*Journal of the American Mosquito Control Association,* 14(4):477–480, 1998


# ORGANOPHOSPHATE AND PYRETHROID SUSCEPTIBILITIES OF *CULEX SALINARIUS* ADULTS FROM TEXAS AND NEW JERSEY[1]

KABKAEW SUKONTASON,[2,3] JIMMY K. OLSON,[2] W. KEITH HARTBERG[4] AND RICHARD E. DUHRKOPF[4]

ABSTRACT. Susceptibilities of adults from newly established colonies of *Culex salinarius* from New Jersey and Texas to commonly used mosquito adulticides were assessed using the insecticide-coated vial bioassay technique. Females from both colonies were similar in their susceptibilities to naled, chlorpyrifos, resmethrin, and permethrin. However, females from the New Jersey colony (established from collections made in Cape May County, NJ) were found to be 9 times more tolerant to malathion than were those from the Texas colony (established from collections made in Chambers County, TX), with median lethal concentration values for malathion tested against these 2 colonies of 0.70 and 0.08 μg malathion/vial, respectively. The differences between these 2 colonies with respect to their tolerances to malathion may be a product of the age of each colony at the time assessments were made and/or the degree to which the parent stock used to start each colony was previously exposed to malathion in the field.

**KEY WORDS** Mosquito adulticides, insecticide tolerance, resistance management, susceptibility baselines

*Culex salinarius* Coq., a major nuisance mosquito in the eastern one half of the United States, has been implicated as a possible vector of St. Louis encephalitis virus (Clark et al. 1977). Thus, not only are new ways to more effectively manage this pest mosquito necessary, but also ways are needed to monitor the effectiveness of the current control tactics used against it. Mosquito abatement programs generally attempt to target both the larval and adult stages of mosquito populations to be controlled. However, the current control strategy for *Cx. salinarius,* particularly as it occurs in the rice-growing coastal region of southeastern Texas, rests almost entirely on the use of effective chemicals against adult populations. The chemical adulticides are most commonly applied at ultra-low volume (ULV) rates (3 oz. active ingredient or less per acre) by truck-mounted, cold aerosol ground units or by aircraft against adult *Cx. salinarius* populations just as they are emerging or as they migrate from their larval development sites toward more human-populated areas. This one-dimensional approach to controlling problems associated with *Cx. salinarius* makes it imperative that the chemical adulticides being used against this mosquito species are the most effective ones available and that the effectiveness of these chemicals be preserved.

Until now, no in-depth laboratory studies have been conducted on the various adulticides currently available for use against mosquitoes as to their relative effectiveness against *Cx. salinarius* adults because of the difficulty in rearing this species under laboratory conditions. However, laboratory colonies of *Cx. salinarius* were recently established at Texas A&M University using wild mosquito stock collected from Cape May County, NJ, and the U.S. Fish and Wildlife Service's (USFWS) Anahuac Wildlife Refuge in Chambers County, TX. The establishment of these colonies made it possible for us to determine some baselines of susceptibility for *Cx. salinarius* to the adulticidal agents most commonly used against this species in the United States.

Mosquito adulticide susceptibility tests were conducted on 3- to 4-day-old females from the 2 *Cx. salinarius* colonies using the insecticide-coated vial bioassay technique of Plapp (1971). The females used in these bioassays were obtained from the 11th laboratory generation of the Texas colony and the 2nd laboratory generation of the New Jersey colony of *Cx. salinarius* established at Texas A&M University. Methods used in establishing and maintaining these colonies will be the subject of a subsequent paper. The 5 currently labeled adulticides included in the bioassays were malathion, naled, chlorpyrifos, resmethrin, and permethrin. Each technical grade insecticide was serially diluted with acetone to the appropriate concentrations. The insecticide dilutions were pipetted into 20-ml glass scintillation vials (5 vials/concentration/mosquito strain) and, if necessary, acetone was added to bring the total volume to 0.5 ml of liquid being placed into each vial. Only acetone (0.5 ml/vial) was placed in the control vials (5 vials/insecticide/mosquito strain). After adding the acetone solutions, all vials were placed on their sides and rolled manually until the solvent completely evaporated leaving the insecticide residue (when present) even-

[1] This study was conducted in cooperation with the U.S. Department of Agriculture (USDA), Agricultural Research Service, as part of USDA, Cooperative State Research, Education, and Extension Service (CSREES) Southern Regional Project S-260 involving State Agricultural Experiment Station personnel in Arkansas, California, Illinois, Louisiana, Mississippi, and Texas.

