Correlation of probit parameters of malathion-resistant *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) determined by topical application and residual methods†

J.L. Zettler and F.H. Arthur*

Abstract

Resistance frequency (R_f) determined by topical application of a diagnostic dose of pesticide can be a useful tool in monitoring the development of pesticide resistance in insects. In order to determine the usefulness of this statistic as a predictor of control failure for malathion-resistant strains of the red flour beetle, *Tribolium castaneum* (Herbst), correlations of resistance frequencies were made with probit parameters obtained by residual and topical tests. Results showed that topically-derived LD_{50} was correlated with residual LD_{50} and LT_{50} values. Further, residual probit parameters were highly correlated when either dose or time were the dependent variables. However, R_f was not correlated with probit parameters regardless of whether they were obtained by topical application or residual exposure. In view of these results, it is unlikely that R_f, can reliably predict malathion control failures due to resistance in this insect.

Introduction

A resistance management program must contain at least three parts. The first is to monitor the suspect population to determine if resistance exists. The second is to characterise the resistance in some way that will determine if control failures will occur following a normal pesticide treatment. Finally, based on this information, the third component, the control strategy, can then be formulated, adjusted or modified, and implemented to achieve some level of acceptable control against the resistant pests. The usefulness of this process is directly related to its timeliness. Therefore, development of resistance test methods which can quickly detect and characterise resistance is a prerequisite for successful resistance management programs. Pesticide resistance may be characterised in a number of ways. For monitoring populations to detect the presence of resistance, the most common method is the diagnostic dose (DD) method (Roush and Miller 1986). This involves testing a population with a minimum dose of pesticide (the diagnostic dose) that will kill susceptible individuals in a population but none of the resistant ones. The proportion of survivors following such treatment gives an estimate of the phenotypic frequency of resistance (R_f) in that population. This technique identifies resistant individuals in the population and, unlike probit parameters, does not measure the resistance intensity. The DD can be quickly carried out on resistant populations with a minimum of insects and time. Thus, timely information on which to base control strategies can be obtained.

As it relates to control failures, resistance is usually expressed in terms of probit parameters like lethal dose or lethal time values (ie., the LD_{50} or LT_{50}), the slope (b) of a regression line, and the resistance ratio (RR) which is the ratio of the LD or LT value (usually at the 50% level) of the suspect field strain to the corresponding lethal value of a standard pesticide-susceptible strain of the same pest. These resistance parameters give a relative estimate of the intensity of resistance in a population. Reliable probit analyses are dependent upon testing many (sometimes hundreds) of individual insects over a very broad range of susceptibility (sometimes as many as 5 or 6 different doses of the pesticide in question). This procedure can be laborious as well as time consuming.

There are many advantages to using the single statistic of R_f, to make predictions about resistance. However, evidence of its reliability as a predictor of control failure is not well established. For example, we showed that there were no correlations of topical application-derived R_fs of malathion-resistant strains of red flour beetle, *Tribolium castaneum* (Herbst), with residual toxicity of malathion on treated surfaces (steel and plywood) simulating warehouse conditions, and concluded that malathion R_fs cannot be used as a predictor of red flour beetle mortality under field conditions (Arthur and Zettler 1991a) . On the other hand, we showed that R_fs of malathion-resistant strains of confused flour beetle, *Tribolium confusum* Jacqueline du Val, can be correlated with residual mortality on malathion treated steel and plywood surfaces (Arthur and Zettler 1991b).

The objective of this study was to determine the relationship between R_fs for malathion-resistant strains of red flour beetle and probit parameters of these resistant strains based on topical application tests and on residual tests using both time and dose as dependent variables. This study is part of a larger study to develop laboratory tests which can accurately predict control failures in stored product insects due to pesticide resistance.

Materials and Methods

Eleven strains of red flour beetle known to be resistant to malathion (Zettler 1991) were tested with malathion in different laboratory tests to characterise malathion resistance. Topical application tests followed the method of Halliday et al. (1988), where individual insects were treated with 0.5 µL of malathion in acetone solutions on the dorsal thorax. At least

* Agricultural Research Service, United States Department of Agriculture, Stored-Products Insects Research and Development Laboratory, 3401 Edwin Street, Savannah, Georgia 31405, USA.

† This paper reports the results of research only. Mention of a pesticide in this paper does not constitute a recommendation for use by the USDA nor does it imply registration under FIFRA as amended. Mention of a proprietary product does not constitute an endorsement by the USDA.

