Comparing Residue Exposure and Topical Application Techniques for Assessing Permethrin Resistance in House Flies (Diptera: Muscidae)

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ABSTRACT A residue on glass exposure technique was compared with topical application as a method of assessing permethrin resistance in a house fly (*Musca domestica* L.) population. Multiple levels of treatment permitted development of significant dosage-mortality lines with both techniques. Average resistance factors were ca. 150 with the topical application technique and 154 with the residue on glass method. The residue on glass technique provided less variable results, more closely reproduced the mode of real-life acquisition of residual insecticides and would be reasonable to use with small and delicate insects for which topical applications are not feasible.

THE STANDARD TOPICAL application technique is widely used for construction of dosage-mortality regression lines and calculation of lethal dosages because it permits quantification of the amount of toxicant received by each individual test subject. However, topical application does not reflect the mode by which residual insecticides are acquired by insects in actual usage. Application of the insecticide to a substrate with subsequent exposure of the test insects allows the insects to pick up the toxicant in a manner analogous to real-life situations.

Exposure of horn flies (*Haematobia irritans* L.) to a series of concentrations of residues of fenvalerate and flucythrinate in glass petri dishes allowed Sheppard (1984) to develop dosage-mortality regression lines for both pyrethroid resistant and susceptible populations. In the study reported here this petri dish residue technique was compared to the widely accepted topical application technique. By using both methods to compare susceptible and permethrin resistant strains of house flies (*Musca domestica* L.), resistance factors were generated which permitted comparison of the two techniques.

Materials and Methods

Two colonies of house flies were used for comparing a residue exposure method and topical application technique. A susceptible strain of house flies which had been colonized for 7 years with no pyrethroid exposure was obtained from Florida (Jerry Butler, Univ. of Florida, Gainesville).

The resistant colony was established in late February 1984, using flies captured from an enclosed caged layer house in Lincoln County, Georgia. Permethrin had been used there for 2 years and was no longer providing effective control. Over the past 4 years natural pyrethrins, permethrin, and combinations of the two had been used as adulticides at this site until control deteriorated to the point at which dimethoate was introduced as a larvicide to supplement control efforts.

The two colonies were maintained under identical conditions in a rearing room with an average temperature of ca. 27°C, ca. 80% RH, and a spring photoperiod. Larvae were reared in CSMA media. Adults were provided water and a 1:1 sugar/powdered milk mixture ad lib. The two strains were maintained separately in screened cages (30 by 33 by 36 cm). Female flies (3-10 days old) were used for all tests; for any given test, flies of the two populations were the same age ±1 day.

Stock solution (10 $\mu g/\mu l$) for topical applications was made by dissolving 0.22 g of 91% permethrin in 20.0 ml acetone. Serial dilutions were prepared to produce five to seven levels of mortality between 10 and 90% when 1.0 μl of each dilution was applied topically to each of 30 female house flies.

Another set of dilutions of permethrin in acetone was prepared using a stock solution prepared by dissolving 0.068 g 91% permethrin in 100.0 ml acetone. This solution was calculated to produce a residue of 10.0 $\mu g/cm^3$ when 1.0 ml was evenly dried in a 100 mm diameter glass petri dish bottom. Serial dilutions were prepared to produce five to seven levels of residue which would cause mortality between 10 and 90% when female house flies of the appropriate strain were exposed for 24 h. For each concentration 1 ml was applied to the bottoms of the three petri dishes and distributed uniformly while drying on an oscillating platform modified from an Ames aliquot mixer (Miles Laboratories, Elkhart, Ind.). Acetone alone was ap-

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Table 1. Slopes of lines estimated by probit regression, LD_{50} and LC_{50} values, and resistance ratios of female house flies treated with permethrin

Test date (1984)	Strain	Slope (SEM)	LD_{50}[a] LC_{50}[b]	(95% CI)	R/S
			Topical tests		
3 Apr.	Resistant	2.31 (0.28)	2.7463	(2.2719–3.3581)	
	Susceptible	3.37 (0.42)	0.0275	(0.0238–0.0323)	100
10 May	Resistant	1.56 (0.31)	1.4847	(0.9036–3.0503)	
	Susceptible	3.85 (0.49)	0.0057	(0.0048–0.0068)	261
30 May	Resistant	2.10 (0.23)	1.8274	(1.4972–2.2370)	
	Susceptible	4.25 (0.53)	0.0204	(0.0178–0.0236)	90
					$\bar{x}$ = 150
			Residue exposure tests		
2 May	Resistant	3.02 (0.47)	2.2345	(1.8894–2.7437)	
	Susceptible	3.97 (0.61)	0.0128	(0.0110–0.0150)	175
3 May	Resistant	2.84 (0.59)	0.9609	(0.7053–1.1809)	
	Susceptible	4.97 (0.86)	0.0064	(0.0054–0.0073)	150
30 May	Resistant	2.67 (0.32)	1.3620	(1.1400–1.5990)	
	Susceptible	3.76 (0.56)	0.0099	(0.0085–0.0114)	138
					$\bar{x}$ = 154

