium castaneum* (Herbst), and 10 strains of confused flour beetle, *Tribolium confusum* Jacqueline DuVal, collected from flour mills were tested for resistance to malathion and dichlorvos by topical application. Lethal dose estimates showed that all strains of *T. castaneum* were resistant to malathion and dichlorvos. Some strains showed the highest malathion resistance ever reported for this insect. Approximately half the strains of *T. confusum* tested were resistant to malathion and dichlorvos. *T. castaneum* was more resistant than *T. confusum* to both malathion and dichlorvos. There was a positive correlation of the lethal dose values for insecticide resistant strains of both species when compared with the survival of insects exposed to diagnostic doses. Therefore, survival of insects at a diagnostic dose may be used to predict field efficacy of malathion and dichlorvos in these 2 pest species if resistance levels are low.

KEY WORDS *Tribolium castaneum*, *Tribolium confusum*, lethal doses, pesticide exposure, organophosphates, resistance

THE MOST COST-EFFECTIVE method used to detect or monitor pesticide resistance in insects is the diagnostic dose test (Roush and Miller 1986), where a population is treated with a minimum dose of a pesticide so that susceptible individuals are killed and resistant ones survive. The percentage of individuals surviving such a treatment estimates the phenotypic frequency of resistance (R_p) in that population. However, relating the results of such tests to meaningful decisions regarding control failures in field populations is difficult for the following 2 reasons: (1) diagnostic dose tests cannot estimate the genetic resistance frequency in a population; and (2) the diagnostic dose test may not simulate actual exposure of insects in the field (Subramanyam and Hagstrum 1996). Thus, follow-up studies are frequently required to actually measure the level of resistance found in a population and to subsequently forecast the success of future control efforts.

Diagnostic dose tests indicated that pesticide resistance was common in adults of *Tribolium castaneum* (Herbst) and *Tribolium confusum* Jacqueline du Val collected from flour mills in the United States (Zettler 1991). Both species were resistant to malathion, dichlorvos, chlorpyrifos-methyl, and phosphine; and some of the resistant strains showed high R_p s. The objectives of this study were to further characterize the pesticide resistance in

those flour mill strains by performing dose-response tests and constructing dose-response regression lines with malathion and dichlorvos, and to establish the relationship between lethal dose estimates and R_p s.

Materials and Methods

Original insect collections and laboratory rearing conditions were reported earlier (Zettler 1991). Laboratory cultures were reared on a standard diet (Boles and Marzke 1966) at 27°C, 60% RH, and a photoperiod of 12:12 (L:D) h. At the time of testing, adults were sifted from laboratory cultures with a standard laboratory sieve (U.S. Standard Sieve, mesh size 0.85 mm) and placed in petri dishes. Malathion (95.0%, American Cyanamid, Princeton, NJ) and dichlorvos (98.6%, Amvac, Los Angeles, CA) were formulated in acetone.

Adults of both species were briefly anesthetized in a Buchner funnel with carbon dioxide and then picked up individually with a vacuum wand. A 0.5- μ L droplet of malathion or dichlorvos solution was placed directly on the thorax of each beetle with an ISCO Model M microapplicator (Instrumentation Specialties, Lincoln, NE) (Zettler 1974). Dosages were based on the average weight of each strain, and at least 4 dosages producing >0 and <100% mortality were run for each strain. At least 80 insects were tested at each dose. Treated beetles were placed in petri dishes, 10 insects per dish, and held for 5 d at rearing conditions to determine

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Table 1. Dose-response estimates for malathion against resistant strains of *T. castaneum* collected from flour mills in the United States and assayed by topical application