80 insects were used for each dose and at least 5 doses were used for each strain. Treated insects were placed in petri dishes, 10 insects per dish, and held at 27 ± 1°C and 60 ± 2% r.h. for 3 days at which time mortality was assessed.

For residual tests, Whatman no. 1 filter paper (7-cm diameter) was treated with 0.5 mL ethanol solution of malathion. The papers were allowed to air dry and were then placed under glass cylinders (2.5 cm high × 6.6 cm diameter) forming an open arena with treated paper as the floor. At least 100 adult beetles of each strain, 10 adults per arena, were exposed to each dose and at least 5 doses were tested against each strain. Mortality was recorded periodically for 7 days or until all insects had died. Mortality data based on dose and time were subjected to probit analysis (SAS Institute 1987) and probit parameters such as LD_{50} and LD_{95}, LT_{50} and LT_{95}, and the slopes (*b*) were taken from the resulting regression lines. Correlation coefficients (SAS Institute 1987) were determined for probit parameters. The analysis included R_fs for the malathion resistant strains which had been determined in earlier tests (Zettler 1991).

Results

Twelve of 16 resistance parameters and resulting statistics were used in correlation analysis of 11 malathion-resistant strains of red flour beetles (Table 1). Parameters determined by topical application (R_f and *RR*) varied more than those obtained with residual exposure. Among the 11 strains R_fs averaged 74.5 with a range of 8–100. *RR*s averaged 619.4 with a range of 8–2693. LD values determined by residual exposure varied less than those determined by topical application but more than LT values. Resistance responses were least variable for LT values (mean = 15.2 hours, range = 6–34 hours). Variability of residually obtained LD values was inversely related to exposure time, ie., the longer the exposure period, the smaller the range of the LD response.

Topically-derived *RR* was correlated with the residually obtained LD_{50} when it was based either on dose or time (Table 2). *RR* is correlated with the length of LD exposure periods, increasing from 0.6682 (P = 0.0246) at 24 hours to 0.8533 (P = 0.0008) at 72 hours exposure. *RR* was most highly correlated with the LT_{50}(r = 0. 8843, P = 0. 0193). In addition, *RR* was correlated with the slope (*b* (24)) of the regression line for the LD_{50}(24) (r = 0.6825, P = 0.0207). R_f was not significantly correlated (P > 0.21) with any resistance parameter.

Generally, residually-derived LD values correlated well with each other. The highest correlations occurred at the longest exposure period (LD_{50}(72)) with the 24-and the 48-hour exposures (r = 0.8053, P = 0.0028 and r = 0.9305, P = 0.0001, respectively). The only LD_{95} parameter which showed a significant correlation with any other parameter was the LD_{95}(48) and that was correlated with the LD_{50}(24) (r = 0.7151, P = 0.0134) . The slope parameter (b(24)) was correlated with 5 other parameters, being highest with the LT_{50} (r = 0.9073, P 0.0125).

The parameter which showed the highest correlations was the LT_{50}. This parameter was correlated with 6 others, being near unity with the LD_{50}(24) and the LD_{50}(72) (r = 0.9714, P = 0.0012 and r = 0.9686, P = 0.0015 respectively). The slope parameter from these regression lines (*b* (LT)) was correlated with its LT_{50} (r = 0.8119, P = 0.0497) and the LD_{50}(72) (r = 0.8241, P = 0.0437).

Discussion

Developing a simple and timely diagnostic test method for predicting pesticide control failures on stored-product insect pests is complicated by many variables. The nature and duration of the exposure period, the species of pest and it's life stage, the nature of the resistance of the pest, the storage structure, and the commodity itself contribute to the biological response of the target pest to the pesticide. For example, the red flour beetle may be exposed to pesticidal treatments in a number of ways. It can walk and forage through a treated commodity (chemical grain protectant on grains or oilseeds) or walk on different treated substrates (concrete, wood, etc.) and may be exposed to pesticide residues for many months during storage of the treated commodity. It may consume a treated commodity on a daily basis during much of its lifetime. In addition, this insect may come in contact with insecticidal

Table 1. Probit parameters correlation statistics for malathion-resistant strains of red flour beetle, *Tribolium castaneum* (Herbst) obtained with topical application and residue tests.

