RELATIVE EFFECTS OF INSECTICIDE USAGE IN LOUISIANA MOSQUITO CONTROL PROGRAMS ON THE SUSCEPTIBILITY OF ADULT FEMALE *CULEX PIPIENS QUINQUEFASCIATUS* POPULATIONS

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ABSTRACT. A wind tunnel device was utilized in laboratory tests to determine the susceptibility to 6 selected insecticides of natural populations of adult female *Culex pipiens quinquefasciatus* Say collected from 6 southern Louisiana parishes. Mosquitoes collected from locations having mosquito control programs were significantly less ($P<0.05$) susceptible to the organophosphate insecticides temephos, chlorpyrifos, fenthion, malathion and naled than those populations from areas having no mosquito control programs. The *C. p. quinquefasciatus* adults exposed to propoxur showed little variation in susceptibility between the populations collected from the 6 locations.

Propoxur caused excellent knockdown and high mortality in 1-hour knockdown tests on mosquitoes collected from all areas sampled in this study. Naled caused excellent knockdown and high mortality to *C. p. quinquefasciatus* populations collected from areas where no organophosphate tolerance existed.

The southern house mosquito, *Culex pipiens quinquefasciatus* Say is an important vector of St. Louis encephalitis (Chamberlain et al. 1959). In 1975, outbreaks of St. Louis encephalitis occurred in various locations in North America from Ontario, Canada, diagonally across the United States to Houston, Texas. Preliminary data provided by the Viral Diseases Division, Bureau of Epidemiology, Center for Disease Control, Atlanta, Georgia, indicate that 1367 confirmed cases plus 574 cases with some serologic evidence of infection were reported from 29 states and the District of Columbia. Louisiana had 7 confirmed cases and 6 suspected cases; Mississippi had 109 confirmed cases and 80 suspected; and Texas reported 37 confirmed cases. In 1975, more than 90 deaths from St. Louis encephalitis were reported in the United States.

Culex pipiens quinquefasciatus is the most important vector of the dog heartworm, *Dirofilaria immitis* (Leidy), in Louisiana (Villavaso and Steelman 1968). An average of 63.3 *C. p. quinquefasciatus* adult females were captured per night in Louisiana with 1.45% containing the infective stage of *D. immitis* larvae.

Steelman et al. (1967) reported that *C. p. quinquefasciatus* larval populations greater than 1500 per dip occurred in waste disposal lagoons and septic ditches throughout Louisiana. The extensive use of organophosphate insecticides by mosquito control agencies has frequently led to the development of resistance to these compounds by many mosquito species throughout the world. In Louisiana, Steelman and Devitt (1976) reported the development of organophosphate tolerance in field populations of *C. p. quinquefasciatus* larvae.

Dosage-mortality data for mosquito species relative to currently labeled insecticides are of primary concern in planning and executing mosquito control programs. These data are especially important where the target species is an important vector of disease-causing organisms.

MATERIALS AND METHODS. Mosquitoes were collected as 3rd or 4th instar larvae from septic ditches throughout the year and transported to the laboratory according to the method described by Craven and Steelman (1968). Each group of larvae was collected from three or more sites within each major area.

Natural populations of mosquitoes were collected from Cameron, Calcasieu, Jefferson, St. Tammany, East Baton Rouge, and Tangipahoa Parishes. The first four of

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these had organized mosquito control districts. Collections were also made in 1 parish which had a Parish Health Unit Vector Control Program and 1 parish which had no mosquito control program.

In the laboratory larvae were divided into groups of 300 and along with 1,000 ml of water collected in the field were immediately transferred to aerated 30.5 x 19 x 5 cm enamel pans. The larvae in each pan were fed 0.25 g of finely ground commercial rabbit pellets daily. Water treated with sodium thiosulfate to remove chlorine was added to the pans to replace water lost by evaporation. Pupae were transferred into 0.25 liter glass jars filled with sodium thiosulfate treated water which were then placed in cages constructed from 3.785 liter ice cream cartons. Raisins used as a carbohydrate source were placed on top of each cage for adult nourishment and a 9 cm^2 piece of water-soaked cellucotton was placed on top of the raisins to maintain humidity. A temperature of 24± 2° C and 9 hr photoperiod was maintained in the laboratory. Wind tunnel and subsequent tests described by Mount and Pierce (1973) and Mount et al. (1976) were conducted with 3- to 7-day old adult females.