Strain	Avg wt, mg	% survivors ^a	n	Slope ± SE	LD ₅₀ ^b (95% CL)	RR ^c	LD ₉₉ ^b (95% CL)	RR ^c
Laboratory ^d	3.23	0	800	4.46 ± 0.47	0.03 (0.02-0.03)	1	0.09 (0.75-0.155)	1
16	1.63	8	240	2.21 ± 0.29	0.22 (0.08-0.34)	8	1.2 (0.71-1.52)	13
15	1.82	69	280	2.06 ± 0.30	2.27 (1.5-3.0)	84	14.2 (10.6-22.6)	158
34	1.74	89	440	0.82 ± 0.09	2.47 (1.21-4.14)	91	1,528.4 (536.7-9,872.0)	16,962
4	1.84	83	400	1.68 ± 0.16	5.4 (4.2-6.8)	200	51.9 (36.4-84.5)	577
6	1.57	73	440	1.53 ± 0.18	6.9 (4.5-9.2)	256	81.7 (58.3-133.3)	908
35	1.41	84	440	1.24 ± 0.16	7.0 (4.9-9.5)	259	147.4 (97.4-258.8)	1,638
28	1.91	97	200	2.21 ± 0.31	8.6 (6.8-10.6)	319	47.9 (32.6-91.4)	532
14	1.74	80	480	1.26 ± 0.11	11.0 (7.8-14.5)	407	221.6 (148.7-379.0)	2,462
13	1.56	100	440	1.31 ± 0.14	16.8 (11.9-22.3)	622	303.5 (197.6-558.3)	3,372
24	1.36	98	440	1.40 ± 0.13	23.4 (17.4-30.0)	867	351.5 (236.0-610.5)	3,406
7	1.47	99	440	1.64 ± 0.14	35.5 (28.3-43.7)	1,315	355.7 (250.7-568.0)	3,952
31	1.74	100	440	1.46 ± 0.15	46.5 (35.6-58.2)	1,722	622.7 (644.0-7,470.0)	6,919
29	1.39	35	440	1.40 ± 0.12	72.7 (53.8-90.5)	2,693	1,060.9 (715.1-1,793.4)	11,788
36	1.66	99	440	1.17 ± 0.11	104.0 (72.0-140.4)	3,852	2,617.3 (1,707.4-4,679.5)	29,081

^a Percentage of survivors (phenotypic resistance frequency, R_p) from a diagnostic dose of 0.2 mg/g (Zettler 1991).

^b Dose, milligrams of malathion per gram of insect applied topically.

^c RR, ratio of lethal dose value for resistant strain to that of pesticide-susceptible laboratory strain.

^d From Zettler (1991).

mortality. Dose-response regression lines were estimated (LeOra Software 1987) for the pesticide-susceptible laboratory strains of *T. castaneum* and *T. confusum* and were compared with those determined for each of the field-collected strains. Resistance ratios (RRs) were determined by dividing the appropriate lethal dose values (LD₅₀ and LD₉₉) of the field-collected strain by the corresponding lethal dose value of each laboratory strain. Spearman rank order correlations (Jandel Scientific 1995) were used to compare R_p s obtained from the original resistance survey (Zettler 1991) with probit parameters (LD₅₀, LD₉₉ and slope) obtained from the regression lines.

Results

Dose-response regression estimates for malathion against 14 resistant strains of *T. castaneum* are shown in Table 1. LD₅₀ values ranged from 0.22 to

104 mg (AI)/g of insect, and LD₉₉ values ranged from 1.2 to 2,617.3 mg/g. The steepest slope was 2.21 (strains 16 and 28) and the flattest was 0.82 (strain 34); all were flatter than that of the pesticide-susceptible laboratory strain. The LD₅₀ and LD₉₉ for the laboratory strain were 0.03 and 0.09 mg/g, respectively, and the slope was 4.46. The largest resistance ratio at LD₅₀ was 3,852, and the largest at LD₉₉ was 29,081 (strain 36). The LD₅₀ for the most resistant strain was 104 mg/g. Malathion LD₅₀s for 10 *T. confusum* field strains ranged from 0.05 to 0.43, and LD₉₉s ranged from 0.17 to 2.07 mg/g (Table 2). Slopes were flatter than that of the laboratory strain and ranged from 1.83 to 4.32. Maximum resistance ratios were 8.3 (LD₅₀) and 10.2 (LD₉₉). The LD₅₀ and LD₉₉ for the laboratory strain were 0.05 and 0.20 mg/g, respectively, and the slope was 4.0.

