Journal of the American Mosquito Control Association, 12(3):487–490, 1996

OPERATIONAL AND SCIENTIFIC NOTES

INSECTICIDE SUSCEPTIBILITY OF *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* IN THE LOWER RIO GRANDE VALLEY OF TEXAS AND MEXICO[1]

W. J. SAMES IV,[2] R. BUENO, JR.,[2] JACK HAYES[3] AND J. K. OLSON[2]

ABSTRACT. In response to a potential dengue fever outbreak in south Texas during 1995, the susceptibilities of *Aedes aegypti* and *Aedes albopictus* to commonly used mosquito adulticides were assessed. Larvae collected from the Lower Rio Grande Valley of Texas and Mexico were reared to adults and tested against susceptible laboratory strains at Texas A&M University. Resistance ratios at both the LC_{50} and LC_{95} rates were all less than 10, indicating that adult populations of both species are still susceptible to malathion, chlorpyrifos, resmethrin, and permethrin.

Dengue fever is not new to Texas. However, due to good public health and mosquito control practices and an improved standard of living, dengue is no longer endemic in Texas. Infected travelers and/or immigrants from countries where dengue fever is endemic occasionally bring the disease into Texas. These people are usually hospitalized and kept away from potential vectors such as *Aedes aegypti* (Linn.) and *Aedes albopictus* (Skuse). This prevents the disease from spreading. Unfortunately, dengue fever epidemics are becoming more common in the Americas and recent epidemics have occurred in Brazil, Venezuela, Central America, the Caribbean, and Mexico (Frankel 1995).

Before 1995, a mosquito surveillance program was lacking in the Lower Rio Grande Valley of Texas and Mexico. In 1995, the Border Health Unit of the Texas Department of Health supported the establishment of a surveillance system on the Texas/Mexico border between McAllen/Reynosa and Brownsville/Matamoros. While seeking surveillance cooperation with Mexico, Mexican officials reported numerous cases of dengue fever in Reynosa and Matamoros. In 1994, they reported more than 600 suspected cases; the number of clinical cases exceeded 2,000 in 1995. Several cases of dengue hemorrhagic fever in Mexico indicated that more than one dengue serotype might be present. The presence of Dengue 2 in Reynosa was confirmed, and Dengue 4 has been confirmed from one patient in Brownsville and one in Matamoros. Dengue fever cases have also been confirmed in Texas, but these cases involved people who were frequent visitors to dengue fever areas in Mexico. However, in September 1995 the Texas Department of Health announced the first Texas-transmitted case in McAllen, TX.

To help the Texas Department of Health combat the spread of dengue fever into Texas, susceptibility of *Ae. aegypti* and *Ae. albopictus* to insecticides (malathion, chlorpyrifos, resmethrin, and permethrin) most commonly used against mosquito adults in the Lower Rio Grande Valley were evaluated. Mosquito larvae and pupae were collected from Matamoros and Reynosa, Mexico, and from Brownsville, Weslaco, Donna, McAllen, Mission and Hidalgo, TX, and reared to the adult stage in the Mosquito Research Laboratory at the Department of Entomology, Texas A&M University in College Station, TX. High larval mortality during the first collecting trip and large numbers of *Culex quinquefasciatus* Say larvae intermixed with *Ae. aegypti* and *Ae. albopictus* larvae required 3 collecting trips to obtain the necessary numbers of adults for resistance testing. Table 1 shows the number of adult female mosquitoes reared from larvae collected at locations in the Lower Rio Grande Valley. These data support the species distributions reported by Francy et al. (1990) and Womak (1993).

Insecticide susceptibilities of field-collected *Ae. aegypti* females were compared with the susceptible, University of Texas Medical Branch (UTMB) strain of *Ae. aegypti* mosquitoes. The UTMB strain, which has been maintained in colony for about 31 years, was obtained from D. W. Micks, University of Texas Medical Branch,

[1] This study was conducted in cooperation with the U.S. Department of Agriculture, Agricultural Research Service, as a part of USDA, CSRS Southern Regional Project S-260.

[2] Department of Entomology, Texas A&M University, College Station, TX 77843-2475.

[3] University of Texas–Houston School of Public Health, 7703 Floyd Curl Drive, San Antonio, TX 78284-7976.

Table 1. Numbers of adult female *Aedes aegypti* and *Aedes albopictus* reared from larvae collected in the Lower Rio Grande Valley of Texas and Mexico by date of collection during 1995.