Test type	Parameter	Number of strains	Mean	SD	Minimum	Maximum
Topical application	R_f^a	11	74.5	33.40	8	100
	RR (LD_{50})	11	619.4[b]	791.30	8	2693
	slope	11	1.6	0.45	1.33	2.23
Residual	LD_{50} (24)[c]	11	31.7	14.50	1.65	60.46
	LD_{50} (48)[c]	11	17.2	10.32	2.34	39.49
	LD_{50} (72)[c]	11	15.8	9.29	1.95	36.25
	slope(24)[c]	11	3.7	1.57	1.56	7.00
	slope(48)[c]	11	4.5	2.45	1.48	10.70
	slope(72)[c]	11	4.3	1.80	1.38	7.24
	LD_{95}(48)[d]	11	48.99	23.63	7.16	78.37
	LT_{50}[d]	6	15.2	10.02	5.79	34.09
	slope LT_{50}	6	3.4	1.08	2.38	5.41

[a]Resistance frequency determined by diagnostic dose of malathion (Zettler 1991)
[b]LD_{50} malathion-susceptible Savannah Laboratory strain = 0.027 mg malathion per g insect; average LD_{50} of 11 resistant strains = 16.72 malathion per g insect
[c]Lethal dose expressed as mg malathion per paper and exposure period (hours)
[d]Lethal time expressed as hours at dose of 50 mg malathion per paper

Table 2. Correlation coefficients (and probabilities) for probit parameters of malathion-resistant strains of red flour beetle, *Tribolium castaneum* (Herbst), obtained by topical application and residue tests.

	R_f[a]	LD_{50} (24)[b]	LD_{50} (48)[b]	LD_{50} (72)[b]	b(24)[b]	LT_{50}[b]	b(LT)[b]	LD_{95} (48)[b]
R_f[a]	1	0.1295	–0.1878	0.0140	–0.2220	–0.5882		
	(0.7043)	(0.5803)	(0.9674)	(0.5118)	(0.2195)			
$RR(LD_{50})$[a]	–0.0614	0.6682	0.7527	0.8533	0.6825	0.8843	–	–
	(0.8577)	(0.0246)	(0.0075)	(0.0008)	(0.0207)	(0.0193)		
LD_{50}(24)	0.1295	1	0.7587	0.8053	0.7948	0.9714	0.7151	
	(0.7043)	(0.0068)	(0.0028)	(0.0035)	(0.0012)	–	(0.0134)	
LD_{50}(48)	–0.1878	0.7587	1	0.9305	0.8328	0.8892	–	
	(0.5803)	(0.0068)	(0.0001)	(0.0015)	(0.0178)			
LD_{50}(72)	0.0140	0.8053	0.9305	1	0.8023	0.9686	0.8241	–
	(0.9674)	(0.0028)	(0.0001)	(0.0030)	(0.0015)	(0.0437)		
slope(24)	–0.2220	0.7948	0.8328	0.8023	1	0.9073	–	–
	(0.5118)	(0.0035)	(0.0015)	(0.0030)	(0.0125)			
LT_{50}	–0.5882	0.9714	0.8892	0.9686	0.9073	1	0.8119	–
	(0.2195)	(0.0012)	(0.0178)	(0.0015)	(0.0125)	(0.0497)		

[a]Topical application test.
[b]Residual exposure test

droplets in the form of aerosols at periodic intervals during the storage period. Thus, the nature of the pesticidal exposure and its duration are critical factors which must be determined in order to meaningfully interpret resistance data in terms of control failure.

Duplicating field conditions in the laboratory to determine a discriminating dose which will identify resistant populations that will survive a normal pesticidal treatment is difficult and time consuming. In addition, mortality from those types of tests are not necessarily related to the R_f and therefore might not be used to accurately predict control failure (Arthur and Zettler 1991a) . Further, some assay methods may not be suitable for describing resistance. For example, Dennehy et al. (1983) demonstrated that results of a topical bioassay method conflicted with those of a residual method when describing mite resistance. Likewise, Roush and Luttrell (1989) showed that topical bioassays could not accurately detect pesticide resistance in tobacco budworm. In addition, Welty et al. (1989) concluded that field efficacy of residual pesticides cannot be based on laboratory bioassays alone. In this test, the *Rf* was shown to be an unreliable predictor of control failures for this insect because it was not correlated with any of the other resistance parameters (Table 2). This fact is not surprising because *Rf*, is a measure only of the frequency with which resistance genes occur in a population; it does not estimate the level of resistance in the population. However, similar field-simulation tests with malathion-resistant strains of confused flour beetle, *Tribolium confusum* Jacquelin du Val, showed that the *Rf*, could be used to predict malathion control failure in this insect early in the development of resistance when resistance levels are low (Arthur and Zettler 1991b) . The *Rf* may have failed as a control failure predictor for the red flour beetle because resistance levels of the 11 strains reported here are some of the highest ever reported for this insect in the United States. In fact the LD_{50} for the most resistant of the 11 strains was 72,711 ppm malathion.