Compared with malathion, dichlorvos resistance in *T. castaneum* was much lower (Table 3). LD₅₀

Table 2. Dose-response estimates for malathion against resistant strains of *T. confusum* collected from flour mills in the United States and assayed by topical application

Strain	Avg wt, mg	% survivors ^a	n	Slope ± SE	LD ₅₀ ^b (95% CL)	RR ^c	LD ₉₉ ^b (95% CL)	RR ^c
Laboratory ^d	2.98	0	660	4.00 ± 0.50	0.05 (0.05-0.06)	1.0	0.20 (0.15-0.24)	1.0
8	2.14	0	320	2.64 ± 0.28	0.05 (0.04-0.06)	1.0	0.21 (0.16-0.31)	1.0
17	2.51	0	360	3.62 ± 0.39	0.06 (0.05-0.07)	1.2	0.17 (0.14-0.22)	0.8
26	1.78	4	400	3.07 ± 0.30	0.08 (0.07-0.09)	1.5	0.27 (0.22-0.36)	1.3
7	2.02	3	400	4.14 ± 0.42	0.09 (0.08-0.10)	1.7	0.22 (0.19-0.29)	1.1
37	1.72	19	540	2.43 ± 0.19	0.11 (0.09-0.12)	2.1	0.51 (0.42-0.67)	2.5
21	2.20	30	480	1.83 ± 0.16	0.11 (0.08-0.13)	2.1	2.88 (1.66-1.31)	6.9
1	2.15	15	400	2.84 ± 0.24	0.13 (0.11-0.14)	2.5	0.47 (0.38-0.63)	2.3
33	2.14	41	540	2.31 ± 0.19	0.21 (0.18-0.24)	4.0	1.08 (0.88-1.42)	5.3
40	2.04	49	530	4.32 ± 0.38	0.43 (0.24-0.57)	8.3	1.04 (0.74-3.53)	5.1
18	2.00	78	500	2.42 ± 0.22	0.43 (0.37-0.49)	8.3	2.07 (1.66-2.79)	10.2

^a Percentage of survivors (phenotypic resistance frequency, R_p) from a diagnostic dose of 0.3 mg/g (Zettler 1991).

^b Dose, milligrams of malathion per gram of insect applied topically.

^c RR, ratio of lethal dose value for resistant strain to that of pesticide-susceptible laboratory strain.

^d From Zettler (1991).

Table 3. Dose-response estimates for dichlorvos against resistant strains of *T. castaneum* collected from flour mills in the United States and assayed by topical application

Strain	Avg wt. mg	% survivors ^a	n	Slope ± SE	LD ₅₀ ^b (95% CL)	RR ^c	LD ₉₉ ^b (95% CL)	RR ^c
Laboratory ^d	3.12	0	1,000	5.58 ± 0.42	0.025 (0.024-0.027)	1.0	0.066 (0.059-0.073)	1
36	1.66	11	480	2.86 ± 0.27	0.056 (0.036-0.095)	2.2	0.21 (0.171-0.283)	3.2
13	1.56	4	400	2.70 ± 0.27	0.056 (0.038-0.073)	2.2	0.22 (0.151-0.473)	3.3
34	1.74	16	400	4.58 ± 0.43	0.068 (0.058-0.093)	2.7	0.16 (0.108-0.499)	2.4
15	1.82	6	480	2.78 ± 0.26	0.071 (0.052-0.095)	2.8	0.28 (0.174-0.881)	4.2
28	1.91	30	480	2.93 ± 0.28	0.072 (0.062-0.081)	2.9	0.26 (0.218-0.339)	3.9
14	1.74	21	440	2.57 ± 0.24	0.094 (0.082-0.107)	3.8	0.41 (0.319-0.578)	6.2
24	1.36	16	480	2.18 ± 0.19	0.097 (0.076-0.127)	3.9	0.55 (0.350-1.242)	8.3
31	1.74	44	400	3.23 ± 0.30	0.099 (0.084-0.117)	4.0	0.32 (0.262-0.429)	4.8

^a Percentage of survivors (phenotypic resistance frequency, R_f) from a diagnostic dose of 0.3 mg/g (Zettler 1991).

^b Dose, milligrams of malathion per gram of insect applied topically.

^c RR, ratio of lethal dose value for resistant strain to that of pesticide-susceptible laboratory strain.