Adults were anesthetized in the 3.785 liter cages within a standard chemical hood by placing a 20 cm^2 piece of cellucotton soaked with 15 ml of ether over the top of the cage for 100 sec. Twenty females were transferred into each of the test cages which consisted of 0.25 liter ice cream cartons with the bottoms and the lids covered with 18 mesh nylon screen. The test cages were placed at the end of the tunnel for exposure to the various insecticides. One ml. of insecticide solution was then pipetted into the nozzle of the insecticide dispersing mechanism and the mosquitoes exposed to the resulting aerosol spray for 10 sec. Each test was replicated 4 times with each replication consisting of 1 cage of 20 female mosquitoes for each of a series of 5–10 insecticide concentrations plus a water check. After treatment, the mosquitoes were again anesthetized and transferred into clean cages. One raisin and a moist cellucotton pad were placed on top of each holding cage. Knockdown and mortality counts were taken at 1 and 24 hr after exposure.

Emulsifiable concentrate formulations of 5 organophosphates (temephos, fenthion, naled, chlorpyrifos, and malathion) and one carbamate (propoxur) were used in the wind tunnel tests. Test concentrations of insecticides were prepared by serial dilution in deionized water.

Data obtained from wind tunnel tests performed on *C. p. quinquefasciatus* from 6 locations and exposed to 6 insecticides were statistically analyzed using a randomized block design. Dosage-mortality regression lines were established by probit analysis described by Daum (1970). An eye-fitted line was constructed on log probit graph paper in order to estimate dosage-mortality lines of tests which were computed to have a non-significant ($P<0.01$) regression coefficient.

RESULTS AND DISCUSSION. Table 1 shows the analysis of variance (ANOV) for the LC50 response of *C. p. quinquefasciatus* to temephos, propoxur, fenthion, naled, chlorpyrifos, and malathion. A highly significant ($P<0.01$) difference was detected at the LC50 responses between chemicals and between locations, indicating that *C. p. quinquefasciatus* populations collected from the same area varied in their susceptibility to the 6 insecticides and that *C. p. quinquefasciatus* populations collected from different locations varied in their susceptibility to the same insecticides.

Table 1. Analysis of variance of LC50 values of 6 insecticides used to test the susceptibility of *Culex p. quinquefasciatus* collected from 6 locations in Louisiana.

Source	df	MS
Location	5	0.1977**
Chemical	5	0.0893**
Error	25	0.0186
Total	35	

** $P<0.01$.

Tables 2–7 show the dosage-mortality responses of *C. p. quinquefasciatus* after exposure to chlorpyrifos, fenthion, propoxur, naled, malathion, temephos, respectively. Significant differences ($P<0.05$) in susceptibility to each compound existed between populations collected from different locations when confidence limits computed from probit analysis were compared.

Culex p. quinquefasciatus populations collected in Jefferson Parish appeared to be more susceptible to chlorpyrifos than any of the other populations of this species tested (Table 2). These data showed that Jefferson and St. Tammany Parishes and in the Zachary and Baker localities of East Baton Rouge Parish for 5 to 8 years.

Populations of *C. p. quinquefasciatus* from Jefferson and Tangipahoa Parishes were the most susceptible mosquitoes to fenthion (Table 3). Susceptibility levels of the mosquitoes from Calcasieu, St. Tammany and the LSU colony were not significantly different ($P>0.05$); however, a significant difference ($P<0.05$) at the LC50 response level was shown between mosquitoes from these locations and those collected in Jefferson and Tangipahoa

Table 2. Susceptibility of *Culex p. quinquefasciatus* collected from 8 locations in Louisiana to chlorpyrifos.