Based on the correlations reported here, parameters which rely on the relative intensity of resistance (ie., LD_{50} LT_{50} *RR*) seem more likely to be important in predicting control failures than are R_fs. In addition, residual exposure parameters may be more useful than those derived by topical application methods. Further, those residual parameters which rely on time (ie., LT_{50}) rather than dose appear to provide the most meaningful information in terms of malathion resistance in the red flour beetle. For other pest species, the situation may be different. For example, Cochran (1994) showed that *RR*s based on LT_{50}s do not provide an adequate assessment of the status of resistance in a given population of German cockroach.

The relationship between laboratory resistance data and control failure is difficult to interpret (Denholm et al. 1984; Welty et al. 1989). Unfortunately, the relationship is a complex one where each situation is unique, and it probably cannot be adequately described by a single laboratory statistic or resistance parameter. A more thorough understanding of this relationship is essential in order to formulate a meaningful resistance management program. However, based on the correlations reported here, the development of LT parameters seems to hold the most promise for assessing malathion resistance in the red flour beetle.

Acknowledgment

The authors appreciate the technical assistance of L. E. Fetzer of this laboratory. This study was partially funded by a Cooperative Agreement between the USDA-ARS and the Southeastern Peanut Association, Albany, Georgia 31707.

References

Arthur, F.A. and Zettler, J.L. 1991a. Malathion resistance in *Tribolium castaneum* (Coleoptera: Tenebrionidae): differences between discriminating concentrations by topical application and residual mortality on treated surfaces. Journal of Economic Entomology, 84, 721-726

Arthur, F.A. and Zettler, J.L. 1991b. Malathion resistance in *Tribolium confusum* Duv. (Coleoptera: Tenebrionidae): correlating results from topical applications with residual mortality on treated surfaces. Journal of Stored Products Research, 28, 55-58.

Cochran, D.C. 1994. Changes in insecticide resistance gene frequencies in field-collected populations of the German cockroach during extended periods of laboratory culture (Dictyoptera: Blattellidae). Journal of Economic Entomology, 87, 1–6.

Dennehy, T.J., Granett, J. and Leigh, T.F. 1983. Relevance of slide-dip and residual bioassay comparisons to detection of resistance in spider mites. Journal of Economic Entomology, 76, 1225–1230.

Denholm, I., Sawicki, R.M. and Farnham, A.W. 1984. The relationship between insecticide resistance and control failure. British Crop Protection Conference—Pests and Diseases, 6A, 527–534.

Halliday, W.R., Arthur, F.H. and Zettler, J.L. 1988. Resistance status of red flour beetle (Coleoptera: Tenebrionidae) infesting stored peanuts in the Southeastern United States. Journal of Economic Entomology, 81, 74–77.

Roush, R.T. and Luttrell, R.C. 1989. Expression of resistance to pyrethroid insecticides in adults and larvae of tobacco budworm (Lepidoptera: Noctuidae): implications for resistance monitoring. Journal of Economic Entomology, 82, 1305–1310.

Roush, R.T. and Miller, G.L. 1986. Considerations for design of insecticide resistance monitoring programs. Journal of Economic Entomology, 79, 293–298.

SAS Institute. 1987. SAS Stat Guide For Personal Computers, 6 Edition. Cary, North Carolina, USA, SAS Institute.

Welty, C., Reissig, W.H., Dennehy, T.J. and Weires, R.W. 1989. Relationship between field efficacy and laboratory estimates of susceptibility to cyhexatin in populations of European red mite (Acari: Tetranychidae). Journal of Economic Entomology, 82, 354–364.

Zettler, J.L. 1991. Pesticide resistance in *Tribolium castaneum* and *T. confusum* (Coleoptera: Tenebrionidae) from flour mills in the United States. Journal of Economic Entomology, 84, 763–767.