^d From Zettler (1991).

values ranged from 0.056 to 0.099 mg/g, and LD₉₉ values ranged from 0.16 to 0.55 mg/g. Slopes were flatter than that of the laboratory strain and ranged from 2.18 to 4.58. The LD₅₀ and LD₉₉ for the laboratory strain were 0.025 and 0.066 mg/g, respectively, and the slope was 5.58. Resistance ratios at LD₅₀ ranged from 2.2 to 4.0, and those at LD₉₉ ranged from 2.4 to 8.3. *T. confusum* showed low levels of resistance to dichlorvos (Table 4). Four strains had lethal dose values lower than the laboratory strain. LD₅₀ values ranged from 0.11 to 0.89 mg/g, and LD₉₉ values ranged from 0.32 to 7.13 mg/g. Slopes were relatively flat, ranging from 1.82 to 3.52. The LD₅₀ and LD₉₉ of the laboratory strain were 0.11 and 1.21 mg/g, respectively, and the slope was 2.17. Maximum resistance ratios at the LD₅₀ and LD₉₉ were 8.4 and 5.9, respectively.

Table 5 shows correlations of R_f and probit parameters for the beetles and insecticide combinations. There was a significant positive correlation ($P \leq 0.05$) between R_f and LD₅₀ values for each of the insect-pesticide combinations for both species. The correlation for *T. castaneum* with malathion was weak ($r = 0.651$), but those for the other combinations were strongly positive, ranging from

0.806 to 0.949. At the LD₉₉, R_f was significantly correlated ($P \leq 0.05$) with each combination except for *T. castaneum* and dichlorvos. The correlations for *T. confusum* were strongly positive ($r = 0.982$ and 0.758). The slope was not significantly correlated ($P \leq 0.05$) with any R_f . When lethal dose values were compared with slope, significant correlations ($P \leq 0.05$) were found only for the LD₉₉. These were negative and were significant for all combinations except that for *T. confusum* and malathion.

Discussion

Malathion resistance in *T. castaneum* was first reported in the United States by Speirs et al. (1967) and Vincent and Lindgren (1967). Since that time, many reports have shown that malathion resistance in this insect pest has grown in both scope and intensity (Zettler 1974, 1982; Bansode and Campbell 1979; Haliscak and Beeman 1983; Horton 1984; Halliday et al. 1988; Subramanyam et al. 1989; and Zettler and Cuperus 1990). It was only recently that malathion resistance was investigated among strains of *T. castaneum* from flour mills in the

Table 4. Dose-response estimates for dichlorvos against resistant strains of *T. confusum* collected from flour mills in the United States and assayed by topical application

Strain	Avg wt. mg	% survivors ^a	n	Slope ± SE	LD ₅₀ ^b (95% CL)	RR ^c	LD ₉₉ ^b (95% CL)	RR ^c
Laboratory ^d	2.98	0	1,200	2.17 ± 0.68	0.11 (0.10-0.12)	1.0	1.21 (0.93-1.55)	1.0
7	2.02	0	440	2.29 ± 0.22	0.11 (0.09-0.12)	1.0	0.57 (0.43-0.84)	0.5
8	2.14	0	480	3.52 ± 0.27	0.11 (0.10-0.12)	1.0	0.32 (0.27-0.40)	0.3
26	1.78	0	400	2.70 ± 0.26	0.13 (0.09-0.14)	1.2	0.53 (0.41-0.73)	0.4
17	2.51	0	400	3.13 ± 0.34	0.13 (0.10-0.15)	1.2	0.43 (0.36-0.56)	0.4
40	2.04	5	400	2.23 ± 0.19	0.37 (0.31-0.43)	3.5	2.02 (1.54-2.90)	1.7
37	1.72	0	400	2.16 ± 0.19	0.37 (0.31-0.43)	3.5	2.14 (1.62-3.11)	1.8
1	2.15	0	400	2.45 ± 0.20	0.42 (0.36-0.48)	4.0	1.95 (1.52-2.72)	1.6
21	2.20	7	320	2.59 ± 0.28	0.46 (0.39-0.53)	4.3	1.99 (1.54-2.90)	1.7
33	2.14	13	400	1.96 ± 0.19	0.54 (0.44-0.64)	5.1	3.72 (2.75-5.66)	3.1
18	2.00	38	550	1.82 ± 0.16	0.89 (0.70-1.09)	8.4	7.13 (5.45-10.21)	5.9

^a Percentage of survivors (phenotypic resistance frequency, R_f) from a diagnostic dose of 0.3 mg/g (Zettler 1991).