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

[3] Present address: Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand.

[4] Department of Biology, Baylor University, Waco, TX 76798–7388.

Table 1. Results of insecticide susceptibility bioassay tests conducted on adult females from laboratory colonies of Texas (TX) and New Jersey (NJ) strains of *Culex salinarius* during 1996.[1]

| Insecticide and mosquito strain[2] | $LC_{50}$ (µg/vial) | $RR_{50}$[3] | $LC_{95}$ (µg/vial) | $RR_{95}$[3] | Slope ± SE |
|---|---|---|---|---|---|
| Malathion | | | | | |
| NJ | 0.701 | 9.22 | 1.159 | 3.38 | 7.527 ± 1.914 |
| TX | 0.076 | — | 0.343 | — | 2.526 ± 0.647 |
| Naled | | | | | |
| NJ | 0.076 | 1.77 | 0.162 | 1.53 | 5.029 ± 1.497 |
| TX | 0.043 | — | 0.106 | — | 4.247 ± 1.132 |
| Chlorpyrifos | | | | | |
| NJ | 0.087 | 2.23 | 0.505 | 2.34 | 2.152 ± 0.357 |
| TX | 0.039 | — | 0.216 | — | 2.209 ± 0.297 |
| Resmethrin | | | | | |
| NJ | 0.033 | 1.83 | 0.111 | 0.57 | 3.124 ± 0.705 |
| TX | 0.018 | — | 0.194 | — | 1.594 ± 0.296 |
| Permethrin | | | | | |
| NJ | 0.039 | 4.33 | 0.101 | 3.88 | 3.996 ± 1.405 |
| TX | 0.009 | — | 0.026 | — | 3.591 ± 0.853 |

[1] Colonies were maintained at 20°C, 80% relative humidity, and 9 h:13 h light:dark with a 2-hr morning and evening crepuscular features. $LC_{50}$s median lethal concentration; $LC_{95}$, 95% lethal concentration.

[2] The NJ and TX colonies were in their $F_2$ and $F_{11}$ laboratory generations, respectively, at the time of testing.

[3] The $LC_{50}$ and $LC_{95}$ resistance ratios ($RR_{50}$ and $RR_{95}$, respectively) were determined by dividing the $LC_{50}$ and $LC_{95}$ values recorded for the New Jersey strain by the comparable values recorded for the Texas strain of *Cx. salinarius*.

ly coated over the inside surfaces of each vial. A small cotton pad (ca. 0.75 $cm^2$) soaked with 10% sucrose solution was placed in the bottom of each vial as a carbohydrate source for the mosquitoes.

Female *C. salinarius* aspirated from one of the laboratory colonies, were lightly anesthetized with carbon dioxide and placed on a chill table (set at ca. 7°C) for counting. Five females were placed into each treated and control vial included in a test set for a given insecticide. Separate test sets were prepared for each of the 2 mosquito colonies. The vials of mosquitoes were plugged with cotton, placed in the holding compartments of a scintillation vial carton, covered with moist paper toweling, and held at room temperature for 24 h.

Mosquito mortality occurring in each vial was recorded at the end of the 24-h exposure period. The resulting data were analyzed using the SAS Probit Program (SAS 1985). The analyses corrected for control mortalities and provided median lethal concentration ($LC_{50}$), 95% lethal concentration ($LC_{95}$), and 95% confidence interval values in micrograms of insecticide per vial. Slopes (indicators of the degree of homogeneity in each population's response to the insecticides tested) were also provided by this program.