Location	Collection dates	*Ae. aegypti* no. (%)	*Ae. albopictus* no. (%)	Habitat
Texas				
Brownsville	Sept. 22–29	81 (53%)	71 (47%)	Urban cemetery
Brownsville	Oct. 8–11	67 (7%)	935 (93%)	Used tires/cemetery
McAllen	Sept. 22–29	65 (89%)	8 (11%)	Backyards
McAllen–Hidalgo	Oct. 8–11	414 (84%)	80 (16%)	Used tires/wrecking yards
McAllen–Hidalgo	Nov. 12–14	189 (100%)	0 (0%)	Used tires
Mission	Sept. 22–29	0 (0%)	6 (100%)	Rural cemetery
Weslaco	Sept. 22–29	21 (16%)	109 (84%)	Suburban cemetery
Weslaco–Donna	Oct. 8–11	87 (34%)	166 (66%)	Cemetery/used tires
Mexico				
Matamoros	Sept. 22–29	226 (99%)	3 (1%)	Urban cemetery
	Sept. 22–29	17 (10%)	154 (90%)	Suburban cemetery
	Sept. 22–29	17 (81%)	4 (19%)	Other sites
Reynosa	Sept. 22–29	66 (78%)	19 (22%)	Backyards/tires
Totals		1,250 (45%)	1,555 (55%)	

Galveston, TX. Robert and Olson (1989) previously used this colony to compare insecticide susceptibility of Texas *Ae. albopictus.*

Insecticide susceptibilities of field-collected *Ae. albopictus* females were compared with the susceptible, Texas A&M University (TAMU) strain of *Ae. albopictus.* The TAMU strain was started at Texas A&M University in 1986 from field-collected mosquitoes from Liberty and Chambers counties, TX. The Liberty County strain tested by Robert and Olson (1989) was the TAMU *Ae. albopictus* strain used in this study.

Field-collected larvae as well as the UTMB and TAMU larvae were reared 10–14 days in a controlled environment at 25°C and 85% RH until there were sufficient numbers to evaluate for resistance. Adults (up to 14 days old) were collected with a mechanical aspirator, anesthetized with CO_2 and placed on a chill table for sorting. Mosquitoes were sorted by sex and species, and only females were used for testing. Insecticide susceptibilities of the adult field-collected and laboratory strains of *Ae. aegypti* and *Ae. albopictus* were determined using the insecticide-coated vial technique of Plapp (1971).

Five scintillation vials (20 ml) were prepared for each of the following insecticide concentrations (in μg/vial): malathion (*Ae. aegypti*)—0.01, 0.03, 0.06, 0.1, 0.3, and 0.6, (*Ae. albopictus*)—0.03, 0.06, 0.1, 0.3, 0.6, and 1.00; chlorpyrifos (both species)—0.006, 0.01, 0.03, 0.06, 0.1, and 0.3; resmethrin (both species)—0.006, 0.01, 0.03, 0.06, 0.1, and 0.3; permethrin (*Ae. albopictus* only)—0.006, 0.01, 0.03, 0.06, 0.1, and 0.3. Dilutions were prepared from technical grade solutions of each insecticide, and the appropriate amount of insecticide was added to each vial. Acetone was then added to the vial to give a total amount of 0.5 ml of solution per vial. The control (no insecticide) for each trial was made by placing 0.5 ml acetone in a vial.

To distribute the test chemical on the inner surface of the glass, vials were placed on their sides and manually rolled until dry. A small piece of cotton saturated in 5% sucrose water was added to each vial. Five females of the same species were placed into each vial, which was then sealed with a cotton ball. This represented 25 mosquitoes per concentration and a total of 175 mosquitoes per test.

Twenty-four hours later, the number of live mosquitoes per vial was counted, and these data were analyzed using SAS probit analysis, which also corrects for mortality in the controls. Control mortalities for the field-collected mosquitoes were 0% except for malathion (4%—*Ae. aegypti*), chlorpyrifos (6%—*Ae. aegypti,* 2%—*Ae. albopictus*), resmethrin (30%—*Ae. aegypti,* 4%—*Ae. albopictus*), and permethrin (8%—*Ae. albopictus*). In the laboratory strains, the only control mortalities were in the resmethrin test (16% for the TAMU strain of *Ae. albopictus*).

Resistance ratios, RR_{50} and RR_{95}, were calculated by dividing the field-strain LC_{50} or LC_{95} by the respective, susceptible-strain LC_{50} or LC_{95}. Based on field experience, our laboratory

Table 2. 1995 Lower Rio Grande Valley dengue vector insecticide resistance screening results.[1]

Insecticide/ species	LC_{50} (μg/ vial)	RR_{50}	LC_{95} (μg/ vial)	RR_{95}	Slope
Malathion					
Aedes aegypti[2]					
TX	0.16	4.00	0.31	1.00	5.74
MX	0.19	4.75	0.58	1.87	3.45
UTMB	0.04	—	0.31	—	1.87
Aedes albopictus[2]					
TX	0.70	5.38	2.35	4.90	3.11
MX	0.77	5.92	1.82	3.79	4.41
TAMU	0.13	—	0.48	—	2.89
Chlorpyrifos					
Ae. aegypti[3]					
TX	0.08	4.00	0.33	8.25	2.64
UTMB	0.02	—	0.04	—	6.76
Ae. albopictus[3]					
TX	0.03	1.00	0.06	1.50	4.41
TAMU	0.03	—	0.04	—	20.04
Resmethrin					
Ae. aegypti[4]					
TX	0.008	1.33	0.08	2.00	1.64
UTMB	0.006	—	0.04	—	1.85
Ae. albopictus[5]					
TX	0.02	0.50	0.20	1.18	1.46
TAMU	0.04	—	0.17	—	2.59
Permethrin					
Ae. albopictus[5]					
TX	0.02	1.00	0.08	1.14	2.48
TAMU	0.02	—	0.07	—	3.17

[1] UTMB and TAMU strains are, in each case, the laboratory strains of "susceptible" mosquitoes used to determine the resistance ratios (RRs) at the LC_{50} (= RR_{50}) and LC_{95} (= RR_{95}) concentrations of each insecticide tested. Resistance ratios exceeding a value of 10 give cause for concern because tolerance is increasing in a given field population of mosquitoes.

