

ORGANOPHOSPHOROUS RESISTANCE IN THE MEMPHIS, TENNESSEE, *CULEX PIPIENS* COMPLEX

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ABSTRACT. Organophosphorous resistance was confirmed in *Culex pipiens* complex mosquitoes from Memphis, Tennessee. Field and laboratory studies with fenthion, malathion, naled, and resmethrin gave comparative results. Birmingham *Culex pipiens quinquefasciatus* were tested in the laboratory against the same 4 compounds and found to be susceptible to all.

The organophosphorous compounds resulted in approximately 30% kill of laboratory and field specimens from Memphis at the highest dosage level. One hundred percent kill of Birmingham *Culex p. quinquefasciatus* was recorded at the same dosage levels in the laboratory. Both *Culex* spp. were susceptible to resmethrin.

INTRODUCTION

Ineffective control of mosquitoes of the *Culex pipiens* complex with ground ultra-low-volume (ULV) malathion applications was first suspected by the Memphis and Shelby County Health Department during the 1975 season. The St. Louis encephalitis epidemic during that year made it imperative that an evaluation of the effectiveness of malathion and other compounds registered for use in ULV equipment be initiated. Field studies were carried out in Memphis during August-September 1976 while laboratory studies were being completed in Atlanta with freshly colonized mosquitoes of the *Culex pipiens* complex from Memphis, Tenn., and Birmingham, Ala.

MATERIALS AND METHODS

FIELD STUDIES. Fenthion, malathion, naled, and resmethrin were the compounds studied. In testing each compound, 25 field-collected adult female mosquitoes from within the City of Memphis were placed in each of 12 clean screen-wire cages and held in plastic bags at 72°F prior to treatment. Five percent sugar-water cotton pads were placed on each cage during holding. Control cages were handled in the same manner and transported to and from the test site.

Three cages from outside the City in Shelby County were prepared for the malathion test, two for the treatment and one as control. The caged mosquitoes were placed on 6-ft stakes at stations 75, 150, and 300 ft from the point of discharge in all tests but the malathion test, where only 150- and 300-ft stations were used. Three stations with one cage each were placed at 100-ft intervals at each distance from the point of discharge. Temperature, humidity, and wind velocity were monitored at the test site. Percent kill was determined at 12 and 24 hr for the organophosphorous tests, and 12 hrs for the resmethrin tests.

Test runs were up to 1/3-mile long at vehicle speeds of from 5 to 10 mph. Approximately 30 min after exposure the cages were removed, replaced in plastic bags, and returned to the holding area. Fenthion (93%), malathion (95%), and naled (10%) were dispensed at 0.9, 3.8, and 9.27 fluid ounces per minute, respectively. These flow rates delivered 0.013, 0.0713, and 0.0152 lb actual insecticide pounds per acre, respectively. Resmethrin (10%) was dispensed at 10.52, 9.08, and 6.53 fluid ounces per min delivering, respectively, 0.007, 0.0035, and 0.0025 pounds per acre. All applications were done with a truck-mounted Leco HC ULV Fog Generator.³

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³ Use of trade names and commercial sources are for identification only and do not constitute endorsement by the Public Health Service or by the U.S. Department of Health, Education, and Welfare.

LABORATORY STUDIES. Mosquitoes of the *Culex pipiens* complex from the city of Memphis, from outside the city in Shelby County, and from Birmingham, Ala., were colonized in the laboratories of the Vector Biology and Control Division, Bureau of Tropical Diseases, Center for Disease Control, Atlanta, Ga. Twenty male genitalia from each of the Memphis and Birmingham colonies were prepared and examined for subspecies identification using Barr's techniques (Barr 1957). The colonies were maintained on chicken blood.

A modified Hoskins-Caldwell Spray Chamber described by McCray and Schoof (1963) was used in these studies. The DeVilbiss No. 631 Atomizer used with 10 lb air pressure produced droplets in the throat of the chamber with a mass median diameter (mmd) of 12 microns. The four insecticides under study were dissolved in ethanol, 85 parts; glycerol, 15 parts, and further diluted with this ethanol-glycerol combination into concentrations of 0.05%, 0.025%, 0.01%, 0.005%, and 0.0025%.

Approximately 50 three-day-old adult female mosquitoes were placed in pint ice cream cartons which were screened on both ends. Three replicates per dosage, 3 checks, and 3 controls were prepared for each test. Carbon dioxide was used to anesthetize the mosquitoes for transfer. The checks were prepared in pint cartons as were the other mosquitoes, then anesthetized and transferred into clean holding cages and placed in the holding room.