Results of the bioassays involving the 2 colonized strains of *Cx. salinarius* are summarized in Table 1. At the $LC_{50}$ level of comparison, the Texas strain of *Cx. salinarius* females tended to be slightly more susceptible to each of the adulticides tested than were the females of the New Jersey strain of this species. This is indicated by the New Jersey strain to Texas strain $LC_{50}$ resistance ratios ($RR_{50}$s) ranging from a low of 1.77 for naled to a high of 9.22 for malathion (Table 1).

To gain further insight as to the meaning of these results, the $LC_{50}$ and $LC_{95}$ values for the insecticides tested against the Texas strain of *Cx. salinarius* were compared, via the computation of additional resistance ratios, to the values recorded for tests conducted during the same time period on females from the colony of the insecticide-susceptible UTMB strain of *Culex quinquefasciatus* Say maintained at Texas A&M University. The results of these comparisons are shown in Table 2 and indicate the Texas *Cx. salinarius* strain was just as susceptible (e.g., to malathion) and in most cases, more susceptible to the adulticides tested than was the UTMB strain of *Cx. quinquefasciatus*. The Texas strain of *Cx. salinarius* was thus deemed the best colony to keep on hand as a reference base for future efforts being planned for monitoring the status

Table 2. Insecticide resistance ratios for adult females of the colonized Texas strain of *Culex salinarius* vs. the colonized, insecticide-susceptible UTMB strain of *Culex quinquefasciatus*.

| Insecticide | $RR_{50}$[1] | $RR_{95}$[1] |
|---|---|---|
| Malathion | 1.07 | 2.43 |
| Naled | 0.57 | 0.82 |
| Chlorphyrifos[2] | — | — |
| Resmethrin | 0.17 | 0.48 |
| Permethrin | 0.05 | 0.06 |

[1] The median lethal concentration ($LC_{50}$) and 95% lethal concentration ($LC_{95}$) resistance ratios ($RR_{50}$ and $RR_{95}$, respectively) were determined by dividing the $LC_{50}$ and $LC_{95}$ values recorded for the Texas strain of *Cx. salinarius* by the comparable values recorded for the UTMB strain of *Cx. quinquefasciatus*.

[2] Chlorpyrifos was not tested against the UTMB strain of *Cx. quinquefasciatus*.

of resistance in wild populations of this mosquito species in Texas and elsewhere in the United States.

Based on the Texas A&M laboratory's experience in monitoring resistance levels in wild populations of *Cx. quinquefasciatus* using the UTMB strain of this species as a reference base, resistance ratio values that begin to approach a value of 10 indicate that the wild populations for which such values are being recorded are beginning to develop a tolerance to the particular pesticide in question. Ratios exceeding 10 indicate that a significant level of insecticide resistance is present in the target population. If the same trend holds true for *Cx. salinarius,* the $RR_{50}$ value of 9.22 recorded for the New Jersey strain of this species against malathion (Table 1) indicates that resistance to this particular chemical is beginning to develop in the New Jersey population of *Cx. salinarius* from which stock was taken to start our laboratory colony.

Judy A. Hansen (Director of the Cape May County Mosquito Control Commission, personal communication) indicated that the particular *Cx. salinarius* population from which our colonizing stock came had been periodically treated with malathion on an annual basis with aerial ULV applications of malathion for several years prior to our investigation. The overall adulticiding strategy used by the Cape May Mosquito Control Commission is to interdict adult populations of *Aedes sollicitans* (Walker) and sometimes those of *Cx. salinarius* as they migrate enmass from their marshland development sites upland and toward more human-populated areas of the county. This exposure, although limited in nature, may have been enough to cause the elevated tolerances to malathion noted for the New Jersey strain of *Cx. salinarius* used in our assessments. If such is the case, it demonstrates how careful mosquito control agencies must be in using any class of insecticides in their programs to avoid the development of resistance.