[a] μg per fly.
[b] μg per cm^2.

plied in a similar manner to each of the three check dishes. Following treatment the dishes were allowed to air dry uncovered for an hour, recovered, and then held until the following day for testing.

Before tests flies were anesthetized with CO_2 for sexing and counting. For residue exposure tests individual groups of 10 female flies were lightly anesthetized, transferred to the treated dishes, and covered. For topical application tests, each group of 10 was lightly anesthetized with CO_2 while 1.0 μl of an appropriate dilution was applied to the notum of each fly using a Hamilton microliter syringe. Flies treated topically were held in untreated petri dishes. Three complete tests were conducted with each technique using three groups of 10 flies of each strain for each treatment level in a test. Flies unable to stand at 24 h were considered dead.

To investigate the possibility that mortality was due to fumigation rather than contact, a test was conducted similar to that described in the residual tests but the flies were prevented from contacting the treated glass surface by a nylon screen fitted inside the petri dish.

Mortality data were analyzed using Daum's computer program for probit analysis ($P = 0.05$) (Daum 1970). Resistance (R) factors (R/S) were calculated by dividing the LD_{50} (or LC_{50}) of the resistant population by the corresponding value of the susceptible population.

Results and Discussion

Table 1 includes slopes of regression lines (with standard errors), estimated LD_{50}'s and LC_{50}'s (with 95% confidence intervals), and derived resistance factors.

In all tests regression line slopes of the two populations were significantly different. Slopes of the susceptible population were invariably steeper than those of the resistant population and slopes obtained for either population were steeper with the residue method than those obtained for the same population using the topical method. R factors obtained using the residue technique were less variable than those estimated by the topical method, though mean R factors estimated by the two methods were similar.

The average slope of the resistant population was 1.99 with the topical technique and 2.85 with the residue method while means of the susceptible population were 3.82 with the topical technique and 4.23 with the residue technique. Differences in slopes and relative susceptibility to permethrin of the two populations were similar with either method, but slopes were steeper with the residue exposure technique. Dahm et al. (1961), in a similar study, concluded that higher slopes indicate a more sensitive technique. Among all tests the highest slope of the resistant population (3.02) was lower than the lowest slope of the susceptible population (3.37).

R factors estimated using the topical application technique ranged from 90 to 261 (mean = 150), while R factors determined by residue exposure ranged from 138 to 175 (mean = 154). Similarity of mean R factors obtained with the two techniques indicates a good approximation of the degree of resistance present while the narrower range of values with the residue exposure technique suggests that this method may yield more reproducible results than the widely accepted topical application method (at least with pyrethroids). Results of the fumigation test yielded no mortality even at rates far in excess of those used in residue tests.

Pyrethroid residues on glass gave good results in this study (house flies) and for Sheppard (1984) (horn flies), but exposure to DDT residues was inferior to DDT topical applications for determining responses of house flies (Dahm et al. 1961). Dahm et al. compared topical application and residue ("film") exposure with DDT on susceptible house flies only and concluded that the topical method was much more likely to produce a significant regression. They also reported that (with DDT) the topical method produced a slope over twice as steep as the "film" method. In our studies the residue method actually produced higher slopes than the topical method, indicating that DDT and pyrethroid residues may "behave" quite differently. Researchers attempting to adapt the residue on glass technique to other classes of insecticides should consider the possibility of other residue–insect systems interacting differently.

Intoxication through tarsal contact of pyrethroid residues closely approximates the mode of acquisition in the field and exposure to several concentrations of residues can produce data to determine a dosage–mortality regression line. This technique should be especially useful for studying control failures of residual pyrethroid treatments on livestock or crops as well as for toxicological studies of small and delicate insects which do not tolerate topical applications of organic solvents.

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