Following the controls which were treated with ethanol: glycerol only, each carton of mosquitoes was treated beginning with the low dosage and continuing to the high dosage. Each carton of mosquitoes was inserted in the throat of the chamber and exposed for 30 sec to 2 ml of the aerosol. After exposure and transfer to clean cages, the mosquitoes were given 5% honey-water cotton pads and held at 75°F for 24-hr mortality counts. The Shelby County mosquitoes were tested as 4-day-olds and 14-day-olds and only against malathion.

RESULTS

FIELD STUDIES. The results of field studies in Memphis are presented in Table 1. Of the organophosphorous compounds tested, naled gave the highest kill which was only 37% in field-collected mosquitoes. Mosquitoes from outside the city which were tested only with malathion were quite susceptible, however, with 82% kill at both stations. Resmethrin at all 3 dosage levels was 79 to 99% effective in these tests. Temperatures averaged 71.5°F, relative humidity 68.5%, and wind velocity 6.8 mph. Mortality in the checks averaged 2% with a range of 0-7%.

LABORATORY STUDIES. Subspecies identification of specimens from the Memphis colony showed that 40% of those examined were *Culex pipiens* hybrids, 30% were *C. p. pipiens*, and 30% were *C. p. quinquefasciatus*. All of the Birmingham mosquitoes were *C. p. quinquefasciatus*.

Table 1. Field studies of four compounds against mosquitoes of the *Culex pipiens* complex, Memphis, Tenn., 1976.

Compound	Fl. oz./min.	Replication	Percent female mortality (24-hour period)		
			75 ft.	150 ft.	300 ft.
fenthion	0.90	3	20	8	10
malathion (city)	3.80	4	-	21	32
malathion (Shelby County)	3.80	1	-	82	82
naled	9.27	3	21	25	37
resmethrin	6.53	3	79	98	85
resmethrin	9.08	3	99	87	84
resmethrin	10.52	3	97	98	99

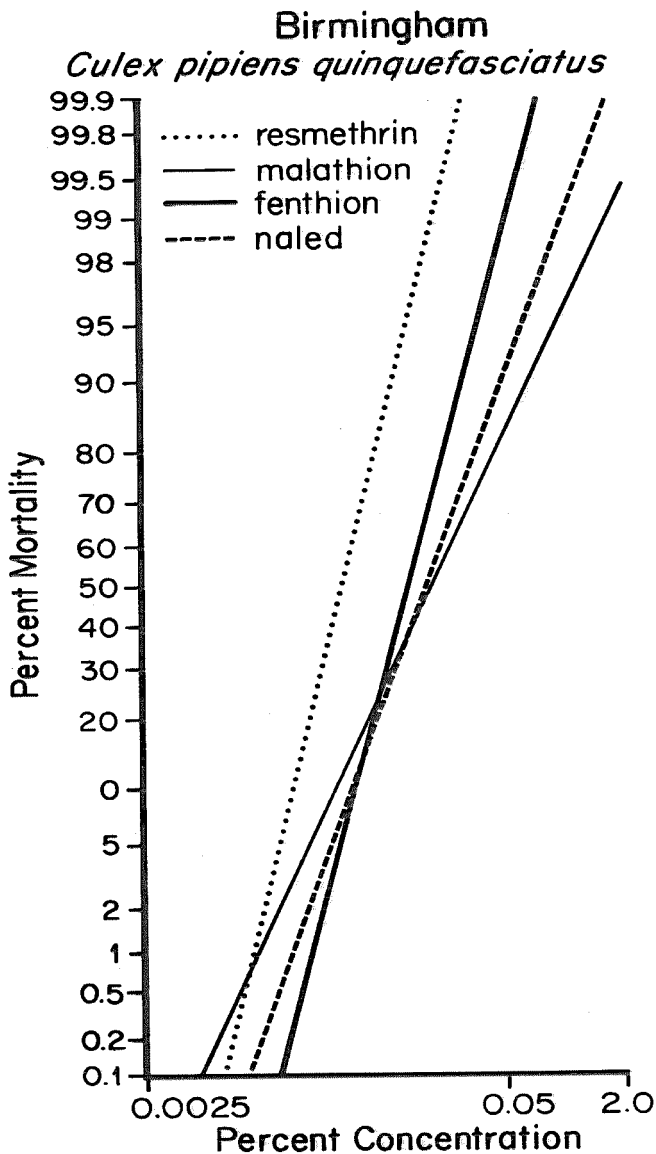


Fig. 1. Dosage-mortality regression line for four space-spray adulticides in laboratory applications against female *Culex pipiens quinquefasciatus* colonized from Birmingham, Ala.