In this regard, the continuous use of only one kind or class of insecticide over long periods of time as a sole tactic for controlling a target mosquito population can put a great deal of insecticidal pressure on the population. Eventually, if such a strategy is continued, resistance to the insecticide or to a whole class of insecticides can be selected for in a manner as described by Frisbie et al. (1987). Indeed, the continuous use of malathion over a period of several years has caused increased incidences of resistance in populations of such mosquito species as *Cx. quinquefasciatus* (Bisset et al. 1991) and *Aedes albopictus* (Skuse) (Herbert and Perkins 1973, World Health Organization 1986). Although no evidence of insecticide resistance has yet to be reported in *Cx. salinarius,* our data indicate that such could happen in Cape May County, NJ, if it were not for the fact that the mosquito control commission in this county is following good insecticide resistance management (IRM) practices by using malathion sparingly and alternating classes of chemicals in its adulticiding program when possible. Even with these IRM practices in place, the tolerances of the Cape May County adult *Cx. salinarius* populations appear to be elevated for malathion and these tolerances need to be regularly monitored as long as this agent is used in the Cape May County mosquito adulticiding program.

In contrast to the situation in New Jersey, the Texas population of *Cx. salinarius* included in our bioassay appears to be quite susceptible to all the adulticides tested. This may be due in part to the fact that the Texas strain of this species had been in colony longer (11 generations) than the New Jersey strain (2 generations) before it was tested against insecticides, thereby giving more opportunity for susceptibility to become reestablished in the colony, as would occur in insecticide-free refugia in nature (Frisbie et al. 1987). Also, the fact that the stock used to establish the Texas strain colony of *Cx. salinarius* came from a wildlife refuge that had not been treated with any type of insecticide for at least 15 years (Hoyt Henry, Director, Chambers County Mosquito Control District, Anahuac, TX, personal communications) could explain why the Texas strain of this species proved so susceptible to the adulticides tested.

The research described herein established baselines of susceptibility for select populations of *Cx. salinarius* to commonly used adulticides and established a colony of a susceptible strain of this species for use in subsequent insecticide resistance monitoring programs. Also, our research points out the care that must be taken in using a given chemical in a mosquito control program and the value of maintaining insecticide-free refugia. Guarded and strategic use of any given insecticide and making provision for refugia where susceptible members of a target insect population can continue to survive and extend their susceptibility back into the whole of the target insect population are essential ingredients in any plan developed to proactively manage insecticide resistance in an insect population (Frisbie et al. 1987). Mosquito populations are no exception in this regard.

## ACKNOWLEDGMENTS

We thank Rudy Bueno, Jr., W. Powell Knight, Jr., and Roy C. Vogtsberger at Texas A&M University for their assistance in the mosquito colonization and bioassay effort and Judy A. Hansen and the personnel under her directorship at the Cape May County Mosquito Commission for their help in collecting mosquito stock in New Jersey.

## REFERENCES CITED

Bisset, J. A., M. M. Rodriguez, J. Hemingway, C. Diaz, G. J. Small and E. Ortiz. 1991. Malathion and pyrethroid resistance in *Culex quinquefasciatus* from Cuba: efficacy of pirimiphosmethyl in the presence of at least

three resistance mechanisms. Med. Vet. Entomol. 5: 223–228.

Clark, G. G., H. L. Pretula, T. Jakubowski and M. A. Hurd. 1977. Arbovirus surveillance in Illinois in 1976. Mosq. News 37:389–395.

Frisbie, R. E., W. P. Morrison, C. E. Hoelscher, S. R. Pullen, F. W. Plapp, Jr., C. T. Allen and J. W. Stewart. 1987. Insecticide resistance management. Texas Agricultural Extension Service Leaflet 500.3–87. Texas Agricultural Extension Service, College Station, TX.

Herbert, E. W. and P. V. Perkins. 1973. Comparative tests of five insecticides against *Aedes albopictus* larvae from South Vietnam. Mosq. News 33:76–78.

Plapp, F. W., Jr. 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.

SAS Institute, Inc. 1985. SAS user's guide statistics, version 5 edition. SAS Institute, Inc., Cary, NC.

World Health Organization. 1986. Resistance of vectors and reservoirs of disease to pesticides. 10th Report of the Expert Committee on Vector Biology and Control. WHO Tech. Rep. Ser. 737. Geneva, Switzerland.