Location	LC50 (%)	95% C.L.[b]	LC90 (%)	95% C.L.[b]	Slope
Jefferson	0.0267	0.0236–0.0300	0.0735	0.0642–0.0860	2.9124
Tangipahoa	0.0424	0.0305–0.0550	0.1254	0.0924–0.2071	2.7236
Calcasieu	0.0622	0.0329–0.0893	0.1736	0.1181–0.4044	2.8763
L.S.U.[a]	0.0950		0.1720		3.9635
St. Tammany	0.1299	0.0988.0.1684	0.5262	0.3477–1.1797	2.1095
Cameron [a]	0.1480		0.5110		0.5475
Zachary	0.7211	0.5216–1.5321	1.5123	1.2381–7.6571	3.4821
Baker [a]	1.3900		2.7000		3.7676

[a] Eye-fitted line.
[b] Confidence limits.

the mosquitoes from Jefferson Parish were 27 to 52X more suspectible at the LC50 response to chlorpyrifos than the populations of *C. p. quinquefasciatus* collected from Zachary and Baker (both in East Baton Rouge Parish), respectively. Although no adulticide treatments of *C. p. quinquefasciatus* with chlorpyrifos has occurred in any of the areas, this compound has been used extensively as a larvicide in Parishes. At the LC50 response level the mosquitoes collected in Jefferson Parish were 30 to 43X more susceptible to fenthion than the populations collected from Zachary and Baker, respectively. The mosquitoes collected in Baker and Zachary were less than 7X as susceptible to fenthion at the LC50 response level as the *C. p. quinquefasciatus* collected from the other 5 locations.

Table 3. Susceptibility of *Culex p. quinquefasciatus* collected from 7 locations in Louisiana to fenthion.

Location	LC50 (%)	95% C.L.[a]	LC90 (%)	95% C.L.[a]	Slope
Jefferson	0.0176	0.0124–0.0239	0.0681	0.0479–0.1132	2.1831
Tangipahoa	0.0307	0.0204–0.0413	0.0886	0.0633–0.1600	2.7822
Calcasieu	0.0775	0.0627–0.0928	0.1629	0.1309–0.2330	3.9751
L.S.U.	0.0916	0.0813–0.1036	0.2811	0.2291–0.3698	2.6306
St. Tammany	0.0904	0.0598–0.1271	0.7260	0.4726–1.5696	1.4439
Zachary	0.5354	0.2670–2.0330	2.4297	1.0154–569.73	1.9510
Baker	0.7710	0.7185–0.8325	1.4297	1.2507–1.7350	4.7785

[a] Confidence limits.

The only record of fenthion use for mosquito control was in Jefferson Parish 5 years prior to the time of this study. Apparently little if any selective pressure on the adult populations occurred as a result of this limited usage in Jefferson Parish.

The susceptibility of *C. p. quinquefasciatus* to the carbamate propoxur is shown in Table 4. Mosquitoes exposed to propoxur showed little variation in susceptibility between 6 collection localities in Louisiana. However, mosquitoes collected from Tangipahoa Parish were significantly ($P < 0.05$) more susceptible than the LSU laboratory colony but the difference in the LC50's was only about 2X. Mosquitoes collected from the various localities appeared to be relatively susceptible to propoxur. At the present time, propoxur is not presently labeled for mosquito control therefore natural populations of *C. p. quinquefasciatus* have not been exposed to the compound and this accounted for less variation between the locations.

Table 4. Susceptibility of *Culex p. quinquefasciatus* collected from 6 locations in Louisiana to propoxur.

Location	LC50 (%)	95% C.L.[a]	LC90 (%)	95% C.L.[a]	Slope
Tangipahoa	0.0837	0.0728–0.0950	0.2314	0.1932–0.2963	2.9011
Zachary	0.1056	0.0080–0.1811	0.2449	0.1510–45.5031	3.5070
St. Tammany	0.1157	0.0932–0.1386	0.3012	0.2376–0.4392	3.0843
Calcasieu	0.1404	0.1051–0.1972	0.3328	0.2257–1.0050	3.4200
Jefferson	0.1604	0.1489–0.1724	0.4000	0.3546–0.4655	3.2297
L.S.U.	0.1782	0.1327–0.2781	0.4852	0.3006–2.2026	2.9465

[a] Confidence limits.