^b Dose, milligrams of malathion per gram of insect applied topically.

^c RR, ratio of lethal dose value for resistant strain to that of pesticide-susceptible laboratory strain.

^d From Zettler (1991).

Table 5. Correlation (r) of survivors of diagnostic dose tests and dose-response regression estimates for malathion and dichlorvos tested against insecticide-resistant strains of *T. castaneum* and *T. confusum*.

Combined ^a	n	r (probability) ^b for			
		R_f ^c and		LD ₅₀ and	
		LD ₅₀	LD ₉₉	Slope	Slope
RFB versus malathion	15	0.651 (0.006)	0.648 (0.009)	-0.453 (0.086)	-0.682 (0.005)
RFB versus dichlorvos	9	0.840 (0.002)	0.561 (0.099)	-0.059 (0.844)	-0.750 (0.016)
CFB versus malathion	11	0.949 (0.001)	0.982 (0.000)	-0.404 (0.210)	-0.518 (0.095)
CFB versus dichlorvos	11	0.806 (0.001)	0.758 (0.006)	-0.558 (0.071)	-0.855 (0.000)

^a RFB, red flour beetle (*T. castaneum*); CFB, confused flour beetle (*T. confusum*).

^b r , Spearman rank order correlation coefficient (probability).

^c R_f , phenotypic resistance frequency (percentage survivors from diagnostic dose tests) (Zettler 1991).

United States (Zettler 1991). The results reported here are follow-up studies on the levels of malathion resistance in these insects from flour mills. Based on the results reported here, malathion resistance has developed to the highest levels ever recorded in *T. castaneum*. The LD₅₀ of the most resistant strain was 104,000 ppm (104 mg/g). The greatly depressed slopes of the regression lines resulted in extremely large resistance ratios. Based on similar lethal dose tests, alarmingly high levels of malathion resistance were reported in *T. castaneum* from peanut storages in the southeastern United States >10 yr ago and control failures caused by resistance had occurred (Zettler 1982). Based on diagnostic dose tests, Halliday et al. (1988) reported that malathion resistance in *T. castaneum* from peanut storages was almost complete but no follow-up studies were done to quantify the resistance levels. With 93% of these flour mill strains resistant to malathion (Zettler 1991), and with the extremely high resistance levels reported here, it is obvious that malathion resistance is virtually complete in flour mill populations of this insect as well.

By contrast, dichlorvos resistance levels in *T. castaneum* were rather low despite the fact that 64% of field strains tested by diagnostic dose tests were shown to be resistant (Zettler 1991). The LD₅₀ of the most resistant strain (0.099 mg/g) was 3 orders of magnitude smaller than the resistance maximum for malathion (104 mg/g). The 1st instance of dichlorvos resistance in U.S. strains of *T. castaneum* occurred in stored peanuts where about half the strains were resistant in diagnostic dose tests (Halliday et al. 1988). There have been no reports of control failures caused by dichlorvos resistance in these insects, however.

Not much is known about insecticide resistance levels in *T. confusum*. Three previous studies (Vincent and Lindgren 1967, Strong et al. 1969, Horton 1984) found no evidence of malathion resistance in this insect. However, in the recent survey of *T. confusum* from flour mills, about one-half and one-fourth of the strains were found to be resistant to malathion and dichlorvos, respectively (Zettler 1991). The levels of resistance reported in our study were fairly low. The *T. confusum* strain most resistant to malathion was about as resistant as the

least resistant strain of *T. castaneum* was to malathion. Despite the fact that about half the *T. confusum* strains were more susceptible to dichlorvos than was the susceptible laboratory strain, dichlorvos resistance was greater in *T. confusum* than in *T. castaneum* when dose comparisons are made.