[2] TX = combined collections from McAllen, Mission, Weslaco, and Brownsville; MX = combined collections from Matamoros and Reynosa (collected as larvae on Sept. 22–29, 1995; tested as adults on Oct. 5–6, 1995).

[3] TX = combined collections from McAllen, Hidalgo, Weslaco, Donna, and Brownsville (collected as larvae on Oct. 8–11, 1995; tested as adults on Oct. 19–20, 1995).

[4] TX = combined collections from McAllen and Hidalgo (collected as larvae on Nov. 12–14, 1995; tested as adults on Dec. 1–2, 1995).

[5] TX = collections from Brownsville only (collected as larvae on Oct. 8–11, 1995; tested as adults on Oct. 25, 1995).

Table 3. Mosquito adulticides being used by Texas Lower Rio Grande Valley municipalities as of September 1995.

County	No. of cities using Malathion	Chlorpyrifos	None
Cameron	7	7	2
Hidalgo	8	10	3
Starr	1	0	2
Willacy	6[1]	0	0

[1] Lyford, TX, using malathion and methoxychlor.

considers ratios that approach 10 to indicate that the test populations are beginning to develop resistance; ratios greater than 10 indicate that a significant level of resistance is present in the population. All ratios presented in Table 2 are below 10, although for chlorpyrifos against *Ae. aegypti*, the LC_{95} is somewhat elevated compared to the other insecticides tested on this species. This elevated ratio may be the result of pooling the data from several cities or it may show that *Ae. aegypti* is beginning to develop resistance to chlorpyrifos in those areas. Despite these latter results, female *Ae. aegypti* and *Ae. albopictus* populations in the Lower Rio Grande Valley of Texas and Mexico still remain fairly susceptible to the insecticides tested in this study, and at the current time, mosquito controllers in this region retain a variety of insecticides from which to choose. The current pattern of mosquito adulticide usage in the Texas Lower Rio Grande Valley is depicted in Table 3.

Insecticide application effects need to be further evaluated under the existing operational conditions in the Lower Rio Grande Valley. Many of the homes from which larvae were collected were built close together and in "U" or "L" configurations. These configurations, which in many cases are used to store items that could hold water, are protected from insecticide drift. It is, therefore, suspected that backyard mosquito populations may be protected from insecticides sprayed from vehicles on the street, and this lack of insecticide exposure may help maintain susceptible mosquito populations in these areas. The building configurations may also prevent "drive-by" sprayings from achieving adequate mosquito control for disease suppression. In addition to using effective insecticides, public education and good insecticide dispersal practices (such as calibrating equipment properly and spraying at the proper time of day) and using pre- and postspray surveillance will help reduce mosquito populations, help eliminate dengue fever from Texas, and hopefully lead to better control of this disease in Mexico.

We thank Powell Knight and Kabkaew Likitvong of Texas A&M University for their assistance in conducting the resistance testing, and Gary Heseltine and Robert Wood of the University of Texas–Houston School of Public Health in San Antonio, TX, and John Goolsby of McAllen, TX, for their assistance in collecting mosquito larvae in the Lower Rio Grande Valley. We also thank Jose Luis Hernandez, Nigacio Estrella, and Juan Camacho of Centro de Salude, de Reynosa for their help in collecting in Reynosa, Mexico. We also thank Roger W. Meola, Patricia Pietrantonio, and George L. Teetes for their helpful comments on this manuscript.

REFERENCES CITED

Francy, D. B., C. G. Moore and D. A. Eliason. 1990. Past, present and future of *Aedes albopictus* in the United States. J. Am. Mosq. Control Assoc. 6:127–132.

Frankel, D. H. 1995. Americas act to combat dengue outbreaks. Lancet 346:957.

Plapp, F. W. 1971. Insecticide resistance in *Heliothis*: tolerance in larvae of *H. virescens* as compared with *H. zea* to organophosphate insecticides. J. Econ. Entomol. 64:999–1002.

Robert, L. L. and J. K. Olson. 1989. Susceptibility of female *Aedes albopictus* from Texas to commonly used adulticides. J. Am. Mosq. Control Assoc. 5: 251–253.

Womak, M. L. 1993. Distribution, abundance and bionomics of *Aedes albopictus* in southern Texas. J. Am. Mosq. Control Assoc. 9:367–369.