Susceptibility of adult *C. p. quinquefasciatus* to naled is shown in Table 5. Less than 2X difference occurred between the LC50 response level of *C. p. quinquefasciatus* collected from Tangipahoa, Calcasieu, and St. Tammany Parishes and the LSU laboratory colony. At the LC50 response level, the LSU colony, St. Tammany, and Cameron Parish mosquitoes differed significantly ($P < 0.05$) from those collected in Calcasieu and Tangipahoa Parishes. The LC50 response levels obtained from mosquitoes collected in Jefferson Parish differed significantly ($P < 0.05$) from those obtained from Tangipahoa, Calcasieu, and St. Tammany and the LSU colony. Naled has been the adulticide used by the Jefferson Parish MCD during the past 5 years. Selective pressure on the adults of *C. p. quinquefasciatus* by naled was apparent and was

Table 5. Susceptibility of *Culex p. quinquefasciatus* collected from 8 locations in Louisiana to naled.

Location	LC50 (%)	95% C.L.[c]	LC90 (%)	95% C.L.[c]	Slope
Tangipahoa	0.0521	0.0187–0.0762	0.1235	0.0834–0.5537	3.4189
Calcasieu	0.0605	0.0431–0.0810	0.1967	0.1346–0.4097	2.6491
St. Tammany	0.1100	0.0824–0.1545	0.5966	0.3466–1.7171	1.7457
L.S.U.	0.1110	0.0858–0.1442	0.2760	0.1964–0.5846	3.2403
Cameron	0.1830	0.0819–1.3241	1.3894	0.4266–18404.02	1.4556
Jefferson	0.3166	0.2237–1.6465	0.7540	0.4119–127.310	3.4004
Baker [a]	0.6820		1.6900		3.3278
Zachary [b]	0.7267	0.5074–	1.7370	0.9316–	3.3867

[a] Eye-fitted line.
[b] Upper confidence limit not computed.
[c] Confidence limits.

reflected in their comparative degree of susceptibility to this compound. Naled has been utilized as an adulticide to some degree in St. Tammany Parish with ground ULV equipment over the last 5 years and extensively used in aircraft applications in Cameron Parish since 1972. Thus, it appears that the use of naled as an adulticide has lowered the susceptibility level of *C. p. quinquefasciatus* to this compound in these parishes.

Susceptibility of *C. p. quinquefasciatus* adult females to malathion is shown in Table 6. Malathion has been used as an adulticide in all of the areas from which mosquitoes were collected except Tangipahoa Parish. Only limited amounts of malathion have been utilized by the Jefferson Parish MCD. This is apparent in the LC50 response levels of the mosquitoes collected from this parish to malathion (identical LC50 response values in the LSU laboratory colony and less than 2X difference in susceptibility from the Tangipahoa populations). Malathion has been used as an adulticide extensively in the Calcasieu, St. Tammany, Cameron, Zachary, and Baker locations in ULV ground and/or aerial applications. Thus, the difference in susceptibility (LC50) ranges from 3X between Tangipahoa and Calcasieu to 9X between Tangipahoa and Baker.

Temephos has been used as a larvicide to control *C. p. quinquefasciatus* in Jefferson Parish and in Zachary. No record of its use as an adulticide was obtained in any of the areas from which *C. p. quinquefasciatus* was collected. The susceptibility of *C. p. quinquefasciatus* to temephos is shown in Table 7. Because no insecticide applications have been made for mosquito control in Tangipahoa Parish, mosquitoes collected in this parish were used as an indicator of the effectiveness of temephos as an adulticide. The LC50 response showed that a 4X difference existed between the mosquitoes collected in

Table 6. Susceptibility of *Culex p. quinquefasciatus* collected from 8 locations in Louisiana to malathion.