The disparity in the levels of malathion resistance between the 2 species of flour beetles is striking. For example, the development of malathion resistance in *T. castaneum* is virtually complete in these populations, and control failures caused by resistance are probably common. However, resistance in *T. confusum* is building although it is presently low and, from a practical standpoint, negligible. Yet, both species of these beetles have been targeted with control efforts using malathion for many years. Obviously, the selection pressure has not been the same. It is possible that *T. confusum*, being found predominately in cooler climates (Good 1936) where pest control efforts might be less intensive than those in a warmer climate, has not experienced as much selection pressure through the years as has *T. castaneum*. This situation is confounded of course by the fact that these pests can survive in heated buildings (i.e., flour mills) in climates where they might not normally be able to survive.

As part of a resistance management program, resistance data are important only in terms of their capacity to predict control, or conversely, control failures under field conditions. However, relating insect resistance data based on laboratory tests to those under actual field conditions is no easy task (Dennehy et al. 1983, Denholm et al. 1984, Subramanyam and Hagstrum 1996). An easily obtained laboratory test statistic for resistance is the R_f . With *Tribolium* spp., as with other pest insects, it can be easily and quickly obtained by lethal dose, lethal concentration, or lethal time tests. However, it can be a poor indicator of control under field conditions. For example, Arthur and Zettler (1991a) subjected the resistant flour mill strains of *T. castaneum* to simulated field exposure tests using malathion as a residual treatment. That study demonstrated that the R_f determined by topical application of a diagnostic dose, was not a good predictor of control under these conditions, probably because resis-

tance was already well established in the populations and the levels were high. However, similar simulated field tests (Arthur and Zettler 1991b) with the malathion-resistant strains of *T. confusum* showed that R_f s obtained by topical application of a diagnostic dose can predict *T. confusum* mortality under simulated field conditions.

Subsequently, Zettler and Arthur (1994) attempted to correlate the resistance ratios (lethal dose tests) from these malathion-resistant flour mill strains of *T. castaneum* with the R_f s, both obtained in laboratory tests using topical application. No significant correlations could be made with the topical data. However, there were significant correlations of R_f s with residually obtained lethal concentration and lethal time data for malathion resistant strains of *T. castaneum* (Zettler and Arthur 1994). In the tests reported here, there was a significant, weakly positive correlation of R_f s with the LD_{50} values of resistant strains of *T. castaneum* obtained by topical application. This correlation differs from that of Zettler and Arthur (1994) who found no significant correlation between the parameters. This difference occurred because our analyses included several additional resistant strains not reported earlier, and, because the R_f s were not normally distributed, a Spearman instead of the Pearson correlation was used. Stronger positive correlations occurred with the other insect-pesticide combinations. In each instance where there was strong correlation of R_f with LD_{50} values, resistance levels were low. R_f s were less strongly correlated with the LD_{99} , except for the *T. confusum* and malathion combination. There was no instance where R_f was significantly correlated with slope. The LD_{99} was significantly correlated with the slope however, but not for all pest-pesticide combinations. The slope of the dose-response regression line provides information only about the phenotypic variation within a population so that if variation is not genetically based, then slope probably is not a good indicator of resistance (Chilcutt and Tabashnik 1995).

Ideally, resistance tests should be designed to simulate field conditions that approximate the manner in which target pests become exposed to a pesticide (Subramanyam and Hagstrum 1996). Generally, resistance data obtained from these simulated field tests can be correlated with field control (Arthur and Zettler 1991b, Rust et al. 1993, Cochran 1996). Our data show that R_f is correlated with LD_{50} data provided that resistance levels are low. Thus, we conclude that R_f can predict field efficacy of malathion and dichlorvos with *T. confusum* and of dichlorvos with *T. castaneum*. However, it has been shown that, based on dose-response regression analysis, substantial variation in insecticide tolerance is common among insect populations (Tabashnik and Cushing 1989, Robertson et al. 1995). Additionally, R_f is a measure of phenotypic variation in population susceptibility to a pesticide and not the true gene frequency for resis-

tance. Further, in addition to the genetic component, biological and operational factors influence the selection of pesticide resistance (Georghiou 1983) and it has been shown that each of these factors must be evaluated along with laboratory bioassays to predict field efficacy (Welty et al. 1989). Thus, reliance on a single test statistic to predict field efficacy is not always valid (Dennehy et al. 1983, Milio et al. 1987) and should be used with caution.

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