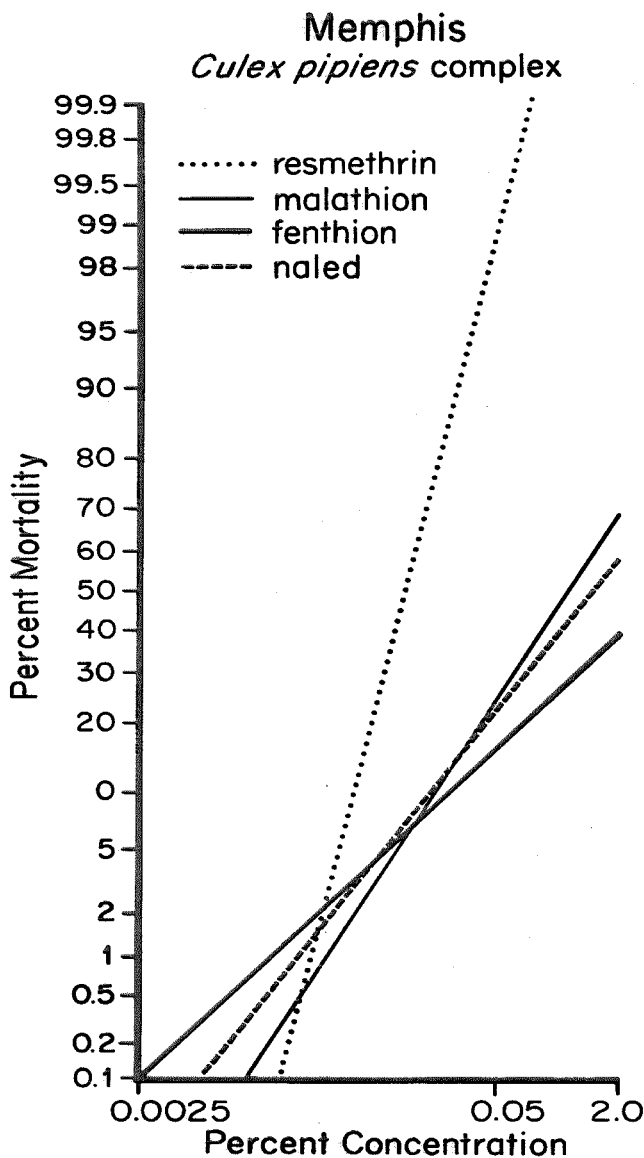


Fig. 2. Dosage-mortality regression lines for four space-spray adulticides in laboratory applications against females of the *Culex pipiens* complex colonized from Memphis, Tenn.

Aerosol test results, Figure 1, show that Birmingham *C. p. quinquefasciatus* are susceptible to all 4 of the test compounds with high mortality at lower dosages. The intrinsic toxicity of resmethrin, however, was somewhat higher than that of the other compounds. In contrast, the Memphis *C. p.* complex results presented in Figure 2 show that fenthion produced less than 20% kill at the highest dosage level of 0.05%, while naled and malathion produced only 30% kill. Regression lines have been projected to 2.0% to dramatize the level of resistance. Resmethrin was effective in all tests at the 0.025% concentration level.

Culex pipiens complex mosquitoes from outside Memphis in Shelby County were not examined for subspecies. They were, however, tested with malathion and found to be susceptible, and 96% kill at the 0.025% level was recorded with both the 4- and 14-day-olds, thus indicating that age is not a factor in susceptibility under the conditions of these tests.

DISCUSSION AND CONCLUSIONS

The use of malathion as a mosquito adulticide was initiated in Memphis-Shelby County, Tenn., during 1968 in connection with control of early spring floodwater adult mosquitoes. Initial application of adulticides was accomplished by use of 1200-B Dyna-Fog Thermal applicators. During 1970-71, Leco ULV HD nonthermal fog applicators were added. From 1969 through 1973, adult mosquito control was directed chiefly

toward the control of adult floodwater species.

In 1974 and 1975, intensive adult mosquito control activities were conducted in connection with outbreaks of St. Louis encephalitis in several census tracts located in the City of Memphis.

Since the inception of the program no larviciding operations had been undertaken utilizing an organophosphorous compound.

It is evident that the *Culex pipiens* complex population in Memphis is resistant to the organophosphorous compounds tested in this study. It is surprising, however, that malathion adulticiding pressure on that population over a period of 8 summers was sufficient to select for resistance.

ACKNOWLEDGMENT

The authors wish to thank Theresa B. Blue, Mary S. Crawford, and Gregory S. Matsunaga for their assistance in the laboratory phase of these studies. Drs. George Hardy and Michael Maetz, and Messrs. Paul Pate and Phil Walkley of the Jefferson County Health Department, Birmingham, Ala., very kindly provided *Culex p. quinquefasciatus* egg rafts for the Birmingham colony used in this study.

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