Location	LC50 (%)	95% C.L.[b]	LC90 (%)	95% C.L.[b]	Slope
Tangipahoa [a]	0.1410		0.2750		4.1176
Jefferson	0.2599	0.1297–0.2461	0.3524	0.2662–0.7072	4.7245
L.S.U.	0.2599	0.2341–0.2904	0.7015	0.5878–0.8815	2.9720
Calcasieu	0.4340	0.3753–0.5300	0.8854	0.6690–1.7565	4.1385
St. Tammany	0.5243	0.4345–0.6915	1.4819	0.9985–3.4919	2.8400
Cameron	0.5259	0.3703–1.7887	1.3840	0.7586–164.020	3.0497
Zachary	0.7644	0.5668–1.5038	2.1031	1.1905–25.6215	2.9157
Baker [a]	1.3000		2.7800		6.0187

[a] Eye-fitted line.
[b] Confidence limits.

Table 7. Susceptibility of *Culex p. quinquefasciatus* collected from 6 locations in Louisiana to temephos.

Location	LC50 (%)	95% C.L.[b]	LC90 (%)	95% C.L.[b]	Slope
Tangipahoa	0.1620	0.1310–0.2089	0.3227	0.2390–0.7172	4.2837
Calcasieu	0.2557	0.2362–0.2777	0.6263	0.5416.0.7564	3.2943
Jefferson [a]	0.3790		0.6580		5.1673
St. Tammany	0.4834	0.3589–0.7860	1.0353	0.6850–5.1517	3.8745
L.S.U.	0.5102	0.3441–0.9882	1.7329	0.9252–20.7582	2.4135
Zachary	0.6889	0.5739–0.9111	1.5013	1.0644–4.0334	3.7879

[a] Eye fitted line.
[b] Confidence limits.

Table 8. One hour mortality of *Culex p. quinquefasciatus* treated with propoxur.

Location	LC50 (%)	95% C.L.[b]	LC90 (%)	95% C.L.[b]	Slope
Zachary [a]	0.0820		0.1540		4.2604
Tangipahoa	0.0914	0.0803–0.1028	0.2304	0.1837–0.2944	3.1920
St. Tammany	0.0996	0.0742–0.1242	0.2220	0.1725–0.3451	3.6814
Jefferson	0.1429	0.1208–0.1653	0.3284	0.2694–0.4478	3.5467
Calcasieu	0.1516	0.1144–0.2189	0.3218	0.2218–0.9666	3.9216
L.S.U.	0.1518	0.1219–0.1934	0.3796	0.2719–0.7786	3.2203

[a] Eye fitted line.
[b] Confidence limits.

Tangipahoa as compared to those collected from Zachary. Since a portion of the mosquitoes used to start the LSU colony were collected in Zachary in 1968 before it was known that the area had been treated with temephos, this probably explains the lack of susceptibility of the LSU colony to temephos and other insecticides tested.

The results of 1-hr knockdown and mortality tests conducted with propoxur are shown in Table 8. Propoxur showed excellent knockdown qualities on *C. p. quinquefasciatus* collected from all 5 areas. Mosquitoes collected from Zachary which had the highest degree of tolerance to the organophosphate insecticides were highly susceptible to propoxur in the 1-hr knockdown tests. These data indicate that in an emergency situation (i.e., an epidemic of St. Louis encephalitis) propoxur could be used effectively to control the vector *C. p. quinquefasciatus* even in areas where organophosphate tolerance exists.

Table 9 shows the 1-hr mortality response of *C. p. quinquefasciatus* to naled. This chemical caused excellent knockdown and high mortality to the mosquito populations collected from Calcasieu, Tangipahoa and St. Tammany Parishes where no tolerance existed and could be used effectively to control the mosquito vector of St. Louis encephalitis in such areas.

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Table 9. One hour mortality of *Culex p. quinquefasciatus* treated with naled.

Location	LC50 (%)	95% C.L.[b]	LC90 (%)	95% C.L.[b]	Slope
Calcasieu	0.0844	0.0618–0.1083	0.1765	0.1328–0.3179	3.9975
Tangipahoa	0.0868	0.0606–0.1244	0.1711	0.1207–0.6049	4.3478
St. Tammany [a]	0.2664	0.1555–	0.8637	0.3844–	2.5090
Zachary	3.2592	1.4075–69.5407	37.120	6.6904–22567.9	1.2130

[a] Upper confidence limit not computed.
[b] Confidence limits.

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